Claudins and the Kidney

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ABSTRACT
Claudins are tight-junction membrane proteins that function as both pores and barriers in the paracellular pathway in epithelial cells. In the kidney, claudins determine the permeability and selectivity of different nephron segments along the renal tubule. In the proximal tubule, claudins have a role in the bulk reabsorption of salt and water. In the thick ascending limb, claudins are important for the reabsorption of calcium and magnesium and are tightly regulated by the calcium-sensing receptor. In the distal nephron, claudins need to form cation barriers and chloride pores to facilitate electrogentic sodium reabsorption and potassium and acid secretion. Aldosterone and the with-no-lysine (WNK) proteins likely regulate claudins to fine-tune distal nephron salt transport. Genetic mutations in claudin-16 and -19 cause familial hypomagnesemic hypercalciuria with nephrocalcinosis, whereas polymorphisms in claudin-14 are associated with kidney stone risk. It is likely that additional roles for claudins in the pathogenesis of other types of kidney diseases have yet to be uncovered.


The renal tubule efficiently reabsorbs the bulk of filtered salt and water, and accurately fine-tunes the concentrations of many different solutes in the urine. This challenging task is accomplished by a combination of transcellular and paracellular transport. The paracellular pathway is a route for passive transport that passes between tubule epithelial cells,1 with the tight junction constituting the primary permeability barrier.2–4 Claudins are members of a family of tight-junction membrane proteins that act simultaneously as paracellular pores and barriers and determine the selectivity to small ions and neutral solutes.5

STRUCTURE AND FUNCTION OF CLAUDINS
There are 27 mammalian claudin genes, although the homology and nomenclature of the more distantly related genes are not completely settled.6–7 Claudin proteins have four predicted transmembrane helical domains with a short intracellular N-terminus, two extracellular loops, and a long C-terminal tail. Claudins are thought to polymerize to form continuous strands along the lateral membrane of one cell while the extracellular domains of claudins on adjacent cells bridge the paracellular space to interact with each other, much like the teeth of a zipper. The first extracellular loop appears to line the paracellular pore and determine its selectivity,8,9 while the second extracellular loop mediates trans interactions.10,11 The C-terminal tail plays roles in protein trafficking to the tight junction and protein stability,12 contains phosphorylation13 and palmitoylation sites,14 and has a conserved hydrophobic dipeptide motif that binds to PDZ domains on tight-junction scaffolding proteins, including ZO1, ZO2, ZO3,15 and MUPP1.16 The three-dimensional structure of claudin-15 has recently been solved at a resolution of 2.4 Å.17 The structure reveals a characteristic β-sheet fold comprising the two extracellular segments, which is anchored to a transmembrane four-helix bundle. This β-sheet forms a palm-like region that likely lines the paracellular pore (Figure 1).

EXPRESSION OF CLAUDINS IN THE KIDNEY
Most claudins are expressed in the renal tubule. Each segment and cell expresses multiple isoforms (Figure 2, Table 1). It is widely believed that the specific set of claudins expressed by each nephron segment determines the unique paracellular permeability properties of that segment. In addition, the glomerulus also expresses claudins. Parietal epithelial cells express claudin-1.18,19 Mature podocytes form slit diaphragms, which are a specialized form of intercellular junction, but tight junctions are also present during fetal development and reappear during podocyte injury.20,21 Claudin-5 and -6 have both been detected in podocytes.22,23
PROPERTIES OF CLAUDINS

The function of individual claudins in determining paracellular permeability and selectivity has been investigated primarily by overexpression and knockdown experiments in epithelial cell lines. The interpretation of such studies is complicated because, unlike transmembrane transport proteins, claudins must simultaneously function as both the barrier and the pores. Moreover, all epithelia already express multiple endogenous claudins, so the function of a heterologously expressed (or a knocked down) claudin gene must be superimposed on this background. As a consequence, the apparent function of claudin is highly dependent on the properties of the host cell line. For example, claudin-15 decreases Cl permeability when overexpressed in Na-selective, leaky MDCK II cells, whereas in Cl-selective and less leaky LLC-PK1 cells, claudin-15 increases Na⁺ permeability.²⁴

