Claudins at the gate: determinants of renal epithelial tight junction paracellular permeability

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The first description of TJ structure and function in the kidney was provided by Farquhar and Palade (15) in 1963. Ultrathin section electron microscopic images demonstrated that renal TJs form a continuous beltlike structure on the lateral membrane domain closest to the apical membrane. The TJ structure appears as a zone of fusion of the adjacent cell membranes, resulting in obliteration of the intercellular space over variable distances. In the fusion zone, the outer leaflets of the adjoining cell membranes converge to form a single, intermediate line. Hemoglobin tracer experiments provided evidence for the control of movement of molecules between epithelial cells via the paracellular pathway (6, 23, 56). Two major functions of the epithelial TJ are simply characterized as the gate and fence functions (13). The TJ gate function refers to the control of movement of molecules between epithelial cells via the paracellular route. The TJ is essential for the barrier function of epithelia by restricting paracellular diffusion. TJs encircle the epithelial cells at the apical end of the lateral membrane and form the boundary between the apical and basolateral membrane surfaces. The TJ fence function refers to the maintenance of epithelial cell polarity. TJs are also crucial for the development and maintenance of epithelial cell surface polarity, because they form an intramembrane diffusion fence that restricts diffusion of membrane proteins and/or lipids in the exoplasmic leaflet of the plasma membrane. The ability of the TJ protein complex to form a seal is dependent on the interaction of the TJ protein complex with structurally organized actin filaments in the cytoskeleton. The role of claudins in the TJ fence function is not known. Assembly and disassembly of the TJ between epithelial cells are also partially controlled by E-cadherin-mediated cell-cell adhesion in the adherens junction (27). The molecular composition of tight junctions includes an array of transmembrane proteins and cytosolic plaque proteins and has been recently reviewed (23, 56).

THE REABSORPTION AND SECRETION of various solutes across the renal tubular epithelium constitute a major aspect of normal renal physiology. Typically, solutes cross the renal tubular epithelium by either the transcellular or the paracellular route (depicted in Fig. 1). In the transcellular route, the solutes cross the apical membrane, traverse through the cytoplasm, and then cross the basolateral membrane. This process is frequently facilitated by specific membrane transporters or channels on the apical and/or basolateral surface. When crossing the epithelium by the paracellular route, solutes cross by going through the tight junction (TJ) between adjacent epithelial cells. In this review, we focus on the role of the recently identified claudin family of transmembrane TJ-associated proteins as determinants of paracellular transport across the renal tubular epithelium.

The zonula occludens (ZO), or TJ, is one of the vertebrate epithelial cell junctional complexes that are important for the development of polarized epithelium and an intact barrier (see Fig. 1). These junctional complexes also include focal adhesions (mediated, in part, by integrin molecules), which attach the cells to the extracellular matrix; the adherens junction (mediated, in part, by E-cadherin), which is localized to the lateral membranes of adjacent cells; and gap junctions (mediated by connexins), which are important for cell-cell communication. The TJ is located at the most apical region of the lateral membrane in epithelial cell sheets. TJs are composed of transmembrane and cytosolic proteins and seal the intercellular space between adjacent cells to create a primary barrier against the diffusion of fluid, electrolytes, macromolecules, and patho-

gens via the paracellular pathway (6, 23, 56). Two major functions of the epithelial TJ are simply characterized as the gate and fence functions (13). The TJ gate function refers to the control of movement of molecules between epithelial cells via the paracellular route. The TJ is essential for the barrier function of epithelia by restricting paracellular diffusion. TJs encircle the epithelial cells at the apical end of the lateral membrane and form the boundary between the apical and basolateral membrane surfaces. The TJ fence function refers to the maintenance of epithelial cell polarity. TJs are also crucial for the development and maintenance of epithelial cell surface polarity, because they form an intramembrane diffusion fence that restricts diffusion of membrane proteins and/or lipids in the exoplasmic leaflet of the plasma membrane. The ability of the TJ protein complex to form a seal is dependent on the interaction of the TJ protein complex with structurally organized actin filaments in the cytoskeleton. The role of claudins in the TJ fence function is not known. Assembly and disassembly of the TJ between epithelial cells are also partially controlled by E-cadherin-mediated cell-cell adhesion in the adherens junction (27). The molecular composition of tight junctions includes an array of transmembrane proteins and cytosolic plaque proteins and has been recently reviewed (23, 56).

