Biological Functions and Mechanisms of Tight Junctions in Intestinal Mucosal Epithelial Tissue[1]Postprint

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Abstract

Tight junctions (TJ) are the primary mode of intercellular connection between intestinal mucosal epithelial cells, playing a crucial role in maintaining epithelial cell polarity and regulating intestinal barrier permeability. TJ form a cellular barrier between mucosal epithelial cells that restricts the movement of solutes and substances. Overall, the structure of TJ can be summarized as a junction composed of transmembrane barrier proteins and peripheral scaffolding proteins. Within this complex network, there are numerous associated signaling proteins that influence barrier function and broader cellular activities. This article provides a comprehensive review of the biological functions, molecular regulatory mechanisms, and current research status of TJ in the intestinal mucosal epithelium.

Full Text

Biological Function and Mechanism of Tight Junctions in Intestinal Mucosal Epithelial Tissue

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Abstract: Tight junctions (TJs) represent the primary mode of intercellular connection in intestinal mucosal epithelium, playing a crucial role in maintaining epithelial cell polarity and regulating intestinal barrier permeability. TJs form a cellular barrier between mucosal epithelial cells that restricts the paracellular movement of solutes and materials. Structurally, TJ architecture can be conceptualized as compartments comprising transmembrane barrier proteins

linked to peripheral scaffolding proteins. Within this complex network, numerous associated signaling proteins influence both barrier function and broader cellular activities. This review provides a comprehensive overview of the biological functions, molecular regulatory mechanisms, and current research status of TJs in intestinal mucosal epithelium.

Keywords: intestinal mucosal epithelial cell; tight junction; mucosal barrier; mechanism

The mucosa serves as a critical barrier in the intestinal epithelium, composed of interconnected intestinal epithelial cells (IECs) that regulate trans-epithelial transport of substances such as monosaccharides, amino acids, nucleotides, vitamins, and hormones. Multiple types of intercellular junctions exist between intestinal epithelial cells, including tight junctions (TJ), gap junctions (GJ), adherens junctions (AJ), and desmosomes. Among these, TJs constitute the most important connection mode, forming a cellular barrier that restricts solute and material movement between mucosal epithelial cells. Numerous proteins have been identified as TJ components, and understanding their organizational structure and interactions is essential for comprehending the biological role of the barrier. The structural framework can be summarized as protein complexes composed of transmembrane barrier proteins [such as claudin [1], occludin [2], and junctional adhesion molecules (JAM) [3]] and peripheral scaffolding proteins [such as zonula occludens-1 (ZO-1) and afadin [4]]. Within this intricate network, many associated signaling proteins affect barrier function and broader cellular activities. Paracellular transport requires sealing structures between cells to enable directional movement of ions and solutes across cellular layers. These sealing structures are formed by TJs, which create lateral connections through a series of cellular contacts at the most apical junctions between adjacent cells. The TJ structure is illustrated in Figure 1 [Figure 1: see original paper].

Figure Legend: tight junctions: tight junctions; lumen: lumen; mucus layer: mucus layer; apical side: apical side; basolateral surface: basolateral surface; protein complex: protein complex; occludin: occludin; claudin-1: claudin-1; E-cadherin: E-cadherin; ZO-1: ZO-1; JAM-1: JAM-1; catenins: catenins; cingulin: cingulin; actin: actin; paracellular space: paracellular space; plasma membrane: plasma membrane; basolateral side: basolateral side.

