Transmembrane Proteins in the Tight Junction Barrier

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Abstract. Three types of transmembrane proteins have been identified within the tight junction, but it remains to be determined how they provide the molecular basis for regulating the paracellular permeability for water, solutes, and immune cells. Several of these proteins localize specifically within the continuous cell-to-cell contacts of the tight junction. One of these, occludin, is a cell adhesion molecule that has been demonstrated to influence ion and solute permeability. The claudins are a family of four-membrane spanning proteins; unexpectedly, other members of this family have already been characterized without recognizing their relationship to tight junctions. Junction adhesion molecule, the most recently identified tight junction component, is a member of the Ig superfamily and influences the paracellular transmigration of immune cells. A plaque of cytoplasmic proteins under the junction may be responsible for scaffolding the transmembrane proteins, creating a link to the perijunctional actin cytoskeleton and producing regulatory signals that control the paracellular barrier.

Tight junctions form continuous circumferential intercellular contacts between epithelial cells and create a regulated barrier to the paracellular movement of water, solutes, and immune cells (1). They also provide a second type of barrier that contributes to cell polarity by limiting exchange of membrane lipids between the apical and basolateral membrane domains. Numerous proteins within the junction have been identified (Figure 1); however, our understanding of their functions remains limited. In this review, we will focus on insights gained by the recent identification of three types of transmembrane proteins located at the junction. We consider how they might provide a molecular explanation for some of the barrier’s physiologic properties, and how the cortical cytoskeletal network may organize and regulate these proteins. The reader is referred elsewhere for detailed reviews on the molecular architecture of the tight junction (2–5), its physiologic properties (6–10), and the very important role of the actin cytoskeleton in regulating structure and function (11,12). Several recent reviews have focused on the implications of this work for normal renal physiology and changes in acute tubular ischemia (13–15). To understand the potential role of the transmembrane proteins, we begin with a brief review of the major physiologic properties of the tight junction.

Physiologic Characteristics of the Paracellular Pathway

The net transepithelial transport across an epithelium is the sum of two distinct components: transcellular and paracellular transport. Transcellular transport results from the regulated movement of solutes and water across the apical and basolateral membranes. A specific profile of functionally distinct transporters and channels is distributed in a cell type-specific manner on the apical and basolateral membranes and accounts for the unique transport characteristics of each epithelium and organ. Examples of specialization include the ability of the renal collecting duct to resorb water in response to ADH and the ability of the hepatocyte to transport bile acids. Transport rates are subject to a high degree of regulation and can be turned off or on by second-messenger signaling pathways, transmembrane voltage, pH, and many other factors. Transcellular transport is active, dependent either on hydrolysis of ATP or on the electro-osmotic gradient generated by basolaterally positioned Na\(^+\)/K\(^+\)-ATPase. In summary, transcellular transport has a very high degree of molecular specificity, is tightly regulated, and is variable among different epithelia.

The paracellular component of transport is distinct from transcellular transport in several ways. It is a completely passive process, resulting from paracellular dissipation of the electro-osmotic gradients established by transcellular transport. It also lacks the vast functional diversity and molecular discrimination of the transcellular component. Paracellular transport has two basic characteristics: (1) permeability – the magnitude of the barrier; and (2) permselectivity – the ability to discriminate molecular size and ionic charge. In practice, most investigators quantify permeability by two complementary techniques. The first is by measuring transepithelial electrical resistance (TER). This is a measure of the barrier to small ions (predominately Na\(^+\) and Cl\(^-\)) in an experimentally applied electrical field in the bathing media. The second is by measuring the flux of tracer solutes, such as radiolabeled inulin or mannitol, which only traverse the epithelia through the intercellular space.

