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SLC26 Chloride/Base Exchangers in the Kidney in Health and Disease

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Solute-linked carrier 26 (SLC26) isoforms are members of a large, conserved family of anion exchangers, many of which display highly restricted and distinct tissue distribution. Cloning experiments have identified 10 SLC26 genes or isoforms (SLC26A1-11). Except for SLC26A5 (prestin), all function as anion exchangers with versatility with respect to transported anions. Modes of transport mediated by SLC26 members include the exchange of chloride for bicarbonate, hydroxyl, sulfate, formate, iodide, or oxalate with variable specificity. Other anion exchange modes not involving chloride also have been reported for some of the members of this family. Several members of SLC26 isoforms are expressed in the kidney. These include SLC26A1 (SAT1), SLC26A4 (pendrin), SLC26A6 (putative anion transporter [PAT1] or chloride/formate exchange [CFEX]), SLC26A7, and SLC26A11. Each isoform displays a specific nephron segment distribution with a distinct subcellular localization. Coupled to expression studies and examination of genetically engineered mice deficient in various SLC26 isoforms, the evolving picture points to important roles for the SLC26 family in chloride absorption, vascular volume homeostasis, acid-base regulation, and oxalate excretion in the kidney. This review summarizes recent advances in the identification and characterization of SLC26 family members, with specific emphasis on their distribution and role in kidney physiology. Specifically, the roles of A4 (pendrin), A6 (PAT1), and A7 (PAT2) in chloride homeostasis, oxalate excretion, and acid-base balance are discussed.
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Anion exchangers are essential for a number of cell functions, including intracellular chloride, pH and volume regulation, and homeostatic maintenance of certain monovalent and divalent anions. Different functional isoforms of anion exchangers have been identified in in vivo and in vitro systems. These include Cl^-/HCO_3^- (bicarbonate), Cl^-/OH^- (hydroxyl), Cl^-/formate, Cl^-/oxalate, Cl^-/sulfate, and sulfate/oxalate/bicarbonate exchange.[1-5] In epithelial tissues, anion exchangers are expressed in apical and basolateral membranes and predominantly are involved with vectorial transport of various anions including chloride and bicarbonate.[1-5]

Cloning and functional expression studies over the past 2 decades have identified the solute-linked carrier (SLC)4 gene superfamily.[6-9] This family is composed of at least 3 well-known Na-independent chloride-bicarbonate exchanger genes (SLC4A1, A2, and A3) and multiple Na-bicarbonate cotransporter and Na-dependent anion exchanger genes (SLC4A4 to A11). The SCL4A1, A2, and A3, also known as AE1, AE2, and AE3, respectively, function as Cl^-/HCO_3^- exchangers.[6-9] AE1 and AE3 show a highly selective tissue-distribution pattern, whereas AE2 is expressed widely in both epithelial and nonepithelial tissues.[4-9] The AE1 gene encodes erythrocyte AE1 (band 3), the major intrinsic protein of the red blood cell, and kidney AE1, the basolateral Cl^-/HCO_3^- exchanger of the acid-secreting α-intercalated cell in the collecting duct.[7] The AE2 gene is distributed widely and is involved mostly with cell volume and/or intracellular pH (pHi)

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regulation.[4-9] The AE3 gene shows abundant expression in the heart and in a few other tissues, but less is known about its role and regulation. The epithelial cells AE1, AE2, and AE3 are all expressed in the basolateral membrane domain and presumably are involved with vectorial transport of chloride and/or bicarbonate.[4-9] One newly identified member of the SL4A family, namely SLC4A9, also mediates Cl^-/HCO_3^- exchange and is referred to as AE4.[10] This exchanger is expressed in the kidney cortical collecting duct, albeit with conflicting reports on its subcellular distribution.[11,12]

Two lines of evidence suggested that anion exchangers distinct from the AE family (AE1, 2, and 3) are expressed in the kidney and other epithelial cells and play important roles in electrolyte balance and acid-base homeostasis. First, immunolocalization studies could not unequivocally localize any of the AE members (AE1-3) in the apical membrane of the kidney nephron segments, where many functional isoforms of anion exchangers are detected.[4-9] Second, AE1 to 3 isoforms have a poor affinity for certain established modes of anion exchange such as chloride/oxalate or oxalate/bicarbonate exchange, which are well described in the kidney nephron segments. Taken together, these studies pointed to the presence of anion exchangers distinct from the AE family members in the kidney tubules.

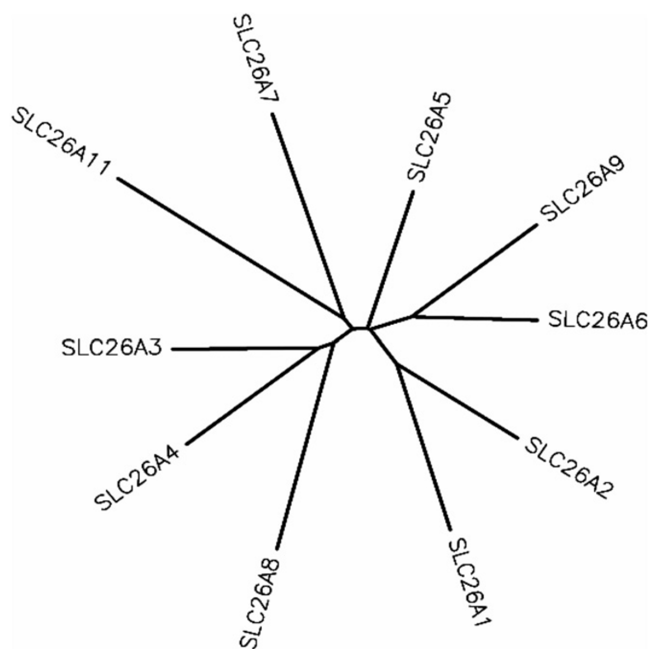

Figure 1 A phenotypic dendrogram illustrating the relationships of the SLC26 anion exchanger family.

