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Endocytosis of tight junction proteins and the regulation of degradation and recycling

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Abstract

Internalization of tight junction (TJ) proteins from the plasma membrane is a pivotal mechanism regulating TJ plasticity and function in both epithelial and endothelial barrier tissues. Once internalized, the TJ proteins enter complex vesicular machinery, where further trafficking is directly dependent on the initiating stimulus and downstream signaling pathways that regulate the sorting and destiny of TJ proteins, as well as on cell and barrier responses. The destiny of internalized TJ proteins is recycling to the plasma membrane or sorting to late endosomes and degradation. This review highlights recent advances in our knowledge of endocytosis and vesicular trafficking of TJ proteins in both epithelial and endothelial cells. A greater understanding of these processes may allow for the development of methods to modulate barrier permeability for drug delivery or preventing barrier dysfunction in disease states.

Keywords

early endosomes; late endosomes; recycling; degradation; claudin

Introduction

Barrier-forming epithelial cells and endothelial cells (i.e., the cerebral endothelial cells that form the blood–brain barrier) have tight junctions (TJs) that greatly limit paracellular diffusion. TJ complexes are large, multimeric, and highly elaborate structures, arranged as a series of multiple anastomosing strands localized on the cell lateral membrane to completely seal the intercellular cleft.^{1,2} Structurally, such complexes are composed of three components.¹ The first are the transmembrane adhesion proteins occludin, the claudins (e.g., claudin-1, -3, and -5), tricellulin and MarvelD2 (TAMP-proteins), and an IgG type of

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Competing interests

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protein, junctional adhesion molecule (JAM-A, -B and -C) ESAM and CAR, which physically interact with their counterparts on the plasma membrane of adjacent cells.^{3,4} It should be noted that some selected claudins (e.g., claudin-2) are pore- rather than barrier-forming.⁵ A second TJ complex component involves the cytoplasmic plaque proteins (e.g., ZO-1, ZO-2, ZO-3, afadin-6, cingulin, 7H6, Par3, Par6, PKC ζ) that provide a link between transmembrane proteins and the actin cytoskeleton and participate in intracellular signaling.^{6,7} A third component is the actin cytoskeleton, which provides essential physical support for the complex.^{8,9}

The morphological appearance and properties of the TJ complex in tissue barriers suggest a very stable and rigid seal of the paracellular space. However, recent observations challenge this belief, suggesting that the TJ complex is a very dynamic structure capable of responding to various biochemical and mechanical stimuli through reshaping and remodeling. In general, as with other protein complexes, two mechanisms are indicated as predominantly regulating the dynamics of TJ proteins: diffusion and endocytosis.

Diffusion is the dynamic movement of proteins in steady-state conditions as well as, to some degree, under certain external stimuli when rapid remodeling of protein complexes is required. In the case of the TJ complex, diffusion may take place within the plasma membrane.¹⁰

In contrast, endocytosis is an active process by which cells absorb molecules by engulfing them with their cell membranes. It involves an extensive cellular machinery to mediate the formation of membrane transport vesicles to enable the internalization, fusion with endosomes, and entry into the endo-lysosomal membrane system. The process is tightly regulated by variety of enzymes, adaptor and motor proteins, signaling molecules (small G proteins) and the actomyosin cytoskeleton.^{11–13} Endocytosis is mostly implicated in the response to external stimuli, although it can occur under steady-state conditions as a process for TJ protein turnover. Recent evidence also indicates that endocytosis is a cell signaling event, signaling that is resolved in space and time.^{11,12} Thus, TJ protein/complex endocytosis is a key regulator of other cell–cell interactions, barrier properties, and remodeling.^{14–16}

Endocytosis pathways for TJ proteins

The initial step in the endocytosis pathway is the internalization of membrane-located proteins. There are several routes involved in such internalization. The general division includes two types of endocytosis: clathrin dependent and clathrin independent. The latter includes lipid raft-dependent and lipid raft-independent endocytosis. Lipid raft-dependent endocytosis includes caveolae- flotillin-, GTPase regulator associated with focal adhesion kinase-1 (GRAF1)-, adenosine diphosphate-ribosylation factor 6 (Arf6)-, and RhoA-dependent endocytosis. Non-raft-dependent endocytosis includes macropinocytosis.¹²

Clathrin-dependent internalization is mediated by small clathrin-coated vesicles formed in plasma membrane domains termed *clathrin-coated pits*. Plasma membrane phosphatidylinositol-(4,5)-bisphosphate (PIP₂), adaptor proteins, and clathrin have essential

roles in forming vesicles, while the GTPase dynamin is involved in budding the vesicles off of the plasma membrane.^{17,18} In epithelial cells, clathrin-mediated endocytosis is critical for the removal of transmembrane TJ (claudins, occluding, and JAM-A) proteins from the plasma membrane, causing TJ complex dissociation. Examples include mAChR-mediated internalization of claudin-4 in submandibular epithelial (SMG-C6) cells;¹⁹ TGF- β 3-induced uptake of JAM-A and occludin in Sertoli cells;^{20,21} claudin-1 and peptide C21C2 (from the C-terminal half of claudin-1's first extracellular loop) uptake in human embryonic kidney (HEK)-293 cells;²² poly-L-arginine (PLA)-induced internalization of occludin, ZO-1, claudin-4 and tricellulin in Caco-2 cells;²³ epidermal growth factor (EGF)-induced, clathrin-dependent internalization of claudin-2 in MDCK cells;²⁴ Ca²⁺-induced occludin, JAM-1, ZO-1 and claudins-1 and -4 uptake in colonic (T84) epithelial cells;²⁵ hepatitis C virus (HCV) particle-mediated/facilitated internalization of claudin-1 and occludin;^{26–28} occludin internalization in Madin–Darby canine kidney (MDCK) cell migration during epithelial wound healing;²⁹ and hypotonic stress-induced endocytosis of claudin-1 and -2 in renal tubular epithelial cells (Table 1).³⁰

Caveolae-mediated internalization occurs in cell membrane microdomains: the membrane/lipid rafts (MLR). Caveolae are plasma membrane flask-like invaginations containing the coat protein caveolin-1 (Cav-1). Phosphorylation of Cav-1 (on Tyr14 residues) triggers the formation and fusion of caveolae.^{12,31} Caveolae-mediated internalization is considered to be the predominant form of TJ endocytosis in endothelial cells. For example, it is involved in the internalization of occludin and claudin-5 upon exposure to the chemokine CCL2 in brain endothelial cells;³² acute brain endothelial barrier injury during subarachnoid hemorrhage and claudin-5, JAM-1, occludin and zonula occludens-1 degradation;³³ claudin-5 internalization in acute ischemic brain endothelial injury (murine stroke model)³⁴ and Tat-induced alterations in occludin, ZO-1 and ZO-2.³⁵ It is also involved in some cases in epithelial cells, such as TNF- α -induced occludin internalization in enterocytes³⁶ and in MDCK cells;³⁷ claudin-2, -3, -4, -7 internalization in enterocytes;³⁸ coxsackievirus-induced internalization of occludin in Caco-2 cells;³⁹ *Escherichia coli* cytotoxic necrotizing factor-1 (CNF-1) or IFN- γ - and LIGHT (lymphotoxin-like inducible protein that competes with glycoprotein D for herpes virus entry on T cells)-mediated endocytosis of occludin in intestinal epithelial cells;^{40,41} and high glucose-induced claudin-1 and occludin internalization in retinal pigment epithelial monolayers (Table 1).⁴²

Macropinocytosis is the invagination and ruffling of the cell membrane to form a pocket-like structure through the polymerizing of actin filaments.⁴³ It forms a vesicle for large cargo (> 1 μ m) and is mostly involved in the uptake of extracellular fluid and molecules.