In general, claudins regulate a pore pathway that is selectively permeable to small ions and neutral solutes. Unlike most transmembrane channels, claudins do not tend to be highly selective but they do exhibit charge preference. Thus, claudins that are more cation-selective preferentially permeate Na⁺, K⁺, and other monovalent cations but also divalent cations such as Ca²⁺. Anion-selective claudins permeate Cl⁻ but also other halides and small anions. Claudins differ in the magnitude of their permeability, with some predominantly acting as pore-formers and others acting mostly as barrier-formers. The underlying mechanism for this is unknown but likely involves intrinsic differences in the size and conformation of the pore formed by each claudin isoform.

The pore diameter has been estimated for claudin-2 and is 6.5–8 Å, making it permeable to small organic ions, neutral molecules, and even water. In addition to this pore pathway, the tight junction exhibits a low-capacity, size-independent permeability to uncharged macromolecules, such as dextrans. The latter has been dubbed the “leak pathway” and appears to be dependent on occludin and ZO-1 but not on claudins. Table 1 summarizes the functional properties of claudins of particular relevance to the kidney. A more detailed and comprehensive catalog of claudins and their properties may be found in recent general reviews.³⁴,³⁵

IN VITRO PERMEABILITY PROPERTIES OF CLAUDINS

The primary role of the proximal tubule (PT) is bulk reabsorption of filtered solutes, including Na⁺, K⁺, Cl⁻, and Ca²⁺, and water. The PT is the leakiest nephron segment in the renal tubule, with transepithelial resistances of 5–7 Ω·cm². The driving forces for paracellular NaCl reabsorption in the late superficial PT have been well studied. Late PT fluid has relatively high concentrations of Cl⁻ and low concentrations of HCO₃⁻ because of early PT transcellular reabsorption of Na⁺, coupled largely to HCO₃⁻ or organic solutes, together with isosmotic water reabsorption. The late PT is more permeable to Cl⁻ than HCO₃⁻ (PₐCl/PₐHCO₃ ratio ranging from 2 to 18). This permits net passive reabsorption of Cl⁻, presumably via paracellular diffusion, and generates a lumen-positive electrical potential. This voltage in turn provides the driving force for passive reabsorption of Na⁺, again presumably via the paracellular pathway. An estimated 32%–64% of superficial PT NaCl reabsorption is passive and presumably paracellular (reviewed in reference 42).

Claudin-2 is the main claudin responsible for reabsorption of Na⁺. Claudin-2 is highly expressed in the PT, with highest levels in the late PT and early segment of the thin descending limb of long loops of Henle. In vitro overexpression and knockdown studies have shown that claudin-2 forms high-conductance, cation-selective paracellular pores. Muto et al. generated a constitutive knockout of claudin-2 in mice and showed a significant decrease in net Na and water reabsorption in the PT S2 segments and loss of Na⁺ selectivity. The mice had normal fractional excretion of Na⁺ on a normal diet but had excessive natriuresis in response to a saline load. Thus, claudin-2 likely plays an important role in PT paracellular salt reabsorption.

The PT also reabsorbs a substantial portion of filtered K⁺ and Ca²⁺ and this is thought to be mostly passive. There are two proposed mechanisms: (1) passive diffusion, driven by a lumen-to-bath K⁺ and Ca²⁺ concentration gradient (generated by net water reabsorption) and lumen-positive electrical gradient in the mid-late PT, or (2) convection (solvent drag). It has always been assumed (as for NaCl) that the route of this passive reabsorption is paracellular, but this has never been definitively proven. Claudin-2 is highly permeable to K⁺ and also moderately permeable to Ca²⁺. Claudin-2 null mice do not have any abnormality in...
K⁺ excretion, but they are hypercalciuric, suggesting the possibility that claudin-2 may mediate PT paracellular Ca reabsorption.

