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Occludin Protein Family: Oxidative Stress and Reducing Conditions

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Abstract

The occludin-like proteins belong to a family of tetraspan transmembrane proteins carrying a marvel domain. The intrinsic function of the occludin family is not yet clear. Occludin is a unique marker of any tight junction and is found in polarized endothelial and epithelial tissue barriers, at least in the adult vertebrate organism. Occludin is able to oligomerize and to form tight junction strands by homologous and heterologous interactions, but has no direct tightening function. Its oligomerization is affected by pro- and antioxidative agents or processes. Phosphorylation of occludin has been described at multiple sites and is proposed to play a regulatory role in tight junction assembly and maintenance and, hence, to influence tissue barrier characteristics. Redox-dependent signal transduction mechanisms are among the pathways modulating occludin phosphorylation and function. This review discusses the novel concept that occludin plays a key role in the redox regulation of tight junctions, which has a major impact in pathologies related to oxidative stress and corresponding pharmacologic interventions. *Antioxid. Redox Signal.* 15, 1195–1219.

Introduction

Occludin is a unique and redox-sensitive marker of tight junctions (TJs). TJs form the most apical cell–cell contact in the lateral membrane of epithelial and endothelial cells. Depending on their protein composition, TJs show tissue-specific differences in tightness, ranging from almost complete tightening of the paracellular cleft for solutes (92) to the formation of paracellular pores for specific ions (193). Some evidence suggests that the disruption of TJs and the resulting loss of barrier function play a crucial role in a variety of diseases, including those caused by oxidative stress.

In transmission electron microscopy, TJs appear as fusion of the plasma membranes of opposing cells. Freeze–fracture electron microscopy displays intramembranous networks of anastomosing strands (79). TJ strands represent multiprotein complexes of transmembrane proteins, such as claudins (99), occludin-like proteins (55, 81, 182) or junctional adhesion molecules (JAMs) (13a), and membrane-associated proteins, such as *Zonula occludens* (ZO) proteins recruiting the TJ proteins (67a). A tissue-specific combination of members of the claudin protein family constitutes the backbone of the TJs. Some claudins are shown to enhance the tightness, and others reduce it, thus defining the specific paracellular barrier characteristics of tissues (143a). Occludin was the first identified (55) transmembrane protein of the TJs, is specific for TJs, and plays a regulatory role (190). Nevertheless, its physiologic functions and molecular structure (197) are still not fully understood, with contradictory results being reported (153).

The occludin family of TJ-associated marvel proteins comprises occludin, tricellulin, and marvelD3 (Fig. 1). The family shares a conserved four-transmembrane marvel domain (MAL and related proteins for vesicle trafficking and membrane link) and is best considered as a group with parallel, but nonredundant, functions. The marvel domain is involved in apposition to cholesterol-rich membrane microdomains (163), originally discovered in proteins involved in membrane apposition and fusion events, as also observed for occludin, tricellulin, and marvelD3 in TJs. The domain covers a segment spanning the first to the last transmembrane domain (182) and might enable homologous or heterologous associations with other membrane proteins (Fig. 2). Marvel proteins possess intracellular termini, a short intracellular loop, and two extracellular loops (ECLs). The N-terminus of occludin is much shorter than that in tricellulin or marvelD3. The C-termini of occludin and tricellulin are longer than those in marvelD3. The four transmembrane domains are predicted to form α-helices spanning the membrane. Although drawn in line, the helices are thought to be tightly packed, as reported for gap-junction proteins (114) (Fig. 3).

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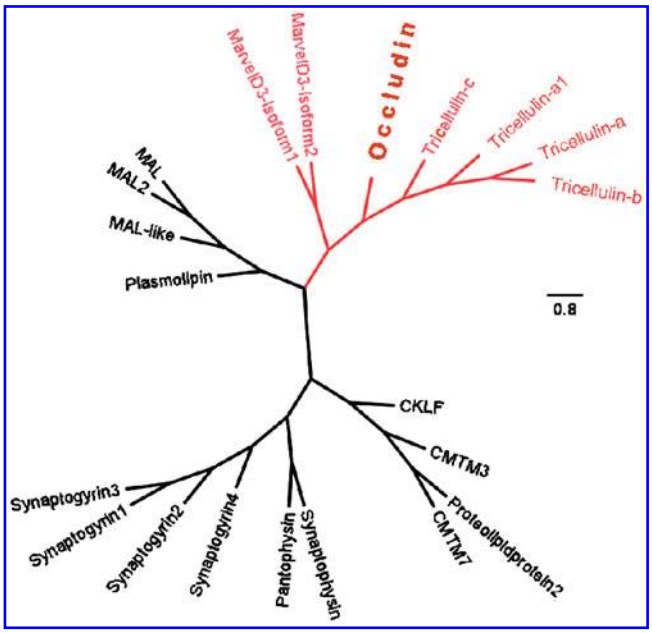

FIG. 1. Phylogenetic tree of the marvel-domain–containing protein superfamily with special consideration of the tight junction–associated occludin family (red). Human sequences were aligned by the program clustalW2 (http://www.ebi.ac.uk/Tools/clustalw2/). Bar, phylogenetic distances (indicated by line length and branching) were calculated with the program Jalview (99) and depicted with the program figtree (http://evolve.zoo.ox.ac.uk/software.html?id=figtree). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

In recent years, an increasing number of observations showed that not only occludin but also TJ barriers are redox sensitive. Occludin has been reported to be an early and specific target for redox-reactive species (113). Claudins are primarily responsible for the TJ function, but are less sensitive to oxidative stress (67, 75). We therefore review the structure and properties of occludin-like proteins and present the new view that occludin acts as a redox sensor and mediator of redox-mediated changes in the TJ. First, the structural domains, posttranslational modifications, binding partners, and their functional roles are addressed. Second, we show how these properties are modified by redox changes, which redox-dependent signaling pathways and mediators are involved, and which redox-related pathologic conditions affect occludin.

Occludin

General properties. The exact mechanism of how occludin supports the TJ function is unclear. It mediates intercellular adhesive interactions, and modulation of occludin expression affects TJ barrier function. Occludin-derived peptides were found to disrupt TJ (12, 55, 123, 210, 215). Internalization of occludin has been associated with pathophysiologic and pharmacologically induced TJ-barrier loss (35, 169, 172, 188). In contrast, occludin-knockout mice are viable and fail to display defective barrier function. However, the animals display signs of pathologic disorders, such as small size, testicular atrophy, male infertility, salivary gland dysfunction, atrophic gastritis, thinning of compact bone, or brain calcification, which are probably due to secondary effects (161). It therefore appears that occludin expression is not essential for TJ formation and function, although virtually all TJs contain occludin. In conclusion, occludin influences epithelial and endothelial TJ function by indirect mechanisms, such as protein–protein interactions (see subsequent sections).

Knockdown of occludin in cultured keratinocytes during differentiation blocked the formation of the paracellular seal (213), underlining a role of occludin in cell differentiation. In addition, occludin knockdown weakens the tricellular localization of tricellulin (82). Conversely, tricellulin or marvelD3 partially compensate for the loss of occludin, which also explains why the knockout mice lack distinct TJ defects. Consistent with this observation, a study was performed on the barrier injury of jejunal epithelial cells by a proinflammatory cytokine. Here, the redistribution of occludin from the TJ into cytosolic vesicles is counterbalanced by the enrichment of marvelD3 and tricellulin in the TJ (153). Conversely, deafness caused by mutations in tricellulin is obviously not compensated by occludin (153).

Human occludin contains 522 amino acids (aa); the isoelectric point is 5.77, and the calculated molecular mass is 59.1 kDa; N- (66 aa) and C-terminus (256 aa) are cytosolic. The use of antibodies and freeze–fracture studies demonstrated TJ localization (55). The ECL1 (about 50 aa) is rich in glycine and tyrosine. Tyr residues form hydrophobic interactions and H-bonds, whereas Gly residues provide flexibility. The ECL2 (about 45 aa) contains two cysteines (Fig. 3) which are assumed to form disulfide bridges in the oxidizing environment of the interstitium. The short intracellular loop ($\sim$10 aa) reveals an excess of basic aa (Fig. 2). The exact structure and function of the loops remain unclear.

Occludin is highly dynamic within the TJs and exhibits a mobile fraction of 71% and a diffusion constant of 0.011 μm^2/s. In contrast, claudin-1 is much more stably localized at the TJ, with a mobile fraction of only 24% (173). This is in accordance with the view that claudins, in contrast to occludin, directly assemble the TJ barrier and that, for example, claudin-1 has a tightening function (97), whereas occludin has a more regulatory and supportive role.

Functions of different occludin segments: results from chimerae, splice variants and peptides. Studies with an exogenous occludin expression and peptide exposure have provided details about cellular distribution and protein interactions that help to elucidate the molecular behavior and the controversial role of this protein in TJs. Overexpression of occludin in TJ-free cells (*e.g.*, L-fibroblasts) results in TJ-like strand formation (59). Expression in rat lung endothelial cells (occludin-free but containing ZO-1) leads to junctional localization without changes in the paracellular permeability. Accumulation of occludin at the junctions is accompanied by the accumulation of actin. Depolymerization of actin interrupted the junctional localization of occludin. These effects point to an interrelation between occludin, ZO-1, and actin (104).

Chimerae were prepared in which the occludin cytosolic C-terminal tail is N-terminally fused with ecto- and transmembrane parts of the IgG receptor and then transfected in Madin-Darby canine kidney (MDCK) cells. The results show that the C-terminus of occludin mediates basolateral localization in the plasma membrane (120). Moreover, constructs glycosylated at the ECLs accumulate basolaterally. In both