Macropinocytosis shares some interfaces with caveolae but also requires unique machinery, including Ras GTPase, phosphatidylinositol (3,4,5)-trisphosphate (PIP₃) enzymes, sorting nexin (SNX) family (SNX5) adaptors, and motor proteins.^{44–46} There is prevalent macropinocytosis in IFN- γ -induced disassembly of TJs and bulk uptake of occludin, claudin, and JAM-A uptake in epithelial barrier disassembly in inflammatory bowel disease.⁴⁷ Similarly, chemokine CCL2-induced relocalization of JAM-A also involves macropinocytosis.⁴⁸ Coxsackievirus modulates barrier function and occludin internalization by macropinocytosis in concert with caveolae-mediated endocytosis.³⁹

Among the other processes potentially involved in TJ protein internalization, only flotillin-dependent internalization has shown a close association with TJ transmembrane protein uptake.^{12,49} That form of endocytosis is lipid raft dependent, but the vesicles contain flotillin-1 (Reggie-2) and -2 (Reggie-1) proteins rather than Cav-1.^{12,49} Such internalization is regulated by the Src family kinase Fyn⁵⁰ and is involved in the internalization of occludin and ZO-1 in enteropathogenic *E. coli* (EPEC)–induced barrier dysfunction and in IFN γ -treated Caco-2 cells.^{51,52} It is also involved in the internalization of occludin; claudin-1, -3, -5, and -8; and ZO-1 that occurs in 2,4,6-trinitrobenzenesulfonic acid–induced colitis in rats.⁵³ Although flotillin and caveolin have distinct localizations and do not physically associate, flotillin and caveolin may cooperate in controlling internalization.⁵⁴ Thus, caveolin- and flotillin-dependent TJ protein internalization involves an interplay between these two pathways.

Endocytic trafficking and sorting of TJ proteins

After internalization, TJ proteins continue to move through dynamic and interconnected membrane-bound endosome compartments. Those compartments allow the collection, sorting, and transfer of cargoes (e.g., TJ proteins) to their final destinations. These vesicles play an essential role in regulating cell surface molecule expression, spatial restriction of signaling, and control of signaling fate.^{12,13} Endosomes are identified as being early, late, or recycling based on luminal pH, alterations in phosphatidylinositol lipids, and differential recruitment and activation of members of the Ras superfamily of monomeric G proteins (Rab-GTPases), as well as a member of the ADP ribosylation factors (ARFs) family of small GTPases, like Arf1 or Arf6.^{12,13} Established routes of endocytotic sorting include the collection of cargo in early endosomes and their further redirection towards four main pathways: recycling, recycling by bulk membrane flow, degradation, or transport to the trans-Golgi network (TGN) (Fig. 1).

So far, the endocytic sorting process has been dissected in the greatest depth for clathrin-dependent endocytosis. However, a growing body of evidence suggests that multiple and functionally distinct types of internalization merge to the same vesicular tools and have the same sorting processes. Some early studies reported that, after caveolae internalization and micropinocytosis, cargo was collected in caveosomes and macropinocytotic vesicles. From caveosomes and macropinosomes, cargo was directly delivered to late endosomes, bypassing the early endosomes.^{12,55,56} However, this has recently been challenged. Caveosomes and macropinosomes are now considered as transformation steps of internalized vesicles toward early endosomes, where all postinternalization sorting decisions are made.^{12,31,57}

Early endosomes: a center of sorting

Early endosomes (Rab5⁺, EEA1⁺, phosphoinositide kinase Vps34⁺, and enriched in phosphatidylinositol 3-phosphate PI(3)P) represent the central sorting station for cargo. Early endosome formation is a two-step process. There is an initial step involving the fusion of internalized vesicles/cargo and accumulation, until an ill-characterized switch limits further progress. There is then maturation of the endocytic vesicles—the actual sorting process.^{12,13,58,59} Sorting is an active and complex process directly dependent upon the

physicochemical characteristics of the vesicles themselves (pH and endosomal geometry), the cargo (sorting signals encoded within the amino acid sequences of C-termini, such as lipid-binding motifs like BAR domain-containing proteins and nexin family (SNXs)), and supporting and signaling molecules, which include adaptor proteins (i.e., APPL1, APPL2); membrane proteins, such as soluble NSF attachment protein receptors (SNAREs), involved in fusion events in membrane trafficking; and PtdIns3P kinase, VASP34, Rab subfamily GTPases (Rab5, Rab5 effector Rabenosyn-5, Rab4), which enable and facilitate sorting decisions (Fig 1).^{58,60,61}

An important regulator of sorting that needs to be highlighted is the Rab family of small GTPases. The members of this large family of small GTPases (~ 60 members in humans), together with supporting molecules (coat components, motors, and SNAREs), serve as multifaceted organizers of membrane trafficking.⁶² Rab proteins are localized on the membrane compartment of endosomes and function as molecular switches, cycling between GTP (active) and GDP (inactive) forms, thereby regulating formation, transport, tethering, and fusion of transport vesicles as a general mechanism for regulating traffic between organelles.^{58,63} Early endosomes express Rab5, and most mammalian cells have three Rab5 isoforms, Rab5A, Rab5B and Rab5C.^{64,65} Activation of these isoforms directly or indirectly via a network of interacting proteins (i.e., Rabex5, Gapex-5, Rin1, Rin2, Rin3, Als2, EEA1, and Rabenosyn-5) serve in sorting and decision-making processes.^{65–67}

TJ proteins accumulate in early endosomes as a first station after internalization. Several reports indicate that claudin-1, claudin-5, claudin-16, occludin, JAM-A, and ZO-1 accumulate in early endosomes in both epithelial and endothelial cells.^{23,25,32,40,48,68–71} Their presence in early endosomes is closely associated with diminished barrier function (hyperpermeability).^{32,40,47,71} Furthermore, Rab5A depletion using small interfering RNA (siRNA) increased claudin-1 association with the plasma membrane in the human endothelial cell line EA hy.926 and also stabilized brain endothelial barrier function owing to an inability to further process internalized junction proteins.⁷²

From the Rab5⁺ early endosomes, cargo can be sorted in four potential directions: to recycling and degradation pathways, to a bulk-flow recycling pathway, and toward the trans-Golgi network (Fig. 1). However, how this sorting process occurs for TJ proteins is still unclear. There is a lack of evidence about specific binding motifs on TJ proteins that could recognize microdomains on the Rab5⁺ endosomes, as well any specific regulatory and adaptor proteins associated with the junction protein–sorting process.

