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Citi, Sandra; Cordenonsi, Michelangelo

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Review

Tight junction proteins[1]

Sandra Citi [a,b,*], Michelangelo Cordenonsi [b]

[a] *Department of Molecular Biology, University of Geneva, Geneva, Switzerland*
[b] *Dipartimento di Biologia, Università di Padova, Padua, Italy*

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Contents

1. Introduction

Epithelial cells separate us from the external environment, and separate different compartments within our body. In order to cross intact epithelial and endothelial cell sheets, molecules and cells have two options: (1) go through cells (transcellular pathway) or (2) go between cells (paracellular pathway). In vertebrate organisms, the tight junction (TJ) is the cellular structure which prevents the free passage of molecules and cells through the paracellular pathway, thereby ensuring the maintenance of compositionally distinct body compartments. This 'barrier' function is essential in tissues, such as the brain, where it is critical to maintain a tight separation between blood vessels and interstitial fluids (blood-brain barrier), but is also necessary in all epithelial tissues involved in vectorial absorption and secretion. A second function of TJ is to maintain a different protein and lipid composition between the apical and basolateral plasma membrane domains of polarized epithelial cells ('fence' function). Excellent reviews in recent years have discussed the structure, function and regulation of TJ [1–7]. The reader is referred

* Corresponding author;
E-mail: Sandra.Citi@molbio.unige.ch and citi@civ.bio.unipd.it

[1] This review is dedicated to the memory of Thomas Kreis.

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Table 1
A list of TJ-associated proteins, with their apparent molecular size (M_r) (kDa), and essential references[a]

Name	M_r	References
ZO-1	220–225	[8],[51,52]
Cingulin	140–160	[57]
BG9.1	192	[111]
ZO-2	160 kDa	[49,53]
7H6	155–175	[61]
ZO-3	130	[19,50]
Occludin	58–82	[15,30]
Rab8	24	[112]
Rab13	41	[39]
Rab3B	25	[38]
Symplekin	150	[43]
Protein kinase Cζ	81	[36]
G protein α	39–41	[36,37]
19B1	210	[65]
AF-6	180–195	[67]
Claudins	22	[16]
JAM	36–41	[17]

[a]See text for additional references. The variability in apparent molecular size of some TJ proteins is due to tissue- or species-specific variations, and in some cases (for example occludin) can also depend on phosphorylation levels.

to these reviews for areas which are not covered in detail here.

Since the first discovery of a TJ-localized protein in 1986 [8], many proteins have been shown to be associated with TJ, either by direct immunoelectron microscopic localization or by their physical or topological association with known TJ proteins (Table 1). This minireview focuses on recent advances in the identification and characterization of TJ proteins, and how these advances may help to answer key questions about TJ function and regulation.

2. Membrane proteins: occludin, claudins, and JAM

The membrane domain of TJ has a characteristic ultrastructure. Transmission electron microscopy shows focal sites of intimate contact between the apposed plasma membranes of adjoining cells [9]. Freeze-fracture shows a fascinating network of fibrils (also called strands) and grooves on the two faces of the fractured membranes, corresponding to the sites of membrane apposition [10,11]. The fibrils are thought to be the structural counterpart of the barrier function, and to contain molecules which form a 'pore' through which paracellular flux occurs. For some time, it was debated whether the fibrils were the result of a special configuration of lipids, or whether they were proteinaceous in nature [12–14]. We now know, through the work of the Tsukita and Dejana groups, that the TJ membrane domain contains at least three distinct proteins, named occludin [15], claudin [16], and JAM [17]. Occludin was identified by raising monoclonal antibodies against a chicken liver junctional membrane fraction [15], and claudin (actually two similar proteins, claudin-1 and claudin-2) was identified by sequencing a M_r 22 kDa polypeptide present in the same membrane fraction [16]. JAM was identified by raising monoclonal antibodies against endothelial cells [17]. Studies on these three molecules are starting to provide answers to some important questions. Which proteins form TJ fibrils, and which are responsible for