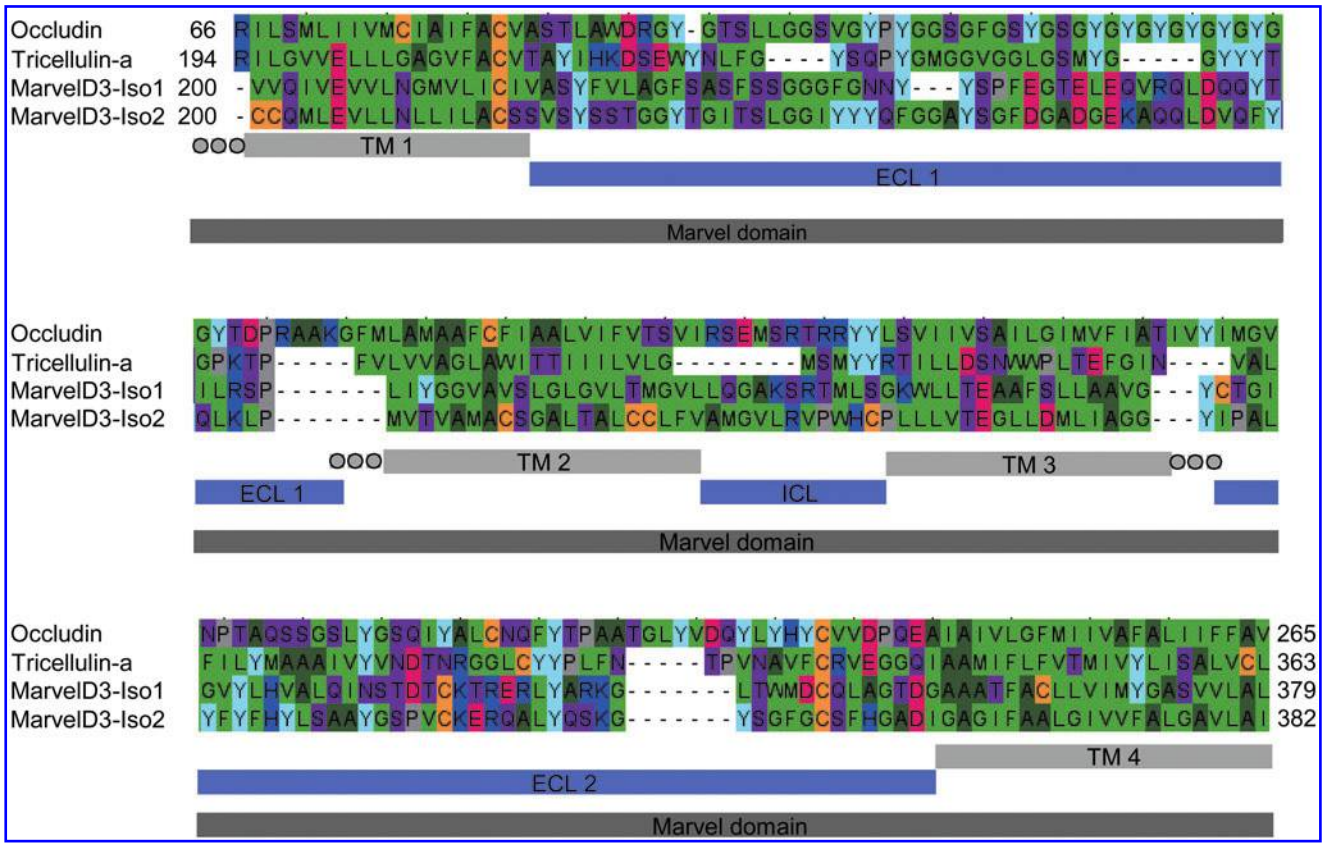

FIG. 2. Alignment of the marvel-domains of the human tight junction–associated marvel proteins occludin (UniProtKB/Swiss-Prot Q16625), tricellulin-a (Q8N4S9-1), marvelD3-Iso1 (Q96A59-1), and -Iso2 (Q96A59-2). The marvel domains include segments from first to fourth transmembrane domain (TM); ICL/ECL, intra-/extracellular loop. Alignment is based on sequences taken from UniProtKB/Swiss-Prot (5/3/2010), calculated by ClustalW2 (http://www.ebi.ac.uk/Tools/clustalw2/index.html), and edited by Jalview (http://www.jalview.org/). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

cases, the molecules became too large for integration into the TJ or the binding properties of the constructs were altered or both. In contrast, connexin chimera with the ZO-1 associating C-terminus of occludin localize to the TJs (130). After N-terminal fusion with hemagglutinin and C-terminal deletion, occludin exhibited discontinuous distribution at the TJs. This indicates that the N-terminal or transmembrane region or both contribute to the TJ localization (78). Truncation after the 14^{th} aa of the ECL2 decreased paracellular tightness; in freeze–fracture replicas, strong fragmentation of the TJ strands occurred. These data demonstrate that the N-terminus or the first three transmembrane segments or both play a role in the barrier function of the TJ (13).

In the splice-variant occludin 1B, described in canine MDCK cells only (UniProtKB/Swiss-Prot Q9N0W3), the first 17 aa of the N-terminus are replaced by 56 aa. This variant colocalizes with endogenous occludin in murine intestine and T84 (human colonic adenocarcinoma) cells (135). However, the corresponding exon exists neither in the human genome nor in mouse cDNA (65). Some splice variants lack the fourth transmembrane sequence (TM4) and some subsequent aa; consequently, this C-terminus is extracellular. The latter variants do not localize to the TJs (116). Another TM4-deletion mutant is slightly expressed in subconfluent but not in confluent cells (65). Overexpression of occludin in epithelial cells increased transcellular electrical resistance (TER), but unexpectedly, increased rather than decreased paracellular flux (11, 123). These contradictory actions are related to the expression of different splice variants (Fig. 3).

Studies with ECL-derived peptides are also inconsistent because of species heterology between the peptides and cells applied (Table 1), as well as folding problems when using synthetic peptides. An ECL2-derived peptide (chicken occludin$^{184\text{-}227}$) reversibly disrupted the permeability barrier in A6 (*Xenopus* kidney epithelial) cells. It decreased cellular levels and junctional localization of occludin, whereas an ECL1 peptide showed no effect (210). Other authors found the opposite: only ECL1-derived chicken-occludin$^{100\text{-}108/109}$ peptides influenced the TJ integrity of *Xenopus* cells (194). In a homologous system, human occludin$^{90\text{-}103}$ (ECL1) increased the permeability of human Caco-2 (colon carcinoma) cells, however, only when applied basolaterally. Human lipoamide-occludin$^{90\text{-}103}$, protected against degradation, caused rapid apical opening of the TJ (187). The lipoamide construct led to redistribution of occludin and reduced barrier function in human airway epithelial cells, but did not change distribution/expression of ZO-1 or claudin-1 or -4 (50). Rat occludin$^{209\text{-}230}$ (ECL2) reversibly disrupted the TJ-barrier of rat Sertoli cells but did not open the blood–testis barrier (BTB) (34). The follicle-stimulating hormone conjugated peptide rat-FSH-occludin$^{209\text{-}230}$ showed transient and reversible disruption of the rat BTB (207).

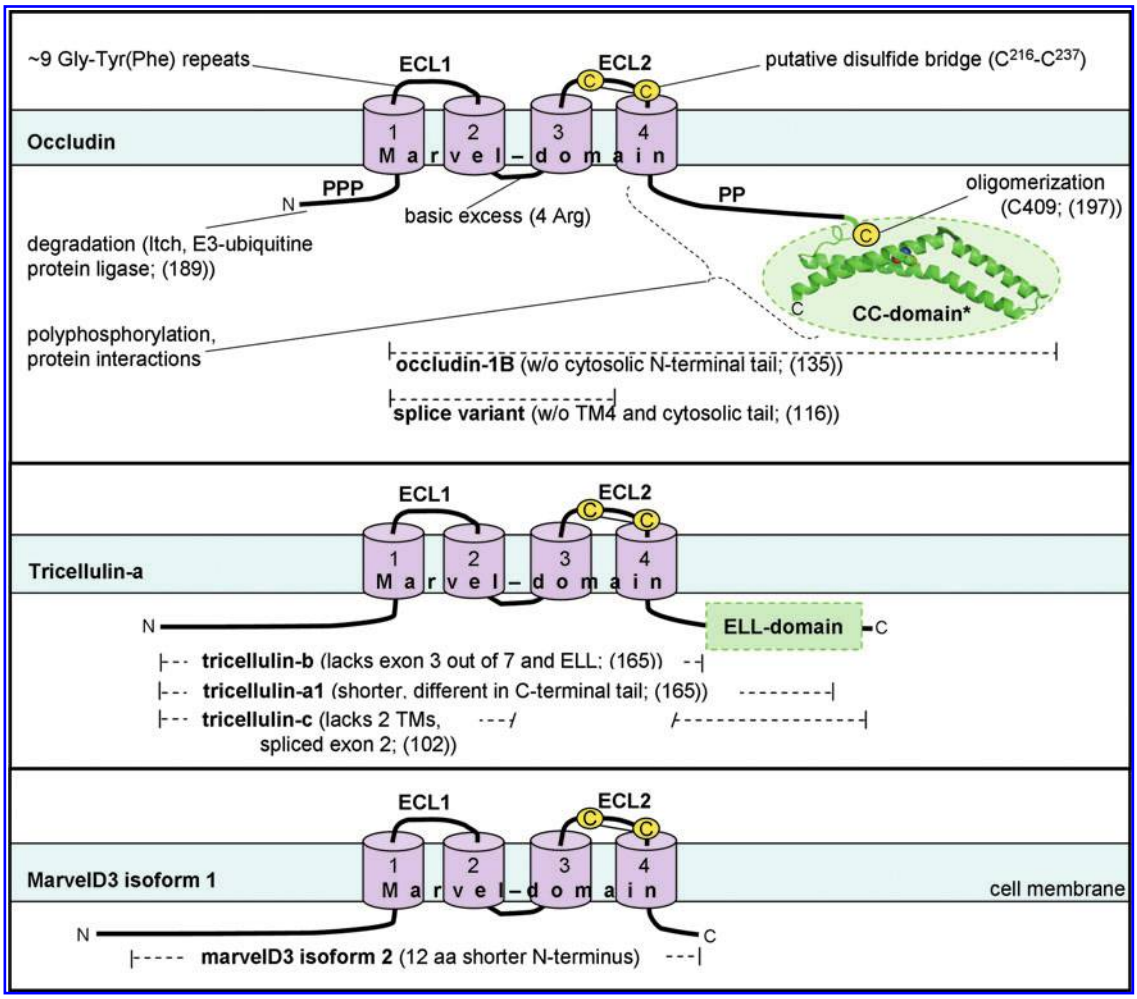

FIG. 3. Topology and functions of the occludin protein family (human sequences). ECL, extracellular loop; TM, transmembrane domain; P, proline-rich motif; C, cysteine; aa, amino acid. *The coiled coil (CC) domain is homologous with the ELL domain, a conserved region in occludin proteins (109) and an RNA polymerase II elongation factor encoded by the human ELL gene (176). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

In general, occludin localizes specifically to the TJ, helps to connect TJs and cytoskeleton, but has no direct tightening function. Moreover, the data provide evidence that the ECLs mediate intercellular *trans*-interaction (150) and modulate paracellular barrier functions.

Posttranslational modifications. The molecular behavior of occludin, its cellular distribution, and its interactions within the TJs are regulated through modifications of its phosphorylation status. Occludin is phosphorylated by various protein kinases (PKs) at different sites (Table 4), partially with opposing functional effects. Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) reveals multiple bands for occludin (62–82 kDa) because of multiple phosphorylations (5, 6, 31, 162, 185, 209). PKs identified include conventional Ca^{2+}-dependent (cPKC) and novel diacylglycerol-dependent PKCs (nPKCs) (5, 6), casein kinase 1 (CK1) (48, 125), CK2 (41, 48, 179), $p34^{cdc2}$/cyclin B-complex (40, 41), ERK1 (15), and nonreceptor TyrK c-Yes (31). Rho-associated kinase (ROCK) (212) and Rab13 (132) are also involved in occludin regulation and signaling. In epithelial cell lines, highly phosphorylated occludin is enriched in TJs, and less phosphorylated occludin localizes in the cytoplasm (5, 162, 186). The total extent of phosphorylation correlates with the concentration of Ca^{2+}, as shown by depletion and reintroduction of Ca^{2+} (5, 6). The addition of peptides derived from the ECL2 but not ECL1 of occludin reduce its phosphorylation in MDCK-I cells (210). Increased occludin phosphorylation in retinal cell monolayers resulted in increased permeability (7), and involvement of cPKCβ in the peptide effect was demonstrated (71).

Identified sites in human occludin are $T^{403/404}$ phosphorylated by nPKCη (186), T^{305} (119), and S^{408} (71) phosphorylated by *Ataxia telangiectasia* mutated and Rab3-related Ser/ThrPK (ATR). Phosphorylation of S^{490} (Act1-mediated) (184) and ubiquitination are caused by VEGF (vascular endothelial growth factor) in retinal cells, concomitant with TJ-fragmentation and occludin trafficking to endosomes (134). Phospho-S^{490} attenuated interaction with ZO-1 (180). Dephosphorylation is caused by protein phosphatases PP1 (174) and PP2A (72).

