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The Tight Junction (TJ) Protein, Occludin, Is Degraded and the Products Released Preferentially From the Basolateral (BL) Surface of MDCK Cells During Incubation with Methyl β Cyclodextrin (MBCD)
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After 2h of incubation with 10 mM MBCD, the cholesterol (CH) content of MDCK cell monolayers decreases by ~70%, transepithelial electrical resistance falls and amounts of the tight junction (TJ) protein occludin, and some members of the claudin family (claudin-2 and claudin-7) decline. We examined the extent to which MBCD-induced CH efflux might stimulate the release of TJ proteins into the medium. After a 2h incubation with MBCD, we treated apical (AP) and BL solutions with TCA and analyzed the precipitates using electrophoresis and Western Blot analysis. Occludin, 59 kDa, and two C-terminal fragments (48 and 41 kDa), derived from it, were present in AP and BL solutions. However, the amount of each in the BL solution was 2, 6 and 4 times, respectively, of that released into the AP solution. These peptides continued to accumulate in the medium when MBCD was replaced with control medium after 1h. Galardin (GM6001), a metalloprotease inhibitor, suppressed formation of the 48 and 41 kDa fragment by 70% and 50 % respectively. Trace quantities of claudins-2, 3, 4, and 7 were present in the AP and BL solutions, with degradation products being evident for claudin-2 and 7 only. Nearly half of the occludin related peptides in the MBCD solution was recovered in the pellet after overnight centrifugation at 107,600 x g. Intact GP-135, actin and caveolin-1 were also elevated in the AP medium after MBCD treatment; none of them were found in the BL medium. Our data suggest that reducing cell CH activates a pathway that preferentially degrades occludin via a metalloprotease and releases vesicles containing its degradation products into the BL medium.

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cAMP Perturbs Inter-Sertoli Tight Junction Permeability Barrier *in vitro* via its Effects on Proteasome-sensitive Ubiquitination of Occludin
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Throughout spermatogenesis, inter-Sertoli tight junctions (TJs) that constitute the blood-testis barrier must be disassembled and reassembled to permit the timely movement of preleptotene and leptotene spermatocytes from the basal to the adluminal compartment of the seminiferous epithelium. However, the mechanism and the participating molecules that regulate the bioavailability of TJ proteins are entirely unknown. Using Sertoli cell culture, it was shown that there was an increase in occludin level, concomitant with a reduction of an E3 ubiquitin ligase, Itch, at the time when inter-Sertoli TJs were assembled. By co-immunoprecipitation, occludin was shown to associate with Itch at the TJs. A novel interaction between Itch and UBC4, an ubiquitin-conjugating enzyme was identified. When TJs were disrupted by dibutyryl-cAMP (db-cAMP), an increase in protein levels of Itch and UBC4 along with a significant reduction in endogenous occludin was detected. These results seemingly suggest that the interaction of Itch and UBC4 on occludin is potentially involved in regulating Sertoli TJ dynamics. Addition of a proteasome inhibitor, MG-132, into Sertoli cells cultured with db-cAMP blocked the db-cAMP-induced occludin loss *in vitro*. Accumulations of ubiquitin-conjugated and Itch-conjugated occludin were detected in Sertoli cells cultured in the presence of both MG-132 and db-cAMP. These results suggest that MG-132 prevented db-cAMP-induced TJ disruption by altering the rate of occludin degradation. Taken collectively, the results reported herein support the notion that db-cAMP-induced TJ disruption was mediated by an induction of Itch protein expression, which in turn triggered the ubiquitination of occludin resulting in TJ disruption.

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Identification and Characterization of Peptides that Modulate Epithelial Tight Junctions
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Tight junctions play an important role in regulating paracellular drug transport. The aim of this study was to identify peptides that rapidly and reversibly alter tight junction permeability in epithelial tissue. Using 3 different cell lines, MDCK, Caco-2, and 16HBE14o-, and primary respiratory epithelial tissue (EpiAirway from MatTek), as model systems, we discovered a group of peptides that effectively modulate tight junction permeability. Compounds were characterized by their effects on transepithelial electrical resistance (TER), the passage of different molecular weight FITC-dextrans through tissue monolayers, and cell viability. Transcellular and paracellular transport pathways were differentiated by fluorescence microscopy. The concentration of peptides that reduced TER ranged from 10 μM to 500 μM. TER changes were observed as early as 15 mins after treatment for some peptides, followed by recovery between 3 and 21 hours, *in vitro*. Different peptides and treatment conditions showed distinguishable permeability changes to different sized FITC-dextrans. Peptides were also characterized by their effects on tight junction structure as assessed by fluorescence microscopy using labeled antibodies against specific tight junction components. In conclusion, we have identified peptides that transiently alter tight junction permeability in epithelial cell layers. Select peptides may have utility for improving transepithelial drug delivery.