Studies on the function of Rab5 isoforms suggest that Rab5A and Rab5B are involved in sorting toward degradation pathways, while Rab5C is mostly involved in recycling.⁶⁵ Our preliminary study suggests that claudin-5 sorting could be associated with activation and interaction with the isoforms Rab5A and/or Rab5C, which have the ability to direct claudin-5 toward degradation or recycling pathways, respectively. Further studies should elucidate how decision-making processes on the fate of TJ proteins occur in the early endosome (Fig. 2).

Recycling pathways for TJ proteins

One potential route in endocytic sorting is recycling. In general, recycling pathways can be fast and Rab4 dependent or slow and Rab8/Rab11 and ARF6 dependent.^{13,73} Each recycling route is a multistep process that depends on the cargo and its recycling capacity, based on the existence of a recycling motif on the C terminus (i.e., a PDZ barcode), an endosomal complementary domain, trafficking and transformation of Rab proteins (Rab4 and Rab11), promoters of the translocation carrier (i.e., nexin protein SNX4, SNX17, ACAP1 BA2R), or other members of the retromer complex (VPS26, VPS29, and VPS35) that are able to select and package cargo proteins into transport carriers that export cargo from the endosome.^{12,13,73}

The decision for a slow or fast recycling route is more dependent on the set of regulator proteins than on the cargo capacity and endosomal domain.⁷³ This suggests that the type of stimulus and the signaling pathways engendered in particular cell types may tightly regulate the outcome of endocytosis. In fibroblasts and epithelial cells, occludin can undergo continuous recycling.^{40,74–76} This is associated with a fast exchange between cytosol and membrane pools in steady-state conditions as well as during dynamic and rapid remodeling of epithelial polarity and TJs.¹⁰ Similar to occludin, claudin-1 and claudin-2 show constant endocytosis and recycling back to the plasma membrane in a range of epithelial cells (kidney, colon, lung).^{22,77–79} Intriguingly, constitutive recycling of claudin-1 is dependent on endosomal sorting complex required for transport (ESCRT).^{80,81} ESCRT is known to be involved in the trafficking of transmembrane proteins to the lysosome, as well as other processes, including autophagy and TGN trafficking.⁸² In kidney epithelial cells, claudin-16 recycling is dependent on Rab11 and STX8.⁷⁰

In contrast, in brain endothelial cells, the chemokine CCL2 induces claudin-5 and occludin accumulation in Rab4 and Rab11 vesicles, and there is fast and slow recycling back to the plasma membrane upon CCL2 removal in order to reseal TJs and recover barrier properties.³² Consequently, inhibition of Rab4 or Rab11 vesicles via siRNA may prevent the recovery of the barrier and prolong hyperpermeability (Fig. 4).⁶⁶ In the case of JAM-A, CCL2-induced vesicular accumulation predominantly occurs in Rab4⁺ but not in Rab11⁺ vesicles. This is associated with fast delivery to the cell apical surface (rather than the lateral membrane), where JAM-A gains a role as a leukocyte adhesion molecule.⁴⁸

Late endosomes: a degradation route

Degradation is another sorting option. It is characterized by the movement of cargo toward late endosomes (Rab7⁺, VPS34⁺, focal high concentration enriched in LAMP1 and MLN64, enriched in CD63 and lysobisphosphatidic acid, mannose-6-phosphate receptor⁺, CD63⁺).^{83,84} The late endosomes are generated from early endosomes through a transformation process that includes an increase in luminal acidification, endosome movement from the cell periphery toward a juxtanuclear localization, and the switching of two key endosome identity cues—Rab5 to Rab7.^{58,83} Sorting of TJ proteins toward late endosomes has been reported in a few studies. In MDCK II cells, Rab14 sorts the pore-forming claudin claudin-2 to late endosomes and lysosomes, preventing claudin-2 inclusion

in the TJ complex and barrier leakage.⁷⁷ In the same cell type, claudin-1 localization in late endosomes was associated with E3 ubiquitin ligase ligand of Numb-protein X1 (LNXP80).⁸⁵ In brain endothelial cells, CCL2-induced endocytosis with claudin-5 and occludin was mostly associated with recycling (80%), while 20% was associated with Rab7.³⁴

An important mechanism closely associated with sorting toward late endosomes is ubiquitination.^{86,87} In order for cargo to be sorted by ubiquitination, the presence of ubiquitin (UB)-binding domains is critical, as is the escort complex formed by the ESCRT family of proteins (particularly ESCRT III) and Vps4.⁸⁸ These guide cargo along the degradation route to the lysosomes.^{89,90}

A sorting pathway for occludin toward late endosomes and degradation has been reported. In retinal endothelial cells, occludin phosphorylation on Ser490 after vascular endothelial growth factor (VEGF) treatment is associated with ubiquitination and degradation, leading to subsequent vascular permeability.^{71,91} Similarly, a study by Traweger *et al.* reported that occludin is subjected to ubiquitination by the E3 ubiquitin protein ligase Itch and degradation by the proteasome, resulting in increased permeability after cAMP stimulation in Sertoli cells.^{92,93}

Other sorting routes: bulk-flow recycling and the trans-Golgi network

Bulk-flow recycling is characterized by narrow tubules with large surface area. Continuous fission and recycling of these tubules provide a geometric basis for nutrient receptors to be recycled to the cell surface during periods of exceptional need.^{11,12} Currently, there is no solid evidence regarding TJ recycling via the bulk-flow recycling pathway.

Endosome-to-TGN retrograde sorting is similarly complex, with contributions from clathrin in conjunction with adaptor proteins (AP1, PACS1, epsinR, and SNARE), and it is closely associated with biosynthetic processes.⁹⁴ Rab13 is implicated as being involved in TGN–endosome or endosome–TGN retrieval pathways. However, regarding the TJ proteins occludin and claudin-1, Rab13 is involved in recycling rather than endosome-to-TGN sorting.^{74,79,95} There is currently no evidence that TJ proteins are recycled toward the TGN network.

Signaling pathways in endocytic sorting of TJ proteins

Multiple signaling pathways are involved in regulating the steps in TJ protein endocytosis. Cellular signaling events occur on internalization vesicles and endosome membranes, controlling both internalization and postinternalization sorting. While general principles of endosome-based signaling are emerging, the significance of signaling on the endocytic sorting process still remains poorly understood.

The intercellular signaling involved in internalization depends on the mechanism of uptake. In clathrin-dependent internalization, activation of MAP kinases (Mek/ERK1/2 in concert with Akt), protein kinase A (PKA) or protein kinase C (PKC), and RhoGTPase (RhoA-ROCK, Cdc42) plays critical roles in internalization and activation of signaling

endosomes.^{96–98} Regulators of caveolin-dependent internalization include MAP kinase ERK1/2 and p38, in concert with c-Jun N-terminal kinase (JNK) and PKC- α , and small GTPase RhoA (RhoA, Rac1 Cdc42).^{99–102} In addition, several tyrosine kinases (c-Abl, Src, and Fyn) that phosphorylate caveolin-1 residue Tyr14 play critical roles in caveolae dynamics.^{103,104} Flotillin-mediated internalization is regulated predominantly by small Rho GTPases and the actin organization machinery.¹⁰⁵ Macropinosome formation is also tightly associated with actin organization, and Rac-GTPase-, myosin light chain kinase (MLCK)-, and Ras-dependent actin signaling is critical for this type of internalization.^{105–107} However, recent evidence suggests that macropinocytosis could share some raft components, and raft-associated signaling may be additionally involved¹⁰⁸.