In conclusion, phosphorylation at certain sites [*e.g.*, T^{404} (human)] leads to junctional distribution of occludin, associ-

TABLE 1. EFFECTS OF OCCLUDIN-DERIVED PEPTIDES

ECL	Peptide	Species	Barrier	Species	References
1	^{81}DYGYGLGGAYGTGLGGFYGSNYYGSGLSYSYGYGGYYGGVNQRT^{125}	Chicken	ø	*Xenopus*	(210)
1	^{100}SNYYGSGLSY^{109}	Chicken	↓	*Xenopus*	(105)
1	^{100}SNYYGSGLS^{108}	Chicken	↓	*Xenopus*	(105)
1	C_{14} lipoamide acid conjugated ^{90}DRGYGTSLLGGSVG^{103}	Human	↓	Human	(50, 187)
1	^{90}DRGYGTSLLGGSVGYPYGGSGFGSYGSGYGYGYGYGGYTDPR^{135}	Human	↓	Human	(187)
1	^{90}DRGYGTSLLGGSVG^{103}	Human	↓*	Human	(187)
1	^{90}DRGYGTSLLG^{99}	Human	ø	Human	(187)
1	^{98}LGGSVG^{103}	Human	ø	Human	(187)
1	^{102}VGYPYGGSGFGS^{113}	Human	ø	Human	(187)
1	^{106}YGGSGFGSYGSGYGYGYGYGGYTDPR^{135}	Human	ø	Human	(187)
1	^{109}SGFGSYGSGYGYGY^{122}	Human	ø	Human	(187)
2	^{184}GVNPQAQMSSGYYYSPLLAMCSQAYGSTYLNQYIYHYCTVDPQE^{227}	Chicken	ø	*Xenopus*/m	(194, 209)
2	^{184}GVNPQAQMSSGYYYSPLLAMCSQAYGSTYLNQYIYHYCTVDPQE^{227}	Chicken	↓$^{+}$	*Xenopus*	(209)
2	^{210}STYLNQYIYN^{219}	Chicken	ø	*Xenopus*	(105)
2	^{196}GVNPTAQSSGSLYGSQIYALCNQFYTPAATGLYVDQYLYHYCVVDPQE^{243}	Human	ø	Human	(187)
2	^{209}GSQIYTICSQFYTPGGTGLYVD^{230}	Rat	↓	Rat	(34)
2	FSH-fused ^{209}GSQIYTICSQFYTPGGTGLYVD^{230}	Rat	↓	Rat	(207)

↓, cell barrier decreased; ø, no effect; *, basolateral effect only; $^{+}$, Cys protected; ECL, extracellular loop; m, mouse; FSH, follicle-stimulating hormone.

ation with TJ proteins, and barrier function (186). Interestingly, T^{404} is localized at the N-terminus of the coiled coil (CC)-domain close to the redox-sensitive dimerization site Cys^{409} (cf. subsequent sections). At the C-terminal end of the domain, at S^{490}, phosphorylation has an opposite effect (184). Taken together, occludin phosphorylation plays an indirect role in the regulation of TJs. One potential way is the interaction with claudins, as pointed out recently (74). However, the exact molecular mechanisms and their functional consequences remain to be defined.

Tight junction– and regulatory proteins binding to occludin. Full-length occludin self-associates (23, 197), and interacts with tricellulin (204) and marvelD3 (153). As the lengths of the C- and N-termini of the occludin family members differ considerably, it is likely that their heteromeric binding area involves their highly homologous marvel domains. Direct association of occludin with claudins 1, 4, 6, 9, 11, 12, and 17 has been indicated by fluorescence resonance energy transfer (74).

The intracellular N-terminus of occludin bears a type I WW-binding motif (PPXY) and interacts with itch, an E3-ubiquitin protein ligase involved in occludin degradation (189). Occludin 1B lacks PPXY and, consequently, is degraded differently.

Within the occludin cytosolic C-terminal tail, a proteolytically stable structure (148) forms an oligomerizing CC-domain [murine occludin$^{406\text{-}521}$ (133)] (Fig. 3). Here, crystallography shows one longer α-helix antiparallel to two shorter α-helices. The domain is homologous to the ELL domain (human occludin$^{416\text{-}522}$), a conserved region in eukaryotic occludins (109) and the RNA polymerase II elongation factor encoded by the human ELL gene (176).

The recombinant CC-domain also self-associates (133, 197) and binds the junctional recruiting proteins ZO-1 (51, 58), ZO-2 (83, (206), and ZO-3 (76). It interacts with the gap-junction protein connexin 26, the protooncogene TyrK c-Yes, Ser/Thr kinase atypical PKCζ (aPKC) being independent of Ca^{2+} and diacylglycerol, phosphoinositol-3 kinase (140), TyrK c-Src (49), CK1ε (125), and CK2 (179). Further binding partners are cingulin, a cytoplasmic plaque protein of the TJs also containing a CC-domain (41), and F-actin (104, 206), which establishes occludin as a link between TJs and the cytoskeleton.

Chicken occludin$^{358\text{-}504}$ binds VAP-33, both colocalizing at TJs. VAP-33 is also found in intracellular vesicles, suggesting that occludin together with VAP-33 is targeted for vesicular transport to the membrane (106). The TJ-protein JAM (18) and the gap-junctional connexin 32 (96) are potential interaction partners of occludin. JAM supports the localization of occludin to the TJs, as cotransfection enhanced accumulation at the cell–cell contacts (18). However, it is unclear whether the two proteins bind directly to each other. Some evidence suggests that occludin also associates with caveolin, the marker of caveolae (141), the internalization mediator clathrin (84), and the transforming growth factor (TGF) β-receptors I and II (14).

Most of the binding proteins mentioned above are of structural or regulatory significance for TJs (Fig. 4). The occludin marvel domain or CC-domain interacts with the majority of the TJ proteins, which supports the view that occludin has a central structural position and a key regulatory function in the TJs.

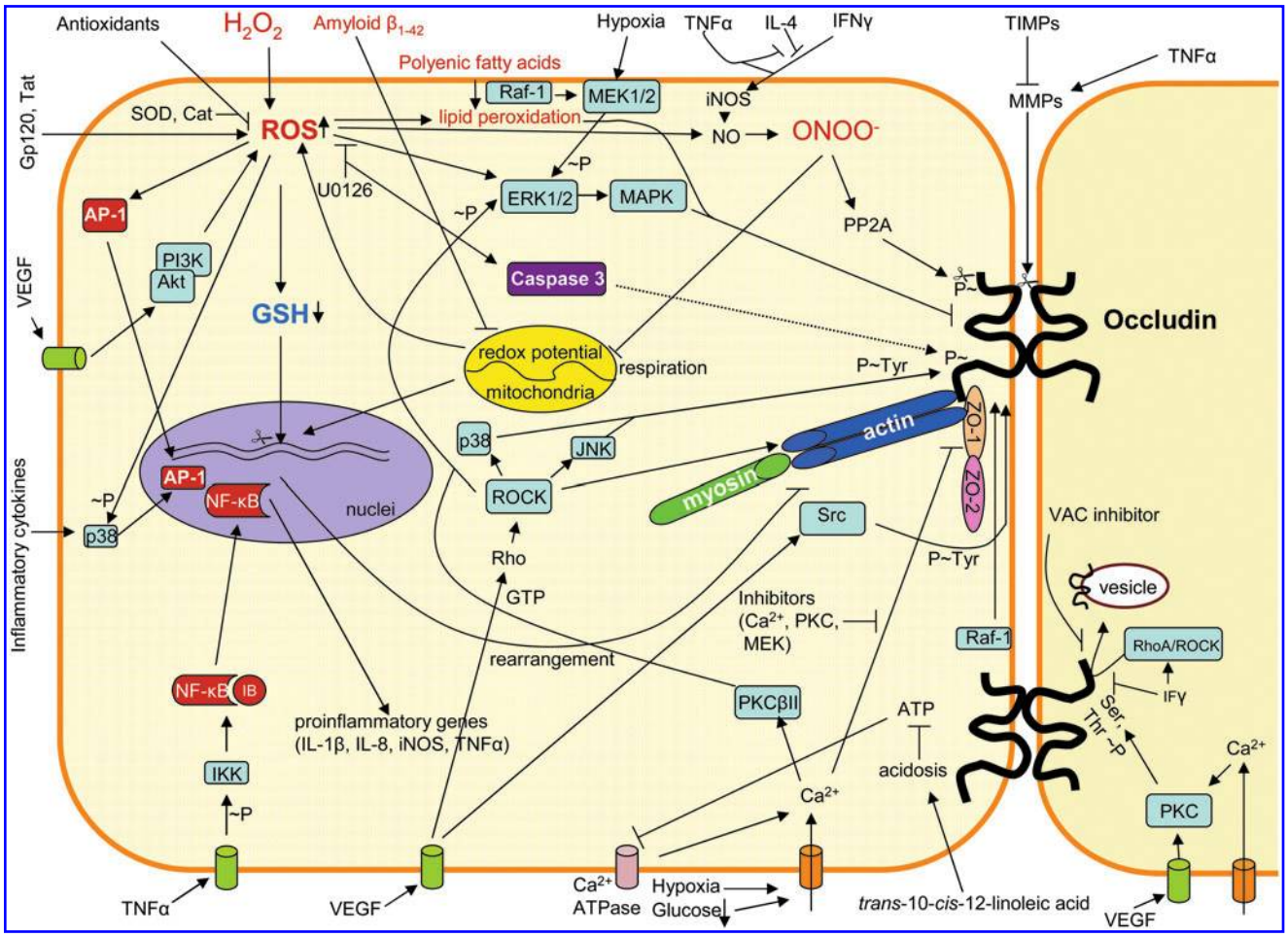

FIG. 4. Signal-transduction pathways influencing occludin, with special consideration of redox-dependent processes. TNF, tumor necrosis factor; IL, interleukin; IFN, interferon; ROS, reactive oxygen species; GSH, reduced glutathione; PKC, protein kinase C; AP-1, activator protein 1; NF-κB, nuclear factor κB; Rho, Ras homologue; p38, mitogen-activated protein kinase; ROCK, rho-associated kinase; Src, Src kinase; ~P, phosphorylation; iNOS, inducible NO-synthase; VEGF, vascular endothelial growth factor; VAC, vesicular apical complex; TIMPs, tissue inhibitors of matrix metallopeptidases (MMPs); MAPK, mitogen-activated protein kinase; MEK, MAP/ERK-kinase; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; IKK, inhibitory κB kinase; IB, inhibition unit of NF-κB; Akt, protein kinase B; Raf-1, MAPK-kinase-kinase-kinase. Light blue boxes, kinases; red boxes, transcription factors; green cylinders, receptors; orange cylinders, ion channels; UO126, MEK inhibitor; Gp120, envelope protein of human immunodeficiency virus (HIV); Tat, transcription-activating factor of HIV. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

Tricellulin: barrier function at tricellular contacts and occludin substitution at bicellular contacts

Like occludin, tricellulin (marvelD2) knockdown interferes with TJ assembly and enhances solute diffusion across epithelial monolayers (81), whereas overexpression enhances barrier function (102). Human tricellulin mutations are associated with nonsyndromic deafness (32, 156). Obviously, tricellulin is not essential for the function of epidermal, respiratory, renal, or intestinal TJs.

