Biochimica et Biophysica Acta 1788 (2009) 761–767

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Review

Tight junctions and the regulation of gene expression

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ARTICLE INFO

Article history:
Received 8 May 2008
Received in revised form 25 November 2008
Accepted 26 November 2008
Available online 11 December 2008

Keywords:
ZO-1
ZO-2
ZO-3
ZONAB
DbpA
Cell cycle
Cyclin D1
PCNA
erbB-2
CDK4
Apg-2
Symplekin RalA
Occludin Claudin
JAM
Cingulin GEF-H1
Beta-catenin
E-cadherin
VE-cadherin
Ras
TGF-beta
Snail
Slug
Glucocorticoid
Gene methylation
Epithelial–mesenchymal transition

ABSTRACT

Cell adhesion is a key regulator of cell differentiation. Cell interactions with neighboring cells and the extracellular matrix regulate gene expression, cell proliferation, polarity and apoptosis. Apical cell–cell junctions participate in these processes using different types of proteins, some of them exhibit nuclear and junctional localization and are called NACos for Nuclear Adhesion Complexes. Tight junctions are one type of such cell–cell junctions and several signaling complexes have been identified to associate with them. In general, expression of tight junction components suppresses proliferation to allow differentiation in a coordinated manner with adherens junctions and extracellular matrix adhesion. These tight junction components have been shown to affect several signaling and transcriptional pathways, and changes in the expression of tight junction proteins are associated with several disease conditions, such as cancer. Here, we will review how tight junction proteins participate in the regulation of gene expression and cell proliferation, as well as how they are regulated themselves by different mechanisms involved in gene expression and cell differentiation.

Contents

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doi:10.1016/j.bbamem.2008.11.024

1. Introduction

For the development and function of epithelial tissues, the interactions of epithelial cells with each other and the extracellular matrix via specialized adhesive structures play a critical role. Therefore, during the last years a key area of cell and developmental biology has been to understand how epithelial cells interact with their neighbors and the extracellular matrix, to regulate intracellular signal transduction, as well as transcriptional and translational regulatory mechanisms of gene expression involved in the control of cell proliferation and differentiation. The apical epithelial intercellular complex consists of tight junctions (TJs), adherens junctions, and desmosomes. As reviewed in other chapters of this series, adherens junctions and desmosomes are adhesive junctions that are linked to the actin and intermediate filament cytoskeleton, respectively [1–3]. TJs also interact with the actin cytoskeleton and function as selective barriers that restrict paracellular diffusion – the gate function – as well as apical/basolateral intramembrane diffusion of lipids — the fence function [4–7]. A fourth type of intercellular junctions are gap junctions, which allow cell to cell communication via the exchange of small diffusible molecules [8,9]. At the basal membrane, epithelial cells adhere to the extracellular matrix mainly via integrins and syndecans [10]. All of these adhesion complexes consist of particular sets of transmembrane proteins that interact extracellularly with ligands and intracellularly with generally large multimeric protein complexes consisting of cytoskeletal linkers, signal transduction proteins as well as factors involved in DNA transcription and RNA processing.

The transmission of signals from adhesion complexes occurs according to two different principles: regulation of signaling cascades that transmit signals via several intermediates, and regulation of specific proteins that shuttle between sites of adhesion at the plasma membrane and the nucleus, a class of proteins we have previously proposed to call NACos [11–17].

We have previously reviewed how epithelial TJs and cell adhesion use these dual localization proteins to regulate gene expression in the context of G1/S phase transition and crosstalk with Ras signaling [11,18,19]. Here, we will provide an update on the role of TJs in the regulation of gene expression and review some recent observations on how the expression of TJs proteins can be controlled at the transcriptional level.

2. Tight junction associated proteins

The identification of TJ associated proteins has been a key area of cell biology during the last twenty years. Occludin, claudins, tricellulin, JAMs (Junction Adhesion Molecules), CRB-3 (a human homologue of *Drosophila* Crumbs) and Bves (blood vessel/epicardial substance) are the TJ-associated transmembrane proteins that have been identified [7,20–25]. We are only just starting to understand how each of these transmembrane proteins interacts with components of the cytoplasmic plaque and how these interactions affect cell functions.

