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Tight Junctions

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1. Introduction

Epithelial cells cover the body (e.g. skin), cavities (e.g. stomach, uterus, bladder) and ducts (e.g. renal tubules, intestine) of multicellular organisms, and thus constitute the frontier between the individual and the external milieu. In areas that withstand strong mechanical or chemical stress (e.g. skin, esophagus, cornea, vagina) epithelia are stratified, whereas in the rest of the body, the epithelia independently of their morphology (e.g. columnar of the intestine, tubular of renal tubules, squamous of the lung), are organized in monolayers (Fig 1A). Epithelia protect the tissues that lie beneath, from microorganisms, toxins, trauma and water evaporation, and regulate the exchange of substances between the content of body cavities and ducts and the underlying tissues. Transport across epithelia occurs through the transcellular and paracellular pathways and requires the presence in epithelial cells of two basic features: a polarized plasma membrane and tight junctions (Fig. 1B).

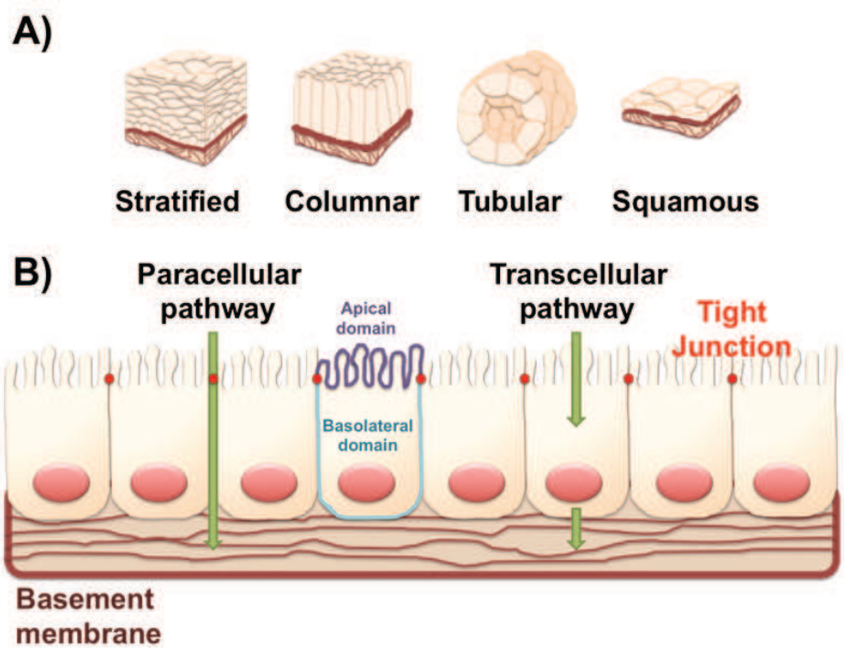

Fig. 1. Schematic representation of epithelia and transport pathways across a monolayer.

2. What are tight junctions?

Tight junctions are cell-cell adhesion structures present in epithelial cells at the limit between the apical plasma membrane that faces the exterior environment or the lumen of cavities and ducts, and the basolateral plasma membrane in contact with the internal milieu.

Tight junctions were first observed by transmission electron microscopy in thin sections of epithelial cells, as points of cell contact where the exterior membrane leaflets of the neighboring cells appeared to fuse, occluding in consequence the paracellular space (Fig 2A). When the interior of the membrane is observed by physically breaking apart a frozen biological sample with the freeze fracture technique, the tight junction is observed as a network of linear fibrils or chains of particles, termed TJ strands located bellow the apical microvilli (Fig 2B). By immunofluorescence, tight junction proteins display a cell border distribution that forms a chicken fence pattern when the sample is observed from above (Fig. 2C), while dots concentrated at the uppermost portion of the lateral membrane are seen when the tissue is viewed from the side (Fig. 2D)

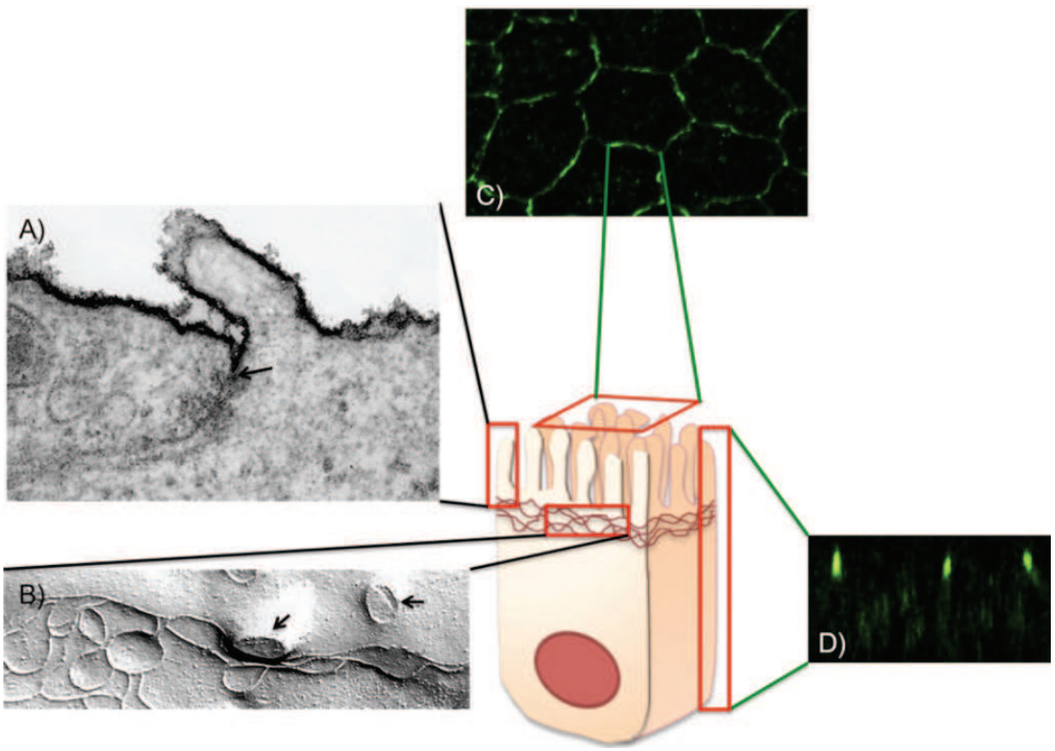

Fig. 2. Tight junction can be viewed by electron microscopy and immunofluorescence. A) By transmission **e**lectron microscopy, the tight junction appears in thin sections as points of cell-cell contact where the membranes of the adjacent cells appeared to fuse (arrow). Observe how the electrodense marker ruthenium red, added to the apical surface, does not stain the paracellular pathway bellow the tight junction. B) By freeze fracture, the tight junction appears as a network of strands bellow the apical microvilli (arrows). By immunofluorescence tight junction proteins give a chicken fence pattern when seen from above (C) while dots at the limit of the apical and the basolateral membrane are detected on a lateral view (D).

3. What are the canonical functions of tight junctions?

Tight junctions have two canonical functions that resemble those of a gate and a fence. The gate function refers to the capacity of tight junctions to regulate the passage of ions, molecules and water through the paracellular pathway. The gate function can be detected measuring the transepithelial electrical resistance (TER) of the tissue. The electric circuit in figure 3 shows how TER is the result of two resistances in parallel: the transcellular and the paracellular. The transcellular resistance is due to the resistance in series of the apical and the basolateral membranes. However since the electrical resistance across the plasma membrane is very high (2 GΩ) the current flows through the paracellular pathway regulated by the resistance offered by the tight junction. Therefore, the value of TER reflects the resistance of the tight junction. The gate function can also be evaluated by detecting the paracellular transit of molecules that are unable to cross through the transcellular pathway. Hence, these molecules must not be lipophilic, a target of carriers, pumps or co-transporters and subjected to a minimal degree of fluid phase endocytosis. When the observation is done by transmission electron microscopy, electrodense molecules like ruthenium red and lanthanum are chosen, whereas when the transit is evaluated across monolayers plated on transwell filters, molecules with fluorescent (e.g. FITC-dextran) or radioactive labels (^{3}H-mannitol) are used (Fig. 4).

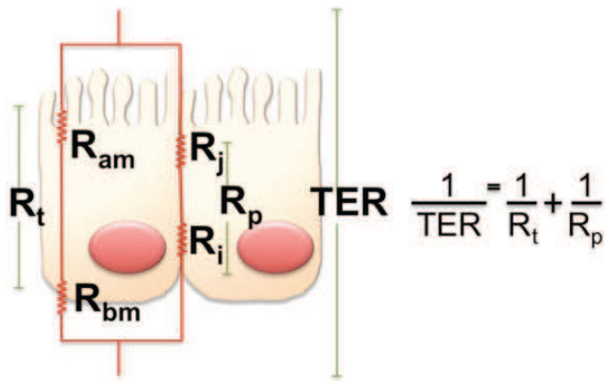

Fig. 3. Electrical circuit diagram and transepithelial electrical resistance equation of an epithelial monolayer. TER, transepithelial electrical resistance; R_t, transcellular resistance; R_{am}, apical membrane resistance; R_{bm}, basolateral membrane resistance; R_p, paracellular resistance; R_j, tight junction resistance; R_i, intercellular space resistance.

The fence function refers to the ability of tight junctions to restrict the movement of lipids and proteins within the membrane from the apical to the basolateral domains and vice versa. This function maintains the polarity of the plasma membrane and thus allows the vectorial transit of molecules across epithelia. The fence function of tight junctions is evaluated in monolayers cultured in transwell filters, by inserting a fluorescent lipid (e.g. fluorescent sphingomyelin) into the apical membrane and detecting if the fluorescent label reaches the basolateral membrane, or by chemically biotinylating the external domains of membrane proteins present at one of the cell surfaces (Fig. 4). In the latter, after generating a cell lysate, the polarized distribution of a particular membrane protein is detected by an immunoprecipitation with a specific antibody for the target protein, followed by a western blot with labeled (e.g. horse radish peroxidase or fluorescein) streptavidin, a molecule with an extraordinarily high affinity for biotin.