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Actin depolymerization disrupts tight junction structure and function via caveolae-mediated endocytosis
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The tight junction (TJ) is a protein complex that determines epithelial barrier function and is structurally linked to the actin cytoskeleton. Pharmacological actin depolymerization disrupts TJ structure and epithelial barrier function, but the mechanisms by which these changes occur incompletely defined. OBJECTIVE: To define the mechanisms that link actin depolymerization to disruption of TJ structure and barrier function. METHODS: The actin binding drug latrunculin A (LtA) was used to depolymerize actin and disrupt the TJ. For live cell studies monolayers of MDCK cells stably expressing EGFP- or mRFP1-fusion proteins of β-actin, occludin, claudin-1, or ZO-1 were plated on semi-permeable supports and imaged by time-lapse multi-dimensional (x/y/z) fluorescence microscopy with simultaneous measurement of transepithelial electrical resistance (TER). Immunostained fixed monolayers were imaged by deconvolution microscopy. RESULTS: Live cell imaging demonstrated occludin internalization into vesicular structures that coincided with initial TER loss after LtA addition. Redistribution of ZO-1 and claudin-1 was not apparent until later time points, well after TER loss occurred. Both occludin internalization and TER loss, but not actin depolymerization, were inhibited at 13°C, suggesting that membrane traffic is required for LtA-induced TER loss. Inhibition of endocytosis by hypertonic buffer or plasma membrane cholesterol extraction, but not macropinocytosis inhibitors, also blocked both LtA-induced TER loss and occludin internalization. Internalized occludin colocalized with caveolin-1 and dynamin-2, but not clathrin heavy chain. Finally, dominant negative (K44A) dynamin-2 blocked occludin internalization. CONCLUSION: LtA causes barrier dysfunction by inducing caveolae-mediated endocytosis of tight junction components including occludin.

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Differential Regulation of ROCK1 and ROCK2 by Dexamethasone and Rnd3/RhoE during Epithelial Tight Junction Formation
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Tight Junctions and Adherens Junctions control intercellular adhesion and barrier formation that regulate the permeability properties of epithelial and endothelial cells. We have previously shown that tumorigenic rat mammary epithelial cells can be induced to form Adherens and Tight Junctions upon treatment with dexamethasone. Apical complex remodeling can be achieved via steroid-mediated protein downregulation of the small GTPase RhoA, or by stable expression of the RhoA antagonist Rnd3/RhoE. Both pathways for apical junction formation require signaling of the downstream RhoA targets ROCK1 and/or ROCK2, since Adherens and Tight junction formation can be abolished with the Rho kinase inhibitor Y-27632. ROCK1 and ROCK2, which share 92% homology in their kinase domain, are considered to have similar function and downstream targets. Here we show evidence that ROCK1 and ROCK2 are differentially regulated in regards to protein expression and kinase activity during steroid- and Rnd3/RhoE-induced Adherens and Tight Junction formation. Our observations suggest that ROCK1 and ROCK2 have different upstream regulators and possibly, downstream targets, and both kinases have to be investigated separately.

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The N-terminus of mammalian Scribble binds hLGL2
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Scribble is a multi-domain protein that localizes to the basolateral surface of epithelial cells. Disruption of Scribble causes severe neural tube defects in mouse and loss of epithelial cell polarity in Drosophila indicating that Scribble has diverse functions. It is conserved throughout evolution and belongs to the LAP (for LRR (leucine rich repeat) and PDZ) family of proteins, which contain 16 LRRs at their N-termini followed by two LAP-specific domains, LAPa and LAPb, and 0-4 PDZ domains in their C-termini. Prevously, we demonstrated that the PDZ domains of hScrib bind the C-terminal tail of an integral membrane protein, Vangl2. Others have reported that the PDZ domains of hScrib binds betaPIX, a guanine nucleotide exchange factor. Here, we report that full-length hScrib and the N-terminal half of hScrib (encoding the LRR and the LAP domains) bind hLGL2. The C-terminal half (encoding the 4 PDZ domains) does not bind hLGL2. We also identified two hLGL2 mutations (A566D and Q618K) that disrupt binding to hScrib. Currently, we are mapping the hLGL2 binding site within the N-terminus of hScrib.

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Novel Role of Claudin 16 Tight Junction Protein in the Goblet Cells of Chick Intestine
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Tight junction (TJ) proteins form major barriers in epithelial and endothelial tissues and are responsible for regulating paracellular transport. Claudin protein