The cytoplasmic plaque associated with TJs is formed by multiple adaptor and scaffold proteins (e.g., ZO-1/2/3, PATJ, Pals1, PAR-3 and PAR-6) as well as different types of signaling components such as GTP-binding proteins, protein kinases and phosphatases as well as transcriptional and post-transcriptional regulators [6,19,26,27]. Cytoplasmic plaque components interact with the membrane proteins as well as each other, resulting in a protein network that controls paracellular permeability, gene expression, junctional dynamics, proliferation and polarity. Although we start to know more about particular proteins and their interactions and functions, how these proteins work in the normal cellular context and which of their interaction partners are relevant for particular functions are still poorly understood.

ZO-1, the first TJ protein identified [28], can serve as a typical example. It is an adaptor protein that belongs to a family of proteins that contain different types of protein/protein interaction domains such as three PDZ and an SH3 domain, a domain homologous to yeast guanylate kinase (GUK domain) and an alternatively spliced large C-terminal domain that interacts with the actin cytoskeleton [29–31]. Other family members include PSD-95 (post-synaptic density-95) and DlgA (Discs large A), a *Drosophila* tumor suppressor. ZO-1 interacts with many different TJ proteins and at least some of these interactions are mutually exclusive; hence, ZO-1 seems to participate in distinct protein complexes that also might have distinct functions. Functionally, ZO-1 has been linked to both, assembly of functional junctions as well as signal transduction [26,32–34]. This is not restricted to vertebrate cells, the *Drosophila* homologue of ZO-1, Tamou/Polychaetoid, associates with adherens junctions and regulates dorsal closure, epithelial migration, and cell fate determination in sensory organs [35,36], and is required for cell specification and rearrangement during *Drosophila* tracheal morphogenesis [37]. In agreement, knockout of ZO-1 in mice has recently also been shown to cause an embryonic lethal phenotype associated with defects in yolk sac angiogenesis and apoptosis of embryonic cells [38].

3. ZO-1 and ZONAB in the control of cell proliferation and gene expression

Several of the TJ-associated adaptor proteins have been linked to the regulation of epithelial proliferation, and two of them, ZO-1 and ZO-2, have been shown to regulate transcription factors (Fig. 1). In the case of ZO-1, reduced expression correlates with increased proliferation of epithelial cells and/or transformation. For example, in proliferative cells, during corneal wound repair and in colorectal epithelial cells transformed by overexpression of beta-catenin, ZO-1 is downregulated [39,40]. Similarly, ZO-1 is downregulated in breast cancer tissues[41], in primary and metastatic pancreatic cancer [42], in brain microvascular endothelial cells from human brain tumors [43] as well as several models of epithelial mesenchymal transition [44–47]. In agreement, increased expression of ZO-1 in MDCK cells reduces cell proliferation [48].

The mechanism by which ZO-1 regulates proliferation may involve nuclear translocation as ZO-1 has been reported to accumulate transiently in the nucleus of proliferating cells. However, the role of nuclear ZO-1 is not clear as not all investigators have found it in the nucleus, suggesting that additional unknown parameters might affect its nuclear distribution [49–54]. In fact, ZO-1 inhibits cell proliferation outside of the nucleus by cytoplasmic sequestration of the Y-box transcription factor ZONAB, which interacts with the cell cycle regulator CDK4 and controls expression of cell cycle regulators such as cyclin D1 and PCNA [48,50,55]. ZO-1 also interacts with the NaCo ubinuclein, a protein that interacts with viral transcription factors in the nucleus and ZO-1 at epithelial TJs [56]. Little is known about the functional relevance of the interaction between ZO-1 and ubinuclein; however, it may play a role during differentiation as their interaction is cell density dependent, similarly to the one with ZONAB.

ZONAB is a Y-box transcription factor, a class of multifunctional regulators of gene expression and cell proliferation [57,58]. The Y-box factor family includes DbpA/ZONAB, DbpB/YB-1 and DbpC/contrin. DbpA and DbpB were originally identified as DNA binding proteins of the promoters of EGF receptors and MHC II [59,60]. DbpB/YB-1 is the most extensively studied Y-box factor. Overexpression of DbpB/YB-1 increases the expression of genes involved in proliferation, including cyclin A, cyclin B1, EGFR and erbB2 [61–63]. Although DbpB/YB-1 does not seem to associate with cell junctions as DbpA/ZONAB does [50] (Matter and Balda, unpublished), the two Y-box factors seem to be functionally redundant to some extent. Knockout experiments in mice have shown that ZONAB/MSY3-deficient animals develop normally but, if combined with a DbpB/YB-1 knockout, die earlier than animals with a DbpB/YB-1 knockout alone [64]. Nevertheless, the molecular basis for this redundancy is not known.