Fig. 1 Protein structure of TJ [5]

Biological Characteristics of TJ

1.1 Protein Types

Intestinal mucosal epithelial cells are organized into distinct compartments including the mucus layer, apical surface, and basolateral surface. TJs represent the most apical structure of the junctional complex in epithelial and endothelial cells, forming barrier structures and paracellular spaces from the apical to baso-

lateral plasma membrane surfaces through numerous proteins and their linkers. Research has demonstrated that TJs contain a multitude of proteins, and the multi-molecular TJ complex can be categorized into three groups: (1) TJ integral proteins (occludin and claudin) that connect apical intercellular spaces and form regulated permeability barriers; (2) TJ plaque proteins [E-cadherin, ZO-1, JAM-1], most of which express PDZ domains that serve as linkers between integral TJ proteins and the actin cytoskeleton and as adaptors for cytoplasmic molecules involved in cell signaling; and (3) other proteins [catenins, cingulin, actin, etc.], including regulatory proteins, tumor suppressors, and transcriptional and post-transcriptional factors that interact directly or indirectly with TJ plaque proteins to coordinate diverse functions such as regulation of extracellular solute permeability, cell proliferation, cell polarity, and tumor suppression.

1.2 Major Protein Structures

As a barrier, TJs regulate channels for ions and small molecules through the paracellular pathway (barrier function) and restrict lateral diffusion of membrane lipids and proteins between apical and basolateral surfaces to maintain cell polarity (fence function). Among the numerous TJ proteins, integral proteins are the most important, primarily including occludin and claudin. Occludin is a 64 kDa four-transmembrane protein forming two extracellular loops separated by short cytoplasmic turns, with both amino- and carboxy-terminal domains located in the cytoplasmic region (Figure 2 [Figure 2: see original paper]). The carboxy-terminal domain is rich in serine, threonine, and tyrosine residues, serving as a target for many protein and tyrosine kinases. The two extracellular loops exhibit unusual amino acid compositions: the first loop contains a high content (61%) of tyrosine and glycine residues, while the second loop is enriched in tyrosine residues (18%). Very few charged amino acids exist in either loop, and they are predicted to have no net charge at neutral pH. Based on database searches and genomic cloning studies, 24 claudin family members have been identified, encoding proteins of 20–27 kDa. Claudins share no homologous sequences with occludin but, similar to occludin, are four-transmembrane proteins with relatively short cytoplasmic amino- and carboxy-terminal flanking regions, a first extracellular loop of 53 amino acids, and a second shorter loop of 24 amino acids (Figure 2).

The ZO protein family serves as important scaffolding proteins for TJs, including ZO-1 [6-7], ZO-2, and ZO-3 [8]. These three multi-modular proteins belong to the membrane-associated guanylate kinase-like (MAGUK) family and structurally contain an amino-terminal PDZ domain, a central Src homology 3 (SH3) domain, a guanylate kinase-like (GUK) domain, and a proline-rich region [9]. These protein-protein interaction domains form stable structures through binding to the cytoplasmic tails of TJ transmembrane proteins and filamentous actin, which is critical for strand assembly. Beyond scaffolding functions, ZO proteins exhibit regulatory roles in actomyosin modulators, signaling proteins, transcription factors, and cell polarity [10]. Additionally, under conditions of

low cell confluence or junction remodeling, some ZO proteins can shuttle between TJs and the nucleus [11]. These functions enable ZO proteins to act as mechanosensors for dynamic extracellular impacts on TJs, coordinating fundamental cellular processes such as polarization, junctional assembly, proliferation, and differentiation.

Junctional adhesion molecules (JAMs) are glycoproteins belonging to the immunoglobulin superfamily (IgSF), composed of two extracellular immunoglobulin-like domains (v-c2 type Ig domains) [12], a transmembrane region, and a variable cytoplasmic tail. The JAM family consists of three closely related proteins (JAM-A [13-14], JAM-B [15-16], and JAM-C [17-18]) that share 35% amino acid identity and possess a short, variable intracellular domain (40–50 residues) at the carboxy-terminus containing a class II PDZ-binding motif [19]. Through class I and class II PDZ-binding motifs, JAMs and related proteins interact with scaffolding proteins, the actin cytoskeleton, and signaling pathways, localizing to different cytoplasmic proteins responsible for distinct functional connections. Claudin and claudin-1 are four-transmembrane proteins that do not share sequence homology. The first extracellular loop of claudin is rich in tyrosine and glycine residues, and both loops contain few charged amino acid residues. In contrast, the amino acid composition of the two extracellular loops of claudin-1 varies significantly, resulting in a wide range of isoelectric points. JAM-1 spans the plasma membrane once and possesses two extracellular immunoglobulin-like domains, with the first amino-terminal loop interacting with homophilic partners on adjacent cells.

