MULTIFUNCTIONAL STRANDS IN TIGHT JUNCTIONS

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Tight junctions are one mode of cell–cell adhesion in epithelial and endothelial cellular sheets. They act as a primary barrier to the diffusion of solutes through the intercellular space, create a boundary between the apical and the basolateral plasma membrane domains, and recruit various cytoskeletal as well as signalling molecules at their cytoplasmic surface. New insights into the molecular architecture of tight junctions allow us to now discuss the structure and functions of this unique cell–cell adhesion apparatus in molecular terms.

EPITHELIAL CELLS
Closely packed cells, arranged in one or more layers, that cover the outer surfaces of the body or line any internal cavities or tubes (except the blood vessels, heart and serous cavities).

ENDOTHELIAL CELLS
Thin, flattened cells of mesoblastic origin that are arranged in a single layer lining the blood vessels and some body cavities, for example those of the heart.

MESOTHELIAL CELLS
Flat cells derived from mesoderm that are arranged in a single layer, found lining some body cavities.

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The existence of separate fluid compartments with different molecular compositions is particularly important for the development and maintenance of multicellular organisms. These compartments are delineated by various cellular sheets, which function as barriers to maintain the distinct internal environment of each compartment. For example, renal tubules, blood vessels and the peritoneal cavity are lined with EPITHELIAL, ENDOTHELIAL and MESOTHELIAL cellular sheets, respectively. Within these sheets, individual cells are mechanically linked with each other to maintain the structural integrity of the sheet, and the intercellular space between adjacent cells is sealed to prevent the diffusion of solutes through the intercellular space.

The junctional complex of simple epithelial cells is located at the most-apical part of the lateral membrane and consists of three components: tight junctions, ADHERENS JUNCTIONS and DESMOSOMES[1] (FIG. 1). On ultrathin section electron micrographs, tight junctions appear as a series of apparent fusions ('kissing points'), involving the outer leaflets of the plasma membranes of adjacent cells (FIG. 1b and FIG. 2b). At the kissing points, the intercellular space is completely obliterated, whereas in adherens junctions and desmosomes, the apposing membranes are 15–20 nm apart (FIG. 1b). In simple epithelial cellular sheets, adherens junctions and desmosomes mechanically link adjacent cells, whereas tight junctions are responsible for intercellular sealing[2,3]. But many physiological situations require that various materials are selectively transported across cellular sheets, and this occurs either by TRANSCELLULAR TRANSPORT through the cell or by paracellular flux through tight junctions[4] (BOX 1). So tight junctions are not simply impermeable barriers: they show ion as well as size selectivity, and vary in tightness depending on the cell type[3,5].

In addition to the 'barrier function', tight junctions are thought to function as a 'fence'[2,3]. Plasma membranes are functionally divided into apical and basolateral domains that face the luminal and serosal compartments, respectively. Apical and basolateral membrane domains differ in the compositions of integral membrane proteins and lipids. However, because integral membrane proteins and lipids can diffuse laterally within the plane of the plasma membrane, some diffusion barrier is required at the border between apical and basolateral membrane domains. Tight junctions look like a fence in the most apical part of the lateral plasma membrane and they probably are the morphological counterpart of a localized diffusion barrier.

In recent years, information on the molecular components of tight junctions, and in particular their cell- adhesion molecules, has accumulated. Here, we present an overview of our current understanding of the structure and functions of tight junctions in molecular terms.

The composition of tight-junction strands

The morphology of tight junctions has been intensively analysed by FREEZE-FRACTURE REPLICA ELECTRON MICROSCOPY.

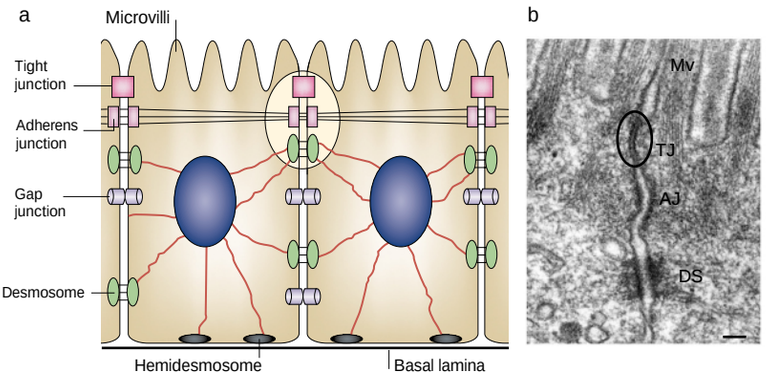


Figure 1 | **Junctional complex and tight junctions**. **a** | Schematic drawing of intestinal epithelial cells. The junctional complex, which is located at the most apical region of lateral membranes, is circled. **b** | Electron micrograph of the junctional complex in mouse intestinal epithelial cells. The tight junction is circled. (Mv, microvilli; TJ, tight junction; AJ, adherens junction; DS, desmosome.) Scale bar, 200 nm.

ADHERENS JUNCTIONS
Cell–cell adhesive junctions that are linked to cytoskeletal filaments of the microfilament type.

DESMOSOMES
These patch-like intercellular junctions are found in vertebrate tissue, and are particularly abundant in tissues undergoing mechanical stress. The central plaque contains adhesion molecules, and represents an anchorage point for cytoskeletal filaments of the intermediate filament type.

On freeze-fracture replica electron micrographs, tight junctions appear as a set of continuous, ANASTOMOSING intramembranous particle strands or fibrils (tight-junction strands) on the P FACE with complementary vacant grooves on the E FACE[6] (FIG. 2a). The number of tight-junction strands as well as the frequency of their ramification vary notably depending on cell type, producing marked variation in the morphology of tight-junction strand networks. These observations led to our understanding of the three-dimensional structure of tight junctions (FIG. 2c). Each tight-junction strand within the plasma membrane associates laterally with another tight-junction strand in the apposing membrane of adjacent cells to form 'paired' tight-junction strands, where the intercellular space is obliterated.