SLC26 Isoforms: Cloning, Structural Features, and Tissue Distribution

SLC26 family members first were identified as sulfate transporters in Neurospora. Subsequent studies have established their presence in mammalians. To date, 10 different genes from the SLC26 family have been cloned.[13-26] An unrooted phenotypic dendrogram illustrating the relationships of the SLC26 anion exchanger family is shown in Figure 1. Although all SLC26 isoforms display a high degree of amino acid identity with one another, the dendrogram in Figure 1 shows striking homology among certain SLC26 members. As noted in this diagram, A1 and A2 display the highest degree of homology with each other, whereas A3 shows the closest homology to A4. Interestingly, A6 shows the highest sequence homology to A9, and A7 is closest to A11. The first 5 members (A1-A5) were identified by expression cloning (A1), linkage disequilibrium mapping (A2-4), or tissue-specific array (A5).[13-16,18,20] The rest, A6-A11, were cloned by wide genome scanning using homology-based searches.[21,22,24,26] In the following sections, we discuss the similarities and differences among SLC26 members with regard to their functional properties, tissues distribution, and subcellular localization, with specific emphasis on their expression and role in the kidney physiology.

Conserved Motifs in SLC26 Isoforms

SLC26 isoforms in general have 10 to 14 predicted membrane-spanning helixes followed by a long hydrophilic carboxyl terminal region,[13-16,18,20-26] which contains a unique signature sequence known as the sulfate transporter and anti-sigma (STAS) domain. The functional role of the STAS domain with respect to the transport of anions remains unknown. However, a recent report indicated that the STAS domain of the human SLC26A3 (downregulated in adenoma [DRA]) interacts with the cystic fibrosis transmembrane conductance regulator, thus resulting in mutual activation of both DRA and the cystic fibrosis transmembrane conductance regulator.[27] In addition to the STAS domain, SLC26 isoforms have unique short regions referred to as *sulfate transport* consensus signature in their transmembrane region,[13-16,18,20-26] which likely are important for the transport of anions.

Expression of SLC26 Isoforms in the Kidney

Figure 2 highlights the SLC26 isoforms that are expressed in the kidney. It further depicts those SLC26 isoforms that can function in Cl^-/HCO_3^- exchange mode. As indicated, SLC26 isoforms expressed in the kidney include SLC26A1, A2, A4, A6, A7, A8, and A11. As further indicated, SLC26A3 (DRA), SLC24A4 (pendrin), SLC26A6 (putative anion transporter 1 [PAT1]), SLC26A7 (PAT2), and SLC26A9 (PAT4) can function in Cl^-/HCO_3^- exchange mode.[19,25,28,29] SLC26A3 and SLC26A9 are not expressed in the kidney. In the following sections, the cloning, tissue distribution, and functional properties of renally expressed SLC26 isoforms are discussed. Further, the expression, role, and regulation of each isoform in the kidney in health and disease states are elaborated.

SLC26A1 (Sulfate/Anion Transporter-1)

The first evidence in support of a new family of anion exchangers came from studies of the liver. By using rat liver messenger RNA, Bissig et al[13] identified a single complemen-

	GI	Kidney	Cl^-/HCO_3^- exchange
SLC26A1 (SAT1)	+	+	no
SLC26A2 (DTDTS)	+@	+@	no
SLC26A3 (DRA; CLD)	+	-	yes
SLC26A4 (Pendrin; PDS)	-	+	yes
SLC26A6 (PAT1; CFEX)	+	+	yes
SLC26A7 (PAT2)	+	+	yes
SLC26A9 (PAT4)	+	-	yes
SLC26A11 (PAT5)	+@	+	yes@

@ : personal observation

Figure 2 Expression of SLC26 isoforms in the kidney and gastrointestinal tract.

tary DNA encoding a liver sulfate transporter using *Xenopus laevis* oocyte as the functional expression system. This transporter is an anion exchanger and likely mediates sulfate/oxalate or sulfate/bicarbonate exchange.[30] It was annotated sulfate/anion transporter 1 (SAT-1) in consonant with its ability to transport various anions in addition to sulfate. It encodes 701 amino acids in human beings (NM_022042) and 704 amino acids in the mouse (BC032151).

SAT-1 is expressed highly in the liver and kidney, with lower levels of expression in the brain and lung.[13] Based on functional studies showing the presence of a sulfate/anion exchanger with affinity for oxalate, it was concluded that SAT-1 is the liver canalicular sulfate transporter.[13] In the kidney, immunolabeling studies localized SAT-1 in the basolateral membrane of the proximal tubule,[31] where it probably is involved with the excretion of oxalate or sulfate.

SLC26A2 (Diastrophic Dysplasia Sulfate Transporter)

This gene was first identified by linkage disequilibrium studies and positional cloning in patients with diastrophic dysplasia (DTD) which is a well-characterized autosomal-recessive osteochondrodysplasia with clinical features including dwarfism, spinal deformation, and specific joint abnormalities.[14] Based on sequence homology to SLC26A1, it is suggested that SLC26A2 is a sulfate transporter, and impairment in its function likely leads to undersulfation of proteoglycans in cartilage matrix and thereby causes the clinical phenotype DTD. Indeed, functional studies in vitro show the transport of sulfate by SLC26A2.[32] Because of its role in the pathogenesis of DTD, SLC26A2 also is referred to as the DTD sulfate transporter. Both human (accession number NM_000112) and mouse (accession number NM_007885) SLC26A2 encode a 739 amino acid protein. Aside from the growing osteoblasts, thymus, and testis[14] Slc26a2 is detected readily in the kidney (data not shown), however, little is known about its function, nephron segment distribution, and subcellular distribution in this tissue.

SLC26A4 (PDS, pendrin)

SLC26A4 (PDS) is structurally the most closely related isoform to SLC26A3 (DRA). PDS is located in a head-to-tail arrangement in close vicinity to DRA on chromosome 7,[18] suggesting an ancient gene duplication. PDS is expressed at extremely high levels in the thyroid, where it is thought to be involved in iodide transport across apical membranes of the thyroid follicular epithelial cells.[18,33,34] PDS also is expressed in the inner ear,[35] but its function in this organ is not well known. PDS showed moderate expression in the kidney.[19] Mutations in PDS cause Pendred's syndrome, an autosomal-recessive hereditary disorder characterized by goiter and profound deafness.[18,36-38] Indeed, SCl26A4 was cloned by linkage analysis in families with Pendred's syndrome.[18] Both the human and mouse SLC26A4 (accession numbers NM_000441 and NM_011867, respectively) encode a 780 amino acid protein.