Values for the paracellular element of electrical resistance in natural epithelia vary enormously and with important functional consequences. For example, “leaky” mammalian proximal renal tubules (6 to 7 \(\Omega \times \text{cm}^2\)) permit rapid paracellular...
reabsorption of most of the glomerular filtrate. Collecting ducts maintain electro-osmotic gradients used for transport and have paracellular barriers of approximately 300 $\Omega \times \text{cm}^2$. The very “tight” epithelium of the bladder (6,000 to 300,000 $\Omega \times \text{cm}^2$) can preserve the composition of either highly dilute or concentrated urine (reviewed in reference (6)). In contrast to the wide variation in permeability, the size cutoff for permselectivity is in a rather narrow range, 5 to 18 Å, for most natural epithelia and cultured epithelial monolayers (6,16). One interpretation of the variable resistance and fixed size is that the barrier includes a variable number of aqueous channels of predetermined size. Charge selectively of the paracellular pathway also varies little among different epithelia and in most cases shows a slight cation selectivity. For example, the relative ion permeabilities for the dog proximal renal tubule for ($P_{K^+} : P_{Na^+} : P_{Cl^-}$) are (1.10 : 1.00 : 0.72), and for the rabbit ileum are (1.14 : 1.00 : 0.55) (6). This small cation selectivity suggests that the paracellular pathway (the tight junction or lateral interspace) is predominantly lined by negative charges. The very limited ability to discriminate charge is also consistent with the presence of large aqueous channels within the tight junction barrier. This lack of paracellular discrimination can again be contrasted with the character of transcellular transport, for example, the exquisite molecular selectively of $K^+$ channels, amino acid transporters, or anion exchangers.

**Experimental Problems in Measuring the Tight Junction Barrier**

Caution is required when using either TER or flux to characterize the tight junction or changes in its sealing properties in response to experimental manipulations. Both methods are influenced by many factors other than those attributable specifically to the molecular components of the tight junction (10). Electrical resistance methods model an epithelial monolayer as a circuit of parallel resistors composed of all the individual transcellular and paracellular elements. Because the total resistance is a function of the inverse sums of individual resistances, ($1/R_{\text{total}} = 1/R_1 + 1/R_2 ...$), TER is dominated by elements with the lowest resistance. Since membrane resistance is generally much higher than intercellular resistance, it is usually safe to assume that a change in resistance after an experimental manipulation represents a change in the paracellular pathway (16). Unfortunately, this method is excessively sensitive to small regions with low resistance, such as crushed cells near the edge of a mounting apparatus or a patch of dead cells within the monolayer. Another problem is that the lateral interspace also contributes to the paracellular resistance, directly by its length and inversely by its width. Both of these dimensions can be affected by seemingly trivial factors, such as changing culture media or the time a cultured epithelial monolayer has been in culture (1).

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**Figure 1.** Model of the molecular interactions of proteins within the tight junction. Transmembrane proteins occludin, claudin(s), and junctional adhesion molecule (JAM) function to define barrier properties of the paracellular space. While presently unproven, they are shown as interacting in a homophilic manner. ZO-1, -2, and -3 are homologous, and all bind the cytoplasmic tail of occludin. ZO-1 is known to bind actin, AF-6, ZO-associated kinase (ZAK) fodrin, and $\alpha$-catenin, although the last three proteins have yet to be localized to the junction by immunoelectron microscopy. Other proteins that have been localized to junctions but which presently lack functions and known molecular interactions include symplekin, cingulin, and 7H6. Several signaling proteins are found within the apical junction complex but are not unique to the tight junction.
Permeability, measured by tracer flux, is linearly proportional to the area across which diffusion occurs. Thus, it is theoretically less sensitive to trivial defects in the monolayer and more reliably reports changes induced in the junction by experimental manipulations. Flux has the added advantage that small changes in the tight junction barrier can be distinguished from defects in the monolayer by the ability of the monolayer to maintain an upper size limit for diffusion. As future experiments attempt to define the specific contribution of transmembrane proteins to the tight junction barrier, the limitations of presently available methods should be taken into account. Consideration should be given to the less commonly used methods of alternating current impedance (17) and microelectrode surface scanning conductance (18) or, ideally, a combination of several complementary methods.

Structure–Function Correlation

Claude and Goodenough (19) were the first to point out a correlation between the ultrastructure of tight junctions and their permeability as defined experimentally in natural epithelia. In transmission electron micrographic images, the tight junction appears as several points of intercellular membrane contact at the apical end of the lateral interspace. In freeze fracture images, these contacts correspond to fibril-like rows of intramembrane particles that form a continuous branching network around the cell (Figure 2). Evidence that the fibrils are the sites of resistance comes from numerous observations in which disruption of the fibrils correlates with loss of the paracellular barrier. This is seen following both experimental manipulations of cultured epithelial monolayers and in disease states in natural epithelia (2,20). If each row of intramembrane proteins creates a fixed resistive barrier positioned along the intercellular space, then the overall electrical resistance is predicted to be linearly proportional to the number of barriers. In contrast, Claude noted that fibril number was actually proportional to the logarithm of the paracellular resistance when comparing epithelia throughout the body (21). She proposed that the empirical data were better explained by a model that assumed the fibrils were not fixed barriers but contained individual channels that flicker open and closed (see reference (21) for mathematical justification). A solute molecule traversing the junction would need to move in a stepwise manner along a series of intercellular spaces enclosed by fibrils. Passage between each fibril-enclosed space would require an open channel (Figure 2). The molecular basis for this behavior remains to be determined. In addition, there are exceptions to this simple structure–function correlation, suggesting that other factors also contribute to defining the magnitude of the barrier.