Fig. 2 Protein structures of occludin, claudin-1 and JAM-1 [1-3]

Functions of Major TJ Proteins

Mucosal epithelium forms a selective barrier between cellular compartments with different fluid and solute compositions, controlled essentially through two pathways: (1) the transcellular pathway, regulated by energy-dependent transporters and asymmetrically distributed channels at the apical surface; and (2) the paracellular pathway, where integral TJ proteins span apical intercellular spaces and regulate passive diffusion of ions and small uncharged solutes through paracellular spaces [20]. In addition to serving as a regulated barrier in the paracellular pathway, TJs function as a fence in the plane of the plasma membrane, helping maintain asymmetric distribution of integral membrane proteins and lipids.

2.1 Claudin Involvement in Formation of Ion-Selective Pores

Recent studies using cell membrane-impermeable polyethylene glycol oligomers have demonstrated the existence of restrictive pores within TJs of enterocyte monolayers, with radii of 0.43–0.45 nm [21]. Although this data indicates that the TJ barrier is permeable to small uncharged solutes, TJ pores can discriminate between similarly charged ions, and their permeability depends on ion

concentration and environmental pH [22-23]. With few exceptions, most TJs in mucosal epithelia are cation-selective. However, TJ ion permeability (TER) and solute permeability vary considerably across different tissues and even among cells within the same tissue [24-25]. The presence of only two claudin isoforms and few charged residues in the two extracellular domains of TJs suggests that claudins may not directly participate in forming ion-selective pores. In contrast, the quantity and isoforms of claudins differ, and their extracellular loops exhibit a wide range of isoelectric points, suggesting that charged amino acid side chains in the extracellular domains of one or more claudins may form selective pores in given TJs. Experimental evidence indicates that the extracellular domains of TJ proteins are sufficient to form ion-selective pores in the paracellular pathway [26]. However, expression of the claudin-4 extracellular domain produces greater electrophysiological effects on claudin-2 than overexpression of native claudin-4, suggesting that other domains, including the carboxy-terminus, may play a role in regulating ion selectivity.

Studies of confluent monolayers under steady-state conditions with minimal perturbation associated with changes in medium, pH, and temperature have revealed that TJs share biophysical properties with ion channels, including size and charge selectivity, dependence on ion concentration and pH, and ion competition. These observations suggest that specific claudins determine the ion selectivity of pores within TJs [27].

2.2 Role of Occludin in TJs

The role of occludin in TJs and its contribution (if any) to the function and/or regulation of ion pores remains unclear. Overexpression of claudin in Madin-Darby canine kidney (MDCK) cells increases TER but paradoxically increases transepithelial flux of mannitol. Similar functional changes have been observed in MDCK cells expressing prostaglandin receptors coupled to G proteins following activation of Ras homolog gene family member A (RhoA)-GTPase, while overexpression of ras-related C3 botulinum toxin substrate 1 (Rac1) or RhoA-GTPase disrupts TJ structure and function. Notably, overexpression of a recently identified TJ-associated Rho-GTPase-specific guanine nucleotide exchange factor (GEF/H1) increases paracellular permeability to hydrophilic solutes without affecting TER. However, the effects of GEF/H1 activity on claudin phosphorylation status and TJ function were not examined. The possibility that p160ROCK (a key effector of RhoA) may regulate claudin phosphorylation and TJ permeability has been investigated in endothelial cells. Experimental observations indicate that claudin may be a target of receptor-initiated signaling and that claudin phosphorylation can regulate TJ permeability independently of cytoskeletal activity. These observations suggest that occludin may coordinate cytoskeletal activity with various signaling pathways essential for maintaining epithelial cell phenotype.