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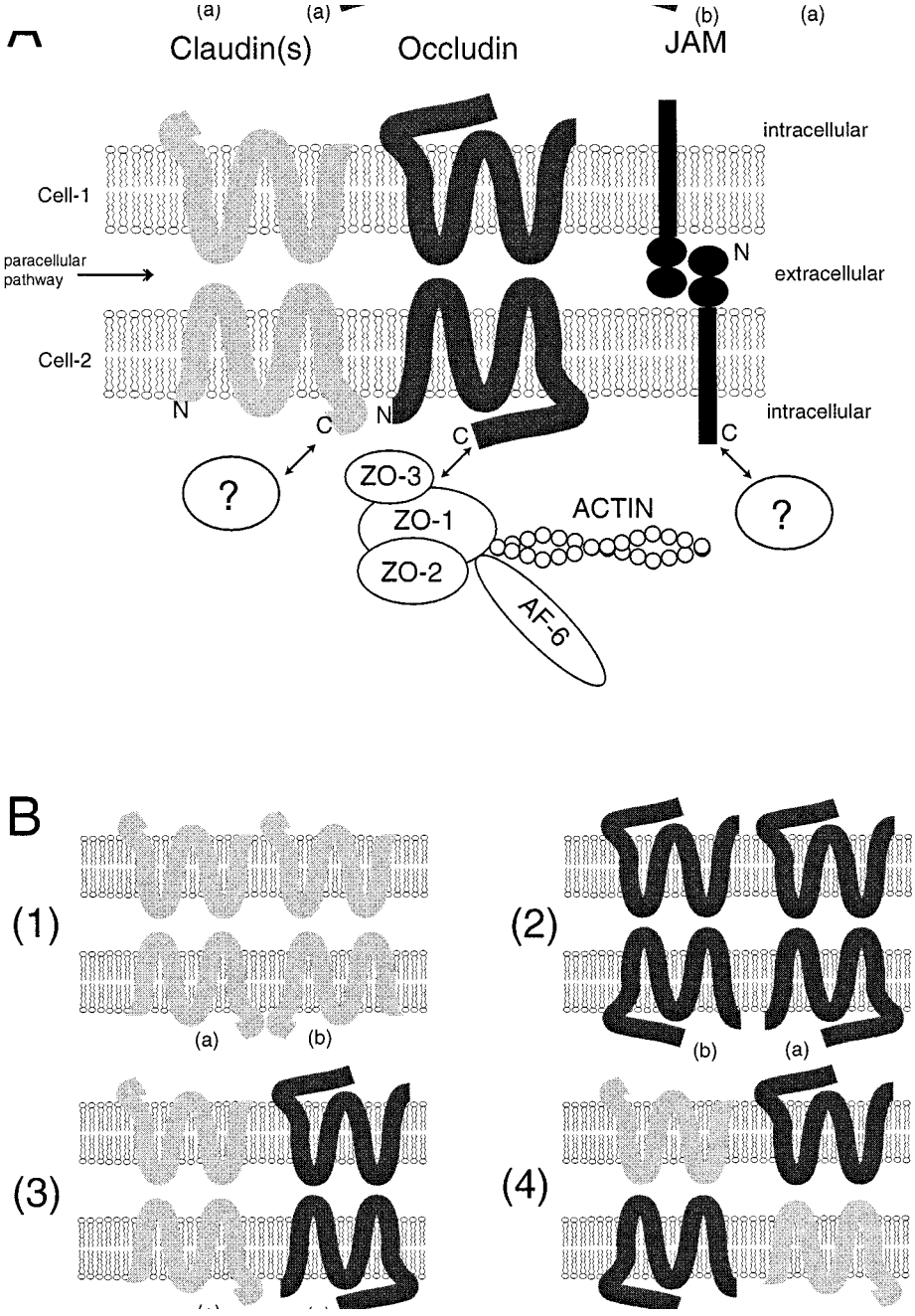
Fig. 1. A schematic representation of TJ, with emphasis on proteins of the TJ membrane domain. (A) Claudin(s) (light gray) and occludin (dark gray) are depicted as chains with four transmembrane regions embedded in the plasma membrane. JAM is depicted as a protein with one transmembrane domain. The plasma membranes of adjoining cells (Cell-1 and Cell-2) are schematically represented and define the paracellular pathway (extracellular space). The N-terminal and C-terminal ends of occludin, claudin and JAM are indicated with N and C, respectively. The C-terminal domain of occludin interacts with cytoplasmic plaque components of TJ (ZO-1 and ZO-3), whereas nothing is known yet on cytoplasmic interactions of claudin(s) and JAM. ZO-1, ZO-2 and ZO-3 are shown as a complex. ZO-1 and AF-6 interact one with the other and with actin. (B) Four hypothetical modes of polymerization of claudin(s) and occludin. (1) Homopolymerization of claudin(s) and homotypic interaction with claudin(s) of adjoining cell. (2) Homopolymerization of occludin, and homotypic interaction with occludin of adjoining cell. (3) Heteropolymerization of claudin(s) and occludin, and homotypic interactions with identical molecules of adjoining cell. (4) Heteropolymerization of claudin(s) and occludin, and heterotypic interactions with different molecules of adjoining cell. Also note that (a) and (b) in each panel refers to hypothetical alternative ways of homophylic and heterophylic interactions between the extracellular loops of claudins and occludin. (a) extracellular loop-1 associates with extracellular loop-2 of opposing molecule; (b) extracellular loop-1 associates with extracellular loop-1 of opposing molecule. The shapes and sizes of the proteins are imaginary and out of scale, and other TJ proteins of the plaque domain (Table 1) have been omitted for clarity.

the barrier function of TJ? Are the membrane proteins of TJ involved in homotypic cell-cell adhesion?