Two models have been proposed to explain the chemical nature of tight-junction strands (FIG. 3). In the 'protein model', tight-junction strands represent units of integral membrane proteins that are polymerized linearly within lipid bilayers whereas, in the 'lipid model', lipids organized in inverted cylindrical micelles are proposed to constitute tight-junction strands[7]. Recent identification of tight-junction-specific integral membrane proteins strongly supports the 'protein' model, although we cannot exclude the possibility that specific lipids might also be important for the formation of tight-junction strands.

Occludin (~60 kDa) was identified as the first integral membrane protein localized at tight junctions in chicken[8], and then also in mammals[9]. Occludin has four transmembrane domains, a long carboxy-terminal cytoplasmic domain and a short amino-terminal cytoplasmic domain (FIG. 4a). No occludin-related genes have been identified yet, but two isoforms of occludin are generated by alternative splicing[10].

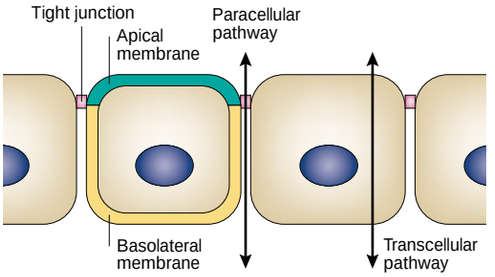
In IMMUNO-REPLICA ELECTRON MICROSCOPY, antibodies against occludin exclusively labelled tight-junction strands[11], indicating that occludin is probably incorporated directly into tight-junction strands. Furthermore, as the intensity of immunostaining with antibodies against occludin in various tissues correlates well with the number of tight-junction strands, the density of occludin molecules in tight-junction strands seems almost constant[11]. But tight-junction strands can also be formed without occludin, as in some cell types such as endothelial cells in non-neuronal tissue and in SERTOLI CELLS in some species, occludin was not detected in tight-junction strands[12,13]. More importantly, VISCERAL ENDODERM cells originating from occludin-deficient embryonic stem cells have well-developed networks of tight-junction strands[14]. At present, the physiological functions of occludin are not well understood. Its possible functions will be discussed in detail below.

More recently, two other transmembrane proteins — claudin-1 and claudin-2 — were identified as integral components of tight-junction strands[15]. These proteins also have four transmembrane domains, but do not show any sequence similarity to occludin (FIG. 4b). So far, 24 members of the claudin family have been identified in mouse and human, mainly through database searches[16,17] (TABLE 1).

There is accumulating evidence that claudins constitute the backbone of tight-junction strands. Immuno-replica electron microscopy revealed that claudins are exclusively localized on tight-junction strands[16,18,19]. Exogenously expressed claudins conferred cell-aggregation activity on L FIBROBLASTS with their concomitant concentration at cell–cell contact planes[20] and led to the formation of a large network of structures that look like tight-junction strands[21] (FIG. 4d). Occludin itself cannot reconstitute such well-organized strands, but when it was introduced into claudin-expressing L transfectants, it was incorporated into reconstituted claudin-based strands[21].

The expression pattern of claudins varies considerably among tissues[15,16] (TABLE 1). Some claudins are expressed in specific cell types; for example claudin-

Box 1 | Two distinct pathways across cellular sheets



There are two pathways through which materials cross epithelial and endothelial cellular sheets: the transcellular and paracellular pathways. The transepithelial electric resistance of epithelia from different tissues varies by two orders of magnitude, which is thought largely to reflect variations in the permeability of tight junctions[91]. Tight junctions show charge and size selectivity in their permeability. Ion transport across tight junctions is cation selective[92]. Anion-selective transport was found in a few cases, such as the rabbit colon and frog skin[92]. Non-charged materials such as water and sucrose also move across tight junctions[4,5]. Under various physiological conditions, material transport across tight junctions occurs in a regulated fashion, and its regulation in certain states might be coupled to transcellular transport[91,93]. For example, during absorption of glucose from the intestine, a large fraction of glucose might be transported across tight junctions, particularly when luminal glucose concentrations are elavated, saturating the Na^+–glucose co-transporter in the apical plasma membrane[91]. Activation of the Na^+–glucose co-transporter is thought to alter the structure and function of tight junctions, but the molecular mechanism underlying this coupling remains unclear.

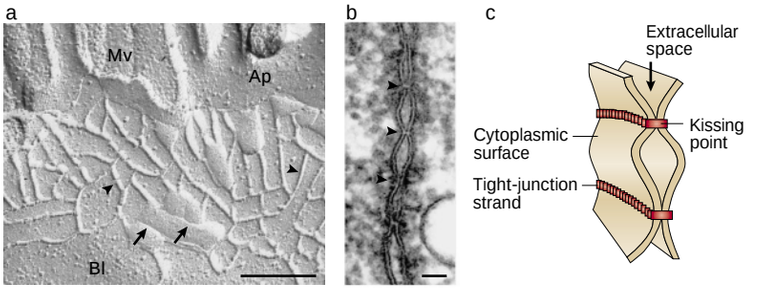


Figure 2 | **Structure of tight junctions. a** | Freeze-fracture replica electron microscopic image of intestinal epithelial cells. Tight junctions appear as a set of continuous, anastomosing intramembranous particle strands or fibrils (arrowheads) on the P face with complementary vacant grooves on the E face (arrows). (Mv, microvilli; Ap, apical membrane; Bl, basolateral membrane.) Scale bar, 200 nm. **b** | Ultrathin sectional view of tight junctions. At kissing points of tight junctions (arrowheads), the intercellular space is obliterated. Scale bar, 50 nm. **c** | Schematic of three-dimensional structure of tight junctions. Each tight-junction strand within a plasma membrane associates laterally with another tight-junction strand in the apposed membrane of an adjacent cell to form a paired tight-junction strand, obliterating the intercellular space (kissing point).

