Tubular Transport: Core Curriculum 2010

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INTRODUCTION

The renal tubular epithelial cells that line the multiple distinct nephron segments stretching beyond the glomerulus confront the extraordinary daily task of converting 180 L of glomerular filtrate into 1-2 L of urine. Beyond simple reabsorption of solutes and water, nephron epithelial cells respond to and control the overall organismal balance of acid, solutes, fluid, hormones, vitamins, and xenobiotics. Transport functions of these epithelial cells are accomplished by solute-specific transporters and channels that, aided by specific accessory proteins, provide translocation pathways across the permeability barriers posed by the phospholipid bilayer of the plasma membrane. Transepithelial transport depends on the establishment and maintenance of epithelial cell polarity. Insults to epithelial cell polarity, such as ischemic kidney injury, can lead to loss of transport function. Normal nephron function also requires the collective and consecutive efforts of axially heterogeneous nephron segments of differing water permeabilities and energy requirements, expressing distinct profiles of transporters, channels, and other determinants of epithelial permeability. Thus, the effectiveness of diuretics is determined not only by inhibition of specific transporters, but also by the consequences of increased solute delivery to downstream nephron segments.

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TRANSTUBULAR MOVEMENT OF SUBSTANCES

- Transcellular pathway, which can occur by means of any of a number of mechanisms (Box 1; Fig 1)
  - Using passive or active transporters
  - Through channels
  - Through receptor-mediated endocytosis
- Paracellular pathway

General Factors Influencing Tubular Transport of Substances

- Electrochemical gradients
- Endocrine or paracrine hormones
- Tubular flow

SUGGESTED READING

» Koeppen BM, Stanton BA. *Renal Physiology*. 4th ed. Philadelphia, PA: Mosby Elsevier; 2007.
» Moe OW, Giebisch GH, Seldin DW. Logic of the kidney. In: Lifton RJ, Somlo S, Giebisch GH, Seldin DW, eds. *Genetic Diseases of the Kidney*. 1st ed. San Diego, CA: Academic Press; 2009:39-73.
» Rose BD, Post TW. *Clinical Physiology of Acid-Base and Electrolyte Disorders*. 5th ed. New York, NY: McGraw-Hill Medical Pub Division; 2001.

EPITHELIAL CELL POLARITY

The ability of epithelial cells to convert a glomerular filtrate of plasma-like solute composition into urine reflects a complex choreography of solute reabsorption, secretion, and recycling mediated by a tightly regulated polarized arrangement of solute transport proteins. With the help of regulated tight junctions between epithelial cells, this polarized arrangement allows for the establishment and maintenance of transepithelial electrochemical gradients for solutes. These gradients facilitate reclamation of 99% of glomerular filtrate volume and solute-specific fractions of individual filtrate components (Box 2). Alterations in cell polarity caused by either genetic loss of function or acquired dysfunction, such as in ischemic injury, lead to impaired epithelial function and nephron dysfunction.

Box 1. Channels, Transporters, and Endocytic Receptors Providing Permeation Pathways Across Tubular Epithelial Cells

Channels

- Transport through channel proteins can be electrogenic (as for ions) or electroneutral (as for H_2O and NH_3)
- Can be ion-selective or nonselective
- Ion channels may be open or closed (gated)
  * Once open, a channel can pass many ions through its pore before closing again; thus, ion translocation rates through ion channels are usually much higher than those through ion transporters
  * Electroneutral solute channels may be constitutively open
- Regulated by the probability that the closed channel will open, by the probability that the open channel will close, and by the number of functional channels in the membrane

Transporters

- Must undergo ≥1 conformational change to mediate each cycle of transmembrane solute translocation and thus mediate lower rates of solute/ion transport than channels
- Can move 1 type of solute (uniporter) or >1 type of solute in the same (cotransporter or symporter) or opposite direction (antiporter or exchanger)
- Facilitated diffusion (passive) transporters move solutes only as determined by their electrochemical gradients
- ATPases are primary active transporters, able to transport ions against their electrochemical gradient, fueled by ATP hydrolysis
- Secondary active transporters use the favorable (downhill) chemical or electrochemical gradient of 1 solute to drive transport of another solute against its unfavorable (uphill) gradient, either as cotransport or antiport
- Activity regulated by amount of polypeptide in membrane (through retrieval, production, and stability), transport substrate affinity, and regulation of rate of the conformational change(s) that mediate ion/solute translocation

Endocytic Receptors

- Substrates or substrates bound to their carrier proteins can bind to receptors, which then are internalized as a complex through endocytosis
- Depend on intracellular endocytic machinery through 2 general pathways: the clathrin-coated pit pathway or the caveolar/lipid raft pathway
- Fusion with acidifying endosomes often is needed to release endocytic cargo and recycle its receptor; fusion with lysosomes can degrade both receptor and cargo proteins
- Regulated by biosynthetic rate and rate of delivery to membrane, stability at the membrane, and retrieval from the membrane; ubiquitination of receptors, transporters, and channels can also signal endocytic retrieval and degradation

Note: See Fig 1 for more information about transporters.
Abbreviation: ATPase, adenosine triphosphatase.

Specialized Properties of Renal Epithelial Cells

- Polarized arrangement of transporters and channels for reabsorption of needed solutes and water and secretion of excess salt and acid, toxic metabolites, drugs, and xenobiotics

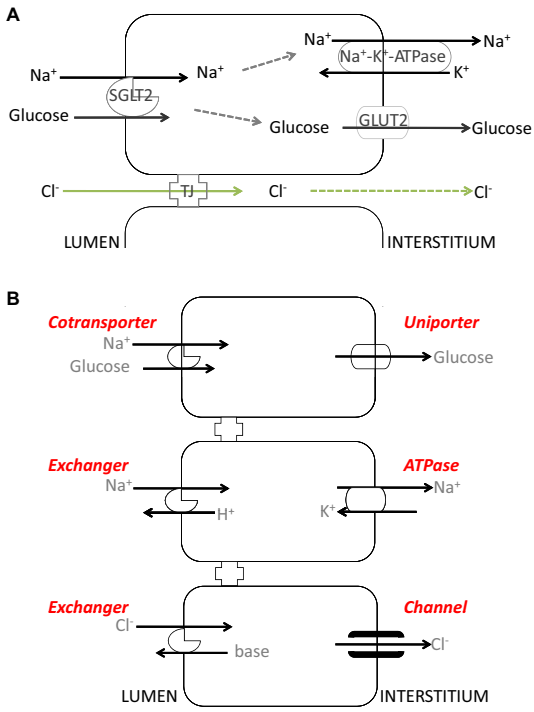

Figure 1. (A) Schematic of transcellular versus paracellular transport of ions. In the proximal tubule, Na^+ is transported across the luminal membrane together with glucose by SGLT2, diffuses across the cell, and then exits through the basolateral Na^+-K^+-ATPase. (Na^+ also enters the cell through many other Na^+-solute cotransporters and the Na^+-H^+ exchanger NHE3; Na^+ also leaves across the cell's basolateral membrane through the Na^+-HCO_3^- cotransporter NBCE1). Proximal tubular paracellular transport of Cl^- and, to a lesser extent, Na^+ involves traversal of the intercellular pore created by claudin proteins of adjacent cells that constitute the permeability barrier of epithelial tight junctions (TJ). (B) Schematic of channel and transporter characteristics. The example shown for a cotransporter is SGLT2, which transports Na^+ and glucose. GLUT2, by which glucose exits the basolateral side, exemplifies a uniporter. NHE3 is an example of an exchanger, in which Na^+ can enter the cell in exchange for H^+. A prototypical ATPase (adenosine triphosphatase) is the Na^+-K^+-ATPase, through which Na^+ can exit the cell against its chemical gradient. Another example of an exchanger is shown for chloride, namely, the Cl^--base exchanger SLC26A6, by which Cl^- enters cells. An example of a channel is the basolateral Cl^- channel, by which Cl^- exits the cell. Further information on channels and transporters is provided in Box 1.