Tricellulin is mainly localized at tricellular cell–cell contacts, and to a lesser extent in bicellular TJs. The longest human isoform (156), tricellulin-a (558 aa, UniProtKB/Swiss-Prot Q8N4S9-1), contains seven exons and a C-terminal ELL sequence, with 51% sequence similarity to occludin. In tricellulin-al (546 aa, UniprotKB/Swiss-Prot Q8N4S9-3), aa 383–394 are missing. Tricellulin-b (457 aa, UniprotKB/Swiss-Prot Q8N4S9-2) lacks exon 3 and ELL and is expressed in the basal cell layers of keratinocyte cultures or epidermis (165). For tricellulin-c (442 aa, NCBI ABG 89106.1), a loss of at least two transmembrane domains is predicted, with an alternatively spliced exon 2 (102) (Fig. 3). Tricellulin is found in a wide variety of epithelial tissues, the expression levels of tricellulin, marvelD3, and occludin are highly correlated (153).

It is debated whether tricellulin is phosphorylated (48, 81) or binds ZO-1 (82, 153, 156). Both tricellulin and occludin form homomeric lateral complexes in the membrane. However, dimerization of tricellulin is not mediated by the C-terminal domain, as reported for occludin (109, 133, 204).

Suppression of tricellulin-a results in compromised barrier function and disorganized tricellular and bicellular TJs, indicating an essential role in barrier formation (81). In the absence of tricellulin, the continuity of the TJ network disappeared, and bicellular TJ appeared to be poorly developed. In occludin-

knockdown cells, tricellulin was spread out in bicellular TJs, indicating that occludin supports tricellular TJ localization of tricellulin at tricellular contacts by excluding it from bicellular TJs (82). When overexpressed in bicellular TJs, tricellulin decreases permeabilities to ions and midsize or large solutes. In tricellular TJs, overexpression of tricellulin affects the permeation of macromolecules, but not of ions, indicating that, at low physiologic tricellulin expression levels, the central tube in tricellular TJ formed by tricellulin provides a pathway for macromolecules (102).

N- or C-terminal deletion analysis suggested that the tricellulin C-terminus is important for efficient transport and targeting to the plasma membrane, whereas the N-terminus is involved in targeting to tricellular TJs (204). Heteromeric tricellulin–occludin complexes have been observed after overexpression in human embryonic kidney cells. This observation is consistent with the model that occludin excludes tricellulin from bicellular TJ (82), and suggests that, in the initial phase of their transport to the cell surface during TJ assembly, occludin and tricellulin are directed to bicellular TJs in common complexes, which subsequently dissociate (204) when tricellular contacts are formed. However, the attempted detection of endogenous heteromeric tricellulin–occludin complexes has failed (153), whereas overexpressed tricellulin was coprecipitated with endogenous occludin (204).

In summary, tricellulin and occludin share similar but not completely overlapping protein-binding properties. Similar to occludin, cysteine-containing sequences are conserved in tricellulin at both the beginning and the end of the cytosolic C-terminal ELL domain, which is homologous to the occludin CC-domain. However, it still remains to be clarified whether tricellulin is redox sensitive.

MarvelD3

The marvelD3 isoforms 1 (410 aa, 46 kDa) and 2 (401, 45) exhibit broad distribution in epithelial and endothelial tissues. They colocalize with occludin at TJs in intestinal and corneal epithelial cells. MarvelD3 expression is not required for the formation of functional TJ, whereas depletion results in enhanced TER. Taken together, marvelD3 functions are a determinant of epithelial paracellular permeability properties (182). Analysis of RNA and protein tissue distribution, as well as trafficking and protein interactions, shows that marvelD3, occludin, and tricellulin have distinct but overlapping functions at the TJs (153). Redox sensitivity at the cytosolic C-terminus (20 aa) is unlikely, because marvelD3 lacks the ELL domain and any cysteine (Fig. 3). It remains to be investigated whether conserved cysteine residues in the cytosolic N-terminus affect the redox sensitivity of marvelD3.

Redox Sensitivity of the Occludin Oligomerization

Many observations document that occludin is redox sensitive (Tables 2 through 6). Under oxidative stress, occludin appears as an early and specific target for reactive species (115). Disulfide bond formation is important (122) for the self-association of full-length occludin and of its cytosolic C-terminal CC-domain (23) (Fig. 3). The dimerization depends on the concentration of reduced glutathione (GSH), on Cys^{409} in the CC-domain (human), and is prevented by the aa exchange C409A (197), strongly arguing for intermolecular disulfide bridge formation within the cell. Similarly, lipophilic thiols prevent oligomer formation, indicating oligomerization potential of the marvel domain with its highly conserved cysteine residues, especially in the transmembrane domains (122). Furthermore, oxidative stress during inflammation (121) and radical generation in hypoxia/reoxygenation (113) reduce occludin oligomerization. The redox sensitivity explains the failure of oligomer detection in Western blots (5), because either the antibody-binding sequence on occludin is partially covered within the disulfide-dimer or as the SDS-PAGE used dissolves the oligomer. Occludin and GSH are highly sensitive to oxidative stress (10, 68, 115, 121). The redox sensitivity of the CC- and marvel domains of occludin, therefore, reveals important mechanisms in the dysregulation of endothelial and epithelial barriers under oxidative stress. As occludin interacts via both domains with the most important structural and functional proteins of the TJs, the redox sensitivity controls key processes of cell–cell contacts. Disulfide bridge formation of many intracellular proteins perturbs their function and controls multiple processes (80). Thus, occludin is considered a TJ-protein in which dimerization of the cytosolic C-terminal CC-domain and oligomerization of the full-length protein are directly regulated by redox processes. Consequently, on oxidative stress, the regulatory functions of occludin are affected as well as its heterologous interaction with other TJ proteins, such as ZO proteins (58, 76, 83) or claudins (74).

Evidence indicates that oxidation-driven oligomerization supports TJ assembly, whereas reducing conditions, as found in hypoxia, result in the dissociation of occludin oligomers, which can disassemble TJs. Interestingly, the effective thiol concentrations determining the state of oligomerization of occludin are >0.5 m*M* (198), which is in the range of the normal intracellular concentrations of GSH (1–10 m*M*), the main determinant of the cellular thiol level (155). Although the cytosol has a reducing environment (80) that should prevent stable disulfide bridges, numerous cytosolic proteins undergo disulfide bond formation (42). Thus, intracellular GSH regulates oligomerization of occludin and, in consequence, TJ assembly. As monomers and oligomers coexist in similar amounts under normal conditions (198), the oligomerization is sensitive to redox changes under physiologic conditions and is also relevant for reversible pathologic situations.

The direct redox-dependent changes in occludin oligomerization reviewed previously influence the functions of occludin, and, *inter alia*, its interaction with claudins (74), which directly influences the constitution and function of the TJ. Indirect ways to modulate occludin and, consequently, the TJ function in response to redox processes are discussed later.

Redox Changes and Functional Consequences in Tissue Barriers *via* Signaling to Occludin

Oxidative and antioxidative interventions act in opposite manners on TJ barriers. Claudins form the functional backbone of the TJs. However, the data available ascribe redox sensitivity of TJs rather to occludin, which responds earlier to oxidative stress than claudins (67), with some claudins not responding at all (75). Oxidants downregulate occludin, reduce its specific membrane localization and regulatory contribution to barrier tightness (Table 2), which are prevented by antioxidants (Table 3). In addition to the direct redox sensitivity described in preceding sections, different redox-sensitive signal-transduction pathways modulate occludin indirectly (Table 4; Fig. 4).

Table 2. Effect of Oxidative Mechanisms on Occludin

Oxidant	*Nature, mode of action*	*Occludin*					*References*
		Localization	*Expression*	*Barrier*	*Subject*	*Remarks*	
OxPAPC	Induces $\cdot O_2^-$ generation				BAEC	Occ∼P↑; Occ release by vascular EC	(44)
SOD1 mutant	Enzyme activity↓→ $\cdot O_2^-$ excess		↓	↓	Murine brain	Mutation → model of amyotrophic lateral sclerosis	(220)
			↓	↓	Rat brain	Mutation → model of amyotrophic lateral sclerosis	(137)
XO	Generates $\cdot O_2^-$ and H_2O_2	Cytosol		↓	Caco-2	ZO-1/Occ complex↓; Tyr∼P↑; Tyr-Kin inhibitor blocks	(154)
		Membr.↓	↓	↓	GP8	Occ ruffling; PKB∼P↑, PI3-Kin inhibitor blocks; RhoA↑	(168)
DMNQ	Free radical initiator		↓	↓	Brain EC	Pronounced in absence of glucose	(100)
Efavirenz	$\cdot O_2^-$ formation; HIV inhibitor		↓	↓	HCAEC	MAPK JNK∼P↑, IκBα↑ →transcription↑	(85)
H_2O_2	ROS, GSH↓	Cytosol	ø	↓	HUVEC	Ser∼P↑ (similar in MDCK); cytoskeleton rearranged	(90)
		Cytosol	↓	↓	MDCK-II		(67)
		Cytosol		↓	RPEC	PEDF prevents effects; HSP27∼P↑, p38↑	(77)
		Cytosol		↓	MDCK-II	Cat protective→ TJ reassembly, protein synthesis ø	(127)
	TJ↓	Cytosol		↓	MDCK	P∼$Y^{398/402}$ intensify effect; Y∼P→ ZO-1(Src) binding↓	(49)
H_2O_2/MMP-2		Cytosol	↓	↓	PBEC	Proteolysis of Occ	(111)
NO	Oxidative via $ONOO^-$ (high NO conc.)	Cytosol	↓	↓	MDCK	NO released after cytokine stimulation	(43)
					GP8.3	Occ proteolysis↑ (similar in mouse)	(95)
Fe^{2+}/Cu^+	Oxidative (via redox cycling)	ø	ø	↓	Caco-2		(52)
Abrasion	Fe/Cu/Mn → ox. stress↑; GSH↑	Cytosol	↓	↓	A549		(60)
CeO_2	Particle stress		↓	↓	A549	Disrupts TJ	(159)
$CdCl_2$	GSH peroxidase↑; GSSG reductase↓		↓	↓	Sertoli	Disrupts TJ	(34)
Pb	Cytotoxic		↓	↓	Rat brain	Protection by Fe^{2+} → Occ expression normalized	(201)
Amyloid $\beta_{1\text{-}42}$	Mitoch. redox pot.↓, ROS↑, LPO↑	Cytosol	↓	↓	ARPE-19	VEGF↑, PEDF↓; antioxidant reduced effects	(24)
		Cytosol	↓		Brain EC		(117)
Cigar. smoke	Myosin phosphatase∼P↑ →ROCK↑			↓	Calu-3	Occ/ZO-1 interaction↓; Occ-Tyr∼P↑	(143)
Dichromate	LPO↑, TJ↓	Cytosol	↑		Tubules	Vit. E protects renal tubules (via ERK1/2); Occ∼P↑	(8)

ROS, reactive oxygen species; LPO, lipid peroxidation; GSH/GSSG, reduced/oxidized glutathione; Occ, occludin; ∼P, phosphorylation; ZO-1, zonula occludens protein 1; Kin, kinase; MAPK, mitogen-activated protein kinase (PK); p38, mitogen-activated PK; ROCK, Rho-associated kinase; ERK1/2, extracellular signal-regulated kinase; Cat, catalase; MMP, matrix metallopeptidase; TJs, tight junctions; PEDF, pigment epithelium-derived factor; OxPAPC, oxidized 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine; DMNQ, dimethoxynaphthoquinone; SOD, superoxide dismutase; XO, xanthine oxidase; m, murine;↑, increase;↓, decrease; ø, no change.

