Functional Characterization of Three Novel Tissue-specific Anion Exchangers SLC26A7, -A8, and -A9*

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A second distinct family of anion exchangers, SLC26, in addition to the classical SLC4 (or anion exchanger) family, has recently been delineated. Particular interest in this gene family is stimulated by the fact that the SLC26A2, SLC26A3, and SLC26A4 genes have been recognized as the disease genes mutated in diastrophic dysplasia, congenital chloride diarrhea, and Pendred syndrome, respectively. We report the expansion of the SLC26 gene family by characterizing three novel tissue-specific members, named SLC26A7, SLC26A8, and SLC26A9, on chromosomes 8, 6, and 1, respectively. The SLC26A7–A9 proteins are structurally very similar at the amino acid level to the previous family members and show tissue-specific expression in kidney, testis, and lung, respectively. More detailed characterization by immunohistochemistry and/or in situ hybridization localized SLC26A7 to distal segments of nephrons, SLC26A8 to developing spermatocytes, and SLC26A9 to the luminal side of the bronchiolar and alveolar epithelium of lung. Expression of SLC26A7–A9 proteins in *Xenopus* oocytes demonstrated chloride, sulfate, and oxalate transport activity, suggesting that they encode functional anion exchangers. The functional characterization of the novel tissue-specific members may provide new insights to anion transport physiology in different parts of body.

The systematic characterization of gene families using full genome sequences provides a rich source for expanding our physiological understanding of body functions. Recently, a second distinct family of anion exchangers, SLC26, has been delineated. The members of the SLC26 family are well conserved across different species and can mediate the electroneutral exchange of Cl− for HCO₃⁻ across the plasma membrane of mammalian cells like members of the classical SLC4 (anion exchanger) family (1–3). Specific interest in the SLC26 gene family is stimulated by the fact that the first three human genes are associated with phenotypically distinct recessive diseases. The SLC26A2, SLC26A3, and SLC26A4 genes have been recognized as disease genes mutated in diastrophic dysplasia, congenital chloride diarrhea, and Pendred syndrome, respectively (4–6). Thus, the three closely related but highly tissue-specific human anion transporters play central roles in the etiology of phenotypically very different recessive diseases.

In human, six tissue-specific genes of the SLC26 family have been cloned so far, namely SLC26A1–A4 (previously known as SAT-1, DTDST, CLD or DRA, and PDS, respectively), SLC26A6, and TAT1. The SLC26A2–A4 members have been shown to transport, with different specificities, the chloride, iodide, bicarbonate, oxalate, and hydroxyl anions (7–12). SLC26A5 has been cloned from gerbil and rat and shown to act as a motor protein of cochlear outer hair cell; it is sensitive to intracellular anions but has not been found to act as a transporter (13, 14). The SLC26A6 protein is expressed at highest levels in the kidney and the pancreas and suggested SLC26A6 as a candidate for a yet unknown cystic fibrosis transmembrane-regulated protein responsible for the luminal anion exchanger activity in pancreas (7, 15). The newest member, TAT1, has been shown to act as a sulfate transporter in human male germ cells and has been linked to RhoGTPase signaling (16).

Our observation that the first fully sequenced animal, *Caenorhabditis elegans*, has seven members of this gene family prompted us to hypothesize that many more than three genes with important physiological functions might exist also in human (2). By a homology approach, five novel loci were identified with distinct tissue expression patterns (7). In this study, we have characterized two previously mapped candidates in the chromosomes 8 and 6 and a novel gene in chromosome 1, named SLC26A7–A9, respectively (Table I). The SLC26A7–A9 proteins show a high degree of similarity to the previous tissue-specific family members and are expressed at the highest level in kidney, testis, and lung, respectively. Functional expression of SLC26A7–A9 in *Xenopus laevis* oocytes demonstrated chlo-

diothiocyanato[sulfonfyl]stilbene; PDZ, PSD-95/Disc-large/Z-1; STAS, sulfate transporter and anti-σ-factor antagonists; ST, sulfate transporter family domain; aa, amino acid; RT, reverse transcriptase.
Characterization of Three Tissue-specific Anion Exchangers

The chromosomal locations of SLC26A10 and -A11 are known, but the genes remain poorly characterized.