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Evidence that the TJ blocks paracellular movement across the TJ into the intercellular space beyond it (15).

**DISCOVERY OF CLAUDINS**

The identification of claudins was the result of an intensive search for integral transmembrane proteins localized to the TJ of chicken liver by Tsukita’s laboratory (21) in the 1990s. In 1993, occludin was identified as the first integral membrane protein exclusively localized to the tight junction (20). However, when occludin-deficient embryonic stem cells are differentiated to epithelial cells during embryogenesis, well-developed TJs formed between adjacent epithelial cells (54). This finding indicated that occludin is not necessary for TJ formation. Moreover, occludin-deficient mice are viable but showed significant postnatal growth retardation (55). At this time, the function of occludin in the TJ is not known. Because of the vital importance of TJ gate and fence functions, the viable phenotype of the mice lacking occludin also suggested the presence of other transmembrane proteins important for TJ structure and function.

The first claudin isoforms were discovered in 1998 when Tsukita’s laboratory (21) reported the identification of two novel 22-kDa integral membrane proteins localized to the TJ. These proteins exhibited no sequence similarity to occludin, contained four putative transmembrane domains, and were named claudin-1 and -2. The name claudin was chosen from the Latin word “claudere” (to close) (21). Epitope-tagged mutants of both claudin-1 and -2 isoforms were targeted and incorporated into the TJs of Madin-Darby canine kidney (MDCK) II epithelial cells. Northern blot analysis showed expression of claudin-1 in all tissues examined (heart, brain, spleen, lung, liver, skeletal muscle, kidney, and testes), whereas claudin-2 expression was primarily restricted to the kidney and liver (21).

A recent database search indicates the existence of 24 claudin isoforms (23). All claudins encode 20- to 27-kDa proteins with four transmembrane domains, two extracellular loops, and a short COOH intracellular tail (62). Because the existence of claudin proteins in the TJ has only been known for the last 7 years, the precise role of these proteins in renal physiology as well as human renal disease remains largely unknown. Examples of human renal diseases related to abnormal expression or function of claudin proteins have been identified. Abnormalities in paracellular transport of chloride ions have been implicated in the pathogenesis of pseudohypoaldosteronism type II (PHAII), a disease characterized by hypertension and hyperkalemia (30). This report provided evidence that the kinase WNK4 regulates paracellular ion flux. PHAII mutant WNK4 further augmented paracellular chloride transport to cause hypertension and hyperkalemia. Yamauchi et al. (69) have shown increased phosphorylation of claudin-1 through -4 in the presence of PHAII mutant WNK4. Collectively, these findings implicate WNK4-mediated phosphorylation of claudins in the mechanism of altered paracellular transport.

Mutations in the gene coding for the renal TJ protein claudin-16 (also called paracellin-1) cause familial hypomagnesemia with hypercalciuria and nephrocalcinosis, an autosomal recessive disorder of renal calcium and magnesium handling that progressively leads to chronic renal failure, with nephro lithiasis having been reported in heterozygous carriers (57). In these patients, there is a selective defect in paracellular magnesium and calcium reabsorption in the thick ascending limb of Henle. This has led to the theory that claudin-16 functions as a paracellular channel selective for magnesium and calcium.

Renal ischemia alters the expression pattern of renal claudins. Claudin-1, -3, and -7 mRNA levels are increased in response to experimental murine renal ischemia-reperfusion injury (33). This same study also demonstrated a 1.9-fold reduction in claudin-2 mRNA expression. These observations raise the possibility of a role for claudins in the development of or recovery from ischemic acute renal failure.

