Distribution of the tight junction proteins ZO-1, occludin, and claudin-4, -8, and -12 in bladder epithelium


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Submitted 24 September 2003; accepted in final form 4 April 2004


In mammals, the bladder stores urine without permitting the passage of urine contents into the bloodstream, a function, in part, of the uroepithelial-associated tight junction complex. The protein constituents that make up this high-resistance barrier in the bladder are currently unknown, although the claudins, a multigene family, are thought to govern paracellular transport in other epithelia. Reverse transcriptase-polymerase chain reaction analysis was used to define that mRNA for claudin-2, -4, -8, -12, and -13 was expressed in mouse bladder tissue. No claudin-2 staining was associated with tight junctions of rat, mouse, and rabbit epithelium. Claudin-4, -8, and -12 were found in the umbrella cell tight junction; however, additional staining of claudin-4 was observed along the sites of cell-cell contact in the underlying cell layers of rat, mouse, and rabbit tissue. No claudin-2 staining was associated with tight junctions in the uroepithelium. Our results indicate that claudin-4, -8, and -12 are expressed in umbrella cells, where they may impart the high-resistance phenotype associated with this cell type, and that in some instances tight junction proteins are also associated at the sites of cell contact of the underlying cell layers, perhaps playing some role in cell-cell adhesion.

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ascending limb of Henle and the distal convoluted tubule (37). Hereditary defects in expression of functional claudin-16 lead to a loss of paracellular recovery of Mg²⁺ resulting in severe hypomagnesaemia (37). Different claudins can exist within the same junction, where they interact in both a homotypic and heterotypic manner, and permutations of claudins expressed in each cell or tissue may define the unique paracellular properties of each epithelium (14, 40). In this regard, it has been shown that overexpression of claudin-1, -4, -8, or -15 in Madin-Darby canine kidney (MDCK) cells increases TER (7, 8, 27, 42, 45). In contrast, expression of claudin-2 decreases TER in high-resistance variants of this cell line that do not normally express this claudin isoform (1, 11). Electrophysiological studies confirm that claudin-2, -4, and -8 act to regulate paracellular ion conductivity (1, 7, 8, 42). In claudin-4, this regulation is dependent on charged residues in the first extracellular loop (7). Similarly, substitutions of negative-charged amino acids with positive-charged ones in the first extracellular loop of claudin-15 convert the paracellular pathway from one selective for Na⁺ over Cl⁻ to one that is selective for Cl⁻ over Na⁺ (7).

At present, little is known about the specific tight junction proteins that form the high-resistance paracellular pathway that is associated with the bladder epithelium. ZO-1 and occludin have been localized to the umbrella cell layer (21, 22), and expression of claudin-4 has been described in the underlying intermediate and basal cell layers (29). However, it is unknown whether other claudins are expressed in the bladder or whether expression of tight junction proteins is limited to specific cell layers or regions of the bladder, or whether there is species variation in the expression and distribution of these proteins. We observed that mRNA for claudin-2, -4, -8, -12, and -13 was present in mouse bladder tissue. When immunolocalized, claudin-4 was expressed in the tight junctions of umbrella cells as well as along the sites of cell-cell contact in all three epithelial cell layers, and claudin-8 and -12 were localized to the tight junctions of the umbrella cell layer. Claudin-2 was not found associated with cellular junctions. ZO-1 and occludin were found in the tight junctions of the umbrella cells; however, in some instances they were also found at sites of cell-cell contact. Our results indicate that the high-resistance paracellular pathway observed in bladder epithelium may reflect the expression of claudin-4, -8, and -12. In addition, the localization of ZO-1, occludin, and claudin-4 to the sites of cell-cell contact provides evidence that tight junction proteins may also play some role in cell adhesion.