Box 2. Daily Urinary Solute Excretion

Daily Mass of Urinary Solute Excretion

Sodium: 100-250 mEq or >2,300 mg
Chloride: 100-250 mEq or >2,300 mg
Potassium: 40-120 mEq or >1,560 mg
Calcium: 100-200 mg (2.5-5 mmol)
Phosphate: 500-700 mg (5.3-7.4 mmol)
Urea: 27-32 g (450-530 mmol)

Urinary Fractional Excretion of Solutes

Sodium: <1%
Chloride: <1%
Potassium: 5%-15%
Calcium: ~5%
Phosphate: 5%-20%
Urea: 50%-60%
Magnesium: 3%
Citrate: 10%-35%

Note: Sodium, chloride, and potassium levels expressed in mEq and mmol are equivalent.

- Segregation of luminal from basolateral plasma membrane domains by the tight junctional barrier of the epithelium
- Ability to regulate this polarized arrangement of transporters and channels, as in response to changing conditions or demands (eg, for H^+ reabsorption or secretion in the collecting duct)
- Restricted expression of certain transporters in specific nephron segments (eg, aquaporin 1 [AQP1] water channels in the descending thin limb, but not in the luminal membrane of the ascending limb of the loop of Henle) or specific membrane domains of the cell (eg, adenosine triphosphatase sodium-potassium pump [Na^+-K^+-ATPase] in basolateral, but not luminal, membrane)
- Axial heterogeneity of tight junctions along the nephron, allowing for segment-specific magnitude and ion selectivity of paracellular transport of water and salt (eg, 1/3 of proximal tubular Na^+ reabsorption is paracellular, but very little Na^+ reabsorption is paracellular in the distal nephron)

Two Main Routes of Solute Transport

- Transcellular transport, in which solute enters and traverses the cell and exits the cell on the side opposite that which it entered (Fig 1)
  - For example, luminal Na^+ entry into the proximal tubular epithelial cell across the luminal membrane through the Na^+-H^+ exchanger NHE3 (encoded by the *SLC9A3* gene) or Na^+-glucose cotransporter SGLT2 (*SLC5A2*), diffusion across the cell, and extrusion across the basolateral membrane through the Na^+-HCO_3^- cotransporter or Na^+-K^+-ATPase into the interstitial fluid
- Paracellular transport, in which a luminal solute traverses epithelial tight junctions of segment-specific ion selectivity into the interstitial fluid without passing through the cell cytoplasm
  - For example, paracellular transport of Cl^- across proximal tubular tight junctions
- Solutes can use both transcellular and paracellular routes, either across the same nephron segment (Cl^- in the proximal tubule) or in different parts of the nephron (paracellular Ca^{2+} reabsorption in the thick ascending limb [TAL], transcellular Ca^{2+} reabsorption in the distal convoluted tubule [DCT])

Establishment and Maintenance of Epithelial Cell Polarity

Establishment of Polarity

- After insertion of transmembrane proteins into the endoplasmic reticulum membrane and biosynthetic delivery to the Golgi apparatus, specific sorting signals within the protein sequence target the polypeptides in vesicles to the basolateral membrane (eg, the vasopressin V2 receptor), the luminal membrane (eg, AQP2), or sometimes both membranes (eg, Na^+-K^+-ATPase in fetal kidney and some tissue culture epithelial cells)
- The vesicular delivery pathways allow for regulation of targeting and delivery of only a subset of luminal proteins (ie, only the peptide transporter and not all other luminal transporters)
- Biosynthetic sorting (trafficking) is dependent on
  - The presence of specific targeting or retrieval sequences in the amino acid sequence of the sorted protein
  - Posttranslational modification of the sorted protein using glycosylation, phosphoryla-

tion, or conjugation with small sorting signal proteins, such as ubiquitin and SUMO (small ubiquitin-like modifier) proteins
  - Association of the protein with specialized lipid membrane patches called "lipid rafts"
  - Interaction with specific adaptor proteins of vesicular sorting compartments (eg μ-adaptins and Rab-type small GTPases [guanosine triphosphatases]); adaptin expression can be nephron segment–specific, resulting in occasional segment-specific sorting differences for the same protein

Mechanisms of Polarity Maintenance

- Membrane protein stabilization by interference with its endocytic removal (eg, Na^+-K^+-ATPase stabilization by attachment to the actin cytoskeleton at the basolateral membrane)
- Selective membrane protein retrieval from only the luminal membrane or only the basolateral membrane
- Prevention by the tight junctions of mixing of transmembrane proteins of luminal and basolateral membranes

Defects in Polarity

Genetic

- Some mutations in the AE1 (anion exchanger 1; *SLC4A1*) HCO_3^--Cl^- exchanger of the type A intercalated cell lead to distal renal tubular acidosis (RTA) due to accumulation of this normally basolateral membrane protein in the luminal membrane or both membranes, effectively short-circuiting collecting duct H^+ excretion
- Mutations in the autosomal dominant polycystic kidney disease (ADPKD) genes polycystin 1 and polycystin 2 lead to functional polarity defects:
  - Loss of "planar cell polarity" (required for restriction of the axis of tubular epithelial cell division to the tubular axis, such that growing tubules elongate without increasing local tubular diameter)
  - Downregulation of reabsorptive transporters and channels paralleled by upregulation of secretory transporters and channels, leading to transition from the normally net reabsorptive phenotype to the net secretory phenotype required for cyst enlargement
- ARC (arthrogryposis, renal dysfunction, and cholestasis) syndrome, most often caused by mutations in the *VPS33B* gene product (a "sec-MUNC18" protein that binds the sorting GTPase RAB11A to regulate SNARE-mediated intracellular vesicle membrane fusion and trafficking), leading to generalized epithelial cell polarity defects in kidney tubules, liver, and other epithelia

Acquired

- Ischemic injury leads to loss of normal epithelial morphologic characteristics, including the polarized arrangement of transporters and tight junctions
- On regenerative repair, epithelial polarity is re-established

SUGGESTED READING

» Angelow S, Ahlstrom R, Yu AS. Biology of claudins. *Am J Physiol Renal Physiol.* 2008;295(4):F867-876.
» Cullinane AR, Straatman-Iwanowska A, Zaucker A, et al. Mutations in VIPAR cause an arthrogryposis, renal dysfunction and cholestasis syndrome phenotype with defects in epithelial polarization. *Nat Genet.* 2010;42(4):303-312.
» Kuwahara M, Asai T, Terada Y, Sasaki S. The C-terminal tail of aquaporin-2 determines apical trafficking. *Kidney Int.* 2005;68(5):1999-2009.
» Weisz OA, Rodriguez-Boulan E. Apical trafficking in epithelial cells: signals, clusters and motors. *J Cell Sci.* 2009;122(pt 23):4253-4266.

TRANSPORT IN THE PROXIMAL TUBULE

The proximal tubule reabsorbs up to 60% of the glomerular filtrate. Most of this transport is coupled to sodium reclamation, either by secondary active sodium-solute cotransport driven by the favorable transluminal electrochemical gradient for sodium ion entry across the luminal membrane or by allowing for passive gradient-driven paracellular solute movement. The low intracellular concentration of sodium ion is maintained by the basolateral Na^+-K^+-ATPase and Na^+-HCO_3^- cotransporter, which thereby provide the driving force for much of the proximal tubular solute transport.