Postinternalization protein sorting includes a variety of signaling molecules that regulate recognition of compatible membranes and membrane fusion SNAREs, recruitment of cargo in endosomes (nexin SNX), endosomal sorting complex required for transport (ESCRT), and Rab GTPases. These are key regulators of vesicular trafficking between endosomal compartments.^{11,58,60,62,82}

Regarding TJ protein internalization and sorting, several signaling pathways are indicated. Occludin endocytosis in intestinal anoxia/reoxygenation injury and TNF- α - and LIGHT-induced caveolae internalization is regulated by MLCK.^{36,41,109} RhoA and Rho-associated kinase (ROCK) are key regulators of IFN- γ -induced endocytosis of epithelial TJ proteins and JAM-A macropinocytosis, while C-Jun N-terminal kinase regulates caveolae- and clathrin-dependent endocytosis and macropinocytosis of occludin.^{48,110–112} The MAP kinase ERK1/2 modulates internalization and degradation of TJ proteins claudin-2, claudin-4, occluding, and ZO-1.¹¹³ In TJ endocytic sorting, a recent study showed a close association between ZO-2 and SNX27 in an epithelial mpkCCD cell line, suggesting a role in endocytic pathways.¹¹⁴

The RabGTPases have important roles in regulating TJ protein endocytosis. They include the Rabs associated with early endosomes, like Rab5, Rab4, and Rab11, as well as Rab7, associated with late endosomes where TJ proteins accumulate after internalization.^{62,63} It is also important to highlight one member of the Rab family: Rab13. In concert with JRAB/MICAL-L2, Rab13 regulates transport and delivery of TJ proteins at the lateral membrane, controlling TJ complex assembly.^{79,115}

It should be noted that the pathways involved in modulating TJ protein endocytosis are also involved in regulating other epithelial and endothelial cell functions. For example, the MAP kinases ERK1/2 and JNK are involved in many cell stress responses. One goal of an in-depth understanding of the signaling that regulates TJ protein endocytosis and sorting is to elaborate ways whereby the potentially beneficial effects of manipulating signaling can be separated from potentially harmful effects (e.g., inhibiting ERK1/2 may stabilize TJs in disease states but also prevent other cellular protective effects).

Conclusions

Future characterization of the mechanism of endocytic sorting will provide important information about the dynamic behavior of TJ proteins and the molecular characterization of TJ regulation. A greater understanding of these processes may allow researchers to create methods to enhance barrier permeability, thereby increasing drug delivery to tissues (e.g., to brain across the blood–brain barrier). In addition, knowledge of the mechanisms determining TJ recycling and degradation may provide methods of preventing barrier dysfunction in disease states (e.g., the blood–brain barrier disruption and associated brain edema and leukocyte infiltration that occurs in stroke).

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References

1. Van Itallie CM, Anderson JM. Architecture of tight junctions and principles of molecular composition. *Seminars in cell & developmental biology*. 2014; 36:157–165. [PubMed: 25171873]
2. Bazzoni G, Dejana E. Endothelial cell-to-cell junctions: molecular organization and role in vascular homeostasis. *Physiological reviews*. 2004; 84:869–901. [PubMed: 15269339]
3. Haseloff RF, Dithmer S, Winkler L, et al. Transmembrane proteins of the tight junctions at the blood-brain barrier: structural and functional aspects. *Seminars in cell & developmental biology*. 2015; 38:16–25. [PubMed: 25433243]
4. Martin-Padura I, Lostaglio S, Schneemann M, et al. Junctional adhesion molecule, a novel member of the immunoglobulin superfamily that distributes at intercellular junctions and modulates monocyte transmigration. *The Journal of cell biology*. 1998; 142:117–127. [PubMed: 9660867]
5. Rosenthal R, Gunzel D, Krug SM, et al. Claudin-2-mediated cation and water transport share a common pore. *Acta physiologica*. 2016
6. Gonzalez-Mariscal L, Betanzos A, Avila-Flores A. MAGUK proteins: structure and role in the tight junction. *Seminars in cell & developmental biology*. 2000; 11:315–324. [PubMed: 10966866]
7. Luo Y, Fukuhara M, Weitzman M, et al. Expression of JAM-A, AF-6, PAR-3 and PAR-6 during the assembly and remodeling of RPE tight junctions. *Brain research*. 2006; 1110:55–63. [PubMed: 16859655]
8. Van Itallie CM, Tietgens AJ, Anderson JM. Visualizing the dynamic coupling of claudin strands to the actin cytoskeleton through ZO-1. *Molecular biology of the cell*. 2016
9. Ivanov AI. Structure and regulation of intestinal epithelial tight junctions: current concepts and unanswered questions. *Advances in experimental medicine and biology*. 2012; 763:132–148. [PubMed: 23397622]
10. Shen L, Weber CR, Turner JR. The tight junction protein complex undergoes rapid and continuous molecular remodeling at steady state. *The Journal of cell biology*. 2008; 181:683–695. [PubMed: 18474622]
11. Barbieri E, Di Fiore PP, Sigismund S. Endocytic control of signaling at the plasma membrane. *Current opinion in cell biology*. 2016; 39:21–27. [PubMed: 26872272]
12. Doherty GJ, McMahon HT. Mechanisms of endocytosis. *Annual review of biochemistry*. 2009; 78:857–902.
13. Elkin SR, Lakoduk AM, Schmid SL. Endocytic pathways and endosomal trafficking: a primer. *Wiener medizinische Wochenschrift*. 2016; 166:196–204. [PubMed: 26861668]
14. Shen L. Tight junctions on the move: molecular mechanisms for epithelial barrier regulation. *Annals of the New York Academy of Sciences*. 2012; 1258:9–18. [PubMed: 22731710]