MATERIALS AND METHODS

Materials, antibodies, and other labeled reagents. Unless specified otherwise, all chemicals were obtained from Sigma (St. Louis, MO) and were of reagent grade or better. Rabbit antisera to claudin-2, -4, -8, or -12 were characterized previously (10, 11, 20, 32) and were graciously donated by Drs. M. Furuse and S. Tsukita (Department of Cell Biology, Kyoto University, Japan). Claudin-2-specific antiserum was used at a 1:250–1:500 dilution, whereas claudin-4, -8, and -12 antisera were used at dilutions of 1:500–1:5,000. Purified rabbit polyclonal anti-claudin-2 or claudin-11 antibody (Zymed Laboratories, San Francisco, CA) was used at a final concentration of 5–10 μg/ml; mouse monoclonal anti-claudin-4 antibody (Zymed Laboratories) was used at a final concentration of 5 μg/ml; rat anti-occludin hybridoma MOC37 supernatant was used at a 1:1 dilution (kindly provided by Drs. M. Furuse and S. Tsukita); rabbit anti-occludin polyclonal antibody (Zymed Laboratories) was used at a final concentra-
Table 1. Primers used for RT-PCR analysis

<table>
<thead>
<tr>
<th>Claudin Subtype</th>
<th>Sequence of Primers (5’→3’)</th>
<th>Expected Product Size, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AGGCTGCTGGGACATGACTGG</td>
<td>204</td>
</tr>
<tr>
<td>2</td>
<td>GTGGCTGTTAGGTGGAGAT</td>
<td>178</td>
</tr>
<tr>
<td>3</td>
<td>GCAACCCCAAGATCCTCTTA</td>
<td>247</td>
</tr>
<tr>
<td>4</td>
<td>ATGCCTCTATGGAAGACTACA</td>
<td>633</td>
</tr>
<tr>
<td>5</td>
<td>CCTCTCTGGACAAACAGATC</td>
<td>203</td>
</tr>
<tr>
<td>6</td>
<td>GTCTCGTGTTCTAGCAGCA</td>
<td>660</td>
</tr>
<tr>
<td>7</td>
<td>TTTCAATGGGAGCTTG</td>
<td>158</td>
</tr>
<tr>
<td>8</td>
<td>GCCAAGATCGCTTGGAA</td>
<td>208</td>
</tr>
<tr>
<td>9</td>
<td>AAGAGGAGACTGGGCTTG</td>
<td>148</td>
</tr>
<tr>
<td>10</td>
<td>GTCCTGATTACGCGCACTC</td>
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</tr>
<tr>
<td>11</td>
<td>CTTGCCGCTCTGATTG</td>
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</tr>
<tr>
<td>11-alt</td>
<td>AGGCTAGGAAAGGCTGCGG</td>
<td>474</td>
</tr>
<tr>
<td>12</td>
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<td>148</td>
</tr>
<tr>
<td>13</td>
<td>TGTGATTTCAAGGCAAGGAG</td>
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</tr>
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<td>14</td>
<td>GCCAGCAATGCTGTTTAAGA</td>
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<td>15</td>
<td>ACGGTGAGTGTGAGTGAAGG</td>
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<td>17</td>
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</tr>
<tr>
<td>18</td>
<td>AGATGGGAGGCTGAGTGAAGAAG</td>
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</table>

Primers were designed using Primer3 software (www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). The top sequence is the left primer and the bottom sequence is the right primer. Two different primer pairs were used for claudin-11.

whole mount tissue and cryosections, paraformaldehyde was quenched and the cells were permeabilized with PBS containing 20 mM glycine, pH 8.0, 75 mM ammonium chloride, and 0.1% (vol/vol) Triton X-100 for 10–15 min at room temperature. The cells were washed with block solution [PBS containing 0.7% (wt/vol) fish skin gelatin and 0.025% (wt/vol) saponin] and then incubated in block solution containing 10% (vol/vol) newborn calf serum for 15 min at ambient temperature. Tissue was subsequently washed in block solution three times over 15 min and then incubated for 60–120 min at ambient temperature with primary antibody diluted in block solution. After incubation with the primary antibody, the tissue was washed three times with block solution over a 15-min period of time. The tissue was then incubated with the secondary antibody for 60–120 min at ambient temperature and then washed three times with block solution over a 15-min time period. After three washes with PBS, the tissue was postfixed for 10–15 min in 4% (wt/vol) parafomaldehyde in 100 mM sodium cacodylate, pH 7.4. After a final wash with PBS, coverslips were placed over the stained cryosections using p-diaminobenzidine-containing mounting medium (3). In the case of whole mounts, it was necessary to place plastic spacers (50 µm high) underneath the coverslip to raise it above the tissue.

