# The Biology of Epithelial Cell Tight Junctions in the Kidney

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## ABSTRACT

Nearly 50 years have lapsed since the tight junction between epithelial cells was first identified by electron microscopy. The tight junction was once viewed as a static structure providing a barrier to paracellular movement and restricting proteins to the apical or basolateral membrane. Recent insights into the molecular composition of tight junctions reveal surprising complexity and dynamic regulation. Epithelia along the nephron exemplify a diversity of tight junctions that contribute to more than a 100-fold difference in permeability from the proximal tubule to the collecting duct. Tight junctions along the nephron form during kidney development and must reassemble after tubular injury. Hereditary diseases, animal models, and cell culture studies provide a variety of new perspectives on the function of tight junctions in health and disease.

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The tight junction (TJ), first identified by electron microscopy, is the most apical landmark of the epithelial junctional complex composed of the TJ, adherens junction, and desmosome.[1] Despite differences in size of TJs in various epithelia, the prevailing view was these connectors formed static structures and, once assembled, provided the fence separating apical from basolateral domains and a barrier to paracellular movement of water and ions. The TJs along renal tubules[2] and the glomerular slit diaphragm[3] contain a rich array of proteins that include integral membrane proteins such as claudins, occludin, and junctional adhesion molecules, scaffolding proteins such as zona occludens-1 (ZO-1), and signaling proteins from all major families of G proteins, kinases, and phosphatases (Figure 1A).

Scaffolding proteins such as ZO-1 are characterized by PDZ domains (protein interaction domain named for the founding members of the family, PSD-95, discs large A, and ZO-1) and link TJ proteins to each other, to the actin cytoskeleton, and to signaling molecules. Additional adaptor complexes such as $\alpha$-protein kinase C (PKC)-Partitioning Defective Proteins3; 6 complexes are important in the generation of apical-basolateral polarity.[4] The major permeability characteristics of epithelia are defined by the unique composition of claudins (a 24-member family of tetramembrane spanning proteins that interact in the paracellular space (Figure 1A).[5] Signaling proteins interact with TJ proteins to regulate baseline permeability, and distinct signaling pathways are important to disruption of the TJ complex and its reassembly after injury (Figure 1). The TJ is also a signaling hub integrating local signals to modulate the barrier as well as providing nuclear signals for gene expression through the shuttling of specific DNA-binding proteins from the TJ to the nucleus, particularly ZO-1-associated nucleic acid-binding protein,[6] which senses epithelial cell density and regulates proliferation.[7]

## COMPONENT OF THE TIGHT JUNCTION

In the mammalian kidney, paracellular permeability decreases from the proximal tubule to the collecting duct (Figure 1B) because of the unique array of claudins expressed in each segment[8] and higher levels of occludin and ZO-1 in the distal segments.[9] Paracellular transport through the TJ is passive and is driven by electro-osmotic gradients produced by transcellular transport.

In the proximal tubule, a leaky epithelium with low transepithelial resistance (TER) and high paracellular transport, one-third of total fluid reabsorption occurs through the paracellular pathway. Claudin-2, -10, and -11 are highly expressed, and claudin-2 forms high-conductance cation pores permitting large amounts of paracellular $Na^+$ reabsorption.[10] Physical factors such as peritubular protein concentration and renal hydrostatic pressure are important determinants of paracellular transport, and nitric oxide, prostaglandins, and cAMP also increase paracellular permeability.[11]