15. Chalmers AD, Whitley P. Continuous endocytic recycling of tight junction proteins: how and why? *Essays in biochemistry*. 2012; 53:41–54. [PubMed: 22928507]
16. Utech M, Mennigen R, Bruewer M. Endocytosis and recycling of tight junction proteins in inflammation. *Journal of biomedicine & biotechnology*. 2010; 2010:484987. [PubMed: 20011071]
17. Lampe M, Vassilopoulos S, Merrifield C. Clathrin coated pits, plaques and adhesion. *Journal of structural biology*. 2016; 196:48–56. [PubMed: 27431447]
18. Taylor MJ, Lampe M, Merrifield CJ. A feedback loop between dynamin and actin recruitment during clathrin-mediated endocytosis. *PLoS biology*. 2012; 10:e1001302. [PubMed: 22505844]
19. Cong X, Zhang Y, Li J, et al. Claudin-4 is required for modulation of paracellular permeability by muscarinic acetylcholine receptor in epithelial cells. *Journal of cell science*. 2015; 128:2271–2286. [PubMed: 25948584]
20. Xia W, Wong EW, Mruk DD, et al. TGF-beta3 and TNFalpha perturb blood-testis barrier (BTB) dynamics by accelerating the clathrin-mediated endocytosis of integral membrane proteins: a new concept of BTB regulation during spermatogenesis. *Developmental biology*. 2009; 327:48–61. [PubMed: 19103189]
21. Yan HH, Mruk DD, Lee WM, et al. Blood-testis barrier dynamics are regulated by testosterone and cytokines via their differential effects on the kinetics of protein endocytosis and recycling in Sertoli cells. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 2008; 22:1945–1959. [PubMed: 18192323]
22. Staat C, Coisne C, Dabrowski S, et al. Mode of action of claudin peptidomimetics in the transient opening of cellular tight junction barriers. *Biomaterials*. 2015; 54:9–20. [PubMed: 25907035]
23. Yamaki T, Kamiya Y, Ohtake K, et al. A mechanism enhancing macromolecule transport through paracellular spaces induced by Poly-L-Arginine: Poly-L-Arginine induces the internalization of tight junction proteins via clathrin-mediated endocytosis. *Pharmaceutical research*. 2014; 31:2287–2296. [PubMed: 24590880]
24. Ikari A, Takiguchi A, Atomi K, et al. Epidermal growth factor increases clathrin-dependent endocytosis and degradation of claudin-2 protein in MDCK II cells. *Journal of cellular physiology*. 2011; 226:2448–2456. [PubMed: 21660968]
25. Ivanov AI, Nusrat A, Parkos CA. Endocytosis of epithelial apical junctional proteins by a clathrin-mediated pathway into a unique storage compartment. *Molecular biology of the cell*. 2004; 15:176–188. [PubMed: 14528017]
26. Schwarz AK, Grove J, Hu K, et al. Hepatoma cell density promotes claudin-1 and scavenger receptor BI expression and hepatitis C virus internalization. *Journal of virology*. 2009; 83:12407–12414. [PubMed: 19776133]
27. Farquhar MJ, Hu K, Harris HJ, et al. Hepatitis C virus induces CD81 and claudin-1 endocytosis. *Journal of virology*. 2012; 86:4305–4316. [PubMed: 22318146]
28. Eyre NS, Drummer HE, Beard MR. The SR-BI partner PDZK1 facilitates hepatitis C virus entry. *PLoS pathogens*. 2010; 6:e1001130. [PubMed: 20949066]
29. Fletcher SJ, Poulter NS, Haining EJ, et al. Clathrin-mediated endocytosis regulates occludin, and not focal adhesion, distribution during epithelial wound healing. *Biology of the cell*. 2012; 104:238–256. [PubMed: 22187938]
30. Fujii N, Matsuo Y, Matsunaga T, et al. Hypotonic Stress-induced Down-regulation of Claudin-1 and -2 Mediated by Dephosphorylation and Clathrin-dependent Endocytosis in Renal Tubular Epithelial Cells. *The Journal of biological chemistry*. 2016; 291:24787–24799. [PubMed: 27733684]
31. Nabi IR, Le PU. Caveolae/raft-dependent endocytosis. *The Journal of cell biology*. 2003; 161:673–677. [PubMed: 12771123]
32. Stamatovic SM, Keep RF, Wang MM, et al. Caveolae-mediated internalization of occludin and claudin-5 during CCL2-induced tight junction remodeling in brain endothelial cells. *The Journal of biological chemistry*. 2009; 284:19053–19066. [PubMed: 19423710]
33. Li Z, Liang G, Ma T, et al. Blood-brain barrier permeability change and regulation mechanism after subarachnoid hemorrhage. *Metabolic brain disease*. 2015; 30:597–603. [PubMed: 25270004]

34. Knowland D, Arac A, Sekiguchi KJ, et al. Stepwise recruitment of transcellular and paracellular pathways underlies blood-brain barrier breakdown in stroke. *Neuron*. 2014; 82:603–617. [PubMed: 24746419]
35. Zhong Y, Smart EJ, Weksler B, et al. Caveolin-1 regulates human immunodeficiency virus-1 Tat-induced alterations of tight junction protein expression via modulation of the Ras signaling. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2008; 28:7788–7796. [PubMed: 18667611]
36. Marchiando AM, Shen L, Graham WV, et al. Caveolin-1-dependent occludin endocytosis is required for TNF-induced tight junction regulation in vivo. *The Journal of cell biology*. 2010; 189:111–126. [PubMed: 20351069]
37. Shen L, Turner JR. Actin depolymerization disrupts tight junctions via caveolae-mediated endocytosis. *Molecular biology of the cell*. 2005; 16:3919–3936. [PubMed: 15958494]
38. Bergmann KR, Liu SX, Tian R, et al. Bifidobacteria stabilize claudins at tight junctions and prevent intestinal barrier dysfunction in mouse necrotizing enterocolitis. *The American journal of pathology*. 2013; 182:1595–1606. [PubMed: 23470164]
39. Coyne CB, Shen L, Turner JR, et al. Coxsackievirus entry across epithelial tight junctions requires occludin and the small GTPases Rab34 and Rab5. *Cell host & microbe*. 2007; 2:181–192. [PubMed: 18005733]
40. Hopkins AM, Walsh SV, Verkade P, et al. Constitutive activation of Rho proteins by CNF-1 influences tight junction structure and epithelial barrier function. *Journal of cell science*. 2003; 116:725–742. [PubMed: 12538773]
41. Schwarz BT, Wang F, Shen L, et al. LIGHT signals directly to intestinal epithelia to cause barrier dysfunction via cytoskeletal and endocytic mechanisms. *Gastroenterology*. 2007; 132:2383–2394. [PubMed: 17570213]
42. Rosales MA, Silva KC, Duarte DA, et al. Endocytosis of tight junctions caveolin nitrosylation dependent is improved by cocoa via opioid receptor on RPE cells in diabetic conditions. *Investigative ophthalmology & visual science*. 2014; 55:6090–6100. [PubMed: 25190662]
43. Lim JP, Gleeson PA. Macropinocytosis: an endocytic pathway for internalising large gulps. *Immunology and cell biology*. 2011; 89:836–843. [PubMed: 21423264]
44. Sharma M, Henderson BR. IQ-domain GTPase-activating protein 1 regulates beta-catenin at membrane ruffles and its role in macropinocytosis of N-cadherin and adenomatous polyposis coli. *The Journal of biological chemistry*. 2007; 282:8545–8556. [PubMed: 17255093]
45. Lim JP, Wang JT, Kerr MC, et al. A role for SNX5 in the regulation of macropinocytosis. *BMC cell biology*. 2008; 9:58. [PubMed: 18854019]
46. Yang Z, Vadlamudi RK, Kumar R. Dynein light chain 1 phosphorylation controls macropinocytosis. *The Journal of biological chemistry*. 2005; 280:654–659. [PubMed: 15504720]
47. Bruewer M, Utech M, Ivanov AI, et al. Interferon-gamma induces internalization of epithelial tight junction proteins via a macropinocytosis-like process. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 2005; 19:923–933. [PubMed: 15923402]
48. Stamatovic SM, Sladojevic N, Keep RF, et al. Relocalization of junctional adhesion molecule A during inflammatory stimulation of brain endothelial cells. *Molecular and cellular biology*. 2012; 32:3414–3427. [PubMed: 22733993]
49. Staubach S, Hanisch FG. Lipid rafts: signaling and sorting platforms of cells and their roles in cancer. *Expert review of proteomics*. 2011; 8:263–277. [PubMed: 21501018]
50. Riento K, Frick M, Schafer I, et al. Endocytosis of flotillin-1 and flotillin-2 is regulated by Fyn kinase. *Journal of cell science*. 2009; 122:912–918. [PubMed: 19258392]
51. Li Q, Zhang Q, Wang C, et al. Invasion of enteropathogenic *Escherichia coli* into host cells through epithelial tight junctions. *The FEBS journal*. 2008; 275:6022–6032. [PubMed: 19016848]
52. Bowie RV, Donatello S, Lyes C, et al. Lipid rafts are disrupted in mildly inflamed intestinal microenvironments without overt disruption of the epithelial barrier. *American journal of physiology Gastrointestinal and liver physiology*. 2012; 302:G781–793. [PubMed: 22241861]