Characterization of the predicted tertiary structure of occludin, claudin and JAM provides clues to understanding their organization in the TJ membrane. Occludin and claudin share a common membrane topology, with four major putative transmembrane domains, whereas JAM shows only one putative transmembrane sequence (Fig. 1A). The transmembrane domains of occludin and claudin are separated by hydrophilic regions, which are believed to form one cytoplasmic loop and two extracellular loops. In addition, occludin contains a hydrophilic N-terminal region of 60 residues, and a hydrophilic C-terminal region of about 250 residues, which are believed to be exposed on the cytoplasmic



side of the junctional membrane [15] (Fig. 1). The C-terminal domain of occludin may be important in anchoring it to the cytoplasmic plaque domain of TJ, through the interaction with proteins such as ZO-1 [18] and ZO-3 [19] (Fig. 1A), and mediating basolateral targeting [20]. Claudin contains a short (20–40 amino acids, depending on the claudin isoform) C-terminal domain [16], and JAM shows a putative intracellular domain of 45 residues [17]. The extracellular portion of JAM (215 residues) contains two domains with intrachain disulfide bonds typical of immunoglobulin-like loops of the V type [17] (Fig. 1A).

Immunolabeling of freeze-fracture replicas of normal or transfected cells shows that occludin and claudin are components of TJ fibrils [16,21], whereas similar studies have not been performed yet with JAM. Only claudin appears to induce the formation of a developed network of TJ fibrils in the plasma membrane of transfected L-cells [113], which is possibly the ultimate test to determine whether a TJ membrane protein actually forms the fibrils. These results allowed Tsukita's group to formulate the hypothesis that TJ fibrils are assembled by a process similar to that of intermediate filament assembly, whereby several proteins (occludin, claudin, others?) sharing a common structural motif (for example, the four transmembrane domains) can give rise to a similar polymer embedded in the plasma membrane [16]. To test this model it is necessary to address a number of questions. First, what moieties of the claudin and occludin molecules are involved in the polymerization process (Fig. 1B)? It was shown that occludins from different species can oligomerize in vivo [22], and C-terminal truncations of exogenous occludin are targeted to TJ [22,23], suggesting that the C-terminal domain is not involved in occludin polymerization. On the other hand, the four transmembrane domains do not appear to be sufficient to act as structural motifs for TJ assembly, since other membrane proteins, such as connexins, have four transmembrane domains but do not accumulate in TJ. Second, can occludin in one cell form heteropolymers with claudin in the same cell and interact heterotypically with claudin on the adjoining cell (Fig. 1B)? Occludin behaves as a cell-cell adhesion molecule [24], and transfection of truncated occludin induces redistribution of occludin in neighboring cells [23], suggesting that the extracellular domains of occludin on adjoining cells somehow interact. However, it is not clear whether occludin interacts laterally with claudin molecules, or whether it can interact with claudin on an opposing cell (Fig. 1B). JAM has also been reported to mediate homotypic cell-cell adhesion [17].

The organization of the fibril polymer within one cell and its association with the polymer of the adjoining cell is probably at the core of TJ barrier function. Indeed, a perturbation of the occludin-containing polymer has effects on the barrier and fence properties of TJ, as shown by the observation that heterologous expression of truncated occludin results in an increase in transepithelial resistance and loss of the fence function in cultured MDCK cells [23,25] and in a decreased barrier function in *Xenopus* embryos [22]. In addition, peptides mimicking the sequence of the second extracellular loop of chicken occludin decrease transepithelial resistance in *Xenopus* A6 cells [26]. All these observations suggest that occludin is important for the barrier function of TJ. However, recent knockout experiments in mouse embryonic stem cells have demonstrated that occludin is not necessary to form functionally competent TJ [27]. In fact, occludin-deficient epithelial cells show normal polarity, normal number and morphology of TJ fibrils, normal localization of ZO-1, and normal diffusional barrier to a labelled tracer [27]. This raises the possibility that occludin is not a key component of TJ fibrils, nor does it have a critical role in the barrier function of TJ, and in anchoring the membrane domain of TJ to the plaque domain. So, is occludin a redundant protein, and are all of these functions performed by claudin and JAM? While further transfection and knockout studies are being carried out, it is interesting to note that when JAM is transfected into CHO cells, the permeability of a cell monolayer to dextran is decreased by 50% [17]. Since dextran is a large molecule (M_r 38 900) and the same effect is produced by transfection of cadherins [17], it remains unclear whether JAM could form structures capable of forming a barrier to the flux of ions and small solutes. In principle, the homotypic interaction of JAM molecules on adjoining cell membranes may contribute to regulating the passage of large molecules and cells through endothelial sheets. Indeed, the observation that an anti-JAM monoclonal anti-