Expression, Regulation, and Role of Pendrin in the Kidney

Acid-Base Homeostasis

Messenger RNA expression of SLC26A4 in the kidney is detected predominantly in the cortical collecting duct (CCD), with lower levels in the proximal tubule.[19] However, SLC26A4 protein is detected only in the CCD.[19,39] Immunofluorescence and

immunohistochemical staining localized SLC26A4 in the apical membrane of B and non-A–non-B–intercalated cells.[39,40] Functional studies in in vitro expression systems showed that SLC26A4 mediates Cl^-/HCO_3^- exchange.[19] Based on its immunolocalization in the kidney and function, it was postulated that SLC26A4 is an apical Cl^-/HCO_3^- exchanger in intercalated cells and may play an important role in bicarbonate secretion in the collecting duct.[3,19,39] In support of this possibility, pendrin expression was found to be decreased in potassium depletion but increased in bicarbonate loading.[41] Although the activity of the apical Cl^-/HCO_3^- exchanger in pendrin-expressing cells was not examined, these results[41] possibly point to an important role for pendrin in systemic acid-base homeostasis by regulating bicarbonate secretion in the collecting duct. It was hypothesized that pendrin downregulation may contribute to the maintenance of metabolic alkalosis in hypokalemia by blunting bicarbonate secretion in the collecting duct, and its upregulation may contribute to enhanced bicarbonate secretion and prevention of metabolic alkalosis in bicarbonate loading.[41] Essential to the earlier-described conclusions is, of course, the assumption that the activity of apical Cl^-/HCO_3^- exchanger in B (and non-A–non-B)-intercalated cells directly correlates with the expression of pendrin. This relationship was tested in metabolic acidosis, which is associated with decreased net bicarbonate secretion and enhanced bicarbonate reabsorption in the CCD.[42] In rats with metabolic acidosis, the expression of pendrin in B-intercalated cells decreased significantly and correlated with decreased activity of apical Cl^-/HCO_3^- exchanger in B-intercalated cells, which was measured in microperfused tubules.[42] The definitive answer with regard to the role of pendrin in bicarbonate secretion was ascertained in pendrin null mice. Bicarbonate-loaded pendrin null mice showed impaired bicarbonate secretion in their collecting duct, providing strong evidence that Slc26a4 indeed plays an important role in bicarbonate secretion in the collecting duct.[39]

Vascular Volume Homeostasis and Blood Pressure Regulation

Recent studies have examined the role of pendrin in mineralocorticoid-mediated hypertension. In a mouse model of aldosterone-induced hypertension (salt loading plus deoxycorticosterone pivalate (DOCP), the expression of pendrin increased significantly and animals developed hypertension. However, pendrin null mice had a blunted blood pressure response to the same treatment.[43] It was postulated that pendrin plays an important role in chloride absorption and its absence may impair coordinated NaCl absorption in the collecting duct, resulting in prevention of hypertension in response to mineralocorticoids.[43]

The role of pendrin in blood pressure and fluid and electrolyte homeostasis was explored further during NaCl restriction, a treatment known to increase aldosterone. Slc26a4 expression in the apical plasma membrane increased significantly in type B intercalated cells and was associated with increased chloride absorption in the CCD in mild NaCl restriction.[44] However, Slc26a4(−/−) mice failed to increase chloride absorption in their CCDs under the same maneuver.[44] In moderate NaCl restriction, Slc26a4 null mice displayed increased urinary volume and Cl^- excretion relative to wild-type (WT) animals, and developed volume depletion along with metabolic alkalosis.[44] In severe NaCl restriction, Slc26a4(−/−) mice became hypotensive relative to WT mice. These results strongly suggest that Slc26a4 is upregulated with NaCl restriction and is critical in the maintenance of acid-base balance and renal conservation of Cl^- and water during NaCl restriction. Taken together, these results show that pendrin plays an essential role in vascular volume homeostasis in NaCl restriction.

The schematic diagram in Figure 3 illustrates the role of pendrin in coordinated absorption of sodium and chloride in CCD in response to mineralocorticoid excess. According to this diagram, activation of pendrin and sodium channel in response to aldosterone results in enhanced chloride and sodium absorption in the CCD. In normal subjects this leads to the expansion of vascular volume and generation of hypertension. Under NaCl restriction, this results in enhanced volume and salt absorption and prevents volume depletion and hypotension.

SLC26A6 (PAT1, CFEX)

SLC26A6 was cloned from the pancreas based on homology to DRA (SLC26A3) and pendrin (SLC26A4).[21] Human SLC26A6 (Genebank accession number AF279265) maps to chromosome 3 and encodes a 738 amino acid protein.[21] Mouse Slc26a6 Genebank accession number AY032863) encodes a 735 amino acid protein.[23] Functional expression studies in in vitro systems showed that human or mouse PAT1 can mediate various anion exchange modes including Cl^-/HCO_3^-, Cl^-/oxalate, Cl^-/hydroxyl, and Cl^-/formate exchanges.[21,23,25] Anion exchangers with similar functional properties have been identified in apical membranes of kidney proximal tubule and villi of small intestine[1-5,45,46] where SLC26A6 is abundantly detected by immunohistochemical studies.[25] Based on its ability to transport anions, it was annotated *putative anion transporter 1* (PAT-1), and based on its ability to exchange chloride for formate it was named *CFEX* (chloride/formate exchange). In addition to the kidney and gastrointestinal tract, SLC26A6 shows expression in the heart, brain, skeletal muscle, and lung.[21,25]

Expression, Regulation, and Role of PAT1 in the Kidney

In the kidney, SLC26A6 is located in the apical membrane of the proximal tubules.[23,47] Based on its apical localization in the kidney and its ability to transport various chloride/anion exchange modes, it was postulated that SLC26A6 is a major contributor to NaCl absorption in the proximal tubule.[23,47] To study its role in kidney physiology, a gene-targeting strategy was used in our laboratory to prepare mice lacking Slc26a6.[48]

