The unusually low 78% amino acid identity between the orthologous human SLC26A6 and mouse slc26a6 polypeptides prompted systematic comparison of their anion transport functions in Xenopus oocytes. Multiple human SLC26A6 variant polypeptides were also functionally compared. Transport was studied as unidirectional fluxes of $^{36}\text{Cl}^-$, $[^{14}\text{C}]\text{oxalate}$, and $[^{35}\text{S}]\text{sulfate}$; as net fluxes of $\text{HCO}_3^-$ by fluorescence ratio measurement of intracellular pH; as current by two-electrode voltage clamp; and as net $\text{Cl}^-$ flux by fluorescence intensity measurement of relative changes in extracellular and intracellular $[\text{Cl}^-]$. Four human SLC26A6 polypeptide variants each exhibited rates of bidirectional $[^{14}\text{C}]\text{oxalate}$ flux, $\text{Cl}^-/\text{HCO}_3^-$ exchange, and $\text{Cl}^-/\text{OH}^-$ exchange nearly equivalent to those of mouse slc26a6. $\text{Cl}^-/\text{HCO}_3^-$ exchange by both orthologs was $\text{cAMP}$-sensitive, further enhanced by coexpressed wild type cystic fibrosis transmembrane regulator but inhibited by cystic fibrosis transmembrane regulator domain human-mouse chimeras revealed cosegregation of the high $^{36}\text{Cl}^-$ exchange transport phenotype with the transmembrane domain of mouse slc26a6. Mouse slc26a6 and human SLC26A6 each mediated electroneutral $\text{Cl}^-/\text{HCO}_3^-$ and $\text{Cl}^-/\text{OH}^-$ exchange. In contrast, whereas $\text{Cl}^-/\text{oxalate}$ exchange by mouse slc26a6 was electronegenic, that mediated by human SLC26A6 appeared electroneutral. The increased currents observed in oocytes expressing either mouse or human ortholog were pharmacologically distinct from the accompanying monovalent anion exchange activities. The human SLC26A6 polypeptide variants SLC26A6c and SLC26A6d were inactive as transporters of oxalate, sulfate, and chloride. Thus, the orthologous mouse and human SLC26A6 proteins differ in anion selectivity, transport mechanism, and acute regulation, but both mediate electroneutral $\text{Cl}^-/\text{HCO}_3^-$ exchange.

$\text{Cl}^-/\text{HCO}_3^-$ exchange plays important roles in cell pH and volume regulation. In polarized epithelial cells, $\text{Cl}^-/\text{HCO}_3^-$ exchange mediates transepithelial secretion of acid and base and contributes to fluid and volume secretion and reabsorption. Two gene superfamilies encode $\text{Na}^+$-independent $\text{Cl}^-/\text{HCO}_3^-$ exchangers, SLC4 and SLC26. The SLC4 family includes, in addition to the electroneutral AE anion exchangers, electronegenic and electroneutral $\text{Na}^+$-bicarbonate cotransporters, and $\text{Na}^+$-dependent $\text{Cl}^-/\text{HCO}_3^-$ and $\text{Cl}^-$/base exchangers (1, 2). Many SLC26 transporters mediate $\text{Cl}^-/\text{HCO}_3^-$ and/or $\text{Cl}^-$/OH$^-$ exchange, and some transport many additional anions, including sulfate, formate, oxalate, nitrate, and iodide. SLC26A1/Sat-1 (3) and SLC26A2/DTDST (4) transport sulfate but not $\text{Cl}^-$, and SLC26A5/prestin is thought to serve as a transducer of intracellular chloride and/or bicarbonate concentration signals without itself mediating anion transport (5, 6).

The SLC26 family includes four human disease genes. SLC26A2/DTD mutations are associated with diastrophic dysplasias (7, 8), believed to be secondary to defective sulfate uptake by chondrocytes. SLC26A3/DRA mutations are associated with congenital chloride-losing diarrhea (9, 10) due to deficient lower ileocolonic $\text{Cl}^-$ reabsorption via apical $\text{Cl}^-/\text{HCO}_3^-$ exchange (11, 12). The slc26a3 knock-out mouse is also reported to show impaired intestinal luminal fluid reabsorption (13). SLC26A4/pendrin mutations cause a syndrome of deafness and variably penetrant goiter (14). The former has been attributed to defective cochlear $\text{Cl}^-/\text{HCO}_3^-$ exchange (15, 16), and the latter has been attributed to deficient $\text{I}^-$ secretion across the apical membrane of the thyrocyte into the colloid space (17, 18). Although the deaf pendrin (−/−) mouse lacks apparent thyroid pathology, it exhibits impaired up-regulation of renal bicarbonate secretion in response to systemic bicarbonate loading (19) and impaired mineralocorticoid-induced up-regulation of renal NaCl reabsorption (20). SLC26A5/prestin mutations cause human deafness (21), and the prestin (−/−) mouse is similarly deaf (22).

Additional SLC26 gene products reported to mediate anion transport include SLC26A6 (23, 24, 25, 3, 26, 27, 28), SLC26A7 (29, 30, 31), SLC26A8 (32, 30), SLC26A9 (30), and SLC26A11 (33). None of these have yet been defined as human disease genes.

SLC26 polypeptides expressed in apical membranes of epithelial cells (among them SLC26A3 (34), SLC26A4 (19), and SLC26A6 (23)) have generated considerable interest by virtue of their co-localization with cystic fibrosis transmembrane reg-

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Indeed, cAMP-stimulated Cl⁻/HCO₃⁻ exchange associated with overexpression of any of these three apically localized SLC26 polypeptides is enhanced when co-expressed with wild type but not with mutant CFTR in HEK-293 cells (28) or (in the case of SLC26A3/DRA) in Xenopus oocytes (12). In addition, SLC26 polypeptides can interact with and potentiate activation of CFTR (28, 35). These data, together with evidence that cAMP-dependent epithelial HCO₃⁻ secretion is impaired or absent in cystic fibrosis (CF) and in cystic fibrosis mouse models (28, 36), have focused attention on SLC26 transporters as leading candidates to mediate the major apical HCO₃⁻ permeability pathways of HCO₃⁻-secreting epithelial cells.

Recent reports have suggested that Cl⁻/HCO₃⁻ exchange activities of murine slc26a3/DRA (28) and slc26a6 (3, 28) are electrogenic.² Whereas bath Cl⁻ substitution by gluconate depolarized Xenopus oocytes expressing slc26a3, oocytes express-

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¹ The abbreviations used are: CFTR, cystic fibrosis transmembrane regulator; aa, amino acid(s); CFEX, chloride/formate exchanger; BCECF-AM, 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein acetoxymethyl ester; MQAE, N-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide; DIDS, 4,4'-disothiocyanostilbene-2,2'-disulfonic acid; L, long N-terminal; S, short N-terminal; IBMX, isobutylmethylxanthine; DRA, down-regulated in adenoma.

² Ko et al. (28) described human SLC26A6 cDNA obtained as the RZPD EST AL036079. This DNA sequence (GenBank™ AB102713) encodes a previously unreported SLC26A6 variant polypeptide (BAC56861). However, the transport function expressed by Ko et al. (28) in both Xenopus oocytes and HEK293 cells was that of mouse slc26a6/CFEX from the Mammalian Gene Collection (S. Muallem and K. Ishibashi, personal communication). The functional properties of the novel human SLC26A6 variant BAC56861 remain unreported.
ing slc26a6 were hyperpolarized by Cl⁻ removal. Ko et al. concluded that these two polypeptides mediate Cl⁻/HCO₃⁻ exchange of opposite stoichiometries (28) and proposed that the full range of electrogenic and electroneutral HCO₃⁻ secretion observed in epithelia can be explained by varying proportional contributions of different SLC26 anion exchangers, with or without invocation of HCO₃⁻-permeable secretory anion channels (28, 36, 37, 63).

Unlike the high degree of amino acid identity between orthologous mouse and human SLC4 bicarbonate transporters, orthologous mouse and human SLC26 polypeptide sequences (with the notable exception of the cochlear outer hair cell mechanism transducer, SLC26A5/prestin) diverge to a much greater degree. Mouse slc26a6 and human SLC26A6 share only 78% aa identity. A single demonstration of unidirectional isotopic influx has been reported for the human SLC26A6 (25) after an earlier report failed to detect evidence of function in Xenopus oocytes (38). Therefore, we examined systematically the functional properties of several human SLC26A6 polypeptide variants and compared them with the only functionally studied mouse SLC26A6 polypeptide variant, also known as the chloride/ formate exchanger, CFEX (23).