5/TMVCF is expressed primarily in endothelial cells of blood vessels[19], and claudin-11/OSP is expressed primarily in OLIGODENDROCYTES and Sertoli cells[18]. Most cell types, however, express more than two claudin species in various combinations to constitute tight-junction strands: within individual single strands, distinct species of claudin are polymerized together to form 'heteropolymers', and between adjacent strands within 'paired' strands, claudins adhere with each other in a homotypic as well as a heterotypic manner[22,23].

The last transmembrane component of tight junctions is JAM (junctional adhesion molecule; ~40 kDa)[24]. There are three JAM-related proteins[25,26], which belong to the immunoglobulin superfamily: they have a single transmembrane domain and their extracellular portion is thought to be folded into two immunoglobulin-like domains (FIG. 4c). Preliminary freeze-fracture replica electron microscopy revealed that exogenously expressed JAM does not reconstitute tight-junction strands in L transfectants and that it associates laterally with the claudin-based backbone of tight-junction strands in epithelial cells. JAM is involved in cell–cell adhesion/junctional assembly of epithelial/endothelial cells[24,25,27,28] as well as in the EXTRAVASATION of MONOCYTES through endothelial cells[24], but our knowledge on its function is still fragmentary.

A ziplock with diversified permeability

Tight junctions vary in tightness in a tissue-dependent manner[2,3]. Their tightness can be directly measured as TRANSEPITHELIAL ELECTRIC RESISTANCE (TER). The number of tight-junction strands was found to correlate well with the TER values of tight junctions in various tissues[29,30]. For example, in the kidney, epithelial cells of the proximal and distal tubules have 1–2 and 4–7 tight-junction strands, respectively, and the epithelial cells of the distal tubules have a much higher TER than those of the proximal tubules. However, exceptions to this correlation have also been reported[31,32]. For example, the two existing strains of Madin–Darby canine kidney (MDCK) epithelial cells — MDCK I and MDCK II — show marked disparity in their TER. Stevenson *et al.* reported that MDCK I cells have a 30–60-fold higher TER than MDCK II cells, but the number of tight-junction strands in these strains is very similar[33]. These observations indicate that individually paired tight-junction strands vary not only in number, but also in quality.

The number of strands. The number of tight-junction strands is an important factor in determining the barrier properties of tight junctions, but the molecular mechanism underlying regulation of the strand number remains unknown. When MDCK I cells, which express claudin-1 and claudin-4, were specifically depleted of claudin-4, a marked decrease was observed in the number of tight-junction strands and in their barrier function[34]. Mice lacking claudin-11/OSP, which is expressed specifically in oligodendrocytes and Sertoli cells in wild-type mice, were recently generated, and in these mice tight-junction strands were absent in MYELIN SHEATHS as well as in Sertoli cells[35]. Furthermore, when claudins were overexpressed in L fibroblasts, a large network of tight-junction strands was formed[21]. In addition, overexpression of occludin in MDCK cells was also shown to cause a slight increase in the number of tight-junction strands[36]. These findings indicate that the number of tight-junction strands might be determined by the total amount of expressed claudins (and occludin) in individual cells. However, the regulation of the number of tight-junction strands is probably more complicated. In epithelial cells that already express claudins, overexpression of claudins did not lead to an increase in the number of tight-junction strands[37], indicating that an upper limit might exist. Interestingly, when a claudin-1 mutant that cannot bind the underlying cytoskeleton was overexpressed in MDCK cells, aberrant tight-junction strands were formed[37]. This finding indicates the possible involvement of the underlying cytoskeleton in the regulation of the tight-junction strand number, but how the upper limit is set remains a mystery.

In addition to the strand number, the complexity of the network pattern might also be an important factor for determining the barrier properties of tight junctions[30].There were marked differences in the network

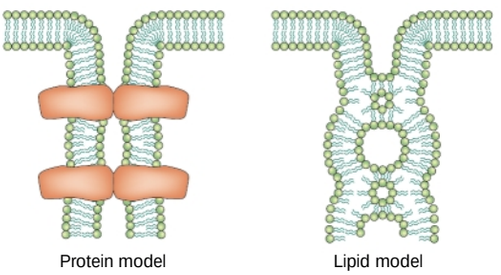


Figure 3 | **Protein versus lipid models.** In the protein model, tight-junction strands represent units of integral membrane proteins polymerized linearly within lipid bilayers, whereas in the lipid model inverted cylindrical lipid micelles constitute tight-junction strands.

TRANSCELLULAR TRANSPORT
Transport of macromolecules across a cell, including transport through channels, pumps and transporters, as well as transcytosis (endocytosis of a macromolecule at one side of a monolayer and exocytosis at the other side).

FREEZE-FRACTURE REPLICA ELECTRON MICROSCOPY
An electron-microscopic method that uses metal replicas to visualize the interior of cell membranes. This technique provides a convenient way to visualize the distribution of large integral membrane proteins as intramembranous particles in the plane of a membrane.

ANASTOMOSIS
Cross-connection between adjacent channels, tubes, fibres or other parts of a network.

P AND E FACE
When membranes are freeze-fractured, fracture planes run between the cytoplasmic and extracytoplasmic leaflets of plasma membranes, giving the P- or E-face images of membranes. The P (protoplasmic) face is the inner leaflet viewed from the outside, whereas the E (extracytoplasmic) face is the outer leaflet viewed from the inside.

