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Review

Molecular physiology and genetics of Na^+-independent SLC4 anion exchangers

Seth L. Alper

Renal Division and Molecular and Vascular Medicine Unit, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, USA

e-mail: salper@bidmc.harvard.edu

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Summary

Plasmalemmal Cl^-/HCO_3^- exchangers are encoded by the *SLC4* and *SLC26* gene superfamilies, and function to regulate intracellular pH, $[Cl^-]$ and cell volume. The Cl^-/HCO_3^- exchangers of polarized epithelial cells also contribute to transepithelial secretion and reabsorption of acid–base equivalents and Cl^-. This review focuses on Na^+-independent electroneutral Cl^-/HCO_3^- exchangers of the SLC4 family. Human SLC4A1/AE1 mutations cause the familial erythroid disorders of spherocytic anemia, stomatocytic anemia and ovalocytosis. A largely discrete set of AE1 mutations causes familial distal renal tubular acidosis. The *Slc4a2/Ae2*$^{-/-}$ mouse dies before weaning with achlorhydria and osteopetrosis. A hypomorphic *Ae2*$^{-/-}$ mouse survives to exhibit male infertility with defective spermatogenesis and a syndrome resembling primary biliary cirrhosis. A human SLC4A3/AE3 polymorphism is associated with seizure disorder, and the *Ae3*$^{-/-}$ mouse has increased seizure susceptibility. The transport mechanism of mammalian SLC4/AE polypeptides is that of electroneutral Cl^-/anion exchange, but trout erythroid Ae1 also mediates Cl^- conductance. Erythroid Ae1 may mediate the DIDS-sensitive Cl^- conductance of mammalian erythrocytes, and, with a single missense mutation, can mediate electrogenic SO_4^{2-}/Cl^- exchange. AE1 trafficking in polarized cells is regulated by phosphorylation and by interaction with other proteins. AE2 exhibits isoform-specific patterns of acute inhibition by acidic intracellular pH and independently by acidic extracellular pH. In contrast, AE2 is activated by hypertonicity and, in a pH-independent manner, by ammonium and by hypertonicity. A growing body of structure–function and interaction data, together with emerging information about physiological function and structure, is advancing our understanding of SLC4 anion exchangers.

Key words: SLC4, chloride/bicarbonate exchange, renal tubular acidosis, spherocytosis, stomatocytosis.

Introduction

Electroneutral Cl^-/HCO_3^- exchange is widely expressed in diverse cell types. Cl^-/HCO_3^- exchange in these cells regulates intracellular pH (pH_i) and cell volume, and contributes to the regulation of membrane potential through its contribution to the transmembrane Cl^- gradient.

Cl^-/HCO_3^- exchangers are encoded by two evolutionarily unrelated gene superfamilies, *SLC4* and *SLC26*. The anion exchanger polypeptide products of these genes exhibit distinct phylogenetic relationships and distinct patterns of tissue and subcellular distribution, anion selectivity, transport mechanisms, and regulatory properties. Deficiencies in expression of SLC4 and SLC26 Cl^-/HCO_3^- exchanger polypeptides lead to characteristic phenotypes. This brief review will focus on electroneutral anion exchangers of the *SLC4* gene family, highlighting genetics, aspects of molecular mechanism and regulation.

The AE anion exchangers among the anion transporters of the *SLC4* gene family

The *SLC4* gene family comprises three major clades (Alper, 2002; Alper, 2006; Cordat and Casey, 2009; Romero et al., 2004; Stewart et al., 2007). The Na^+-independent, electroneutral Cl^-/HCO_3^- exchangers include SLC4A1/AE1, SLC4A2/AE2 and SLC4A3/AE3. The Na^+-dependent SLC4 HCO_3^- transporters include electrogenic (SLC4A4/NBCe1, SLC4A5/NBCe2) and electroneutral Na^+/HCO_3^- cotransporters (SLC4A7/NBCn1, SLC4A10/NBCn2), and a Na^+–$2HCO_3^-/Cl^-$ exchanger (SLC4A8/NDCBE). Also part of the Na^+-dependent clade is SLC4A9/AE4, but varying Na^+ dependence of function has been reported. SLC4A11/BTR1 is the lone mammalian member of a third clade that includes borate transporters of plants and fungi. SLC4A11 has been reported to mediate electrogenic Na^+–borate cotransport (Park et al., 2004), but its yeast Bor1p homolog has been proposed to mediate a different physiological function, despite its association with boron efflux driven by the inward proton gradient (Jennings et al., 2007). *SLC4* gene products from multiple mammalian species, teleost fish (Guizouarn et al., 2005; Paw et al., 2003; Shmukler et al., 2008; Shmukler et al., 2005), marine invertebrates, insects (Romero et al., 2000; Sciortino et al., 2001), ascidians, and the roundworm *C. elegans* (Sherman et al., 2005) have also been cloned, localized and functionally expressed (Romero et al., 2004). For many years *SLC4* genes were believed to be restricted to eukaryotic cells. However, the recent appearance of *SLC4* homologs among emerging genomes of marine bacteria has expanded the domain of this gene superfamily.

Structure of SLC4 AE polypeptides

All SLC4 polypeptides have in common three structural domains. An N-terminal hydrophilic, cytoplasmic domain of 400–700 amino acids is followed by a hydrophobic, polytopic transmembrane domain of ~500 amino acids, and completed by a C-terminal cytoplasmic domain of ~30–100 amino acids. Most *SLC4* genes express 5′-variant transcripts from alternate promoters to generate multiple polypeptide isoforms with distinct N-terminal amino acid

sequences. The *AE1* gene encodes the longer erythroid AE1 (eAE1, historically known as 'red cell band 3') and the shorter kidney AE1 (kAE1) which in human initiates at Met66 and in mouse at Met79 or Met80. The mouse *AE2* gene encodes five N-terminal variant polypeptides (the human gene encodes only four), while the *AE3* gene encodes two variant N-terminal and two variant C-terminal polypeptide sequences (Fig. 1).

Most structural information comes from four decades of study of the abundant, native erythroid AE1 protein and from more recent mutagenesis studies of recombinant AE1. A current topographical model of the AE1 monomer is shown in Fig. 2. AE1 is a dimer or tetramer in the membrane, and a detergent-solubilized dimer. The extreme N-terminal sequence of human AE1 binds multiple glycolytic enzymes (Campanella et al., 2008; Chu and Low, 2006) and hemoglobin (Chu et al., 2008) under the control of hemoglobin oxygenation. Other parts of the N-terminal cytoplasmic domain provide binding sites for the erythroid cytoskeletal proteins ankyrin-1 (Chang and Low, 2003; Stefanovic et al., 2007), protein 4.2 (Toye et al., 2005), the ERM protein 4.1R (Salomao et al., 2008), and integrin-linked kinase (Keskanokwong et al., 2007). Solution of a 2.6 Å X-ray structure of the dimeric human erythroid AE1 cytoplasmic domain (amino acids 1–379) required crystallization at pH 4.8 and resolved residues 55–201 and 212–356 within a globular domain (Zhang et al., 2000). This structure, although at odds with the elongated shape earlier predicted from the cytoplasmic domain's migration during gel filtration, has been supported by the techniques of electron paramagnetic (EPR) and double electron electron resonance (DEER) spectroscopies performed at neutral pH (Zhou et al., 2005). The portion of the eAE1 crystal structure that is absent from kAE1 encodes a central core β-sheet. Absence of that β-sheet in the recombinant kAE1 cytoplasmic domain, although without consequence for the circular dichroism spectrum or oligomeric state, was associated with decreased thermal stability and increased intrinsic fluorescence refractory to further dequenching by urea, and was consistent with a less compact structure (Pang et al., 2008).