Transfection of cDNA for claudins 1–3, -5, or -11, but not occludin, into TJ-deficient fibroblasts resulted in the formation of TJ strands (21, 46, 47). These data suggest that claudins, but not occludin, are necessary for the formation of TJ strands. Individual claudins interact with each other within and between TJ strands in a specific manner. Cells transfected with single claudin isoforms are able to form strands, indicating that homopolymers of claudin form strands in these cells. In addition, the claudin homopolymers of claudin-1, -2, or -3 were able to associate laterally with both different and the same claudin homopolymers in adjacent cells. Claudin-1 homopolymers were able to laterally associate with both claudin-1 and -3 homopolymers, and claudin-2 homopolymers were able to laterally associate with both claudin-2 and -3 homopolymers. Claudin-1 and claudin-2 homopolymers did not associate with each other.
MDCK CELLS AS A MODEL TO INVESTIGATE THE ROLE OF CLAUDINS IN TJs OF RENAL EPITHELIAL CELLS

A PubMed search for the terms MDCK and tight junction conducted in March 2005 showed 213 publications and illustrates the utility of the MDCK cell culture system in the analysis of epithelial TJs. The original population of MDCK cells was isolated in 1957 by Madin et al. (36, 41) from a heterogeneous population of tubules obtained from ~25 g of dog kidney cortex and is one of the best-characterized and most widely used renal epithelial cell lines (61). When grown on permeable supports, MDCK cells form a well-polarized epithelial monolayer that reconstitutes a simple in vitro polarized epithelial tissue. Two strains of MDCK cells have since been established and are termed MDCK I and II (53). Several studies report that MDCK II cells exhibit properties of proximal tubule epithelium, whereas MDCK I displays properties of distal tubule epithelium. MDCK II cells possess measurable activities of proximal tubule markers alkaline phosphatase and γ-glutamyl transpeptidase, whereas MDCK I cells do not (53). MDCK I cells form epithelial monolayers displaying a high transepithelial resistance (TER) (~4,100 Ω·cm²) and respond to antidiuretic hormone (ADH). MDCK II cells form epithelial monolayers of low TER (~200 Ω·cm²) and are insensitive to ADH (53). Na⁺/H⁺-ATPase activity is twofold greater in MDCK II than MDCK I cells (53), similar to the distribution of Na⁺-K⁺-ATPase in vivo where proximal tubules have higher activity than that of the collecting tubule (31). Phospho-ERK1/2 (active) is observed only in the distal nephron of human kidneys (42), and MDCK I cells express high basal levels of phospho-ERK1/2 whereas MDCK II cells have lower basal phospho-ERK1/2 levels (40). The TJ-associated protein claudin-2 is expressed in MDCK II cells under routine culture conditions but not in MDCK I cells (17). Claudin-2 expression is restricted to the “leaky” nephron segments, including the proximal tubule and thin descending limb of Henle, and is absent in the remaining distal nephron, a “tight” epithelium (14, 35, 51). These properties of MDCK I and II cells account for why they are thought to represent renal epithelial cells from different segments of the nephron (16, 53).

The MDCK epithelial cell line has been particularly useful in the study of TJs in a cell culture model of a simple renal epithelium (10, 24, 27). TJ permeability in cultured MDCK cells has been generally measured using either of two techniques: TER or paracellular tracer (e.g., dextran) flux (8, 32). Paracellular tracer flux measures TJ permeability of a monolayer over a time course of several hours, whereas TER represents an instantaneous measurement. MDCK I cells show a relatively higher (10- to 100-fold) TER than MDCK II cells while displaying little differences between these two strains in their morphology or the number of TJ strands (25, 59). Thus with regard to relative TER values, MDCK I cells represent a tight epithelium while MDCK strain II cells represent a leaky epithelium. While differences in MDCK I and II cell TER has been known for over 20 years, only recently has the molecular nature accounting for the differences in TER between different strains of MDCK cells been understood. Furuse et al. (17) studied MDCK I and II cells with special attention to claudins and found that claudin-2 was expressed in MDCK II but not in MDCK I cells. When exogenous claudin-2 was introduced into MDCK I cells, the TER of the cells dropped dramatically (similar to those found in MDCK II cells). While introduction of claudin-2 into MDCK I cells resulted in a dramatic reduction in TER, the paracellular flux of 4- and 40-kDa-labeled dextran molecules across monolayers of mock-transfected and claudin-2-transfected MDCK I cells was not significantly different.