53. Li Q, Zhang Q, Zhang M, et al. Effect of n-3 polyunsaturated fatty acids on membrane microdomain localization of tight junction proteins in experimental colitis. *The FEBS journal*. 2008; 275:411–420. [PubMed: 18167140]
54. Vassilieva EV, Ivanov AI, Nusrat A. Flotillin-1 stabilizes caveolin-1 in intestinal epithelial cells. *Biochemical and biophysical research communications*. 2009; 379:460–465. [PubMed: 19121286]
55. Wong AW, Scales SJ, Reilly DE. DNA internalized via caveolae requires microtubule-dependent, Rab7-independent transport to the late endocytic pathway for delivery to the nucleus. *The Journal of biological chemistry*. 2007; 282:22953–22963. [PubMed: 17562704]
56. Hewlett LJ, Prescott AR, Watts C. The coated pit and macropinocytic pathways serve distinct endosome populations. *The Journal of cell biology*. 1994; 124:689–703. [PubMed: 8120092]
57. Falcone S, Cocucci E, Podini P, et al. Macropinocytosis: regulated coordination of endocytic and exocytic membrane traffic events. *Journal of cell science*. 2006; 119:4758–4769. [PubMed: 17077125]
58. Huotari J, Helenius A. Endosome maturation. *The EMBO journal*. 2011; 30:3481–3500. [PubMed: 21878991]
59. Jovic M, Sharma M, Rahajeng J, et al. The early endosome: a busy sorting station for proteins at the crossroads. *Histology and histopathology*. 2010; 25:99–112. [PubMed: 19924646]
60. Platta HW, Stenmark H. Endocytosis and signaling. *Current opinion in cell biology*. 2011; 23:393–403. [PubMed: 21474295]
61. van Weering JR, Verkade P, Cullen PJ. SNX-BAR-mediated endosome tubulation is co-ordinated with endosome maturation. *Traffic*. 2012; 13:94–107. [PubMed: 21973056]
62. Agola JO, Jim PA, Ward HH, et al. Rab GTPases as regulators of endocytosis, targets of disease and therapeutic opportunities. *Clinical genetics*. 2011; 80:305–318. [PubMed: 21651512]
63. Wandinger-Ness A, Zerial M. Rab proteins and the compartmentalization of the endosomal system. *Cold Spring Harbor perspectives in biology*. 2014; 6:a022616. [PubMed: 25341920]
64. Candiello E, Kratzke M, Wenzel D, et al. AP-1/sigma1A and AP-1/sigma1B adaptor-proteins differentially regulate neuronal early endosome maturation via the Rab5/Vps34-pathway. *Scientific reports*. 2016; 6:29950. [PubMed: 27411398]
65. Chen PI, Schauer K, Kong C, et al. Rab5 isoforms orchestrate a “division of labor” in the endocytic network; Rab5C modulates Rac-mediated cell motility. *PloS one*. 2014; 9:e90384. [PubMed: 24587345]
66. Nielsen E, Christoforidis S, Uttenweiler-Joseph S, et al. Rabenosyn-5, a novel Rab5 effector, is complexed with hVPS45 and recruited to endosomes through a FYVE finger domain. *The Journal of cell biology*. 2000; 151:601–612. [PubMed: 11062261]
67. Mattera R, Arighi CN, Lodge R, et al. Divalent interaction of the GGAs with the Rabaptin-5-Rabex-5 complex. *The EMBO journal*. 2003; 22:78–88. [PubMed: 12505986]
68. Harhaj NS, Barber AJ, Antonetti DA. Platelet-derived growth factor mediates tight junction redistribution and increases permeability in MDCK cells. *Journal of cellular physiology*. 2002; 193:349–364. [PubMed: 12384987]
69. Collier KE, Berger KL, Heaton NS, et al. RNA interference and single particle tracking analysis of hepatitis C virus endocytosis. *PLoS pathogens*. 2009; 5:e1000702. [PubMed: 20041214]
70. Ikari A, Tonegawa C, Sanada A, et al. Tight junctional localization of claudin-16 is regulated by syntaxin 8 in renal tubular epithelial cells. *The Journal of biological chemistry*. 2014; 289:13112–13123. [PubMed: 24659781]
71. Murakami T, Felinski EA, Antonetti DA. Occludin phosphorylation and ubiquitination regulate tight junction trafficking and vascular endothelial growth factor-induced permeability. *The Journal of biological chemistry*. 2009; 284:21036–21046. [PubMed: 19478092]
72. Asaka M, Hirase T, Hashimoto-Komatsu A, et al. Rab5a-mediated localization of claudin-1 is regulated by proteasomes in endothelial cells. *American journal of physiology Cell physiology*. 2011; 300:C87–96. [PubMed: 20926780]
73. Grant BD, Donaldson JG. Pathways and mechanisms of endocytic recycling. *Nature reviews Molecular cell biology*. 2009; 10:597–608. [PubMed: 19696797]