### Table I

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Chromosome location</th>
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### EXPERIMENTAL PROCEDURES

#### Computational Sequence Analysis—The SLC26A7 and -A8 genes were identified and mapped as described before (7). SLC26A9 was first found by searching for the sulfate transport motif (PS01130) of the human SLC26A3 protein against NCBI’s high throughput genomic sequence (htgs) data base with TBLASTN algorithm (17–19). GENSCAN was used to predict the coding regions from genomic clones (20–22). Multiple alignment of the protein sequences was done by ClustalW and BoxShade Server 3.21 (www.ch.embnet.org/software/BOX_form.html). Putative N-glycosylation sites were analyzed by the PROSITE program (23). Transmembrane topologies of the SLC26A7–A9 proteins were predicted by the TMHMM (24) and PSIPred programs (25).

#### Cloning of SLC26A7–A9 cDNAs—The coding region of each gene was assembled from several overlapping PCR fragments, which were amplified by gene-specific primers designed to GENSCAN-predicted exons. The primer sequences can be obtained from the authors (hannes.lohi@helsinki.fi and juha.kere@biosci.ki.se). The overlapping PCR fragments were amplified by PCR from the first strand cDNAs of human kidney, testis, and lung. The first strand cDNAs were synthesized from 1 μg of poly(A) RNA (CLONTECH, Palo Alto, CA) by the SMART RACE cDNA Amplification Kit (CLONTECH) according to the manufacturer’s instructions. The SMART RACE method was used for amplification of the 5’ and 3’ ends of the SLC26A7–A9 cDNAs by PCR. The following antisense primers were used with the universal primer mix for 5’ rapid amplification of cDNA ends: 5’-ATA AGA ATT CAA GCT TGT ACA TTG-3’ and 5’-ACT TTG AAA TGC AAA GGA TCC ACG-3’ for A7; 5’-GGA CTT AGA CCT GAT GAA GCC AGA GAT-3’ and 5’-CTG AAG GTG GTG ATG TTG ATC TTG-3’ for A8; and 5’-GCC GGT TGG TGT GTA TGT ACA ATG ATT CCA ATC-3’ and 5’-CTG GGC TTG TTG CTT TCA CCA CCA GCT-3’ and 5’-CTT GTG GGA CCT GGA TGC ATT CCG-3’ for A9. The following sense primers were used for 5’ rapid amplification of cDNA ends: 5’-ACC CTG CAG CAG GTG AAA ATT ATC-3’ and 5’-CTA ATT CAG TAT AGA TTA TCC TGG GCA-3’ for A7; 5’-CTG GCC TAT GTA TCA TCA TTC GTC TAT GTC TTC for A8; and 5’-GGA GAT CCT ACA GGA GCC TCA GGA ATT TCC-3’ and 5’-GTT TGA AGC AGG AAA GAT GGA GCC AA-3’ for A9. The cloning of the 3’ ends of the genes was also verified by PCR with primers designed to the regions, which matched the expressed sequence tags (EST) annotated in GenBank79. PCR were done in 25-μl volumes using 5 μl of cDNA as template, 10 pmol of each primer, 1× reaction buffer, 0.2 mM of each nucleotide, and 1 unit of Advantage Polymerase Mix (CLONTECH) using the following conditions: 94°C for 3 min, 35 cycles of 94°C for 30 s, and 68°C for 1 min–2 min, followed by 72°C for 10 min. PCR products were subcloned to PCR-2.1 plasmid (Invitrogen) and sequenced using dye-terminator chemistry (26) and an automated sequencer (ABI 373A, Applied Biosystems, Inc.).

### Northern Analysis—Northern analysis was done using the CLONTECH (Palo Alto, CA) MTN Northern blots (MTN 7760-1 and MTN 7760-1). A 1984-bp PCR-amplified probe corresponding to nt 198–2181 of SLC26A7, a 551-bp probe corresponding to the 5’ sequence from nt 156–707 of SLC26A8, and a 2362-bp probe corresponding to the open reading frame sequence from nt 115–2476 of SLC26A9 were radiolabeled with [32P]dCTP with Rediprime Kit (Amersham Biosciences) according to the manufacturer’s instructions. The specific probes were hybridized to Northern blot filters in ExpressHyb solution (CLONTECH) for about 2 h to overnight, followed by washes with 2× SSC, 0.1% SDS at room temperature to 65°C for several hours. Autoradiography was performed on x-ray films at –20°C.