**RESULTS**

RT-PCR reveals expression of claudin-2, -4, -8, -12, and -13 in mouse bladders. Our initial goal was to define which claudins were expressed in the mammalian bladder. Mice were chosen for this analysis because many of the claudin isoforms have been identified and cloned in this species, thus facilitating primer design for the PCR analysis (see Table 1). Unambiguous mouse sequences or no sequences were available for claudin-17 and -20–24. PCR products of the expected size were obtained for claudin-2, -4, -8, -12, and -13 (Fig. 1A). The product for claudin-2 was not abundant. The PCR fragments...
were purified, and DNA sequencing confirmed that the products corresponded to the known DNA sequence of the appropriate claudin isoform.

Positive control reactions for claudin-1-16 were also performed (Fig. 1B). Products of the expected sizes were observed for claudin-1-8, -10, and -12-16, confirming that primers for these particular isoforms were able to amplify claudin isoform-specific products from tissues known to express these proteins. However, we were unable to obtain positive controls for claudin-9, -11, -18, or -19. Claudin-9 and -18 were recently identified as messengers for claudin-11, a protein known to be expressed in the nephron of the kidney (20); however, all attempts were unsuccessful. No expression was observed when attempts were made to determine whether there were any interspecies differences in expression of these proteins. Furthermore, rabbit bladders were subdivided into three regions, i.e., neck, equatorial, and dome, to determine whether there were any regional differences in expression of these proteins. The neck region was defined as the third of the bladder, just above the urethra, that included the sites of ureter insertion. The equatorial region included the middle third of the bladder and the dome region of the last third of the organ. Finally, to define the regions of cell-cell contact in the different cell layers and to identify the apicolateral junction in the umbrella cell layer, the tissue was stained with rhodamine-phalloidin. This phalloidin labels the filamentous actin-rich cortical cytoskeleton of the cells and pronounces labels the actin-rich junctional ring at the apicolateral border of the cells. ToPro-3 was used to label cell nuclei.

A three-dimensional reconstruction of the umbrella cell layer from the equatorial region of whole mounted rabbit bladder tissue is shown in Fig. 2A. ZO-1 was localized at the periphery of the large polyhedral umbrella cells where it colocalized with actin at the apicolateral junction of the cells. Colocalization was apparent when the tissue was viewed in tilted reconstructions (Fig. 2A, right) or in x-z cross section (arrows in Fig. 2B). Similar results were observed for the other two regions of the rabbit bladder and for mouse and rat bladders. In preliminary experiments, we observed that some of the antibodies used in our analysis penetrated poorly into the underlying cell layers of whole mounted tissue. To obviate this problem, we also examined the localization of these proteins in tissue cryosections. The three cell layers of the cryosectioned rabbit uroepithelium are annotated in Fig. 2, C-E, middle: umbrella cells (UC) are marked by long thin arrows, intermediate cells by filled circles, and basal cells by filled triangles. In the neck and equatorial regions of the rabbit bladder, ZO-1 was localized to the tight junctions of the umbrella cell layer (Fig. 2, C and D). ZO-1 staining was also observed in the capillary endothelial cells that underlie the basal cell layer (marked with * in Fig. 2, C and D). In the dome region of rabbit bladder, ZO-1 was localized to both the apicolateral tight junctions (marked with arrows in Fig. 2E, right and left) as well as to the bladder tissue was stained with a commercially available claudin-11-specific antibody (data not shown). There are no published reports of claudin-19 tissue distribution.

Distribution of ZO-1 and occludin in rabbit, mouse, and rat uroepithelium. Before characterizing the specific claudin proteins expressed by the bladder epithelium, we first examined the expression and distribution of well-characterized tight junction proteins, ZO-1 and occludin, in rabbit, mouse, and rat bladder tissue. Bladders from multiple species were examined to define whether there were any interspecies differences in expression of these proteins. Furthermore, rabbit bladders were subdivided into three regions, i.e., neck, equatorial, and dome, to determine whether there were any regional differences in expression of these proteins. The neck region was defined as the third of the bladder, just above the urethra, that included the sites of ureter insertion. The equatorial region included the middle third of the bladder and the dome region of the last third of the organ. Finally, to define the regions of cell-cell contact in the different cell layers and to identify the apicolateral junction in the umbrella cell layer, the tissue was stained with rhodamine-phalloidin. This phalloidin labels the filamentous actin-rich cortical cytoskeleton of the cells and pronounces labels the actin-rich junctional ring at the apicolateral border of the cells. ToPro-3 was used to label cell nuclei.