We found that four active human SLC26A6 polypeptide variants exhibit similar functional properties in Xenopus oocytes. All differed from mouse slc26a6/CFEX most prominently in their very low rates of 36Cl⁻/36Cl⁻ transport in Cl⁻/Cl⁻ exchange assays and in their very low rates of 35SO₄²⁻ transport. However, these same polypeptides mediated bidirectional [14C]oxalate fluxes as well as Cl⁻/HCO₃⁻, Cl⁻/OH⁻, and oxalate/HCO₃⁻ exchange activities (measured as dpH/dt) at rates approaching those of mouse slc26a6/CFEX. Wild-type CFTR, but not CFTR ΔF508, stimulated Cl⁻/HCO₃⁻ and Cl⁻/OH⁻ exchange by human SLC26A6 and enhanced its cAMP sensitivity. High rates of 36Cl⁻ transport cosegregated with the transmembrane domain of mouse slc26a6 in studies with chimeric proteins. The orthologous polypeptides of both species mediated electroneutral Cl⁻/HCO₃⁻ and Cl⁻/OH⁻ exchanges, although expression was accompanied by oocyte current with pharmacological properties distinct from those of the corresponding anion exchange activities. In marked contrast, Cl⁻/oxalate exchange by mouse slc26a6/CFEX was clearly electrogenic, whereas that mediated by human SLC26A6 appeared electroneutral. Two unusual human variant SLC26A6 polypeptides previously reported to be functional (25) were nonfunctional as anion transporters. These data emphasize the complexity of SLC26A6-mediated anion exchange. They suggest an experimental path toward definition of amino acid residues important for Cl⁻ binding and/or translocation, provide an unusual example of a single polypeptide able to exchange different substrates with either electroneutral or electrogenic mechanisms, and suggest reexamination of current hypotheses of epithelial HCO₃⁻ secretion.

**EXPERIMENTAL PROCEDURES**

Materials—Na36Cl and Na235SO₄²⁻ were obtained from ICN (Irvine, CA). Na36Cl and H36Cl were also purchased from Amersham Biosciences. [14C]oxalate originally from PerkinElmer Life Sciences was the generous gift of C. Scheid and T. Honeyman (University of Massachusetts Medical Center). Restriction enzymes and T4 DNA ligase were from New England Biolabs (Beverly, MA). The EXPAND High Fidelity PCR system was obtained from Roche Applied Science. 2',7'-Bis(carboxyethyl)5-(and-6)-carboxyfluorescein acetoxymethyl ester (BCECF-AM), lucigenin (bis-N-methylacridinium nitrate), and N-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide (MQAE) were from Molecular Probes, Inc. (Eugene, OR). 4,4'-Diisothiocyanato-stilbene-2,2'-disulfonic acid (DIDS) was from Calbiochem. Tenidap was from Dr. Chris Gabel of Pfizer (Groton, CT). N8-3623 was from Dr. Palle Christophersen of Neurosearch (Copenhagen, Denmark). S20787 was from Dr. Elisabeth Scalbert of I.R.I.S. Servier (Courbevoie Cedex, France). All other chemical reagents were from Sigma or Fluka (Milwaukee, WI) and were of reagent grade.

cDNA Clones—The cDNAs studied are presented in the schematic of Fig. 1. Human SLC26A6 L-Q was obtained in pCMV-SPORT6 from the Mammalian Genome Collection (MGC_21068, IMAGE:4398446, BC017697) and was resequenced in entirety. In Fig. 1, Gln632 (Q632) is numbered as in the long N-terminal (L) sequence. The corresponding short N-terminal (S) position is Gln611 (Q611). The cDNA encoding this Gln can be retained (L+Q, S+Q) or excluded (L-Q, S-Q) from the 5' end of its exon by elective utilization of an alternate S' splice-acceptor splice site (3, 38, 39). The MGC_21068 human cDNA lacks the optional 13-aa insert (beginning at L aa 108 as in the human SLC26A6 variant, GenBank™ BAC56861) and contains in its C-terminal cytoplasmic domain the 30-aa insert (beginning at L aa 602-631) rather than the alternative 11-aa insert present in the human SLC26A6 variant, GenBank™ BAC56861.

Human SLC26A6 L-Q subcloned in the Xenopus β-globin oocyte expression vector pGEMHE was obtained from D. Mount. Human SLC26A6a (equivalent to S-Q), SLC26A6c (which has the short N terminus and lacks Gln632), and SLC26A6d (also with the short N terminus), originally studied as subclones of pCDNA3.1 (39, 25) (Fig. 1), were subcloned into pCMV-SPORT6 for the current study.

Mouse slc26a6/CFEX was obtained in pCMV-SPORT7 from the
Mammalian Genome Collection (MGC_25824, IMAGE:4165725, BC028856 from the FVB/N strain; it corresponds to human S/H11002Q, since the equivalent residue to human Gln611 is absent from the mouse gene). This murine slc26a6 cDNA (also studied by Ko et al. (28)) encodes the polymorphic amino acid residues Glu2 and Arg549 in place of the alternative Gly2 (23) and Pro549 (3). The genomic origins of the long (L) (38) and short (S) N termini (39) in humans and in mice were described by Xie et al. (3).

cDNAs encoding wild type human CFTR and CFTR disease mutant S/H9004F508 (12) in pBluescript were from M. Drumm (40). Mouse slc26a3/DRA cDNA in pcDNA3.1 was obtained from J. Melvin (11). Human SLC26A3/DRA cDNA was described previously (12).

Mutagenesis—From the MGC_21068 human SLC26A6 L/H11002Q cDNA, we generated the L/H11001Q (38), S/H11001Q (39), and S/H11002Q (3) variants of human SLC26A (Fig. 1) in the same pCMV-SPORT6 vector, using a four-primer PCR method (12). Oligonucleotide primers were obtained from BioSynthesis (The Woodlands, TX); sequences are available upon request.

Mutagenized PCR products were ligated into appropriately engineered host plasmids to reconstruct the desired open reading frame, and recombination was confirmed by diagnostic restriction digestion. Plasmid DNA sequences of the PCR-amplified regions and their ligation junctions were confirmed on both strands. The variable presence of Glu611/Gln632 (Fig. 1) was noted previously (38, 39).

Antibodies—Rabbit polyclonal anti-human SLC26A6a (S/H11001Q) aa 725–738 (C-terminal sequence) antiserum was described previously (25). Rabbit polyclonal anti-mouse slc26a6/CFEX aa 701–715 (C-terminal sequence) antiserum was generated against synthetic peptide cross-linked via an added N-terminal cysteine to keyhole limpet hemocyanin by maleimidobenzoyl-N-hydroxysuccinimide.

Solutions—ND-96 (pH 7.40) consisted of 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, 5 mM HEPES, 2.5 mM sodium pyruvate, and 100 mg/ml gentamicin. ND-96 flux medium and other flux media lacked sodium pyruvate and gentamicin. In Cl-/H11002-free solutions, 96 mM NaCl was replaced with 96 mM sodium isethionate. The Cl− salts of K+, Ca2+, and Mg2+ were substituted with the corresponding equimolar gluconate salts. Heps-free CO2/HCO3-/H11002-buffered solutions of pH 7.4 were saturated with 5% CO2, 95% air at room temperature for 1 h and differed from Cl-/H11002-free ND-96 in replacement of 24 mM sodium isethionate with 24 mM NaHCO3. The pH of CO2/HCO3-/H11002-buffered solutions was verified prior to each experiment. The addition to flux media of the weak acid salt sodium butyrate (40 mM) or NH4Cl (20 mM) was in equimolar substitution for NaCl.

cRNA Expression in Xenopus Oocytes—Capped cRNA was transcribed from linearized cDNA templates with SP6, T7, or T3 RNA polymerase (Ambion, Austin, TX). RNA integrity was confirmed by agarose gel electrophoresis in formaldehyde. Mature female Xenopus (NASCO, Madison, WI) were maintained and subjected to partial ovariectomy under Tricaine/hypothermia anesthesia as described (12), conforming to methods approved by the Institutional Animal Care and Use Committee of Beth Israel Deaconess Medical Center. Stage V–VI oocytes were manually defolliculated following incubation of ovarian fragments with 2 mg/ml collagenase A or collagenase B (Roche Applied Science) for 60 min in ND-96. Oocytes were injected on the same day or the following day with cRNA (10 ng in most experiments; as little as 50 pg when indicated) or with water in a volume of 50 nl. Injected oocytes were then maintained for 2–6 days at 19 °C.