Chloride Absorption in the Proximal Tubule

The Slc26a6 null mice appear normal, with normal growth, blood pressure, and serum electrolyte profile. Studies in microperfused kidney proximal tubule showed that the apical

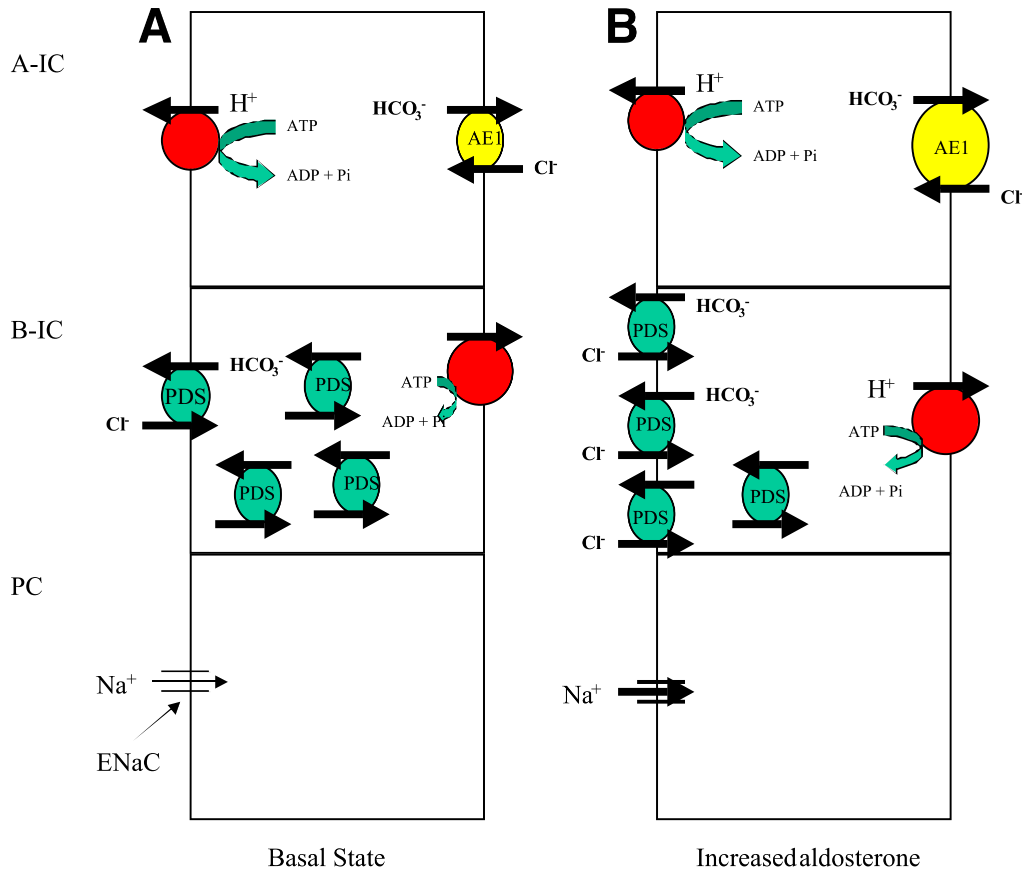

Figure 3 Schematic diagram showing the role of pendrin (SLC26A4) in coordinated absorption of sodium and chloride in CCD. As indicated in A and B, aldosterone increases the abundance of pendrin in the apical membranes of cortical B-IC cells and in conjunction with Na channel activation results in enhanced chloride and sodium absorption in the CCD. See Wall et al[40,44] and Verlander et al[43] for more detail.

Cl-base exchanger activity was reduced by approximately 60% in Slc26a6 null animals.[48] Further, oxalate-stimulated NaCl absorption, which is an important mechanism for electrolyte and fluid reabsorption in the proximal tubule, was abolished completely in Slc26a6 null mice.[48] Formate-stimulated NaCl absorption, however, was not affected significantly in proximal tubules of Slc26a6 null mice.[48] Taken together, these results show that Slc26a6 is a major apical chloride-absorbing transporter in the proximal tubule. Interestingly, urine chloride excretion in Slc26a6 null mice is comparable with that in WT animals,[48] suggesting the presence of either compensatory upregulation of other chloride-absorbing transporters in the kidney or enhanced chloride absorption via paracellular pathways. It should be noted that unlike the known tight junction molecules (claudins), which regulate paracellular absorption of cations, no anion-specific tight junction molecules have been identified. Further, molecular analysis of the distal kidney segments did not show upregulation of any known chloride-absorbing transporters (ie, BSC1, NCC, and pendrin) in PAT1 knockout (KO) mice (personal observation). Last, a recent study showed that the apical chloride/formate exchanger activity is intact in the kidney proximal tubule in PAT1 KO animals (see later). The absence of enhanced chloride excretion, coupled with the lack of compensatory upregulation in distal chloride-absorbing transporters, raises a strong possibility that another, yet to be identified, chloride-absorbing transporter is present in the kidney proximal tubule. Whether this other transporter is upregulated in PAT1 KO mice remains speculative at the present.

Oxalate Excretion in the Kidney

Slc26a6 is the most abundant apical anion exchanger in the small intestine.[25,48,49] Given its ability to transport chloride/oxalate exchange, the unidirectional and net fluxes of oxalate across the distal ileum of WT mice and Slc26a6 null mice were examined.[50] The selection of distal ileum for these studies was because this segment of the small intestine is the major site of oxalate transport in the gastrointestinal tract. In addition, urinary oxalate excretion was measured in both groups. In WT mouse ileum, there was a small net secretion of oxalate, whereas in KO mice, net oxalate was significantly absorptive, which was the result of a large mucosal-to-serosal oxalate flux, leading to the reversal of the direction of net oxalate transport from secretion to absorption.[50] Interestingly, urinary oxalate excretion was about 4-fold greater in KO mice compared with WT littermates, as a result of en-