EC, endothelial cell; BAEC, primary bovine arterial EC; HCAEC, human coronary artery EC; HUVEC, human umbilical vein EC; RPEC, retinal pigment epithelial cell; GP8, rat brain EC line; MDCK, Madin-Darby canine kidney cell line; PBEC, porcine brain EC; GP8.3, rat brain EC; ARPE-19, A549, human lung carcinoma cell lines; Caco-2, human colon carcinoma cell line; Sertoli, Sertoli cells; Calu-3, airway epithelial cell line.

Table 3. Effect of Antioxidative Mechanisms on Occludin

Antioxidant	*Properties*	*Oxidat. stress (noxa)*	*Occludin*					*Ref.*
			Localization	*Expression*	*Barrier*	*Subject*	*Remarks*	
GSH	Main intracell., mitoch. respirat.↑					HEK	Dimers↓ (C-terminal domain)	(197)
Ascorbate	Hydrophilic, endogenous	Brain trauma		Norm	Norm	Rat cortex	Blood-brain barrier injury↓	(110)
		IFN-γ + LPS	Norm		Norm	MVEC	Prevented Ser/Thr∼P↓	(72)
γ-Linolenate	Lipophilic, endogenous	Estrogen stress	Norm	ø	Norm	HVEC	Estrogen damaged TJ	(118)
Dithiothreitol	Reducing agent					HEK	Dimers↓ (C-terminal domain)	(197)
		H_2O_2	ø		ø	MDCK-II		(128)
Gingkolide B	Plant antioxidant, GSH↑	Eotaxin→ ROS↑		ø	ø	HCAEC	Blocks TJ disruption	(86)
MnTBAP	SOD mimetic, GSH↑	Eotaxin → ROS↑		ø	ø	HCAEC	Blocks TJ disruption	(86)
Cu,Zn-SOD	Disproportionates $\cdot O_2^-$	SOD mutant^{G93A}		↓		Spinal cord	Transgenic rats with SOD mutant	(137)
		oxPAPC		Norm	Norm	BAEC	Occ∼P↑	(45)
		H_2O_2			Norm	MDCK-II		(128)
Catalase	Decomposes H_2O_2	H_2O_2		Norm	Norm	MDCK-II		(128)
		oxPAPC			ø	BAEC		(45)
Pyruvate	Reduction of H_2O_2 (decarboxylat.)	H_2O_2	ø	ø	ø	MDCK-II	Tyr∼P↑	(128)
Na_2SeO_4	Se cofactor in antiox. enzymes	Estrogen stress	ø	Norm	Norm	HVEC	Estrogen damaged TJ	(118)
SERPINA3K	GSH↑, SOD↑; Ser protease↓	Hypoxia/reoxyg.		ø	ø	Rat retina	→Retinopathy, RPEC injury	(218)
XO inhibitor	Xanthine oxidase→ $\cdot O_2^-$, H_2O_2	Brain trauma		Norm	Norm	Rat cortex	Blood-brain barrier injury↓	(110)
NADPHO inhib.	NADPH oxidase→ $\cdot O_2^-$	Brain trauma		Norm	Norm	Rat cortex	Blood-brain barrier injury↓	(110)
GSNO	Inducible NO-synthase↓	Brain trauma		Norm	Norm	Rat brain		(91)
Triamcinolone acetonide	Preserves GSH/GSSG	H_2O_2 + myristate			ø	ECV-304	Prevents dislocalization in cytosol	(131)

GSNO, *S*-nitrosoglutathione; MnTBAP, Mn(III)tetrakis (4-benzoic acid); IF; interferon; LPS, lipopolysaccharide; norm, normalized; HEK, human embryonic kidney cell line 293; MVEC, microvascular endothelial cell (EC); HVEC, human vein EC line; ECV-304, human umbilical vein EC line. For further symbols and abbreviations, see Table 2.

TABLE 4. REDOX-RELEVANT SIGNAL-TRANSDUCTION PATHWAYS INFLUENCING OCCLUDIN

		Occludin					
Pathway	*Mode of action/approach*	*Localization*	*Phosphorylation*	*Expression*	*Barrier*	*Subject*	*Remarks*	*Ref.*
panPKC activation	O_2 chelator*/aglycemia → Ca↑				↓	HMEC-1	Occ/actin complex↓	(147)
panPKC inhibition	Ca chelator/O_2 chelator*/aglycemia				ø	HMEC-1	Prevents Occ/actin complex↓, P↑	(147)
	Low Ca + myristate/diacylglycerol	Membrane↑	↑			MDCK	Inhibitor → Ca↑ →PKC↑;~P↓, TJ↓	(5)
Conventional cPKC	Low Ca → cPKCs↓	Membrane↓			↑	MDCK	cPKCα→ TJ↓; cPKC inhibitor→ ~P↑, P↓	(6)
	~P of recombinant Occ by cPKC		Ser^{258}~P			m kidney		(5)
novel PKC (nPKC)	High Ca after low Ca	Membrane↑	↑		↑	MDCK	nPKCε→ TJ↑; nPKC inhibitor→ ~P↓, P↑	(6)
nPKCη	Occ-$T^{403/404}$~P↑		↑		↑	Caco-2	Inhibitor, $T^{303/304}A$ → ~P↓,P↑	(186)
MAPK inhibitor	+O_2 chelator*/aglycemia				ø	HMEC-1	Inhibitor → Occ/actin complex↓, P↑	(147)
PKG inhibitor	+O_2 chelator*/aglycemia				ø	HMEC-1	PKG inhibitor prevents P↑	(147)
ERK1/2 inhibitors	Hypox./reox./aglycem. →ERK1/2~P			↓	↓	BEC	Inhibitors → reduce Occ down-regulation	(101)
	Cell stretch → JNK↑, p38↑, ERK~P↑	TJ stain↓		↓	↓	rAEC	Inhibitors → JNK~P↓ →Occ loss↓	(38)
	H_2O_2 → ERK1/2↑, p38↑, TJ↓			↓	↓	MDCK-II	Inhibitor (caspase 3↑) →TJ, ~P recovers	(67)
Raf-1 (transfected)	Oncogene	Indetectable		↓	↓	Pa4	Occ overexpression reassembles TJ	(203)
SV-40	→PP2A↓ from cell contacts	Membrane↓		↓		MDCK	Rac1↑, Cdc42↑; RhoA↓	(139)

*, thioglycolic acid; PK, protein kinase; PKG, cGMP-dependent PK; Raf-1, protooncogene Ser/Thr-PK; SV-40, simian vacuolating virus 40; JNK, c-Jun N-terminal kinase; PP2A, protein phosphatase 2. P, permeability; Rac1, Ras-related C3 botulinum toxin substrate 1; Cdc42, cell-division control protein 42 homologue; RhoA, Ras homologue gene family member; BEC, primary brain EC; HMEC-1, human dermal microvascular EC; rAEC, primary rat alveolar epithelial cell; Pa4, rat salivary gland epithelial cell line; nPKC, novel protein kinase c. For further symbols and abbreviations, see Table 2.

For PKCs, it was found that cPKCα participates in TJ disassembly, whereas nPKCδ (6) and nPKCη (186) support TJ formation in MDCK cells. Both groups of PKCs are redox dependent, as the cellular levels of their regulators (Ca^{2+}, diacylglycerol) are influenced by the redox state of the cell. Moreover, stress-induced activation of mitogen-activated protein kinase (MAPK)-related pathways, in particular of ERK1/2 and p38, lead to decreased expression of occludin and increased paracellular permeability. Conversely, inhibition of the latter enzymes results in recovery of the barrier (67, 100). MAPK activation is also involved when alveolar epithelial cell monolayers are subjected to mechanical stress. Stretch-induced permeability was reversed by inhibition of ERK or JNK (c-Jun N-terminal kinase); JNK inhibition alone prevented the loss of occludin from the cell–cell contacts (38). In endothelial cells, stretch stress decreased the Tyr-phosphorylation of occludin in a PKC-dependent manner (39). Oxidative burden in mechanical cell stress is caused by the concomitant production of reactive oxygen species (ROS) (63), which also activate ERK1/2 (160). Further redox-relevant pathways affecting occludin are given in Table 4.

In general, oxidative stress influences different signal pathways that modify expression of occludin, its enrichment in cell contacts and cell-barrier properties, with PKC and MAPK playing a central role. Nevertheless, counter-regulating pathways exist with compensatory or repair functions (6, 93) that attenuate the stress-induced alterations. Later we review oxidative and antioxidative mechanisms interfering with the complex network of regulatory pathways of occludin and TJ barriers.

Reactive oxygen species

$^{\cdot}O_2^-$ and H_2O_2 are the reactive oxygen species of most importance in oxidation-dependent signaling of occludin (Table 2). The relatively low oxidative reactivity of $^{\cdot}O_2^-$ and its efficient removal by superoxide dismutase (SOD) often prevent the observation of direct effects of $^{\cdot}O_2^-$ or reaction products thereof. SOD is of vital importance for barrier functions. SOD1 mutations linked to amyotrophic lateral sclerosis (ALS) show a disrupted blood–spinal cord barrier in mice as a consequence of reduced levels of ZO-1, occludin, and claudin-5 (220). The mRNA levels of occludin are decreased in the spinal cord of symptomatic (but not presymptomatic) animals (137), implying that the barrier opening might be a consequence rather than the cause of ALS.

Oxidized-1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine (OxPAPC) and oxidatively modified low-density lipoprotein in atherosclerotic lesions reduce total occludin protein and increase occludin phosphorylation in aortic endothelial cells (45). These effects, accompanied by doubling of $^{\cdot}O_2^-$ generation, were inhibited by SOD or catalase or both. Interestingly, these enzymes increased the expression of occludin, even in unaffected cells. Catalase attenuated the OxPAPC-induced increase in occludin phosphorylation and permeability even more effectively than SOD. This suggests that H_2O_2 formed by $^{\cdot}O_2^-$ disproportionation is the more potent species. In addition, signaling pathways (*e.g.*, PKCs, Raf/MEK1,2/ERK1/2 MAPK cascade, JNK MAPK, tyrosine phosphorylation) known to be activated by OxPAPC (21) must be considered.

Superoxide generated by xanthine/xanthine oxidase induces Tyr-phosphorylation and redistribution of occludin in

Caco-2 cells (154). It was concluded that tyrosine kinase–dependent dissociation of occludin/ZO-1 and adherence junction protein complexes from the cytoskeleton is the main mechanism involved in oxidative-stress–induced barrier disruption.

A decrease in occludin expression was observed in brain endothelial cells exposed to hypoxia/reoxygenation or to 2,3-dimethoxy-1,4-naphthoquinone, which induces intracellular $^{\cdot}O_2^-$ formation. This effect was strongly dependent on the presence of glucose and was paralleled by activation of ERK1/2 (100). $^{\cdot}O_2^-$ generated by the antiretroviral drug efavirenz was recently found to increase the permeability of human coronary artery endothelial cells, to decrease the levels of TJ proteins including occludin, and to activate JNK and NF-κB pathways (85).