Transmembrane Components of the Tight Junction

The discussion above supports the existence of both sealing and channel elements within the tight junction, both of which may be regulated. Many tight junction proteins have now been identified (Figure 1), and most of these are located in the cytoplasmic plaque under cell–cell contacts. Some of these are unique to the tight junction, whereas others are also located elsewhere in the cell. Several categories of transmembrane proteins have been identified that appear to be unique to tight junctions. Because they extend into the paracellular space, they are candidates for creating the seal and channels. Most experimental evidence addresses the role of occludin, the first of these proteins to be identified.

Occludin

Occludin is an approximately 65-kD type II transmembrane protein composed of four transmembrane domains, two extracellular loops, and a large C-terminal cytosolic domain (22) (Figure 3). This topology has been confirmed by antibody accessibility studies (23). The extracellular loops are chemically quite distinctive, particularly the first, which contains approximately 65% tyrosine and glycine residues. Although the presence of alternating tyrosine and glycine residues is conserved in all five animal species presently cloned, the functional significance of this peculiar sequence is unclear (24).

When observed by immuno-freeze fracture electron microscopy, occludin is concentrated directly within the tight junction fibrils (24). Interestingly, immunofluorescence localization reveals an additional minor pool of occludin along the lateral membrane that is more easily extracted in nonionic detergents, less phosphorylated, and not assembled into fibrils (25,26). Conceivably, the lateral pool represents a reservoir of subunits available for dynamic regulated expansion of junctional com-
plexity. The capacity for rapid increases in fibril number was demonstrated in experiments predating the discovery of occludin. These include the observation that when trypsin is applied to the basolateral surface of cultured Madin-Darby canine kidney (MDCK) cells there follows a dramatic and rapid increase in fibril number and increase in transmonolayer electrical resistance (27). The role of occludin phosphorylation and functional significance of the extrajunctional occludin in fibril dynamics deserves investigation. Although the junctional pool appears to be more phosphorylated, there is no proven causal relationship with assembly, at this time.

Occludin is a $\text{Ca}^{2+}$-independent intercellular adhesion molecule (23). When expressed in fibroblasts, which lack endogenous occludin, it confers adhesiveness in proportion to the level of occludin expressed and the adhesiveness is blocked by peptides corresponding to either of the two extracellular loops. It remains to be determined whether occludin is a homotypic adhesion molecule or has a yet unidentified counter-receptor. Occludin is also capable of lateral oligomerization through side-to-side associations, perhaps within the membrane bilayer (28).

Given its adhesive properties, occludin might create a paracellular barrier by polymerizing laterally in the membrane to create a continuous line of adhesion between cells. Several experiments are at least partly consistent with this model. For example, when occludin levels are increased in cultured monolayers of MDCK cells through transfection, this results in an increase in the number of fibrils and an increase in transmonolayer electrical resistance (29,30). Conversely, a decrease in transmonolayer electrical resistance and an increase in permeability to tracer molecules can be induced by adding a synthetic peptide corresponding to the second extracellular loop of occludin to monolayers of *Xenopus* A6 cells (31). The soluble peptide is presumably interfering with occludin’s extracellular contacts. This effect requires several days, and loss of the barrier coincides with loss of occludin as detected by both immunofluorescence staining and immunoblotting. Not surprising, forced expression of mutated occludin can produce a dominant-negative effect on barrier properties. For example, expression in *Xenopus* embryos of truncated occludin constructs lacking the large C-terminal cytoplasmic domain results in increased permeability of tight junctions to a biotin tracer molecule (28). However, expression of similar constructs in MDCK cells resulted in increases in TER similar to the increase seen with expression of full-length occludin (29,30).

Although overexpression of C-terminal truncated occludin in MDCK monolayers increases the electrical barrier, it also disrupts the intramembrane barrier and allows diffusion of lipids between the apical and basolateral membrane domains (29). Dissociation of the intercellular (gate) and intramembrane (fence) barriers was previously observed to occur in MDCK monolayers in response to acute ATP depletion (32). However, in the latter situation the membrane polarity barrier remains after the paracellular barrier is lost. Hypothetically, the two barriers could be based on lateral and cell–cell adhesive contacts, respectively, and these are differentially affected in the transfection and ATP-depletion experiments.