IMMUNO-REPLICA ELECTRON MICROSCOPY
A form of electron microscopy, combining freeze-fracture replica electron microscopy and immune labelling of proteins.

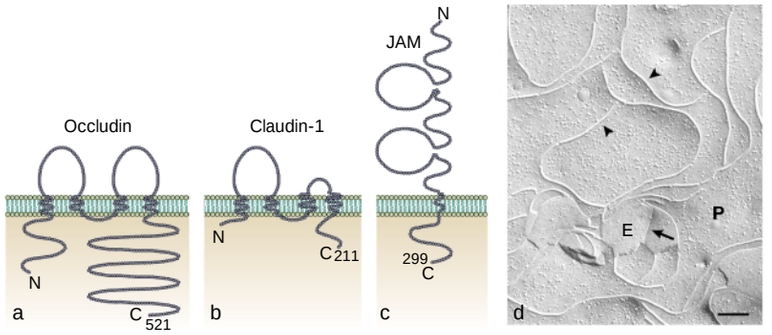


Figure 4 | **Integral membrane proteins localized at tight junctions. a** | Occludin has four transmembrane domains with two extracellular loops. The first loop is characterized by a high content (~60%) of glycine and tyrosine residues. **b** | Claudin-1 also has four transmembrane domains, but shows no sequence similarity to occludin. Note that the cytoplasmic tail of claudin-1 is shorter than that of occludin. **c** | Junctional adhesion molecule (JAM) has a single transmembrane domain, and its extracellular portion bears two immunoglobulin-like loops that are formed by disulphide bonds. **d** | Freeze-fracture replica electron microscopy of L cell transfectants expressing exogenous claudin-1. At cell–cell contact planes, a huge network of tight-junction strand-like structures was reconstituted; strands (arrowheads) on the P face and complementary grooves (arrows) on the E face. Scale bar, 100 nm.

patterns of tight junctions reconstituted by expression of different types of claudin in fibroblasts. For example, claudin-1-induced tight-junction strands form a large network with frequent ramifications[21], whereas claudin-11/OSP-induced strands scarcely branched, running parallel to each other[18]. This observation is likely to be relevant *in vivo*, as claudin-11/OSP-based tight-junction strands in myelin sheaths and Sertoli cells are mostly parallel with little branching[18,35]. It is tempting to speculate that the complexity of the tight-junction strand network is determined by the combination and the mixing ratio of the different claudins.

Extracellular aqueous pores. The extracellular portion of tight-junction strands probably functions as a ziplock to create a primary barrier against paracellular diffusion (FIG. 5). By comparing the TER and the morphology of tight-junction strands in various epithelia, Claude *et al.* found that as tight-junction strands increase in number, the TER value increases logarithmically[30]. To explain this relationship, the existence of aqueous pores, taking both open and closed states, was postulated within the paired tight-junction strands[5,30,38] (BOX 2). However, some exceptions to the relationship between the number of tight-junction strands and the TER value have been found[31,32]. The difference in tightness of individual tight-junction strands could be explained by the heterogeneity of aqueous pores in terms of their probability of being open or closed[33].

What is the chemical nature of these aqueous pores? Recent studies on hereditary hypomagnesemia have provided a clue to this question[39]. Most Mg^{2+} is resorbed from the urine through the paracellular pathway in the thick ascending LIMB OF HENLE, but in patients with hereditary hypomagnesemia, this reabsorption is reduced. Positional cloning identified claudin-16/paracellin-1 as the gene responsible for this disease. Consistently, claudin-16/paracellin-1 is exclusively expressed in the thick ascending limb of Henle. This finding indicated that claudin-16/paracellin-1 might form aqueous pores that function as Mg^{2+} paracellular channels.

The difference between MDCK I and MDCK II cells might be due to the expression of different claudins[40]: MDCK I express primarily claudin-1 and claudin-4, whereas MDCK II cells also express large amounts of claudin-2 in addition to claudin-1 and claudin-4. When claudin-2 was introduced into MDCK I cells, the TER value of these MDCK I transfectants fell to the level of MDCK II cells without any changes in the number of tight-junction strands. By contrast, exogenously expressed claudin-3 did not affect the TER value of MDCK I cells. Therefore, it is likely that claudin-2 constitutes aqueous pores with high conductance within paired tight-junction strands of MDCK II cells.

These findings led to the speculation that claudins not only form the backbone of tight-junction strands but also form extracellular aqueous pores, and that the

SERTOLI CELLS
A supporting cell of the mammalian testis that surrounds and nourishes developing sperm cells.

VISCERAL ENDODERM
Cells that delineate the yolk sac cavity together with parietal endoderm cells in the egg cylinder stage of the mammalian embryo.

L FIBROBLASTS
A mouse fibroblast line derived from connective tissue that does not show adhesion activity.

OLIGODENDROCYTES
A type of glial cell that forms and supports the myelin sheath around axons in the central nervous system of vertebrates.

EXTRAVASATE
Let or force out something from a vessel that naturally contains it.

MONOCYTES
Large leukocyte of the mononuclear phagocyte system found in bone marrow and the bloodstream. Monocytes are derived from pluripotent stem cells and become macrophages when they enter the tissues.

TRANSEPITHELIAL ELECTRIC RESISTANCE
Electric resistance across epithelial sheets, measured across the apical–basolateral axis of the cell.