H_2O_2, the most abundant ROS in pathophysiology, compromises TJ barriers by diverse mechanisms (Table 2). The H_2O_2-induced increase of permeability observed in the retinal pigment epithelium (RPE) barrier was prevented by RPE-derived trophic factor (PEDF) (77). The PEDF effects are linked to p38 and HSP27, as indicated by the diminished activation of these mediators of actin cytoskeleton rearrangement. Human umbilical vein endothelial cells (HUVECs) treated with H_2O_2 showed increased permeability, increased Ser-phosphorylation of occludin, and its redistribution from cell–cell junctions (90). These effects were found to depend on ERK1/2 activation and were blocked by ERK inhibition, which even enhanced the junctional organization of occludin. Similarly, a protective effect on the recovery of MDCK-II cells after H_2O_2 exposure was observed after inhibition of the activation of MAPK enzymes ERK-1/2 and p38 (67). H_2O_2 treatment of MDCK cells also markedly reduced TER and caused disrupted occludin staining, which was reversed by catalase (128).

Tyr-phosphorylation at the lateral membrane was detected during reassembly of the TJs, and tyrosine kinase inhibitors inhibited the recovery of TER and perturbed the relocalization of occludin to the TJs. Dephosphorylation of occludin threonine residues was also found in H_2O_2-treated Caco-2 cells (175). Increased association of the phosphatase PP2A with occludin by a Src kinase–dependent mechanism indicated that PP2A activity is involved in H_2O_2-induced disruption of TJ in Caco-2 monolayers. c-Src–mediated (tyrosine) phosphorylation was investigated (49) with respect to a highly conserved Y_{398}ETDY_{402}TT sequence of human occludin. Y398A and Y402A mutations abolished c-Src–mediated phosphorylation, whereas expression of the Y398D/Y402D phosphorylation-mimicking mutant of occludin sensitized MDCK cells for H_2O_2-induced barrier disruption (Table 2).

Other oxidative effectors

In addition to ROS, further factors, in particular, NO and transition metal ions, modulate TJ function (Table 2). NO is a reactive species involved in signaling processes and oxidative/nitrosative stress. Deleterious effects are attributed mostly to reaction products, such as $ONOO^-$. Direct effects of NO on occludin have not been reported so far, but barrier functions are affected indirectly: VEGF receptor 2 activation may result in NO release by endothelial NO synthase (eNOS), which, in turn, activates signaling pathways (*e.g.*, Rho-Rac), with subsequent involvement of junctional proteins including occludin (16). *In vivo* experiments demonstrate that thiamine depletion induces region-selective eNOS expression in murine brain. Collateral release of NO was considered a major factor leading to cerebrovascular alterations, such as decreased expression of TJ proteins including occludin (19). NO generation also resulted in loss of occludin immunoreactivity at TJs in MDCK cells and in redistribution of occludin into the cytoplasm (43).

Transition metal ions, specifically Fe^{2+}/Fe^{3+} (180), play a central role in redox-dependent and free radical–mediated reactions (144). The Janus-faced role of transition metals is illustrated by increased transcellular permeability of Caco-2 cells after treatment with iron (or copper) ions (52) and by the protective effect of iron supplementation on the lead-induced disruption of the blood–brain barrier (BBB) during rat development (201).

Lipid peroxidation (LPO) contributes significantly to the impairment of tissue barriers (22); however, the interplay of LPO and membrane-embedded TJ proteins is widely unknown. Inconsistent results are described in reports on effects of fatty acids added to cells. After addition of γ-linoleic acid and eicosapentaenoic acid to ECV304 cells, upregulation and more intense staining at cell contacts of occludin were observed (87), whereas positional and geometric isomers of linoleic acid provoked redistribution of occludin in Caco-2 cells (157). Treatment of Caco-2 cells with docosahexaenoic acid increased LPO and disrupted the barrier, which was partly attributed to activation of the phospholipase C/Ca^{2+}/PKC pathway and formation of eicosanoids (158).

The mechanism(s) responsible for the toxic effects of amyloid-β (Aβ) are not fully understood. A dramatic loss of occludin was observed in postmortem brain tissue obtained from patients with capillary cerebral amyloid angiopathy (28a), and addition of Aβ(1-42) to brain capillary endothelial cells induced ROS formation. Increased formation of ROS was found after administration of the oligomeric form of Aβ(1–42) to RPE cells (24), which was accompanied by a decrease in the expression of occludin. This was also found *in vivo* and in primary rat brain endothelial cells (117).

Cadmium and its salts are highly toxic, and this is, at least partly, based on effects on cell–cell contacts in different organs. The $CdCl_2$-induced disruption of the BTB of rats (208) is accompanied by loss of occludin and ZO-1 from the BTB. The barrier disruption was associated with transient induction of testicular TGF-β2 and TGF-β3 and phosphorylation of p38 MAPK. Administration of a specific p38 MAPK inhibitor blocked $CdCl_2$-induced occludin and ZO-1 loss from the BTB. TJ disruption by $CdCl_2$ was observed in Sertoli cells (34), and reassembly of inter-Sertoli TJs was accompanied by partial restoration of the occludin expression. Acute renal failure due to dichromate was associated with oxidative damage, as assessed by renal LPO. In the tubuli, occludin was hyperphosphorylated; it appeared disrupted, and its quantity increased. TJ dislocation and downregulation were diminished by α-tocopherol in an ERK1/2-dependent mechanism (9).

Increasing interest exists in stress-inducing reactions in the presence of nanoparticles and their molecular determinants. Lung epithelial cells challenged with CeO_2 nanoparticles responded with loss of occludin density at the cell–cell contacts and decreased TER, accompanied by increased oxidative DNA damage (159). Exposure of these cells to freshly generated brake-wear particles (consisting predominantly of iron and lower fractions of copper and manganese) caused a

decrease in occludin levels, but only at high particle concentrations, which, in parallel, led to increased production of ROS (60). Cigarette smoke also increases the permeability of lung epithelium, probably caused by occludin redistribution and subsequent loss of occludin/ZO-1 interaction (143). Activation of ROCK indicates that these processes are linked to alterations in the cytoskeleton.

Independent of the different mechanisms (Table 2), agreement exists that oxidants reduce the expression of occludin and disturb its membrane localization, which affects TJ function *in vitro* and *in vivo*. These alterations play a role under pathologic conditions and are mediated via stress-dependent pathways (Table 4, Fig. 4).

Antioxidative approaches

Antioxidants counteract the deleterious consequences of oxidative stress on occludin and preserve barrier functions (Table 3). They operate on different levels (*e.g.*, by scavenging free radicals, terminating oxidative chain reactions, or inhibiting radical-generating enzymes.

Sustained but moderate epidural compression of the somatosensory cortex of rats caused a short-term sensory deficit, a marked increase in BBB permeability, and upregulation of occludin (and claudin-5) in the injured cortex. Administration of ascorbic acid prevented both compression-induced BBB disruption and sensory impairment. Protection was also induced by apocynin and allopurinol (NADPH- and xanthine oxidase inhibitor, respectively) (110). Similarly, cytokine-stressed microvascular endothelial cells were protected by ascorbate and dehydroascorbate from serine/threonine dephosphorylation, redistribution of occludin and barrier disruption. Inhibition of NADPH oxidase and PP2A showed analogous effects (72).

Increased paracellular permeability was provoked in human coronary artery endothelial cells by the chemokine eotaxin (86). This was accompanied by decreased mRNA and protein levels of TJ molecules, including occludin and claudin-1. The permeability increase was blocked by MnTPAP, a manganese-containing porphyrin considered to be Mn-SOD mimetic. MnTPAP also prevented the decrease in intracellular GSH induced by eotaxin.

Reassembly of the TJs is of crucial functional importance after oxidative stress. The reassembly of occludin in Caco-2 cells is determined by phosphorylation of its threonine residues and by the phosphatases PP2A and PP1 (171). Administration of H_2O_2 to MDCK-II cells (128) leads to markedly reduced TER and disrupted staining patterns of occludin. Besides reversion of these effects by catalase, it has been found that pyruvate [reduces H_2O_2 levels (66, 177)] protected the cells from loss of TER.

17β-Estradiol induces concentration- and time-related effects on TJ functions and expression of occludin in endothelial cells, and it has been shown (214) that estradiol-induced perturbation of TJ functions may have implications in mastalgia. Strengthening the antioxidative potential of HUVECs by γ-linolenic acid, selenium and iodine completely reversed the permeability increase and the occludin relocation from the cell–cell contacts induced by 17β-estradiol (118).

Indirect antioxidative strategies, directed at occludin-containing barriers, focus on the increase or, at least, the conservation of the endogenous antioxidative potential (*e.g.*, intracellular GSH or SOD levels) (Table 3). Treatment of RPE cells with H_2O_2 decreased the staining of occludin at the intercellular contacts and increased the permeability of the monolayer (131). Both effects were inhibited by triamcinolone acetonide (corticosteroid administered in ocular inflammation), which concomitantly preserved the GSH/GSSG ratio. To study oxygen-induced retinopathy (OIR), rat retinal cells exposed to 75% O_2 were treated with SERPINA3K, a serine protease (219) and Wnt pathway inhibitor (217). SERPINA3K prevented the OIR-induced decrease of occludin in the rat retina, in cultured retinal capillary endothelial cells, as well as in RPE cells. The SERPINA3K action was attributed to a decreased ROS generation and upregulated GSH and Mn-SOD levels (218).

S-Nitrosoglutathione (GSNO) is a metabolite formed from GSH and NO (167). In traumatic brain injury, treatment of the rats with GSNO improved BBB integrity and restored the expression of occludin (and ZO-1) that was diminished after controlled cortical impact (91). The mechanism(s) behind these protective effects seem to be complex, because GSNO releases NO and, conversely, inhibited inducible NO-synthase expression. Protective effects of NO at the BBB have also been reported in a cellular BBB model (192).

These examples prove that antioxidant action protects occludin from oxidative stress and normalizes morphologic alterations, disturbed occludin expression, and TJ function. These effects influence occludin indirectly via manifold signal-transduction pathways or simply reduce the levels of stress factors affecting occludin directly.

Cytokine effects on occludin and tight junctions

Inflammatory cytokines are inevitably connected with oxidative stress, play an important role in the regulation of cellular barrier functions, and influence occludin in a variety of ways (Table 5). Cytokine production/release and oxidative stress are well documented in neurologic (38a) and intestinal inflammation (88a) or in cancer (29). This interplay is illustrated by the influence of cytokine-activated factors on the cellular level of GSH/GSSG (149) and by the dependence of interleukin (IL)-12 production on the redox equilibrium (2). Interferon (IFN)-γ, ILs, tumor necrosis factor (TNF)-α, and chemokines are generally considered proinflammatory cytokines. Growth factors belonging to the cytokine group reduce barrier integrity *per se*; however, they also provide conditions for the formation and repair of TJs.

IFN-γ induces TJ disassembly and a leaky barrier in T84 epithelial cells. The subsequent internalization of TJ proteins (early/recycling endosomes) is macropinocytosis-like, and not clathrin or caveolae mediated (25). Occludin was internalized into large actin-coated vacuolar apical compartments, which required myosin II motor ATPase (191). IFN-γ treatment resulted in activation of Rho GTPase and upregulation of ROCK, which was shown to mediate the endocytosis. In addition, the cellular energy sensor, AMP-activated protein kinase (AMPK), is involved, because AMPK knockdown prevented epithelial leakiness and the loss of occludin and ZO-1 (164). In contrast, IFN-γ improved the barrier function in human lung epithelial cells compromised by pretreatment with IL-4 and IL-13, which was accompanied by reduced expression of ZO-1 and occludin (1).