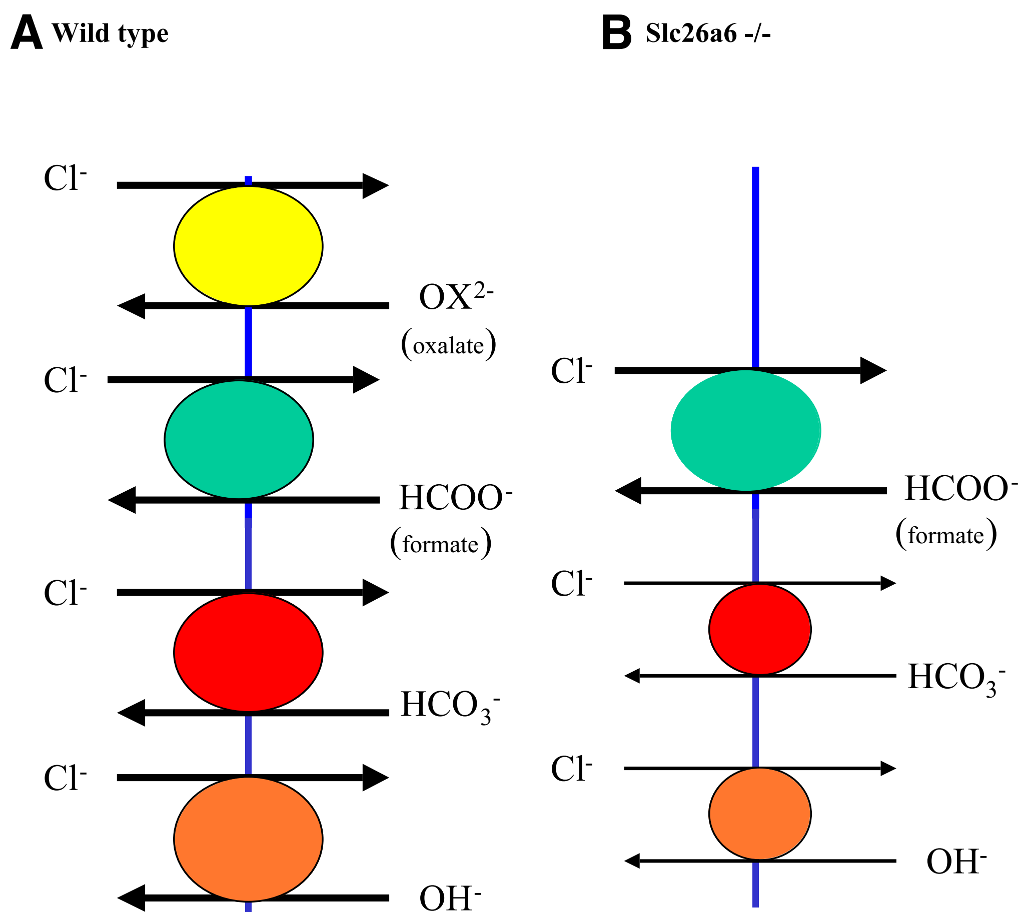

Figure 4 Schematic diagram showing the role of PAT1 (SLC26A6) in mediating various apical anion exchangers in the kidney proximal tubule. Apical anion exchange modes in WT (A) and Slc26a6 KO mice (B). As shown, the Cl^-/oxalate exchange is abolished completely and Cl^-/OH^- and Cl^-/HCO_3^- exchanges are downregulated significantly in Slc26a6 KO mice (B). Interestingly, Cl^-/formate exchange activity is completely intact and, as displayed, a significant portion of Cl^-/OH^- and Cl^-/HCO_3^- exchange activities are retained in Slc26a6 null mice. See Wang et al[48] and Jiang et al[51] for more detail.

hanced oxalate absorption in the intestine. We conclude that PAT1 mediates a significant fraction of oxalate secretion across the intestinal apical membranes in exchange for Cl^-.[50] The increased absorption of oxalate from the small intestine and its increased excretion from the kidney was confirmed in another independently generated Slc26a6 null mouse.[51] Further, increased renal excretion of oxalate was associated with a high incidence of calcium oxalate urolithiasis.[51] Taken together, these studies show that Slc26a6 plays an important role in oxalate homeostasis and its deletion results in enhanced oxalate absorption from the intestine with increased blood levels, resulting in increased renal excretion of oxalate and urolithiasis (see Fig 4).

The schematic diagrams in Figure 5 illustrate the anion exchange modes in normal kidneys (left panel) and kidneys of Slc26a6 KO mice (right panel). As shown, the Cl^-/oxalate exchange is abolished completely and Cl^-/OH^- and Cl^-/HCO_3^- exchanges are downregulated significantly in Slc26a6 KO mice (right panel). Interestingly, Cl^-/formate exchange activity is completely intact and, as displayed, a significant portion of Cl^-/OH^- and Cl^-/HCO_3^- exchange activities are retained in Slc26a6 null mice. Taken together, these results suggest that although Slc26a6 mediates the entire Cl^-/oxalate exchange, other anion exchanger(s) mediate the Cl^-/formate exchange and the remaining $Cl^-/OH^-/HCO_3^-$ exchange activities. Of interest is the issue of electrogenicity of SLC26A6/Slc26a6. Although this exchanger is electrogenic in its chloride/oxalate exchange mode,[23,52] studies on the electrogenicity of its Cl^-/HCO_3^- exchange mode are conflicting.[52,53] Figure 4 illustrates the role of SLC26A6 in oxalate transport in the ileum. According to this proposed model, PAT1 mediates oxalate excretion in the ileum[50] and its deletion leaves the oxalate-absorbing transporter(s) unopposed,[50] resulting in enhanced oxalate absorption, increased serum oxalate concentration, and enhanced oxalate excretion in the kidney,[50] therefore promoting urolithiasis[51]

SLC26A7

SLC26A7 was cloned based on homology to DRA (SLC26A3) and pendrin (SLC26A4).[21,24] There are 2 SLC26A7 variants. Human SLC26A7 variant A (Genebank accession number