Table 1 | Claudin gene family

Claudins*	Alternative names	Expression‡						
		Heart	*Brain*	*Lung*	*Liver*	*Kidney*	*Testis*	*Other*
Claudin-1	–	+	+	+	+	+	+	
Claudin-2	–	–	–	–	+	+	–	
Claudin-3	RVP1 (REF. 94)	–	–	+	+	+	±	
Claudin-4	CPE-R[95]	–	–	+	–	+	–	
Claudin-5	TMVCF[96]	+	+	+	+	+	+	Endothelial cells
Claudin-6	–	–	–	–	–	–	–	Embryonic tissues
Claudin-7	–	–	–	+	–	+	±	
Claudin-8	–	–	–	+	±	+	±	
Claudin-10	OSPL	ND	ND	ND	ND	ND	ND	
Claudin-11	OSP[97]	–	+	–	–	–	+	Oligodendrocytes, Sertoli cells
Claudin-14	–	–	–	–	+	+	ND	Hair cells in the Corti organ
Claudin-16	Paracellin-1 (REF. 39)	–	–	–	–	+	ND	Thick ascending limb of Henle

*Claudin-9, -12, -13, -15 and 17–24 have not been characterized well. ‡Detected by northern blotting[15,16,18,39,88]. ND, not determined.

MYELIN SHEATH
The sheath that surrounds the axons of vertebrate nerves to prevent the leakage of electric current. It is formed by Schwann cells in peripheral nerves and by oligodendrocytes in the central nervous system. These cells wrap up to 100 concentric layers of their plasma membrane in a tight spiral around the axons.

LIMB OF HENLE
U-shaped part of a nephron lying in the renal medulla. It comprises a thin descending tubule and an ascending tubule formed of both a thin and a thick segment. It has a role in the selective reabsorption of fluid and solutes.

HYPERPLASIA
The increase in the size of a tissue or organ, resulting from an increase in the total number of cells present. The part that is affected retains its normal form.

GAP JUNCTION
A junction between two cells consisting of pores that allow passage of molecules (up to 9 kDa).

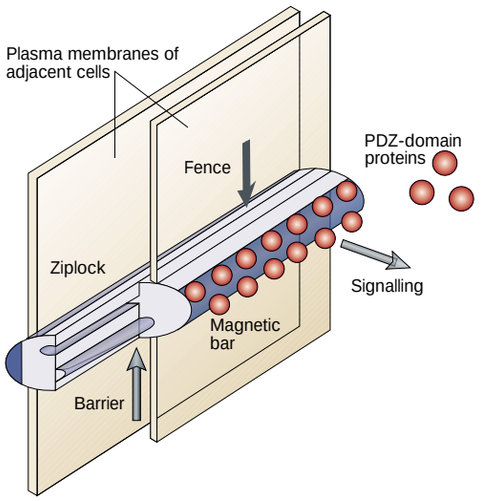


Figure 5 | **Multiple functions of tight-junction strands.** The extracellular and cytoplasmic portions of tight-junction strands function as a ziplock with diversified permeability (barrier function) and a magnetic bar attracting various PDZ-containing proteins (signalling function), respectively. Furthermore, within plasma membranes, the strand functions as a 'fence' to establish apical and basolateral membrane domains (fence function).

combination and the mixing ratios of different claudins determine the tightness of individual tight-junction strands[23]. But it is also possible that tight-junction strands are simply repeatedly broken and annealed, and that this contributes to the tightness of individual strands. So far, no information is available about the stability of strands, and the molecular details should be examined in future studies.

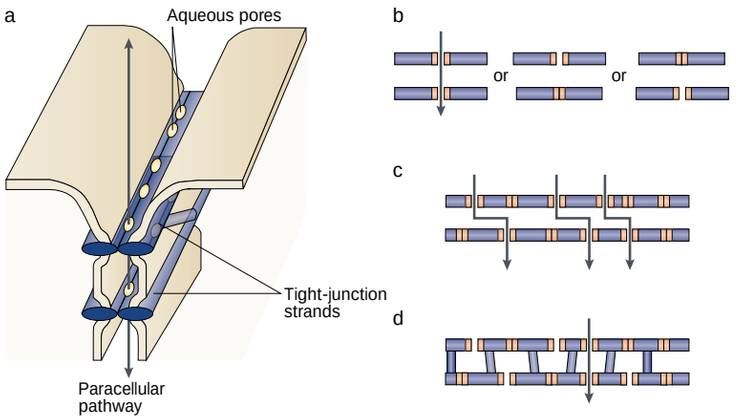
Occludin has also been shown to be involved in the barrier function of tight junctions, but at present, how occludin is involved remains unclear. Transfection of carboxy-terminally truncated occludin into MDCK cells removed endogenous occludin from tight-junction strands. In these transfectants, the TER did not decrease[41]. These findings contradict those of another study, in which endogenous occludin was removed from tight junctions by addition of synthetic peptides corresponding to the second extracellular loop of occludin to the culture medium. This resulted in a marked decrease in the TER, accompanied by an increase in paracellular flux[42]. Interestingly, overexpression of full-length as well as carboxy-terminally truncated occludin in MDCK cells did not affect the TER, but, paradoxically, increased the paracellular flux of mannitol[36,37,41]. Although the precise relationship between occludin and the TER is still a mystery, these observations indicate that occludin might contribute to the electrical barrier function of tight junctions and possibly to the formation of aqueous pores within tight-junction strands through which flux of non-charged solutes occurs.

These conclusions are compatible with those obtained from studies of occludin-deficient mice. These mice were born seemingly normal, but as they grew up, they began to show complex phenotypes, including marked growth retardation, chronic inflammation and HYPERPLASIA of the gastric epithelium, and mineral deposition in the brain[43]. Tight junctions in most organs of occludin-deficient mice that have been studied so far, such as intestinal epithelial cells, seem normal in terms of their morphology and TER. Although the paracellular flux of non-charged solutes has not yet been examined in these mice, it is possible that the defects found in these mice could be caused by the disappearance of occludin-dependent aqueous pores from tight-junction strands. Of course, it is also possible that occludin is important for other functions of tight junctions apart from regulating paracellular flux. These could be related to the fence function of tight junctions or to signalling events that take place at tight junctions, which could explain the pleiotropic defects observed in occludin-deficient mice.