ZONAB localizes to TJs, where it binds to the SH3 domain of ZO-1, and can be in the nucleus where it participates in the regulation of gene expression [48,50]. In MDCK cells, ZONAB's nuclear distribution is

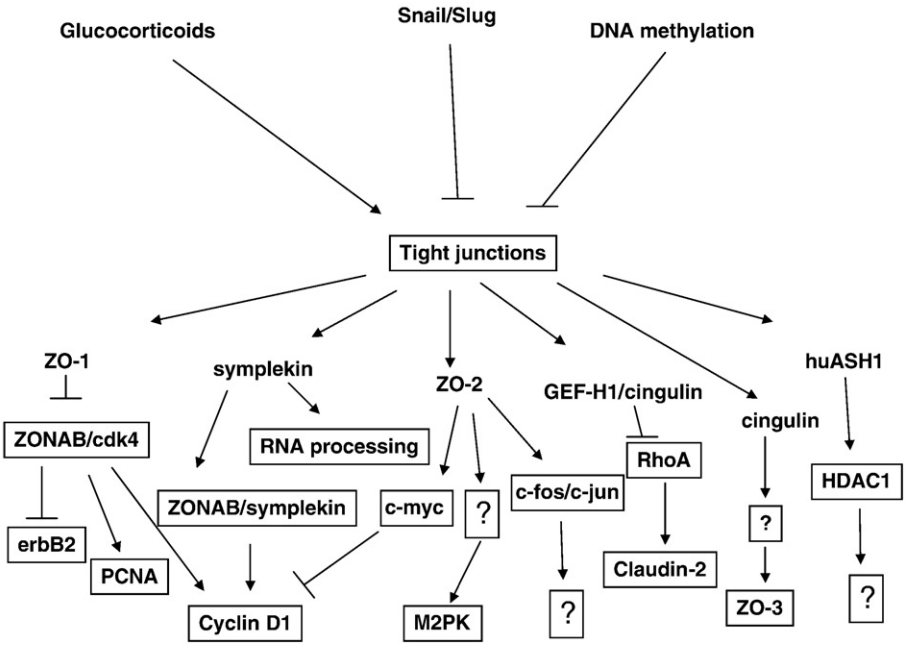

Fig. 1. Tight junction-associated proteins in the control of gene expression and emerging mechanisms that control tight junction protein expression. Indicated are the main transcriptional pathways discussed in this review. In the upper half, some of the most studied mechanisms that can affect TJ proteins expression are pointed out. In the lower half TJ proteins are indicated that have been suggested or demonstrated to regulate gene expression. Arrows do not reflect direct physical interactions, although in some cases this would be justified, but refer to functional interactions. ZO-1, by regulating ZONAB localization, has been linked to decreased erbB-2 but increased cyclin D1 and PCNA expression. Symplekin can regulate gene expression by it interaction with ZONAB but also by regulating RNA processing. The interaction of ZO-2 with c-*fos*/c-*jun* transcription factors has not been linked to a particular gene but the one with c-*myc* seems to repress cyclin D1 expression. Cingulin can regulate gene expression in a RhoA dependent manner by regulating localization of GEF-H1 as well as other unidentified mechanisms.

regulated by the expression levels of ZO-1, which, in turn, is regulated by cell density. Low density and proliferating MDCK cells express high levels of ZONAB and low levels of ZO-1; hence, ZONAB accumulates in the nucleus as well as TJs. In contrast, high density confluent cells, express high levels of ZO-1 and low levels of ZONAB, which results in a predominant junctional localization of the transcription factor. These observations are supported by experiments demonstrating that if the nuclear ZONAB pool is reduced either by overexpression of ZO-1 or by depletion of total ZONAB expression using antisense or RNA-interference techniques, proliferation of MDCK cells is reduced [48,50]. This suggests that the proliferation suppressive function of ZO-1 is based on the cytoplasmic sequestration of ZONAB. In agreement, the SH3 domain of ZO-1, which binds to ZONAB, is necessary and sufficient to reduce proliferation [48]. These data indicate that the cell-density dependent accumulation of ZO-1 at TJs results in the inactivation of the proliferation-promoting ZONAB pathway.