In addition to sodium, all major solutes are reabsorbed to some degree in the proximal tu-

Box 3. Fanconi Syndrome

Features of Renal Fanconi Syndrome

- Glucosuria
- Generalized (nonselective) aminoaciduria and proteinuria
- Hypophosphatemia
- Proximal renal tubular acidosis
- Polyuria
- Absence of azotemia

Causes of Renal Fanconi Syndrome

- Primary, due to NaPi-IIa mutation and at least 1 additional undefined genetic locus
- Secondary, due to
  - Genetic deficiencies (including cystinosis and other lysosomal storage diseases)
  - Monoclonal gammopathies (κ light chain) and amyloidosis
  - Autoimmune disorders (Sjögren syndrome)
  - Toxic metal injuries
  - Drug effects (eg, tenofovir)

Note: Renal Fanconi syndrome is characterized by glycosuria, generalized (nonselective) aminoaciduria, hypophosphatemia, renal tubular acidosis, and polyuria, in the absence of azotemia. Mechanistically, it implies global proximal tubular dysfunction. Partial proximal tubule dysfunction diseases (such as primary aminoacidurias resulting from loss-of-function mutations in individual amino acid transporter genes, or the low molecular weight proteinuria of Dent Disease) do not fulfill the clinical criteria of Renal Fanconi syndrome.

bule, including potassium, chloride, bicarbonate, sulfate, citrate, phosphate, calcium, glucose, and uric acid.

This obligatory coupling of nearly all solute reclamation to sodium leads to increased reabsorption solute in states (such as hypovolemia) requiring increased proximal sodium reabsorption. The proximal tubule also secretes oxalate, organic anions and cations, toxins, and sodium into the lumen. Global dysfunction of the proximal tubule, as in Fanconi syndrome, affects transport of multiple solutes (see Box 3).

Sodium Reabsorption

- The proximal tubule reabsorbs 60%-70% of filtered Na^+ by the co- and countertransport processes summarized next

Bicarbonate Reabsorption

- HCO_3^- reclamation requires coordinated action of luminal NHE3, the vacuolar H^+-ATPase (vH^+-ATPase), cytosolic and membrane-associated carbonic anhydrase, and the basolateral Na^+-HCO_3^- cotransporter NBCE1 (*SLC4A4*; Fig 2)
  - Proximal tubular H^+ secretion by NHE3 and vH^+-ATPase promotes luminal formation of CO_2 from filtered HCO_3^- with the aid of luminal membrane carbonic anhydrase 4 (CA4) and other isoforms
  - CO_2 diffuses into the cell, where cytoplasmic CA2 accelerates its hydration to HCO_3^- and H^+
  - H^+ is recycled out of the luminal membrane in exchange for luminal Na^+ entry through NHE3
  - HCO_3^- exits the basolateral membrane through the electrogenic NBCE1
- This process normally reabsorbs up to 90% of filtered HCO_3^-
  - Mutations in NBCE1 cause autosomal recessive proximal (type 2) RTA with ocular abnormalities, characterized by bicarbonaturia and cataract and/or glaucoma, sometimes accompanied by mental retardation, epilepsy, and/or migraines
  - Mutations in CA2 cause combined proximal-distal (type 3) RTA

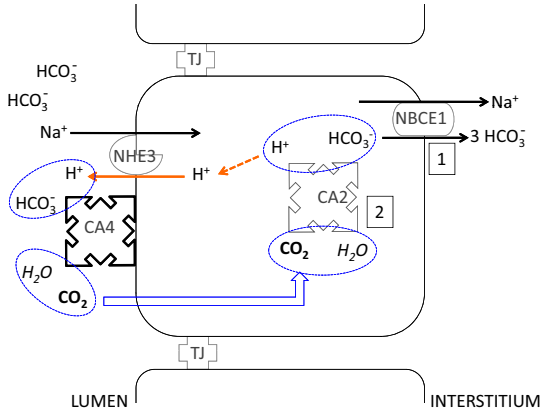

Figure 2. Schematic of proximal tubule HCO_3^- reclamation. Filtered HCO_3^- combines with H^+ secreted by the luminal Na^+-H^+ exchanger NHE3 and the vacuolar H^+-ATPase (the latter not shown), with the aid of luminal membrane carbonic anhydrase (CA4). CO_2 then diffuses into the cell through gas channels and across the lipid bilayer, where intracellular carbonic anhydrase (CA2) generates HCO_3^- and H^+. HCO_3^- exits the basolateral membrane through the Na^+-HCO_3^- cotransporter NBCE1. H^+ is recycled back into the lumen through NHE3. Mutations in NBCE1 (1) cause recessive proximal RTA, whereas mutations of CA2 (2) cause combined proximal-distal RTA.

  - Acquired proximal RTA in adults results most commonly from carbonic anhydrase inhibitors or toxic or ischemic injury to the proximal tubule
- HCO_3^- reclamation is aided by proximal tubular generation of NH_3 by deamination of glutamine and glutamate when intracellular NH_4^+ is secreted into the lumen, likely through luminal NHE3, for delivery to the medullary TAL (discussed later); cytosolic NH_3 can diffuse into the lumen to trap luminal H^+, preventing back leak (re-entry into the cell)

Chloride Reabsorption

- Cl^- reabsorption is driven by both passive and active processes
- Paracellular Cl^- reabsorption across proximal tubular tight junctions is driven by active transcellular Na^+ reabsorption
- The luminal membrane routes of transcellular Cl^- reabsorption include:
  - Cl^--HCO_3^-, Cl^--OH^-, and Cl^--formate exchangers, each coupled to NHE3
  - The Cl^--oxalate exchanger SLC26A6, which effects net (tertiary active) NaCl uptake when coupled to oxalate/sulfate exchange and Na^+-sulfate cotransport
- Basolateral routes contributing to transcellular Cl^- reabsorption include:
  - K^+-Cl^- cotransporters (KCCs)
  - Cl^- channels

Oxalate, Citrate, and Sulfate Transport

Oxalate

- Circulating oxalate levels depend largely on endogenous hepatic biosynthesis from metabolism of glycine, ascorbate, and possibly also hydroxyproline and fructose; dietary oxalate (from foods such as spinach and broccoli) contributes 5%-50% of total oxalate load
- The luminal oxalate absorption pathway is undefined
- The oxalate-sulfate exchanger SLC26A1 mediates basolateral oxalate uptake
- The oxalate-Cl^- exchanger SLC26A6 mediates luminal oxalate secretion; the same polarized arrangement of SLC26A1 and SLC26A6 is found in enterocytes
- Mice lacking either SLC26A1 or SLC26A6 develop urolithiasis secondary to lack of enterocyte oxalate secretion into stool, leading to hyperoxalemia, increased oxalate filtration, and hyperoxaluria (despite the presumed decrease in proximal tubular oxalate secretion)

Citrate

- Citrate reabsorption across the luminal membrane is through the Na^+-dicarboxylate transporter (NaDC1 [*SLC13A2*], which has a preference for divalent citrate)
- Basolateral membrane uptake of citrate into the cell also occurs through a similar Na^+-coupled transporter
- The proximal tubule cell then metabolizes citrate from both sources to HCO_3^- or feeds it into the tricarboxylic acid cycle
- Luminal citrate uptake is increased by
  - Long-term K^+ depletion (by increasing activity of the transporter)
  - Metabolic acidosis (by increased dibasic citrate [substrate] concentration)
  - Limited Ca^{2+} or Mg^{2+} in urine (which normally complexes with citrate and prevents it from being absorbed)
  - Acetazolamide therapy (by inducing mild metabolic acidosis)
- Hypocitraturia is a risk factor for calcium oxalate nephrocalcinosis and nephrolithiasis

Sulfate

- Exclusively reabsorbed by the proximal tubule, mainly through the Na^+-sulfate cotransporter (NaSi-1; *SLC13A1*)
- Other transporters might mediate sulfate back flux into the lumen in the process of Cl^- reabsorption
- Sulfate is extruded across the basolateral membrane by the sulfate-anion exchanger SAT1 (*SLC26A1*)
- Sulfate transport is influenced by
  - Heavy metals (cadmium, mercury, or lead), which can chelate sulfate
  - High-sulfate diet or nonsteroidal anti-inflammatory drugs (by lowering transporter abundance)

- Vitamin D and thyroid hormone (by increasing transporter abundance)

Water and Glucose Reabsorption

Water

- Proximal tubular water reabsorption is through AQP1 water channels present on both luminal and basolateral surfaces
- Water is believed to follow the slight osmotic gradient established by proximal reabsorption of $NaHCO_3$, NaCl, and the numerous other solutes cotransported with Na^+
- Proximal tubular tight junctions permit paracellular water transport
- AQP1 abundance is regulated by angiotensin II