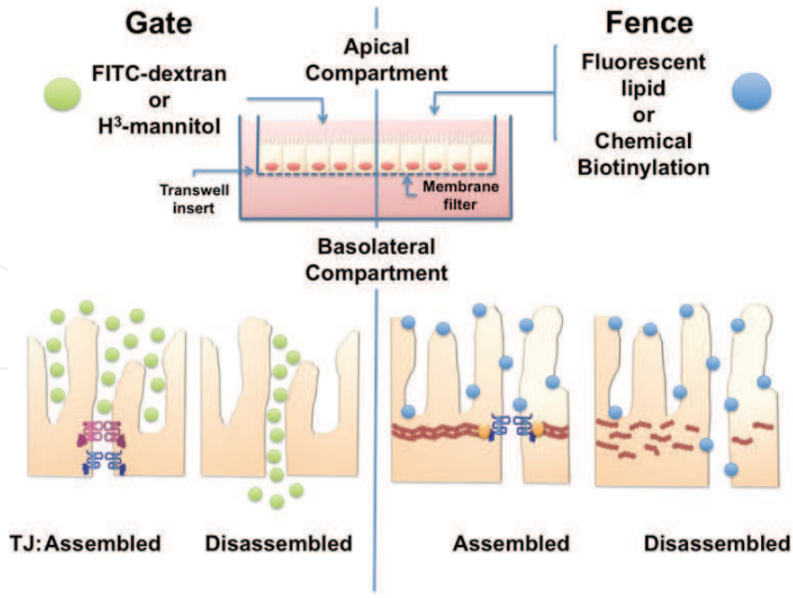

Fig. 4. The tight junction functions as a paracellular gate and a membrane fence. The gate function of the tight junction can be evaluated by measuring the passage of paracellular tracers from the apical to the basolateral compartment, whereas the fence function is determined by evaluating the free diffusion in the membrane plane of incomporated lipids and chemical probes.

4. Tight junctions are key players in the blood-brain, blood-retinal and blood-testis barriers and in the packing of myelin sheets

4.1 The blood-brain and blood-spinal cord barrier

The blood brain barrier separates circulating blood from the brain extracellular fluid and hence provides an optimal medium for neuronal function and protects the brain from fluctuations in ionic composition that occur after a meal or exercise that could perturb synaptic signaling. The blood brain barrier relies on the tight junctions present in brain capillaries. The latter differ from those in other organs for having a low rate of fluid-phase endocytosis, the absence of fenestrations and the presence of "tight" tight junctions. These characteristics restrict ion and fluid movement between the blood and the brain, and rely transendothelial traffic on specific ion transporters and channels. The signals that induce brain endothelial cells to express non-leaky tight junctions result from the specific interactions between capillary endothelial cells and the surrounding perivascular astrocytes and pericytes (Fig. 5A). The blood-spinal cord barrier is similar to the blood brain barrier and prevents the free passage of cells and blood substances to the spinal cord.

4.2 The blood-retinal barrier

The blood-retinal barrier confers protection or "immune privilege" to the ocular microenvironment. It is integrated by two separate anatomical sites: 1) the inner blood retinal barrier formed by the tight junctions present in the capillaries of the retina, and 2) the outer barrier integrated by tight junctions present between the retinal pigment epithelial cells (Fig 5C) that separate the neural retina from the choroidal vasculature.

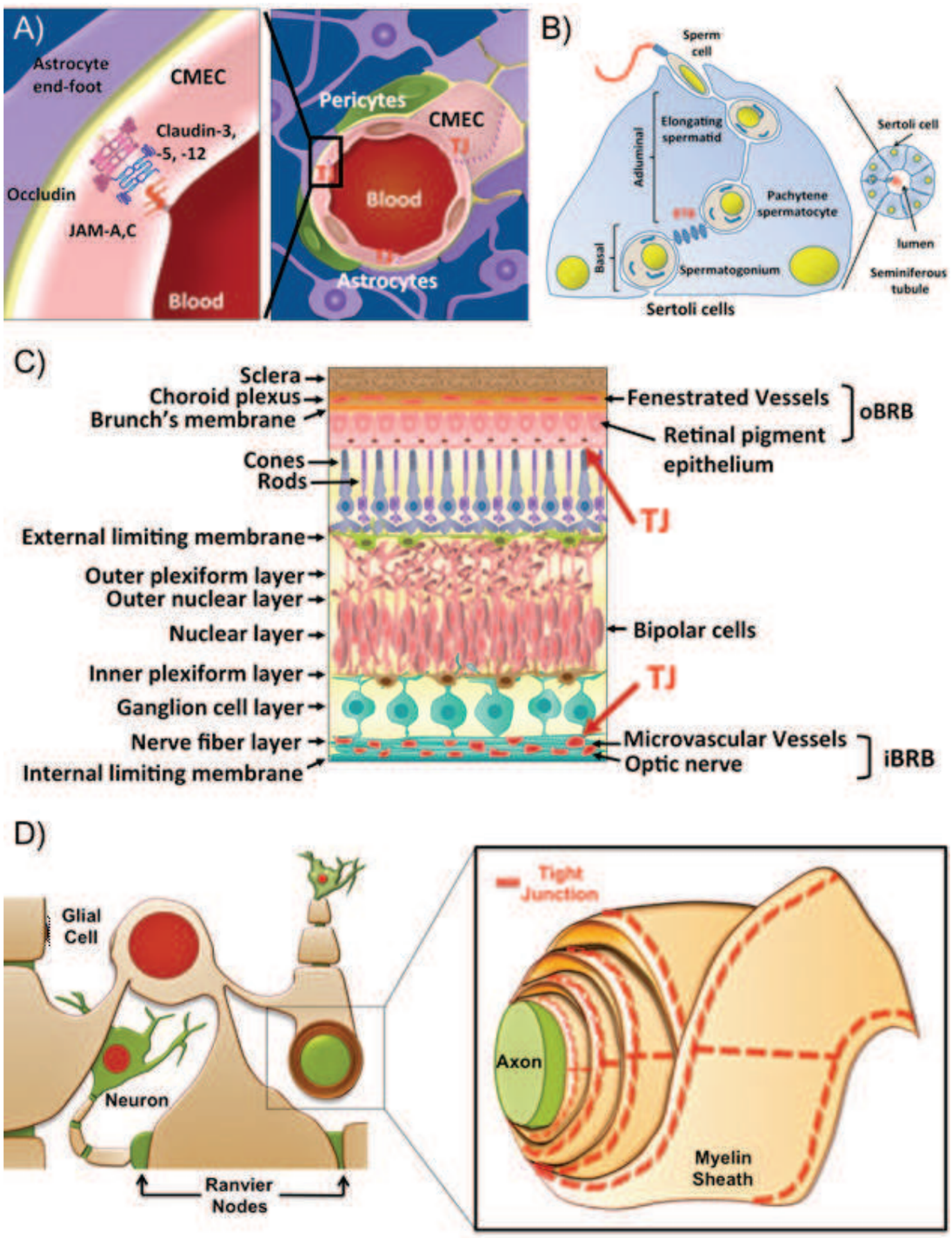

Fig. 5. Schematic representation of the blood-brain, blood-testis and blood-retinal barriers, and of the tight junctions present in myelinated axons. CMEC, cerebral microvascular endothelial cells; TJ, tight junction; oBRB, outer blood retinal barrier; iBRB, inner blood retinal barrier. A) Blood brain barrier B) Blood testis barrier C) Blood retinal barrier D) Glial cells tight junctions around myelin layers.

4.3 The blood-testis barrier

The tight junctions present between the epithelial cells of the testis known as Sertoli cells, form the blood testis barrier that divides the seminiferous epithelium into basal and adluminal compartments (Fig 5B). Germ cells at different stages of development move along the paracellular space of Sertoli cells in a basal to apical direction. Thus in the basal compartment the diploid spermatogonium and preleptotene spermatocytes are found whereas above the blood testis barrier, at the adluminal compartment, pachytene spermatocytes and round spermatids are present. Tight junctions of the blood testis barrier hence prevent the contact of systemic circulation with postmeiotic germ cells. This is important since spermatozoa and their surface antigens arise in puberty, long after self-tolerance is established in the fetus, and thus a compromise in the blood testis barrier could result in the generation of antibodies against the organism own sperm.

4.4 Tight junctions in myelinated axons

Myelinated axons are wrapped by continuous membrane layers derived from individual glial cells, the Schwann cells in the peripheral nervous system and the oligodendrocytes in the central nervous system (Fig 5D). Tight junctions mediate the adhesion among the successive layers of the myelin wrap. The compaction of these layers electrically insulates the axons and permits the saltatory conduction of action potentials that occurs by jumping from node to node, where axon insulation is interrupted.

5. Tight junctions have a complex molecular organization

Integral and peripheral proteins form tight junctions. The former are responsible for establishing cell-cell contact in the intercellular space, while the latter serve as a bridge between the integral proteins and the actin cytoskeleton. Peripheral proteins usually have multiple protein-protein binding domains that allow them to function as scaffolds for the attachment of a variety of signaling proteins (Fig. 6).

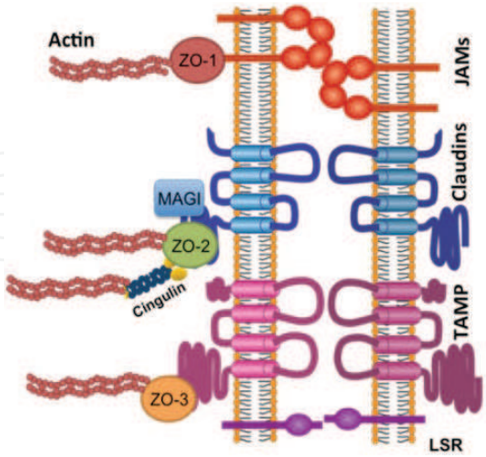

Fig. 6. Tight junctions are formed by a complex array of proteins. The integral proteins of the tight junction are single span like LSR and the family of JAMs, and tetraspan like the family of claudins and the TAMP proteins. Integral proteins establish cell-cell contact in the intercellular space. The peripheral proteins, act as a bridge between the integral proteins and the actin cytoskeleton