Another study showed that exogenous claudin-2 expression in MDCK-C7 cells (a twin to MDCK I cells) also induces cation-selective channels in the TJ (2). These findings suggest that claudin-2 specifically decreases the TER of TJs in renal epithelial cells by increasing the cation permeability across the TJ.

Other groups have also studied the effects of claudins on paracellular transport by expression of individual claudins in MDCK cells. A major limitation of this approach is that MDCK cells already express a background of multiple claudins. Thus one cannot define a specific paracellular conductance property for an individual claudin but can only document changes relative to the background (64).

To investigate the function of claudin-1, Inai et al. (29) expressed myc-tagged claudin-1 in MDCK II cells. The myc-tagged claudin-1 was targeted to the TJ. Myc-tagged claudin-1 expression in MDCK II cells also increased TER approximately fourfold and decreased the paracellular flux of 4- and 40-kDa-labeled dextran molecules. Another group demonstrated that inducible expression of myc-tagged claudin-1 also increased the TER of MDCK II cells in a reversible fashion (43). This same study also showed that overexpression of myc-tagged claudin-1 increased the paracellular flux of dextran and increased the aberrant formation of TJ strands in the lateral membrane.

The role of claudin-4 in influencing paracellular permeability across the TJ was studied by overexpressing human claudin-4 in MDCK II cells using an inducible promoter (63). Overexpression of claudin-4 increased the complexity of the TJ strands without affecting the levels of claudin-1, -2, or -3. The claudin-4 expression appeared to decrease paracellular conductance through a selective decrease in sodium permeability without significant effects on chloride permeability.

Nephron expression of claudin-8 is restricted to the aldosterone-responsive distal nephron (35). This segment of the nephron exhibits relatively cation-impermeable TJ and is capable of maintaining transtubular gradients up to 1,000:1 for H⁺, 20:1 for K⁺, and 28:1 for Na⁺. To test the hypothesis that claudin-8 plays a role in impeding paracellular cation permeability, Yu et al. (70) examined the effects of claudin-8 overexpression in MDCK II cells. In this study, induction of claudin-8 expression was associated with decreased expression of claudin-2 protein. Ussing chamber and radiotracer flux studies demonstrated that claudin-8 expression reduced paracellular permeability to monovalent inorganic and organic cations and to divalent cations but not to anions or neutral solutes. These data are consistent with a model by which claudin-8 expression displaces endogenous claudin-2 and causes a reduction in the function of cation pores in the TJ (70). These data suggest that claudin-8 plays an important role in restricting paracellular transport of cations in the distal nephron.
Claudin-14 is expressed in the sensory epithelium of the organ of Corti in the inner ear. Loss of claudin-14 results in hereditary deafness (68). To explore the role of claudin-14 in the inner ear and in other tissues, a mouse model with targeted deletion of claudin-14 was created (9). In the targeted allele, a lacZ cassette was expressed under the claudin-14 promoter. In claudin-14-lacZ heterozygous mice, β-galactosidase activity was detected in cochlear inner and outer hair cells and supporting cells, in the collecting ducts of the kidney, and around the lobules of the liver (9). Although direct demonstration of claudin-14 expression in the kidney is lacking, this work provides evidence for the expression of claudin-14 in the collecting duct of the nephron. Exogenous expression of claudin-14 in MDCK II cells increased TER sixfold (9). However, the effect of claudin-14 expression on the expression of other claudin isoforms in the kidney was not determined. This study concluded that claudin-14 also acts as a cation-restrictive barrier in the TJ.

Claudin-15 expression alters TJ permeability in MDCK II cells. The effect of claudin-15 expression on paracellular permeability properties was examined using an inducible MDCK II cell line (66). Inducible expression of claudin-15 in cation-selective MDCK II cells had no effect on paracellular permeability of Na\(^+\) but did increase TER values 1.9-fold. In anion-selective LLC-PK\(_1\) cells (another established renal epithelial cell line), expression of claudin-15 increased the permeability to Na\(^+\) and lowered the TER.