Fig. 2. Distribution of zonula occludens-1 (ZO-1) in rabbit bladder epithelium. A and B: whole mounted epithelium from the equatorial region of rabbit bladder was labeled with antibodies to the tight junction protein ZO-1 (left) and with rhodamine-phalloidin to label the actin cytoskeleton (red; middle) and TO-PRO3 to label the nuclei (blue; middle). Right: merged image. A: images are a 3-dimensional reconstruction of a z-series collected with a confocal microscope. The images were tilted 65° about the x-axis to allow for better appreciation of the 3-dimensional organization of the umbrella cell (UC) layer. The grid is a 3-dimensional scale marker, with each side of the square equivalent to ~18.5 μm. B: x-z confocal section of whole mounted bladder epithelium. The arrows mark the location of tight junctions. C-E: cryosections of bladder tissue from rabbit neck region (C), rabbit equatorial region (D), or rabbit dome region (E) were labeled with anti-ZO-1 antibodies (left) or rhodamine-phalloidin and TO-PRO3 (middle). Right: merged images. Images were collected as a z-series with a confocal microscope and then summed and displayed as a single composite projection. Right and left: arrows show location of tight junctions. Middle: UCs are labeled with arrows, intermediate cells with filled circles, and basal cells with filled triangles. *Mark small blood vessels underlying the uroepithelium. Bar = 25 μm.
sites of cell-cell contact in the intermediate and basal cell layers (Fig. 2E).

Compared with rabbit bladder, the mouse epithelium was relatively thin and comprised only a few cell layers (which like Fig. 2 are annotated in Fig. 3A). In this tissue, ZO-1 was localized to punctate structures at the apicolateral borders of adjacent umbrella cells (see arrows in Fig. 3A, right and left), consistent with localization to tight junction. However, significant ZO-1 staining was also found associated with the underlying cell layers in what appeared to be a cytoplasmic distribution. The rat uroepithelium was also relatively thin, and ZO-1 was observed in the superficial umbrella cell layer at the
apicolateral junction (see arrows in Fig. 3B, right and left) and to a lesser extent along the basolateral surface of this cell layer (Fig. 3B).

In most simple epithelia, occludin is concentrated in the tight junctional ring at the apex of the cell. This expected distribution was observed in all three layers of rabbit bladder epithelium (Fig. 4, A–C). Unlike ZO-1, significant occludin was not observed in the capillary bed. In rat and mouse bladder, occludin was also found in an apicolateral distribution (marked with arrows in Fig. 4, D and E). However, significant occludin was also expressed along the basolateral surface of the umbrella cell layer and on the plasma membranes of the underlying cell layers (Fig. 4, D and E).

Localization of claudin-4, -8, and -12 to bladder epithelium. Because whole mouse bladders were used in the RT-PCR analysis, immunofluorescence was used to determine which claudins were expressed in the uroepithelium. The distribution of claudin-2, -4, -8, or -12 was examined in mouse and rat tissue using purified rabbit polyclonal antibodies or rabbit polyclonal antibodies found in serum, whereas claudin-4 expression was examined in rabbit tissue using a commercially available mouse monoclonal antibody. No antibodies are currently available for assessing the distribution of claudin-13.

When mouse or rat bladder tissue was stained with claudin-2-specific antiserum, expression was limited to the uroepithelium. The distribution of claudin-2, -4, -8, or -12 was examined in mouse and rat tissue using purified rabbit polyclonal antibodies or rabbit polyclonal antibodies found in serum, whereas claudin-4 expression was examined in rabbit tissue using a commercially available mouse monoclonal antibody. No antibodies are currently available for assessing the distribution of claudin-13.