Glucose

- Glucose is reabsorbed nearly entirely by the proximal tubule
- Glucose traverses the proximal tubule cell luminal membrane through Na^+-glucose cotransporters (low-affinity/high-capacity SGLT2 proximally and higher affinity/lower capacity SGLT1 [*SLC5A1*] predominating in the straight segment)
- Glucose exits the basolateral membrane by facilitated diffusion down its concentration gradient through the GLUT glucose transporters (GLUT2 [*SLC2A2]* in the convoluted [S1/S2] segments and GLUT1 [*SLC2A1*] in the straight [S3] segment)
  - Transport capacity is saturable; concentrations exceeding T_{max} (transport capacity) lead to glucosuria, as in the hyperglycemia of diabetes
  - Transport is upregulated in states of hyperglycemia
- SGLT2 mutations cause recessive familial renal glucosuria; in some families, this is accompanied by generalized aminoaciduria, for reasons yet unknown
- SGLT2 inhibitors are in development as a novel class of therapeutic agents for treatment of diabetes

Phosphate Reclamation

- Phosphate reuptake occurs through 2 renal-specific Na^+-Pi (inorganic phosphate) cotransporters, NaPi-IIa (*SLC34A1*) and NaPi-IIc (*SLC34A3*), each preferentially binding divalent HPO_4^{2-} rather than monovalent $H_2PO_4^-$, thus allowing nonresorbed urinary phosphate to serve as an H^+ carrier and contribute to net acid excretion
- The roles of other Na^+-Pi cotransporters also present on proximal tubule cells (eg, PIT-2 [sodium-dependent phosphate transporter 2]) in phosphate reabsorption uptake remain unclear
- Phosphate reabsorption is regulated by
  - Parathyroid hormone (PTH), through increased endocytic recycling of cotransporter (with consequent decreased phosphate reabsorption)
  - Fibroblast growth factor 23 (FGF-23; and its receptor and coreceptor, klotho), by a similar mechanism
- One family with a recessive NaPi-IIa mutation encoding a transporter protein that fails to reach the proximal tubular luminal surface shows generalized Fanconi syndrome, by a mechanism yet unknown

Potassium Reabsorption

The proximal tubule reabsorbs 60%-70% of filtered potassium across the paracellular pathway. Luminal membrane potassium channels are believed to function as stabilizers of luminal membrane potential, sustaining continued transcellular solute transport processes described next.

Protein and Amino Acid Reclamation

Amino Acids

- Free amino acids in the filtrate (resulting from cellular metabolism and dietary intake) are fully reabsorbed in the proximal tubule
- Multiple transporter systems exist to ensure reclamation:
  - Coupled to either Na^+ (most) or H^+ gradient (proline, lysine)
  - Transport based on the chemical groups of amino acids: dibasic (lysine, arginine) separate from neutral (leucine, isoleucine) separate from imino (proline), etc
    - Thus, dysfunction of 1 transporter system results in aminoaciduria of related compounds: in classic cystinuria, also lost are arginine, lysine, ornithine

- Near-total reabsorption of an amino acid (eg, glycine, cystine) is ensured by the proximal presence of low-affinity/high-capacity transporters, followed in the straight segment by higher affinity/lower capacity transporters

- Reabsorption is regulated by
  - Dietary availability (through synthesis of the transporters)
  - Osmolarity (for amino acids serving as intracellular osmoles, such as taurine)

Peptides and Proteins

- Peptides and proteins are either broken down at the brush border membrane by peptidases (eg, angiotensin II) for reabsorption by the H^+-peptide cotransporters HPEPT1 (*SLC15A1*) and PEPT2 (*SLC15A2*) or into their component amino acids for reabsorption by specific amino acid transporters
- Proteins and larger peptides also are endocytosed and shuttled to lysosomes for degradation
- Reclamation of vitamins and hormones from urine is another key function of the proximal tubule; reuptake is either
  - For the vitamin molecule itself (eg, vitamin C, through at least 2 different Na^+-dependent transporters) or
  - For the vitamin or hormone together with its binding protein complex
- Many vitamin- and hormone-binding protein complexes are endocytosed by megalin (*LRP2*) and/or cubilin (*CUBN*), large transmembrane members of the low-density lipoprotein–related receptor gene family, which, while interacting with multiple other transporters of the luminal membrane, bind to and facilitate endocytic uptake of
  - Vitamin A bound to retinol-binding protein(s)
  - Vitamin B_{12} bound to transcobalamin
  - Vitamin D bound to vitamin D–binding protein
  - A small proportion of iron-loaded transferrin that (despite its large size) passes through the glomerular filtration barrier
- Dysfunction of megalin and cubulin results in low-molecular-weight proteinuria and albuminuria
- The low-molecular-weight proteinuria and hypercalcemic nephrocalcinosis of Dent disease arise from mutation of the Cl^--H^+ exchanger CLC-5 (*CLCN5*), required for normal acidification of endocytic vesicles and without which megalin and cubulin function is impaired
- Transcellular solute transport also depends on regulated transporter protein internalization, recycling, and/or lysosomal or proteosomal degradation
- Thus, disorders of lysosomal acidification also can lead to dysregulated reabsorption of the mentioned solutes and vitamins

Albumin

- It generally is taught that albumin is not filtered by the glomerulus; however, some albumin is filtered, and the magnitude of normal physiologic glomerular albumin filtration remains controversial
- Mechanisms involved in proximal tubule reabsorption of albumin involve:
  - The megalin/cubulin system, which may target reabsorbed albumin for degradation (and can be impaired by CLC-5 mutations in Dent disease)
  - Receptor proteins related to the major histocompatibility complex Fc receptors, which may participate in albumin retrieval
  - Intact lysosomal activity (affected in Dent disease and possibly by angiotensin-converting enzyme inhibitors)

Organic Ions and Urate Transport

Organic Cations

- Cations, such as creatinine, cimetidine, and trimethoprim, use the polyspecific organic cation transporter OCT1 (*SLC22A1*) and other OCTs to traverse basolateral membrane into the epithelial cell
- Subsequent secretion across the luminal membrane is linked to H^+ exchange by OCT and OCTN (*SLC22A4*) transporters or through MDR (multidrug resistance) protein [ie, P-glycoprotein]) and MRP (MDR-related protein) in a process uncoupled to H^+ transport
- These shared transport mechanisms can lead to competition among organic cations, resulting in increased serum creatinine levels

secondary to decreased creatinine secretion in the presence of cimetidine or trimethoprim
- H^+-cation exchange also mediates tubular reabsorption of filtered organic cations

Organic Anions

- Anions, such as urate, ketoacid anions, salicylate, penicillins, and diuretics, use separate secretory pathways
- Basolaterally, organic anion transporters (OATs and OATPs) facilitate entry into the cell
- Luminally, organic anion secretion occurs through
  - Anion exchange for filtered urate through URAT1 (*SLC22A12*)
  - Anion exchange for Cl^-
  - Facilitated diffusion through luminal OATs (down a concentration gradient)
- Competition among organic anions for shared transport systems can lead to hyperuricemia during fasting, when the increased need for ketone excretion increases urate uptake through the URAT1-mediated ketone-urate exchange
- These relatively nonspecific secretory pathways also eliminate from the body drugs and toxins, including anionic radiocontrast media

Uric Acid

- Uric acid (urate) is the end product of purine metabolism and has low solubility at urine pH <5.5
- Urate transport occurs through several transporters, including:
  - URAT1 and OAT10 (*SLC22A13*), which reabsorb urate across the luminal membrane in exchange for lactate or nicotinate
  - GLUT9 (*SLC2A9*), a urate uniporter that promotes both reabsorption across the luminal membrane and basolateral urate efflux
    - In rodents, Glut9 also is expressed in the DCT
  - Luminal OAT4 (*SLC22A11*) reabsorbs urate in exchange for dicarboxylates
  - Basolateral membrane urate transport is mediated by OAT1 (*SLC22A6*) and OAT3 (*SLC22A8*) in addition to GLUT9
  - Urate secretion across the proximal tubular luminal membrane can be mediated by MDR proteins MRP4 (*ABCC4*) and ABCG2, and is associated with expression of Na^+-Pi cotransporters NPT1 (*SLC17A1*) and NPT4 (*SLC17A3*)
    - These Na^+-Pi cotransporters are different from the major PTH-regulated NaPi-IIa and NaPi-IIc transporters
  - Gout has been associated with polymorphisms in ABCG2; hyperuricemia has been associated with GLUT9 and NPT4 polymorphisms
  - Hereditary hypouricemia in patients with URAT1 loss-of-function mutations is accompanied by susceptibility to exercise-induced acute renal failure, by unclear mechanisms

SUGGESTED READING

» Aalkjaer C, Frische S, Leipziger J, Nielsen S, Praetorius J. Sodium coupled bicarbonate transporters in the kidney, an update. *Acta Physiol Scand.* 2004;181(4):505-512.