A magnetic bar for PDZ-containing proteins

The thickness of tight-junction strands[6] (~10 nm; see FIG. 2a) is similar to the diameter of the GAP JUNCTIONAL channel (connexon), which consists of six connexin molecules that also have four transmembrane domains. Therefore, it is likely that, instead of being aligned in a single line to constitute tight-junction strands, claudins are packed more densely in the strands. It is then expected that the cytoplasmic surface of individual tight-junction strands appears as a toothbrush consisting of densely packed, numerous short carboxy-terminal cytoplasmic tails of claudins. In addition to these claudin tails, relatively long carboxy-terminal tails of occludin are probably intermingled.

Box 2 | Aqueous pores within tight-junction strands



Claude *et al.* found that transepithelial resistance (R_t) increases logarithmically with the number of parallel tight-junction strands (n) that must be crossed by a permeating ion in the transepithelial direction[29,30]. To explain this relationship, they postulated the existence of aqueous pores within tight-junction strands that fluctuate between open and closed states[30] **(a)**. If each parallel strand has one channel, and if the open probability (P_O) of all channels is the same, R_t would depend on P_O^{-n} **(b)**. However, along individual tight-junction strands, there would be a large number of channels, and under this condition R_t would increase linearly with n **(c)**. To maintain the exponential relationship, Cereijido and colleagues suggested compartmentalization, corresponding to the freeze-fracture replica images of tight-junction strand networks[38] **(d)**.

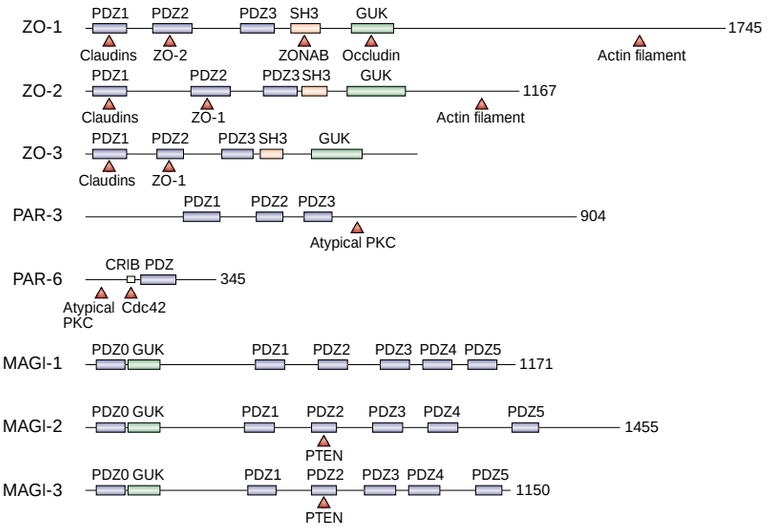


Figure 6 | **PDZ-containing proteins localized at tight junctions.** PDZ domains are represented by closed boxes, and the regions responsible for intermolecular association are indicated by arrowheads. Numbers show amino-acid positions. (GUK, guanylyl-kinase-like domain; MAGI, membrane-associated guanylyl kinase inverted; PKC, protein kinase C; PAR, partitioning defective; SH3, src homology region 3.)

PDZ DOMAIN
Protein–protein interaction domain first described in the proteins PSD-95, DLG and ZO-1.

POSTSYNAPTIC DENSITY
Higher-order protein structure present in postsynaptic membranes that functions to concentrate neurotransmitter receptors.

Many cytosolic proteins have been reported to associate with the cytoplasmic surface of tight junctions. As the first component of tight junctions, a peripheral membrane protein with a molecular mass of 220 kDa was identified through monoclonal antibody production, and was named ZO-1 (zonula occludens-1)[44]. When ZO-1 was immunoprecipitated from cell lysates of MDCK cells, two proteins with molecular masses of 160 kDa and 130 kDa were co-precipitated[45,46]. As these proteins were also localized at tight junctions, they were designated as ZO-2 and ZO-3, respectively. ZO-1, ZO-2 and ZO-3 have sequence similarity with each other[47–50]: they contain three PDZ DOMAINS (PDZ1, PDZ2 and PDZ3), one SH3 domain, and one guanylyl kinase-like (GUK) domain (FIG. 6).

The PDZ domain was initially reported to bind specifically to the carboxy-terminal Glu–Ser/Thr–Asp–Val motif, but it is now known that it recognizes more diverse four amino-acid sequences, most often ending in Val. Interestingly, most claudin tails have a Val at their carboxyl termini[15,16]; the only known exception is claudin-12. This indicates that these carboxyl termini might directly bind to PDZ domains. If so, the cytoplasmic surface of tight-junction strands might function as a magnetic bar that strongly attracts and recruits many PDZ-containing proteins (FIG. 5). Indeed, the PDZ1 domains of ZO-1, ZO-2 and ZO-3 were recently shown to bind directly to the carboxyl termini of claudins[51]. No notable differences were detected in the affinity of different claudins for PDZ1 domains. ZO-1, ZO-2 and ZO-3 also directly bind to the carboxy-terminal tail of occludin, but their GUK domains, not their PDZ domains, are involved in this interaction [50,52–54]. As ZO-1, ZO-2 and ZO-3 are localized to tight junctions in occludin-deficient mice, this interaction might not be essential for their recruitment to tight junctions[43,51]. Furthermore, JAM, which is concentrated around tight-junction strands, also ends in Val, and was recently shown to bind directly to ZO-1[55,56] and other PDZ-containing proteins[57]. So, it is possible that JAM is also involved in the recruitment of various PDZ-containing proteins to tight junctions.