ZO-1 and ZONAB regulate G1/S phase transition of the cell cycle in at least two ways. In one pathway, ZONAB interacts with CDK4, a regulator of G1/S phase transition, which colocalizes with ZO-1 at junctions [48]. A reduction in the nuclear ZONAB accumulation was found to reduce the nuclear pool of CDK4 and cyclin D1, and reduced hyperphosphorylation of the retinoblastoma protein, a nuclear CDK4 substrate [48]. In another pathway, ZONAB functions as a transcription factor and regulates the expression of cell cycle genes [50,55].

Among the known ZONAB target genes are two well-known cell cycle genes. *PCNA* is one of these genes. Its promoter contains an inverted CCAAT box sequence, which functions as a ZONAB binding site. PCNA is an essential eukaryotic DNA replication and repair factor [65,66]. Furthermore, a recent cDNA array analysis suggested that ZONAB overexpression results in the up-regulation of several components that either participate in replication (e.g., RFC40 and replication licensing factors) or in chromatin remodeling (histone H4 and HMG-I) or are part of the cellular machinery for DNA repair (e.g., Rad23A and UBE2A) [55]. It thus seems that ZONAB promotes S-phase progression by up-regulating the expression of proteins that are required for DNA replication. Additionally, it is possible that ZONAB might participate in stress-related signaling responses by up-regulating DNA repair genes.

The PCNA promoter may also serve as an experimental system to determine how ZONAB activates some promoters but represses others. The PCNA promoter can be transcriptionally regulated by various oncoproteins and transcription factors, such as adenovirus oncoprotein E1A, cAMP response element-binding protein (CREB), RFX1 transcription factors, the coactivator CREB-binding protein (CBP), tumor suppressor p107, p53, and E2F [67–69]. The ZONAB binding site is between the p53 (−237), E2F (−84), and ATF-1 (−52) sites. Thus, it will be interesting to determine whether these binding sites and the proteins that bind to them affect each other and ZONAB activity.

The identification of cyclin D1 as a ZONAB target gene is of particular importance as it is unregulated in many tumors. Cyclin D1 plays a central role in cell cycle control, and its expression is regulated at multiple levels, including transcription, mRNA stability, translation, and protein turnover [70,71]. Furthermore, the fact that the cyclin D1 promoter contains several *cis*-acting elements that are controlled by signaling pathways regulated by cell–extracellular matrix and cell–cell adhesion, and its regulation involves integrin-linked kinase, focal adhesion kinase, and beta-catenin [11,72–77] suggests that this promoter represents an important site for crosstalk between pathways originating at different sites of adhesion and, hence, may also affect cell differentiation. A role of ZO-1 and ZONAB in epithelial morphogenesis is supported by results from 3D-culture experiments indicating that manipulation of the ZO-1/ZONAB pathway affects the morphogenetic potential of MDCK cells [55].

Another ZONAB target gene that might be important for the role of this transcription factor in epithelial differentiation is the erbB-2 proto-oncogene. Upregulation of erbB-2 has been linked to organ development and cell differentiation, as well as in certain cellular contexts to tumorigenesis [78–80]. Since overexpression of erbB-2 has been associated with tumorigenesis, it was surprising that overexpression of ZO-1 reduced cell proliferation but increased endogenous erbB-2 expression. However, the levels of erbB-2 overexpression in cancer cells

is around 10–100 times higher than in their non-transformed counterparts and correlates with gene amplification. Furthermore, increased levels of erbB2 expression have been observed in other cells when they start to differentiate[81,82] and during mouse development [78–80]. Moreover, overexpression of erbB-2 does not always correlate with increased cell proliferation or transformation, and the activity of constitutively active alleles depends on the cellular background and environment [83,84]. Therefore, by regulating the expression of erbB-2, ZO-1 and ZONAB could be part of a TJ-associated signal transduction pathway important for differentiation of certain tissues as well as for cross talk with growth factor pathways. Nevertheless, further studies are needed not only to characterize the molecular mechanisms involved in the effect of ZO-1 and ZONAB in epithelial morphogenesis but also to identify more genes regulated by the pathway.