The thick ascending limb expresses a large number of claudins: claudin-10, -16,

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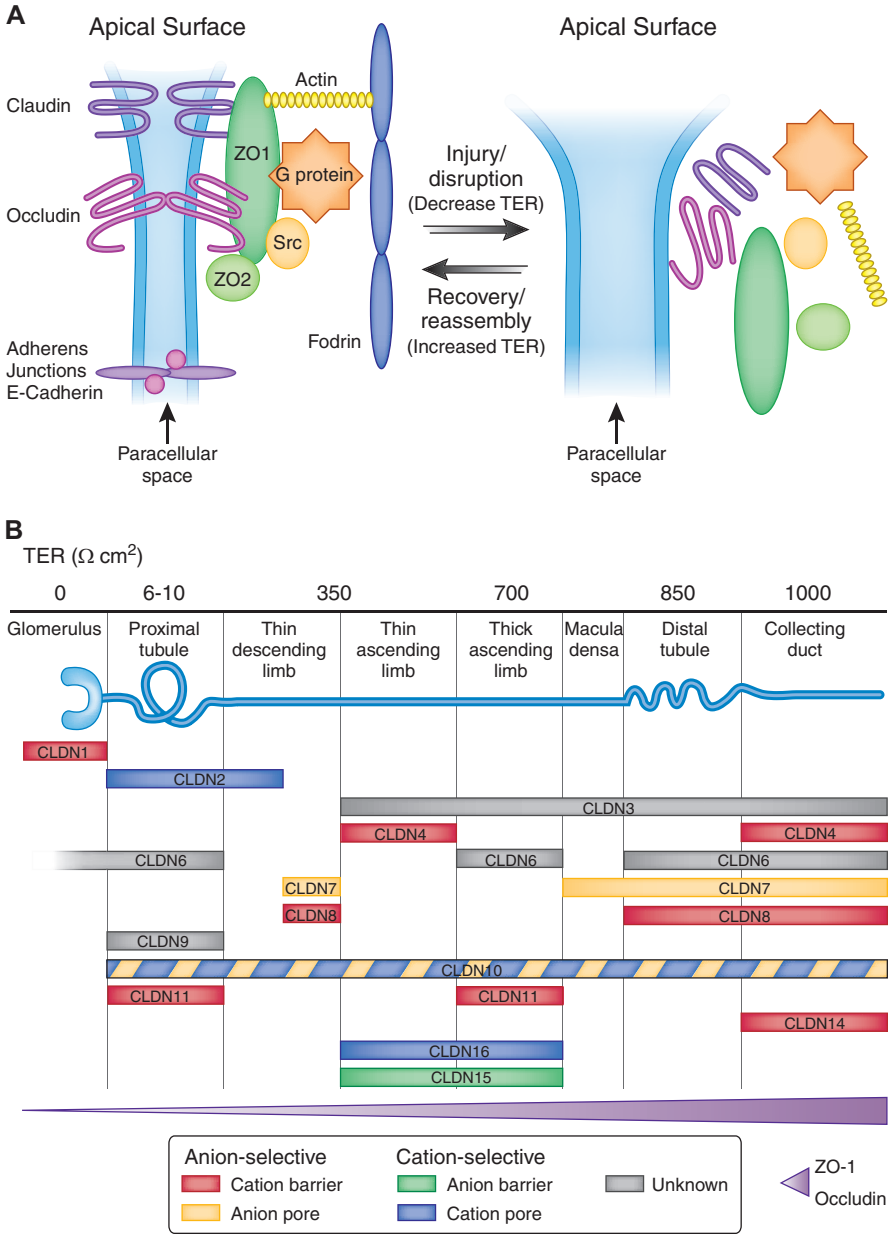


**Figure 1.** (A) Simplified schematic of major tight junction proteins. Claudins are key integral membrane proteins that provide the barrier function and permit selective paracellular transport. Numerous additional proteins modulate the barrier function. With injury, multiple signaling pathways are activated, resulting in phosphorylation of TJ proteins and disruption of the complex. Recovery requires additional signaling to reassemble the TJ complex. Src, Src tyrosine kinase. (B) Schematic of nephron with major claudins (CLDN) expressed in each segment. TER is an indicator of permeability and varies inversely with paracellular flux, and estimates of different nephron segments are shown on top.

and -19 and, depending on the species, also claudin-3, -4, -8, and -11. Paracellular reabsorption of $Ca^{2+}$ and $Mg^{2+}$ occurs in the thick ascending limb and is driven by the lumen-positive transepithelial voltage that is generated by $Na^+$-$K^+$-$2Cl^-$ cotransport and luminal $K^+$ recycling.[12] The interaction between claudin-16 and -19 is critical for $Ca^{2+}$ and $Mg^{2+}$ reabsorption, and mutations in these proteins lead to familial hypomagnesemia with hypercalciuria and nephrocalcinosis.[13]

The distal nephron is considered a tight epithelium with high TER and low passive permeability to cations. In this segment, claudin-4, -7, and -8 function as cation barriers to prevent the dissipation of transtubular $Na^+$, $K^+$, and $H^+$ gradients. Aldosterone and a new class of signaling kinases called WNKs (with no lysine) regulate the transport of $Na^+$ and $K^+$ in this segment. WNK-4 is localized within TJ of the cortical and distal collecting duct epithelia and phosphorylates claudins leading to increased paracellular chloride permeability.[14] Nedd4–2, an aldosterone-sensitive ubiquitin protein ligase that regulates $Na^+$ channel activity, interacts with occludin to increase paracellular permeability in these nephron segments.[15]