Adding to classical proteolysis and chemical modification studies in intact erythrocytes, hydropathy analysis of the AE1 amino acid sequence led to a transmembrane domain model of 14 α-helical transmembrane spans. This model has been updated to include two re-entrant loops in the C-terminal portion of the transmembrane domain as predicted by cysteine scanning mutagenesis studies of partially functional Cys-less AE1 (Zhu and Casey, 2004; Zhu et al., 2003), and slightly modifying predictions from N-glycosylation insertion mutagenesis experiments (Popov et al., 1999). An additional re-entrant loop in the N-terminal region of the transmembrane domain was more recently predicted by N-glycan insertional mutagenesis (Cheung and Reithmeier, 2005).

E681 of human eAE1 (E699 in mouse) has been identified biochemically and by mutagenesis as a likely part of the permeability barrier within the anion translocation pathway (Chernova et al., 1997; Jennings, 1995). Group-specific chemical modification of AE1 in intact red cells has suggested that, in addition to glutamate residues, histidine, arginine and lysine residues each contribute to anion transport and selectivity (Stewart et al., 2007). The binding site for stilbene inhibitors is believed to sit astride the external vestibule leading to the anion translocation pathway. The lysine residues that react covalently with the isothiocyanate groups of the stilbene disulfonate inhibitor of anion exchange, H_2DIDS, have been identified, but other parts of the stilbene disulfonate interaction surfaces of AE1 remain under study (Salhany et al., 2005). Crystals of the AE1 transmembrane domain have been generated, but remain insufficiently ordered for X-ray structure analysis.

The central transmembrane domain of AE1 also includes exofacial and possibly intra-bilayer interaction sites for erythroid glycophorin A, which acts like a 'β-subunit' for AE1 trafficking and optimal function (Williamson and Toye, 2008; Young et al., 2000). Glycosylphosphatidylinositol-linked carbonic anhydrase IV (Sterling et al., 2002) and transmembrane carbonic anhydrase IX (Morgan et al., 2007) also interact with exofacial portions of SLC4 transmembrane domains. The exofacial loops of AE1 carry allo-transplantation antigens (Jarolim et al., 2004; Jarolim et al., 1998a), contribute to the generation of autoimmune disease antigens in NZB mice (Hall et al., 2007), and may also serve as part of the receptor(s) for plasmodial merozoite invasion (Goel et al., 2003).

The C-terminal tail of AE1 and other SLC4 polypeptides contains one or more acidic motifs that may serve as a binding site for cytoplasmic carbonic anhydrase II (CAII) (Sterling et al., 2001; Vince and Reithmeier, 2000). The cytoplasmic binding of CAII and the simultaneous interaction of SLC4 anion exchangers with ecto-carbonic anhydrases has been proposed to constitute a bicarbonate transport metabolon serving to accelerate transmembrane bicarbonate transport by concentrating or consuming transported substrate near internal and external substrate-binding and release sites (Sterling et al., 2002; Sterling et al., 2001). However, others have argued against both the physiological presence and potential importance of the physical

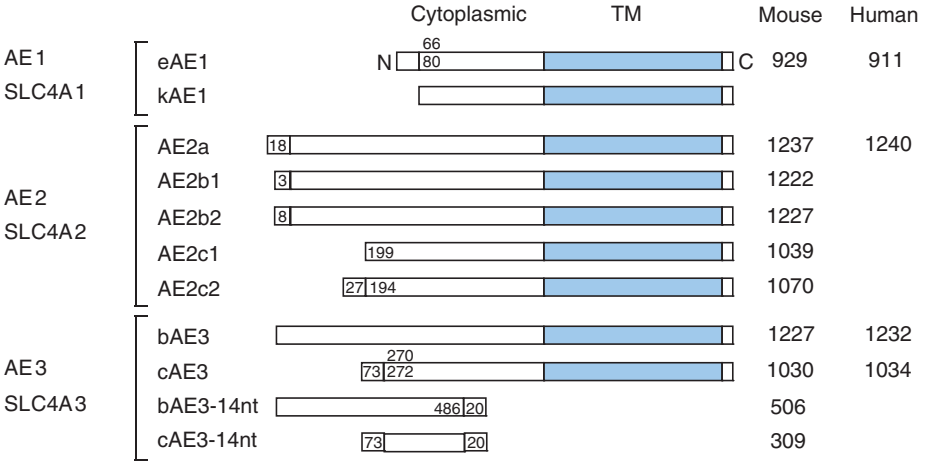

Fig. 1. Schematic diagram of polypeptide variants expressed by the genes encoding the SLC4 Na^+-independent anion exchangers, AE1, AE2 and AE3. Predicted transmembrane (TM) domains are blue. Total polypeptide lengths are on the right. Lengths of variant N-terminal sequences are indicated within the leftmost boxes, and lengths of variant C-terminal domains (for the AE3-14nt variants) in the rightmost boxes. Modified from Stewart et al. (Stewart et al., 2007).