A long-standing controversy in renal physiology concerns whether water is transported paracellularly in the PT. At least 75% of PT water permeability is transcellular and mediated by aquaporin-1. However, the mechanism by which the remaining 25% is transported is unclear. Several investigators have found that the reflection coefficient for NaCl in the PT is <1, consistent with a convective pathway, but others have estimated that paracellular water permeability is very low, whereas those derived from measurements of plasma membrane water permeability have suggested a large contribution from the paracellular pathway. Finally, a number of investigators have observed solvent drag in the PT, which has been attributed to entrainment of solute movement with water through the paracellular shunt.

Claudin-2 has now been shown in cultured cell monolayers to permeate water, providing a potential molecular basis for paracellular water reabsorption in the PT. Like Muto, Schnermann et al. found a substantial (23%) reduction in proximal fractional volume reabsorption in claudin-2 null mice. This could arguably be due to the primary defect in Na reabsorption.

Figure 2. Localization of claudins along the adult mammalian renal tubule. Captions shaded in gray summarize some of the key physiologic functions of each nephron segment. AVP, arginine vasopressin; TGF, tubuloglomerular feedback. Refer to Table 1 for references.

Table 1. Localization and role of claudins in the mammalian renal tubule

<table>
<thead>
<tr>
<th>Claudin</th>
<th>Tubule Localization</th>
<th>Permeability Properties</th>
<th>Physiologic Role</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>PT, tDL, tAL, DCT</td>
<td>Na⁺, K⁺, Ca²⁺, and H₂O</td>
<td>PT Na⁺ and fluid reabsorption</td>
</tr>
<tr>
<td>3</td>
<td>tAL, TALH, DCT</td>
<td>Nonselective barrier</td>
<td>Unknown</td>
</tr>
<tr>
<td>4</td>
<td>tAL, CD</td>
<td>Na⁺ barrier and Cl⁻</td>
<td>Unknown</td>
</tr>
<tr>
<td>7</td>
<td>tDL, DCT, CD</td>
<td>Cl⁻ barrier</td>
<td>PT Cl⁻ reabsorption</td>
</tr>
<tr>
<td>8</td>
<td>tDL, DCT, CD</td>
<td>Na⁺, K⁺, and Cl⁻</td>
<td>PT Al⁻ reabsorption</td>
</tr>
<tr>
<td>10a</td>
<td>PT, TALH, CCD</td>
<td>Na⁺ or Na⁺ and Ca²⁺</td>
<td>PT Ca²⁺ reabsorption</td>
</tr>
<tr>
<td>10b</td>
<td>TALH, MCD</td>
<td>Na⁺ or Na⁺ and Ca²⁺</td>
<td>PT Mg²⁺ reabsorption</td>
</tr>
<tr>
<td>14</td>
<td>TALH, TALH</td>
<td>Na⁺ or Na⁺ and Ca²⁺</td>
<td>PT Mg²⁺ reabsorption</td>
</tr>
<tr>
<td>16</td>
<td>tAL, TALH</td>
<td>Na⁺ or Ca²⁺ and Mg²⁺</td>
<td>TALH reabsorption of divalent cations</td>
</tr>
<tr>
<td>17</td>
<td>PT &gt; tAL, TALH</td>
<td>Na⁺ or Ca²⁺ and Mg²⁺</td>
<td>TALH reabsorption of divalent cations</td>
</tr>
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<td>18</td>
<td>TALH, CD</td>
<td>Na⁺ and H⁺ barrier</td>
<td>TALH reabsorption of divalent cations</td>
</tr>
<tr>
<td>19</td>
<td>tAL, TALH</td>
<td>Cl⁻ barrier</td>
<td>TALH reabsorption of divalent cations</td>
</tr>
</tbody>
</table>

PT, proximal tubule; tDL, thin descending limb; tAL, thin ascending limb; DCT, distal convoluted tubule; CD, collecting duct; CCD, cortical collecting duct; MCD, medullary collecting duct.