Immunoblot—Oocytes previously injected with water or with cRNAs

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Fig. 4. Cl− and sulfate transport by SLC26 variants. A, four human SLC26A6 variants mediate minimal Cl− uptake compared with mouse slc26a6 (CFEX). Values are corrected for uptake by water-injected oocytes. B, time course of 36Cl− efflux by representative oocytes expressing CFEX (mouse slc26a6) or the indicated human SLC26A6 variant polypeptides and by one water-injected oocyte. C, summary of 36Cl− efflux rate constants exhibited by human SLC26A6 polypeptide variants and by mouse slc26a6/CFEX. D, [35S]sulfate uptake by oocytes expressing mouse slc26a6 (CFEX) or human SLC26A6 variants. Values in all bar graphs are means ± S.E. for n oocytes previously injected with 10 ng of the indicated cRNA.

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3 K. Ishibashi, personal communication.
encoding the indicated human SLC26A6 isoforms or mouse slc26a6 (CFEX) were solubilized by gentle homogenization in buffer containing 50 mM Tris-Cl, pH 7.4, 1 mM EDTA, 1% Triton X-100, and protease inhibitor mixture (Roche Molecular Biochemicals), followed by 30 min of incubation at 4 °C. The clarified lysate was fractionated by 8% SDS-PAGE, followed by semidry electrophoretic transfer to nitrocellulose and immunodetection as described (12, 40).

Confocal Immunofluorescence Microscopy—Oocytes previously injected with water or with cRNAs encoding the indicated human SLC26A6 isoforms or mouse slc26a6 (CFEX) were fixed, clarified, and immunostained as described previously (40). Single oocytes were imaged with a Bio-Rad MRC1012 confocal laser-scanning immunofluorescence microscope.

Isotopic Flux Studies—Unidirectional 36Cl\(^{-}/\text{H}^{+}\) influx studies were carried out in ND-96 containing 10 \text{mM} bumetanide for 15-, 30-, or (rarely) 60-min periods as described previously (12, 41). Total bath \([\text{Cl}^{-}]\) was 104 \text{mM}. For unidirectional 36Cl\(^{-}/\text{H}^{+}\) efflux studies (12, 41, 42, 43), individual oocytes in Cl\(^{-}\)-free ND-96 were injected with 50 nl of 130 \text{mM} Na\(^{36}\text{Cl}\) (10,000–12,000 cpm). Following a 5–10-min recovery period, the efflux
Functional Differences between SLC26A6 Orthologs

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Oocyte pH—Oocyte pH was monitored during bath superfusion using BCECF fluorescence excitation ratio imaging, as described previously (12, 44, 45). Cl−/HCO3− exchange activities were assayed by measurement of dpH/dt during bath Cl− substitution with isethionate and during subsequent restoration of Cl−. Initial rates of intracellular alkalization and acidification were computed by linear least squares fit to at least six consecutive fluorescence excitation ratio measurements. Data acquisition and analysis were with MetaFluor software (Universal Imaging, Chester, PA). dpH/dt values were compared by two-tailed t test. Values of initial pH in the presence of Cl− were indistinguishable among groups studied in the presence of CO2 and were similarly indistinguishable among groups in the nominal absence of CO2 (see Supplemental Table I).

Qualitative Estimate of Extracellular [Cl−] by Measurement of Luciferin Fluorescence Intensity—The impermeant chloride indicator dye lucigenin (10 µM) was intravitro calibrated to [Cl−] between 0 and 40 mM in solution balanced with sodium gluconate to achieve a final anion concentration of 110 mM. Using suboptimal filters (λex = 380 nm; λem = 510 nm), the Stern-Volmer constant (Ksv) of 10 µM lucigenin in free solution was measured by linear regression analysis (46, 47) to be 221 M−1 (in contrast to 390 M−1 measured at optimal wavelengths). Perioocyte [Cl−] in droplets was estimated by a modification of previous methods used to measure perioocyte pH (41, 48). Individual oocytes previously injected with water or with crRNA encoding mouse slc26a6CFEX or human SLC26A6 S–Q were placed in a sodium gluconate bath in a coverslip bottom chamber on the microscope stage. The medium was removed by suction and replaced with a -1.2-µl droplet of 5% CO2/24 mM NaHCO3-buffered Cl−-free flux medium containing 10 µM lucigenin. The humidified chamber atmosphere was equilibrated with 5% CO2 and sealed with a coverslip to maintain nominally constant droplet volume. Luciferin fluorescence intensity (F) was monitored and recorded for at least 15 min. Initial droplet fluorescence (F0) was quickly quenched (F′) increased (nominally due to Cl− exit from the oocyte across its plasma membrane) according to the Stern-Volmer equation: 

\[ F'/F = 1 + K_{sv}[Cl^-] \]

Qualitative Estimate of Intracellular [Cl−] by Measurement of MQAE Fluorescence Intensity—Oocytes previously injected with water or with crRNA encoding mouse slc26a6CFEX or human SLC26A6 S–Q were Cl−-depleted and nitrate-loaded by overnight incubation in Cl−-free nitrate medium. Oocytes were then injected with 50 nL of stock solution containing 100 µM MQAE to achieve an estimated final oocyte concentration of 5–10 µM MQAE and allowed to recover for 5–10 min in nitrate medium. One pair of MQAE-injected oocytes was placed in a glass bottom perfusion chamber on the microscope stage. Fluorescence intensity was monitored in two separated regions of interest in each oocyte while the perfusion medium was changed from 96 mM sodium nitrate to NaCl (ND-96). Decreased MQAE fluorescence intensity was interpreted as elevation of intracocyte [Cl−] (49) secondary to Cl− entry across the oocyte plasma membrane. MQAE (λex = 340 nm; λem = 460 nm) was intravitro calibrated to [Cl−] between 0 and 35 mM in balancing gluconate solutions with total anion concentration of 110 mM, yielding Ksv = 206 M−1 (n = 2). However, attempts to calibrate intracocyte MQAE fluorescence intensity were not successful due to low apparent efficacy in oocytes of the Cl−/OH− exchanger ionophore, tributyltin, even at bath concentrations up to 80 µM.

Two-electrode Voltage Clamp Measurements of Oocyte Current—Microelectrodes from borosilicate glass made with a Narashige puller were filled with 3 M KCl and had resistances of 2–3 megohms. Oocytes were placed in a 1-mL chamber (model RC-11, Warner Instruments, Hamden CT) on the stage of a dissecting microscope and impaled with microelectrodes under direct view. Steady-state currents achieved within 2–5 min following bath change or drug addition were measured with a Geneclamp 500 amplifier (Axon Instruments, Burlingame, CA) interfaced to an HP computer with a Digidata 1200 interface (Axon). Data acquisition and analysis utilized pCLAMP 8.0 software (Axon). The voltage pulse protocol generated with the Clampex subroutine consisted of 20-mV steps between −100 and +40 mV, with durations of 738 ms separated by 30 ms at the holding potential of −30 mV. Bath resistance was minimized by the use of agar bridges filled with 3 M KCl, and a virtual ground circuit clamped bath potential to zero.

Standard recording bath solution (24) was 95.5 mM NaCl, 2 mM KCl, 5 mM HEPES, 2.8 mM MgCl2, with pH 7.40. Occasional experiments as
Functional Differences between SLC26A Orthologs

**RESULTS**

**Four Human SLC26A6 Variant Polypeptides Are Expressed at or near the Surface of the Xenopus Oocyte**—Fig. 2A shows that human SLC26A6 polypeptide variants S–Q, S+Q, L–Q, and L+Q each accumulate in eRNA-injected oocytes. Fig. 2B confirms accumulation of mouse slc26a6/CFEX polypeptide in oocytes, as shown previously in COS-7 cells (23). All bands show substantial heterodispersion. The confocal immunofluorescence micrographs of Fig. 3 reveal that all four human SLC26A6 polypeptides tested are expressed at or near the oocyte surface with qualitatively similar abundance.