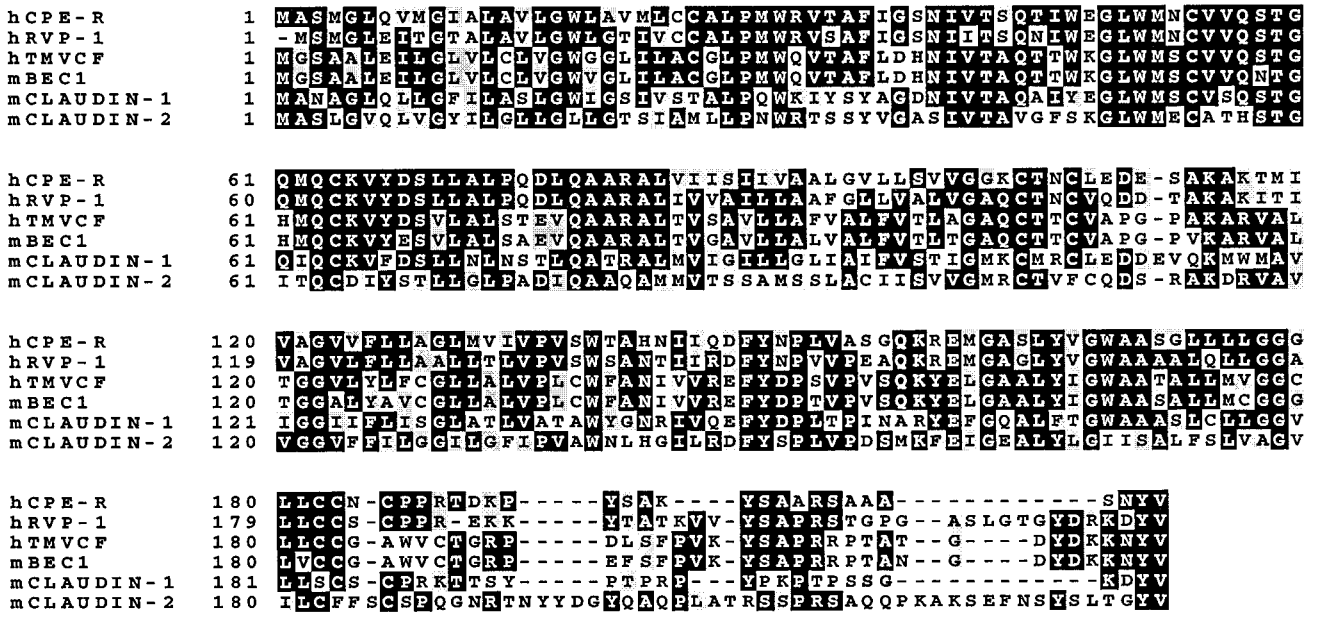


Fig. 2. Multiple alignement of the amino acid sequences of human *Clostridium perfringens* enterotoxin receptor (hCPE-R; accession number AB000712), human androgen withdrawal apoptosis protein (hRVP-1, accession number AB00714), transmembrane protein deleted in velo-cardio-facial syndrome (hTMVCF; accession number AF000959), mouse brain endothelial cell protein BEC1 (MBEC1; accession number AF035814), and mouse claudin-1 and claudin-2 (mCLAUDIN-1, mCLAUDIN-2, [16]).

body inhibits transendothelial migration of monocytes in vitro and in vivo suggests that JAM may actually bind to and direct the migration of monocytes through the intercellular cleft [17].