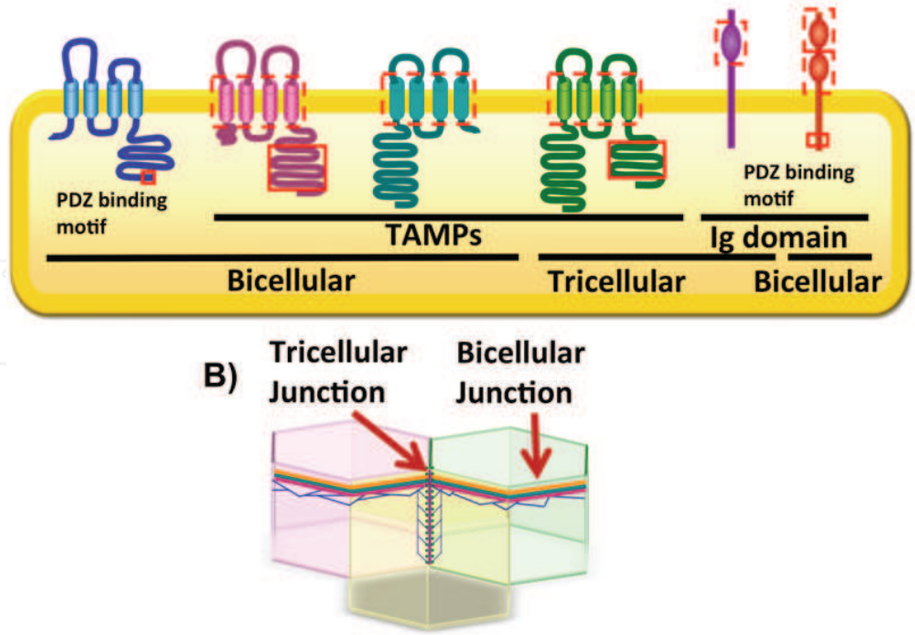

Fig. 7. Six different types of integral proteins constitute bicellular and tricellular tight junctions. A) Claudins and the TAMP proteins occludin, MarvelD3 and tricelullin are the tetraspan proteins of the tight junction. The latter three contain a marvel domain (boxed with discontinuous lines). Occludin and tricellulin share a highly homologous region in their carboxyl tail (boxed with continuous lines). LSR and JAM are single span proteins of the immunoglobulin family (Ig domains in boxes with discontinuous lines). B) Schematic drawing of the organization of bicellular and tricellular tight junctions. Tricellulin and LSR concentrate at the central sealing element of tricellular tight junctions, present at the corner where three cells meet, while the rest of the integral tight junction proteins are present in bicellular borders.

5.1 Integral tight junction proteins

The integral proteins of the tight junction establish cell-to-cell points of contacts or "kisses", where the outer leaflets of the membrane of apposing cells appear to fuse. There are two main classes of transmembrane proteins at the tight junction: the four and the single span proteins. The former include claudins, occludin, tricellulin and MarvelD3 and the latter are namely JAMs and LSR (Fig 7A). Occludin tricellulin and MarvelD3 are collectively called TAMPs (Tight junction associated MARVEL proteins) as they contain a conserved four transmembrane Marvel domain present in proteins concentrated in cholesterol rich microdomains and involved in membrane apposition and fusion events. Occludin and tricellulin carboxyl cytoplasmic tales are long and share a similar domain called ELL, whereas MarvelD3 has a short carboxyl segment and a long amino cytoplasmic domain similar to that in tricellulin. TAMPs have distinct but overlapping functions at the tight junction. JAMs and LSR belong to the immunoglobulin superfamily because they contain Ig domains which are regions of 55 to 75 amino acids separated by two cysteine residues that function as modules for protein-protein interaction. JAMs and LSR do not constitute tight junction strands and instead act as landmarks. Thus while LSR defines cell corners for

tricellular tight junction formation, JAMs are the first integral proteins to appear at the sites where bicellular tight junction will be assembled.

5.1.1 Claudins are integral proteins of the tight junction that regulate paracellular ionic selectivity

The word claudin derives from the Latin word "claudere" that means to close. Around 24 members comprise the claudin family in most vertebrates, although for example the puffer fish has 56 claudins. Since some claudin genes are closely linked, gene duplication is thought to have participated in the expansion of this family. In invertebrates like the fly and the worm *Caenorhabditis elegans*, claudin homologues have also been identified.

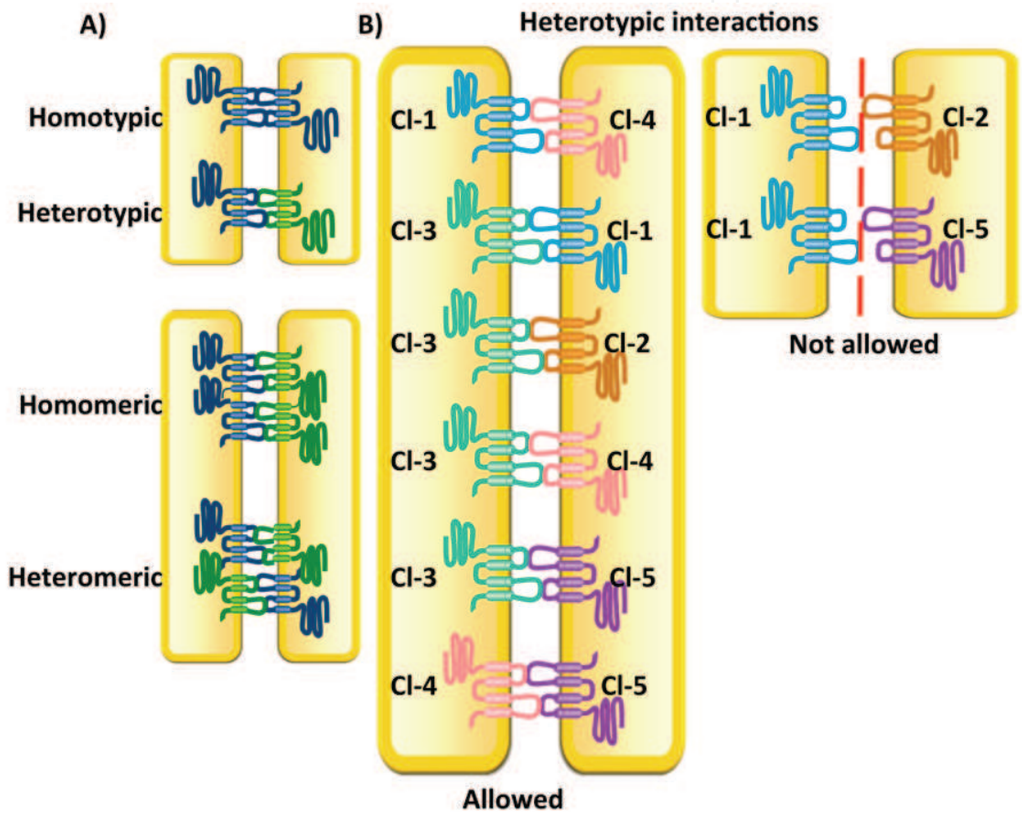

Fig. 8. Schematic representation of the interaction among claudins between neighboring cells. A) Between two cells, claudins establish homotypic and heterotypic interactions and dimerize in homomeric and heteromeric fashion. B) Drawing of the reported heterotypic interactions among claudins and of those proven not to occur.

Claudins like occludin are tetraspan proteins, yet they do not show any sequence similarity. Claudins size ranges from 20 to 34 kDa. The first extracellular loop of claudins is longer than the second one (around 52 Vs 16-33 amino acids) and contains a pair of cysteines that enhance stability by a disulfide bond, and charged amino acids that determine the ionic selectivity of the paracellular pathway. The second extracellular loop interacts with claudin molecules in the apposing cell membrane (trans-interaction) and participates in the side-to-

side oligomerization of claudins within the same membrane (cis-interaction). The cytoplasmic tails of claudins vary considerably among family members, constitute the site of posttranslational modifications that modulate junction tightness and stability, and end with two amino acids that form a PDZ binding motif that associates with adaptor proteins of the tight junction like ZOs and MUPP1.

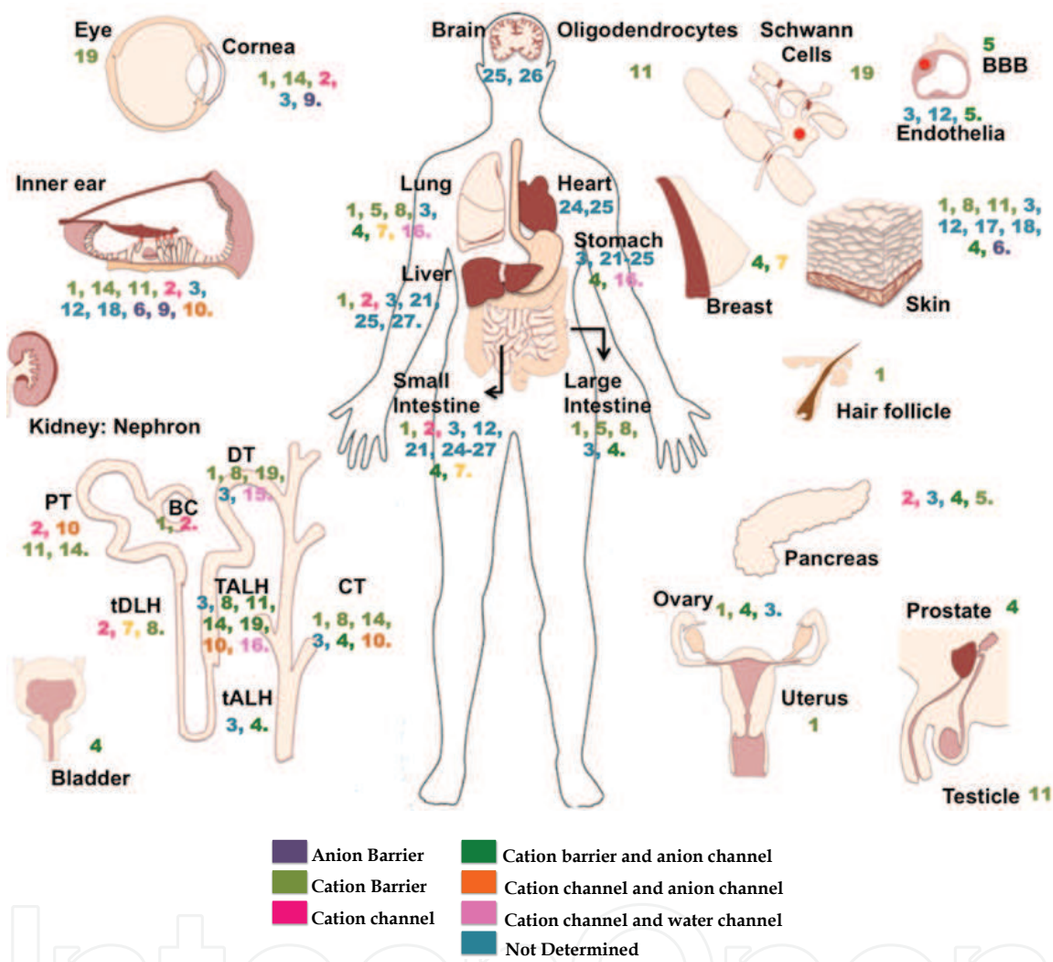

Fig. 9. Expression of different set of claudins in every tissue of the organism. BBB, blood-brain barrier; BC, Bowman capsule; PT, proximal tubule; tDLH, thin descending limb of Henle; tALH, thin ascending limb of Henle; TALH, thick ascending limb of Henle; DT, distal tubule; CT, collecting tubule.