Regulatory Mechanisms of Intestinal Epithelial TJ

Structural alterations, reduction, or loss of intestinal epithelial TJs lead to enlarged intercellular spaces and increased permeability [28], allowing bacteria and endotoxins to enter systemic circulation through paracellular routes and potentially causing intestinal inflammatory diseases such as inflammatory bowel disease. Although the regulatory mechanisms of these TJs remain incompletely understood, several fundamental regulatory pathways have been established.

3.1 Phospholipase C (PLC)-Dependent Signaling Pathway

When intestinal epithelial mucosa is stimulated by exogenous or endogenous factors, intestinal epithelial cells activate PLC through G protein mediation. PLC hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) into 1,2-diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3), subsequently activating protein kinase C isozymes, calmodulin-dependent kinases, and myosin light chain kinase (MLCK). Altered PLC activity induces contraction of the perijunctional actin-myosin ring; this contraction and displacement relax TJ structures, thereby altering their function [29] and changing intestinal mucosal epithelial permeability. Ethanol and medium-chain fatty acids can also disrupt the perijunctional actin-myosin ring through this mechanism, consequently altering intestinal mucosal epithelial permeability.

3.2 Calcium Ion (Ca2+)-E-cadherin Signaling Pathway

E-cadherin regulates TJs by modulating extracellular Ca2+ concentration. The extracellular portion of E-cadherin forms five structural domains containing Ca2+-binding sites. Research indicates that decreased extracellular Ca2+ concentration can activate MLCK, thereby increasing intestinal mucosal epithelial cell permeability. Experimental studies have shown that Ca2+ chelators [such as ethylenediaminetetraacetic acid (EDTA)] increase TJ permeability in intestinal mucosal epithelial cells, likely through a mechanism where EDTA depletes extracellular Ca2+, reducing Ca2+ availability required for E-cadherin [30], leading to disassembly of TJ proteins (occludin and ZO-1) and increased paracellular permeability.

3.3 Tyrosine Kinase-Phosphatase Signaling Pathway

G protein 12 (G 12), a heterotrimeric G protein family member, binds to ZO-1 through its SH3 domain, and G 12 activation enhances paracellular permeability in MDCK cells. Meyer et al. [31] found that MDCK cells exhibiting G 12 activity showed increased Src autophosphorylation activity and elevated tyrosine phosphorylation of -catenin. Confocal microscopy revealed disruption of TJ proteins, altered normal distribution of ZO-1 and Na+,K+-ATPase, loss of cell polarity, and increased actin stress fibers. These changes could be reversed by the tyrosine kinase inhibitor genistein and the Src-specific inhibitor PP-2,

which also prevented increased paracellular permeability. Therefore, G 12 can regulate epithelial cell TJs partially through the Src tyrosine kinase pathway.

3.4 Rho-GTPase Pathway

Rho-GTPases, a family of G proteins with molecular masses of 20–30 kDa, regulate TJ assembly by modulating Rho activity. Studies have found that activation of Rho by Rho-GTPase leads to deposition of ZO-1 and occludin at TJs, thereby maintaining TJ function, whereas inhibition of Rho activity by Rho-GTPase reduces ZO-1 and claudin localization at TJs. Research has also shown that simultaneous inhibition of Rho activity and ATP depletion in transfected cells results in substantial loss of TJ components [32].

Influencing Factors

4.1 Physical and Chemical Factors

Studies investigating changes in blood-brain barrier permeability and JAM-1 expression in rat cerebral cortex and hippocampus under different power densities of microwave radiation have demonstrated that microwave radiation reduces JAM-1 expression levels in rat brain tissue and increases blood-brain barrier permeability. Other studies have shown that oxygen-glucose deprivation/reoxygenation processes in cultured rat brain microvascular endothelial cells can disrupt intercellular TJs [19].