The identification of multiple TJ membrane proteins may provide better tools to address an old problem: what is the relationship between the barrier function of TJ, which is extremely heterogeneous in different tissues and cell types [28], and the molecular composition of TJ? The first issue is to determine whether the tissue distribution of occludin, claudins and JAM parallels that of TJ. This seems to be the case for occludin, which is expressed only in TJ-bearing epithelial and endothelial cells [15,29]. On the other hand, claudin is expressed also in skeletal muscle [16], and JAM is expressed in megakaryocytes [17], which lack TJ. The role of claudin and JAM in tissues lacking TJ remains unclear. It is also unclear why JAM expression is low or absent in hepatocytes, which contain well-characterized TJ [17]. The second issue to determine is whether different isoforms of TJ membrane proteins show tissue-specific expression. This seems to be the case only for claudin, of which there are at least two isoforms (claudin-1 and claudin-2) with different tissue distribution [16], whereas no isoforms have been reported for occludin and JAM.

It is interesting that despite the similarity in membrane topology, occludin and claudin do not show any homology at the amino acid sequence level. This would suggest that they have evolved from unrelated precursor proteins. If occludin function is redundant with respect to claudins, one could speculate that occludin may have appeared later in evolution, to provide some accessory function. However, it is unclear how phylogenetically ancient occludin and claudin are, and how the evolution of TJ morphology correlates with the expression of these proteins or their homologous counterparts in invertebrates or early vertebrates. Some information about evolutionary conservation of occludin is available. Occludin has been detected and sequenced from chicken, mouse, human, rat kangaroo, dog [15,30] and *Xenopus laevis* (Cordenonsi et al., in preparation). Its sequence is relatively well conserved across mammalian species, but diverges significantly from that of chicken [30] and *Xenopus* (Cordenonsi et al., in preparation). Claudins from human and mouse are almost 100% identical, but nothing is known so far about their expression and sequence in other organ-

isms. Occludin amino acid sequence does not show homology to know proteins, whereas claudins (claudin-1 and claudin-2) are homologous to a family of four transmembrane domain proteins, including the rat androgen withdrawal apoptosis protein (RVP-1) [31], the transmembrane protein deleted in velo-cardio-facial syndrome (TMVCF) [32], the *Clostridium perfringens* enterotoxin receptor (CPE-R) [33,34], and the brain endothelial cell protein BEC1 [35] (Fig. 2).

It is likely that the discovery of occludin, claudins and JAM is not the end of the story of TJ membrane proteins. For example, the chicken liver junctional fraction used by Tsukita's laboratory to identify occludin and claudin contains an additional eight polypeptides which remain to be characterized [16].

3. Cytoplasmic plaque proteins: linking membrane to cytoskeleton?

Except for occludin, claudins and JAM, all TJ proteins identified so far (Table 1) are localized in the cytoplasmic plaque domain of TJ. These include the MAGUK proteins of TJ, called ZO-1, ZO-2, and ZO-3, and a more heterogeneous group, including cingulin, symplekin, 19B1, and AF-6. Finally, the cytoplasmic plaque domain of TJ contains proteins involved in signal transduction pathways, such as protein kinases [36], heterotrimeric G proteins [36,37], and small GTP-binding proteins [38,39]. These latter proteins are not uniquely localized at TJ, but their role in the assembly and functional modulation of TJ may be critical. The possible roles of these signaling proteins in TJ assembly have been reviewed recently [40] and will not be discussed here.

Several of the proteins of the cytoplasmic plaque domain of TJ are not exclusively expressed in TJ-bearing cells. For example, ZO-1 and symplekin are expressed in fibroblasts and neurons [41–43]. ZO-1 exists in two isoforms (ZO-1 α+ and ZO-1 α−) which are differentially expressed in tissues showing a different degree of TJ 'plasticity' [44] and show a sequential expression during early mouse development [45]. ZO-1 [46], symplekin [43] and cingulin [47] can show a nuclear immunofluorescent localization, depending on various factors, including degree of confluency of the epithelial monolayer, cell type, and fixation procedure. For example, symplekin is localized in the nucleus in non-epithelial cells, and is localized in TJ and in the nucleus in epithelial cells [43]. The significance of the nuclear localization of TJ proteins is unclear. A possible model could predict that TJ plaque proteins exist in a junction-associated (less soluble) pool in equilibrium with a cytoplasmic (more soluble) pool, and when the equilibrium is shifted towards the soluble pool there is nuclear accumulation of the protein. Whether nuclear TJ proteins may lead to changes in gene expression, similarly to what has been shown for β-catenin [48], remains to be seen.