In addition to ZO-1, ZO-2 and ZO-3, several PDZ-containing proteins are recruited to the cytoplasmic surface of tight junctions, but it remains unknown whether these proteins directly bind to the carboxyl termini of claudins (FIG. 6). Examples include MAGI-1/-2/-3 (MAGUK inverted -1/-2/-3)[58–61], and mammalian homologues of *C. elegans* PAR (partitioning defective) gene products, which function in asymmetric cell division[62–65] (FIG. 6). The list of PDZ-containing proteins localized at tight junctions will probably continue to increase. These proteins might function as adaptors at the cytoplasmic surface of tight-junction strands to recruit other proteins including cytoskeletal and signalling molecules (FIG. 6, TABLE 2).

Through the recruitment of various types of protein to tight junctions using PDZ-containing proteins, a huge macromolecular complex is probably formed at the cytoplasmic surface of tight-junction strands (FIG. 5). What are the physiological functions of this complex? First, as actin filaments bind to the carboxy-terminal portions of ZO-1 and ZO-2 (REFS 53,54,66), this complex probably crosslinks tight-junction strands to actomyosin cytoskeletons, and this interaction might have a role in the regulation of tight-junction functions. Interestingly, similar accumulation of PDZ-containing proteins occurs in the POSTSYNAPTIC DENSITY in neurons, where PDZ-containing proteins are directly involved in synaptic signal transduction and its regulation[67]. In cell–matrix adhesion, a huge macromolecular complex

Table 2 | Proteins lacking PDZ domains recruited to tight junctions

Proteins	Binding partners in tight junctions	Possible functions	References
Cingulin	JAM, ZO-1, ZO-2, ZO-3	Cross-linking tight junctions and the actomyosin cytoskeleton	98, 99
7H6 antigen		?	100
Symplekin		?	101
Heterotrimeric G proteins		Regulation of tight-junction formation	102
Atypical PKC (PKCζ/λ)	PAR-3, PAR-6	Serine/threonine kinase Regulation of epithelial polarization	62
ZONAB	ZO-1	Transcription factor Regulation of ErbB-2 transcription	103
huASH1		Transcription factor	104
Rab3b, Rab-13		Polarized vesicle transport	69, 70
Sec6/Sec8 homologues		Vesicle targetting	71
PTEN	MAGI-2, MAGI-3	Lipid phosphatase Tumour suppressor	60, 61

JAM, junctional adhesion molecule; MAGI, membrane-associated guanylyl kinase inverted; PAR, partitioning defective; PKC, protein kinase C; ZO, zonula occludens; ZONAB, ZO-1-associated nucleic-acid-binding protein.

is formed at INTEGRIN-based adhesion sites, which has crucial roles in extracellular-matrix-dependent signalling, but in this case, complex formation is not based on PDZ-containing proteins[68]. However, such macromolecular complexes are not well developed at CADHERIN-based cell–cell adhesion sites (adherens junctions). Therefore, it is tempting to speculate that the huge macromolecular complex formed at the cytoplasmic surface of tight junctions is central in the intercellular adhesion signalling of epithelial and endothelial cells, and is involved in the regulation of their proliferation, differentiation and polarization. In this context, it is interesting to point out that tight junctions recruit a tumour suppressor gene product (PTEN)[60,61], cell-polarity-related gene products (PAR-3, PAR-6, cdc42)[63–65] and vesicular-transport-related proteins (Rab3b, Rab13, Sec6/Sec8 products)[69–71].

A fence within the plasma membrane

Tight-junction strands are heteropolymers of integral membrane proteins, occludin and claudins, which are embedded within the plasma membrane, and encircle the top of individual epithelial/endothelial cells to delineate the border between the apical and basolateral membrane domains. Therefore, it is likely that tight-junction strands act as a 'fence', limiting the lateral diffusion of lipids and proteins between the apical and basolateral membrane domains (FIG. 5).

The apical membrane of epithelial cells is enriched in GLYCOSPHINGOLIPIDS and SPHINGOMYELIN[72,73]. Interestingly, this membrane has a striking asymmetric organization of lipids across the lipid bilayer, and glycosphingolipids, as well as sphingomyelin, are concentrated in its outer leaflet[74,75]. Such polarized localization of lipids indicates the possible existence of a diffusion barrier, especially for the lipids in the outer leaflet. This was confirmed experimentally. When fluorescently labelled lipids were inserted into the outer leaflet of the apical membrane of cultured epithelial cells, they remained on the apical surface. By contrast, fluorescently labelled lipids inserted into the inner leaflet of the apical membrane quickly redistributed to the basolateral surface[76,77].

It is reasonable to speculate that tight junctions restrict the lateral diffusion of not only lipids but also integral membrane proteins. As the intercellular space is completely obliterated at tight junctions, integral membrane proteins with extended extracellular portions could not cross tight junctions. However, it is also clear that, in addition to tight junctions, there are other mechanisms behind the asymmetric distribution of certain integral membrane proteins within plasma membranes[78]: the cytoskeletal proteins underlying the plasma membranes can restrict the lateral diffusion of proteins within membrane domains, and the targeted delivery of exocytic vesicles is also important. Early studies showed that the disruption of intercellular junctions (for instance, by incubation with low-calcium medium) resulted in the intermixture of membrane proteins from the apical and basolateral domains[79,80]. In another study, the correct polarization of a basolateral protein depended on cell–cell junctions, whereas that of an apical protein did not[81]. However, in these experiments, not only intercellular junctions but also cytoskeletal organization were affected. So, it is difficult to conclude that tight junctions act as a diffusion barrier for membrane proteins. In a more recent study, expression of constitutively active RhoA and Rac1 small GTPases in MDCK cells resulted in the disorganization of tight-junction-strand networks as well as the disruption of the junctional fence for lipids but not for integral membrane proteins[82]. So, the importance of tight junctions in the asymmetric distribution of integral proteins remains controversial.