#### PCR Analyses of SLC26A7–A9 Genes—PCR analyses were done as above using cDNAs from the CLONTECH’s human multiple tissue cDNA panels I and II (K1421–1 and K1421–1) with the following primers: 5’-ATA GGA CAG TAG TAC CCT CCA GGA GGA GAG-3’ and 5’-GGA AGC ATC ATC AAG ATT-3’ for A9. The primers for SLC26A7 were radiolabeled and hybridized to Northern blot filters in ExpressHyb solution (CLONTECH) for about 2 h to overnight, followed by washes with 2× SSC, 0.1% SDS at room temperature to 65°C for several hours. Autoradiography was performed on x-ray films at –20°C.

### Immunohistochemistry—A 538-bp DNA fragment corresponding to positions 1629–2166 of the SLC26A8 cDNA was generated by PCR and was used to transcribe sense and antisense RNA probes (27). Formalin-fixed, paraffin-embedded specimens of adult human testes were used in the Department of Pathology, Haartman Institute, University of Helsinki. Deparaffinized 5-μm tissue sections were digested with 1 μg/ml proteinase K for 30 min at 37°C and treated with 0.1 M triethanolamine buffer containing 0.25% acetic anhydride for 10 min at room temperature. Sections were hybridized with 35S-labeled RNA probes (4 × 105 cpm/μl of hybridization buffer) at 52°C overnight. The slides were then washed under stringent conditions with buffer containing RNase A (28), digested to LM-1 emulsion (Amersham Biosciences), and exposed for 10–30 days at 4°C. The slides were developed and counterstained with hematoxylin and eosin. The sense RNA probe was used as a negative control.
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TABLE II
Conservation of the exon structure of the human SLC26 family members

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<td>72</td>
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</table>

mens of adult human kidney, testis, and lung were obtained from the Department of Pathology, Haartman Institute, University of Helsinki. Deparaffinized 5-μm tissue sections were pretreated in 0.3% H2O2 for 30 min to block the endogenic peroxidase activity. The antisera were diluted 1:1000–1:2000. The peroxidase-antiperoxidase technique was performed utilizing the Vectastain Elite ABC Kit (Vector Laboratories). Diaminobenzidine was used as the chromogenic substrate, and the slides were counterstained with Mayer hematoxylin. Preimmune serum for each immunized rabbit was used as the corresponding negative control, with similar dilutions to the antisera.

Functional Transport Measurements in X. laevis Oocytes—Mature X. laevis females were purchased from the African Xenopus Facility C.C., Noordhoek, South Africa. Stage V and VI oocytes from X. laevis were maintained at 17°C in modified Barth’s solution (89 mM NaCl, 1 mM KCl, 0.82 mM MgSO4, 0.4 mM CaCl2, 0.33 mM Ca(NO3)2, 2.4 mM NaHCO3, 10 mM HEPES/Tris, pH 7.4) or solution B (4 mM calcium gluconate, 10 mM HEPES/Tris, pH 7.4, gentamycin sulfate 20 mg/liter). Oocytes were injected with either 50–100 nl of water (control) or 7–12 ng of SLC26A7–A9 cRNA using a Nanojet automatic injector (Drummond Scientific Co., Broomall, PA). For cRNA synthesis, pcDNA3.1: SLC26A7 or pcDNA3.1: SLC26A7, pcDNA3.1:A8 or pNKS2:SLC26A8, and pcRNA:SLC26A9 plasmids were linearized by EcoRI, NotI, or XhoI digestion; the cDNAs were in vitro transcribed using T7 or SP6 RNA polymers (Promega), and the resulting capped cDNA was dissolved in MilliQ water before use. Transport of 35Sulfate, 35Cl– chloride, and 35Oxalate uptake was performed 3 days after injection. Briefly, 10 oocytes (per data point) were washed at room temperature for 1–2 min in solution A (115 mM sodium gluconate, 2.5 mM potassium gluconate, 4 mM calcium gluconate, 10 mM HEPES/Tris, pH 7.4) or solution B (100 mM choline chloride, 4 mM KCl, 2 mM CaCl2, 2 mM MgCl2, and 20 mM Heps/Tris, pH 7.5) and then placed into 100 μl of solution A containing 2.5 mM NaCl with 35Cl– or 0.1 mM oxalate with 2–5 μCi/ml 35Oxalate, or into 100 μl of solution B containing 0.1 mM KSO4 with 10 μCi/ml 35SO42– (PerkinElmer Life Sciences) for 30–60 min at room temperature. The oocytes were washed 4 times with ice-cold solution B, solubilized with 4% SDS, dissolved in scintillant (BCS, Amersham Biosciences), and counted by liquid scintillation spectrometry. Inhibition of the sulfate uptake of SLC26A9 was performed by adding either 1 mM DIDS, 5 mM thiourea, 5 mM oxalate, or 5 mM glucose to the uptake solution B. All isolates were purchased from PerkinElmer Life Sciences. Statistical analyses of the transport results were performed using the prism statistic package, version 3.0 (GraphPad software Inc., San Diego, CA). The degree of statistical significance between two groups was calculated using the unpaired t test, with p < 0.05 considered significant.