ZO-1, ZO-2 and ZO-3 form a complex on the cytoplasmic side of TJ, and indeed ZO-2 and ZO-3 were first identified as polypeptides present in ZO-1 immunoprecipitates [49,50]. Sequence analysis shows that ZO-1, ZO-2 and ZO-3 are related, and are members of the large family of membrane-associated guanylate kinase (MAGUK) proteins [19,51–53]. MAGUK proteins share several structural motifs, including a varying number (typically 1–3) of PDZ domains (also called GLGL/DHR), one *src* homology 3 (SH3) region, and one guanylate kinase (GUK) homology region [54]. The PDZ domain has been shown to bind to specific sequences at the C-termini of proteins [55], to be involved in the clustering of membrane channels/receptors [56], and to mediate association with other proteins containing a PDZ domain [54]. The SH3 region and the GUK homology region may also involved in interactions with other proteins.

Cingulin was identified by monoclonal antibodies raised against a myosin-enriched fraction of chicken intestinal epithelial cells [57], and has been detected only in TJ-bearing epithelial and endothelial cells [58,59]. Cingulin contains a M_r 108 kDa region with biochemical and physical properties of a coiled-coil dimer [57,58]. Cloning of cingulin cDNA shows that the coiled-coil domain is similar to the coiled-coil regions of myosin heavy chains [60]. In addition to the coiled-coil region, cingulin contains a N-terminal globular region with no significant homology to other known proteins (Cordenonsi et al., in preparation). The observation that cingulin co-purifies with actomyosin [58] suggests that cingulin may link the TJ plaque to the actomyosin cytoskeleton. This hypothesis is supported by in vitro

binding experiments, indicating that cingulin interacts with other TJ proteins and components of the actomyosin cytoskeleton (Cordenonsi et al., in preparation).

Another group of TJ plaque protein comprises 7H6, symplekin and 19B1 and AF-6. 7H6 was identified by raising monoclonal antibodies to a rat liver junctional fraction [61]. 7H6 is a phosphoprotein, and its expression has been studied in normal and cancerous epithelial cells and tissues [62–64]. Symplekin is expressed in epithelial cells, where it is localized in TJ, and in non-epithelial cells, where it is localized in the nucleus [43]. Symplekin sequence shows no homologies to other proteins in databases [43]. 19B1 is co-localized with ZO-1 in cultured *Xenopus* A-6 cells [65], and is detected as a maternal protein in early development of *Xenopus laevis* [66]. AF-6 co-localizes with ZO-1 in cultured epithelial cells [67], and was originally identified as the fusion partner of the human acute lymphoblastic leukemia-1 (ALL-1) protein [68,69]. The deduced amino acid sequence of AF-6 shows one PDZ domain, two Ras-binding domains, and one myosin V- and one kinesin-like cargo-binding domains [69].

Recent studies have begun to clarify the molecular interactions occurring between some of the TJ proteins described above (see also Fig. 1A). The C-terminal domain of occludin binds ZO-1 and ZO-3, based on GST pull-down assays and direct interaction studies [18,19] (Fig. 1A). Pull-down assays also show that occludin binds ZO-2, although it is not clear whether this interaction is direct, or mediated by ZO-1 [18]. ZO-1 interacts with the Ras-binding domain of AF-6, and this interaction is inhibited by activated Ras [67]. In vitro experiments also show that ZO-1 is associated with catenins [70,71]. This latter observation is particularly interesting, in view of the finding that in cells devoid of TJ, such as fibroblasts, ZO-1 is localized at cadherin-dependent adhesion sites [41,42,72]. Thus, it can be envisaged that during epithelial polarization ZO-1 may initially form a complex with the cadherin-based multiprotein complex, and then becomes predominantly localized at TJ only when it is recruited there by TJ-specific protein(s). Taken together, this evidence suggests that ZO-1 is at the center of a network of protein-protein interactions, and may be critical in recruiting proteins necessary to establish TJ and in mediating signal transduction events. However, the precise function of ZO-1 in the TJ is not known, nor is it known whether ZO-2 and ZO-3 play a functionally redundant role, or have functions which are complementary to or distinct from those of ZO-1.