74. Morimoto S, Nishimura N, Terai T, et al. Rab13 mediates the continuous endocytic recycling of occludin to the cell surface. *The Journal of biological chemistry*. 2005; 280:2220–2228. [PubMed: 15528189]
75. Fletcher SJ, Iqbal M, Jabbari S, et al. Analysis of occludin trafficking, demonstrating continuous endocytosis, degradation, recycling and biosynthetic secretory trafficking. *PloS one*. 2014; 9:e111176. [PubMed: 25422932]
76. Su L, Mruk DD, Lee WM, et al. Differential effects of testosterone and TGF-beta3 on endocytic vesicle-mediated protein trafficking events at the blood-testis barrier. *Experimental cell research*. 2010; 316:2945–2960. [PubMed: 20682309]
77. Lu R, Johnson DL, Stewart L, et al. Rab14 regulation of claudin-2 trafficking modulates epithelial permeability and lumen morphogenesis. *Molecular biology of the cell*. 2014; 25:1744–1754. [PubMed: 24694596]
78. Dukes JD, Whitley P, Chalmers AD. The PIKfyve inhibitor YM201636 blocks the continuous recycling of the tight junction proteins claudin-1 and claudin-2 in MDCK cells. *PloS one*. 2012; 7:e28659. [PubMed: 22396724]
79. Yamamura R, Nishimura N, Nakatsuji H, et al. The interaction of JRAB/MICAL-L2 with Rab8 and Rab13 coordinates the assembly of tight junctions and adherens junctions. *Molecular biology of the cell*. 2008; 19:971–983. [PubMed: 18094055]
80. Dukes JD, Fish L, Richardson JD, et al. Functional ESCRT machinery is required for constitutive recycling of claudin-1 and maintenance of polarity in vertebrate epithelial cells. *Molecular biology of the cell*. 2011; 22:3192–3205. [PubMed: 21757541]
81. Raiborg C, Stenmark H. The ESCRT machinery in endosomal sorting of ubiquitylated membrane proteins. *Nature*. 2009; 458:445–452. [PubMed: 19325624]
82. Hurley JH, Emr SD. The ESCRT complexes: structure and mechanism of a membrane-trafficking network. *Annual review of biophysics and biomolecular structure*. 2006; 35:277–298.
83. Gruenberg J. The endocytic pathway: a mosaic of domains. *Nature reviews Molecular cell biology*. 2001; 2:721–730. [PubMed: 11584299]
84. Griffiths G, Hoflack B, Simons K, et al. The mannose 6-phosphate receptor and the biogenesis of lysosomes. *Cell*. 1988; 52:329–341. [PubMed: 2964276]
85. Takahashi S, Iwamoto N, Sasaki H, et al. The E3 ubiquitin ligase LNX1p80 promotes the removal of claudins from tight junctions in MDCK cells. *Journal of cell science*. 2009; 122:985–994. [PubMed: 19295125]
86. Piper RC, Dikic I, Lukacs GL. Ubiquitin-dependent sorting in endocytosis. *Cold Spring Harbor perspectives in biology*. 2014; 6
87. Tanno H, Komada M. The ubiquitin code and its decoding machinery in the endocytic pathway. *Journal of biochemistry*. 2013; 153:497–504. [PubMed: 23564907]
88. Alonso YAM, Migliano SM, Teis D. ESCRT-III and Vps4: a dynamic multipurpose tool for membrane budding and scission. *The FEBS journal*. 2016; 283:3288–3302. [PubMed: 26910595]
89. Bruns AF, Bao L, Walker JH, et al. VEGF-A-stimulated signalling in endothelial cells via a dual receptor tyrosine kinase system is dependent on co-ordinated trafficking and proteolysis. *Biochemical Society transactions*. 2009; 37:1193–1197. [PubMed: 19909245]
90. Woodman P. ESCRT-III on endosomes: new functions, new activation pathway. *The Biochemical journal*. 2016; 473:e5–8. [PubMed: 26733719]
91. Muthusamy A, Lin CM, Shanmugam S, et al. Ischemia-reperfusion injury induces occludin phosphorylation/ubiquitination and retinal vascular permeability in a VEGFR-2-dependent manner. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 2014; 34:522–531.
92. Traweger A, Fang D, Liu YC, et al. The tight junction-specific protein occludin is a functional target of the E3 ubiquitin-protein ligase itch. *The Journal of biological chemistry*. 2002; 277:10201–10208. [PubMed: 11782481]
93. Lui WY, Lee WM. cAMP perturbs inter-Sertoli tight junction permeability barrier in vitro via its effect on proteasome-sensitive ubiquitination of occludin. *Journal of cellular physiology*. 2005; 203:564–572. [PubMed: 15605377]

94. Chia PZ, Gunn P, Gleeson PA. Cargo trafficking between endosomes and the trans-Golgi network. *Histochemistry and cell biology*. 2013; 140:307–315. [PubMed: 23851467]
95. Yamamoto Y, Nishimura N, Morimoto S, et al. Distinct roles of Rab3B and Rab13 in the polarized transport of apical, basolateral, and tight junctional membrane proteins to the plasma membrane. *Biochemical and biophysical research communications*. 2003; 308:270–275. [PubMed: 12901864]
96. Smith TH, Coronel LJ, Li JG, et al. Protease-activated Receptor-4 Signaling and Trafficking Is Regulated by the Clathrin Adaptor Protein Complex-2 Independent of beta-Arrestins. *The Journal of biological chemistry*. 2016; 291:18453–18464. [PubMed: 27402844]
97. Loebrich S, Djukic B, Tong ZJ, et al. Regulation of glutamate receptor internalization by the spine cytoskeleton is mediated by its PKA-dependent association with CPG2. *Proceedings of the National Academy of Sciences of the United States of America*. 2013; 110:E4548–4556. [PubMed: 24191017]
98. Zhang Q, Hong M, Duan P, et al. Organic anion transporter OAT1 undergoes constitutive and protein kinase C-regulated trafficking through a dynamin- and clathrin-dependent pathway. *The Journal of biological chemistry*. 2008; 283:32570–32579. [PubMed: 18818201]
99. Monturiol-Gross L, Flores-Diaz M, Campos-Rodriguez D, et al. Internalization of *Clostridium perfringens* alpha-toxin leads to ERK activation and is involved on its cytotoxic effect. *Cellular microbiology*. 2014; 16:535–547. [PubMed: 24245664]
100. Zhou F, Lee AC, Krafczyk K, et al. Protein kinase C regulates the internalization and function of the human organic anion transporting polypeptide 1A2. *British journal of pharmacology*. 2011; 162:1380–1388. [PubMed: 21133891]
101. Prieto-Sanchez RM, Berenjeno IM, Bustelo XR. Involvement of the Rho/Rac family member RhoG in caveolar endocytosis. *Oncogene*. 2006; 25:2961–2973. [PubMed: 16568096]
102. Cheng ZJ, Singh RD, Holicky EL, et al. Co-regulation of caveolar and Cdc42-dependent fluid phase endocytosis by phosphocaveolin-1. *The Journal of biological chemistry*. 2010; 285:15119–15125. [PubMed: 20228056]
103. Williams TM, Lisanti MP. The Caveolin genes: from cell biology to medicine. *Annals of medicine*. 2004; 36:584–595. [PubMed: 15768830]
104. Biedi C, Panetta D, Segat D, et al. Specificity of insulin-like growth factor I and insulin on Shc phosphorylation and Grb2 recruitment in caveolae. *Endocrinology*. 2003; 144:5497–5503. [PubMed: 12960075]
105. El-Sayed A, Harashima H. Endocytosis of gene delivery vectors: from clathrin-dependent to lipid raft-mediated endocytosis. *Molecular therapy : the journal of the American Society of Gene Therapy*. 2013; 21:1118–1130. [PubMed: 23587924]
106. Egami Y, Taguchi T, Maekawa M, et al. Small GTPases and phosphoinositides in the regulatory mechanisms of macropinosome formation and maturation. *Frontiers in physiology*. 2014; 5:374. [PubMed: 25324782]
107. Araki N, Hatae T, Furukawa A, et al. Phosphoinositide-3-kinase-independent contractile activities associated with Fcγ-receptor-mediated phagocytosis and macropinocytosis in macrophages. *Journal of cell science*. 2003; 116:247–257. [PubMed: 12482911]
108. Lajoie P, Nabi IR. Regulation of raft-dependent endocytosis. *Journal of cellular and molecular medicine*. 2007; 11:644–653. [PubMed: 17760830]
109. Jin Y, Blikslager AT. Myosin light chain kinase mediates intestinal barrier dysfunction via occludin endocytosis during anoxia/reoxygenation injury. *American journal of physiology Cell physiology*. 2016; 311:C996–C1004. [PubMed: 27760753]
110. Utech M, Ivanov AI, Samarin SN, et al. Mechanism of IFN-γ-induced endocytosis of tight junction proteins: myosin II-dependent vacuolarization of the apical plasma membrane. *Molecular biology of the cell*. 2005; 16:5040–5052. [PubMed: 16055505]
111. Zhang J, Zhu X, Jin Y, et al. Mechanism study of cellular uptake and tight junction opening mediated by goblet cell-specific trimethyl chitosan nanoparticles. *Molecular pharmaceutics*. 2014; 11:1520–1532. [PubMed: 24673570]
112. Naydenov NG, Hopkins AM, Ivanov AI. c-Jun N-terminal kinase mediates disassembly of apical junctions in model intestinal epithelia. *Cell cycle*. 2009; 8:2110–2121. [PubMed: 19502798]