**Human SLC26A6 Variant Polypeptides Transport 36Cl\(^{-}\) at Very Low Rates and Sulfate at Low Rates in Comparison with Mouse slc26a6/CFEX**—Fig. 4 confirms and extends previous reports (3, 23) that mouse slc26a6 mediates robust influx of 36Cl\(^{-}\) (Fig. 4A) and of 35SO\(_4\)\(^{2-}\) into Xenopus oocytes (Fig. 4D).

Fig. 4 further shows with improved time resolution a rapid, trans-anion-dependent 36Cl\(^{-}\) efflux from oocytes expressing mouse slc26a6 (Fig. 4, B and C). In contrast, all four tested human SLC26A6 polypeptides mediate 35SO\(_4\)\(^{2-}\) influx 10 times more slowly than mouse slc26a6/CFEX (Fig. 4D) and 36Cl\(^{-}\) influx 20 times more slowly than the mouse polypeptide (Fig. 4A). In numerous experiments performed by four individuals over a 2-year period, the maximum value observed for human SLC26A6-mediated influx of 36Cl\(^{-}\) was 0.9 nmol/30 min. Even more remarkable was that human SLC26A6-mediated 36Cl\(^{-}\) efflux was undetectable in most experiments (Fig. 4, B and C) and only rarely exceeded values observed with water-injected oocytes (see Fig. 9A).

**Human SLC26A6 Variant Polypeptides Transport [14C]Oxalate at Rates Almost as Fast as Those of Mouse slc26a6/CFEX but Differ in Regulation**—The human SLC26A6 polypeptides were next examined for their ability to transport oxalate, a previously reported property of mouse slc26a6 (3, 23, 24). Consistent with the apparent surface expression of human SLC26A6 polypeptides (Fig. 3), [14C]oxalate uptake rates in oocytes expressing human SLC26A6 were up to 40% of those...
exhibited by mouse slc26a6/CFEX (Fig. 5A). [14C]Oxalate efflux rates into Cl⁻/HCO₃⁻ bath reached 80% of those exhibited by the orthologous mouse protein (Fig. 5, B and C).

Human SLC26A6-mediated [14C]oxalate efflux into a bath containing 1 mM oxalate (with balancing Cl⁻/free isethionate) at a rate 81% of that into ND96 (n = 5; not shown). Human SLC26A6/DRA did not mediate [14C]oxalate efflux from Xenopus oocytes at detectable rates (n = 5, not shown).

Oxalate transport by human SLC26A6 was moderately inhibited by niflumate (Fig. 5, B and D) and inhibited by tenidap...
Human SLC26A6 L/H11001 Q was not inhibited by 5/H9262 M S20787 (n = 6, not shown), a concentration adequate to inhibit cardiac DBDS-sensitive, DIDS-insensitive Cl/H11002/OH/H11002 exchange (50).

Mouse slc26a6/CFEX-mediated 36Cl/H11002 influx is insensitive to extracellular pH, but regulation of Cl/H11002/Cl/H11002 exchange by intracellular pH has not been reported. The bath addition of the weak acid butyrate acidifies oocytes, and its removal from the bath produces intracellular alkalinization (12, 43, 44). Such intracellular alkalinization activates SLC26A3/DRA and SLC4A2/AE2. In contrast, bath addition of NH4Cl activates anion exchange by SLC26A3/DRA (12) and by SLC4A2/AE2 (52) despite acidification of the oocyte's intracellular pH to levels that would be inhibitory if produced by other means (65). Mouse slc26a6/CFEX-mediated efflux of [14C]oxalate and of 36Cl/H11002 were minimally enhanced by butyrate removal-induced intracellular alkalinization and unaffected by NH4Cl (Fig. 5E). In parallel experiments performed at the same time with the same group of oocytes (not shown), NH4Cl also stimulated AE2/SLC4A2-mediated 36Cl/H11002 efflux as previously reported (51).

Two Unusual SLC26A6 Polypeptide Variants Are Inactive—SLC26A6d encodes the N-terminal cytoplasmic tail and transmembrane domain of SLC26A6a ((S/H11002 Q), but with a unique C-terminal cytoplasmic tail sequence replacing the evolutionarily conserved STAS domain. SLC26A6c is characterized by in-frame deletion of exons encoding two putative transmembrane domains, with retention of the STAS domain (Fig. 1). Xenopus oocytes injected with either of these variant cRNAs were reported to exhibit DIDS-sensitive uptake of 36Cl/H11002 and of 35SO4/H110022 equivalent in magnitude to that of SLC26A6a (25). We found that oocytes expressing SLC26A6a mediated low rates of 36Cl/H11002 influx and very low efflux rates, as did oocytes expressing the SLC26 variants shown in Fig. 4. Moreover, oocytes expressing SLC26A6a or SLC26A6d cRNAs exhibited no detectable 36Cl/H11002 uptake or efflux (not shown).

Therefore, we compared the A6a, A6c, and A6d variants of human SLC26A6 as transporters of oxalate and sulfate. Con-
focal immunofluorescence microscopy revealed SLC26A6 expres-
sion at or near the oocyte surface (not shown). The alter-
native C-terminal sequence of SLC26A6 released it
undetectable with the C-terminal antibody employed. Fig. 6A
shows that SLC26A6a mediated $[^{14}]$Cl\textsubscript{oxalate} uptake at rates
comparable with those of SLC26A6(S–Q) (Fig. 4), whereas the
SLC26A6c and A6d variant polypeptides were both inactive.
SLC26A6c and A6d were similarly inactive as sulfate trans-
porters, whereas SLC26A6a exhibited activity comparable with
that observed for all human SLC26A6 isofoms studied in Fig.
4 (Fig. 6B).

Human SLC26A6 Variant Polypeptides Mediate Apparent
Cl\textsuperscript{-}/HCO\textsubscript{3}{-} Exchange Activity, Further Enhanced by
CFTR Coexpression—We have shown in Xenopus oocytes that
co-expressed CFTR enhances and confers Cl\textsuperscript{-} sensitivity
upon human DRA/SLC26A3-mediated Cl\textsuperscript{-}/HCO\textsubscript{3}{-}
exchange activity (12). Ko et al. (28). showed in HEK-293 cells that CFTR
co-expression with mouse slc26a6/CxFEX similarly stimulates
cAMP-sensitive Cl\textsuperscript{-} transport (Fig. 8, A and C). The relative quench was 3-fold greater in
oocytes expressing human SLC26A6 and its
robust Cl\textsuperscript{-} transport exhibited by human SLC26A6 compared with that of mouse
slc26a6/CxFEX (Fig. 7D). These relative rates paralleled those of
[14]Cl\textsubscript{oxalate} efflux (Fig. 5) but differed from the very low
36Cl\textsubscript{fluoroacetate} flux results obtained previously injected with
10 ng of mouse slc26a6/CxFEX cRNA was 4.3 ± 0.92 (n = 8, p < 0.001 versus water). 
Relative quench in the droplets surrounding oocytes injected
with 10 ng of human SLC26A6(L+Q) cRNA was 1.8 ± 0.18 (n = 8, p < 0.02 versus water), considerably lower than that of mouse
slc26a6/CxFEX (p < 0.003).

We measured net Cl\textsuperscript{-} influx into Cl\textsuperscript{-}-depleted, nitrate-con-
taining oocytes as quench of intracellular MQAE fluorescence
upon substitution of bath nitrate with bath Cl\textsuperscript{-} (Supplemental
Fig. 1, C and D). The relative quench was 3-fold greater in
oocytes expressing mouse slc26a6/CxFEX than in those express-
ing human SLC26A6(S–Q) (n = 4, p < 0.001), which in turn
increased by 5 min in percent competition compared with
injected oocytes (n = 4, p < 0.001) in a separate, paired experiment. Thus, both
net Cl\textsuperscript{-} influx and net Cl\textsuperscript{-} efflux in exchange for HCO\textsubscript{3}{-} and Cl\textsuperscript{-} influx
in exchange for nitrate as estimated by fluorescence quenching
were 3–4-fold higher in oocytes expressing mouse slc26a6/
CxFEX than in oocytes expressing human SLC26A6.