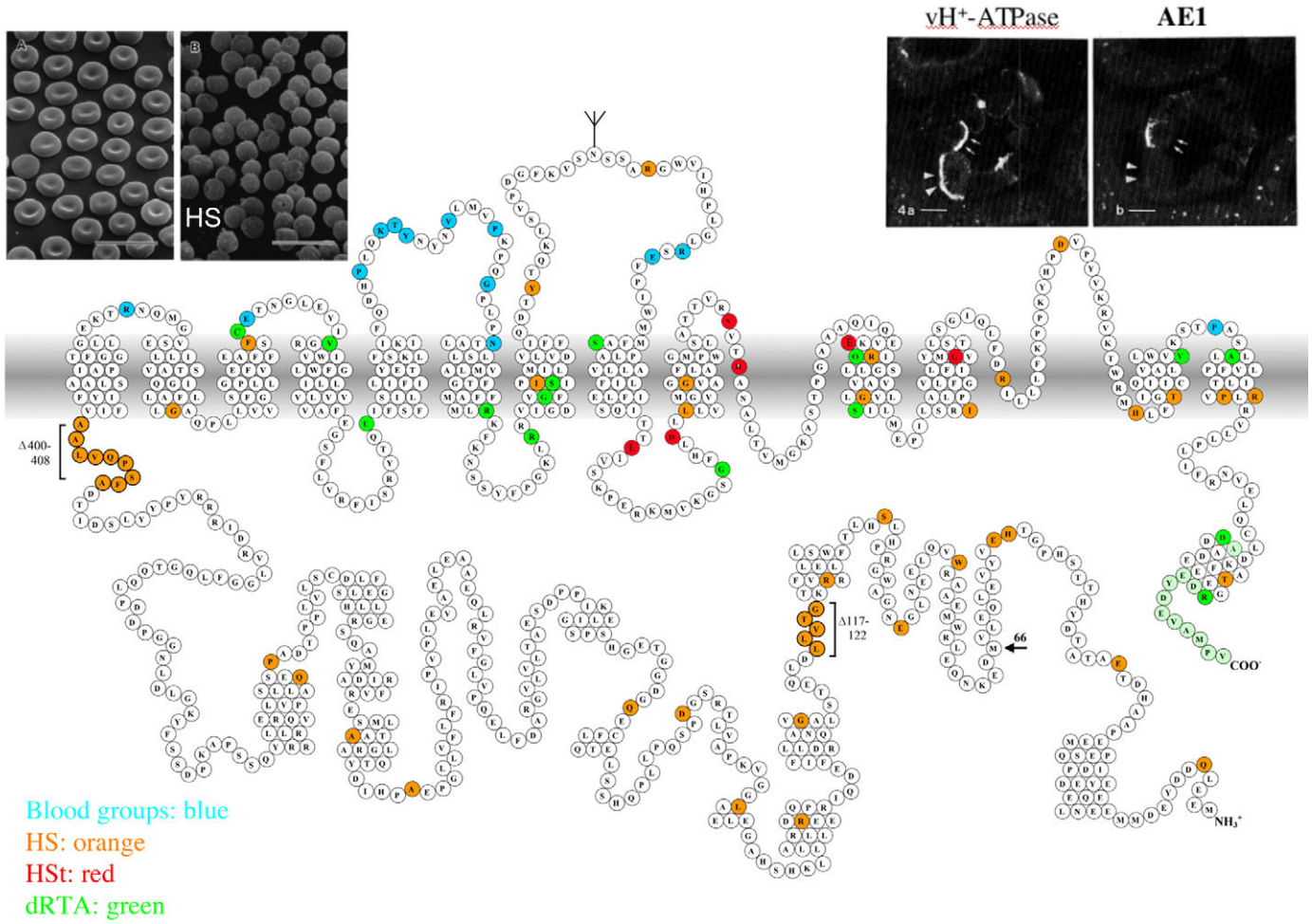

Fig. 2. Proposed topographical model for the human SLC4A1/AE1 Cl^-/HCO_3^- exchanger polypeptide, after Zhu et al. (Zhu et al., 2003). Met66 (arrow) marks the start of kidney AE1. Polymorphisms encoding blood group antigens are blue. The mutations associated with hereditary spherocytic anemia and ovalocytosis are orange, and include missense, nonsense, splicing and deletion mutations. Missense mutations associated with hereditary stomatocytosis and xerocytosis are red. Mutations associated with dominant and recessive distal renal tubular acidosis are green. Terminal deletions are in lighter orange and green. Upper left: scanning electron micrographs of wild-type erythrocytes and $AE1^{-/-}$ bovine spherocytes (HS) (Inaba et al., 1996). Upper right: consecutive semithin sections from rat kidney cortex immunostained with antibodies recognizing vH^+-ATPase (left) and kAE1 (right). Only the Type A intercalated cell with apical vH^+-ATPase expresses basolateral kAE1 (Alper et al., 1989). HS, hereditary spherocytic anemia; HSt, hereditary stomatocytosis; dRTA, distal renal tubular acidosis. Scale bars 10 μm at top left; 7 μm, top right. Modified from Shayakul and Alper, and Stewart (Shayakul and Alper, 2004; Stewart et al., 2007).

interaction between SLC4 transporters and carbonic anhydrases (Lu et al., 2006; Piermarini et al., 2007). The AE1 C-terminal tail may provide a second binding site for the glycolytic enzyme GAPDH (Su et al., 2008), as well as a binding site for the glomerular podocyte protein nephrin (F. Wu, 2008). Human eAE1 is N-glycosylated on N642 in the fourth extracellular loop and in erythrocytes is polylactosaminylated. The physiological significance of N-glycosylation remains elusive, but the extent of terminal glycan processing varies at N-glycosylation sites engineered in different extracellular loops (Li et al., 2000). Pharmacological inhibition of N-glycosylation in AE2 had neglible consequences for function and apparent surface expression (Fujinaga et al., 2003). Human eAE1 C843 is palmitoylated in erythrocytes, but neither human nor mouse Ae1 is detectably palmitoylated in recombinant expression systems, and mutation of the target Cys does not alter expression, folding or targeting (Cheung and Reithmeier, 2004). Human eAE1 phosphorylation at Y8 and Y21 controlled by cell volume and/or tonicity and by cell oxidation state can regulate binding and activity of glycolytic enzymes (Campanella et al., 2005), but likely not anion transport (Brunati et al., 2000). Phosphorylation at Y359 and Y904 regulates trafficking in polarized epithelial cells, as will be discussed below.

Mechanism of electroneutral anion exchange by SLC4/AE polypeptides

SLC4A1/AE1 mediates 1:1 electroneutral exchange of many monovalent anions, but Cl^- and HCO_3^- are its major physiological substrates. Red cell eAE1 has also been shown to mediate H^+/sulfate and H^+/oxalate cotransport in electroneutral exchange for Cl^- (Jennings and Adame, 1996). These latter transport modes may be of importance *in vivo* in the setting of pathologically acidic pH. Monovalent anion exchange can be modeled by ping-pong kinetics, with alternating access of substrate-binding sites and with modifications for a second, possibly regulatory anion-binding site. The equilibrium distribution of inward-facing and outward-facing

conformations is anion substrate dependent in symmetrical anion solutions, but this conformational regulation is not thought to be important in physiological solutions (Jennings, 2005; Knauf and Pal, 2003).

The molecular basis of anion selectivity within AE1 and other anion exchangers remains poorly understood. Expression in *Xenopus* oocytes of AE1 deletion or missense mutants of the C-terminal tail lacking the putative CAII-binding site led to loss of Cl^-/HCO_3^- exchange without inhibition of Cl^-/Cl^- exchange. The loss of Cl^-/HCO_3^- exchange activity was rescued completely by coexpression of the binding site carried on either of two surface expression-competent AE1 polypeptides devoid of their own transport activity (Dahl et al., 2003). If indeed the oocyte lacks CAII (Nakhoul et al., 1998), then the C-terminal cytoplasmic tail may play a direct role in maintaining HCO_3^- selectivity, and can do so in *trans* (from one protomer to another within a dimer). Alternatively, bound CAII (apparently endogenous to the oocyte) might be crucial for HCO_3^- transport, and binding to an adjacent protomer within a dimer brings CAII into proximity sufficient to play this role (Dahl et al., 2003). Consistent with the latter hypothesis, dominant negative carbonic anhydrase II (CAII) coexpression in HEK-293 cells inhibits Cl^-/HCO_3^- exchange by the overexpressed SLC4 polypeptides AE1, AE2 and AE3. The inability of overexpressed CAII to stimulate Cl^-/HCO_3^- exchange activity in SLC4-transfected cells has been attributed to high endogenous CAII expression, perhaps consistent with the failure of an SLC4–CAII fusion protein to accelerate Cl^-/HCO_3^- exchange (Lu et al., 2006). The functional importance of the Ca2/Ae1 transport metabolon has not yet been tested in intact red cells of wild-type and *Ca2*$^{-/-}$ mice. However, the distal renal tubular acidosis of *Ca2*$^{-/-}$ mice is at least consistent with a requirement for Ca2, whether bound to Ae1 or not, in Cl^-/HCO_3^- exchange by renal collecting duct Type A intercalated cells at rates normally required to preserve systemic pH.

Trout AE1 expressed in *Xenopus* oocytes increased constitutively active Cl^- conductance with properties similar to that of the intact trout red cell. The expression of Cl^- conductance has been mapped to two discreet regions of the trout AE1 transmembrane domain, and in engineered mutants need not be tightly linked to the anion exchange mechanism (Borgese et al., 2004). In contrast, expression in *Xenopus* oocytes of AE1 polypeptides from mouse, zebrafish or skate (Borgese et al., 2004) or of AE2 polypeptides from mouse or zebrafish (Shmukler et al., 2008; Shmukler et al., 2005) does not increase Cl^- conductance. Trout AE1 expression in resting oocytes also increases transport of small neutral or zwitterionic osmolytes, whereas osmolyte transport associated with skate AE1 expression requires activation by hypotonic swelling (Koomoa et al., 2005). Thus among AE1 orthologs tested to date in *Xenopus* oocytes, only trout AE1 mediates detectable conductive transport and exchange of anions.