Claudins are the building blocks of tight junction strands and upon transfection of claudins' cDNAs into cells that lack tight junctions like L-fibroblasts, well developed networks of filaments are formed, similar to *in situ* tight junctions. Claudins interact through homotypic and heterotypic associations and dimerize in homomeric and heteromeric manner (Fig. 8). Although all epithelial cells express claudins, each tissue exhibits a particular set of claudins, and some claudins are more ubiquitous than others (Fig. 9). For example claudins 1, 3 and 4 are present in a wide array of tissues, while claudin-5 is the dominant claudin in endothelia, and claudin-11 in ear stria vascularis basal cells, Sertoli cells and oligodendrocytes. The

expression of claudins is a dynamic process that responds to physiological and pathological conditions. For example, in the esophagus of subjects with reflux disease (Barrett's), a specialized columnar epithelium that expresses claudin-18 as the dominant claudin develops as replacement of the damaged squamous epithelia. Claudin-18 increases TER and reduces the paracellular permeability to H^+, making the columnar epithelium in comparison to the squamous epithelium, more resistant to acid. A somewhat similar case is found in the alveolar epithelium that after acute lung injury expresses claudin-4, a cation barrier forming claudin, as a mechanism to limit pulmonary edema. Another interesting example of a specific change in tight junction protein expression is observed in pouchitis, a remanifestation of the inflammatory bowel disease in the ileoanal pouch of patients that have undergone surgical treatment for severe therapy refractory ulcerative colitis. In these patients the expression in the pouch of claudin-1 decreases while claudin-2 increases. This change has a pathophysiological relevance as an elevated expression of the cation pore forming claudin-2 is observed in the colon of patients suffering from ulcerative colitis, Crohn's and celiac disease (Table 1).

Claudins might become major targets of drug development for electrolyte disorders since they regulate the ionic selectivity of the paracellular route due to their function as cation barriers (claudins 1, 4, 5, 8, 11, 14 and 19), cation channels (claudins 2, 10b, 15 and 16), anion barriers (claudins 6 and 9) and anion pores (claudins 4 and 10a) (Figure 9). It is noteworthy that claudin-18 forms paracellular water channels that mediate paracellular water transport in leaky epithelia and that the presence of certain claudins regulates the absorption of particular ions. In this respect it should be mentioned that claudin-16 was the first claudin identified as a paracellular channel, however recent data indicate that claudin-16 instead of being a channel for Mg^{2+}/Ca^{2+} as initially reported, forms a non-selective paracellular cation channel, whose absence results in a collapse of the transepithelial voltage, which is the driving force for Mg^{2+} and Ca^{2+} absorption in the thick ascending loop of Henle.

The intestinal tract and the nephron display specific expression of claudins in each of their respective segments. Thus in the proximal portion of these organs, namely duodenum and jejunum and the proximal tubule and thin descending limb of Henle's, a strong expression of paracellular channels like claudin-2 is observed, whereas in the more distal parts like the colon, distal tubule and collecting duct, more tightening claudins like 3, 4 and 8 are present (Figure 9).

The development of claudin knock out, knock down, transgenic, and mutated mice, with a particular phenotype, together with the identification of human and bovine hereditary diseases affecting the expression of claudins, has unveiled unique and non redundant role of certain claudins (Table 2). Hence it is observed that claudins 1 and 6 regulate the skin barrier in mice, whereas in humans, claudin-1 controls the permeability of liver biliary ducts. Claudin-5 is essential for the blood brain barrier, claudins 9 and 14 for the sensory epithelium of the cochlea, claudin-11 for the blood testis barrier and oligodendrocyte wrapping and claudins 2, 7, 16 and 19 for the renal reabsorption of ions. Claudin-15 promotes the proliferation of crypt cells of the small intestine and claudin-19 in mice is fundamental for Schwann cell wrapping, while in humans it is critical for the organization and development of the retina.

Tissue	Disease	TJ d Upregulation
Brain	Alzheimer's disease and vascular dementia	Occ, Cl-2, -5 and -11
	Cerebral aneurysm	
	Chronic inflammatory pain	Cl-3 and -5
	Edema	
	Epilepsy	
	Massive intracraneal hemorrhage and congenital cataracts.	
	Multiple sclerosis model (autoinmune encephalomyelitis)	
	Multiple sclerosis	Cl-1 and -5 *
	Rehumatoid arthritis (RA)	
Ear	Age related nonsyndromic deafness (DFNA51)	ZO-2 genomic duplicarion
	Nonsyndromic deafness (DFNB49)	
Esophagus	Barrett's esophagus	Cl-18
	Reflux esophagitis	Occ, Cl-1, JAM-A and ZO-1
Eye	Diabetic retinopathy	Cl-1 **
	Dry eye	
	Gelatinous drop-like corneal dystrophy	
	Retina pigmentosa	ZO-1
Intestine	Celiac disease	Cl-2, -3 and -4
	Collagenous colitis	
	Crohn's disease	Cl-2
	Non alcoholic fatty liver disease	
	Obstructive jaundice	Cl-4
	Pouchitis	Cl-2
	Type I diabetes	Cl-2
	Ulcerative colitis	Cl-2
Liver	Intrahepatic cholestasis	
	Necrotizing enterocolitis	
	Primary biliary cirrhosis	
	Primary sclerosing cholangitis	
Lung	Acute lung inflammation	
	Acute lung injury	Cl-4
	Asthma	
	Chronic alcoholic ingestion	Cl-5
	Sepsis	
Spinal cord	Amyotrophic lateral sclerosis (ALS)	
	ALS-mutant mice	
Vessels	Atherosclerosis	

* In peripheral blood leukocytes (PBLs), predominantly in B and T lymphocytes and monocytes.

** In retinal pigment epithelium (oBRB).

Table 1. Pathologies related to tight junction dysfunction.

Table 2. Genetic alterations of claudins that impact mice development and human or bovine health. B, bovine; BBB, blood-brain barrier; DFNB29, non syndromic deafness; FHHNC, familial hypomagnesemia with hypercalcuria and nephrocalcinosis; H, human; KD, knock-down; KO, knock-out; MT, mutation; NA, not available; ND, not determined; TAL, thick ascending limb of Henle; TG, transgenic; TMD, transmembrane domain.

Claudin	KO, KD, TG and MT mice phenotype	Hereditary hu
1	KO: lethal, loss of skin barrier	H: 2 bp deletion → absen ch
2	KO: Defective reabsorption of NA^+,Cl^- and H_2O at proximal tubule	
3	NA	
4	NA	
5	KO: lethal, permeable BBB	
6	TG: permeable skin barrier	
7	KO: lethal, renal salt wasting and dehydratation	
8	NA	
9	MT: Deafness	
10a	NA	
10b	NA	
11	KO: Male sterility, hind limb weakness and deafness.	
12	NA	
14	KO: deafness	H: Single nucleotide del protei H: T254A/V85D → disrupt →
15	KO: Megaintestine	
16	KD: FHHNC, no accumulation of Cl-19 at TAL	H B: chronic interstitial nep creatinin, ↑
17	NA	
18	NA	
19	KO: disorganized Schwann cells TJs, abnormal animal behavior and peripheral neuropathy KD: FHHNC, no accumulation of Cl-16 in TAL	H: FHHNC and s H: Mut G20D → disturba perinu H: Mut Q57E (within W-GI d H: Mut L90P → dis

5.1.2 Occludin and MarvelD3

From a group of monoclonal antibodies generated against a junctional fraction from chicken liver, three were selected for specifically recognizing an integral tight junction protein thereafter named occludin for the Latin word "occludere" that means to close up. Occludin has four membrane spanning domains and two loops with a high content of tyrosine and glycine residues exposed towards the extracellular space and flanked by a short amino and a large carboxyl terminal tail oriented towards the cytoplasm. The distal C-terminus of occludin forms a coiled-coil region essential for ZO-1 binding, while the amino tail associates to the E3 ubiquitin protein ligase Itch that regulates occludin degradation at the proteosome.

Transfection of occludin cDNA into L-fibroblasts induces the formation of only a small number of short strands, and is not only after the fibroblasts are co-transfected with claudin that well developed strands are formed, hence indicating that occludin is an accessory protein and not the main builder of tight junction strands.

The function of occludin has remained uncertain. On one hand the evidence indicates that occludin mediates adhesion at the tight junction since lowering the expression level of occludin, deleting the carboxyl tail of the protein, treating epithelia with peptides homologous to occludin extracellular loops and occludin endocytosis, disrupt the barrier function of tight junctions. In the other hand, the results with occludin knockout mice, reveal that the animals are viable with healthy epidermal, respiratory, renal and intestinal function but with small size, testicular atrophy, male infertility, gastritis, salivary gland dysfunction, thinning of compact bone and brain calcifications. These contrasting results indicate that although occludin is important for the establishment of cell-cell adhesion at the tight junction, other proteins can somehow replace its function. Thus it seems that the tretraspan proteins MarvelD3 and tricellulin, that respectively concentrate at bicellular (where two cells meet) and tricellular (where three cells meet) tight junctions, can partially compensate for occludin loss. In this respect for example it is observed that tricellulin is displaced to bicellular tight junctions upon occludin knockdown.

Recent evidence suggests a role for occludin in growth regulation. Thus occludin regulates the directional migration of epithelial cells by promoting the leading edge localization of the polarity proteins aPKC, Par3 and PATJ, and controls cell cycle progression by regulating centrosome separation and mitotic entry. This capacity is due to occludin localization at centrosomes during interphase and occludin phosphorylation at serine 490.

By SDS-PAGE occludin appears as a set of bands between 62 and 82 kDa. The lower bands are detergent soluble and correspond to the protein that distributes along the basolateral membrane, while the higher bands are insoluble, highly phosphorylated in serine residues and belong to the form of occludin that concentrates at tight junctions. The carboxyl terminal tail of occludin is a phosphorylation target of novel PKCδ and casein kinases 2 and Iε, and of protein phosphatases 2A and 1 that respectively dephosphorylate occludin in threonine and serine residues. Through this action both phosphatases negatively regulate the assembly of tight junctions. In contrast, in retinal pigment epithelial cells, endothelia and in the blood brain barrier, tyrosine phosphorylation of

occludin, induced by hepatocyte growth factor, ischemia and shear or oxidative stress, impairs the barrier integrity of tight junctions, and this process is accompanied by a concomitant increase in the activity of c-Src and FAK tyrosine kinases. Interestingly, the *in vitro* phosphorylation of occludin carboxyl terminal tail by c-Src, diminishes the capacity of occludin to interact with ZO proteins.