A major surprise was the finding that while overexpression of occludin in cultured MDCK cells increases the electrical barrier, it simultaneously decreases the barrier to noncharged solutes like mannitol and dextrans (29,30). This suggests that the barriers for charged and noncharged species are somehow different. This seemingly paradoxical effect is even more pronounced when an occludin construct lacking the C-terminal cytoplasmic domain is expressed in cultured monolayers (29). Whatever the molecular explanation, occludin appears to be more than a barrier-forming glue, and may instead act more like the regulated channel protein predicted by Claude (21). A greater understanding of occludin function will require detailed structure–function analysis of mutant forms of occludin expressed in cultured cells or transgenic animals.

Since its identification, multiple lines of evidence suggested...
that occludin was not the only integral membrane protein in the tight junction. Overexpression of an occludin construct that lacks the C-terminal cytosolic domain results in a dramatically discontinuous pattern of both the endogenous and exogenous occludin polypeptides, visualized at the light microscopic level (29). Despite this, there is no parallel effect on the number and organization of tight junction fibrils visualized by freeze fracture methods at the ultrastructural level. Furthermore, although the addition of a synthetic peptide corresponding to the second extracellular loop to cultured A6 cells results in the disappearance of occludin from the plasma membrane, it does not appear to alter gross cell morphology or affect the distribution of other proteins of the apical junction complex, such as ZO-1 (31). The most direct implication for the existence of a second barrier protein came with the observation that embryonic stem cells, from which occludin was deleted through homologous recombination, could still establish tight junctions with characteristic freeze fracture fibrils, and a paracellular barrier to low molecular weight tracers (33).

**Claudin**

Two additional integral membrane proteins of the junction were recently identified by direct biochemical fractionation of junction-enriched membranes from chicken liver (34). After removal of peripherally attached proteins with guanidine, claudin-4 was recently identified by direct biochemical fractionation of junction-enriched membranes from chicken liver (34). After removal of peripherally attached proteins with guanidine, followed by sonication and stepwise sucrose density gradient centrifugation, claudin-1 and claudin-2 (from the Latin claudere meaning to close) were found to copurify with occludin as a broad approximately 22-kD gel band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Peptide microsequencing permitted cloning of these two closely related proteins from a mouse cDNA library. The deduced sequences predict four transmembrane helices, two short extracellular loops, and short cytoplasmic N- and C-termini (Figure 3). Despite topologies similar to that of occludin, they share no sequence homology. Subsequently, six more claudin gene products were identified based on homology to sequences in the expressed sequence tagged database (35). These have now all been cloned and have been shown to localize within tight junction fibrils, as determined by immunogold freeze fracture labeling (35). No further functional data are available; however, given that a barrier remains in the absence of occludin, they must be considered candidates for the primary seal-forming elements of the extracellular space. Consistent with this role, when either claudin-1 or -2 is expressed in fibroblasts, these proteins are capable of assembling into long branching fibrils reminiscent of their organization in the tight junction of epithelial cells. In contrast, occludin has a limited ability to self-organize into fibrils in transfected fibroblasts, but will join the fibrils when claudin is cotransfected (36).

Interestingly, the eight known claudins show strikingly different mRNA expression patterns among different tissues, suggesting that they may contribute to functional differences among different epithelia (35). Consistent with the idea that differential expression of junction proteins may have functional implications, other biochemical differences among tight junctions have already been documented. For example, the cytoplasmic protein ZO-1 has two major alternatively spliced isoforms; one is expressed in epithelial cells and the other in endothelial cells (37,38). Another cytoplasmic protein, 7H6, appears to be restricted to epithelial cell tight junctions (39). Presumably, these structural differences will be found to underlie functional diversity.

Several claudins were previously cloned and characterized without the investigators’ realizing that they were part of the tight junction. Among these is claudin-4, previously referred to as the murine Clostridium perfringens enterotoxin receptor (CPE-R), which is 46.9% identical to claudin-1 (40). The CPE-R is the high-affinity receptor for an enterotoxin produced by the intestinal pathogen Clostridium perfringens (41). Although the mechanism of action is not fully known, binding of toxin to the CPE-R results in formation of cytotoxic pores in the plasma membrane (42). It is not clear whether the CPE-R is a component of the pore or simply serves as a receptor for the toxin. A more distant branch of the family is represented by oligodendrocyte-specific protein (OSP) (43), which is 33.8% identical to claudin-1. This claudin was originally characterized as a component of myelin in the mouse central nervous system (43). Interestingly, rows of freeze fracture fibrils are found in Schwann cell membranes where they spiral around axons. Although the subcellular location of OSP in Schwann cells is unknown, it is tempting to speculate that OSP is the glial equivalent of epithelial claudin. Located in fibrils, it could function to maintain the electrically resistive wrapping required to accelerate electrical conduction along the nerve. Clearly, much remains to be learned about the claudins, their role in forming variable barrier properties, and their potential role in diseases affecting the barrier.