The mature TJ is a structure dynamically responsive to changing physiologic conditions from circulating cytokines, oxidative stress, and hormones. However, many of the details of this regulation remain unclear. Recent observations suggest that ZO-1 and ZO-2 are critical regulators of TJ assembly because these proteins recruit and activate the assembly of actin filaments and other TJ proteins during epithelial morphogenesis.[16,17] Targeted disruption of either ZO-1 or ZO-2 correlates with disruption of the cell junction and the paracellular barrier in mice that is lethal to the embryo.[18] ZO-1- and ZO-2-deficient cells have defects in TJ assembly and show a severely disrupted paracellular barrier with diffuse distribution of occludin and claudins along the basolateral membrane.[19]

## BIOGENESIS OF TIGHT JUNCTIONS

The initial phase of TJ biogenesis is characterized by interactions of ZO-1 with cadherins at the primordial adherens junction (AJ) that gradually fuse to form belt-like tight junctions. At this stage, claudins, occludins, and junctional adhesion molecules are recruited and polymerize to form the TJ strands that eventually segregate from the AJ. In the mature TJ, ZO-1 is completely excluded from the AJ and found exclusively at the TJ.[20] Oc-

cludin has an important role in maintaining the integrity of the TJ; however, occludin-null mice reveal it is not required for TJ assembly.[21] Phosphorylation of occludin regulates TJ permeability through c-Src, αPKC, and protein phosphatases.[22]

Multiple signaling pathways regulate TJ disruption with injury and reassembly. Heterotrimeric G proteins control cellular responses through Gα and Gβγ subunits, and several Gα subunits are localized in the TJ. $G\alpha_o$, $G\alpha_{i2}$, and $G\alpha_s$ stimulate TJ assembly, whereas $G\alpha_{12}$ interacts with ZO-1,[23] and its activation leads to TJ disruption and increased paracellular permeability by tyrosine phosphorylation of TJ proteins through c-Src and Hsp90. $G\alpha_{12}$ activates during TJ assembly in the calcium switch model of TJ biogenesis, suggesting an important role for this G protein in regulating both baseline TJ permeability and its assembly or disassembly.[24] Rho GTPases are also major regulators of the TJ and the actin cytoskeleton. The inhibition of Rho leads to disassembly of TJ in various epithelial cell lines, and basal Rho activity is required for normal TJ function.[25] PKC has multiple effects on the phosphorylation of TJ proteins and diverse roles in TJ assembly. Protein phosphatase 2A is a serine-threonine phosphatase that localizes to the TJ and regulates the phosphorylation of ZO-1 and occludin, antagonizing αPKC phosphorylation of these proteins.[26] Various additional studies implicate other tyrosine kinases, phosphatases, and signaling proteins in the regulation of TJ assembly.

## TIGHT JUNCTIONS IN DISEASE

The tight junction is a pivotal structure required for healthy epithelial function and is altered in numerous disease processes. Precise assembly of TJs is required during renal development when segmentation of the renal vesicle leads to the establishment of unique nephron segments.[27] Toxic and ischemia/reperfusion injuries are the most common causes of acute kidney injury and result in disassembly of TJs, increased apoptosis, and tubular cell detachment. Studies of delayed graft function in human allografts reveal that TJ disruption is an early and potentially reversible target of ischemia/reperfusion injury.[28] ATP depletion and reactive oxygen species in cultured epithelial cells reveal altered distribution of ZO-1, ZO-2, and occluding and loss of TJ barrier function.[29,30] Once injured, renal tubular epithelia may recover or progress to a fibrotic phenotype. Recovery of functional epithelia requires numerous integrated processes including re-establishment of the TJ. RhoA activation with injury protects the TJ and actin cytoskeleton and in combination with tyrosine phosphorylation is a necessary step for TJ recovery.[31] Genetic studies also reveal that claudins are important in both renal and extrarenal diseases. In addition to mutations in *CLD-16* and *-19* leading to familial hypomagnesemia with hypercalciuria and nephrocalcinosis, some polymorphisms in *CLD-14* associate with increased risk of nephrolithiasis, and recessive nonsyndromic deafness results from *CLD-14* mutations.[32]

Finally, the TJ is likely to be important in other renal diseases such as autosomal dominant polycystic kidney disease where polycystin-1 localizes to the lateral membrane, and changes in claudin expression may contribute to cyst function during tubulogenesis.[33] As progress on understanding these mechanisms advances, it seems certain that novel therapeutic strategies targeting the TJ will emerge for a variety of renal and nonrenal diseases.

## DISCLOSURES

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