5.1.3 Tricellulin and LSR are proteins present in tricellular tight junctions

Freeze-fracture images show how at tricellular tight junctions, the most apical elements of the strands in bicellular tight junctions from both sides turn to and extend in the basal direction. Hence three pair of central sealing elements form a narrow tube in the extracellular space at the center of each tricellular contact. Short strands connect the bicellular tight junctions to the central sealing elements giving this structure an image that somehow resembles that of a fish skeleton placed upside down (Fig 7B).

Tricellulin and LSR concentrate in the central sealing elements of tricellular tight junctions. Tricellulin is a 65 kDa protein, structurally similar to occludin. Loss of the conserved carboxyl cytosolic domain of tricellulin is a cause of nonsyndromic deafness. Suppression of tricellulin expression compromises the barrier function of epithelial cells and overexpression of tricellulin increases the barrier towards ions and larger solutes. Tricellulin is excluded from bicellular tight junctions by occludin and is recruited to tricellular tight junctions by LSR, the receptor for triacylglyceride-rich proteins. The latter is a single span 585 amino acid protein with an extracellular Ig domain. LSR defines tricellular contacts in epithelial sheets, therefore LSR knockdown prevents the accumulation of tricellulin, while LSR accumulates at tricellular contacts even when tricellulin is knocked down.

5.1.4 JAMs are single span proteins important for tight junction assembly, cell migration, leukocyte transmigration, platelet activation and angiogenesis

JAM proteins constitute a family whose members exhibit two extracellular Ig domains, a single transmembrane region and a cytoplasmic tail with a canonical PDZ binding motif (Fig 7A). The family has two groups. One integrated by JAM-A, JAM-B and JAM-C with a short cytoplasmic tail of 45-50 residues and a type II PDZ binding motif, and another that includes CAR, ESAM, JAM4, CRTAM and BT-IgSF with cytoplasmic domains of 80-165 amino acids and type I PDZ domains. JAM-A is present in hematopoietic cells including monocytes, lymphocytes and red blood cells. Epithelial and endothelial cells exhibit JAM-A and CAR, whereas JAM-B, JAM-C and ESAM are expressed only in endothelial cells, and JAM4 and CRTAM only in epithelia. With the exception of JAM-B and CRTAM that localize along the lateral membrane and BT-IgSF that is present in neuron and glial cells, the rest of the proteins of the JAM family concentrate at the tight junction.

JAM proteins establish homophilic (e.g. endothelial JAM-A with platelet or endothelial JAM-A) and heterophilic interactions (Fig 10). The latter, with other members of the JAM family (e.g. endothelial JAM-B with endothelial or leukocyte JAM-C) and with other types of cell adhesion molecules such as integrins (e.g. endothelial JAM-A with integrin LFA-1 in leukocytes and integrin $\alpha v\beta 3$ in endothelial cells; endothelial JAM-B with integrin VLA4 in

leukocytes; and endothelial JAM-C with integrin MAC1 in leukocytes, and platelet JAM-C with integrins αxβ2 and MAC1 in leukocytes).

Crystal structural analysis of the extracellular regions of JAM-A reveals that the membrane distal V type Ig domain of two JAM proteins interconnect forming a U shaped cis homodimer, through a dimerization motif [R(V,I,L)E] also conserved in JAM-A JAM-B, JAM-C. These cis dimers are proposed to then interact between cells in trans, forming a zipper type seal (Fig. 10).

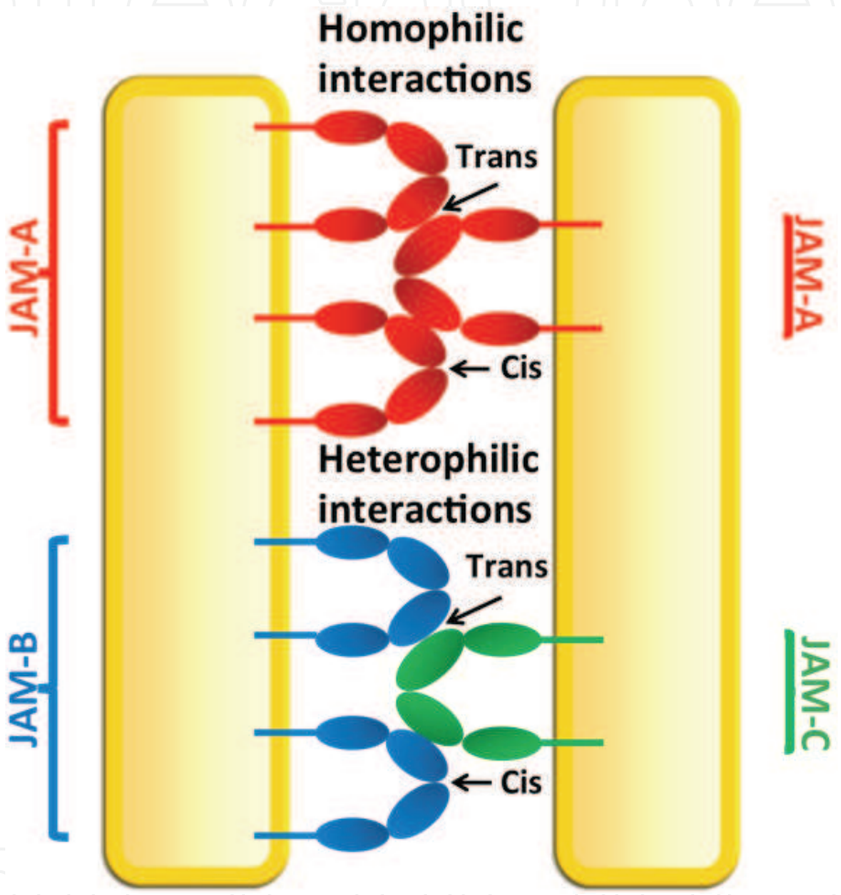

Fig. 10. Schematic representation of the homophilic and heterophilic interactions between JAM proteins. The scheme shows the homophilic interaction between JAM-A proteins and the heterophilic association between JAM-B and JAM-C. The cis interaction between the distal Ig domains of JAMs in the same cell forms a U shaped homodimer and the trans interaction between JAMs in neighboring cells forms a seal that resembles a zipper.

The function of JAMs is complex as they are important for tight junction assembly, cell migration, leukocyte transmigration, platelet activation, angiogenesis and virus binding.

Leukocytes bind JAMs present on the endothelial surface, adhere to the endothelia and then transmigrate interacting with JAMs proteins that form an adhesive "tunnel" at the paracellular pathway between endothelial cells.

JAMs are the first integral proteins to appear at tight junctions, where they tether other proteins to this location. Thus JAM-A transfected into fibroblasts promotes the localization of ZO-1, AF6, CASK and occludin to points of cell-cell contact, and in epithelial cells JAM association with Par3, tethers the complex Par3/Par6/aPKC to tight junctions. The correct location of this complex is crucial for the establishment of the apical-basal polarity of epithelial cells.

Decreasing or ablating the expression of JAMs has been another strategy recently employed to evaluate the roles of these proteins. Thus, for JAM-C, it was observed that most null mice die during postnatal development due to infections and that the surviving males are infertile and fail to produce mature sperm cells. This agrees with previous observations showing that in order for germ cells to move along Sertoli cells they need to establish various types of trans homo and heterodimers including that formed between JAM-C present on the spermatids and JAM-B present in Sertoli cells.

JAM-A knock out mice have a distinct phenotype characterized for an increased gastrointestinal permeability, colon neutrophil infiltration, increased colonic cell proliferation and a higher sensitivity for the development of experimental colitis when compared to wild type animals. JAM-A silencing in epithelial cell lines results in an impaired tight junction barrier function, an increased expression of the leaky claudins 10 and 15 and a concomitant decrease in claudin-1. Interestingly, the expression of JAM-A dominant negative mutants that are dimerization defective or lack the PDZ binding domain, reduces the rate of cell migration, which is important in endothelial cells for the promotion of angiogenesis and in epithelial sheets for the wound healing process. Apparently this is due to the fact that the down regulation of JAM-A reduces the level of active GTP-bound Rap1, which in turn reduces the stability of β1 integrin, which is necessary for cell migration. Similarly, it was observed that ESAM knock out mice have a retarded tumor growth that is associated to a diminished vascular density. This agrees with observations in ESAM null endothelial cells showing less migratory and angiogenic activity. These results hence indicate that ESAM is critical for blood vessel assembly.

5.2 Peripheral tight junction proteins

At the submembranous region of the tight junction more than 40 proteins have been identified. Some are signaling proteins that under certain circumstances concentrate at the tight junction. Such is the case of kinases, phosphatases, phospholipases, G proteins and transcription factors. In this section however we will only describe peripheral proteins essential for tight junction assembly and function. The latter can be classified based on the presence in their sequence of a domain known as PDZ. This domain establishes homotypic interactions with other PDZ domains and heterotypic associations with precise motifs (e.g. S/TXV or φXφ, where φ corresponds to a hydrophobic amino acid and X to any amino acid) present at the carboxyl terminal region of certain proteins. Some tight junction proteins like PAR6 contain a single PDZ domain, while others like PATJ and MUPP1 contain ten or more PDZ domains (Fig 11).

In addition to the PDZ domain, various peripheral proteins of the tight junction contain SH3 and guanylate kinase (GK) domains that serve as protein-protein binding domains with no

inherent catalytic activity. The presence of PDZ, SH3 and GK domains is the characteristic feature of proteins that belong to the MAGUK family. Among these proteins, ZO-1, ZO-2 and ZO-3, as well as Pals1, are present at the tight junction while other members of the MAGUK family localize at the adherens (e.g. Disc large) and synaptic (e.g. PSD95, SAP97 and Chapsyn-110) junctions. At the cytoplasmic region of the tight junction proteins named inverted MAGUKs or MAGIs are also present. These proteins contain six PDZ domains, a GK module and a WW domain instead of the SH3 region (Fig 11). Both the SH3 and WW domains establish molecular interactions with motifs with a precise proline consensus.