A single missense mutation can, however, render human or mouse AE1 electrogenic under defined conditions. Chemical modification of human glutamate to hydroxynorvaline in position 681 at the inner face of putative transmembrane span 8, or mutation of the corresponding mouse Ae1 residue E699 to glutamine, creates transporters which mediate electrogenic 1:1 exchange of internal SO_4^{2-} for extracellular Cl^-. Mouse Ae1 E699Q also mediates electroneutral sulfate homoexchange, but cannot mediate detectable efflux of intracellular Cl^- in exchange for any extracellular anion. Sulfate transport by these mutant AE1 polypeptides is unaccompanied by H^+ cotransport, whether in electrogenic or electroneutral modes. These properties together suggest that human AE1 E681 and mouse Ae1 E699 serve as the H^+-binding site during H^+/SO_4^{2-} cotransport, and as part of the permeability barrier within the AE1 anion translocation pathway (Chernova et al., 1997; Jennings, 1995). Modification of these Glu residues may also increase the binding affinity of an apparent second external anion-binding site (Chernova et al., 2008; Jennings, 2005; Salhany et al., 2000).

Human erythroid Cl^- conductance, functioning in concert with the erythroid KCa3.1 Ca^{2+}-activated K^+ channel, is central to the control of red cell volume during passage through capillaries, during oxidative stress of hemoglobinopathies, and during intraerythrocytic replication of malarial parasites. The DIDS-sensitivity of a major portion of the basal erythroid Cl^- conductance has long suggested that AE1 might mediate this conductance, perhaps by a 'tunneling' mechanism. The DIDS-sensitive component of anion conductance is indeed lacking in murine *Ae1*$^{-/-}$ erythrocytes, but many other membrane proteins are also reduced in abundance in these fragile spherocytic cells. Thus, although AE1 expression is required for expression of erythroid Cl^- conductance, that conductance may still be mediated by a distinct ion channel polypeptide (Alper et al., 2008). A comprehensive understanding of AE1 anion selectivity and transport mechanism will await higher resolution structural information about its transmembrane domain. The recent emergence in marine bacterial genome sequences of SLC4 homologs offers a new route to crystallization and high resolution structure determination of an SLC4 superfamily member.

eAE1 erythroid disease phenotypes

Erythrocytes and Type A acid-secreting intercalated cells of the renal collecting duct are the sites at which AE1 polypeptide accumulates in greatest abundance. In the erythrocyte, eAE1 stabilizes the lipid bilayer and its linkage to the underlying cytoskeleton, and increases the CO_2 carrying capacity of blood to allow up to a 10-fold increase in oxygen consumption and CO_2 exhalation during peak exertion. In the kidney, intercalated cell kAE1 reabsorbs HCO_3^- during apical H^+ excretion into the urine. Mutations of the human *AE1* gene (Fig. 2) are thus, not surprisingly, characterized by erythroid and renal phenotypes [for lists of these mutations, see Kurschat and also Stewart (Kurschat, 2007; Stewart et al., 2007)].

One group of polymorphisms (blue in Fig. 2) encodes almost entirely asymptomatic blood group antigen variants recognized on intact erythrocytes by patient alloantisera. These serological reactivities have established the surface topography of the first several extracellular loops of the AE1 transmembrane domain. The largest group of mutations (orange in Fig. 2) is associated with autosomal dominant hereditary spherocytic anemia (HS), a hemolytic anemia associated with reticulocytosis, hyperbilirubinemia, jaundice, gallstones and splenomegaly. HS red cells are characterized by reduced surface area and osmotic fragility. Many of these mutant alleles generate unstable mRNA. The resulting erythrocytes express reduced total levels of wild-type eAE1 polypeptide, but often in the setting of dosage compensation by the wild-type allele. Splenectomy can ameliorate the anemia for some patients. Autosomal dominant HS patients almost always have an apparently normal renal acidification phenotype. eAE1 is part of a multi-component megadalton protein macrocomplex in the erythroid plasma membrane (Bruce et al., 2003). Thus, similar HS syndromes in dominant and recessive forms are also caused by mutations of the genes encoding the macrocomplex proteins spectrin, ankyrin

and partial or complete deficiency of protein 4.2 or of RhAG polypeptide, a component of the putative gas transporter Rhesus antigen (An and Mohandas, 2008).

Southeast Asian Ovalocytosis (SAO) is caused by an autosomal dominant, heterozygous in-frame deletion of hAE1 amino acids 400–408 (orange in Fig. 1). Homozygotes have not been found, and are presumed to be embryonic lethal. The stable mutant polypeptide is present at normal abundance in the membrane, where it heterodimerizes at apparent normal affinity with wild-type polypeptide (Jennings and Gosselink, 1995). Although AE1 SAO is itself functionally inactive in both Cl^-/Cl^- and Cl^-/HCO_3^- exchange (Dahl et al., 2003), the minimal impact of its dominant effects upon the wild-type monomer within the SAO/wt heterodimer (Cheung et al., 2005; Kuma et al., 2002) explain its lack of renal phenotype. Heterozygote red cell membranes exhibit increased rigidity and cold-induced cation permeability, and the allele seems to confer protection against cerebral complications of malaria.

Only two AE1 mutations have been found in severe, early-onset recessive HS, in each case in progeny of consanguineous parents with mild autosomal dominant HS. Band 3 Neapolis (Perrotta et al., 2005) is an intron 2 splice donor site mutation resulting in skipping of exon 2 and unstable mRNA encoding an AE1 polypeptide lacking the N-terminal 11 amino acids, and present at only 12% of the normal level. Homozygosity for Band 3 Coimbra (AE1 V488M) is associated with the complete absence of AE1, and causes severe neonatal hemolytic spherocytic anemia and recessive distal renal tubular acidosis (dRTA) (Ribeiro et al., 2000).

The consequences of several HS mutations associated with protein 4.2 deficiency have been assessed on the structure of the recombinant AE1 cytoplasmic domain. HS AE1 mutants E40K and G130R exhibited no detectable structural change in their cytoplasmic domains. The recombinant eAE1 P327R cytoplasmic domain maintained a normal large scale structure and dimeric state, but with slightly reduced thermal stability (Bustos and Reithmeier, 2006), accompanied by subtle EPR and DEER spectral changes in residues surrounding the mutation site (Zhou et al., 2007).

A minimally overlapping set of AE1 mutations (red in Fig. 2) is found in families with the disorder of hereditary stomatocytosis with cation leak (Bruce et al., 2005; De Falco, 2008). The mutations cluster between the fourth cytosolic loop and putative re-entrant loop 2 of AE1. One of the mutations had been previously classified as causing HS. The AE1 stomatocytosis mutants are characterized by red cell cation leak of complex and distinct profiles of temperature dependence and cell volume, accompanied variably by hemolytic anemia and pseudohyperkalemia. SAO may also be part of this group, as SAO erythrocytes exhibit cation leak after cold storage. When expressed in *Xenopus* oocytes, AE1 stomatocytosis mutants mediate a non-specific cation conductance, often in the setting of loss or reduction of Cl^- transport and Cl^-/HCO_3^- exchange (Guizouarn et al., 2007). However, this cation conductance is stilbene disulfonate insensitive, and can be accompanied by a range of greatly increased anion permeabilities of varied pharmacological sensitivity (A. K. Stewart, D. H. Vandorpe, P. G. Gallagher and S.L.A., unpublished data).

kAE1 renal disease phenotypes

A distinct set of AE1 mutations causes distal renal tubular acidosis (dRTA; green in Fig. 2). As noted above for AE1 V488M and possibly for Band 3 Neapolis, these mutations in the homozygous state are only rarely accompanied by an erythroid phenotype. dRTA is characterized by impaired urinary acid excretion in the setting of metabolic acidosis (complete dRTA) or imposed acid loading (incomplete dRTA). That metabolic acidosis retards growth, and can be accompanied by hypercalciuria and hypokalemia. Inadequate treatment with bicarbonate supplementation can lead to osteomalacia, nephrocalcinosis and nephrolithiasis. The overexpressed mutant polypeptides can express distinct, characteristic trafficking phenotypes depending on host cell type, plating matrix, and degree of confluence and polarization (Cordat et al., 2006; Kurschat, 2007).