When mouse or rat bladder tissue was stained with claudin-2-specific antiserum, expression was limited to the uroepithelium. However, the staining pattern was not as expected for a tight junction-associated protein. In both mouse and rat bladder, claudin-2 was found under the apical membrane of the surface umbrella cells, as well as to a lesser extent in what appeared to be the cytoplasm of the underlying intermediate and basal cell layers (our unpublished observations). No accumulation of claudin-2 was observed in the expected location of the tight junctions. Staining was not significantly diminished when GST-claudin-2 protein was used to immunodeplete serum of claudin-2-specific antibodies, indicating that the staining was likely to be nonspecific. Use of other tissue preparation methods, such as freezing tissue, followed by fixation of cryosections with methanol, also resulted in no specific staining (our unpublished observations). In addition, no staining was observed when a commercially available purified polyclonal antibody to claudin-2 was used, and expression of claudin-2 protein could not be detected when rat or mouse bladder lysates were screened by Western blot analysis (our unpublished observations).

Claudin-4 was previously localized to the intermediate and basal cell layers of mouse uroepithelium, but not the umbrella cell layer (29). Consistent with this previous observation, we found that claudin-4 was distributed at regions of cell-cell contact in the intermediate and basal cell layers of all three regions of rabbit bladder uroepithelium (Fig. 5, A–C) and of the mouse (Fig. 6A) and rat (Fig. 6B) uroepithelium. Little staining was observed when GST-claudin-4 protein (but not GST alone) was used to immunodeplete serum of claudin-4-specific antibodies. In contrast to previous reports, we also observed claudin-4 association with the lateral surface of adjacent umbrella cells. This was readily apparent when the distribution of ZO-1 and claudin-4 was examined in the umbrella cell layer of whole mounted rabbit (Fig. 5, D–F) or mouse tissue (Fig. 6C).
Fig. 4. Distribution of occludin in bladder epithelium. Cryosections of bladder tissue from rabbit neck region (A), rabbit equatorial region (B), rabbit dome region (C), mouse (D), or rat (E) were labeled with antibodies to label occludin (left) or rhodamine-phalloidin and TO-PRO3 (middle). Right: merged images. E: mucosal fold. Images were collected as a z-series with a confocal microscope and then summed and displayed as a single composite projection. Arrows show location of tight junctions. Bar = 25 μm.
Fig. 5. Distribution of claudin-4 in rabbit bladder epithelium. A-C: cryosections of bladder tissue from the rabbit neck region (A), rabbit equatorial region (B), or rabbit dome region (C) were labeled with antibodies to claudin-4 (left) or rhodamine-phalloidin and TO-PRO3 (middle). Right: merged images. Images were collected as a z-series with a confocal microscope and then summed and displayed as a single composite projection. Left and right: arrows show location of tight junctions. Bar = 25 μm. D-I: whole mounted epithelium from the neck region (D and G), equatorial region (E and H), or dome region (F and I) was labeled with antibodies to ZO-1 (red) and claudin-4 (green). A merged image is shown in each of the panels. D-I: images are 3-dimensional reconstructions of z-series collected with a confocal microscope. The grid is a 3-dimensional scale marker, with each side of the square equivalent to ~16.8 μm (D), ~18.3 μm (E), or ~17.9 μm (F). G-I: x-z confocal sections of whole mounted bladder epithelium. The arrows mark the location of tight junctions.
or when the distribution of actin and claudin-4 was examined in whole mounted rat tissue (Fig. 6D).

Furthermore, we observed that claudin-4, in a region- and species-specific manner, was concentrated at the apicolateral junction of the umbrella cell layer, consistent with localization to tight junctions. In the dome region of the rabbit bladder, there was significant localization of claudin-4 to the tight junction (examples of these regions are marked with arrows in