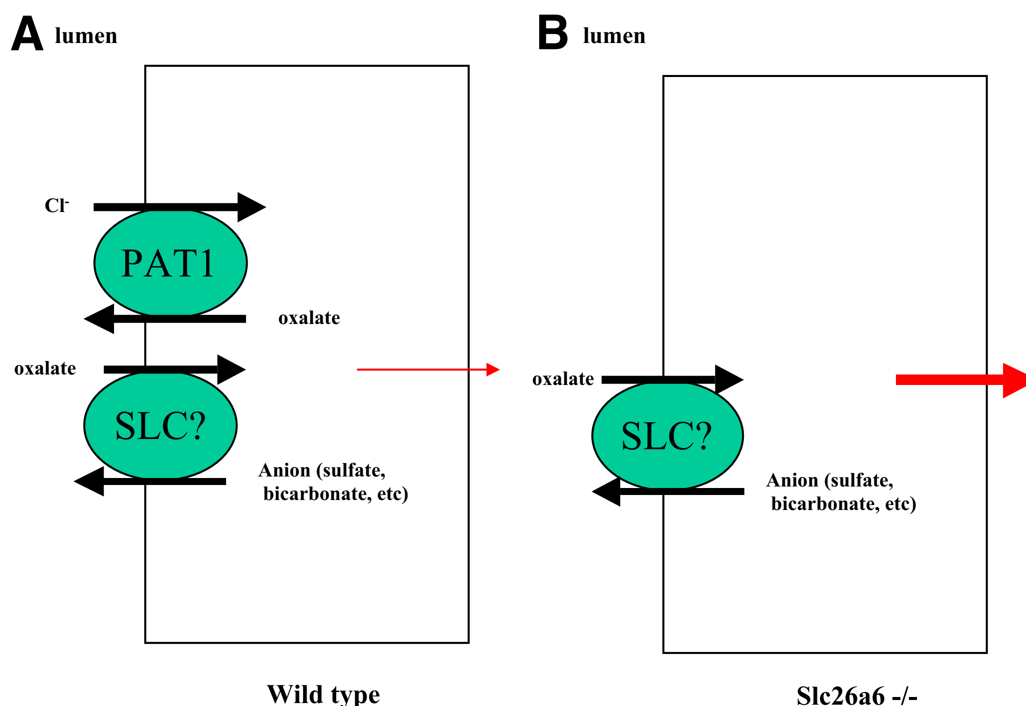

Figure 5 Proposed model depicting the role of PAT1 (SLC26A6) in oxalate transport in the ileum. According to this proposed model, PAT1 mediates oxalate excretion in the ileum (A) and its deletion leaves the oxalate-absorbing transporter(s) unopposed (B), resulting in enhanced oxalate absorption, increased serum oxalate concentration, and enhanced oxalate excretion in the kidney, therefore promoting urolithiasis. Thick red arrow in Slc26a6 KO mouse denotes enhanced oxalate absorption across the basolateral membrane. See Freel et al[50] for more detail.

AF331521) maps to chromosome 8 and encodes a 656 amino acid protein.[22] Mouse Slc26a7 (Genebank accession number NM_145947) encodes a 656 amino acid protein.

The original cloning articles detected the expression of SLC26A7 in the kidney and placenta with lower levels in testis.[21] Functional expression studies showed that SLC26A7 can transport sulfate, chloride, and oxalate.[21] Subsequent pHi studies showed that SLC26A7 can mediate Cl^-/HCO_3^- exchange.[28,29] A recent report indicated that SLC26A7 can function as a pH-sensitive anion channel.[54]

Human SLC26A7 gene encodes 2 different splice variants (A and B), however, only a single mouse Slc26a7 isoform (A) has been identified. The difference in the coding regions of the 2 SLC26A7 variants reside in their C-terminal ends, where the last 11 amino acids in variant A are replaced with 18 amino acids in variant B. In addition, the C-terminal noncoding region of the 2 variants are significantly diverse in their sequence. A wide genome search identified Chimpanzee as the only other species with 2 variants, indicating that the splicing of the SLC26A7 is specific to primates.

Expression, Regulation, and Role of SLC26A7 in the Kidney

Recent studies showed high expression levels of SLC26A7/Slc26a7 in the stomach and kidney.[28,29] Subsequent studies in our laboratory examined the expression and localization of Slc26a7 in the kidney in more detail, as discussed later.

Expression of SLC26A7 in the Kidney

In the kidney, Northern and Western hybridizations localized Slc26a7 predominantly to the outer medulla with lower expression levels in the inner medullas and no expression in the cortex.[29] Immunocytochemical staining showed the localization of SLC26A7 in the basolateral membrane of a subset of the outer medullary collecting duct (OMCD) cells.[29] No labeling was detected in the cortex. Double immunofluorescence labeling with AQP2 and SLC26A7 antibodies identified the SLC26A7-expressing cells as acid-secreting A-intercalated cells.[29] Immunofluorescent labeling showed the colocalization of AE1 and SLC26A7 in the basolateral membrane of A-intercalated cells.[29] We concluded that Slc26a7 is located in the basolateral membrane of acid-secreting intercalated cells (A-ICs) in the OMCD.[29]

Basolateral Cl^-/HCO_3^- Exchange Activity in A-ICs in OMCD

Functional studies in microperfused kidney collecting ducts have identified a basolateral Cl^-/HCO_3^- exchanger in A-IC cells of the cortical and OMCD.[55,56] Immunohistochemical studies have localized AE1 to the basolateral membrane of the α-intercalated cells in both the CCD and OMCD.[57,58] Based on functional studies in microperfused tubules and immunolocalization studies, it was suggested that AE1 is the main basolateral Cl^-/HCO_3^- exchanger in A-intercalated cells in CCD and OMCD.[57,58] However, deletion of AE1 in

mouse does not completely abrogate the basolateral Cl^-/HCO_3^- exchanger activity in A-ICs in OMCD, strongly supporting the existence of another Cl^-/HCO_3^- exchanger on the basolateral membrane of these cells.[59] Based on functional studies showing mediation of Cl^-/HCO_3^- exchange and immunolocalization studies, it was proposed that SLC26A7/Slc26a7 contributes to basolateral Cl^-/HCO_3^- exchange and bicarbonate absorption in OMCD.[29]

Differential Regulation of SLC26A7 and AE1 in Kidney OMCD In Vivo

The presence of 2 distinct Cl^-/HCO_3^- exchangers, SLC26A7 and AE1, in the basolateral membrane of A-IC cells in OMCD raises the possibility of differential regulation between these 2 exchangers in pathophysiologic states. Specifically and based on functional studies showing the activation of SLC26A7 by high osmolarity[29] and the fact that the outer medulla is exposed to a hypertonic medium in vivo, it was postulated that SLC26A7 may be more active in conditions associated with increased medullary interstitial osmolarity. This hypothesis was tested in rats subjected to 3 days of water deprivation, a condition known to increase the osmolality of the medulla. Northern and Western hybridization showed that SLC26A7 expression was increased whereas the expression of AE1 decreased in water-deprived rats.[60] Interestingly, the expression of SLC26A7 remained unchanged in the kidney cortex and stomach in water deprivation, indicating the specificity of SLC26A7 upregulation in the outer medulla.[60] It was proposed that SLC26A7 might play an important role in bicarbonate reabsorption and/or cell-volume regulation in OMCD (specifically under hypertonic conditions).