The AE1 mutants associated with dominant dRTA expressed in *Xenopus* oocytes usually exhibit normal or modestly reduced Cl^- and HCO_3^- transport function inadequate to explain the renal phenotype. Defective urinary acidification arises from trafficking defects of these mutant polypeptides in polarized epithelial cells. One class of dominant dRTA mutations such as AE1 R589H (Jarolim et al., 1998b) and S613F is retained in the endoplasmic reticulum, and exerts a dominant negative trafficking phenotype within heterodimers with the wild-type AE1 polypeptide. A second class of dominant dRTA mutation is exemplified by AE1 901X, lacking the C-terminal 11 amino acids. This mutant accumulates either uniquely in the apical membrane or in both apical and basolateral membranes of polarized epithelial cells, apparently due to the loss of a sorting or retrieval signal related to residues 904–907 (Cordat et al., 2006; Devonald et al., 2003; Toye et al., 2004). The presence of functional, mistargeted kAE1in the apical membrane of the Type A intercalated cell likely short-circuits acid secretion through a codominant mechanism. However, substantial intracellular retention of this mutant has been observed (Cordat, 2006) and more extensive, engineered truncation of the C-terminal tail further increased intracellular retention of the mutant protein (Cordat, 2006; Dahl et al., 2003).

All above observations about dRTA-associated AE1 mutant targeting are based on studies in confluent MDCK cell monolayers. Only two reports of AE1 immunolocalization in dRTA have appeared. In a renal biopsy from a patient with the autosomal dominant mutant AE1 R589H (retained in the endoplasmic reticulum of MDCK cells), chronic scarring from pyelonephritis and nephrocalcinosis complicated interpretation of small Type A intercalated cells with undetectable kAE1 (Shayakul et al., 2004). In a renal biopsy from a patient with the autosomal dominant mutant AE1 S613F (partially mistargeted to the apical membrane of MDCK cells), all AE1 was again intracellular within a reduced number of small Type A intercalated cells (Walsh et al., 2007).

Delocalization of human kAE1 basolateral targeting or mistargeting to the apical membrane can be caused by mutation of sequence elements in both the N-terminal cytoplasmic domain and in the C-terminal cytoplasmic tail (Toye et al., 2004). The N-terminal cytoplasmic domain of the chicken kidney AE1 variant ‘AE1-4’ contains targeting information for polarized basolateral expression within its N-terminal 60 residues, including a YXXΦ motif (Adair-Kirk et al., 2003) required for caveolin-dependent sorting in MDCK cells (Dorsey et al., 2007). Basolateral localization of human kAE1 requires two phosphorylatable tyrosines, Y359 in the N-terminal cytoplasmic domain and Y904 in the C-terminal tail. kAE1 phosphorylation state is stimulated by hypertonicity, by pervanadate inhibition of phosphatases and by extreme elevation of bicarbonate concentration. The kinases responsible for kAE1 phosphorylation have not been identified, but may be those functional in erythrocytes. Regulated tyrosine phosphorylation likely governs trafficking of kAE1 in intercalated cells (Williamson et al., 2008).

Homozygous AE1 mutants causing recessive dRTA are generally found in Thailand (Tanphaichitr et al., 1998), Malaysia and New Guinea (Bruce et al., 2000). As exemplified by recessive dRTA mutant AE1 G701D, these homozygous mutant polypeptides are retained inside the cell, but their trafficking to the cell surface can be rescued by coexpression of the 'eAE1 β-subunit' glycophorin A. This rescue by an erythroid protein not expressed in renal intercalated cells can explain the normal erythroid AE1 expression in these patients (Tanphaichitr et al., 1998). AE1 G701D reaches the Golgi compartment in polarized MDCK cells. Coexpression with wild-type AE1 has demonstrated hetero-oligomer formation with rescue to the basolateral plasma membrane, consistent with the lack of renal phenotype among heterozygotes. The AE1 G701D trafficking defect is reproduced by substitution with any charged residue, but not by substitution with the uncharged residues Ala or Leu (Cordat, 2006; Cordat et al., 2006). Most recessive dRTA mutants of AE1 exhibit this conditional loss-of-function phenotype sensitive to rescue by glycophorin A. However, glycophorin A only partially rescues activity of the recessive mutant AE1 S667P, associated with both HS and complete dRTA. In addition, glycophorin A cannot rescue at all the most clinically severe homozygous mutation yet reported in humans, V488M (Toye et al., 2008). This mutation found in a single family presented perinatally with combined severe hemolytic anemia and renal tubular acidosis, associated with complete absence of eAE1 polypeptide in erythrocytes (Ribeiro et al., 2000).

Recessive dRTA caused by compound heterozygosity of AE1 mutations is sometimes accompanied by SAO or HS. The functionally inactive SAO allele fails to complement a recessive loss-of-function allele in the Type A intercalated cell (Bruce et al., 2000). The G701D allele cannot be rescued to the basolateral membrane by a mutant allele product such as C479W that is retained in the endoplasmic reticulum (Woods, 2008). Recently, selected AE1 dRTA mutants of both recessive and dominant type, when expressed in *Xenopus* oocytes were found to confer increased non-specific cation conductance (Walsh et al., 2008). The proposal that the cation leak of AE1 G701D might contribute to the potentially severe hypokalemia of dRTA would be unlikely if, as in MDCK cells, AE1 G701D is not expressed at any surface membrane in renal collecting duct Type A intercalated cells. Moreover, hypokalemia has not been reported in AE1 G701D obligate heterozygotes, despite the ability of wild-type AE1 partially to rescue AE1 G701D to the basolateral membrane in MDCK cells. Although recessive dRTA tends to be clinically more severe than the dominant form, specific genotype–phenotype correlations have yet to emerge within the sets of dominant and recessive AE1 mutations causing dRTA.

AE1 deficiency diseases show similar phenotypes in animal models. The *Ae1*$^{-/-}$ mouse has runting with combined severe hemolytic anemia (Peters et al., 1996) and a hypercoagulable state (Hassoun et al., 1998), accompanied by complete dRTA (Stehberger et al., 2007). Interestingly, the isolated medullary collecting ducts from the 10–20% of *Ae1*$^{-/-}$ mice surviving to 12 weeks of age retained 80% of wild-type Cl^-/HCO_3^- exchange activity, but with novel pharmacosensitivity. The Type A intercalated cell basolateral membrane Cl^-/HCO_3^- exchanger Slc26a7 upregulation in the setting of Ae1 deficiency (Sun and Petrovic, 2008) may account for this, but the pharmacological sensitivity of the activity upregulated in the *Ae1*$^{-/-}$ mouse argues against it (Stehberger et al., 2007). The *Ae1*$^{-/-}$ mouse also exhibits a more severe concentrating defect than that usually present in humans with AE1-related dRTA, perhaps secondary to the severe nephrocalcinosis and hemosiderosis of the mouse model. *Ae1*$^{-/-}$ mice also exhibit variable albuminuria at 12 weeks age, perhaps related to loss of Ae1 expression in glomerular podocytes (F. Wu, 2008) in addition to the oxidative damage accompanying hemosiderosis. Two additional, spontaneous mouse models of HS with severe Ae1 deficiency in red cells have not yet been examined for distal renal tubular acidosis (Stewart et al., 2007).

Ae1$^{-/-}$ mice exhibit a dilated, fibrotic cardiomyopathy with increased heart weight to body weight ratio in the setting of runting and increased left ventricular mass), but compensatory changes in mRNA levels of alternate regulators of pH_i were absent (Alvarez et al., 2007b). The accompanying high-output cardiac insufficiency is likely secondary to severe hemolysis and resultant oxidative stress. Systemic volume depletion secondary to the urinary concentrating defect and polyuria (Stehberger et al., 2007), if accompanied by increased serum aldosterone, might further contribute to this cardiac fibrotic change. AE1 is expressed at a low level in normal cardiac sarcolemma, but the cardiac AE1 isoform remains undefined, and its role in cardiac function is unclear.