Since the level of ZO-1 expression can affect the activity of ZONAB, it is important to understand how expression of ZO-1 is regulated. ZO-1 is expressed at low levels in proliferating low density MDCK cells and becomes upregulated with increasing cell density [50]. At least in part, this appears to be mediated by stabilization of the protein at the forming junctions since its half-life increases with cell density [85]. Because ZO-1 interacts with several junctional membrane proteins, it is likely that stabilization is mediated by the junctional accumulation of transmembrane components. Nevertheless, it is likely that the contribution of transcriptional mechanisms is also important for the regulation of ZO-1 expression. For example, ZO-1 mRNA is down-regulated during corneal wound repair [39], by overexpression of beta-catenin [40] as well as upon cell transformation induced by Ras and TGF-beta [44,86–88]. Future studies will have to address whether and how signaling pathways that regulate ZO-1 expression affect ZONAB localization and transcriptional activity.

ZO-1 knockout by homologous recombination has recently been shown to retard TJ assembly in calcium switch experiments in the mouse mammary epithelial cell line Eph4 with not change in cell density and proliferation [32]. In agreement, we and others also observed retardation of TJ formation in MDCK cells upon depletion of ZO-1 by RNA interference [26,34,89]. In contrast to Eph4 cells, we have observed increased cell densities in both MDCK and MCF10-A-95 cells if ZO-1 was down-regulated by RNA interference [48,55], suggesting that proliferation control in EpH4 cells is differently regulated. Interestingly, oncogenic Ha-Ras induces increased cell proliferation in EpH4 mammary epithelial cells only if they were cultivated as organotypic structures in three-dimensional collagen-Matrigel matrices but not in standard two-dimensional cultures as in the one used by Umeda et al. [32], suggesting that EpH4 cell proliferation depends on the culture condition [84]. It is thus striking that modulation of the ZO-1/ZONAB pathway only significantly affected the differentiation potential of MDCK cells in three dimensional cultures and not when grown as monolayers. Therefore, it will be interesting to test whether ZO-1 knockout in Eph4 cells will have a stronger phenotype in cell proliferation when cultivated in three-dimensional collagen-Matrigel matrices. Knockout of ZO-1 in mice has recently been shown to cause an embryonic lethal phenotype. This was associated with defects in yolk sac angiogenesis and apoptosis of embryonic cells [38]. However, what caused lethality and the relevant downstream pathways are not known.

4. ZONAB and ZO-1 associated proteins and regulation of the ZONAB pathway

ZONAB interacts with a number of different proteins apart from ZO-1. The first one identified was CKD4, resulting in regulation of the nuclear accumulation of the cell cycle kinase [48]. In contrast, symplekin is another interaction partner and seems to cooperate with ZONAB in the nucleus.

Symplekin is a nuclear protein that can associate with TJs and has been linked to the machinery involved in 3′-end processing of pre-mRNA and polyadenylation [90–92] and thereby promotes gene expression. In agreement, symplekin interacts with HSF-1 (heat shock inducible transcription factor one) to regulate hsp70 mRNA polyadenylation in stressed cells [93]. Although it will be necessary to characterize other mRNA transcripts that are regulated by symplekin, this might represent a mechanism by which intercellular junctions participate in the regulation of mRNA processing.

ZONAB and symplekin form a nuclear complex in kidney and intestinal epithelial cells. Symplekin depletion by RNA interference reduces ZONAB nuclear accumulation and transcriptional activity in colon adenocarcinoma cells, resulting in inhibition of cell proliferation and reduced of expression the ZONAB-target gene cyclin D1. Thus, symplekin and ZONAB cooperate in the regulation of transcription, they promote epithelial proliferation and cyclin D1 expression [94]. ZONAB is a Y-box factor, a family of proteins that are multifunctional and can interact with DNA as well as RNA [57]. It is possible that the interaction between symplekin and ZONAB is not only functionally important for transcription but also for RNA processing, stability, and/or localization.

Two other proteins have been linked to the regulation of the ZO-1/ZONAB pathway in the cytoplasm. The first was RalA [95]. Ral proteins are members of the Ras superfamily of small GTPases and are involved in actin cytoskeleton remodeling, cell cycle control, cellular transformation and vesicle transport. The GTP-bound form of RalA binds to ZONAB, resulting in inhibition of the transcription factor and increasing amounts of RalA–ZONAB complexes at cell–cell junctions with cell confluence. Ral proteins become activated in response to Ras activation; consequently, oncogenic Ras alleviates transcriptional repression by ZONAB in a RalA-dependent manner [95]. Because ZO-1 and RalA interaction results in similar inhibition of ZONAB transcriptional activity, it will be necessary to further understand in which cellular context ZO-1 or RalA interaction takes place and whether RalA also affects ZONAB's transcriptional activity when ZONAB is an activator instead of repressor of transcription.