Positional cloning identified mutations in the gene for claudin-16, also known as paracellin, as the cause of familial hypomagnesemia-hypercalciuria syndrome in humans (57). RT-PCR analysis of mRNA from nephron segments of the rabbit (57) and rat (67) has demonstrated that claudin-16 is expressed in the thick ascending limb and the distal convoluted tubule and appears to mediate resorption of both Mg\(^{2+}\) and Ca\(^{2+}\) (57). Flag-tagged claudin-16 expressed in MDCK II cells localizes to the TJ and is associated with ZO-1. MDCK II cell monolayers expressing claudin-16 exhibited higher activities of Ca\(^{2+}\) transport from the apical to the basolateral compartment (28). These data are consistent with the notion that the claudin-16/ZO-1 complex is important in the reabsorption of divalent cations by renal epithelia.

**MOUSE CLAUDIN KNOCKOUTS**

To date, targeted gene disruption of claudin-1, -5, -11, and -14 has provided important information about the function of these claudin isoforms in the skin (18), blood-brain barrier (50), central nervous system/testes (26), and cochlear hair cells (9), respectively. Of these claudins, only claudin-1 and -11 are expressed in nephron epithelial TJs (35, 51), and information from these claudin knockout animals on the role of claudins in renal TJ function is lacking. In the case of mice lacking claudin-1, newborn mice exhibited no overt histological abnormality in the kidney where claudin-1 is expressed in large amounts in wild-type mice (19). However, a detailed analysis of renal tubular function in claudin-1 knockout mice has not been published. A recent abstract on disruption of the claudin-2 gene in mice showed that net absorption of Na\(^+\) and water in the proximal nephron was reduced compared with wild-type littermates (48).

**DISTRIBUTION OF CLAUDIN ISOFORMS IN THE MAMMALIAN NEPHRON**

Several studies have reported on the expression patterns of claudin isoforms in the mammalian nephron (14, 35, 38, 51). One study examined the expression of claudin-1 to -16 in mouse kidney by Northern blotting (35). This same study also examined the distribution of claudin-1, -2, -3, -4, -8, -10, -11, and -16 in mouse nephron segments by immunofluorescence microscopy. This study by Kiuchi-Saishin et al. (35) provides a summary figure illustrating the nephron segment-specific expression patterns of individual claudin isoforms. In another study, the nephron segment distribution of claudin-1 and -2 in newborn and adult rabbits was determined using immunofluorescence and Western blot detection (51). In addition, this study examined the expression of claudin-1 to -8 in isolated rabbit renal tubules by RT-PCR. The distribution pattern of claudins in the nephron is complicated. In agreement with the previous two investigations on claudin localization in the nephron, Enck et al. (14) reported that claudin-2 expression is restricted to the proximal nephron. Table 1 summarizes the localization of claudins in nephron segments as determined by these and other studies. Clearly, further work will be required...