A bovine cohort presenting with severe hemolytic anemia, likely renal tubular acidosis and perinatal death was found to harbor the homozygous recessive bovine AE1 mutation 664X (Inaba et al., 1996). This mutant also causes autosomal dominant HS, and is subject to ubiquitin-independent proteosomal degradation in the endoplasmic reticulum (Ito et al., 2007). The severely anemic zebrafish *retsina* mutants are also Ae1 loss-of-function mutations, with severe anemia (Paw et al., 2003). Erythroid precursors exhibit a cytokinesis defect in nearly all late nucleated erythroid precurors of zebrafish, and in a small percentage of precursors in the mouse. The resulting binucleate cells resemble those of type II congenital dyserythropoietic anemia (CDA), HEMPAS disease (Paw et al., 2003). A patient of dyserythropoietic phenotype was recently reported to carry a novel AE1 mutation in association with dehydrated stomatocytosis (De Falco, 2008), but none of the three types of CDA maps to the AE1 locus. Nonetheless, red cell AE1 abundance is reduced in patients with CDAII secondary to mutations in two distinct genes (Zdebska et al., 2007).

Disease phenotypes associated with SLC4A2/AE2 and SLC4A3/AE3 gene products

AE2 is the most widely expressed of the electroneutral, Na^+-independent SLC4/AE anion exchangers, present at highest levels in the epithelial cell basolateral membrane of choroid plexus, gastric parietal cells, throughout the GI tract, and in some cell types of respiratory and genital tracts. AE2 in exocrine glands is expressed in acinar cell basolateral membranes, but minimally in duct epithelial cells. It is also expressed throughout the nephron, but most abundantly in the medullary thick ascending limb and the inner medullary collecting duct. AE2 is a major Cl^-/HCO_3^- exchanger of the osteoclast contralacunar membrane (Wu et al., 2008; Josephsen et al., 2009) and in the lateral membrane of dental secretory ameloblasts (Lyaruu et al., 2008). However, hepatobiliary epithelial cells exhibit apical (Aranda et al., 2004; Martinez-Anso et al., 1994) or subapical (Tietz et al., 2003) immunolocalization of AE2. Apical membrane AE2 was also detected in the ameloblast of the *Ae2*$_{a,b}^{-/-}$ mouse (Paine et al., 2008) in unfixed sections, but without the accompanying *Ae2*$^{-/-}$ tissue controls included in the study detecting lateral localization in the complete Ae2 knockout mouse (Lyaruu et al., 2008).

No hereditary human diseases have been mapped to the *AE2* gene, but a synonymous cSNP (coding single nucleotide polymorphism) in the *AE2* gene has been strongly associated with

clinical response to ursodeoxycholic acid therapy in primary biliary cirrhosis (Poupon et al., 2008). The *Ae2*$^{-/-}$ mouse dies at or before weaning, and exhibits severe growth retardation, failure of tooth eruption, osteopetrosis and gastric achlorhydria with gastric mucosal dysplasia (Gawenis et al., 2004). This severe phenotype contrasts with the milder phenotype of a mouse engineered to lack Ae2a, Ae2b1 and Ae2b2, while nominally retaining expression of Ae2c. The grossly normal *Ae2*$_{a,b}^{-/-}$ mouse exhibits male infertility associated with testicular dysplasia (Medina et al., 2003). However, the *Ae2*$_{a,b}^{-/-}$ mouse is also hypomorphic for other phenotypes of the complete knockout mouse, including osteopetrosis (Josephsen et al., 2009), failure of dental enamelization (Lyaruu et al., 2008), and reduced stimulated gastric acid secretion in the setting of preserved basal acid secretion, accompanied by chronic, mild mucosal degeneration (Recalde et al., 2006).

The *Ae2*$_{a,b}^{-/-}$ mouse also exhibits characteristics resembling human primary biliary cirrhosis, including a high prevalence of anti-mitochondrial antibodies, splenomegaly, $CD8^+$ T lymphocyte expansion, and $CD4^+$ Treg lymphocyte depletion. Since siRNA-mediated suppression of Ae2 in rat cholangiocytes decreased secretin- and taurocholate-stimulated apical HCO_3^- secretion (Banales et al., 2006), decreased Ae2-mediated bile secretion secondary to inflammatory and cytokine-mediated damage may contribute to primary biliary cirrhosis in mouse and human (Arenas et al., 2008). Apical SLC26 Cl^-/HCO_3^- exchangers and HCO_3^- channels may also contribute to bile secretion. One of three *Ae2*$^{-/-}$ mice develop periportal inflammation with $CD8^+$ inflammation surrounding damaged biliary ducts (Salas et al., 2008). Ae2 expression in lymphoid cells (Alper et al., 1988) may thus be of immunological consequence. Treatment with combined ursodeoxycholate (UDC) and glucocorticoids can retard the progression of primary biliary cirrhosis in humans, and can attenuate the reduction in hepatic AE2 polypeptide associated with the disease (Medina et al., 1997). Glucorticoids have long been known to increase intestinal mucosal Ae2 mRNA levels (Chow et al., 1992). The combination of UDC and glucocorticoids increases transcription from the overlapping intron 2 promoters driving expression of the liver-enriched Ae2 variants Ae2b1 and AE2b2, resulting in increased Ae2 polypeptide and Cl^-/HCO_3^- exchange activity. Increased transcription results from enhanced p300-related interaction of HNF-1 and glucocorticoid receptor at promoter-binding sites (Arenas et al., 2008). The synonymous polymorphism of AE2 linked to ursodeoxycholate responsiveness in primary biliary cirrhosis may be part of a haplotype that includes polymorphisms within the AE2b1/2 promoter, or intronic polymorphisms governing mRNA stability.

SLC4A3/AE3 is expressed at the highest abundance in brain and heart, but is also present in gut and kidney. Human AE3 mutations have not been directly linked to disease, but the AE3 A867D polymorphic variant has been found with elevated frequency among patients with idiopathic generalized epilepsy (Sander et al., 2002). In support of this association, the grossly normal *Ae3*$^{-/-}$ mouse exhibits enhanced susceptibility to pharmacologically induced seizures (Hentschke et al., 2006), and the human AE3 A867D variant exhibits decreased Cl^-/HCO_3^- exchange activity in HEK-293 cells (Vilas et al., 2008). These data, together with the finding that *Ae3*$^{-/-}$ mouse hippocampal neurons lack detectable Cl^-/HCO_3^- exchange activity (Hentschke et al., 2006), suggest that Ae3 plays a critical role in the maintenance of the chloride equilibrium potential and/or pH_i in these neurons in the mouse, and perhaps also in humans. Ae3 may play a similar role in neurons controlling respiratory rate, which in *Ae3*$^{-/-}$ mice is lower but more sensitive to hypercapnia than in wild-type mice (Meier et al., 2007).

AE3 is expressed in cardiomyocytes (Papageorgiou et al., 2001; Yannoukakos et al., 1994), but at lower apparent abundance than the Cl^-/HCO_3^- exchanger SLC26A6 (Alvarez et al., 2004). Two independently derived *Ae3*$^{-/-}$ mice have exhibited no cardiac phenotype, but cardiac contractility *in vivo*, in perfused heart, and in isolated cardiomyocytes was impaired in the combined genetic absence of the two chloride loaders Ae3 and Nkcc1 (Prasad et al., 2008). Distinct Ae3 isoforms are also expressed in Muller cells and horizontal neurons of the retina (Kobayashi et al., 1994). One line of *Ae3*$^{-/-}$ mice exhibits late onset retinal degeneration and progressive blindness at 4–6 months of age, accompanied by upregulation of NBCe1/SLC4A4, CAII and CAIV (Alvarez et al., 2007a). Retinal Ae3 deficiency might alter chloride equilibrium potential to decrease GABA- or glycine-mediated inhibitory input, as postulated for the *Ae3*$^{-/-}$ brain.