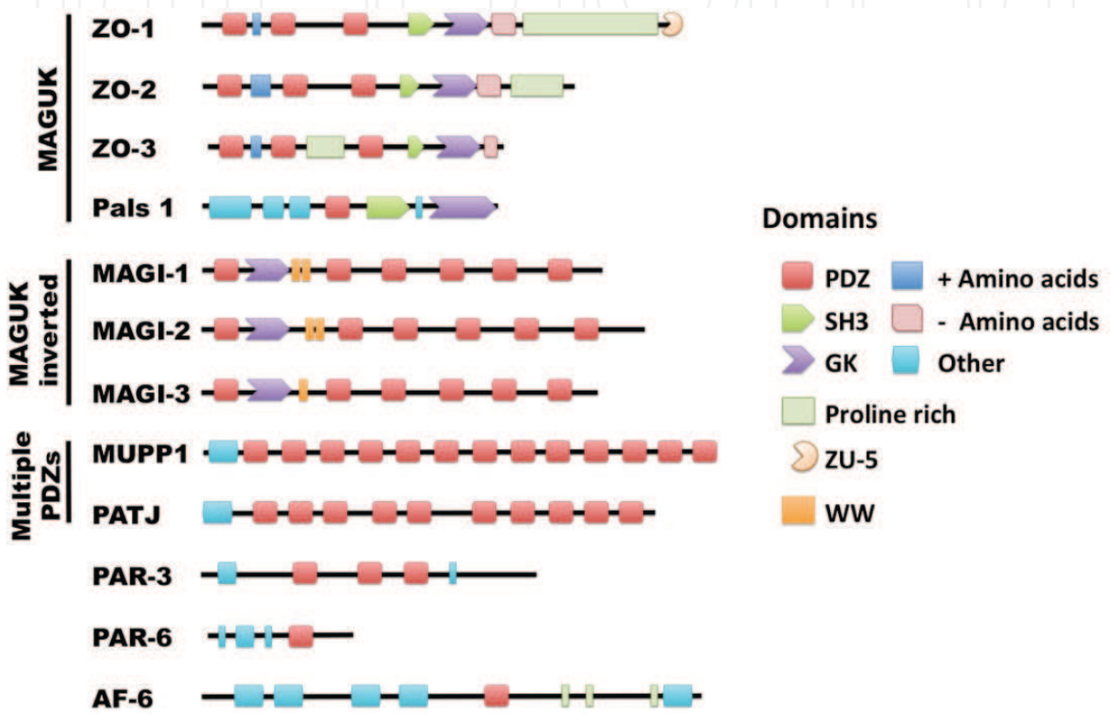

Fig. 11. Schematic representation of peripheral tight junction proteins with PDZ domains. Proteins that belong to the MAGUK protein family contain SH3 and GK domains in addition to PDZ modules. ZO proteins have 3 PDZ domains whereas Pals1 has a single one. MAGI proteins have an inverted organization since the GK domain is present before the majority of PDZ domains. MAGIs have WW domains instead of SH3. Multiple PDZ containing proteins include MUPP1 and PATJ that respectively have 13 and 10 PDZ domains.

In the peripheral tight junction proteins, the presence of multiple protein-protein binding domains like PDZ, SH3, WW and GK explains their function as molecular scaffolds for the formation of multiprotein complexes and for the linkage of the transmembrane proteins of the tight junction to the actin cytoskeleton.

5.2.1 ZOs are scaffolding proteins essential for claudin polymerization

The word ZO is an acronym of Zonula Occludens, the Latin name for tight junctions and number one in ZO-1 reveals that it was the first tight junction protein ever identified.

Although the genes of the MAGUK proteins MAGI and DLG are already present in unicellular protists, those of the ZO proteins are absent in unicellular organism. In the sponges, multicellular animals with no distinct embryonic cell layers and no true organs, the ancestral gene for ZO proteins named CARMA like is present and contains in addition to the PDZ, SH3 and GK domains a CARD region. The ZO gene first appears in the Placozoa, a flat multicellular animal that lacks tight junctions and instead exhibits adherens junctions. In *Hydra*, a Cnidaria with two embryonic cell layers, the endoderm and the ectoderm, and in the Bilateria *Drosophila* and *Caenorhabditis* that have the additional embryonic cell layer of the mesoderm, a ZO protein is present at the zonula adherens and no tight junction structure is distinguishable. In the Craniata an expansion of the gene gave rise to three ZO paralogues: ZO-1, ZO-2 and ZO-3 present at tight junctions. The ZO gene present in Placozoa, Cnidaria and Bilateria contains a ZU5 domain, similar to that present in Craniata ZO-1, hence suggesting that ZO-2 and ZO-3 arose as an expansion of ZO-1 that lost the ZU5 domain.

ZO proteins act as a bridge between the integral proteins of the tight junction and the actin cytoskeleton (Fig. 12). Thus through their first and third PDZ domains they respectively associate with claudins and JAMs, and by the SH3-GK region with occludin. ZOs interact with actin and actin binding proteins like 4.1, through a proline rich region that in ZO-1 and ZO-2 is located at the carboxyl segment, whereas in ZO-3 is present between PDZ-2 and PDZ-3. ZO-1 and ZO-2 also associate to myosin IIa.

ZO proteins have the capacity to form through their second PDZ domains homo and heterodimers. With respect to the latter, ZO-1/ZO-2 and ZO-1/ZO-3 but not ZO-2/ZO-3 interactions have been detected. ZO proteins also bind to other tight junction peripheral proteins like cingulin, and in the case of ZO-1 this association occurs through the GK domain.

In vertebrates, ZO proteins are not exclusively present in tight junctions. Thus in fibroblasts, and in epithelial cells, at the initial stages of assembly of the apical junctional complex, ZOs are detected at adherens junctions, where ZO-1 binds to afadin and α and γ catenins, ZO-2 to α-catenin and ZO-3 to afadin and p120 catenin. ZO proteins also interact with a wide variety of gap junction conexins.

ZO-1 and ZO-2 play a crucial role in the polymerization of claudins, as no tight junction strands are formed in ZO-1 knock out/ZO-2 knock down cells. They however appear to have a redundant role since the reintroduction of one protein or the other, provokes the reappearance of tight junction strands. Although claudins associate to the first PDZ domain of ZO-1 and ZO-2, a construct containing only the PDZ domains of ZO-1 (N-ZO-1) is incapable of inducing claudin polymerization, even when it is forcibly recruited to the plasma membrane by the introduction of a myristoylation sequence. Only after the addition of a homodimerizer, the N-ZO-1 segment is capable of producing the appearance of TJ strands throughout the lateral membrane. Instead, when a longer construct that includes the SH3-GK domain is used, claudins polymerize at the correct site, the limit between the apical and the lateral membrane. Taken together these results indicate that the dimerization of ZO-1 or ZO-2, mediated by PDZ-2, at the TJ region, determined by the SH3-GK domain, induces the polymerization of claudins.

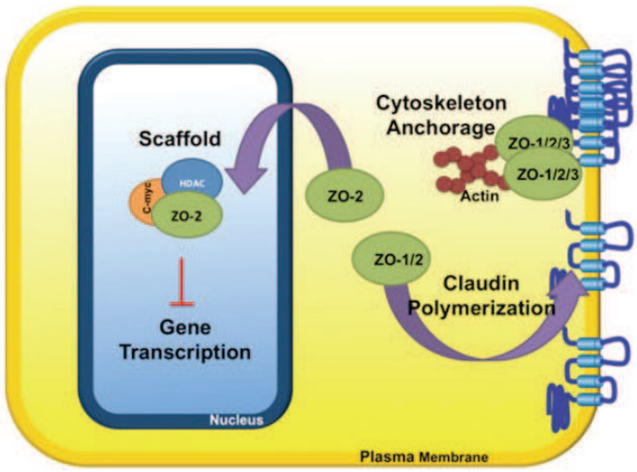

Fig. 12. ZOs form scaffolds that regulate claudin polymerization, integral proteins anchorage to the cytoskeleton and gene transcription. ZO-1 and ZO-2 dimerization and localization at the tight junction is required for claudin polymerization into strands. ZOs act as a bridge between the integral proteins of the tight junction and the actin cytoskeleton. At the nucleus ZO-2 associates to factors that regulate gene transcription.

During development ZO-1 and ZO-2 are essential as KO mice embryos are not viable. ZO-1 KO dies around embryonic stage 10.5 due to massive apoptosis at the neural tube, notochord and allantois and defective angiogenesis in the yolk sac. ZO-2 KO mice die shortly after implantation around E7.5 due to an arrest in early gastrulation. ZO-2 mice chimeras, obtained after injecting ZO-2 KO embryonic stem cells into wild type blastocyst, are viable, yet the males are infertile due to a defective blood testis barrier. Thus, indicating that ZO-2 is essential for the development of the extraembryonic tissue, and for the proper barrier function of tight junctions between Sertoli cells. In contrast, ZO-3 KO mice lack an obvious phenotype. This is interesting since in zebra fish ZO-3 KO embryos develop edema, loss of blood circulation, tail fin malformations and loss of the epidermal barrier.

In cultured epithelial monolayers, silencing of ZO-1 or ZO-2 retards tight junction formation and disorganizes the cortical ring of actin.

ZOs contain several putative nuclear localization (NLS) and exportation signals (NES). The four NES of ZO-2 are functional and the activity of NES-1 is regulated by PKCε phosphorylation. The presence of ZO-1 and ZO-2 at the nucleus is determined by the state of confluence of the culture. Thus, in sparse monolayers ZO-1 and ZO-2 concentrate at the tight junction and the nucleus, whereas in confluent cultures almost no nuclear staining of these proteins is detected. ZO-2 enters the nucleus at late G1 and departs at mitosis, thus explaining why in confluent quiescent cells the protein is absent from the nucleus. At the nucleus ZO-2 associates with nuclear lamina β1 and is distributed in speckles, rich in the essential splicing factor SC-35. ZO-1 and ZO-2 have the capacity to interact with transcription factors and to regulate gene transcription (Fig. 12). Thus ZO-2 associates with C/EBP, Jun and Fos transcription factors and inhibits the transcription of artificial promoters regulated by AP-1 sites, and through its association with c-myc negatively

regulates the transcription of cyclin D1. In accordance ZO-2 overexpression blocks cell cycle progression from G1 to the S phase of the cell cycle and inhibits cells proliferation. ZO-1 instead, through its SH3 domain, sequesters away from the nucleus, the Y box transcription factor ZONAB. Nuclear ZONAB stimulates cell proliferation by interacting with the cyclin D1 binding kinase CDK4, by promoting its nuclear accumulation and by inducing the transcription of CD1 and PCNA, a DNA replication and repair factor.