High Rates of 36Cl Flux Are Encoded by the Transmem-
brane Domain of Mouse slc26a6/CxFEX in Chimeras—We rea-
soned, despite the similarity of measured Cl\textsuperscript{-} exchange
rates, that observed differences in transport activity by human
SLC26A6 in contrast to high exchange rates of intracellular
[36]Cl\textsuperscript{-} with 96 mM extracellular Br\textsuperscript{-}, 96 mM nitrate, or
(at considerably lower rates) 24 mM HCO\textsubscript{3}{-} exhibited by mouse
slc26a6/CxFEX, human SLC26A6 exhibited very low rates of these
exchange activities (Fig. 9A). Both human
SLC26A6(L+Q) and mouse slc26a6/CxFEX exhibited substan-
tial temperature sensitivity, but the former was not activated
to a greater degree than was the latter (Fig. 9, B and C). Thus, differential temperature sensitivities of 36Cl\textsuperscript{-}/Cl\textsuperscript{-}
exchange did not explain the different 36Cl\textsuperscript{-} transport rates by the ortholo-
gous polypeptides.

We therefore tested the possibility that the reduced rate of
36Cl\textsuperscript{-} transport by human SLC26A6 compared with that of mouse
slc26a6 would be reflected by fluorometric measures of Cl\textsuperscript{-}
transport. Cl\textsuperscript{-} efflux from oocytes into a Cl\textsuperscript{-}-free surrounding
droplet containing gluconate/HCO\textsubscript{3}{-} was estimated from the rate and magnitude of quench of lucigenin fluorescence in the droplet (49) (Supplemental Fig. 1). When the fluorescence
quench in droplets surrounding water-injected oocytes (n = 5)
was normalized to a value of 1, the relative quench in droplets
around oocytes previously injected with 10 ng of mouse slc26a6/
CxFEX cRNA was 4.3 ± 0.92 (n = 8, p < 0.001 versus water). 
Relative quench in the droplets surrounding oocytes injected
with 10 ng of human SLC26A6(L+Q) cRNA was 1.8 ± 0.18 (n = 8, p < 0.02 versus water), considerably lower than that of mouse
slc26a6/CxFEX (p < 0.003).
rates, that the remarkable species difference in rates of $^{36}\text{Cl}^-$/HCO$_3^-$ exchange and of $^{35}\text{SO}_4^{2-}$/HCO$_3^-$ transport by mouse slc26a6/CFEX and human SLC26A6 should be explained by the divergent amino acid sequences of these orthologs. We therefore compared unidirectional isotopic fluxes of wild type mouse slc26a6/CFEX and wild type human SLC26A6(S→Q) (h), the chimera of the mouse transmembrane domain attached to the human C-terminal cytoplasmic domain (mh), and the chimera of the human transmembrane domain attached to the mouse C-terminal cytoplasmic domain (hm). Results were normalized to values of 100% for mouse slc26a6/CFEX. Values are means ± S.E. for n oocytes from two or more frogs.

![Diagram](image)

**FIG. 10.** Magnitude of $^{36}\text{Cl}^-$ flux by SLC26A6 polypeptides cosegregates with the transmembrane domain. $^{36}\text{Cl}^-$ influx (Cl$^-$ in) and efflux (Cl$^-$ eff), [14C]oxalate influx (ox) and $^{35}\text{SO}_4^{2-}$ influx were measured in oocytes expressing wild type mouse slc26a6/CFEX (m), wild type human SLC26A6(S→Q) (h), the chimera of the mouse transmembrane domain attached to the human C-terminal cytoplasmic domain (mh), and the chimera of the human transmembrane domain attached to the mouse C-terminal cytoplasmic domain (hm). Results were normalized to values of 100% for mouse slc26a6/CFEX. Values are means ± S.E. for n oocytes from two or more frogs.

Expression in Xenopus Oocytes of Human SLC26A6 or of Mouse slc26a6/CFEX Is Associated with Increased Current, but This Current Does Not Represent Electrogenic Exchange of Monovalent Anions—The initial report of Cl$^-$/HCO$_3^-$ exchange by mouse slc26a3/DRA expressed in HEK-293 cells showed no functional consequence of cell depolarization with a high K$^+$ bath (11). In addition, membrane potential of oocytes expressing human SLC26A3/DRA was no more sensitive to bath Cl$^-$ removal than was that of water-injected oocytes (12). These data suggested that DRA-mediated anion exchange is electro-neutral. Similarly, Jiang et al. (24) observed that the modest hyperpolarization of water-injected oocytes and oocytes expressing mouse slc26a6 upon bath Cl$^-$ removal was indistinguishable. In contrast, Ko et al. (28) reported that both murine slc26a3/DRA and murine slc26a6/CFEX expressed in Xenopus oocytes generate voltage clamp currents with properties of electrogenic Cl$^-$/base exchange. Whereas unclamped oocytes expressing mouse slc26a3 depolarized during bath Cl$^-$ removal (28), oocytes expressing mouse slc26a6 hyperpolarized in response to the same stimulus (3, 28). Interestingly, however, nominal Cl$^-$/OH$^-$ exchange mediated by mouse slc26a6 was not accompanied by consistent, bath Cl$^-$ removal-induced hyperpolarization (3).

These varied results prompted our systematic electrophysiological comparison of oocytes expressing mouse slc26a6/CFEX and human SLC26A6. Fig. 11A shows that expression of mouse slc26a6/CFEX modestly increased oocyte current measured at −100 mV to −282 ± 41 nA (n = 8 from two frogs and to −275 ± 8 nA in 33 additional oocytes from five additional frogs (not shown) subjected to different solution change protocols). During this series of sequential bath changes from Cl$^-$ to gluconate and then to gluconate/HCO$_3^-$ and finally into Cl$^-$/HCO$_3^-$, the maximum change in reversal potential (Δ$E_{rev}$) was +3.3 mV.
Fig. 11. Oocyte currents associated with expression of human SLC26A6 and of mouse slc26a6 do not represent electrogenic exchange of monovalent anions. A, I-V curves of oocytes expressing mouse slc26a6/CFEX (n = 8) recorded during sequential bath changes from Cl\(^-\) to gluconate to 24 mM HCO\(_3\)\(^-\), 5% CO\(_2\) (plus 72 mM gluconate) to 24 mM HCO\(_3\)\(^-\), 5% CO\(_2\) (plus 72 mM Cl\(^-\)). The I-V curve of water-injected oocytes (control, n = 4) was recorded in Cl\(^-\) bath. B, I-V curves of oocytes expressing mouse slc26a6/CFEX (n = 12) recorded during bath change from Cl\(^-\) to gluconate, followed by the addition of 1 mM oxalate, mean reversal potential (E\(_{\text{rev}}\)) changed −24 mV upon the addition of oxalate. C, I-V curves of oocytes expressing human SLC26A6(L\(^-\)Q) (n = 6) recorded during sequential bath changes from Cl\(^-\) to gluconate to 24 mM HCO\(_3\)\(^-\), 5% CO\(_2\) (plus 72 mM gluconate) to 24 mM HCO\(_3\)\(^-\) plus 5% CO\(_2\) (plus 72 mM Cl\(^-\)). D, I-V curves of oocytes expressing human SLC26A6(L\(^-\)Q) (n = 10) recorded during bath change from Cl\(^-\) to gluconate, followed by the addition of 1 mM oxalate. The I-V trace of water-injected oocytes (control, n = 4) is reproduced from A.

and current magnitudes did not change. Mouse slc26a6/CFEX-associated currents in 18 additional oocytes from three more frogs subjected only to Cl\(^-\)-gluconate bath shifts in the absence of CO\(_2\)/HCO\(_3\)\(^-\) exhibited similar minimal change in ΔE\(_{\text{rev}}\). Thus, slc26a6/CFEX-mediated monovalent anion exchange was not detectably electrogenic, whether in the absence or presence of CO\(_2\)/HCO\(_3\)\(^-\).³

The lack of evidence supporting electrogenic exchange of monovalent anions by mouse slc26a6/CFEX was not attributable to inadequate functional expression, as shown in Fig. 11B. The mean change in E\(_{\text{rev}}\) upon bath change from Cl\(^-\) to gluconate was −5.9 ± 1.1 mV (n = 12), consistent with the results of Fig. 11A. However, upon subsequent bath addition of 1 mM oxalate, mean E\(_{\text{rev}}\) shifted a further −23.9 ± 2.5 mV (n = 12). An additional group of seven oocytes (from a different frog) expressing mouse slc26a6/CFEX also exhibited a mean ΔE\(_{\text{rev}}\) of −35 mV in response to the oxalate addition to a gluconate bath (as a sole maneuver, not shown). This evidence for electrogenic oxalate/chloride exchange by mouse slc26a6/CFEX is consistent with a previous report of CFEX-mediated electrogenic oxalate/HCO\(_3\)\(^-\) exchange (24). The data further strengthen the conclusion from Fig. 11A that monovalent anion exchange by mouse slc26a6 is electroneutral.