No phenotype has as yet been reported for the grossly normal *Ae4*$^{-/-}$ mouse (Simpson et al., 2007).

Acute regulation of SLC4/AE-mediated anion exchange

As regulators of cellular and systemic pH, SLC4/AE anion exchangers might be expected to be sensitively regulated by pH_i and/or local extracellular pH (pH_o). However, this may not be true in the physiological pH range for eAE1, which must mediate high rates of transport in both the acidic environment of respiring tissues and the neutral pH of arterial blood. Tyrosine phosphorylation of skate eAE1 has been proposed to play a role in regulatory volume decrease mediated by AE1-associated osmolyte transport in skate erythrocytes (Musch and Goldstein, 2005). As noted above, tyrosine phosphorylation of kAE1 likely regulates polarized targeting to the cell surface. Extracellular hormonal or paracrine regulators of AE1 activity are yet to be described. kAE1 is upregulated by chronic metabolic acidosis (Da Silva Júnior et al., 1991), and downregulated by chronic metabolic alkalosis (Sabolic et al., 1997). kAE1 is also chronically downregulated by water deprivation (Barone et al., 2004) and upregulated by chronic dietary potassium depletion (Barone et al., 2007).

SLC4A2/AE2 expressed in *Xenopus* oocytes exhibits several modes of acute regulation absent or attenuated in SLC4A1/AE1, including inhibition by protons and activation by hypertonicity, NH_4^+ and calmidazolium. Intracellular and extracellular protons each inhibit AE2-mediated Cl^- exchange by independent mechanisms. pH_i is changed independently by isohydric addition and removal of the permeant weak acid butyrate, itself neither a transport substrate nor a competitive inhibitor of SLC4/AE polypeptides. pH_o is regulated independently by bath pH change during butyrate clamp of oocyte pH_i (Stewart et al., 2002). The independent inhibitory effects of pH_o and pH_i on AE2-mediated Cl^- exchange are evident within the physiological range, but inhibition of AE1 in oocytes requires extracellular proton concentrations nearly 100-fold higher (such as might be found at the basolateral membrane of renal Type A intercalated cells during hypoxic antidiuresis). Whereas the $pH_o(50)$ value for AE2 (pH at which maximal Cl^- efflux is 50% inhibited) is 6.8–6.9, that for AE1 is $\leq$5.5–5.0. Lowering pH_i from 7.3 to 6.8 at constant pH_o by exposure to 40 mmol l^{-1} butyrate inhibits AE2 by 80–90%, but AE1 remains completely uninhibited. The sigmoidal pH dependence of AE2 Cl^- transport in the physiological pH range requires the AE2 transmembrane domain. Substitution of any AE2 transmembrane domain region with the corresponding segment of pH-insensitive AE1 attenuates pH sensitivity of AE2. Evaluation of individual

transmembrane domain His residues indicates that they play a cooperative role in their contribution to pH-sensitivity, but do not suffice to control the full response. Most charged residues of the transmembrane domain can also be individually neutralized without alterating either basal transport activity or the independent inhibition by acidic pH_o or pH_i. However, one small region of the AE2 transmembrane domain corresponding to putative AE1 re-entrant loop 1 plays an important role in anion transport regulation by both pH_o and pH_i (Stewart et al., 2008).

AE2 residues of the intracellular N-terminal cytoplasmic domain also contribute to setting the pH sensitivity of anion transport. Mutation of some residues alters responses to both pH_o and pH_i, whereas mutation of others alters only one or the other response. Of particular note is the physiological AE2 variant polypeptide AE2c1, which lacks the N-terminal 199 amino acids of the longest of the five murine AE2 polypeptides, AE2a. The $pH_o(50)$ of AE2c1 is 7.7, compared with 6.8 for AE2a. The basis for this difference is found in two groups of AE2a residues within amino acids 120–150 of the region missing from AE2c1. In contrast, responses of the two AE2 isoforms to acidic pH_i are indistinguishable. The pH_o and pH_i responses of the AE2 isoforms AE2b1 and AE2b2 do not differ from that of AE2a. AE2c1 is predominantly expressed in the gastric parietal cells which alkalinize the mucosal interstitial fluid during stimulated gastric acid secretion. The expression in the parietal cell basolateral membrane of AE2 polypeptide variants with overlapping pH_o sensitivities serves to broaden the pH_o range over which parietal cell basolateral Cl^-/HCO_3^- exchange can be regulated by pH_o, while allowing other mechanisms of pH_i homeostasis (Kurschat et al., 2006).

Several regions of the N-terminal cytoplasmic domain shared by all AE2 polypeptide variants have been characterized as critical for pH regulation of anion exchange activity. Among these is the N-terminal cytoplasmic domain sequence most highly conserved among the entire *SLC4* gene family, including among the Na^+-dependent HCO_3^- transporters AE2a 336–347. Within this stretch, Ala substitution of three residues selectively abolishes regulation by pH_i without altering regulation by pH_o. Additional residues in the region between AE2a amino acids 200 and 500 have been shown to contribute to the independent regulation of anion transport rate by pH_o and by pH_i. Together these residues are modeled to form contiguous patches on the surface of the AE2 N-terminal cytoplasmic domain (Fig. 3) (Stewart et al., 2004). The contribution of AE2 N-terminal cytoplasmic domain residues to AE2 regulation by intracellular protons could involve intramolecular conformational changes upon sidechain protonation, or interaction with undefined AE2-binding proteins. Extracellular protonation of AE2 might induce conformational changes normally sensed by these cytoplasmic domain residues, or these residues might be targets of an independent signal transduction system itself sensitive to pH_o.

Many amino acid residues of the transmembrane domain of AE2 also contribute to pH sensing and regulation of transport activity in response to independent variation of pH_o and pH_i (Fig. 4). A particularly important contribution is made by residues in putative re-entrant loop 1 of AE2 that are not conserved in the corresponding region of the much less pH-sensitive AE1. These re-entrant loop 1 residues cooperate with still undefined residues within transmembrane spans 1–6 to decrease the rate of AE2-mediated anion exchange in response to intracellular protons and, independently, to extracellular protons. These two regions of the AE2 transmembrane domain suffice to explain the transmembrane domain contribution to AE2 regulation by pH_i (Stewart et al., 2008).

Hypertonic activation of AE2 in parallel with NHE1 activation mediates coordinated regulatory volume increase (Humphreys et al., 1995). Similar parallel activation fosters directional cell