5.2.2 Cingulin and JACOP/paracingulin are tight junction proteins that regulate the activity of Rho GTPases

Cingulin is a 140 kDa protein exclusive of vertebrate tight junctions. Cingulin was identified with a monoclonal antibody raised against a preparation of chicken brush border myosin. As the immunofluorescence showed that the antigen localized as a belt below the apical surface it was named cingulin from the Latin word "cingere" meaning to encircle. Cingulin forms parallel homodimers, where each subunit consists of a large globular amino terminal head, a small globular carboxyl terminal tail and a coiled-coil rod domain homologous to the one present in conventional nonmuscle myosins (Fig 13). Cingulin is not an actin dependent motor protein but at least in vitro displays actin bundling activity. Through the head cingulin interacts with actin, myosin, ZO-1, ZO-2, ZO-3, afadin and the carboxyl terminal regions of JAM-A and occludin and through both rod and tail domains with non-muscle myosin II and ZO-3.

Disruption of cingulin gene does not prevent tight junction formation but alters the expression of other junctional proteins. Thus, in mouse embryoid bodies an increased expression of ZO-2, occludin and claudin-6 is observed concomitant with a decreased expression of ZO-1, whereas in MDCK cells, cingulin silencing increases protein levels of ZO-3 and claudin-2. The latter effect is mediated by an increment in RhoA activity. In this respect it is important to highlight that binding of cingulin at the tight junction to the guanine nucleotide exchange factor that activates RhoA named GEF-H1, results in downregulation of RhoA signaling and inhibition of cell proliferation.

Employing a monoclonal antibody against a chicken cytoplasmic antigen that localizes at the apical junctional complex, a protein similar to cingulin was identified and named JACOP for junction associated coiled-coil protein. Later, by searching EST sequences for their homologies to cingulin, a cDNA was identified and named paracingulin. JACOP and paracingulin are the same protein. JACOP/paracingulin is a 160 kDa protein with a domain organization similar to cingulin and 40% sequence identity. JACOP/paracingulin besides acting as a down regulator of RhoA through the recruitment of GEF-H1, promotes the activation of Rac1 by recruiting to junctions the GEF activator for Rac1 named Tiam1. In epithelial cells this is important since the acquisition of confluence is paralleled by a reduction of active Rho A levels and the activation of Rac1 and Cdc42, other members of the Rho family of small GTPases.

Unlike cingulin which is a tight junction specific protein, paracingulin localizes at both tight and adherens junctions. At the tight junction JACOP/paracingulin is recruited through ZO-1, whereas at the adherens junction it interacts with PLEKHA7, a protein that associates to the E-cadherin binding protein catenin p120.

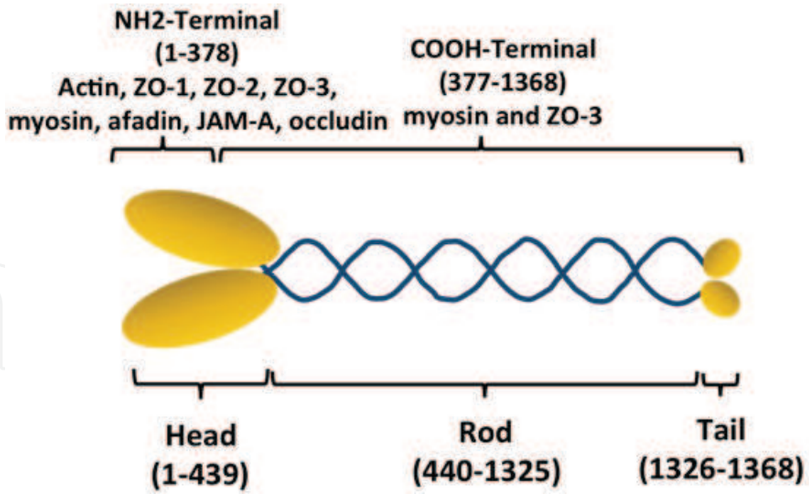

Fig. 13. Cingulin forms parallel homodimers. Each subunit of cingulin has a large globular amino terminal head, a small globular carboxyl terminal tail and a coiled-coil rod domain homologous to nonmuscle myosins.

6. Pathologies related to tight junction dysfunction

As table 1 shows, a wide array of pathologies affecting different organs are related to tight junction dysfunction. For example, the development of a leaky blood brain barrier is associated to various pathologies including stroke, cerebral aneurysms, cerebral edema, multiple sclerosis, epilepsy and Alzheimer disease. Frequently, the decreased expression of tight junction proteins precedes the onset of the clinical disease. Thus, brain edema surges as a consequence of impaired capillary endothelial cells; tight junction destruction is proposed to facilitate aneurism formation, and abnormal blood flow is thought to down regulate tight junction proteins, which leads to leakage of blood components into the central nervous system tissue and to the development of autoimmune diseases like multiple sclerosis and amyotrophic lateral sclerosis. Interestingly, the chronic ingestion of saturated fatty acids appears to generate blood brain barrier dysfunction enhancing the brain delivery of amyloid-β and exacerbating the amyloidogenic cascade that facilitates the development of Alzheimer disease. In other pathologies instead, a compromised blood brain barrier is a direct consequence of the disease. Such is the case of epilepsy, where seizures trigger the down regulation of tight junction proteins.

Disruption of the blood retinal barrier occurs in several blinding ocular diseases. For example in diabetic retinopathy breakdown of the inner blood retinal barrier is one of the initial alterations. Therefore strategies to revert the damage, have focused on the employment of drugs like the pseudo sugar derivative of cholesterol Sac-0601, the statin lovastatin, calcium dobesilate and rottlerin, that target VEGF, TNFα, MAPK and NF-κB, and PKCδ respectively, which are factors that promote tight junction protein breakdown in retinal endothelial cells.

In the intestine, the development of an impaired barrier function has two main consequences, leak flux diarrhea and the uptake of antigens that under normal conditions are prevented from entering the body, and that can aggravate or initiate inflammation as

well as autoimmune diseases. In pathological conditions of autoimmune origin like type I diabetes, ulcerative colitis, Crohn's and celiac disease, the transepithelial electrical resistance of the intestine is markedly reduced and an increase in the pore forming claudin-2 is generally observed.

Sometimes the failure of one organ leads to a major damage in another. For example, during acute liver failure, the generation of tumor necrosis factor α produces the disruption of the blood brain barrier and the consequent cerebral edema that leads to cerebral herniation and death, and non alcoholic liver failure and obstructive jaundice produce an increased intestinal permeability coupled to the loss of tight junction proteins.

Carcinoma	Claudins				TAMPs	JAMs	ZOs
	2	3	4	7			
Intestinal	↑	↑	↑	↑	↓		↑↓
Prostate	↓	↑	↑	↑	↓		↓
Renal		↑	↑	↑		↓	
Colorectal	↑↓	↑	↑	↑↓	↓		↓
Endometrial	↑↓	↑	↑		↓	↓	
Pleura	↓	↑	↑				
Uterus		↑	↑	↓			
Breast	↓	↑	↑↓		↓		↓
Gastric	↑	↑	↑↓	↑	↓		↑↓
Head and neck			↑	↑↓			
Nasopharingeal			↑	↓			
Tongue			↑	↑↓	↓		
Esophageal	↑	↑↓	↑↓	↑↓			
Ovarian		↑↓	↑↓	↑			
Pancreatic	↑	↑↓	↑↓	↑	↓		↑↓
Bladder			↑↓			↑	
Epidermoid			↑↓		↓		↑↓
Cervical	↑		↑↓	↑↓	↓		
Thyroid			↑↓	↑↓			↑
Lung		↓	↑↓	↑↓	↓	↓	↓
Biliary Tract	↓	↓	↑↓	↓	↓		
Liver	↑			↑↓	↓		↓
Melanoma				↓		↑	↑
Meningioma			↓				
Oral				↓			
Testis					↓		↓

↑ Upregulated
↑↓ Up/ Downregulated
↓ Downregulated

Table 3. Regulation of tight junction proteins in cancer

Tight junctions are lost during cellular transformation. Therefore it came as a surprise that while the expression of TAMPs decreases in all types of cancers studied, that of other tight junction proteins like JAMs and ZOs is up or down regulated, depending on the type of cancer, and the stage of development of the disease. In the case of certain claudins like 2, 3, 4 and 7 the result is more surprising as they are generally overexpressed in cancerous tissue (Table 3). Downregulation of tight junction proteins in cancer is easy to understand, as the loss of cell adhesion promotes the transformation of polarized polygonal cells into mobile elongated cells with invasive potential. Instead the upregulation of tight junction proteins in cancer is more difficult to comprehend. Several explanations can be given. For example, the

aberrant expression might reflect cytosolic and nuclear accumulation of tight junction proteins, in a scenario where these molecules could serve as oncogenic factors. In this respect, it should be mentioned that claudin expression increases migration and motility, and promotes activation of metalloproteinases.

7. Pathogenic organism that affect the tight junction

Epithelia block the entry of microorganisms into the body, hence it is not surprising to find that a variety of pathogenic microorganisms have evolved mechanisms to control tight junctions in order to obtain a gateway of access to the underlying tissue. Some pathogens use tight junction proteins as their receptors for attachment and subsequent internalization. Such is the case of hepatitis C virus with occludin, claudins 1, 6 and 9; reovirus and feline calicivirus with JAM-A, and Coxsackie and adenovirus with CAR.

Viruses promote their spreading through the disruption of endothelial or epithelial barriers. Some viruses disrupt epithelial tight junctions by sequestering PDZ containing proteins. Such is the case for example of cancer causing human papillomaviruses types 16 and 18, whose E protein that contains a PDZ binding motif, targets MAGI-1 for degradation producing mislocalization of ZO-1 and loss of tight junction integrity. In a similar fashion the small envelope protein E of SARS coronavirus, the causative agent of severe respiratory infections, has a PDZ binding motif that targets PALS1, a tight junction associated protein member of the Crumbs-PALS1-PATJ complex, to the endoplasmic reticulum-Golgi region, disrupting in consequence tight junctions and the respiratory tract epithelial integrity.

HIV virus proteins Tat and gp120 open the blood brain barrier, the blood retinal barrier and the tight junctions of the intestine and female genital tract, allowing the microbial translocation to the brain, eye and body interstitium.