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**Fig. 6.** Distribution of claudin-4 in rat and mouse bladder epithelium. A and B: cryosections of mouse (A) or rat (B) bladder epithelium were labeled with antibodies to claudin-4 (left) or rhodamine-phalloidin and TO-PRO3 (middle). Right: merged images. Images were collected as a z-series with a confocal microscope and then summed and displayed as a single composite projection. Left and right: arrows show location of tight junctions. Bar = 25 μm. C-F: whole mounted epithelium from the mouse (C and E) was labeled with antibodies to ZO-1 (red) and claudin-4 (green), whereas rat tissue (D and F) was labeled with antibodies to claudin-4 (green) and rhodamine-phalloidin (red). A merged image is shown in each of the panels. C and D: images are 3-dimensional reconstructions of z-series collected with a confocal microscope. The images were tilted 65° about the x-axis. The grid is a 3-dimensional scale marker, with each side of the square equivalent to ~18.8 μm (C) or ~12.5 μm (D). E and F: x-z confocal sections of whole mounted bladder epithelium. The arrows mark the location of tight junctions.
Fig. 7. Localization of claudin-8 in bladder epithelium. A and B: whole mounted epithelium from rat bladder was labeled with antibodies to claudin-8 (green) and rhodamine-phalloidin (red). A merged image is shown. A: image is a 3-dimensional reconstruction of a z-series collected with a confocal microscope. The image was tilted 65° about the x-axis. The grid is a 3-dimensional scale marker, with each side of the square equivalent to ~12.3 μm. B: x-z confocal section of the tissue. The arrows mark the location of tight junctions. C and D: cryosections of bladder tissue from mouse (C) or rat (D) were labeled with antibodies to label claudin-8 (left) or rhodamine-phalloidin and TO-PRO3 (middle). Right: merged images. Images were collected as a z-series with a confocal microscope and then summed and displayed as a single composite projection. Arrows show location of tight junctions. Bar = 25 μm.
Fig. 8. Distribution of claudin-12 in bladder epithelium. A-D: whole mounted epithelium from the mouse (A and C) was labeled with antibodies to ZO-1 (red) and claudin-12 (green), whereas rat tissue (B and D) was labeled with antibodies to claudin-12 (green) and rhodamine-phalloidin (red). A merged image is shown in each of the panels. A and B: images are 3-dimensional reconstructions of z-series collected with a confocal microscope. The images were tilted 65° about the x-axis. The grid is a 3-dimensional scale marker, with each side of the square equivalent to ~13.8 μm (A) or ~12.5 μm (D). C and D: x-z confocal sections of whole mounted bladder epithelium. The arrows mark the location of tight junctions. E-H: cryosections of bladder tissue from mouse (E) or rat (F) were labeled with antibodies to label claudin-12 (left) or rhodamine-phalloidin and TO-PRO3 (middle). Right: merged images. Left and right panels: arrows show location of tight junctions. G and H: cryosections were labeled with TO-PRO3 and antisera preincubated with glutathione-S-transferase (GST; G) or GST-claudin-12 (H). Staining for claudin-12 is in green and staining for TO-PRO3 is in red. G: short arrows indicate localization of claudin-12 to tight junctions. When antiserum was preabsorbed with GST-claudin-12, no staining at tight junctions was observed. E-H: all images were collected as a z-series with a confocal microscope and then summed and displayed as a single composite projection. Bar = 25 μm.
with the high-resistance, low-permeability barrier associated with this cell type (23). Claudin-4 is found in other tight epithelial cells such as those that line the collecting duct of the kidney (20). Overexpression of claudin-4 in MDCK cells increases TER by approximately two- to threefold and reduces transmonolayer conductance by decreasing paracellular Na⁺ permeability without a significant effect on Cl⁻ permeability (42). In addition, when high-resistance type 1 MDCK cells are incubated with a claudin-4-binding peptide, claudin-4 is removed from TJ strands, resulting in a significant increase in TJ permeability (39). Similar to claudin-4, claudin-8 is expressed in “tight” segments of the nephron including the distal tubule, in the collecting duct, and in the thin descending limb of Henle (20, 24a). When claudin-8 is expressed in MDCK cells, it is localized to tight junction strands and reduces paracellular permeability to monovalent inorganic and organic cations and to divalent cations but not to anions or neutral solutes (45). Interestingly, claudin-8 expression is associated with a decrease in expression of endogenous claudin-2, which is thought to form a low-resistance cation-selective pore (45). Finally, claudin-12 was recently localized to the blood-brain barrier, which is composed of high-resistance endothelial cells (32). Because of a lack of reagents, little is presently known about the function or distribution of claudin-13.