RESULTS

Characteristics of SLC26A7—Previously, the SLC26-related EST AA992554 was identified and mapped to chromosome 8. Its expanded sequence matched the human genomic draft sequence (18). Exons predicted by GENSCAN were verified by sequencing the overlapping PCR fragments. The 5′- and 3′-regions of the gene were expanded by RT-PCR. The combined data revealed an open reading frame of 1956 bp encoding a 656-amino acid protein. The complete cDNA sequence has been submitted to GenBankTM (accession number AF331521) and designated SLC26A7 (nomenclature for all three genes verified with the HUGO Nomenclature Committee).

The sequence flanking the putative ATG translation start site (GAAAATGACA) contains the −3 purine but not +4 guanine residues of the Kozak consensus sequence (29), and four in-frame stop codons precede that methionine. A 3024-bp 3′-untranslated region precedes the consensus polyadenylation signal AATAAA. However, several alternative poly(A) signals were identified at the 3′ end of the SLC26A7 composite sequence. Exon-intron boundaries were determined by aligning the cDNA sequence with genomic clones from the Human Genome Project and Celera Genomics (PAC clone RP11–353D5, GenBankTM accession number AC017061 and Celera’s clone GA_12HITB2W902, respectively) (30). The open reading frame was distributed across 19 exons ranging in size from 55 to 306 bp. All exon-intron boundaries obey the general AG-GT rule, and 10 of 19 exons are exactly the same size as those of the SLC26A3 and SLC26A4 genes that share 15 exons of similar size with each other (Table II). The total length of the gene spans about 100 kb of genomic sequence.

The SLC26A7 protein shows 50% similarity to SLC26A2 and A3 proteins (BLASTP E value < 10−30), and the multiple alignment of the proteins reveals a large number of conserved residues (Fig. 1). PROSITE predicted two putative N-glycosylation sites for SLC26A7 at Asn125 and Asn131. The 10- and 12-transmembrane structure with intracellular N- and C-terminal domains was suggested by the TMHMM and PSIPred program, resembling the topology predicted for the SLC26A3 protein (2). ProfileScan analysis of the SLC26A7 protein sequence revealed two domains, which are also commonly shared within the other SLC26 family members (Table III): sulfate transporter family domain (ST family, predicted between aa 200 and 500, PF00916) and sulfate transporters and antiporter family domain (apolipophosphatase, predicted between aa 500 and 700, PS580801). The putative NTP-binding STAS domain was suggested that anion transport could be regulated by intracellular nucleotides (31). In addition, C terminus of the SLC26A7 protein (SEV) comprises the conserved PDZ interacting motif (T/S)XΦ, where Φ is a hydrophobic amino acid (32). The PDZ domain plays an essential role in maintaining the cell polarity and function (33–35).