**Junction Adhesion Molecule**

The recently identified junctional adhesion molecule (JAM) is a novel member of the Ig superfamily and localizes to the tight junctions of both epithelial and endothelial cells (44). The resolution of ultrastructural localization is presently inadequate to determine whether JAM is located specifically within the fibrils with occludin and the claudins, but it is at least limited to the membrane regions containing fibrils. A CDNA encoding JAM predicts an approximately 32-kD type I transmembrane protein with two extracellular Ig-like domains and two sites for N-glycosylation (Figure 3). Experimental evidence suggests that JAM can mediate both homotypic adhesion and influence monocyte transmigration. When JAM is expressed in COS cells, which do not have tight junctions and do not normally express this protein, it accumulates at sites of cell–cell contact and induces cell–cell adhesion. Addition of a monoclonal antibody against JAM to the media bathing cultured endothelial cells inhibits both spontaneous and chemokine-induced transmigration of monocytes. This observation suggests that JAM might function in normal transmigration across epithelial monolayers, perhaps by providing an adhesive contact required for monocytes to find the intercellular pathway (analogous to a homing receptor), or by transducing a signal within the endothelial cell to open the junctional space. Previous studies have demonstrated that a large flux of immune cells can traverse...
such a monolayer without decreasing the transepithelial electrical resistance (45). This raises the possibility that JAM, or a similar protein, creates a seal with the immune cell as it passes through the junction. JAM is the first molecular component of tight junctions that has been demonstrated to have a direct role in the regulation of immune cell transport across the paracellular seal and should provide important insights into the interaction between immune cells, epithelia, and endothelia.

Assembly and Scaffolding of the Paracellular Sealing Proteins

For the transmembrane proteins to provide a paracellular seal, they must be properly targeted to and positioned at the tight junction. Assembly, scaffolding, and regulation of the paracellular seal are presumably accomplished by the cytosolic plaque of proteins associated with the inner surface of tight junction contacts (Figure 1). Among the presumed structural proteins are ZO-1 (46), ZO-2 (47,48), ZO-3 (49), cingulin (50), 7H6 (39), and symplekin (51). The last three have no known function, binding interactions, or informative amino acid sequence homologies. Several presumed signaling proteins have also been localized at the resolution of light microscopy to the apical junction complex, including several heterotrimer G-proteins (52,53), an isofrom of PKC (53), vertebrate homologs of the yeast secretory mutants Sec6 and Sec8 (54), and the nonreceptor tyrosine kinases src and yes (55). The Ras-binding protein AF6 (18) and ZA-kinase (56) both bind to ZO-1 but have not yet been immunolocalized at the ultrastructural level. The potential role of intracellular signaling pathways in regulating junctional properties has been frequently reviewed (2,57), although the specific molecular targets controlling barrier function remain largely unknown.

Occludin binds directly to ZO-1 (58), ZO-2 (B. R. Stevenson, unpublished observation), and ZO-3 (49), and there is evidence that the interaction with ZO-1 recruits and organizes occludin at the junction. ZO-1, like its homologues ZO-2 and ZO-3, is a member of the MAGUK protein family (membrane-associated guanylate kinase homologues) (48,49,59). Other members of the MAGUK family are clearly responsible for tethering transmembrane protein at specific sites of cell–cell contact or to specific cell membrane domains. Coupling is typically based on interaction between a protein binding domain in the MAGUK protein, called a PDZ domain, and a short specific peptide motif at the extreme C terminus of the transmembrane protein (60). However, in the case of ZO-1, the interaction with occludin is not mediated by a PDZ domain (61). Assuming this is also true for ZO-2 and ZO-3, then the nine total PDZ domains present in ZO-1, -2, and -3 are available to recruit and coordinate presently unknown proteins under the junctional contact.