<table>
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<tr>
<th>Claudin</th>
<th>Distribution (Ref.)</th>
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<tbody>
<tr>
<td>1</td>
<td>Primarily distal and collecting segments (rabbit by IF) (51)</td>
</tr>
<tr>
<td>2</td>
<td>Bowman’s capsule (mouse by IF) (35)</td>
</tr>
<tr>
<td>3</td>
<td>Bowman’s capsule, proximal tubule, thin descending limb (mouse by IF) (35)</td>
</tr>
<tr>
<td>4</td>
<td>Proximal segment (rabbit by IF) (51)</td>
</tr>
<tr>
<td>5</td>
<td>Proximal tubule (mouse by IF) (14)</td>
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<tr>
<td>6</td>
<td>Thin ascending limb, thick ascending limb, distal tubule, and collecting duct (mouse by IF) (35)</td>
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<tr>
<td>7</td>
<td>Proximal and collecting ducts (rabbit by RT-PCR) (51)</td>
</tr>
<tr>
<td>8</td>
<td>Thin ascending limb of Henle and collecting duct (mouse by IF) (35)</td>
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<tr>
<td>9</td>
<td>Proximal tubules (rabbit by RT-PCR) (51)</td>
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<tr>
<td>10</td>
<td>Thin ascending limb of Henle and collecting duct (mouse by IF) (35)</td>
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<tr>
<td>11</td>
<td>Proximal and Henle’s loop; collecting segment (rabbit by RT-PCR) (51)</td>
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<tr>
<td>12</td>
<td>Bladder (rat, mouse, and rabbit by IF) (1) and (mouse by IF) (49)</td>
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<tr>
<td>13</td>
<td>Endothelial cells (mouse by IF) (35)</td>
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<tr>
<td>14</td>
<td>Endothelial cells (rabbit by IF) (51)</td>
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<tr>
<td>15</td>
<td>Absent from adult kidney (rabbit by RT-PCR) (51)</td>
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<tr>
<td>16</td>
<td>Absent from adult kidney (mouse by Northern blotting) (35)</td>
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<tr>
<td>17</td>
<td>Distal nephron on basolateral membrane of epithelial cells (mouse by IF) (38)</td>
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<tr>
<td>18</td>
<td>Proximal tubules (rabbit by RT-PCR) (51)</td>
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<td>Thin ascending limb of Henle and collecting duct (mouse by IF) (35)</td>
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<td>20</td>
<td>Proximal and Henle segments (rabbit by RT-PCR) (51)</td>
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<td>21</td>
<td>Aldosterone-sensitive distal nephron (mouse by IF) (38)</td>
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<tr>
<td>22</td>
<td>Bladder (rat, mouse, and rabbit by IF) (1)</td>
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<tr>
<td>23</td>
<td>Not detected (mouse by Northern blotting) (35)</td>
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<tr>
<td>24</td>
<td>Proximal and thick ascending limb of Henle segment (mouse by IF) (35)</td>
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<tr>
<td>25</td>
<td>Proximal and thick ascending limb of Henle segment (mouse by IF) (35)</td>
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<tr>
<td>26</td>
<td>Absent from adult kidney (mouse by Northern blotting) (35)</td>
</tr>
<tr>
<td>27</td>
<td>Absent from adult kidney (mouse by IF) (1)</td>
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<td>Endothelial cells (mouse by IF) (35)</td>
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<td>30</td>
<td>Thick ascending limb of Henle (mouse by IF) (35)</td>
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<tr>
<td>31</td>
<td>Thick ascending limb of Henle (human by IF) (57)</td>
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IF, immunofluorescence microscopy.

*Invited Review*
FUNCTIONAL CHARACTERISTICS OF RENAL CLAUDINS

Table 2 summarizes the functional properties of individual claudins in the TJ of renal epithelia. These results strongly suggest that the specific physiological properties of different nephron segments are determined by the expression of specific claudin isoforms.

An interesting hypothesis has emerged where the determination of TJ paracellular conductance characteristics is determined, in part, by the amino acid composition of the extracellular loop domains of the claudin isoforms. Claudins are four-transmembrane proteins with two extracellular loops. Recent work has suggested that the amino acid composition of the extracellular loops of individual claudins determines, in part, the paracellular conductance characteristics of individual claudins (64). For example, claudin-4 (63) and claudin-8 (70) confer reduced paracellular conductance because of reduced conductance of cations. The low-conductance characteristics of claudin-4 and claudin-8 are consistent with the protein distribution as determined by immunofluorescent microscopy to the distal tight epithelium of the murine nephron (35). However, another study found claudin-4 mRNA expression in both leaky and tight segments of the mammalian (rabbit) nephron and claudin-8 mRNA in leaky segments of the rabbit nephron (51). These differences in claudin isoform expression in the mammalian nephron may be due to species differences between the expression of claudin isoform mRNA and protein expression. Replacement of particular negative amino acid residues in the extracellular loop of claudin-15 converted it from having cation-selective to anion-selective TJ paracellular permeability (12). This has led to the proposal that the extracellular loop of claudins interacts with the extracellular loop of claudins on the adjacent cell to create TJ pores. The amino acid composition of the extracellular loops determines the relative charge in the pore and ion conductance selectivity through the pore. Claudin-16 appears to confer permeability to divalent cations (e.g., Mg$^{2+}$ and Ca$^{2+}$). Curiously, the first extracellular loop of claudin-16 contains relatively more negative amino acids than do the other claudin isoforms, to possibly favor selective permeability for divalent cations (64).