Characteristics of SLC26A8—Previously, using a homology approach we identified an SLC26-related genomic sequence (PAC clone 179N16) in chromosome 6, which predicted a homologous 300-amino acid N-terminal sequence of the protein by GENSCAN (7). Later, another genomic sequence (PAC clone RP11–482O9, accession AL133507) appeared in GenBankTM to the same region allowing us to predict the C terminus of the gene. Primers were designed for the predicted exons, and they were verified by sequencing the overlapping PCR fragments. The 5′- and 3′-regions were expanded and verified by RT-PCR. Sequence analysis revealed an open reading frame of 1971 bp encoding a 797-amino acid protein. The complete cDNA sequence has been submitted to GenBankTM (accession number AF331522), and the gene was designated SLC26A8. While our work was in progress, another group (16) reported also the characterization of the TAT1 gene and protein (for testis-specific anion transporter-1), which is identical to our SLC26A8. The full-length cDNA sequence of the SLC26A8 gene spans about 80 kb of genomic sequence. The open reading frame is distributed across 20 exons ranging in size from 49 to 369 bp.
FIG. 1. Multiple alignment of the human SLC26 family. Identical and similar amino acids are shaded in black and gray, respectively.
Exon-intron boundaries obey the general AG-GT rule. The sequence around the putative ATG translation start site (AG-GAATGGCA) contains the purine and guanine residues of the Kozak consensus sequence (29). A 361-bp 3'-untranslated region precedes the consensus polyadenylation signal AATAAA. A number of exons show conservation of the size, although there are more variations when compared with the other family members (Table II).

The SLC26A8 protein shows the best sequence similarity to SLC26A3 and SLC26A6 (BLASTP E value 2.1×10^{-10} and 1×10^{-73}, respectively). The overall amino acid similarity of SLC26A8 to the known members is over 50%, and multiple alignment of the proteins (Fig. 1) demonstrates the presence of conserved blocks among the proteins. SLC26A8 protein has 200–300 amino acids more than the other group members, and SLC26A8-specific extra sequences were found between amino acids 600–652 and C-terminal regions. However, comparison of the SLC26A8 protein with other family members reveals several unconserved residues (Fig. 1). Altogether eight putative N-glycosylation sites were found along the protein at Asn52, Asn192, Asn277, Asn384, Asn595, Asn651, Asn687, and Asn688. The 11-transmembrane structure of the SLC26A8 protein with intracellular N-terminal domain and extracellular C-terminal domain was predicted by the TMHMM program, whereas

![Figure 2](http://www.jbc.org/)

**TABLE III**

Analysis of the conserved domains within the SLC26 family members by ProfileScan

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<td>PROK lipopro</td>
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<td>PDZ</td>
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The expressions of β-actin and GADPH genes are shown as controls for Northern blots and multiple tissue panels, respectively. S. muscle, skeletal muscle; S. intestine, small intestine.

More detailed descriptions of the profiles can be found by searching the prosite and pfam databases (www.expasy.ch/prosite/ and www.sanger.ac.uk/Software/Pfam/index.shtml, respectively) with the profile accession numbers provided in the second column. For PDZ motif see Ref. 32.
Characterization of Three Tissue-specific Anion Exchangers

PSIpred failed to predict any structures for the protein. ProfileScan analysis of the SLC26A8 protein sequence found ST (aa 212–521) and STAS (aa 544–791) domains. The SLC26A9 protein is highly conserved with the known family members as shown in multiple alignment of the proteins (Fig. 1). SLC26A9 has two putative N-glycosylation sites at Asn153 and Asn156. ProfileScan analysis of the SLC26A9 protein sequence revealed ST (aa 187–497) and STAS (aa 520–733) domains. The C terminus of the SLC26A9 protein (TAL) comprises the consensus PDZ-protein interacting motif (Table II). The 9-transmembrane structure of the protein with intracellular N-terminal domain and extracellular C-terminal domain was suggested by the PSIpred and TMHMM programs.