» Aronson PS. Essential roles of CFEX-mediated Cl(−)-oxalate exchange in proximal tubule NaCl transport and prevention of urolithiasis. *Kidney Int.* 2006;70(7):1207-1213.

» Bouley R, Palomino Z, Tang SS, et al. Angiotensin II and hypertonicity modulate proximal tubular aquaporin 1 expression. *Am J Physiol Renal Physiol.* 2009;297(6):F1575-1586.

» Christensen EI, Verroust PJ, Nielsen R. Receptor-mediated endocytosis in renal proximal tubule. *Pflugers Arch.* 2009;458(6):1039-1048.

» Comper WD, Russo LM. The glomerular filter: an imperfect barrier is required for perfect renal function. *Curr Opin Nephrol Hypertens.* 2009;18(4):336-342.

» Corpe CP, Tu H, Eck P, et al. Vitamin C transporter Slc23a1 links renal reabsorption, vitamin C tissue accumulation, and perinatal survival in mice. *J Clin Invest.* 2010;120(4):1069-1083.

» Dinour D, Gray NK, Campbell S, et al. Homozygous SLC2A9 mutations cause severe renal hypouricemia. *J Am Soc Nephrol.* 2010;21(1):64-72.

» Kleta R. Fanconi or not Fanconi? Lowe syndrome revisited. *Clin J Am Soc Nephrol.* 2008;3(5):1244-1245.

» Ludwig M, Utsch B, Monnens LA. Recent advances in understanding the clinical and genetic heterogeneity of Dent's disease. *Nephrol Dial Transplant.* 2006;21(10):2708-2717.

» Magen D, Berger L, Coady MJ, et al. A loss-of-function mutation in NaPi-IIa and renal Fanconi's syndrome. *N Engl J Med.* 2010;362(12):1102-1109.

» Markovich D, Aronson PS. Specificity and regulation of renal sulfate transporters. *Annu Rev Physiol.* 2007;69:361-375.
» Mather A, Pollock C. Renal glucose transporters: novel targets for hyperglycemia management. *Nat Rev Nephrol.* 2010;6(5):307-311.
» Razzaque MS. The FGF23-klotho axis: endocrine regulation of phosphate homeostasis. *Nat Rev Endocrinol.* 2009;5(11):611-619.
» Sakhaee K. Recent advances in the pathophysiology of nephrolithiasis. *Kidney Int.* 2009;75(6):585-595.
» Santer R, Calado J. Familial renal glucosuria and SGLT2: from a mendelian trait to a therapeutic target. *Clin J Am Soc Nephrol.* 2010;5(1):133-141.
» So A, Thorens B. Uric acid transport and disease. *J Clin Invest.* 2010;120(6):1791-1799.
» Tencza AL, Ichikawa S, Dang A, et al. Hypophosphatemic rickets with hypercalciuria due to mutation in SLC34A3/type IIc sodium-phosphate cotransporter: presentation as hypercalciuria and nephrolithiasis. *J Clin Endocrinol Metab.* 2009;94(11):4433-4438.
» Unwin RJ, Capasso G, Shirley DG. An overview of divalent cation and citrate handling by the kidney. *Nephron Physiol.* 2004;98(2):15-20.
» Verrey F, Singer D, Ramadan T, Vuille-dit-Bille RN, Mariotta L, Camargo SM. Kidney amino acid transport. *Pflugers Arch.* 2009;458(1):53-60.
» Zelikovic I. Aminoaciduria and glucosuria. In: Avner ED, Harmon N, Niaudet P, Yoshikava N, eds. *Pediatric Nephrology*. 6th ed. Heidelberg: Springer-Verlag; 2009:889-927.

TRANSPORT IN THE LOOP OF HENLE

Transport in the loop of Henle is characterized by differential permeability to water, sodium, and urea, which allows generation of a hypo-osmolar filtrate while maintaining a hyperosmolar interstitium. Generation of a hypo-osmolar filtrate is made possible by the ascending loop's ability to reabsorb sodium chloride in excess of water. Thus, impairment of sodium chloride transport in the loop leads not only to inability to maximally concentrate urine, but also to impairment of maximal urinary dilution. Medullary sections of the loop function in a hypoxic environment and are at increased risk of ischemic injury. Genetic disruption of thick limb sodium reabsorption manifests as a group of functionally related disorders known as Bartter syndromes. These syndromes directly affect transcellular sodium and chloride reabsorption and indirectly affect paracellular calcium reabsorption (Fig 3).

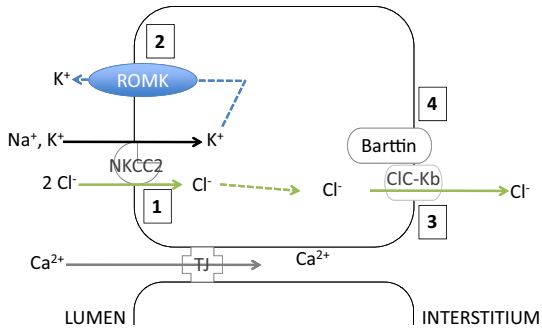

Figure 3. Schematic of thick ascending limb NaCl transport. Luminal Na^+ uptake is coupled to uptake of Cl^- and K^+ through the Na^+-K^+-$2Cl^-$ (NKCC2) transporter. Cl^- exits the cell through the basolateral channel ClC-Kb, whereas K^+ is recycled back into the lumen through the luminal secretory K^+ channel ROMK. Mutations in NKCC2 cause Bartter syndrome type 1 (1), whereas mutations in ROMK cause Bartter type 2 (2). Mutations in ClC-Kb cause Bartter syndrome type 3 (3), whereas mutations in the Cl^- channel auxiliary subunit Barttin cause Bartter syndrome type 4 (4). The thick ascending limb tight junctions (TJ) show preferential permeability to Na^+, Ca^{2+}, and Mg^{2+}.