Our ability to detect message for claudin-2 by PCR, but our inability to detect claudin-2 by Western blot analysis or to immunolocalize the protein to tight junctions, indicates that there might be low levels of claudin-2 mRNA translation into protein, rapid protein turnover, or low amounts of claudin-2 protein in bladder tissue. The apparent lack of expression of claudin-2 in the bladder epithelium is not surprising, as this claudin is associated with “leaky” epithelia such as those found in the proximal tubule of the kidney where the glomerular filtrate is almost fully reabsorbed (20, 45). Furthermore, when expressed in high-resistance MDCK cell lines, claudin-2 forms a cation-selective pore that increases paracellular permeability (1). However, it is worthwhile noting that claudin-2 overexpression results in some increase in TER in low-resistance strains of MDCK cells (7).

An additional finding of our analysis is that many of the “tight junction” proteins we studied were localized not only in tight junctions but in a species- and region-dependent manner were also found along the sites of cell-cell contact in the umbrella, intermediate, and basal cell layers. One example is claudin-4, which was previously localized to the periphery of the basal and intermediate cell layers (29). Our analysis indicates that claudin-4 is also found along the basolateral margins of the umbrella cell layer. Localization of claudins to sites of cell-cell contact has been noted previously in other tissues and cell lines (21). Claudin-1 is localized along the entire basolateral surface of the epithelium lining the epididymis (16), and this claudin also circumscribes the plasma membrane of all of the cellular layers of the epidermis (12). Similarly, claudin-4 is found along the basolateral surfaces of enterocytes in the small and large intestine (33), in the second/third layers of the stratum granulosum of the epidermis (12), and in cultured MDCK cells (7). Finally, claudin-7 is localized to the basolateral surface of the aldosterone-sensitive distal nephron and in the thin descending limb of Henle (24a).

Localization at sites of cell-cell contact was also noted for ZO-1 in mouse bladders and the dome region of rabbit bladders
and for occludin in rat and mouse bladders. The localization of occludin to the lateral margins of the cells was surprising as in most epithelial tissues this protein localizes exclusively to the tight junctions (12, 13); however, occludin staining was recently observed in the suprabasal cell layers of some stratified epithelia (21). Why association of ZO-1 and occludin at the sites of cell-cell contact showed species and regional differences is unknown, but it may reflect species and regional differences in factors such as rates of bladder filling, hydrostatic pressure, bladder tone, or innervation.

One potential function for tight junction protein association at sites of cell-cell contact is to promote cell adhesion. Such interactions would be important in tissues like the skin, gut, and bladder that are constantly exposed to mechanical forces such as compression, distention, and hydrostatic pressure. Consistent with this hypothesis is the observation that ZO-1 is associated with adherens junction proteins during the initial steps of epithelial cell polarization (34). Furthermore, VAB-9, a tetraspanning claudin-like protein in Caenorhabditis elegans, associates with HMR1 (C. elegans cadherin), localizes to adherens junctions, may modulate the association of the actin cytoskeleton with the adherens junction, and may also regulate some aspects of cell adhesion (38). At present, it is unknown if claudins interact directly with cadherin complexes in mammalian cells.

Our results better define the proteins that may comprise the high-resistance paracellular barrier associated with the uroepithelium. In addition to their established roles as tight junction proteins, occludin, claudin-4, and ZO-1 may have additional functions in bladder epithelium, including a possible role in cell-cell adhesion. This information not only serves as a launching point for study of the uroepithelial barrier under normal conditions, but it may also further our understanding of disease processes such as bacterial cystitis, interstitial cystitis, and spinal cord injury. Each of these conditions is characterized by the disruption of the epithelial barrier of the bladder (4, 22, 28, 29).

ACKNOWLEDGMENTS

We thank Drs. M. Furuse and S. Tsukita for graciously providing us with claudin-specific antisera and cDNAs encoding GST-claudin fusion proteins.

GRANTS

This work was supported by National Institutes of Health (NIH) grants to G. Apodaca (RO1-DK-054425 and RO1-DK-051970) and to L. Birder (RO1-DK-54824 and RO1-DK-57284), a training award from NIH to E. Wang (T32-DK-01296), a predoctoral fellowship from the American Heart Association to R. Rojas (0315353U), and an equipment grant from Dialysis Clinics.

REFERENCES


TIGHT JUNCTION PROTEINS IN BLADDER