Fig. 11C reveals that oocytes expressing human SLC26A6 exhibited a similar lack of change in current magnitude and E\(_{\text{rev}}\) in response to bath changes from Cl\(^-\) to gluconate and then to HCO\(_3\)\(^-\)/gluconate and finally to HCO\(_3\)\(^-\)/Cl\(^-\). Human SLC26A6-associated currents in 21 additional oocytes from four more frogs subjected only to Cl\(^-\)-gluconate bath shifts exhibited similar minimal changes in ΔE\(_{\text{rev}}\) (not shown). These data strongly suggest that monovalent anion exchange by human SLC26A6 is also electroneutral.

Fig. 11D confirms the finding of Fig. 9A for mouse slc26a6/CFEX that expression of human SLC26A6 L\(^-\)Q modestly increased oocyte inward current in Cl\(^-\) bath (measured at −100 mV) from the control value of −91 ± 26 nA to −363 ± 108 nA (n = 10 oocytes from two frogs). When these SLC26A6-expressing oocytes were subjected to sequential bath changes, the mean reversal potential (E\(_{\text{rev}}\)) changed −2.2 ± 1.7 mV from Cl\(^-\) into gluconate bath. However, upon the subsequent addition to the gluconate bath of 1 mM oxalate, E\(_{\text{rev}}\) shifted only −2.8 ± 2.1 mV (p < 0.003 compared with the oxalate-induced shift in E\(_{\text{rev}}\) in oocytes expressing mouse slc26a6/CFEX). Thus, in contrast to mouse slc26a6, human SLC26A6-mediated oxalate/Cl\(^-\) ex-

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³ Ko et al. (28) also supported the claim of voltage-sensitive anion transport with their observation of asymmetric tail currents (holding potential was 0 mV, occupying 67% of clamp time). In contrast, oocytes expressing mouse slc26a6 in our experiments (with holding potential of −30 mV occupying 4% of clamp time) exhibited symmetrical tail currents (not shown whether in the absence or presence of CO\(_2\)/HCO\(_3\)\(^-\)). The longer and more extreme depolarization of oocytes in the former protocol may account in part for this difference.
change did not exhibit evidence of electrogenicity.\(^7\)

The voltage clamp results supporting electroneutrality of monovalent anion exchange were corroborated by measurement of \(^{36}\text{Cl}^-\) efflux rate constants and of \(\text{Cl}^-/\text{HCO}_3^-\) exchange rates in BCECF-loaded oocytes during depolarization with high \(K^+\). The rate constant for \(^{36}\text{Cl}^-\) efflux into ND96 from oocytes expressing mouse slc26a6 was \(0.044 \pm 0.005\) min\(^{-1}\), and after shift into \(K^+\) bath it was \(0.049 \pm 0.005\) min\(^{-1}\) (means ± S.E., \(n = 7\)). The 20-fold lower \(^{36}\text{Cl}^-\) efflux rates in oocytes expressing human SLC26A6(S Q) also did not change in response to high \(K^+\) depolarization (\(n = 3\), not shown).

dpH/\(dt\) upon bath \(\text{Cl}^-\) removal in BCECF-loaded oocytes expressing mouse slc26a6/CFEX was \(0.0185 \pm 0.00034\) units/min (S.E., \(n = 9\)) in the presence of \(\text{Na}^+\) and \(0.0187 \pm 0.00033\) units/min (\(n = 13\)) in the same oocytes subsequently exposed to high \(K^+\) (\(p > 0.7\)). Oocytes expressing human SLC26A6(S Q) exhibited dpH/\(dt\) values of \(0.0153 \pm 0.00033\) and \(0.0163 \pm 0.00040\) during \(\text{Cl}^-\) removal first in the presence of \(\text{Na}^+\) and then in high \(K^+\) bath (\(n = 11\), \(p > 0.05\)). dpH/\(dt\) values during bath \(\text{Cl}^-\) restoration were also unchanged by bath \(\text{Na}^+\) substitution with \(K^+\) in all experiments (not shown). Initial values of pH, prior to \(\text{Cl}^-\) removal and restoration were indistinguishable in \(\text{Na}^+\) and \(K^+\) baths for all oocyte groups (Supplemental Table 1). Thus, high \(K^+\) depolarization did not alter rates of \(\text{Cl}^-/\text{Cl}^-\) exchange or of \(\text{Cl}^-/\text{HCO}_3^-\) exchange by oocytes expressing mouse slc26a6 or human SLC26A6.

**DISCUSSION**

Functional study of mouse slc26a6/CFEX has led to novel models of human pathophysiology, but anion exchange mechanisms and biological functions of SLC26 transporters remain controversial. The remarkable degree of sequence difference between human SLC26A6 and mouse slc26a6 and the lack of functional data on SLC26A6 variant polypeptides prompted us to perform the first comprehensive functional comparison of these two orthologous gene products. Our results differ in some important ways from those previously reported and encourage reevaluation of the pathophysiological models built upon those earlier results.

**Orthologous Mouse and Human SLC26A6 Polypeptides Differ in Anion Selectivity**—Four of the human SLC26A6 polypeptide variants revealed similar activity profiles, but these profiles for some functions differed dramatically from those of their murine ortholog (Figs. 4 and 5). Two additional human polypeptide variants previously reported to be functional were shown to be inactive (Fig. 6). Previous \(^{38}\text{Cl}^-\) flux studies of human SLC26A6 revealed low level activity (25) or no function (38). These studies were confirmed by our observations of low \(^{38}\text{Cl}^-\) influx activity and very low \(^{36}\text{Cl}^-\) efflux activity by human SLC26A6, despite robust bidirectional transport of \(^{38}\text{Cl}^-\) by mouse slc26a6 (Fig. 4) as previously reported (3, 23). Sulfate influx exhibited a qualitatively similar pattern: low activity in oocytes expressing human SLC26A6 and high activity for mouse slc26a6 (Fig. 4). However, tests of oxalate transport confirmed that human SLC26A6 at or near the oocyte surface (Fig. 3) was indeed highly functional. Human SLC26A6-mediated oxalate efflux was 40–80% that of mouse slc26a6, and oxalate influx was nearly so (Fig. 5). Chimera studies attributed most of the large species difference in \(^{36}\text{Cl}^-\) transport rate to the transmembrane domain of SLC26A6, whereas the difference in sulfate transport appeared to reside to a greater degree also in the N-terminal cytoplasmic domain (Fig. 10). This species difference in anion selectivity will provide a useful route to definition of the specific amino acid residues in SLC26 polypeptides that govern or modulate binding, translocation, and release of substrate anions.

**Differences in Acute Regulation between Mouse and Human SLC26A6 Orthologs**—Human SLC26A6-mediated \(^{14}\text{C}\)oxalate influx was increased acutely both by the alkalizing stimulus of bath butyrate removal and by the acidifying stimulus of \(\text{NH}_4^+\) exposure (Fig. 5). In contrast, mouse slc26a6-mediated transport of \(^{14}\text{C}\)oxalate or \(^{38}\text{Cl}^-\) was insensitive to \(\text{NH}_4^+\) and only minimally stimulated by butyrate removal, consistent with previous reports that mouse slc26a6-mediated transport of \(^{38}\text{Cl}^-\) and of \(^{14}\text{C}\)oxalate are insensitive to acidic bath \(pH\) (3, 24, 23). Stimulation of human SLC26A6-mediated oxalate influx by butyrate removal (increasing \(pH\) from \(~6.6\) to \(~7.1\)) was not due to increased concentration of the divalent oxalate anion, with \(pK\) values of 1.3 and 4.3.