A different type of regulator is the heat shock protein Apg-2 that binds to the SH3-domain of ZO-1 [96]. In agreement with the observation that the SH3 domain of ZO-1 is sufficient to inhibit ZONAB activation, binding of Apg-2 to the SH3 domain of ZO-1 activates ZONAB. This seems to be due to direct competition for the same or overlapping binding sites. Consequently, depletion of Apg-2 in MDCK cells not only inhibits ZONAB but also G1/S phase progression in similar manner to overexpression of ZO-1 or the SH3 domain.

Apg-2 represents a molecular mechanism that links TJs and ZONAB to the cellular stress response. Apg-2 coimmunoprecipitates with ZO-1 and partially localizes to intercellular junctions. In normally cultured MDCK cells only a small amount of Apg-2 is associated with ZO-1; in heat shocked cells, however, the interaction with ZO-1 is stimulated, triggering ZONAB transcriptional activity [96]. Thus, Apg-2 regulates activation of the ZO-1/ZONAB pathway in response to heat shock.

Apg-2 not only regulates ZONAB activation but also TJ assembly in calcium switch experiments. The effect of Apg-2 depletion is similar to the one of ZO-1 depletion, as the consequence of depletion is a kinetic one; hence, neither ZO-1 nor Apg-2 is required for assembly of functional TJs [89]. Nevertheless, whether Apg-2 only regulates junction assembly due to its interaction with ZO-1 is not clear. Apg-2 depletion has much more drastic consequences on epithelial morphogenesis in a three-dimensional culture system, in which it is required for the development of well-organized polarized cysts [89]. Again, the same observations have been made for ZO-1 [55]. It remains to be determined, however, whether ZO-1 is the only functionally relevant interaction partner of Apg-2 during epithelial morphogenesis and how their interaction is regulated.

5. ZO-2 and gene expression

ZO-2 is a protein with a similar domain structure to ZO-1 and was identified because it binds to the latter protein [85,97]. ZO-2

expression is also reduced in certain tumors [98–100] and has been also reported to accumulate transiently in the nucleus of proliferating cells [53,101,102]. ZO-2 as well as MAGI-1 (which is an inverted MAGUK) and MUPP1 (a multi-PDZ domain protein) have been shown to bind and inactivate oncogenic viral proteins [51,103,104]. Furthermore, ZO-2 binds to DNA scaffolding factor SAF-B [53] and the transcription factors Fos, Jun, C/EBP and c-*myc* (Fig. 1) [105,106].

ZO-2 and c-Myc can be found associated by immunoprecipitation, and ZO-2 reduces cyclin D1 transcription via an E box myc binding site which also recruits histone deacetylases [106]. It is difficult to reconciliate these data in a working model in which nuclear localization of ZO-2 repress cyclin D1 expression but increases cell proliferation. Therefore, the functionally relevant genes regulated by nuclear ZO-2 might not include cyclin D1. However, one possible candidate is M2 pyruvate kinase; expression of which is also stimulated by nuclear ZO-2 and correlates with increase proliferation in epithelial and endothelial cells [107]. By regulating the expression of M2 type pyruvate kinase, ZO-2 might directly contribute to the switching of metabolic pathways observed in tumor cells (i.e., the Warburg effect) [108].

An alternative mechanism by which ZO-2 might regulate gene expression represents ARVCF. ARVCF is an armadillo-repeat protein of the p120 (catenin) family that can associate with adherens junctions and translocate to the nucleus [101]. Nuclear translocation can be mediated by ZO-2 whereas ZO-1 may regulate association with forming junctions at the plasma membrane [101]. However, the role of nuclear ARVCF is not known.

Similar to ZO-1, ZO-2 deficiency in mice causes embryonic lethality. In the absence of ZO-2, embryos show decreased proliferation at embryonic day 6.5 and increased apoptosis at embryonic day 7.5; they die shortly after implantation due to an arrest in early gastrulation [109]. Although the molecular mechanisms responsible for the observed phenotypes is not known, it will be interesting to analyze gene expression in these embryos to find out whether they have different expression of cyclin D1 and M2 type of pyruvate kinase as suggested from cell culture experiments. Furthermore, developmental arrest prior to gastrulation has been described for conventional deletion of several cell cycle regulators (e.g. ATR, Chk1, Mad2, NBS, Rad50, BRCA1, BRCA2, or Rad51) [110]; hence, it could be that ZO-2 is involved in the regulation of such genes.