Expression Profiles of SLC26A7–A9—The tissue distribution of the SLC26A7 gene was determined by Northern hybridization and PCR using CLONTECH’s multiple tissue cDNA panels with 16 different tissues. The 5250-bp full-length cDNA of SLC26A7 corresponds well with the observed size (~5 kb) in Northern analysis, and it revealed a specific tissue distribution, with most abundant expression in the kidney (Fig. 2A). A weaker ~3.0-kb transcript was also observed, and it might be derived from an alternative polyadenylation of the gene. The
long 3′-untranslated region of the SLC26A7 gene contains a number of consensus AUAAA or AUUAAAs that may serve as alternative polyadenylation signals in a reasonable agreement with the size of the less abundant ~3.0-kb transcript. PCR analysis also showed the most abundant expression in kidney, placenta, and testis supported consistently by the Northern analysis. Immunohistochemical experiments were performed in order to localize the expression of SLC26A7 in the human kidney. Positive signal was consistently detected in distal segments of nephrons, whereas the proximal tubules remained negative (Fig. 3a).

Both Northern and PCR analyses revealed a testis-specific distribution for SLC26A8 (Fig. 2B). The 3371-bp full-length cDNA of SLC26A8 corresponded with the observed size (~3.5 kb) in Northern analysis. More detailed cell-specific localization of SLC26A8 was analyzed by both in situ hybridization and immunohistochemistry in human testis. In situ hybridization with SLC26A8 antisense cRNA probe revealed abundant expression in the seminiferous tubules. The SLC26A8 mRNA was concentrated on the luminal side of the tubuli harboring the spermatocytes and the spermatids, whereas the peripheral side of the tubuli, containing the spermatogonia, appeared negative (Fig. 3, c–e). Consistently, immunohistochemistry showed distinct SLC26A8 protein expression in developing spermatocytes and spermatids (Fig. 3, f and g). Altogether, our data suggest that the expression of SLC26A8 is restricted to the meiotic phase of the germ cells in the human testis.

PCR analysis suggested that SLC26A9 is expressed predominantly in the lung, although some expression was found also in pancreas and prostate. The lung-specific expression of SLC26A9 was confirmed by Northern blotting (Fig. 2C). The 4815-bp full-length cDNA of SLC26A9 corresponded with the observed size (~4.8 kb) in Northern analysis. To refine the expression results, we used RT-PCR to study two human lung-specific epithelial cell lines, NCI-H358 and A549, from bronchoalveolus and alveolus, respectively (Fig. 2C). Both cell lines expressed SLC26A9. Furthermore, immunohistochemical stainings with SLC26A9 antisera revealed strong cytoplasmic staining in the bronchial (Fig. 3h) as well as the alveolar epithelium (Fig. 3j). Apparent membrane-associated accumulation of the signal was observed in the alveoli.

Functional Analyses of SLC26A7–A9—To characterize the function of the proteins encoded by SLC26A7–A9, in vitro transcribed cRNAs of SLC26A7–A9 were injected into X. laevis oocytes, and function was measured by [35S]sulfate, [36Cl]chloride, and [14C]oxalate uptakes. The expression of all three proteins separately led to the induction of chloride, sulfate, and oxalate transport above water-injected (control) oocytes (Fig. 4, a–c). Moreover, the SLC26A9-mediated sulfate transport was inhibited by the anion exchanger inhibitor DIDS and thiosulfate but not by oxalate or glucose (Fig. 4d). Similar inhibition of sulfate transport of SLC26A8 by DIDS was observed previously (16). These results demonstrate that SLC26A7–A9 proteins function as anion exchangers mediating at least chloride, sulfate, and oxalate transport.

**DISCUSSION**

A growing interest in the SLC26 family of anion exchangers is stimulated by the involvement of the first three members, SLC26A2–A4, in distinct human genetic diseases, the existence of new tissue-specific members, additional functionalities such as the motor activity of SLC26A5, and the concept that transport proteins may be organized in membrane microdomains through specific interacting proteins (2, 7, 14). In this report, we describe the functional characterization of three new tissue-specific members, designated SLC26A7–A9 (Table I).