Thin Limb

- The descending thin limb of the loop of Henle is permeable to water, urea, and NaCl
- The ascending thin limb is impermeable to water, but actively transports NaCl
- The differential permeability to water is due to expression in the descending limb only of the vasopressin-insensitive water channel AQP1, which also is regulated in this segment by hypertonicity
- AQP1 knockout in the mouse thin descending limb produces a form of diabetes insipidus

Medullary and Cortical TAL

Sodium and Chloride Reabsorption

- The TAL selectively reabsorbs 25%-35% of filtered NaCl without water, which allows establishment and maintenance of the hypertonic medullary interstitium
- Tight junctions help maintain water impermeability, whereas transcellular active ion transport in this segment exceeds the proximal tubule in energy requirement
- Na^+ and Cl^- transport are coupled with K^+ at the bumetanide-sensitive Na^+-K^+-$2Cl^-$ cotransporter 2 (NKCC2; *SLC12A1*) of the luminal membrane

- Three different splice variants of NKCC2, with graded affinities for the transport-limiting Cl^- ion, are coexpressed along the length of the TAL, allowing for NaCl reabsorption across a range of filtrate Cl^- concentrations reflecting progressive dilution of the luminal fluid during transit of the complete TAL
- Uptake activity of this electroneutral transporter, driven by an Na^+ concentration gradient, is regulated by and in coordination with the function of other TAL channels and transporters
- NKCC2 loss-of-function mutations cause antenatal Bartter syndrome type 1
- Luminal ROMK (*KCNJ1*) is a K^+ channel that mediates luminal K^+ recycling to allow sustained NaCl uptake by NKCC
  - Loss-of-function ROMK mutations cause antenatal Bartter syndrome type 2
- Cl^- channels Ka (ClC-Ka; *CLCNKA*) and Kb (ClC-Kb; *CLCNKB*) and their subunit Barttin (*BSND*) are responsible for basolateral Cl^- efflux from the TAL cell into the interstitium
  - Loss-of-function mutation of CLC-Kb causes Bartter syndrome type 3
  - Simultaneous mutation of the adjacent *CLCKNA* and *CLCKNB* genes (usually a deletion) causes infantile Bartter syndrome type 4B with deafness
  - *BSND* mutations cause infantile Bartter syndrome type 4A with deafness

Calcium Transport

- Ca^{2+} and Mg^{2+} transport in the TAL are passive paracellular processes driven by the TAL's lumen-positive transepithelial potential and mediated by the tight junctional permselectivity proteins claudin 16 (*CLDN16*) and claudin 19 (*CLDN19*)
  - Loss-of-function mutations in either gene cause the recessive disease familial hypomagnesemia with hypercalciuria and nephrocalcinosis
- Activation by Ca^{2+} of the basolateral calcium sensing receptor (CaSR) inhibits Na^+ transport by inhibition of NKCC and ROMK, thereby decreasing the transtubular potential gradient driving Ca^{2+} and Mg^{2+} reabsorption
  - Activating mutations of CaSR cause a Bartter-like volume depletion syndrome of autosomal dominant hypercalciuric hypocalcemia

Ammonium

- NH_4^+ generated from glutamine in proximal tubular cells is secreted into the lumen and delivered to the medullary TAL, where it is reabsorbed by NKCC and exits the basolateral membrane into the medullary interstitium through the Na^+-H^+ exchanger NHE4 (*SLC9A4*)
  - NHE4 knockout in the mouse depletes medullary ammonium available for secretion by the collecting duct and thus impairs urinary total acid excretion in response to acid load

Uric Acid

- Tamm-Horsfall protein (uromodulin; *UMOD*)
  - Intracellular retention mutations of the luminally secreted glycosylphosphatidylinositol-linked Tamm-Horsfall protein cause hyperuricemic juvenile nephropathy; the cause of hyperuricemia is not understood
  - Tamm-Horsfall protein mutations also have been associated with glomerulocystic kidney disease

SUGGESTED READING

» Carota I, Theilig F, Oppermann M, et al. Localization and functional characterization of the human NKCC2 isoforms. *Acta Physiol (Oxf).* 2010;199(3):327-338.

» Hou J, Renigunta A, Konrad M, et al. Claudin-16 and claudin-19 interact and form a cation-selective tight junction complex. *J Clin Invest.* 2008;118(2):619-628.

» Huang C, Miller RT. Novel Ca receptor signaling pathways for control of renal ion transport. *Curr Opin Nephrol Hypertens.* 2010;19(1):106-112.

» Kramer BK, Bergler T, Stoelcker B, Waldegger S. Mechanisms of disease: the kidney-specific chloride channels ClCKA and ClCKB, the Barttin subunit, and their clinical relevance. *Nat Clin Pract Nephrol.* 2008; 4(1):38-46.

» Riccardi D, Brown EM. Physiology and pathophysiology of the calcium-sensing receptor in the kidney. *Am J Physiol Renal Physiol.* 2010;298(3):F485-499.

» Schlingmann KP, Konrad M, Jeck N, et al. Salt wasting and deafness resulting from mutations in two chloride channels. *N Engl J Med.* 2004;350(13):1314-1319.

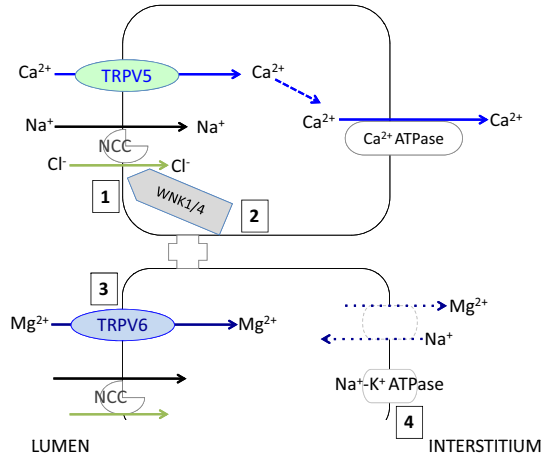

Figure 4. Schematic representation of Na^+, Mg^{2+}, and Ca^{2+} transport in the distal convoluted tubule. Na^+ is reabsorbed through the thiazide-sensitive Na^+-Cl^- cotransporter (NCC). The intracellular kinases WNK1 and WNK4 modulate NCC abundance at the luminal cell surface. Mutations in NCC cause Gitelman syndrome (1), whereas mutations in the kinases cause pseudohypoaldosteronism type 2 (familial hyperkalemic hypertension; 2). The distal convoluted tubule is also the main site for regulated reabsorption of Ca^{2+} through the luminal channel TRPV5. Ca^{2+} then exits the cell through basolateral Na^+/Ca^{2+} exchangers (not shown) or Ca^{2+} ATPases. In addition, Mg^{2+} reabsorption occurs at this site through the related luminal channel TRPV6. Mg^{2+} efflux from the cell may involve an Na^+-Mg^{2+} exchanger (shown with dashed lines). Mutations in the TRPV6 channel cause recessive hypomagnesemia and hypocalcemia (3), whereas mutations in the γ subunit of the Na^+-K^+-ATPase (FXYD2) cause isolated dominant hypomagnesemia (4).

» Seyberth HW. An improved terminology and classification of Bartter-like syndromes. *Nat Clin Pract Nephrol.* 2008;4:560-567.
» Williams SE, Reed AA, Galvanovskis J, et al. Uromodulin mutations causing familial juvenile hyperuricaemic nephropathy lead to protein maturation defects and retention in the endoplasmic reticulum. *Hum Mol Genet.* 2009;18(16):2963-2974.
» Zhai XY, Fenton RA, Andreasen A, Thomsen JS, Christensen EI. Aquaporin-1 is not expressed in descending thin limbs of short-loop nephrons. *J Am Soc Nephrol.* 2007;18(11):2937-2944.

TRANSPORT IN THE DCT AND CONNECTING SEGMENT

The water-impermeable DCT furthers the process of sodium reabsorption and dilution of the tubular urine. It also is the major distal site of magnesium and calcium reabsorption (Fig 4).