113. Rincon-Heredia R, Flores-Benitez D, Flores-Maldonado C, et al. Ouabain induces endocytosis and degradation of tight junction proteins through ERK1/2-dependent pathways. *Experimental cell research*. 2014; 320:108–118. [PubMed: 24140471]
114. Zimmerman SP, Hueschen CL, Malide D, et al. Sorting nexin 27 (SNX27) associates with zonula occludens-2 (ZO-2) and modulates the epithelial tight junction. *The Biochemical journal*. 2013; 455:95–106. [PubMed: 23826934]
115. Terai T, Nishimura N, Kanda I, et al. JRAB/MICAL-L2 is a junctional Rab13-binding protein mediating the endocytic recycling of occludin. *Molecular biology of the cell*. 2006; 17:2465–2475. [PubMed: 16525024]

Figure 1.

Endocytic sorting of tight junction (TJ) proteins. Those proteins may be internalized by different mechanisms: clathrin-, caveolae-, and flotillin-dependent endocytosis or macropinocytosis. The TJ protein (cargo) is then sorted in early endosomes (Rab5⁺, EEA1⁺) and further directed toward either of two pathways: recycling (fast or slow) or degradation.

Figure 2.

Rab5A and Rab5C regulate claudin-5 sorting. In mouse brain endothelial cells, CCL2 treatment (10 ng/mL) induced caveolae-dependent internalization of claudin-5 and its sorting toward Rab5⁺/EEA1⁺ early endosomes.³⁴ (A) In parallel, CCL2 increased activation of Rab5 isoforms Rab5A and Rab5C, measured by Rab5A and Rab5C activation assays (Abcam, MA). The claudin-5 sorting process in CCL2-treated cells is directed from early endosomes primarily toward recycling (Rab4⁺), with a small portion in late (Rab7⁺) endosomes. (B) Analysis of the recycling and late endosomes-isolated fraction showed that, in condition of knockdown of Rab5A (Rab5A siRNA), claudin-5 sorting shifted from Rab7⁺ endosomes to recycling (Rab4⁺) endosomes. In contrast, depletion of Rab5C (Rab5C siRNA) caused accumulation of claudin-5 in Rab7⁺ endosomes. Overexpression of Rab5A and Rab5C induced increased accumulation in Rab7⁺ and Rab4⁺ endosomes, respectively. These data indicate the involvement of Rab5A and Rab5C in claudin-5 sorting, with predominant sorting of Rab5a to late endosomes and degradation and Rab5C to a recycling pathway. IP, immunoprecipitation.

Table 1

A summary of confirmed types of tight junction (TJ) protein internalization in different epithelial and endothelial cells, along with the consequences of that internalization.

Internalization type	Tight junction proteins	Cell type	consequence	Ref.
Clathrin-dependent	Claudin-1	human embryonic kidney (HEK)-293 cells, colonic T84 cells, renal tubular epithelial HK-2, MDCK, HUH 7 hepatocarcinoma cell line	disrupted TJ barrier, virus uptake, transient permeability	23,27,29, 32
	Claudin-2	renal tubular epithelial HK-2 cell, MDCK II	disrupted TJ barrier, balancing barrier integrity	25,32
	Claudin-4	submandibular epithelial SMGC6 cells, Caco-2, T84 cells	transient permeability, disrupted TJ barrier	20,24,27
	Occludin	Caco-2, Sertoli cells, colonic T84 cells, MDCK cells, bovine retinal endothelial cells	transient permeability, cell migration, balancing barrier integrity,	21,22,24,27,31,74
	JAM-A	Sertoli cells, colonic T84 cells	balancing barrier integrity, transient permeability	21,22,26,27
	ZO-1	Caco-2, T84 cells	transient permeability, disrupted TJ barrier	24,26,27
Caveolae-dependent	tricellulin	Caco-2	transient permeability	24
	Claudin-1	retinal epithelial ARPE-19 cells	disrupted TJ barrier	44
	Claudin-2	Intestinal barrier	disrupted TJ barrier	40
	Claudin-3	intestinal barrier	disrupted TJ barrier	40
	Claudin-4	intestinal barrier	disrupted TJ barrier	40
	Claudin-5	brain endothelial cells, blood brain barrier	disrupted TJ barrier, transient permeability,	34–36,
	Claudin-7	Intestinal barrier	disrupted TJ barrier	40
	Occludin	brain endothelial cells, blood brain barrier, intestinal barrier, MDCK, Caco-2, T84 cells, retinal epithelial ARPE-19 cells	disrupted TJ barrier, transient permeability, virus uptake	34,35,37,38,39,41,42,43,44
	JAM-A	blood brain barrier	disrupted TJ barrier	35
	ZO-1	brain endothelial cells, blood brain barrier	disrupted TJ barrier, transient permeability	35,37
Flotillin-dependent	ZO-2	brain endothelial cells	disrupted TJ barrier, transient permeability	37
	Claudin-1	Intestinal barrier	disrupted TJ barrier	56
	Claudin-3	intestinal barrier	disrupted TJ barrier	56
	Claudin-5	intestinal barrier	disrupted TJ barrier	56
	Claudin-8	intestinal barrier	disrupted TJ barrier	56
	Occludin	Caco-2, intestinal barrier	disrupted TJ barrier	54,55,56
	ZO-1	Caco-2, intestinal barrier	disrupted TJ barrier	54,55,56
	claudin-1	T84 cells	disrupted TJ barrier	50
macropinocytosis	occludin	T84 cells, caco-2 cells	disrupted TJ barrier, virus uptake	41,50,
	JAM-A	T84 cells, brain endothelial cells	disrupted TJ barrier, leukocyte adhesion molecule	50,51