Table 5. Effects of Redox-Relevant Cytokines on Occludin

Cyto-kine	Mode of action/approach	Occludin					Ref.
		Localization	Expression	Barrier	Subject	Remarks	
IFN-γ	Endocytosis of Occ↑	Cytosol	ø		T84		(25)
	Internalization↑ (RhoA/ROCK,myosin II-ATPase)			↓	T84	Myosin inhibitor → internalization↓	(191)
	AMPK (cellular energy sensor) activated by ∼P		↓	↓	T84	AMPK↓/inhibition → alterations↓	(164)
	T cells recruit B/mast cells, eosinophils → infiltration/inflammation		↓	↓↑	Calu-3	Wound healing: cell migration↓; reverses IL-4,-13 effects	(1)
IL-4, -13	T → B/mast, eosinophils → infiltrat./inflammation		↓	↓	Calu-3	Wound healing: cell migration↓	(1)
TNF-α	MMP-9↑ →TJ↓ (degradation)		↓	↓	cEND		(53)
			ø		MDCK	Claudin-1↓	(151)
TGF-β	pSmad-2, c-H-Ras, pp38 MAPK, pAkt↑; Smad-interacting protein-1↑, snail↑; E-cadherin↓; epithel-mesenchym transition		↑	↓	Primary hepatocytes	PI3K, PKC inhibitors prevent Occ↑; p38 MAPK, PKC, PI3K inhibitors → P↓	(97)
CCL11	MAPK p38 kinase∼P↑; Stat3↑, NF-κB↑; GSH↓; eosinophils↑		↓	↓	HCAEC	Antiox., p38 inhibitor, anti-CCR3 antibody → P↓	(86)
EGF	MAPK, ERK1 (MEK)∼P↑; (ERK1∼P binds C-terminal Occ)	Membrane		ø	Caco-2	Occ-Tyr∼Pø, -Thr∼P↓, Occ/ZO-1 complex ø (MEK inhibitors → EGF effects↓)	(15)
HGF	Transcription factor	Membrane↓	↓	↓	HVEC		(88)
	oxidative stress↓ (hypoxia)	ø	ø	↓	HCE	Protects against hypoxia	(94)
VEGF	Effect/receptor basolateral		↓	↓	pr.retinal EC	PKC↑ →Occ∼P↑	(73)
	normobaric hypoxia VEGF-mediated		↓	↓	m brain	VEGF inhibition (protective) → P↓	(17)
		Cytosol	↓	↓	prim. BMEC	MAPK involved	(202)
			↑	↓	prim. BREC	Occ-Ser/Thr∼P↑	(7)
			↑	↓	m retina	Intraocular injection	(7)

GF, growth factor; EGF, epidermal GF; VEGF, vascular endothelial GF; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor; TGF, transforming growth factor; CCL, chemokine ligand; PK, protein kinase; AMPK, AMP-activated PK; PI3K, phosphoinositide 3-kinase; p38, mitogen-activated PK; CCR, chemokine receptor; Stat, signal transducers and activators of transcription; NF-κB, nuclear factor κB, P, permeability. T84, human colonic adenocarcinoma cell line; cEND, brain endothelial cell line; BREC, bovine retinal EC; HVEC, human vein EC line; BMEC, brain microvessel endothelial cell; HCE, human corneal epithelial cell. For further symbols and abbreviations, see Table 2.

Similarly, decreased levels of occludin, accompanied by elevated amounts of TNF-α, were found in inflammatory bowel (4, 61) or Crohn's disease (216). However, no change in occludin expression in MDCK (151) and brain endothelial cells occurred after the addition of TNF-α (196). In TNF-α–treated murine brain endothelial cells, a decrease was observed in occludin immunoreactivity and mRNA level (53), both prevented by coapplication of glucocorticoids (which induced *occludin* gene expression when applied alone).

Chemokines are involved in leukocyte migration into the parenchyma. Oxygen–glucose deprivation (OGD) caused secretion of CCL2 (monocyte chemotactic protein-1) by primary brain endothelial cells (47). In parallel, redistribution of occludin and other TJ proteins was observed that could be inhibited under OGD by blocking CCL2. The chemokine induces occludin internalization via caveolae and further processing to early and recycling endosomes (181). CCL11 (eotaxin), produced by IFN-γ–stimulated endothelial cells and TNF-α–activated monocytes, also caused a decrease in occludin mRNA and protein levels in human coronary artery endothelial cells (86). The signaling pathways involved include the eotaxin receptor CCR3, Stat3, NF-κB, and activation of MAPK p38.

The growth factor TGF-β, when elevated in circulation, causes endothelial dysfunction through NADPH oxidase activation–induced oxidative stress (26). Moreover, it is a multifunctional cytokine directly involved in barrier functions (69, 127, 195), because it initiates and maintains epithelial–mesenchymal transition, which also is a crucial step in tumor progression. In hepatocytes, upregulation of occludin protein was observed with 0.1 ng/ml TGF-β (97). However, 10 ng/ml TGF-β downregulated occludin in MDCK-II cells (127).

Although the functions of VEGF have not been completely elucidated, convincing evidence suggests that it plays a pivotal role in vascular barriers, particularly under hypoxia-like conditions. Exposing mice to normobaric hypoxia led to an increase in brain vascular permeability associated with diminished expression of occludin; inhibition of VEGF attenuated vascular leakage (17). This confirms the results of studies in primary brain endothelial cells in which VEGF decreased occludin expression (202).

Hepatocyte growth factor (HGF) provoked a concentration-dependent increase in paraendothelial permeability (88). A protective effect of HGF was observed in human corneal epithelial cells. Hypoxia-induced deleterious effects (*e.g.*, on TJ integrity, paracellular tightness, cytoskeleton, but not on occludin) were inhibited by HGF, probably by stabilizing the ZO-1/cytoskeleton association (94). Application of epithelial growth factor (EGF) to Caco-2 cells prevented the H_2O_2-induced increase in permeability and redistribution of occludin, which was mediated by interaction of ERK with its C-terminal region (15).

In summary, the majority of studies on cytokine effects describe occludin redistribution and downregulation. However, the observed effects depend strongly on the selected model – even barrier-opening cytokines such as IFN-γ or TGF-β exert protective effects in certain cells under certain conditions.

Diseases

Hypoxia-related conditions

Many pathologic states are associated with hypoxic conditions and affect occludin and tissue barriers (Table 6). Mice exposed to hypoxia (hypoxemia) showed reduced expression and membrane localization of occludin in the cerebral endothelium forming the BBB, which is accompanied by deterioration of the TJs and the BBB (17). Reoxygenation of rats after hypoxia opened the BBB and reduced the nonphosphorylated fraction and membrane localization of occludin. However, ZO-1 and claudin-3 levels remained unchanged (205). Similarly, middle cerebral artery occlusion caused dislocation and downregulation of occludin, accompanied by upregulation of NADPH oxidase, ROS generation, matrix metalloproteinase (MMP)-9 activation, and edema formation (112). This demonstrates an important pathogenic role of the oxidase activity in MMP signaling on occludin, as it is fragmented by MMPs, which leads to vascular leakage (17, 111). In addition to cerebral barriers, other organs are affected. In the rat, occludin and other TJ proteins are displaced from membrane fractions of the colon after ischemia/reperfusion injury; the TJs are disrupted; and the intestinal permeability increases (108).

In an opposite approach, normobaric hyperoxia protected the BBB, and the expression and distribution of occludin against MMP-9–mediated effects in cerebral ischemia (112). Conversely, one must consider reports demonstrating that prolonged hyperoxemia diminished the BBB integrity by depression of the endogenous defense against oxidative stress, resulting in free radical–mediated disturbances (138). Moreover, the free radical scavenger tempol prevents alterations in the oligomeric assembly of occludin, its redistribution, and increased BBB permeability after *in vivo* hypoxia/reoxygenation by using *in situ* brain perfusion (113).

In brain endothelial cell cultures, hypoxia/reoxygenation decreased the amount of occludin and the paracellular tightness via the MAPK pathway (100). Hypoxia (O_2-depletion)/aglycemia applied to human dermal microvascular endothelial cells demonstrates the involvement of Ca^{2+}-regulated cPKC, cGMP-dependent PKG, and MAPK in the hypoxia-induced paracellular permeability increase (147). Changes in the binding of occludin to the cytoskeleton observed under the same conditions are inhibited by a Ca^{2+} chelator, as well as by inhibitors of PKC or MAPK (but not of PKG), indicating that both analogies and differences exist in the pathways influencing permeability and cytoskeletal anchoring of the TJs.

Hemorrhagic shock in rats compromised cerebral blood flow and, hence, oxygen supply (101), as well as occludin expression and paracellular tightness. Similar effects were observed in microvessels of human brain tumor tissue (146), which experience hypoxic conditions in the tumor center (62). Further signaling dedicated to occludin and related to oxidative stress refers to tumorigenesis. Raf1, a downstream effector of the *ras* oncogene controlling cellular proliferation/differentiation, is activated in tumorigenesis and mobilizes the redox-related MAPK (ERK) pathway (36). Expression of occludin suppresses Raf1-transformation of epithelial cells, and the ECL2 of occludin is required for reversing changes in the epithelial phenotype (203).

In this context, it is interesting to note that endometrial carcinoma (188), prostate carcinoma (28), and synovial sarcoma (20) show downregulation of occludin. Cancer cells activate glycolysis for their increased energy demand. This is accompanied by the generation of ROS, due to a switch from oxidative phosphorylation to anaerobic glycolysis (62). Accumulation of ROS and oxidative stress also play an important role in carcinogenesis (145). Polychlorinated biphenyls

Table 6. Effect of Hypoxia-Related Conditions on Occludin

Disease	*Mode of action (proposed)*	*Occludin*					*Ref.*
		localization	*Expression*	*Barrier*	*Subject*	*Remarks*	
Hypoxemia	MMP↑ →TJ↓ →BBB↓ →edema	Membrane↓	↓	↓	m brain	Reversed by MMP-9 inhibitor; VEGF inhibitor attenuated MMP-9↑ →TJ↓	(17)
Hypoxemia/reoxygenat.	Oxidative burden		↓	↓	Rat brain	Nonphosphorylated Occ↓	(205)
Hemorrhagic shock	Blood pressure↓ →blood flow↓		↓	↓	Rat brain		(101)
Ischemia	MCAO → MMP-9↑, NADPH oxidase↑ →ROS↑	Membrane↓	↓	↓	Rat brain	Hyperoxia → less MMP-9 up-regulation, barrier↓, brain edema↓	(112)
Ischemia/reperfusion		Cytosol		↓	Rat colon		(108)
Hypoxia (O_2 chelator)/aglycemia	Ca influx↑ → PKC↑; cytoskeleton bound Occ↓			↓	HMEC-1	P ↑ via PKC, PKG, MAPK, Ca-dissociation of Occ/actin complex	(147)
	cPKCβII↑ (mediate damage) nPKCδ↑	Membrane↓		↓	bEND3	Occ/ZO-1↓; cPKCβ inhibitor → ∼P↓, TJ↑; nPKCδ inhibitor → ∼P↓, TJ↓ (protective)	(93)
Hypoxia/reoxygenation	Ox. stress → ERK1/2↑		↓	↓	Brain EC	Aglycemia → intensifies Occ↓,ERK1/2↑	(100)
Endometrial carcinoma		Membrane↓	↓		Tissue	Occ ↓ with increasing grade of cancer	(188)
Prostate carcinoma	CEACAM1↓		↓		Tissue		(28)
Synovial sarcoma		ø	↓		Tissue		(20)
Brain tumor (human)	Septic encephalopathy		↓	↓	μ-Vessels	Cerebral edema; TJ opening	(146)
Brain metastasis	Polybiphenyls (m) →metastases↑		ø	↓	Melanoma	Occ/ZO-1 interaction↑	(170)

EGF, epidermal growth factor; P, permeability; BBB, blood-brain barrier; PK, protein kinase; PKG, cGMP-dependent PK; MAPK, mitogen-activated PK; CEACAM1, carcinoembryonic antigen-related cellular adhesion. bEND3, brain EC; HMEC-1, human dermal microvascular EC. For further symbols and abbreviations, see Table 2.