The knockout mice studies also led to another interesting observation. Although they seem to be redundant in junction formation in tissue culture models, they seem to have non-redundant roles in mouse development, which are likely to involve their roles in signal transduction. In contrast, the third ZO protein, ZO-3, does not seem to play an obvious role in mouse development [109,111]. Nevertheless, ZO-3 is critical for epidermal barrier function in zebrafish embryos and its expression is decreased in breast cancer and squamous cell carcinomas [100,112,113]. However, the molecular mechanisms by which ZO proteins regulate embryonic development in mouse and cause phenotypes in other experimental systems remain to be determined. Moreover, many phenotypes of mice deficient in specific junction-associated proteins might be mild under standard conditions, it will be important to test how these animals respond to challenges such as different types of stress conditions.

6. HuASH1, GEF-H1, cingulin and gene expression

Indirect evidence suggests that several other TJs associated proteins might play a role in gene expression. HuASH1, the human homologue of *Drosophila* ASH1 (absent, small or homeotic discs 1), is a transcription factor that belongs to the trithorax group and that participates in the maintenance of expression of segment-specific homeotic genes. HuASH1 was found to co-localize with TJ markers by immunofluorescence [114]. *Drosophila* ASH1 functions as a histone methyltransferase, an enzymatic activity involved in chromatin remodeling and gene expression [115]. Recent data suggest a model in which *Drosophila* ASH1 is required to coordinate the regulation of Myc trans-activation targets [116]. Furthermore, mammalian ASH1 is also a histone methyltransferase that methylates histone H3 in a subset of genes [117]. Moreover, the C-terminal part of huASH1 interacts with HDAC1 repression complexes [118]. However, how huASH1 becomes recruited to intercellular junctions and the functional relevance of its junctional association in chromatin remodeling and gene expression are unknown.

GEF-H1/Lfc, a guanine nucleotide exchange factor specific for RhoA, is involved in the regulation of paracellular permeability and cell proliferation [119,120]. It is well known that RhoA activation stimulates epithelial cell proliferation, gene expression and differentiation [121–123]. RhoA is downregulated when epithelial cells reach confluence, resulting in inhibition of signaling pathways that stimulate proliferation. GEF-H1/Lfc directly interacts with cingulin, a junctional adaptor. Cingulin binding inhibits RhoA activation and depletion of cingulin by regulated RNA interference results in RhoA activation. These results indicate that forming epithelial TJs contribute to the downregulation of RhoA by inactivating GEF-H1 in a cingulin-dependent manner, providing a molecular mechanism whereby TJ formation is linked to inhibition of RhoA signaling and gene expression [120].

Disruption of the cingulin gene does not affect TJ formation but alters gene expression in mouse embryoid bodies. The absence of cingulin increases the levels of ZO-2, occludin, claudin-2, claudin-6 and claudin-7 but decreases ZO-1, which correlates with increased expression of transcription factors implicated in endodermal differentiation such as GATA-6, GATA-4 and HNF-4alpha [124]. In MDCK cells, increased claudin-2 expression in response to cingulin depletion is RhoA-dependent, suggesting that it might be mediated by activation of GEF-H1 [125]. As Cingulin interacts with ZO-1 and ZO-2 [126], it is tempting to speculate that cingulin could be indirectly involved in regulating the functions of ZO-1 and ZO-2 in gene expression.

7. Regulation of tight junction protein expression

Expression of several TJ proteins is upregulated during differentiation-inducing conditions, such as treatment with glucocorticoids, and is reduced during cancer and epithelial mesenchymal transition. Therefore, differential expression of TJ protein is likely to contribute to the regulation of TJ-associated signaling pathways.

Early studies suggested that dexamethasone stimulates functional TJ structures in hepatoma, mouse mammary epithelial, brain endothelial and intestinal epithelial cells [127–130]. Glucocorticoid-induced expression of TJs in mammary epithelial cells involves the helix–loop–helix protein Id-1 and down-regulation of RhoA by RhoE, and is counteracted by TGF-alpha signaling [131,132]. In an intestinal epithelial cell line, glucocorticoid receptors have been shown to complex to the GRE site on the MLCK promoter, suppressing the TNF-alpha-induced increase in MLCK gene activity, protein expression, and subsequent opening of the intestinal TJ barrier [133]. Additionally, a cellular cascade that includes glucocorticoid-induced protein kinase and Akt is triggered by glucocorticoid and regulates phosphorylation, ubiquitination, and degradation of GSK3, which alters beta-catenin dynamics, leading to the formation of adherens junctions and TJ sealing [134]. It is tempting to suggest that one or more of the mechanisms described could be involved in the increased expression of ZO-1, ZO-2, occludin, claudin induced by glucorticoids [128,135,136].