REGULATION OF CLAUDIN EXPRESSION

Rapid changes in the composition of claudins within the TJ may be an important mechanism by which TJ paracellular permeability properties are regulated. Recent evidence indicates that the ERK1/2 signaling pathway controls, in part, the expression of claudin-2 and tight junction paracellular permeability. The first evidence for regulated changes in claudin-2 expression came from a microarray analysis of hepatocyte growth factor (HGF)-induced changes in gene expression patterns in MDCK II cells. Depending on culture conditions, HGF treatment of MDCK II cells produces pleiotrophic effects on cell movement and morphology (4). These effects include scattering (60) (cells grown at low density; hence the synonym of scatter factor for HGF); tubulogenesis (45) (MDCK II cells grown in collagen gels); or epithelial cell dedifferentiation and loss of contact inhibition of mitosis (3, 7, 37) (MDCK II cells grown on permeant supports). The early genomic responses of polarized MDCK II cell monolayers to HGF activation of the tyrosine kinase c-met were identified using a novel canine DNA microarray (5). Of the 12,473 genes sequences on the microarray, the expression of 125 were differentially regulated by HGF. The most dramatic change in gene expression in MDCK II cells following HGF stimulation was a 23.8-fold reduction of claudin-2 gene expression after 24 h of HGF treatment. HGF activates ERK1/2 in MDCK II cell monolayers by a process that is inhibited by the MEK inhibitor U-0126 (37). HGF-induced inhibition of claudin-2 expression in MDCK II cells requires ERK1/2 activation because U-0126 blocks this process and because expression of dominant, active MEK I constructs activates ERK1/2 and inhibits claudin-2 expression in MDCK II cells (40). HGF treatment of MDCK II cells did not significantly influence the expression of other TJ proteins including claudin-1, -3, -4, -7, occludin, and ZO-1 (40). Claudin-2 is thought to confer a leaky phenotype to epithelial cell TJs.

Recently, Singh and Harris (58) demonstrated that treatment of MDCK II cells with EGF results in a dramatic inhibition of claudin-2 expression. The inhibition of claudin-2 expression by EGF treatment of MDCK II cells was also blocked by the MEK (and thereby ERK1/2) inhibitor PD-98059. In addition, EGF treatment of MDCK II cells induced an increase in TER that

Table 2. Characteristic features of claudin isoforms in renal epithelia

<table>
<thead>
<tr>
<th>Claudin</th>
<th>Characteristics (Ref.)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Present in tight epithelium (e.g., collecting duct) (51). Overexpression in MDCK strain II cells increases TER and decreases paracellular flux of dextran molecules (29).</td>
</tr>
<tr>
<td>2</td>
<td>Present in proximal tubule (leaky epithilia) (14, 35, 51). Present in leaky MDCK II cells and absent in tight MDCK strain I cells (except following ERK1/2 inhibition) (2, 17, 40). Exogenous expression in MDCK strain I cells decreases TER (17) and induces monovalent cation-selective channels in the tight junction (2).</td>
</tr>
<tr>
<td>3</td>
<td>Found in tighter segments of the nephron (thin ascending limb of Henle, thick ascending limb of Henle, distal tubule and collecting duct (35). Exogenous expression of claudin-3 in high-TER MDCK strain I cells does not change TER (17). Claudin-3 is expressed in both low-resistance MDCK strain II and high-resistance MDCK strain I cells (40).</td>
</tr>
<tr>
<td>4</td>
<td>Present in tight nphron segments (thin ascending limb of Henle and collecting duct) (35). Overexpression in MDCK strain II cells decreases paracellular conductance through selective inhibition of sodium permeability (63).</td>
</tr>
<tr>
<td>5</td>
<td>Present in basolateral membrane of distal nephron epithelial cells (38). Also expressed in MDCK strain I and II cells (40).</td>
</tr>
<tr>
<td>6</td>
<td>Present in the distal nephron (38). Claudin-8 overexpression in MDCK strain II cells reduces paracellular permeability to monovalent inorganic and organic cations and to divalent cations but not to anions or neutral solutes (70).</td>
</tr>
<tr>
<td>7</td>
<td>Present in proximal tubule and thick ascending limb of Henle (35). No information on permeability properties at this time.</td>
</tr>
<tr>
<td>8</td>
<td>Present in thick ascending limb of Henle (35, 57). Overexpression of paracellin in MDCK cells increases paracellular transport of divalent cations (magnesium and calcium) (28).</td>
</tr>
<tr>
<td>10 and 11</td>
<td>Present in proximal tubule and thick ascending limb of Henle (35, 57).</td>
</tr>
</tbody>
</table>