SLC26A7 Trafficking in Kidney Cells In Vitro

Immunofluorescent-labeling studies show that the Cl^-/HCO_3^- exchanger SLC26A7 is expressed in the basolateral membrane and cytoplasm of 2 distinct acid secreting epithelial cells: the A-ICs in the kidney OMCD and the gastric parietal cells.[28,29] The intracellular localization of SLC26A7 suggests the possibility of trafficking between the cell membrane and intracellular compartments. To test this hypothesis, full-length human SLC26A7 complementary DNA was fused with green fluorescent protein (GFP) and transiently expressed in Madine Derby canine kidney (MDCK) epithelial cells. In monolayer cells in isotonic media, SLC26A7 showed punctate distribution throughout the cytoplasm.[61] However, in media made hypertonic for 16 hours, SLC26A7 was detected predominantly in the plasma membrane.[61] The presence of mitogen activated kinase (MAP) kinase inhibitors blocked the trafficking of SLC26A7 to the plasma membrane.[61] Double-labeling studies showed the localization of SLC26A7 to the transferrin receptor–positive endosomes.[61] A chimera composed of the aminoterminal fragment of SLC26A7 and carboxyl-terminal fragment of SLC26A1, and a C-terminally truncated SLC26A7 were retained in the cytoplasm in hypertonicity.[61] In separate studies, SLC26A7 showed predominant localization in the plasma membrane in potassium-depleted isotonic media (0.5 or 2 mEq/L KCl) versus cytoplasmic distribution in normal potassium isotonic media (4 mEq/L). We concluded that SLC26A7 is present in endosomes and its targeting to the basolateral membrane is increased in hypertonicity and potassium depletion.[61] The trafficking to the cell surface suggests novel functional upregulation of SLC26A7 in states associated with hypokalemia or increased medullary tonicity, and support the possibility that SLC26A7 may contribute to enhanced bicarbonate absorption in OMCD in hypokalemic states.

Slc26a7 Regulation by Medullary Interstitial Osmolarity

Increased kidney expression of SLC26A7 in water deprivation,[60] which is associated with increased interstitial osmolality in the medulla, raised the possibility that SLC26A7 is an osmotically regulated Cl^-/HCO_3^- exchanger. Alternatively, it is possible that the upregulation of SLC26A7 in water deprivation is caused by factors other than increased medullary osmolarity such as volume depletion, activation of renin angiotensin and sympathetic systems, or decreased kidney perfusion.

Effect of Vasopressin on Slc26a7 Expression in Kidneys of Brattleboro Rats

To examine the effect of interstitial osmolarity on SLC26A7 regulation, our laboratory examined the expression of SLC26A7 under 2 distinct conditions with opposite effects on interstitial osmolarity. In the first series of experiments, Brattleboro rats, which are deficient in the antidiuretic hormone arginine vasopressin (AVP) and have defective urinary concentration, were treated with deamino-dearginino vasopressin (dDAVP) (a vasopressin analog),[62] which has been shown to correct the kidney abnormalities (increased urine output and dilute urine) in this animal model. Immunofluorescence labeling showed that the expression of SLC26A7 was almost undetectable in Brattleboro rats, however, it became significantly upregulated in the basolateral membrane of OMCD A-IC cells in response to 6 days of treatment with vasopressin.[62] The upregulation of SLC26A7 directly correlated with increased interstitial osmolality, as determined by enhanced expression of betain transporter in the outer medulla.[62] Interestingly, the expression of AE1 in the basolateral membranes of the OMCD A-IC cells was actually decreased, albeit mildly, in AVP-treated rats.[62]

Effect of Water Loading on SLC26A7 Expression

The in vitro studies in cultured cells and in vivo studies in Brattleboro rats (see previously) showed that increasing the tonicity increases, whereas decreasing tonicity decreases, the membrane abundance of SLC26A7 in kidney cells.[61,62] To examine the effect of reduced interstitial osmolarity on SLC26A7 expression in a more detailed manner, rats were subjected to water loading for 5 days and then examined by immunofluorescence labeling.[61] Water-loaded rats displayed significant polyuria (increased urine output) and reduced urine osmolarity versus control rats, consistent with pub-