When carboxy-terminally truncated occludin was overexpressed in cultured MDCK cells, fluorescently labelled sphingomyelin added to the apical membrane domain was redistributed to the basolateral surface[41]. The polarized distribution of integral membrane proteins did not seem to be affected. These findings suggest a possible involvement of occludin in the diffusion barrier, especially for lipids. The relationship between claudins and the diffusion barrier in epithelial cells has not yet been examined.

Future directions

Tight junctions have attracted a great deal of interest, but their study has been hampered by lack of information about tight-junction-specific integral membrane proteins. The recent identification of the main components of tight-junction strands has facilitated the molecular assessment of the morphological and physiological observations of tight junctions that have accumulated over years. On the basis of the accumulated information on occludin and claudins, we have discussed here several functions of tight-junction strands at the molecular level; the barrier, signalling and fence functions (FIG. 5). The challenge of answering many other questions lies ahead.

The identification of occludin and claudins raises many basic questions about the structure of the tight-junction strand itself. How are occludin and heterogeneous claudins arranged in individual tight-junction strands? To what extent are tight-junction strands dynamically polymerized and depolymerized? How is this regulated? Are some lipids required for the polymerization of occludin and claudins? How can tight-junction strands restrict the lateral diffusion of lipids only in the outer leaflet of the membrane? As the polymerization of integral membrane proteins in a linear fashion is unique, the elucidation of the basic physico-chemical properties of tight-junction strands might constitute one of the big challenges for years to come.

Several intriguing questions stand out at the cellular level. One of the most pressing questions concerns the molecular mechanism that underlies the polarized formation of tight junctions at the most apical region of the lateral membranes in epithelial cells. What is the relationship between tight junctions, adherens junctions and desmosomes during epithelial polarization? How are occludin, claudins, cadherins and their associated molecules integrated into the polarized junctional

INTEGRINS
A large family of heterodimeric transmembrane proteins that act as receptors for cell-adhesion molecules.

CADHERINS
Calcium-dependent adhesion molecules that mediate homophilic adhesions. There are several subfamilies of cadherin.

GLYCOSPHINGOLIPIDS
Any compound containing residues of a sphingoid and at least one monosaccharide.

SPHINGOMYELIN
Any of a class of phospholipids in which the amino group of sphingosine is in amide linkage with a fatty acid, and the terminal hydroxyl group of sphingosine is esterified to phosphorylcholine.

HETEROTRIMERIC G PROTEINS
Components of receptor-mediated activation or inhibition of adenylyl cyclase and other second messenger systems.

MITOGEN-ACTIVATED PROTEIN KINASE CASCADE
Signalling cascade that relays signals from the plasma membrane to the nucleus. MAPKs, which represent the first step in the pathway, are activated by a wide range of proliferation- or differentiation-inducing signals.

complex during epithelial polarization? It has been hypothesized that ZO-1, ZO-2 and ZO-3 might recruit tight-junction proteins such as claudins and occludin to their final destination at the interface between the apical and basolateral membrane domains, but compelling evidence is lacking so far.

Another outstanding issue concerns the regulation of the tight-junction barrier. As indicated above, tight junctions vary in tightness in a cell-type-dependent manner. This tightness is also dynamically and finely regulated in individual cells, depending on various physiological and pathological requirements[2,3] (BOX 1). The information of the molecular mechanism underlying these regulations is still fragmentary, but several signalling pathways such as serine/threonine phosphorylation, tyrosine phosphorylation, HETEROTRIMERIC G PROTEINS and small G proteins are thought to be involved in their regulation[83]. The transcription of occludin was reported to be downregulated by tumour necrosis factor-α and interferon-γ[84] and/or by activation of the MITOGEN-ACTIVATED PROTEIN KINASE CASCADE[85,86], but there is no information available about the transcriptional regulation of claudins by these or other signalling pathways. The cytoplasmic tail of occludin was shown to be heavily phosphorylated on serine and threonine residues[87], whereas the phosphorylation of claudins has not yet been examined.

Finally, another important challenge for future studies of tight junctions is to examine their possible involvement in various diseases. As mentioned above, mutations in claudin-16/paracellin-1 were shown to cause hereditary hypomagnesemia[39]. Furthermore, recent positional cloning identified claudin-14 as the gene responsible for hereditary deafness[88]. This claudin species is expressed in hair cells in the cochlea of the inner ear. As tight junctions in these cells have crucial roles in the establishment of two compositionally distinct compartments in the inner ear, mutations in the claudin-14 gene would cause deafness. In addition to hereditary diseases, claudins seem to have something to do with various pathological conditions including inflammation[89]. Furthermore, the involvement of occludin[86] as well as claudins[90] in tumorigenesis has been suggested in recent years.

We are only just beginning to understand the functions of tight junctions in molecular terms. Our picture of the molecular architecture of tight junctions remains incomplete, and other important constituents need to be identified. Further development of the molecular biology of tight junctions will lead to a better understanding of their functions, not only in normal physiology, but also in disease.

Links

DATABASE LINKS occludin | claudin-1 | claudin-2 | claudin-5 | connexin | claudin-11 | JAM | claudin-4 | hereditary hypomagnesemia | claudin-16 | claudin-3 | ZO-1 | ZO-2 | ZO-3 | SH3 domain | GUK domain | PDZ domain | claudin-12 | MAGI-1 | MAGI-2 | PTEN | PAR-3 | PAR-6 | cdc42 | Rab3b | Rab13 | Sec6 | Sec8 | tumour necrosis factor-α | interferon-γ | hereditary deafness

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