Table 7. Infection and Inflammation Influencing Occludin

Pathogen/inflammation	*Mode of action*	*Occludin*					*Ref.*
		localization	*Expression*	*Barrier*	*Subject*	*Remarks*	
Pathogenic *Escherichia coli*	Toxin EspF	Membrane↓		↓	T84		(126)
Clostridium difficile	Toxin A and B	Membrane↓	ø	↓	T84	Occ: dissociation of TJ, internal.	(142)
Clostridium perfringens	Enterotoxin (CPE)				Caco-2	Indirect CPE-Occ complexes	(178)
Vibrio cholera	Enterotoxin (HA/Zn-protease)	ø	Cleavage		MDCK-I	Blocked: protease inhibitor	(211)
Helicobacter pylori	*H. pylori*, sonificated	Membrane↓		↓	m stomach	*In vivo*, neutrophil P↑	(185)
Rhesus rotavirus	Metabolic dysfunction	Membrane↓		↓	Caco-2	MVO_2↓, lactate↑, ATP↓	(46)
Trichinella spiralis	Gut P↑ →parasite expulsion	Cytosol	↓Cleaved	↓	m intestine	IL-9 → effect↑, expulsion↑	(124)
Cryptococcus neoformans	→Meningoencephalitis	Membrane↓		↓	HBMEC	Pathogen P↑, Occ extractable*	(30)
Collagenous colitis	NaCl uptake↓, Cl secretion↓		↓	↓	h colon	Diarrhea	(27)
Inflammatory bowel disease	Neutrophil uptake↑, cysts	Membrane↓	↓	↓	h colon	Diarrhea	(103)
Chemical colitis	Rectal trinitrobenzenesulfonate	Membrane↓		↓	Rat ileum		(54)
Irritable bowel syndrome	Proteasome trypsin-like act.↑	Membrane↓	↓	↓	h colon	Occ degradation	(37)
Pancreatitis (by caerulein)	Extravasation → edema	Cytosol	↓		m pancreas	Occ/Claudin-1 ø, Occ/ZO-1↓	(166)
HIV	Causes encephalitis	Membrane↓	↓	↓	h brain	Brain macrophages↑	(44)
	TNF-α↑ by envelope prot. Gp120		↓	↓	T84	Barrier crossing↑ (virus)	(136)
Gp 120, Tat (HIV proteins)	GSH↓, H_2O_2↑; TNF-α↑, IL-2/-4↑	Cytosol	↓	↓	AEC in rats	Intertracheal saline challenge	(107)
Multiple sclerosis (MS)	Serum → IFN-η↑, TNF-α↑, IL-1β↑		↓		SVEC		(129)
	MS-serum	Cytosol	↓	↓	RBE4	Isolated neurons damaged	(152)
HDM allergy	Cys-protease↑ →Occ-ECL1↓	Membrane↓	Cleavage	↓	16HBE14o	Protease inhibitor prevents	(200)
HDM Ser proteases	Occ cleavage	Membrane↓	Cleavage	↓	16HBE14o		(199)

MVO_2, mitochondrial O_2 consumption; h, human; HA, hemagglutinin; *, Triton X-100; P, permeability; ECL1, first extracellular loop; HDM, house-dust mite (*Dermatophagoides pteronyssinus*). T84, human colonic adenocarcinoma cell line; HBMEC, human brain microvascular endothelial cell; SVEC, immortalized murine high endothelial venule cell; RBE4, rat brain endothelial cell line 4; 16HBE14o, immortalized cell line derived from human bronchial epithelium. For further symbols and abbreviations, see Table 2.

stimulating tumor metastasis in the brain have also been shown to induce oxidative stress (33) and enhanced BBB permeability. Although the expression of occludin remained unchanged, interaction with ZO-1 was strengthened in cerebral microvessels (170). Consequently, antioxidative strategies have been suggested to prevent carcinogenesis (70). Conversely, prooxidative action is part of other cancer therapies that specifically intend to obliterate tumor cells (3).

In conclusion, the molecular basis for alterations in occludin and TJs during hypoxia-related injury is poorly understood (108). Characterization of the molecular pathology of occludin will facilitate the design of novel agents against specific tumor tissues or leaky tissue barriers in blood-flow disturbances (146).

Infection and inflammation

Exposure to antigens and exogenous macromolecules often causes inflammatory processes and TJ dysfunction (183). Barrier-forming and mononuclear cells are activated and release reactive species to combat the pathogens. This results in an excess of oxidants and accompanies the inflammation (89). Dislocation from the cell membrane and barrier defects are the main effects of infection and inflammation on occludin (Table 7). This is exemplified by HIV infection of colon epithelial cells *in vitro* (136) and in alveolar epithelial cells of HIV-1 transgenic rats (107). Rotavirus caused similar effects in Caco-2 cells, in which lactate production, O_2 deficiency, and ATP depression indicated oxidative stress (46). *Helicobacter pylori* in mouse gastritis disrupts the epithelial barrier, resulting in a punctuated expression pattern of occludin. Concomitant neutrophil invasion and activation intensify the deterioration via oxidative stress (185). In intestinal epithelial cells, enteropathogenic *Escherichia coli* leads to loss of TER and the redistribution of occludin (126).

Clostridium difficile toxins enhanced paracellular permeability, disorganized F-actin, and dissociated occludin and ZO-1 from the TJs of intestinal epithelial cells (142). *Clostridium perfringens* enterotoxin (CPE) bound to Caco-2 cells demonstrated the presence of occludin in 200-kDa complexes, but failed to do so in TJ-free occludin-transfected fibroblasts (178). This suggested interaction with associating TJ-proteins, such as CPE-binding claudins (56, 74), which causes TJ opening (98). *Vibrio cholerae* produces hemagglutinin/Zn-protease that specifically degrades occludin, which was attenuated by a metalloproteinase inhibitor (211). *Cryptococcus neoformans* binds to brain microvascular endothelial cells; as a consequence, occludin becomes Triton extractable (30), indicating TJ alteration and dephosphorylation of occludin (5).

Inflammatory processes are accompanied by the generation of reactive species (57), downregulation of occludin (121), and cell-barrier defects (Table 7) (88a). Occludin levels were found to be diminished in collagenous colitis (27), inflammatory bowel disease (103), and in a chronic distal colitis model (54). In irritable bowel syndrome, occludin protein but not mRNA expression was reduced, concomitant with mast cell accumulation, proteasome activation in the colonic mucosa, and occludin degradation (37). In an acute pancreatitis model, occludin and claudin-1 are disassembled as an early event, allowing extravasation and edema formation (166).

HIV encephalitis leads to BBB perturbation, TJ disruption, fragmentation or absence of occludin and ZO-1 within cerebral vessels, and accumulation of brain macrophages. Obviously, the TJ disruption in the BBB serves as monocyte entry into the CNS (44). BBB disruption is also a crucial step in multiple sclerosis (MS). Serum from MS patients reduced the expression of occludin in cultured endothelial cells (129). Diminished synthesis, as well as peripheral localization of occludin, are accompanied by a decreased TER (152). It is proposed that the accumulation of cytokines or other serum factors provokes downregulation of occludin and, therefore, contributes to the disruption of the barrier (129).

Allergens contribute to the breakdown of TJs and augment the inflammatory response (64). House dust mite excrement applied to bronchial epithelial cells produced disruption of TJs, accompanied by extensive cleavage of occludin. Ser-peptidases were thought to be responsible for this effect, favoring transepithelial delivery of allergens (199). A Cys-proteinase allergen also disrupted the TJs. Putative cleavage sites were identified in the first ECLs of occludin and claudin-1 (200). Similarly, metalloproteinase-mediated fragmentation of occludin contributes to vascular and epithelial leakage (17, 111).

The findings point to an important role of occludin in infections by many pathogens and various inflammatory diseases. However, the molecular mechanisms remain unclear. Systematic studies of the currently known signal-transduction pathways for occludin will shed more light on the pathogenesis and intervention possibilities.

Conclusions and Future Directions

The function of many organs depends on the integrity of cellular barriers sealed by paracellular tight junctions. Here we review a variety of processes related to oxidative stress and reducing conditions, which impair barrier functions. Convincing evidence indicates that occludin, the marker protein of TJ, is a principal target of redox processes. On the molecular level, numerous reports describe the characteristics of occludin and alterations due to changes in its environmental redox balance. These alterations include structural aspects (oligomerization, protein–protein interactions), signaling pathways (phosphorylation), as well as the downregulation and degradation of occludin. PKC- and MAPK-dependent reactions play a key role in these processes. Wide agreement exists on the consequences of oxidative stress at the functional level: the barrier tightness decreases in parallel with the decline in the abundance and redistribution of occludin from the plasma membrane. Antioxidative strategies were shown to counteract effectively the consequences of oxidative stress at both levels.

However, the unknown ultimate function of occludin within the TJs creates a considerable gap between the expanding knowledge of its molecular characteristics and the barrier properties influenced by different stress-related impacts. Our concept that occludin is a regulator of the TJs with respect to redox processes, under both physiologic and reversible pathologic conditions, is intended to offer an approach to filling this gap. Further systematic investigations are necessary, including study of the recently discovered occludin-like marvel proteins of the TJ. These will deepen our understanding of cellular barriers and may help to develop new approaches directed at pharmacologically influencing their properties.

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Abbreviations Used

Aa = amino acid
BBB = blood–brain barrier
BTB = blood–testis barrier
Caco-2 = human colon carcinoma cell line
CC domain = coiled-coil domain
ECL = extracellular loop
eNOS = endothelial NO synthase
ERK = extracellular signal-regulated kinase
GSH = reduced glutathione
HUVECs = human umbilical vein endothelial cells
JAM = junctional adhesion molecule
LPO = lipid peroxidation
MAPK = mitogen-activated protein kinase
MDCK = Madin-Darby canine kidney cell line
PEDF = pigment epithelial-derived factor
PKC = protein kinase C (isoenzymes: c, convential; n, novel; a, atypical)
PP = protein phosphatase
ROCK = Rho-associated kinase
ROS = reactive oxygen species
RPE = retinal pigment epithelial
SOD = superoxide dismutase
TER = transcellular electrical resistance
TGF = transforming growth factor
TJs = tight junctions
VEGF = vascular endothelium growth factor
ZO = *Zonula occludens*

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