The SLC26A7–A9 proteins are highly homologous to the previously known members as illustrated by multiple alignment of the proteins and also very similar to other genes for their genomic organization (Table II). The SLC26A7–A9 genes have 19–21 coding exons, and most of the exons are of similar size to the corresponding coding exons of the others. Phyloge-
The amino acid sequences of the human, D. melanogaster, and C. elegans reactors were retrieved from GenBank™ and aligned with ClustalX to generate a phylogenetic tree. The length of the branches reflects the number of substitutions between the sequences. Homology searches with the completed genome of D. melanogaster using known amino acid sequences of the SLC26 members revealed that it has nine members of this family. The numbering of the protein sequences of D. melanogaster and C. elegans (DROSO1, DROSO2 . . . , ELE1, ELE2 . . . , respectively) was based on the amino acid similarity with the human SLC26A3 protein by BLASTP E values so that the closest ortholog of SLC26A3 was assigned number 1 and so on. The GenBank™ accession numbers for the protein sequences of D. melanogaster are AAF49285, AAF56989, AAF57797, AAF55195, AAG22176, AAG22321, AAF56347, AAF52515 and AAF57068; for C. elegans T27820, T23629, T23628, T26165, T16077, Q94225, and T32945, respectively.

Phylogenetic analysis of the SLC26 family. The amino acid sequences of the human, D. melanogaster, and C. elegans exchangers were retrieved from GenBank™ and aligned with ClustalX to generate a phylogenetic tree. The length of the branches reflects the number of substitutions between the sequences. Homology searches with the completed genome of D. melanogaster using known amino acid sequences of the SLC26 members revealed that it has nine members of this family. The numbering of the protein sequences of D. melanogaster and C. elegans (DROSO1, DROSO2 . . . , ELE1, ELE2 . . . , respectively) was based on the amino acid similarity with the human SLC26A3 protein by BLASTP E values so that the closest ortholog of SLC26A3 was assigned number 1 and so on. The GenBank™ accession numbers for the protein sequences of D. melanogaster are AAF49285, AAF56989, AAF57797, AAF55195, AAG22176, AAG22321, AAF56347, AAF52515 and AAF57068; for C. elegans T27820, T23629, T23628, T26165, T16077, Q94225, and T32945, respectively.

The kidney is the major organ responsible for maintaining electrolyte balance and acid-base homeostasis in mammals. This is accomplished mainly by absorption of NaCl and secretion of acid or base equivalents in different segments of the nephron (37). Even though functional studies have characterized these exchangers, their molecular identities have remained largely unknown (38–40). We show here that the SLC26A7 protein is principally expressed in the distal segments of the nephrons and mediates chloride, sulfate, and oxalate transport. These results support the hypothesis that SLC26A7 may act as a distal excretory segment-specific anion exchanger, playing a role in the maintenance of the electrolyte and acid-base homeostasis in human kidney.

In situ hybridization and immunodetection experiments showed that SLC26A8 is exclusively expressed in the spermatocytes and in the developing spermatids, since we did not detect significant immunostaining in the spermatogonia. These results suggest that the expression of SLC26A8 is restricted to the meiotic phase of the development of the spermatogenic cells. The extensive morphological changes observed during spermatogenesis suggest that adequate regulation of intracellular ions might be a critical component of this differentiation process. Because SLC26A8 demonstrated chloride, sulfate, and oxalate transport activities, our results suggest its function as a novel male germ cell-specific anion exchanger, which may fulfill critical functions in male germ line. This raises the intriguing possibility that its mutations might result in impaired spermatogenesis in human. Furthermore, our results are consistent with the previous characterization of TAT1, which was published during the processing of this manuscript (16). Our results identify TAT1 and SLC26A8 as the same gene and confirm the previous observation regarding its expression and function.

Functional characterization of SLC26A9 as a DIDS-inhibited anion exchanger mediating chloride, sulfate, and oxalate transport casts more light into the anion transport physiology of human lung. The regulation of ion transport and airway surface liquid is an important part of lung defense mechanisms and may contribute to different airway diseases (41). Recent studies (42) suggest that the composition of airway surface liquid is regulated by active ion transport systems. In cystic fibrosis, abnormal ion transport results in a characteristic syndrome of retained secretions, bacterial infection, and lung destruction (43). Here we show that SLC26A9 is expressed both in the alveolar and the bronchial epithelium of the human lung. Thus, defects in the chloride or sulfate transport function of SLC26A9 in human respiratory epithelium make it a plausible candidate for diseases of the human respiratory system.

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Characterization of Three Tissue-specific Anion Exchangers

REFERENCES

Functional Characterization of Three Novel Tissue-specific Anion Exchangers
SLC26A7, -A8, and -A9
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