Stimulation of human SLC26A6 by \(\text{NH}_4^+\) was not likely to be secondary to depolarization, since \(\text{NH}_4^+\)-sensitive human SLC26A6-mediated oxalate/\(\text{Cl}^-\) exchange was weakly if at all electrogenic, whereas \(\text{NH}_4^+\)-insensitive mouse slc26a6-mediated oxalate/\(\text{Cl}^-\) exchange was strongly electrogenic. Stimulation of human SLC26A6 by \(\text{NH}_4^+\) was not secondary to intracellular acidification, given robust stimulation by the alkalinating stimuli of butyrate removal. The mechanism by which \(\text{NH}_4^+\) stimulates activity of SLC4A2/AE2 but not endogenous currents that differ pharmacologically from SLC26A6-mediated anion exchange. Therefore, the incremental currents and the (electroneutral) monovalent anion exchange measured in these oocytes are not likely to be mediated by the same protein.
SLC4A1/AE1 in Xenopus oocytes also remains unclear. However, elevated NH4+ concentrations are physiologically important not only in the kidney and the portal vascular bed but also in any epithelial lumens colonized by urease-positive bacteria such as Helicobacter pylori and Pseudomonas aeruginosa (52). Inactive Physiological Variants of Human SLC26A6—Four of the six tested human SLC26A6 polypeptides were functionally similar. However, SLC26A6c and SLC26A6d polypeptides expressed no detectable isotopic fluxes (Fig. 6), in contrast to the findings of Lohi et al. (25). This lack of function, reinforced by our observation of SLC26A6a-mediated transport of [14C]oxalate and of [35S]sulfate at levels equivalent to those exhibited by human SLC26A6c and the STAS domain of the C-terminal cytoplasmic tail (absent from SLC26A6d), emphasizes the structural and functional importance of both an intact transmembrane domain (not preserved in the SLC26A6c polypeptide) and the STAS domain of the C-terminal cytoplasmic tail (absent from SLC26A6d). The possibility that one or more inactive SLC26A6 variants might serve as a physiological dominant negative remains to be more thoroughly investigated.

Human SLC26A6 Mediates Robust, Bidirectional Cl-/HCO3- Exchange Despite Its Very Low Rates of 36Cl- Efflux—The vigorous Cl-/HCO3- exchange activity of mouse slc26a6 (Figs. 7 and 8) has been noted previously (3, 24, 26, 28). However, the bidirectional Cl-/HCO3- exchange activity of human SLC26A6 polypeptides at rates ~80% of those exhibited by mouse slc26a6 (Fig. 7) was unexpected in view of the very low 36Cl- transport activity of human SLC26A6 (Fig. 4). Indeed, human SLC26A6(L+Q) was previously reported to lack Cl-/HCO3- exchange activity in Xenopus oocytes (38). Both human SLC26A6 and mouse slc26a6 also exhibited (nominal) Cl-/OH- exchange activity (in ambient CO2) (Fig. 7), a property not uniformly reported in previous studies of mouse slc26a6 (3, 24, 26).

Reconciliation of this robust human SLC26A6-mediated Cl-/HCO3- exchange with very low rates of 36Cl- flux poses a problem not presented by mouse slc26a6 or by anion exchange mediated by human SLC26A3/DRA (12) and human SLC26A4/pendrin (17, 19, 28). In contrast, preliminary expression studies with human SLC26A7, SLC26A8, and SLC26A9 reveal similarly low or undetectable 36Cl- flux activity in concert with substantial Cl-/HCO3- exchange activity. Thus, the property of discordant rates of anion exchange measured by different techniques in different conditions is not unique to human SLC26A6.

note:

8 A. K. Stewart and S. L. Alper, unpublished data.

9 M. N. Chernova, L. Jiang, and S. L. Alper, unpublished results.
HCO_3^− flux (equivalent proton flux, J_{H^+}) by mouse slc26a6 was estimated from dPH/dt × total oocyte buffer capacity^{10} as 6.1 μm/s. The corresponding value for J_{H^+} by human SLC26A6 was 5.2 μm/s. Mouse slc26a6-mediated Cl^−/HCO_3^− exchange was measured as [36Cl]^− efflux into Cl^−-bath in room air was calculated from the efflux rate constant (0.12–0.3 min^{−1}) and an estimated intraoocyte [Cl^−] of 43 mm (native ~30 μM plus the post-36Cl^− injection increment of ~13 μM) to yield Cl^−/HCO_3^− exchange rates of at least 85 μm/s, but often higher. Since the relative rate of [36Cl]^− efflux into the HCO_3^− bath was only 10% of that into the Cl^− bath (Fig. 7A), a low end estimate for Cl^−/HCO_3^− exchange rate derived from [36Cl]^− efflux measurements (for which intraoocyte [Cl^−] is ~30 μM) is 8.5 μm/s, in moderate agreement with the 6.1 μm/s value derived from BCECF fluorescence ratio. Thus, the magnitude of [36Cl]^− transport by mouse slc26a6 in the presence of HCO_3^− agrees moderately well with the rates of fluorometrically measured HCO_3^− transport in response to complete removal and restoration of bath Cl^−.

The large gap between human SLC26A6-mediated HCO_3^− transport at rates ~85% of those of mouse slc26a6 (Figs. 7 and 8) and [36Cl]^− transport at rates 0–10% of those of mouse (Figs. 4 and 9) remains to be explained. Under the conditions tested, mouse slc26a6 mediates [36Cl]^−/Cl^− exchange at rates at least 10-fold greater than those of Cl^−/HCO_3^− exchange measured as J_{H^+}. In contrast, human SLC26A6 mediates [36Cl]^− efflux into Cl^− bath at rates at least 2.5-fold lower than those of fluorometrically measured Cl^−/HCO_3^− exchange (5.2 μm/s) and sometimes at undetectable rates. Cl^− transport by human SLC26A6 may differ from that by mouse slc26a6 in a requirement for CO_3^2−/HCO_3^− for optimal function. The inability to detect such a CO_3^2−/HCO_3^− dependence for human SLC26A6 as a consistent stimulation of [36Cl]^− efflux by bath HCO_3^− (Fig. 9) may reflect effective competition by intracellular HCO_3^− at the protein's internal Cl^− binding site so as to lower [36Cl]^− efflux toward or below the level of detection.

We therefore propose that human SLC26A6 polypeptides mediate very low rates of Cl^−/HCO_3^− homeoexchange while maintaining moderate to high rates of Cl^−/HCO_3^− exchange. This may reflect a species-specific CO_3^2−/HCO_3^−-dependent conformational secondary to altered binding affinities at transport or regulatory anion binding sites.

**Human SLC26A6-mediated Cl^−/HCO_3^− Exchange Is cAMP-sensitive and Further Stimulated by CFTR—Cl^− gradient-dependent HCO_3^− transport by mouse slc26a6 and human SLC26A6 proteins was stimulated by cAMP to similar degrees. This stimulation by cAMP was enhanced for both orthologs by coexpressed wild-type CFTR (Fig. 8), as shown previously for human (12) and mouse DRA (28) and for mouse slc26a6 (28). The stimulation of bidirectional Cl^−/HCO_3^− exchange by CFTR probably reflects direct interaction between CFTR and SLC26 from either species (35). Mechanistic contributions to this stimulation from the abrupt switch in conductive anion selectivity of CFTR induced in oocytes by bath Cl^− removal (37) or from extracellular [Cl^−] control of CFTR gating (64) were neither clearly evident nor possible to rule out. The cAMP sensitivity of both SLC26A6 orthologs is consistent with secretin stimulation of luminal Cl^−/HCO_3^− exchange in the guinea pig pancreatic duct (53). In contrast, mutant CFTR coexpression abrogated cAMP-stimulation of Cl^−/HCO_3^− exchange (Fig. 8), consistent with deficient pancreatic HCO_3^− secretion in cystic fibrosis.

\(^{10}\) Equivalent proton flux rates were calculated as dPH/dt (corrected for values recorded in water-injected oocytes) × total buffer capacity (β_T = β_i + β_CO2), Intrinsic buffer capacity (β_i) was 19 mEq/pH unit (43), and CO_2 buffer capacity (β_CO2) was calculated from the measured pH, by the Henderson-Hasselbalch equation.

**Monovalent Anion Exchange by Mouse slc26a6 and by Human SLC26A6 Is Electroneutral—**Although high K^+ depolarization experiments have not supported an electrogenic Cl^−/HCO_3^− exchange mechanism for SLC26 polypeptides (11, 12), bath Cl^− substitution has been reported to hyperpolarize unclamped oocytes expressing mouse slc26a6 (3, 28) and to depolarize oocytes expressing mouse slc26a3 (28). Ko et al. (28) further reported bath anion-dependent shifts in reversal potential of opposite polarity for mouse slc26a3 and slc26a6 in voltage-clamped oocytes (28). These data were invoked to support the proposal of electrogenic Cl^−/HCO_3^− exchange of opposite stochiometries (>2 HCO_3^−/1 Cl^− for slc26a6/CFEX versus ≥2 Cl^−/1 HCO_3^− for slc26a3/DRA).