Sodium Reabsorption

- The DCT reabsorbs 5%-10% of filtered Na^+ through the thiazide-sensitive electroneutral Na^+-Cl^- cotransporter (NCC; *SLC12A5*)
- Inactivating mutations in the NCC are associated with autosomal recessive Gitelman syndrome (tubular hypokalemia-hypomagnesemia with hypocalciuria)
- NCC activity is regulated by:
  - Distal Na^+ delivery,
  - Aldosterone, and
  - Control of its luminal membrane abundance through action of intracellular kinases WNK1 and WNK4
  - WNK4 and WNK1 mutations that upregulate NCC activity and surface abundance cause pseudohypoaldosteronism type 2 (familial hyperkalemic hypertension)

Calcium and Magnesium Reabsorption

Calcium

- The DCT is the main site of regulated transcellular Ca^{2+} reabsorption through luminal Ca^{2+} entry through the Ca^{2+}-selective TRPV5 channel and basolateral Ca^{2+} efflux through the Ca^+-ATPases and Na^+-Ca^{2+} exchangers
- Ca^{2+} reabsorption is regulated by:
  - PTH (through second messenger cAMP [cyclic adenosine monophosphate])
  - Calcitriol (biosynthesis of transporters and Ca^{2+}-binding proteins)
  - Estrogen/testosterone (biosynthesis of transporters and Ca^{2+}-binding proteins)
  - The TRPV5 auxiliary protein klotho increases TRPV5 stability in the luminal membrane, in part by modifying its glycosylation state
  - pH (alkaline urine increases TRPV5 activity and surface abundance)
  - Dietary Na^+ intake and urinary excretion are correlated with calciuria
  - NCC function has a role because thiazides enhance Ca^{2+} reabsorption by a still uncertain mechanism (heterozygotes for NCC loss-of-function mutations may show increased bone density)

Magnesium

- The DCT is the main site of regulated transcellular Mg^{2+} reabsorption (10%-15%

of the filtered load in DCT vs 70% in the TAL)
- The heteromeric TRPM6/TRPM7 cation channel reabsorbs Mg^{2+} across the DCT luminal membrane
- TRPM6 loss-of-function mutations cause recessive hypomagnesemia with secondary hypocalcemia
- Mg^{2+} reabsorption is regulated by luminal fluid pH (acidosis increases Mg^{2+} excretion) by epidermal growth factor (EGF) in an autocrine/paracrine fashion
  - Hypomagnesemia is a side effect of anti-EGF receptor cancer chemotherapies
- Mg^{2+} exits the DCT basolateral membrane through an unknown pathway believed to mediate Na^+-Mg^{2+} exchange
  - Mutations of the basolateral Na^+-K^+-ATPase γ subunit (FXYD2) are associated with isolated dominant hypomagnesemia by an unknown mechanism, suggesting possible functional linkage between Na^+-K^+-ATPase and the unidentified Na^+-Mg^{2+} exchanger
- FXYD2 transcription is regulated positively by the renal developmental transcription factor HNF1B
  - HNF1B mutations and the associated renal dysgenesis are associated in many cases with hypomagnesemic renal magnesium wasting

SUGGESTED READING

» Bonny O, Rossier BC. Disturbances of Na/K balance: pseudohypoaldosteronism revisited. *J Am Soc Nephrol.* 2002;13(9):2399-2414.

» Glaudemans B, Knoers NV, Hoenderop JG, Bindels RJ. New molecular players facilitating Mg(2+) reabsorption in the distal convoluted tubule. *Kidney Int.* 2010; 77(1):17-22.

» Hsu YJ, Dimke H, Schoeber JP, et al. Testosterone increases urinary calcium excretion and inhibits expression of renal calcium transport proteins. *Kidney Int.* 2010;77(7):601-608.

» Pachulski RT, Lopez F, Sharaf R. Gitelman's not-so-benign syndrome. *N Engl J Med.* 2005;353(8):850-851.

» Riveira-Munoz E, Chang Q, Bindels RJ, Devuyst O. Gitelman's syndrome: toward genotype-phenotype correlations? *Pediatr Nephrol.* 2007;22:326-332.

» Simon DB, Nelson-Williams C, Bia MJ, et al. Gitelman's variant of Bartter's syndrome, inherited hypokalaemic alkalosis, is caused by mutations in the thiazide-sensitive Na-Cl cotransporter. *Nat Genet.* 1996; 12(1):24-30.

TRANSPORT IN THE CONNECTING SEGMENT AND COLLECTING DUCT

The connecting segment, cortical collecting duct, and medullary collecting duct complete sodium chloride and water reabsorption from the glomerular filtrate, converting the hypo-osmotic urine exiting the DCT into concentrated terminal urine into which potassium and hydrogen ions are secreted according to systemic needs. These final adjustments to urinary composition are accomplished despite the comparatively small absorptive and secretory capacity of this nephron segment, reflecting the small delivered urine volume and the preceding actions of upstream transporters.

Sodium Reabsorption

- The connecting segment and cortical collecting duct make final adjustments in urinary Na^+ and Cl^- excretion
- ENaC (epithelial Na^+ channel; *SCNN1B*) in principal cells is the main pathway of Na^+ reabsorption and is transcriptionally regulated by aldosterone (Fig 5A)
- Activating mutations in ENaC subunits leading to increased ENaC abundance in the luminal membrane cause the hypertension of Liddle syndrome, whereas inactivating mutations in ENaC and the aldosterone receptor are associated with the salt-wasting hypotensive pseudohypoaldosteronism type I syndromes
- In addition to aldosterone, regulators of ENaC activity include
  - Urinary proteases, such as bradykinin, prostasin, and channel-activating protease I (CAP), and intracellular processing proteases, such as furins; activate by targeted cleavage of ENaC's large extracellular loops
  - Atrial natriuretic peptide (ANP), which decreases the number of open channels
  - Antidiuretic hormone (ADH), which increases the number of new channels inserted into the membrane
  - Prostaglandin E_2
  - Insulin
  - Fluid flow and shear stress
- ENaC-mediated Na^+ transport is favored by its electrochemical gradient, resulting in a

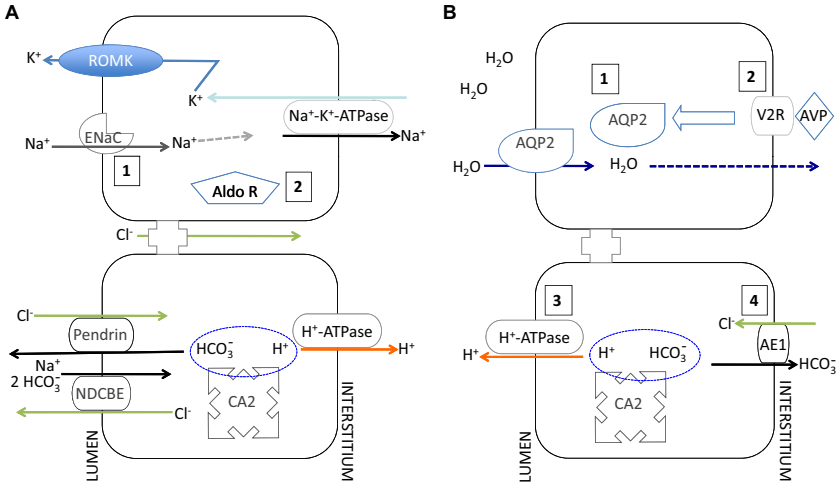

Figure 5. Schematic representation of collecting duct transport. (A) In the principal cell, Na^+ is reabsorbed through the epithelial Na^+ channel (ENaC), regulated by aldosterone. Activating mutations in ENaC cause Liddle syndrome (1), whereas inactivating mutations in ENaC and the aldosterone receptor (aldo R) cause pseudohypoaldosteronism type I (2). K^+ secretion through ROMK also is regulated by aldosterone. K^+ also can be reabsorbed by intercalated cells via H^+-K^+-ATPases (not shown). Na^+ and Cl^- also are reabsorbed across non–type A intercalated cells through the Na^+-dependent Cl^--HCO_3^- exchanger SLC4A8, acting in concert with the Cl^--HCO_3^- exchanger pendrin and aided by intracellular carbonic anhydrase (CA2). Cl^- reabsorption at this site also follows a paracellular route. (B) Water reabsorption in the principal cells of the cortical collecting duct and medullary collecting duct is through the controlled insertion of aquaporin channels in the membrane (AQP2). AVP (vasopressin), through its vasopressin receptor V2R, controls this process. Loss-of-function mutations in V2R (2) lead to X-linked nephrogenic diabetes insipidus, whereas mutations in AQP2 (1) lead to autosomal recessive diabetes insipidus. In addition, H^+ generated intracellularly by CA2 is actively secreted in the type A intercalated cells through luminal vacuolar H^+-ATPase. HCO_3^- is exported to the interstitium through a Cl^--HCO_3^- exchanger (AE1). Familial distal type IV renal tubular acidosis is caused by mutations in the vacuolar H^+-ATPase (3) or AE1 (4).