The zinc-finger transcription factors Snail and Slug are suppressors of the expression of cell–cell adhesion components in different cell types and play a central role in the epithelium-mesenchyme transition. Snail binds directly to the E-boxes of the promoters of claudin and occludin genes, resulting in complete repression of their promoter activity similar to what has been described for E-cadherin

[46,137–140]. Furthermore, tricellulin, a TJ transmembrane protein with a similar domain structure as occludin, was identified as a protein downregulated by Snail [141]. Additionally, Snail and Slug upregulation has been linked to reduced expression of TJ proteins during TGF-beta, HGF and Ras induced transformation [137,138,140,142–144].

Silencing gene expression by methylation is another important regulatory mechanism that controls TJ formation as silencing by methylation of several claudin promoters has been reported in different type of cancer tissues [145]. In MCF7 breast carcinoma cells, for example, claudin-6 expression is partially silenced by promoter CpG island hypermethylation and can be reversed by combining a demethylator and a histone deacetylase inhibitor [146]. Silencing of Claudin-7 expression in breast cancer cell lines also correlates with promoter hypermethylation, suggesting a potential role of Claudin-7 in the progression [147]. Moreover, histone deacetylase inhibitors also stimulate expression of some TJ proteins in HeLa cells [148]. However, the relevance of DNA methylation in TJ proteins gene expression under physiological and developmental conditions remains to be determined.

An elegant example of regulation of the expression of TJ proteins at the transcriptional level by adherens junctions in endothelial has recently been identified. Vascular endothelial (VE)-cadherin upregulates the expression claudin-5 by releasing the inhibitory activity of forkhead box factor FoxO1 and the Tcf-4-beta-catenin transcriptional repressor complex. VE-cadherin acts by inducing the phosphorylation of FoxO1 through Akt activation and by limiting the translocation of beta-catenin to the nucleus what provide an explanation of why VE-cadherin inhibition may cause a marked increase in permeability [149]. Furthermore, in vivo inactivation of beta-catenin causes blood-brain-barrier breakdown with down-regulation of claudin-3 but stabilization of beta-catenin enhances blood-barrier maturation as well as claudin-3 expression. This can also be reproduced in primary brain endothelial cells in vitro, as beta-catenin N-terminal truncation or Wnt3a treatment increases claudin-3 expression and blood-brain-barrier-type tight junction formation. How beta-catenin regulates the expression of claudin-3 needs to be demonstrated, but might involve TCF-LEF binding sites present in the promoter of claudin-3 [150].

In summary, altered expression of TJ proteins seems to be mediated by different mechanisms and occurs during various physiological or pathological states. However, the importance of these changes for disease development and progression, and effects on TJ-associated signaling mechanisms are still to be analyzed in more detail.

8. Conclusions and perspectives

Several TJ-associated proteins have been linked to different types of signaling and transcriptional pathways that modulate cell proliferation, differentiation and gene expression. In general, TJs suppress pathways that stimulate proliferation, allowing differentiation. For example, ZO-1 expression regulates at least two such pathways by cytoplasmic sequestration of the transcription factor ZONAB and the cell cycle kinase cdk4. In agreement, ZO-1 deficiency in mice results in early embryonic lethality. The same is true for ZO-2, which also binds to several transcription factors. However, we are just starting to understand their physiological functions during development and in tissue homeostasis, and we need a better understanding of the roles of each TJ proteins in signaling and transcriptional pathways and how they functionally interact with signaling mechanisms originating at other sites of cell adhesion, such as such as adherens junctions and integrins. TJs seem to function as a sensor of cell density that is critically affected by cell transformation. Therefore, to understand how TJs protein expression is affected during cell differentiation and how these mechanisms affect TJ-associated signaling mechanism will be crucial to understand the role of TJs in various diseases including cancer and inflammation.

Acknowledgments

The research in our laboratories is supported by the Wellcome Trust, MRC, BBSRC, AICR and Fight for Sight.

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