4.2.1 Fatty Acids

Current research indicates that optimal dietary -linolenic acid/linoleic acid (ALA/LNA) ratios increase expression levels of claudin-3, claudin-b, claudin-c, claudin, and ZO-1 in small intestinal mucosa while decreasing claudin-15a expression, suggesting that optimal dietary ALA/LNA strengthens intestinal TJs in fish. Interestingly, claudin-12 expression increased in grass carp intestine at a dietary ALA/LNA ratio of 1.03, which may be related to calcium absorption. Claassen et al. [33] reported that eicosapentaenoic acid and its isomer -linolenic acid (GLA, n-6) can stimulate intestinal calcium absorption in rats. Enhanced calcium absorption helps regulate claudin-12 expression in mouse intestinal mucosal epithelial cells. These data suggest that increased claudin-12 expression at a dietary ALA/LNA ratio of 1.03 may result from stimulated calcium absorption, requiring further investigation. MLCK plays a crucial role in regulating TJs in mice. Current studies show that MLCK transcriptional abundance is significantly reduced at a ratio of 1.03. Further correlation analysis indicates that claudin-3, claudin-b, claudin-c, claudin, and ZO-1 are negatively correlated with MLCK, whereas claudin-15a is positively correlated with MLCK mRNA abundance.

4.2.2 Proteins

Hemoglobin can disrupt intercellular TJs and increase permeability, leading to endothelial barrier dysfunction. Hemoglobin increases intracellular phosphorylated myosin light chain (p-MLC) expression, resulting in extensive formation of intracellular filamentous actin and inducing decreased expression of the intercellular TJ protein ZO-1, which may represent an important pathophysiological mechanism underlying TJ disruption.

Glucagon-like peptide-2 (GLP-2) is an intestinotrophic hormone. Gradient experiments with GLP-2 in isolated intestinal tissue blocks from weaned piglets, involving culture with different GLP-2 concentrations for 72 h, have demonstrated that appropriate GLP-2 concentrations promote expression of ZO-1, occludin, and claudin-1.

Enterotoxin, a 35 kDa protein produced by *Clostridium perfringens* that causes food poisoning in humans, specifically binds to claudin-3 and claudin-4 at its carboxy-terminus [34], while the amino-terminus forms pores in the plasma membrane. To specifically bind TJ proteins and avoid plasma membrane damage, the carboxy-terminal fragment of enterotoxin was transfected into L-cells expressing claudin-1, claudin-2, claudin-3, or claudin-4, or into confluent MDCK I cells expressing claudin-1 and claudin-4. In L-cell transfectants, the enterotoxin fragment selectively bound to claudin-3 and claudin-4 but not to claudin-1 or claudin-2. Notably, in MDCK I cells, TJ disassembly began within 4 h of enterotoxin fragment binding to claudins, with decreased TER and increased paracellular flux. However, it remains unclear whether enterotoxin interaction with claudin-4 leads to disaggregation of proteins from existing TJ strands or, because TJ disruption only occurs when enterotoxin is added to the basolateral surface, whether enterotoxin interaction with claudin-4 monomers in the membrane prevents their incorporation into TJ strands, thereby causing TJ disruption.

CagA is a protein produced by *Helicobacter pylori*. When *H. pylori* adheres to the apical junctional complex of epithelial cells, CagA translocates from bacteria into epithelial cells [35]. Notably, CagA appears to target *H. pylori* to intercellular junctions, where it interacts with ZO-1 and JAM-1 at bacterial attachment sites. Following this interaction, TJ barrier function is disrupted and cell shape is significantly altered [36]. Although the mechanism underlying TJ disruption during *H. pylori* infection remains unclear, perturbation of ZO-1 and JAM-1 may lead to abnormal localization and function of important signaling molecules. If present, the clinical consequences of this abnormal interaction may include gastric ulcers or gastric cancer.

TJs represent one of the most important intercellular connection modes in intestinal mucosal epithelial cells. Their interaction with nutritional factors such as proteins and fatty acids influences TJ protein activity, thereby regulating intestinal epithelial substance transport and permeability. However, the molecular mechanisms underlying TJ regulation remain incompletely un-

derstood. Therefore, in-depth investigation of gene expression regulation of TJ proteins is of significant importance for advancing our understanding of regulatory mechanisms in animal intestinal and tissue barriers.

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