4. The actin cytoskeleton: more muscle, less barrier?

Actin microfilaments have been localized near the cytoplasmic surface of TJ by electron microscopic techniques [73,74]. In addition, actin microfilaments and associated proteins are abundant in a thick circumferential ring associated with the zonulae adhaerentes [75], immediately beneath TJ. There is a vast literature documenting the possible roles of the actin cytoskeleton in the modulation of TJ function (reviewed in [76]). Most of these studies are based on a pharmacological approach, whereby cultured or native epithelial cells are treated with agents which perturb the organization or contractility of the actomyosin cytoskeleton. Such agents include phalloidin [77], cytochalasins [78,79], protein kinase inhibitors and activators [50,80–86], toxins [87], Rho proteins [88,89], and myosin light chain kinase [90]. All of these agents influence TJ barrier function, as assayed by transepithelial resistance and tracer flux studies, and in some cases they also produce changes in TJ ultrastructure and distribution of TJ proteins. Although it is difficult to establish whether these agents may have direct effects on TJ proteins, it appears that all the agents that increase the contractility of the actomyosin cytoskeleton, for example by inducing stress fiber formation [80,91] or increasing myosin light chain phosphorylation [90], cause a decrease in the barrier function of TJ (increased permeability). Vice versa, the agents which negatively affect the contractility of the actomyosin cytoskeleton protect cell monolayers from a fall in the barrier function caused by other treatments (see for example [83,92]). These observations support a model whereby cortical tension generated by the perijunctional actomyosin cytoskeleton physiologically regulates the permeability of TJ (Fig. 3). Under normal circumstances, TJ are subjected to a 'tonic' contraction (Fig. 3A). When the contractility of the circumferential actomyosin ring increases, the tension which is generated induces adjacent cells to pull apart at the

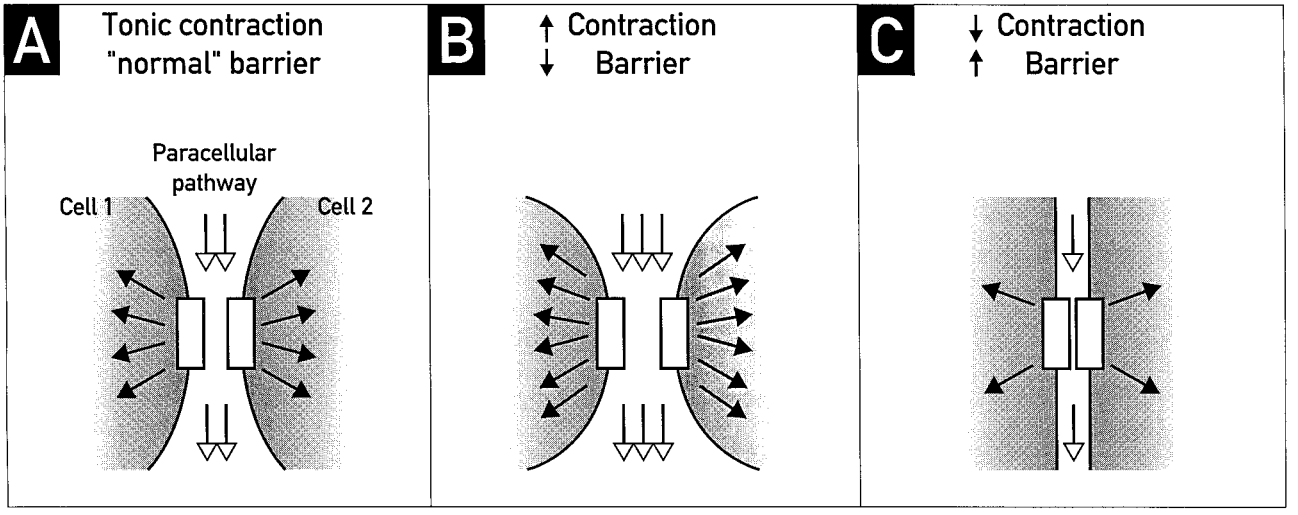


Fig. 3. A schematic representation of how the contraction of the junctional actomyosin ring might regulate the barrier function of TJ. Only the junctional region between two cells is shown in the diagram. Mechanical force pulling the TJ membrane centripetally is represented by oblique arrows on the intracellular side of the TJ. Vertical arrowheads indicate the solute flux through the paracellular pathway.

level of the junctional complex, and reduces TJ barrier (Fig. 3B). Vice versa, a relaxation with respect to the normal 'tonic' state of tension induces a closing of the TJ pore and an increased barrier function (Fig. 3C).

To test the model above, and to understand at the molecular level how the contractility of junctional actomyosin may influence TJ, it is critical to determine which TJ proteins bind to actin or actin-associated proteins. This area of work is still in its infancy. It was recently shown that a C-terminal fragment of ZO-1 cosediments with F-actin [71]. In addition, ZO-1 interaction with catenins [70,71] provides another indirect link to the junction-associated actomyosin cytoskeleton. Another TJ protein which may provide a link to the actin cytoskeleton is AF-6, since the rat homolog of AF-6, called afadin, has been characterized as a junctional protein containing an F-actin-binding region [93].