The mechanisms responsible for occludin targeting have also been examined in numerous transfection studies. When full-length occludin is expressed in fibroblasts that normally do not contain tight junctions, it is targeted to sites of cell–cell contact that contain ZO-1 (23). Occludin lacking the C-terminal ZO-1 binding domain fails to target to these cell–cell contacts (C. Van Itallie, unpublished results), suggesting that interaction with ZO-1 is required for localization to specific sites on the plasma membrane. Consistent with this idea is the observation that when this domain is fused to the nonjunctional protein connexin-32, the chimeric protein becomes positioned precisely within junctional fibrils (62). In contrast, a C-terminal truncated occludin is still recruited into tight junctions in stably transfected MDCK lines, suggesting that lateral interactions with full-length occludin are also sufficient to promote localization (29). Binding to ZO-1 is therefore sufficient but not necessary to assemble occludin into tight junctions, and the ZO-1/occludin link may be responsible for fine-tuning structural aspects of the junction, such as creation of continuous fibrils. Although there is presently no evidence suggesting how claudin or JAM localizes to the junction, it is noteworthy that both proteins have C-terminal motifs predicted to bind PDZ domains like those found in the ZO proteins.

Perijunctional Actin

Throughout the cell, actin filaments are known to serve both structural and dynamic roles. Presumably, this is also true for the thick band of bipolar actin filaments positioned under the apical junction complex. Individual filaments can be observed in ultrastructural images to terminate at the membrane (53,63,64) directly at the points where occludin and claudin are focused into fibrils. ZO-1 binds to actin filaments, although it has not been resolved whether this is direct or through a linking protein. ZO-1 also binds the cytoplasmic tail of occludin (58,61,65,66) and thus is presently the best candidate for coupling both structural and dynamic properties of perijunctional actin to the paracellular barrier.

Several studies suggest that the ability of the tight junction to form a seal is dependent on the structural organization of actin. Treatment of cultured epithelial cells with the actin-disrupting drug cytochalasin causes a decrease in the size and complexity of tight junction fibrils (67–70). There is also considerable correlation between experimentally induced changes in permeability and actin filament organization. For example, experimentally reducing cellular ATP levels results in altered tight junction structure, decreased transepithelial electrical resistance, and an increased association of tight junction proteins like ZO-1 and ZO-2 with the actin cytoskeleton (71,72). Similarly, changes in paracellular permeability induced by the Vibrio cholerae toxin ZOT are also associated with a reorganization of perijunctional actin (73). Finally, activation of small GTP binding proteins of the rho family trigger a marked reorganization of perijunctional actin (74), which coincides with a dramatic increase in permeability of tight junctions (75). Because such manipulations result in widespread alterations to the actin cytoskeleton, these observations must be interpreted with caution. However, they suggest that overall cytoskeletal organization is crucial to tight junction integrity and could be coupled through ZO-1 and regulated by cellular signaling pathways.

A dynamic role for the perijunctional actin ring is supported by its ability to undergo circumferential contraction (17,76). Contraction may be coupled to physiologically relevant sig-
nals. For example, studies in both isolated tissues and cultured cells have demonstrated that activation of the sodium/glucose transporter leads to cytoskeletal contraction and a concurrent increase in paracellular permeability (17,77,78). In cultured cells, this has been shown to occur in concert with the phosphorylation of myosin light chain, which is known to activate nonmuscle myosin contractility (79). In addition, Hecht et al. (80) have shown that the introduction of a constitutively activated myosin light chain kinase into cultured MDCK cells results in a contraction of the cortical cytoskeleton. This is associated with a decreased TER and increased paracellular flux (80). These observations suggest that the force generated by actomyosin contraction can somehow be transmitted to the tight junction and cause a transient physical change in the paracellular space. Such changes could be based on changes in cell–cell or lateral organization of occludin and claudin, mediated through ZO-1.

**Future Directions**

Much work remains to define the individual contributions of occludin, the claudins, and JAM in regulating paracellular permeability, permselectivity, and immune cell transmigration. In addition, there may also be other uncharacterized transmembrane proteins within the tight junction. For example, does the large combinatorial diversity of their expression of claudins lead to physiologic differences among cell types? Organizing the transmembrane proteins is obviously a key aspect in defining the barrier, and the role of plaque proteins in assembly and scaffolding requires more study. Finally, it may now be possible to address whether junctions are physiologically regulated in an acute manner to control transepithelial transport. Identification of transmembrane components of the tight junction has provided new tools with which to examine the molecular basis for paracellular permeability. Cytoplasmic signaling pathways can clearly affect the barrier, and we are now set to reinterpret many past experiments in terms of the effects of these pathways on the transmembrane proteins.

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