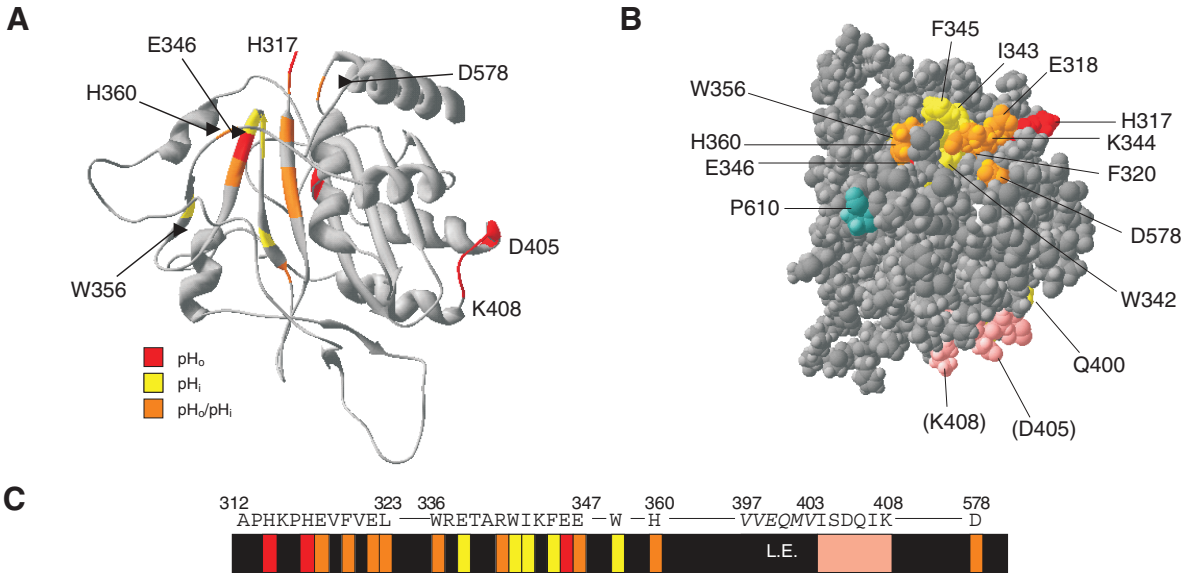

Fig. 3. Model of mouse AE2a NH_2-terminal cytoplasmic domain amino acids 317–623, highlighting conserved residues required for normal regulation of Cl^-/anion exchange by pH_o and pH_i. (A) Ribbon diagram structure of AE2 amino acids 317–623 based on the crystal structure of the corresponding region of human AE1 (Zhang et al., 2000). The structural model (B) and the linear schematic diagram (C) each indicate residues which when mutated alter AE2 regulation by pH_i (yellow), by pH_o (red) or by both pH_i and pH_o (orange). (B) Space-filling structure of AE2 amino acids 317–610, with surface amino acid residues indicated by the same colors. P610 (blue) is the most C-terminal surface residue in this view. Mutation *en bloc* of AE2 amino acids 403–408, at the bottom in pink, altered sensitivity only to pH_o. AE2 amino acids 397–402 are located out of view at the bottom right, adjacent to amino acids 403–408. L323 is modeled to be not at the domain surface. (Modified from Stewart et al., 2004.)

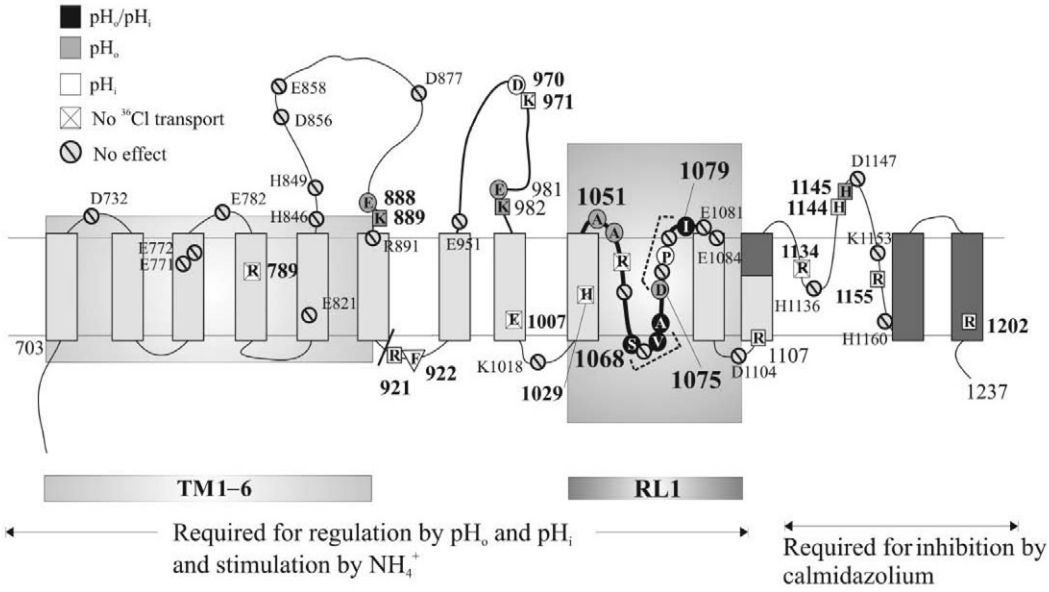

Fig. 4. Re-entrant loop 1 (RL1) of the mouse AE2 transmembrane domain plays a critical role in the acute regulation of anion exchange by pH. Summary of transmembrane subdomains (shaded boxes) and individual amino acid residues identified from mutagenesis studies as contributing importantly to regulation of AE2 activity by pH, NH_4^+ and calmidazolium. Residues of RL1 interact with as yet unidentified amino acids within the TM1–6 region to mediate 'pH sensor' functions in the AE2 transmembrane domain. Residues involved in regulation by pH_o are gray, those involved in regulation by pH_i are white, and those involved in regulation by both pH_o and pH_i are black. White boxes marked with an X are residues that when mutated yielded functional activity too low for study (modified from Stewart et al., 2008).

migration (Klein et al., 2000). Activation of AE2 by ammonium even at acidic pH values (Humphreys et al., 1997) may allow the Cl^--loading and acid-loading functions of AE2 in high [NH_4^+] environments such as the gastrointestinal tract and the renal medulla. AE2 activation by hypertonicity and by ammonium both require the conserved cytoplasmic domain residues 336–347. Both activating stimuli are inhibited by chelation of intracellular Ca^{2+} and by the anti-calmodulin drug calmidazolium. However, this effect is not mimicked by CaM-kinase inhibitors or by other calmodulin modifier drugs (Chernova et al., 2003). The curious insensitivity of kAE1 to regulation by hypertonicity and by ammonium, despite its localization in the hypertonic and ammonia-rich renal medulla, might be compensated in part by sensitivity to hypertonic activation of the medulla-restricted basolateral Cl^-/HCO_3^- exchanger SLC26A7 (Petrovic et al., 2004). However, SLC26A7 has also been described as a HCO_3^--impermeant Cl^- channel devoid of anion exchange activity (Kim et al., 2005).

Tissue culture Cl^-/HCO_3^- exchange and other regulators of pH_i have long been known to be regulated by hormone action (Ganz et al., 1989), but hormonal regulation of defined recombinant Na^+-independent Cl^-/HCO_3^- exchangers remains little studied. AE2-mediated Cl^-/HCO_3^- exchange is stimulated by serum in HEK-293 cells (Jiang et al., 1994). In *Xenopus* oocytes, AE2 is inhibited by phorbol ester activation of endogenous protein kinase C, but this inhibitory effect has yet to be firmly distinguished from the PKC-induced generalized oocyte surface membrane endocytosis. Acute regulation of AE2 activity in oocytes may be achieved in part or in full by control of trafficking. $Ae2_{a,b}^{-/-}$ embryonic fibroblasts with elevated pH_i, likely secondary to the absence of AE2-mediated acid-loading, upregulate bicarbonate-activated soluble adenylyl cyclase. These changes are associated with increased Creb transcription factor phosphorylation, increased Icer1 expression, and consequent marked attenuation of phospho-Creb-mediated transcriptional activation (Mardones et al., 2008).

Conclusion

Understanding of the pathophysiology and genetics of SLC4 anion exchangers is gradually increasing through the study of families with the AE1 deficiency diseases of hereditary spherocytosis and distal renal tubular acidosis, and the AE1 dysregulation disease of hereditary stomatocytosis. This understanding also increases through the continued study of Ae1-deficient mice. Elucidation of AE2 and AE3 pathophysiology will similarly progress through investigation of animal knockout models, and recent associations of human disease occurrence and therapeutic response with polymorphisms in these genes promises stronger ties to human physiology in the near future. Ever more extensive structure–function studies in tandem with investigation of regulatory pathways will refine our view of SLC4 anion exchange mechanisms. The high resolution structural information that may soon emerge from crystallization of recently discovered bacterial SLC4 polypeptides of defined functional properties will likely be an essential component of this progress.

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