The interaction of TJ proteins with the cytoskeleton is interesting to study not only because circumferential contraction of actomyosin may modulate TJ permeability, but also because cytoskeletal interactions may contribute to forming the TJ 'fence', which restricts the mobility and distribution of polarized membrane molecules. This function may be carried out not only by the actomyosin cytoskeleton, but also by the spectrin/fodrin cytoskeleton [94]. In this respect, it is noteworthy that ZO-1 has been reported to interact with spectrin in vitro [18,41,95].

The interaction between TJ proteins and the actomyosin cytoskeleton may be a primary target for physiological or pathological signals. If the model illustrated in Fig. 3 is true, one would predict that any event which would reduce or abolish the interaction of the TJ plaque with the actomyosin cytoskeleton would cause an increase in the barrier function of the epithelium. Vice versa, any event which may strengthen the anchorage of the TJ plaque to the contracting cytoskeleton would reduce TJ barrier function. This hypothesis is currently being tested by a number of laboratories using a number of approaches, including the exogenous expression of TJ proteins fragments in cultured epithelial cells.

5. Protein phosphorylation: a molecular switch to control TJ assembly?

Occludin is phosphorylated on Ser and Thr residues [96,97], and occludin phosphorylation/dephosphorylation has been implicated in the biogenesis of TJ in cultured MDCK cells [96,98] and in *Xenopus laevis* embryos [97]. Using monoclonal antibodies which distinguish between the more phosphorylated and less phosphorylated forms of occludin, it was

shown that increased phosphorylation correlated with (a) decreased extractability of occludin, and (b) TJ assembly by calcium switch [96]. It was thus suggested that occludin phosphorylation is a key step in TJ assembly [96]. The role of phosphorylation may be different in early embryogenesis of *Xenopus laevis*. In this system, a progressive dephosphorylation-dependent downshift in electrophoretic mobility of occludin was observed during the course of development from unfertilized eggs to gastrula stage embryos [97]. The early stages of embryogenesis are correlated with de novo TJ assembly between *Xenopus* blastomeres, as shown by morphological and functional assays [66,99–101]. Thus, occludin dephosphorylation, rather than phosphorylation, appears correlated with TJ assembly during early *Xenopus* development. It is not clear why phosphorylation may play an opposite role in cultured MDCK cells and *Xenopus* embryos. One possibility is that de novo biogenesis of junctions in embryos occurs in a distinct fashion from that of MDCK cells in low calcium. It is also possible that there are multiple occludin phosphorylation sites, not all of which influence electrophoretic mobility, or that there are maternal and zygotic *Xenopus* occludin isoforms, which behave differently. One approach to resolve this apparent discrepancy will be to determine the in vivo phosphorylation sites of occludin and carry out experiments with mutated molecules.

Except for occludin, it is unclear whether phosphorylation of other TJ proteins is related to TJ assembly. ZO-1 and ZO-2 are phosphorylated [102]. ZO-1 is phosphorylated on Ser residues [102], and can be phosphorylated on Tyr residues in cells treated with activators of tyrosine protein kinases or inhibitors of tyrosine protein phosphatases [103–106]. Cingulin is phosphorylated on Ser residues [91]. Changes in total ZO-1 phosphorylation may be correlated with alterations in its distribution [107], but no clear correlation has been established yet between ZO-1 [50,108,109] or cingulin [91] phosphorylation and TJ function. Interestingly, the SH3 domain of ZO-1 binds in vitro to a serine protein kinase that phosphorylates a region immediately C-terminal to this domain [110].

In summary, phosphorylation is an attractive mechanism by which TJ proteins might be regulated in order to coordinate TJ assembly or modulate TJ function. However, since occludin, the only protein for which a clear correlation between level of phosphorylation and TJ assembly has been observed, is not necessary for TJ [27], the effective significance of TJ protein phosphorylation remains to be demonstrated.

6. Future perspectives

In the last few years, the progress in the identification of TJ proteins and in the characterization of their interactions has been quite remarkable. Despite this progress, the function of essentially all TJ proteins is unknown, and the precise molecular mechanisms by which epithelial and endothelial cells establish, maintain and modulate the paracellular permeability barrier remain elusive. The coming years will witness the publication of more studies investigating the roles of known and novel TJ proteins by up- or down-regulation in cultured cells or living animals. These studies, and the further characterization of interactions among TJ proteins and between TJ proteins and the cytoskeleton, will provide much useful information. Ultimately, the targeted disruption of TJ proteins in vertebrate animal systems will be necessary to determine which proteins are necessary and sufficient to form a functional TJ.

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