Some viral proteins like the spike forming VP8 protein of rotavirus, modulates the gate and fence function of tight junctions in epithelial cells.

Other pathogens destroy tight junctions and as a result open the paracellular pathway (Fig 14). For example, enteropathogenic *Escherichia coli* injects into the cytoplasm of host cells, the bacterial receptor Tir that binds to the bacterial adhesin intimin, and the pathogenic protein EspF. The latter that contains PDZ binding motifs and proline rich domains which act as actin nucleation centers, produces dissociation of occludin/claudin-1/ZO-1 complexes from the cell borders and recruits these proteins to actin rich pedestals formed beneath the bacteria, due to the phosphorylation of Tir and the consequent activation of the N-WASP-Arp2/3 pathway. Other bacteria like *Listeria monocytogenes* and *Shigella flexneri*, the respective causative agents of listeriosis and shigellosis, also recruit ZO-1 to the distal portion of actin filaments that like comet tails associate through, N-WASP or its *Listeria* homologue ActA, to the internalized bacteria.

Several bacterial toxins disassemble tight junction proteins. For example, lipopolysaccharide, the main component of the cell wall of Gram-negative bacteria, is an endotoxin that opens the blood brain barrier and induces neurological dysfunction. Other toxins disassemble tight junction proteins by altering the cortical ring of actin-myosin and its key regulators Rho and myosin light chain proteins.

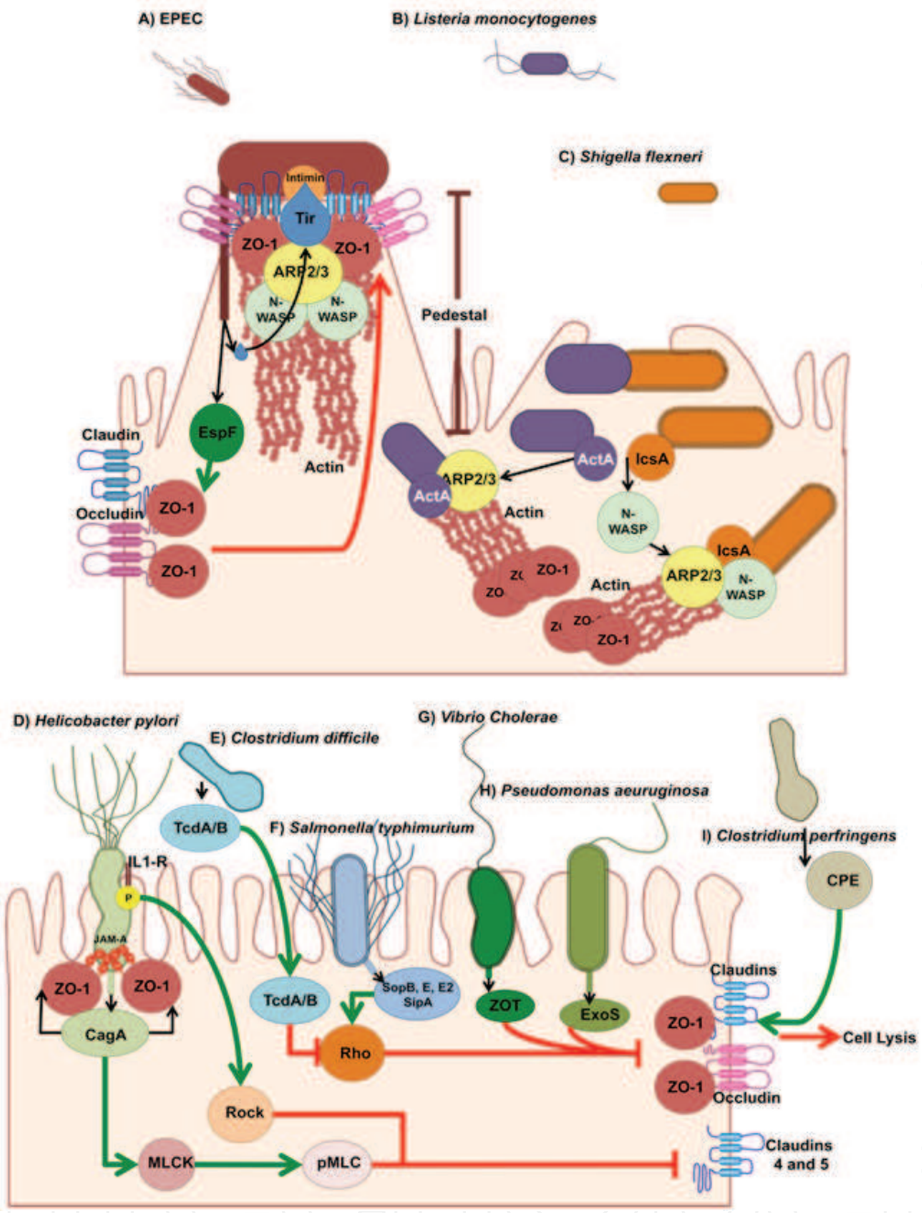

Fig. 14. Pathogens interaction with tight junction proteins A) EPEC produces dissociation of tight junction proteins from the cell borders and recruits these proteins to actin rich pedestals B) *Listeria monocytogenes* and C) *Shigella flexneri*, sequester ZO-1 to their actin comet tails, D) *Helicobacter pylori*, translocates CagA protein, disrupting claudins 4 and 5 expression. E) *Clostridium difficile*, secretes toxins TcdA and B leading to tight junction proteins dissociation. F) *Salmonella typhimurium*, inserts effector proteins that stimulate Rho, disrupting tight junction protein expression. H) *Vibrio cholera*, injects ZOT leading to ZO-1 and occludin disassembly from tight junction. H) *Pseudomonas aeruginosa* translocates toxin ExoS redistributing ZO-1 and occludin. I) *Clostridium perfringens* enterotoxin binds to the second extracellular loop of claudins 3, 4, 6, 7, 8 and 14, producing cell lysis. EPEC, Enteropathogenic *Escherichia coli*; MLCK, myosin light chain kinase; pMLC, phosphorylated myosin light chain; Rock, rho kinase; CPE, *C. perfringens* enterotoxin.

For example: 1) *Helicobacter pylori*, a causative agent of gastritis, gastric ulcers and cancer, translocates CagA protein to the host cell cytoplasm where it activates myosin light chain kinase producing the phosphorylation of myosin light chain and disrupting claudin 4 and 5 expression. In a manner independent of CagA and dependent of interleukin-1 receptor phosphorylation, *H. pylori* is able induce Rho kinase activation that disrupts claudin-4 expression. CagA also ectopically assembles ZO-1 and JAM-A at sites of bacterial attachment 2) In *Clostridium difficile*, the etiologic agent of pseudomembranous colitis, toxins TcdA and B inactivate Rho and hence disorganize the apical ring of actin and dissociate tight junction proteins from the lateral membrane. 3) *Salmonella typhimurium*, a major cause of gastroenteritis, secretes into the host cell the effector proteins SopB, E, E2 and SipA that stimulate Rho proteins and disrupt tight junction protein expression. 4) *Vibrio cholera*, the etiologic agent of cholera injects a protein named ZOT that induces PKCα activation and subsequent phosphorylation of myosin and ZO-1 that provokes the disengagement of ZO-1 from occludin and claudin at the tight junction. 5) *Pseudomonas aeruginosa*, a bacteria frequently present in cystic fibrosis and pneumonia patients, translocates toxin ExoS whose ADP-ribosylating domain disrupts the cytoskeleton and produces redistribution of ZO-1 and occludin.

Other bacterial toxins employ tight junction proteins as their receptors at the plasma membrane. Thus *Clostridium perfringens* enterotoxin binds to the second extracellular loop of claudins 3, 4, 6, 7, 8 and 14, and forms an insoluble complex that alters plasma membrane permeability, producing cell lysis. *Clostridium perfringens* enterotoxin might serve as strategy against cancer as it elicits cytolisis of breast, prostate, ovarian and pancreatic cancer cells that overexpress claudins 3 and 4.

Recommended reading

Claudins

(Furuse *et al.*, 1998), (Furuse *et al.*, 2002), (Nitta *et al.*, 2003), (Turksen and Troy, 2002), (Tatum *et al.*, 2010), (Nakano *et al.*, 2009), (Gow *et al.*, 1999), (Gow *et al.*, 2004), (Ben-Yosef *et al.*, 2003), (Tamura *et al.*, 2008), (Simon *et al.*, 1999), (Miyamoto *et al.*, 2005), (Hou *et al.*, 2009), (Angelow *et al.*, 2008)

Occludin

(Furuse *et al.*, 1993), (Traweger *et al.*, 2002), (Sakakibara *et al.*, 1997), (Saitou *et al.*, 2000), (Furuse *et al.*, 1998), (Du *et al.*, 2010), (Runkle *et al.*, 2011)

MarvelD3

(Raleigh *et al.*, 2010), (Steed *et al.*, 2009)

Tricellulin

(Ikenouchi *et al.*, 2005), (Ikenouchi *et al.*, 2008)

LSR

(Masuda *et al.*, 2011)

JAMs

(Hirabayashi and Hata, 2006), (Weber *et al.*, 2007), (Kostrewa *et al.*, 2001), (Laukoetter *et al.*, 2007), (Pellegrini *et al.*, 2011), (Ishida *et al.*, 2003)

ZO proteins

(de Mendoza *et al.*, 2010), (Gonzalez-Mariscal *et al.*, 2011), (Lopez-Bayghen *et al.*, 2006), (Tapia *et al.*, 2009), (Umeda *et al.*, 2006), (Katsuno *et al.*, 2008), (Xu *et al.*, 2008) (Xu *et al.*, 2009), (McCrea *et al.*, 2009)

Cingulin and Paracingulin

(Guillemont and Citi, 2006), (Guillemot and Citi, 2006), (Guillemot *et al.*, 2008), (Aijaz *et al.*, 2005)

Tight junctions and disease

(Guttman and Finlay, 2009), (Singh *et al.*, 2010), (Gonzalez-Mariscal *et al.*, 2007)

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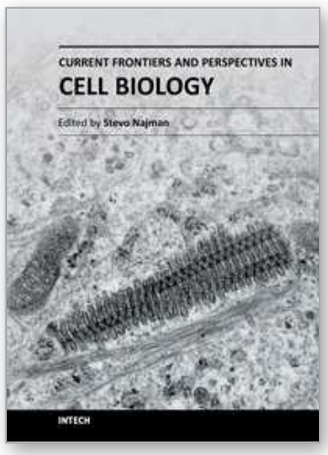

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