lumen-negative transepithelial potential that helps drive
  - Paracellular Cl^- reabsorption
  - K^+ secretion through principal cell ROMK and another K^+ channel
  - H^+ secretion (by intercalated cells)

Chloride Reabsorption

- Two Cl^- transporters participate in amiloride-insensitive electroneutral transcellular NaCl transport across cortical collecting duct type B intercalated cells (Fig 5A)
  - Na^+-dependent Cl^--HCO_3^- exchanger SLC4A8
  - Na^+-independent Cl^--HCO_3^- exchanger pendrin (*SLC26A4*)
- Pendrin secretes HCO_3^- under alkaline loading conditions
- Pendrin also reabsorbs filtered iodide in the cortical collecting duct
- Connecting segment and cortical collecting duct also are believed to mediate paracellular Cl^- reabsorption

Potassium Secretion and Uptake

- Principal cell ROMK activity mediates most cortical and medullary collecting duct K^+ secretion
- ROMK activity is affected by:
  - Dietary K^+ load
  - Aldosterone (through channel abundance)
  - Degree of lumen-negative electrical potential arising from ENaC-mediated Na^+ reabsorption
  - Klotho (through extracellular glycan processing)
  - WNK1 and WNK4 kinases (through increased removal from the membrane)
- K^+ secretion also is mediated by large conductance Ca^{2+}-activated K^+ channels of the intercalated cell luminal membrane, regulated by shear stress/fluid flow
- K^+ homeostasis also is maintained by K^+ reabsorption in states of hypokalemia by an H^+-K^+-ATPase in the intercalated cells

Hydrogen Ion Secretion

- Active H^+ secretion by type A intercalated cells in the medullary and cortical collecting ducts is mediated by luminal vH^+-ATPase in medullary and cortical collecting ducts (Fig 5B)
  - In conditions of systemic K^+ depletion, the H^+-K^+-ATPase of the medullary collecting duct luminal membrane also may contribute
  - Intracellular CA2 generates the H^+ for secretion, and the HCO_3^- produced in the same reaction is reclaimed into the interstitial fluid across the type A intercalated cell basolateral membrane by the kidney AE1 Cl^--HCO_3^- exchanger
- Collecting duct NH_3 secretion through the ammonia channel RHCG increases urinary total acid excretion by being protonated to NH_4^+ by luminal H^+ from the H^+-ATPases
- The vH^+-ATPase is stimulated by aldosterone and angiotensin II
- Impaired distal urinary acidification in these segments characterizes distal (type IV) RTA
  - Familial distal RTA arises from loss-of-function mutations in the vH^+-ATPase cytoplasmic subunit B1 and membrane-spanning subunit a4 or mutations in the kidney AE1 Cl^--HCO_3^- exchanger
  - In mice, knockouts of H^+-K^+-ATPase, the NH_3-transporter RHCG, the basolateral Cl^--HCO_3^- exchanger and Cl^- conductance factor SLC26A7, the K^+-Cl^- cotransporter KCC4 (*SLC12A7*), and the intercalated cell master regulator transcription factor FOXI1 also give rise to distal RTA, but mutations in these genes have yet to be found in humans with distal RTA
- Acquired distal RTA syndromes, such as Sjögren syndrome, are associated with decreased vH^+-ATPase and kidney AE1 levels

Water Reabsorption

- Although the intercalated cells are believed to be impermeable to water, the principal cells of the cortical and medullary collecting ducts, as well as the inner medullary collecting duct cells, express vasopressin-dependent water permeability controlled by luminal insertion of AQP2 water channels (Fig 5B)
- AQP2 channel insertion is controlled by vasopressin through
  - Binding to the vasopressin receptor V2R (*AVPR2*); V2R antagonists are used in the treatment of fluid overload and (still in clinical trial) to retard renal cyst expansion in ADPKD
  - Ligand binding to V2R increases cAMP, promoting fusion of subluminal AQP2-bearing vesicles with the luminal membrane
- Impaired water reabsorption by medullary and inner medullary collecting duct principal cells results in nephrogenic diabetes insipidus
  - X-linked recessive nephrogenic diabetes insipidus is caused by loss-of-function V2R mutations
  - Autosomal recessive diabetes insipidus is caused by loss-of-function AQP2 mutations
  - Lithium therapy can produce acquired nephrogenic diabetes insipidus
    - Li^+ enters the principal cell via ENaC, then interferes with V2R-mediated cAMP generation through inhibition of glycogen synthase kinase b signaling, leading to decreased surface abundance of AQP2

Urea Transport

- Urea contributes half the medullary osmoles in antidiuretic conditions
- The medullary collecting duct participates in medullary urea recycling by reabsorbing urea across the luminal membrane through the urea transporters UTA1 and UTA3 (variants of UTA [*SLC14A2*])
- UTA in the descending thin limb of Henle is thought to secrete interstitial urea into the tubular lumen for delivery to the inner medullary collecting duct, thus recycling urea
- A related transporter, UTB (*SLC14A1*), is found on the vasa recta and red blood cells and also participates in urea recycling
  - In the mouse, the urea transporter UT-B (*Slc14a1*) appears to be predominantly at the basolateral membrane

  - Patients with mutations in UTB have mild concentrating defects
- ADH increases urea transport across the medullary collecting duct by upregulating transporter insertion into the membrane

SUGGESTED READING

» Biver S, Belge H, Bourgeois S, et al. A role for Rhesus factor Rhcg in renal ammonium excretion and male fertility. *Nature.* 2008;456(7220):339-343.

» Deen PM, Verdijk MA, Knoers NV, et al. Requirement of human renal water channel aquaporin-2 for vasopressin-dependent concentration of urine. *Science.* 1994; 264(5155):92-95.

» Fenton RA, Knepper MA. Urea and renal function in the 21st century: insights from knockout mice. *J Am Soc Nephrol.* 2007;18(3):679-688.

» Grunfeld JP, Rossier BC. Lithium nephrotoxicity revisited. *Nat Rev Nephrol.* 2009;5(5):270-276.

» Hamm LL, Feng Z, Hering-Smith KS. Regulation of sodium transport by ENaC in the kidney. *Curr Opin Nephrol Hypertens.* 2010;19(1):98-105.

» Jarolim P, Shayakul C, Prabakaran D, et al. Autosomal dominant distal renal tubular acidosis is associated in three families with heterozygosity for the R589H mutation in the AE1 (band 3) $Cl^-/HCO3^-$ exchanger. *J Biol Chem.* 1998;273(11):6380-6388.

» Kim YH, Pham TD, Zheng W, et al. Role of pendrin in iodide balance: going with the flow. *Am J Physiol Renal Physiol.* 2009;297(4):F1069-1079.

» Leviel F, Hubner CA, Houillier P, et al. The Na^+-dependent chloride-bicarbonate exchanger SLC4A8 mediates an electroneutral Na^+ reabsorption process in the renal cortical collecting ducts of mice. *J Clin Invest.* 2010;120(5):1627-1635.

» Marples D, Christensen S, Christensen EI, Ottosen PD, Nielsen S. Lithium-induced downregulation of aquaporin-2 water channel expression in rat kidney medulla. *J Clin Invest.* 1995;95(4):1838-1845.

» Stover EH, Borthwick KJ, Bavalia C, et al. Novel ATP6V1B1 and ATP6V0A4 mutations in autosomal recessive distal renal tubular acidosis with new evidence for hearing loss. *J Med Genet.* 2002;39(11):796-803.

» Welling PA, Ho K. A comprehensive guide to the ROMK potassium channel: form and function in health and disease. *Am J Physiol Renal Physiol.* 2009;297(4): F849-863.

» Welling PA, Chang YP, Delpire E, Wade JB. Multigene kinase network, kidney transport, and salt in essential hypertension. *Kidney Int.* 2010;77(12): 1063-1069.

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