*Question marks indicate speculative conclusions that have not been studied experimentally or for which multiple studies came to different conclusions.
which would be expected to reduce the osmotic gradient for water reabsorption. However, they found no change in the end-proximal transepithelial osmotic gradient. This suggested that claudin-2 may well mediate a portion of PT water reabsorption. However, the researchers could not demonstrate an additive effect of claudin-2 and AQP1 knockout on proximal fluid reabsorption, as would be expected if they were acting on different routes, so the issue must be regarded as not yet settled.

The molecules that mediate paracellular Cl reabsorption have not been identified yet. Claudin-10a,27,63 and claudin-1764 are both known to form anion-selective paracellular pores and are both expressed in the PT, so they are potential candidates for this role.

**ROLE OF CLAUDINS IN THE THICK ASCENDING LIMB**

The thick ascending limb of Henle (TALH) is an important site for paracellular divalent cation reabsorption that has recently been shown to be highly regulated (Figure 3B). The driving force for paracellular cation reabsorption is a lumen-positive transepithelial voltage. This voltage is generated primarily by the charge movement associated with transepithelial reabsorption of NaCl. Na⁺, K⁺, and Cl⁻ enter the TALH cell through the electroneutral Na-K-2Cl cotransporter at the apical membrane; Na is pumped out through the Na-K-ATPase, and Cl⁻ effluxes basolaterally via Cl⁻ channels; however, K⁺ is largely recycled apically via the renal outer medullary K⁺ channel, thus generating a lumen-positive electrical potential. At high tubule flow rates, this transepithelial electrical potential averages 3–10 mV, lumen-positive.65 At low flow rates, this voltage is augmented by a superimposed NaCl dilution potential. This is caused by the generation of a NaCl concentration gradient (tubule lower than interstitium) across the epithelium, which has a Na⁺ to Cl⁻ permeability ratio (P_{Na}/P_{Cl}) of 2–6,66–68 presumably reflecting a cation-selective paracellular shunt. The magnitude of this dilution potential can be appreciated in isolated TALH tubules in the absence of flow, when transepithelial voltage can reach 10–25 mV.66,68,69

Claudin-16 (originally named paracellin-1) and claudin-19 are expressed in the TALH and are clearly required for paracellular divalent cation reabsorption. Loss-of-function mutations in either gene cause familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) in humans,70,71 while mice with knockdown of claudin-16 or -19 have renal Mg²⁺ and Ca²⁺ wasting.72,73 However, the mechanism of action of these claudins is controversial. Some investigators found that claudin-16 overexpression increased Ca²⁺ and Mg²⁺ permeability74,75 and that claudin-16 knockout mice have reduced TALH Ca²⁺ and Mg²⁺ permeability,76 and argued that this claudin forms the TALH paracellular Ca²⁺ and Mg²⁺ pore itself. By contrast, Hou et al. found that claudin-16 primarily

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**Figure 3.** Physiologic roles of claudins in renal tubule epithelia. (A) Proximal tubule. Na⁺ reabsorption by Na⁺-H⁺ exchange or coupled to organic solutes, such as glucose, generates a high luminal Cl⁻ concentration and negative transepithelial voltage that drives paracellular Cl⁻ reabsorption. The subsequent lumen-positive voltage drives Na⁺ reabsorption through claudin-2. (B) Thick ascending limb. Transcellular Na reabsorption via the Na-K-2Cl cotransporter with K⁺ recycling via an apical K⁺ channel generates a lumen-positive voltage. This is further augmented by the tendency of reabsorbed Na⁺ to backflux through Na⁺-selective paracellular pores, postulated to be formed by claudin-16 and -19. This voltage drives Ca²⁺ and Mg²⁺ reabsorption paracellularly. The CaSR regulates paracellular transport by a cascade involving calcineurin, micro-RNAs, and claudin-14. (C) Collecting duct. Na⁺ is reabsorbed electrogenically via an apical epithelial Na⁺ channel in principal cells. The paracellular pathway, proposed to be constituted by claudin-4, -7, and/or -8, acts as a barrier to prevent backflux of reabsorbed Na⁺ (as well as secreted K⁺ and H⁺) while allowing Cl⁻ to diffuse down its electrical gradient. In each panel, the lumen is on the left and peritubular space on the right.
increased Na permeability while claudin-19 decreased Cl permeability, and that they interacted with each other in order to traffic correctly to the tight junction. They have proposed that the primary role of these genes is to increase \( P_{Na}/P_{Cl} \) in the TALH and thereby allow generation of the NaCl dilution crease. They have proposed that the NaCl dilution potential. In this model, claudin-16 and claudin-19 regulate \( Ca^{2+} \) and \( Mg^{2+} \) transport indirectly, by affecting the electrical driving force.