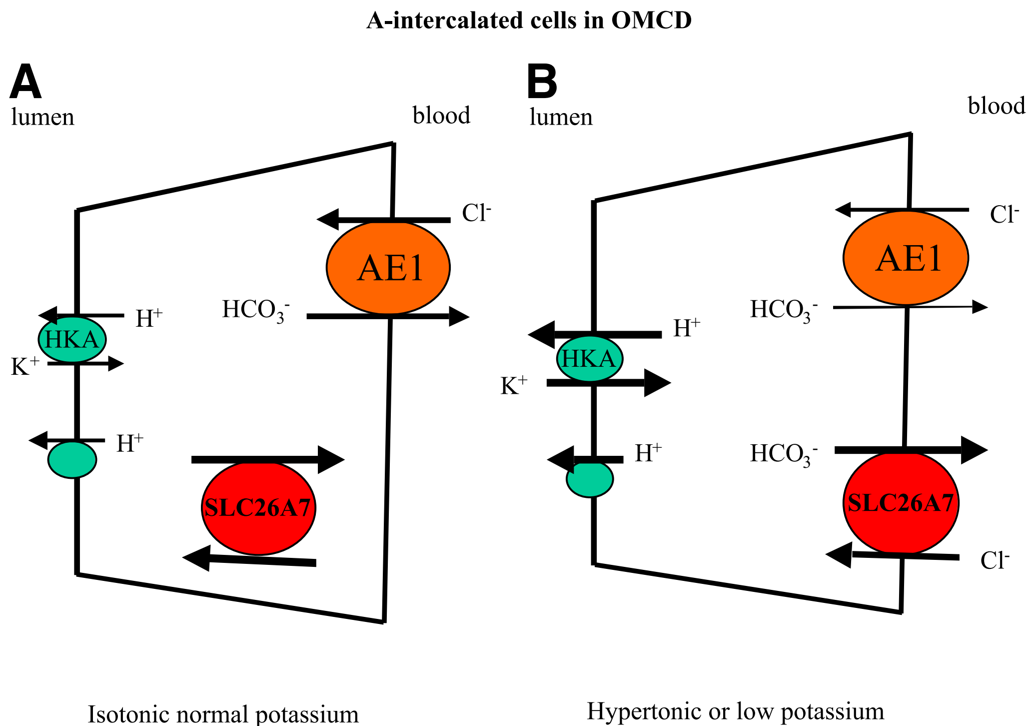

Figure 6 Schematic diagram illustrating the role and regulation of SLC26A7/Slc26a7 in the absorption of bicarbonate in OMCD in normal isotonic states (A) and in the stimulated adaptive state (increased medullary tonicity or hypokalemia). According to this diagram, SLC26A7 and AE1 are located on the basolateral membrane of A-IC cells, with AE1 predominantly detected in the membrane whereas SLC26A7 is expressed predominantly in the cytoplasm in isotonic normal media. AE1 abundance in the membrane decreases mildly but SLC26A7 moves to the basolateral membrane in hypertonic or hypokalemic states (B).

lished reports. Interestingly, when images were acquired of the kidney outer medulla, it became apparent that the number of OMCD cells displaying SLC26A7 expression in the basolateral membrane decreased significantly in water-loaded rats versus normal control rats. The reduction in membrane expression was associated with a reciprocal increase in SLC26A7 abundance in the cytoplasm in OMCD cells.[61] Taken together, these results show that in addition to transcriptional regulation,[60] SLC26A7 shows distinct adaptive regulation through alteration in its trafficking to or from the membrane in pathophysiologic states associated with electrolyte or acid-base perturbation.[61,62]

The schematic diagram in Figure 6 illustrates the role and regulation of SLC26A7/Slc26a7 in absorption of bicarbonate in OMCD in normal isotonic states (Figure 6, left panel) and in the stimulated adaptive state (increased medullary tonicity or hypokalemia). According to this diagram, SLC26A7 and AE1 are located in the basolateral membrane of A-IC cells, with AE1 predominantly detected in the membrane whereas SLC26A7 is expressed predominantly in the cytoplasm in isotonic normal media. AE1 remains in the membrane but SLC26A7 moves to the basolateral membrane in hypertonic or hypokalemic states (Figure 6, right panel).

SLC26A7 in Proximal Tubule

By using antibodies generated against different epitopes, a recent report indicated that SLC26A7 is located in the apical membrane of the kidney proximal tubule and basolateral membrane of the thick ascending limb of Henle.[63] The reason for the discrepancy between previously published reports,[29,60-62] which only detected SLC26A7 in the collecting duct, and the recent report[63] that localizes SLC26A7 in the apical membrane of the kidney proximal tubule and basolateral membrane of the thick limb is not clear. Studies in SLC26A7 KO mice should provide the definitive answer with regard to the localization and role of SLC26A7 in kidney electrolyte and acid-base homeostasis.

SLC26A8 and SLC26A11

A8 was first cloned by a 2-hybrid expression system with *MgcRacGAP*, a new RhoGAP gene [guanosine triphosphate (GTP)ase-activating protein for RhoGTPases], used as bait.[64] In situ hybridization studies show that *SLC26A8* and *MgcRacGAP* genes are co-expressed in male germ cells at the spermatocyte stage.[64] A8 originally was named *testis anion transporter-1* based on its localization and structural similarity to A1.[64] Later, it was named *SLC26A8* when its functional and structural similarities to other SLC26 family members were delineated better.[22] The SLC26A8 protein shows the best sequence similarity to SLC26A3 and SLC26A6. Published functional studies show very limited tissue distribution of SLC26A8[22] and indicate that this transporter can transport sulfate, oxalate, and chloride. Slc26a8, however, shows widespread distribution in mouse tissues including kidney (per-

sonal observation). Its nephron segment distribution and subcellular localization in mouse kidney remain unknown.

SLC26A11 was cloned from human high endothelial venule endothelial cells.[26] Functional expression studies in cultured cells revealed that SLC26A11 can mediate Na+-independent sulfate transport. SLC26A11-mediated sulfate transport is sensitive to the inhibition by anion exchanger inhibitor 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid.[26] Northern blot analysis showed the highest SLC26A11 transcript levels in the placenta, kidney, and brain.[26] SLC26A11 shows wider tissue distribution than was reported originally. In rat and mouse, it is expressed in the kidney and gastrointestinal tract (personal observation) and mediates Cl^-/HCO_3^- exchange in in vitro expression systems (personal observation). Its nephron segment distribution and subcellular localization remain unknown.

Studies on SLC26 anion exchangers are progressing rapidly. The generation of genetically engineered mice deficient in these isoforms has contributed significantly to our understanding and has shed new light on the role of these transporters in health and disease states. Pendrin (SLC26A4) is essential for chloride absorption and bicarbonate secretion in the collecting duct, with resulting regulation of acid-base and vascular volume. PAT1 (SLC26A6) mediates Cl^-/oxalate and Cl^-/base exchange in the kidney proximal tubule and small intestine and its deletion leads to decreased duodenal bicarbonate secretion and increased oxalate absorption, with resulting increased oxalate excretion and urolithiasis. SLC26A7 (PAT2) may contribute to bicarbonate absorption in the OMCD and chloride absorption in the proximal tubule. Investigation into the characterization of SLC26 isoforms and generation of other SLC26 null mice will undoubtedly shed new light on the role of these isoforms in health and disease states.

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