Based on these and other findings, they suggested a model (28, 35) for pancreatic ductal secretion of 140 mM HCO_3^−; axially arrayed, secretin-stimulated, CFTR-enhanced, electrogenic Cl^−/HCO_3^− exchange by SLC26A6 in the proximal pancreatic duct and by SLC26A3/DRA in the distal pancreatic duct could sustain HCO_3^− secretion in the face of high inward HCO_3^− gradients to achieve substitution of most luminal Cl^− (originally secreted by the acinus and early duct) with HCO_3^−. They further noted that regulated expression or activity of multiple SLC26 gene products together with CFTR in the same membrane could explain the full range of electroneutral and/or electrogenic Cl^−-dependent HCO_3^− secretion reported in intact epithelial tissues (28).

This proposal encouraged our further exploration of the electronegativity of anion exchange by human and mouse orthologs. We found that expression of both mouse slc26a6 and of human SLC26A6 in Xenopus oocytes indeed generated currents in Cl^−-bath, albeit of lower magnitude than those reported by Ko et al. (28). However, these oocyte currents were not attributable to Cl^−/CI^− or Cl^−/HCO_3^− exchange, as judged by the following multiple criteria. First, mouse slc26a6-mediated (water control-subtracted) clamp currents measured at ~40 mV (the approximate membrane potential in unclamped water-injected oocytes undergoing flux assays in Cl^−-bath) were only ~25 nA (n = 33; see also Fig. 11A). This current could account for only 5–10% of slc26a6-mediated [36Cl]^− influx (10–20 nmol/h, or 230–560 nA) if all exchange flux were electrogenic, or even less in view of the depolarized resting potential (~26 mV) of slc26a6-expressing oocytes (3). Second, E_rev of the slc26a6/SLC26A6-induced currents did not change significantly in response to bath substitution of monovalent anion transport substrates, whether in the absence or presence of CO_3^2−/HCO_3^−. This lack of change in E_rev contrasted dramatically with the large E_rev shift in oocytes expressing mouse slc26a6 upon the bath addition of divalent oxalate. Third, the induced currents in oocytes expressing either mouse slc26a6 or human SLC26A6 were insensitive to inhibition by several effective blockers of slc26a6-mediated anion flux. Fourth, oocyte depolarization in high K^+ bath did not modify either the [36Cl]^− efflux rate constant for mouse slc26a6 or the rate of Cl^−/HCO_3^− exchange (+dPH/dt) mediated by either mouse slc26a6 or by human SLC26A6. Finally, voltage-clamped currents in oocytes expressing either mouse slc26a3 or human SLC26A3 confirmed the electroneutrality of monovalent anion exchange in the absence and presence of CO_3^2−/HCO_3^−, exhibiting neither change in current magnitude nor E_rev. Expression of mouse slc26a3 in HEK-293 cells similarly failed to produce anion exchange currents detectable above background (35).

**Electroneutral Cl^−/HCO_3^− Exchange and Pancreatic Ductal HCO_3^− Secretion—**The current observations suggest that electrogenic anion exchange by SLC26A6 and SLC26A3/DRA should not be relied on to satisfy the thermodynamic requirements of models of human or mouse pancreatic bicarbonate exchange.
secretion. The secretin-stimulated final [HCO₃⁻] of mouse pancreatic juice remains uncertain (the unlikely value of 30 mM is the only one yet reported (54)). Even if mouse pancreatic [HCO₃⁻] reaches levels comparable with the rat's 70 mM, electroneutral Cl⁻/HCO₃⁻ exchange could suffice to achieve this final concentration (55, 56). However, maximally stimulated human pancreatic juice [HCO₃⁻] resembles more closely the guinea pig's 125–150 mM than the rat's 70 mM. The apical cell membrane potential of isolated guinea pig pancreatic interlobular duct depolarizes only 10 mV, from −60 to −50 mV, during stimulation by cAMP or secretin (57). The latter potential of −50 mV can sustain adequate drive force for electrogenic a luminal [HCO₃⁻] of 125 mM. Moreover, depolarization and hyperpolarization of duct cell apical membrane by changing luminal [K⁺] in the presence of 125 mM luminal HCO₃⁻ were not dependent on the presence of luminal Cl⁻ (58). This finding is consistent with previous observations that high extracellular [HCO₃⁻] inhibits apical Cl⁻/HCO₃⁻ exchange in the pancreatic duct (53, 59) and suggests that conductive HCO₃⁻ secretion by the distal pancreatic duct in the presence of high luminal [HCO₃⁻] is not mediated by anion exchangers.

Recent calculations for the guinea pig distal duct predict that electroneutral Cl⁻/HCO₃⁻ exchange would be of limited consequence (63). The luminal [HCO₃⁻] achievable by apical exchange of two Cl⁻ outward for one Cl⁻ inward (as proposed for slc26a6 (28)) is only 6 mM higher than that achievable by electroneutral exchange. The stoichiometry of 1 HCO₃⁻/2 Cl⁻ (as proposed for slc26a3/DRA (28)) predicts a luminal [HCO₃⁻] 6 mM lower than for electroneutral exchange. The increase in relative HCO₃⁻ permeability of CFTR induced by low bath Cl⁻ (37) and the inhibitory gating of CFTR by extracellular Cl⁻ (62) should also be considered as contributors to the final composition of the pancreatic ductal secretion.

**Human SLC26A6 and Mouse slc26a6 Differ in Electrogenicity of Cl⁻ / Oxalate Exchange—Cl⁻/oxalate exchange by mouse slc26a6 was shown under voltage clamp conditions to be strongly electrogenic (Fig. 11B), and the oxalate-dependent currents resembled [¹⁴C]oxalate fluxes in their DIDS sensitivity (Fig. 12). These observations correspond to those recorded in unclamped oocytes in both the absence and the presence of CO₂/HCO₃⁻ (24). In the current work, both mouse slc26a6 and human SLC26A6 also mediated high initial rates of oxalate/ HCO₃⁻ exchange. However, this exchange slowed considerably within 5 min (Fig. 7), perhaps reflecting a block of HCO₃⁻ efflux by subplasmalemmal accumulation of oxalate, since the affinity of mouse slc26a6 for oxalate greatly exceeds that of HCO₃⁻ (24). The data together support a mechanism in which divalent oxalate can be exchanged for monovalent Cl⁻ or HCO₃⁻ by orthologs of both species. Curiously, however, although human SLC26A6 exhibited [¹⁴C]oxalate influx rates and rates of [¹⁴C]oxalate influx into Cl⁻ bath that were 30–80% those of mouse slc26a6, Cl⁻/oxalate exchange by human SLC26A6 was not detectably electrogenic (Fig. 11D).**

Failure to detect electrogenicity of oxalate/Cl⁻ exchange by human SLC26A6 may represent a mechanistic difference with mouse slc26a6 arising from amino acid sequence divergence. (A single amino acid change suffices to change both anion selectivity and electrogenic activity of the SLC4A14/AE1 anion exchanger (41).) slc26a6 is expressed in mouse proximal tubule brush border (23), where it mediates most or all of the luminal oxalate-dependent chloride reabsorption (66). The difference between electroneutral and electrogenic transport might predict a lower rate of proximal tubular oxalate secretion in humans than in mice, should SLC26A6 be similarly rate-limiting in human proximal tubule. Failure to detect electrogenicity of oxalate/Cl⁻ exchange by human SLC26A6 might alternatively represent activation by human SLC26A6 (in a species-specific manner) of an endogenous oxycte cation current, obscuring detection of oxalate/Cl⁻ exchange current. It seems unlikely that human SLC26A6 expression levels, if adequate for detection of unidirectional oxalate flux, could be insufficient for detection of oxalate-evoked exchange current.

Although the coincidence of low ³⁶Cl⁻ transport rates with robust rates of oxalate flux and Cl⁻/dependent HCO₃⁻ transport in human SLC26A6 remains for now unexplained, the resolution of this contrast will elucidate at least part of the molecular basis of anion selectivity among and translocation by SLC26 anion exchangers. This insight should enhance understanding of human epithelial HCO₃⁻ secretion and its dysfunction in cystic fibrosis and other disorders. Study of SLC26A6-mediated oxalate transport may also improve understanding of familial oxalosis syndromes and of nephrolithiasis-associated hyperoxaluria.

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