**ROLE OF CLAUDINS IN THE ALDOSTERONE-SENSITIVE DISTAL NEPHRON**

The aldosterone-sensitive distal nephron (ASDN), located at the end of the renal tubule, encompasses the distal convoluted tubule, connecting tubule, and collecting duct. In these segments, urine composition is fine-tuned by active re-absorption of \( Na^+ \) and secretion of \( K^+ \) and \( H^+ \) against steep uphill transtubular gradients that can reach lumen to blood ratios of 1:3 (\( Na^+ \)), 20:1 (\( K^+ \)), and 1000:1 (\( H^+ \)). Thus, a major role of the paracellular pathway is to act as a cation barrier to prevent backleak of actively transported cations (Figure 3C). In addition, the cortical collecting duct paracellular pathway is permeable to \( Cl^- \), presumably providing the counterion to accompany electrogenic \( Na^+ \) transport via the epithelial \( Na^+ \) channel, ENaC.

The ASDN expresses claudin-3, -4,-7, -8, and -10. The function of claudin-7 is unsettled. When overexpressed in LLC-PK cells, it appeared to reduce \( Cl^- \) permeability and act as a \( Cl^- \) barrier. However, knockdown of its expression paradoxically also decreased \( Cl^- \) permeability, suggesting that claudin-7 might normally behave as a \( Cl^- \) pore and be responsible for the paracellular \( Cl^- \) conductance in the ASDN. Mice with constitutive knockout of claudin-7 die in neonatal life for the paracellular \( Cl^- \) transport. The ASDN expresses claudin-3, -4, -7, -8, and -10. The function of claudin-7 is unsettled. When overexpressed in LLC-PK cells, it appeared to reduce \( Cl^- \) permeability and act as a \( Cl^- \) barrier. However, knockdown of its expression paradoxically also decreased \( Cl^- \) permeability, suggesting that claudin-7 might normally behave as a \( Cl^- \) pore and be responsible for the paracellular \( Cl^- \) conductance in the ASDN. Mice with constitutive knockout of claudin-7 die in neonatal life.

**REGULATION OF TAL CLAUDINS BY EXTRACELLULAR CALCIUM**

The Ca-sensing receptor (CaSR) plays a major role in regulating whole-body \( Ca^{2+} \) homeostasis. In the kidney, it is primarily expressed on the basolateral membrane of the TALH, where it reduces renal tubular \( Ca^{2+} \) reabsorption and induces calciuresis in response to a \( Ca^{2+} \) load. Although activation of the TALH CaSR may inhibit NKCC2 expression or activity and cause calcium reabsorption, by a loop diuretic-like effect, recent evidence suggests that the main action of CaSR in the tubule is to regulate paracellular permeability.

Loupy et al. showed that a CaSR antagonist increased \( Ca^{2+} \) permeability in isolated perfused TALH, with no change in transepithelial voltage or Na flux. This appears to be mediated by regulation of the expression of claudin-14. Activation of the CaSR causes robust upregulation of claudin-14, which, through physical interaction, inhibits paracellular cation channels formed by claudin-16 and -19. The signaling mechanism seems to involve CaSR somehow inhibiting calcineurin, a phosphatase that normally activates NFAT to increase tran- scription of two micro-RNAs (miR-9 and miR-374), thereby downregulating claudin-14 expression. The central role of claudin-14 is supported by the striking observation that claudin-14 knockout mice are unable to increase their fractional excretion of calcium in response to a high-\( Ca^{2+} \) diet and exhibit complete loss of regulation of urinary \( Ca^{2+} \) excretion in response to a CaSR agonist or antagonist.