MDCK, Madin-Darby canine kidney; TER, transepithelial resistance.
was temporally related to the inhibition of claudin-2 expression. Collectively, these data show that claudin-2 expression is controlled, in part, by the ERK1/2 signaling pathway. Future work is required to determine whether ERK1/2 activity controls claudin-2 expression in the nephron in vivo.

There are other examples of the regulation of claudin expression in other tissues. Expression of claudin-4 is stimulated by indomethacin and appears to be mediated through an increase in intracellular Ca\(^{2+}\) concentration (44). However, non-steroidal anti-inflammatory drugs, including indomethacin, have also been shown to inhibit active ERK1/2 levels (52). Exposure of primary cultured alveolar epithelial cells to EGF led to increases in claudin-4 and -7 and decreases in claudin-3 and -5 (11). Inhibition of protein kinase C by phorbol ester reduced the expression of claudin-2 and -5 in the choroid plexus (39). In T84 intestinal epithelial cells, ERK1/2 activation stimulated expression of claudin-2 (34). This finding suggests tissue-specific differences (renal vs. intestinal) in ERK1/2 regulation of claudin-2 expression.

Another potential mechanism for regulation of TJ permeability appears to be related to the half-life of individual claudin molecules within the TJ. Moreover, it has been proposed that the half-life of individual claudins may be modulated by sequence-specific posttranslational modifications (65). This report provides evidence that the cytoplasmic COOH-terminal domain of claudins determines the protein stability of claudin molecules. For example, in MDCK II cells claudin-2 has a half-life that is more than three times longer than that for claudin-4 (>12 h and ~4 h, respectively) (65). Replacement of the claudin-4 cytoplasmic tail with the claudin-2 cytoplasmic tail stabilized the claudin-4 chimera molecules and augmented the barrier function of claudin-4 molecules within the TJ (65). This study did not determine whether swapping the claudin-2 cytoplasmic tail with the claudin-4 cytoplasmic tail would decrease the half-life of the chimera claudin-2 molecules. Overall, this study established differences in the protein stability of individual claudin molecules and provided a novel potential mechanism by which TJ protein composition may be controlled.

CONCLUSIONS

Recent data from inherited human diseases and epithelial cell culture models have provided strong evidence that claudins are important determinants of renal epithelial TJ gate function. The diverse array of possible combinations of claudin isoforms within an individual epithelial cell TJ provides a rational explanation for the wide variety of TJ paracellular permeability properties of mammalian epithelial organs, including the kidney. There are also data suggesting that acute physiological regulation of TJs can be controlled, in part, by modulation of the composition of individual claudin isoforms within a particular TJ. Aberrant expression of individual claudin isoforms within the TJ of renal epithelial cells has been implicated in renal diseases, and it is very plausible that other pathological renal conditions will be explained by altered expression of claudins within the TJ. Future investigations into the role of claudins in determining renal epithelial cell TJ gate function will provide new information on the contribution of renal epithelial paracellular transport to renal physiology as well as to renal pathophysiological conditions.

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REFERENCES