**REGULATION OF ASDN CLAUDINS BY ALDOSTERONE AND WITH-NO-LYSINE KINASES**

The major function of aldosterone is to stimulate renal salt reabsorption, which is largely mediated by stimulation of ASDN tranacellular transport. However, mineralocorticoids can also regulate paracellular permeability. For example, mineralocorticoids reduce \( Na^+ \) permeability in the inner medullary collecting duct.

Weinstein has estimated that up to two thirds of \( Na^+ \) reabsorbed trancellularly in the cortical collecting duct may back-flux via paracellular pathways. Thus, aldosterone may play a significant role in limiting paracellular backleak of \( Na^+ \) along the collecting duct and thereby enhance net \( Na^+ \) reabsorption. However, the molecular mechanism for this effect is not known. Le Moellic et al. reported another role for aldosterone: to increase paracellular anion permeability in a rat cortical collecting duct cell line. This correlated temporally with phosphorylation of claudin-4 at a threonine residue, although a causal relationship was not established.

With-\( \)-lysine (WNK) 1 and WNK4 are protein kinases that play key roles in switching the ASDN between salt reabsorption and K secretion and are mutated in pseudohypoaldosteronism, type II. In addition to regulating tranacellular transport proteins, WNK4, which is localized to the tight junction in vivo, may also regulate paracellular \( Cl^- \) permeability. When overexpressed in cell lines, WNK4 (particularly the pseudo- hypoaldosteronism, type II mutant) stimulates paracellular \( Cl^- \) conductance. The exact mechanism is unclear. There is evidence both for and against, direct phosphorylation by WNK4 of several claudin isoforms, including claudin-4 and -7.

**CLAUDINS IN HUMAN KIDNEY DISEASES**

The first inherited disorder of claudins to be identified was FHHNC. This
autosomal recessive disease is characterized by renal Mg\(^{2+}\) wasting, hypercalcemia, and nephrocalcinosis. Patients with FHHNC are usually treated with oral Mg\(^{2+}\) supplements, but their serum Mg\(^{2+}\) levels remain very low. The hypercalcemia can be ameliorated with thiazide therapy but generally cannot be completely corrected. Despite treatment, CKD occurs in childhood and adolescence, and progression to ESRD is typical. Cadaveric renal transplantation normalizes the serum Mg\(^{2+}\) concentration and urinary Ca\(^{2+}\) excretion, confirming that the underlying defect is renal in origin. Classic FHHNC is due to mutations in claudin-16. Mutations in claudin-19 cause FHHNC that is associated with ocular abnormalities, including macular coloboma, nystagmus, and myopia. Claudin-19 is normally expressed at high levels in the retina, but why mutations in this protein cause these ocular disorders is unknown.

Claudin-14 has now been implicated in the pathogenesis of hypercalcemia and kidney stones. In a genome-wide association study, a common synonymous variant in the CLDN14 gene, rs219780, was associated with kidney stones. Approximately 62% of the general population is homozygous for the risk variant, which was estimated to confer a 1.64 times greater risk of developing stones. The same variant was also associated with reduced bone mineral density, suggesting that it likely causes hypercalcemia and negative Ca\(^{2+}\) balance. In a recent investigation of rare allelic variants in patients selected from three population-based cohorts, a nonsynonymous variant in CLDN14, rs113831133 (predicted to change threonine to methionine in codon 4), was more common in patients with low urine Ca\(^{2+}\) (4.1%) than in those with high urine Ca\(^{2+}\) (1.1%), suggesting that this mutant might have a dominant negative effect on claudin-14.

The role of claudins in the genetic predisposition to other kidney diseases, as well as in the pathogenesis of acquired kidney diseases, remains largely explored. Given the importance of claudins in the development and maintenance of polarized epithelia, in renal tubule transport function, and potentially in glomerular epithelial cell function, they will probably be found to be involved in many different disease processes within the kidney.

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DISCLOSURES

None.

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of claudin-10 in the kidney create paracellular pores with different ion selectivities. 


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