Epithelial tight junctions, gene expression and nucleo-junctional interplay

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Summary

Tight junctions are components of the junctional complex linking neighbouring epithelial cells and are important for barrier formation. Recent evidence suggests that tight junctions also participate in signal transduction mechanisms that regulate epithelial cell proliferation, gene expression, differentiation and morphogenesis. One important class of tight-junction-associated signal transduction mechanism is based on dual localisation of certain proteins both at junctions and in the nucleus. These proteins and their partners participate in various steps of gene expression, ranging from regulation of transcription and chromatin structure to mRNA processing and translation. In cancer tissues, their expression is often deregulated in a manner that suggests that tight junctions function as suppressors of proliferation and transformation.

Key words: Y-box factor, ZO-1, ZO-2, Symplekin, SAF-B, AP-1

Introduction

Epithelial cells form cellular barriers that separate different tissues and body compartments. This requires that they polarise (i.e. develop distinct cell surface domains) and that they interact with one another through adhesive complexes, called junctions, between the cells. These intercellular junctional complexes are crucial for the biogenesis, maintenance and function of epithelia. They mediate adhesion and provide mechanical strength, they restrict diffusion across epithelia, and they regulate signalling pathways that control cell proliferation, polarisation and differentiation.

In vertebrates, the epithelial junctional complex consists of tight junctions (also known as the zonula occludens), adherens junctions and desmosomes (Cereijido et al., 2004; Farquhar and Palade, 1963; Weis and Nelson, 2006). In some tissues, gap junctions, which form intercellular pores, can also be associated with the junctional complex and can be intercalated with tight junctions (Goodenough and Paul, 2003). Desmosomes and adherens junctions are adhesive junctions. Desmosomes are found all along the lateral membrane and form distinct complexes that are linked to the intermediate filament cytoskeleton (Yin and Green, 2004; Perez-Moreno et al., 2003). Adherens junction components are concentrated close to tight junctions and form a morphologically distinct junction in some epithelia, whereas in other epithelial cell types they can be found along the entire lateral membrane. Both tight junctions and adherens junctions are linked to the actin cytoskeleton and contain several actin-binding proteins. More recently, microtubule-binding proteins have also been found at both types of junction, which suggests that they also interact with microtubules.

Tight junctions have a distinct morphology (Kachar and Reese, 1982; Staehelin, 1973). In ultrathin sections, they appear as close contacts between neighbouring plasma membranes that, depending on the preparation method, can appear as hemifusions (i.e. the outer leaflets of the two neighbouring membranes appear to be continuous) or as closely apposed electron-dense membranes (Fig. 1). These sites of close contact coincide with intramembrane strands that can be visualised by freeze-fracture electron microscopy, a technique that allows visualisation of the hydrophobic membrane surfaces. The strands appear as net-like meshworks and are generally assumed to represent polymers of interacting junctional transmembrane components; however, a contribution of lipids and specialised lipid structures cannot be ruled out (Tsukita et al., 2001).

Tight junctions provide epithelial tissues with a paracellular diffusion barrier that is critical for the normal functioning of organs and tissues. This barrier is not absolute, however: it is semipermeable, allowing the selective passage of certain solutes but not others (Anderson et al., 2004; Cereijido et al., 2004). Tight junctions also help to maintain cell polarity by forming an intramembrane diffusion fence that restricts diffusion of lipids in the exoplasmic leaflet of the plasma membrane (Balda and Matter, 1998; Dragsten et al., 1981; van Meer and Simons, 1986). Recently, tight junctions have been shown to harbour evolutionarily conserved protein complexes that regulate polarisation and junction assembly and to recruit signalling molecules that participate in the regulation of cell proliferation, differentiation and gene expression (Macara, 2004; Matter and Balda, 2003; Shin et al., 2006).

Tight junctions are built according to the same architectural principle as other adhesion complexes. A set of different transmembrane proteins is linked to a cytoplasmic plaque that consists of a network of adaptor proteins that anchor the junction to the cytoskeleton and serve as a scaffold for the recruitment of signalling proteins. The transmembrane proteins mediate cell-cell adhesion and form the paracellular diffusion barrier, and the components of the cytoplasmic plaque function as cytoskeletal linkers, scaffolds, and regulators of junction

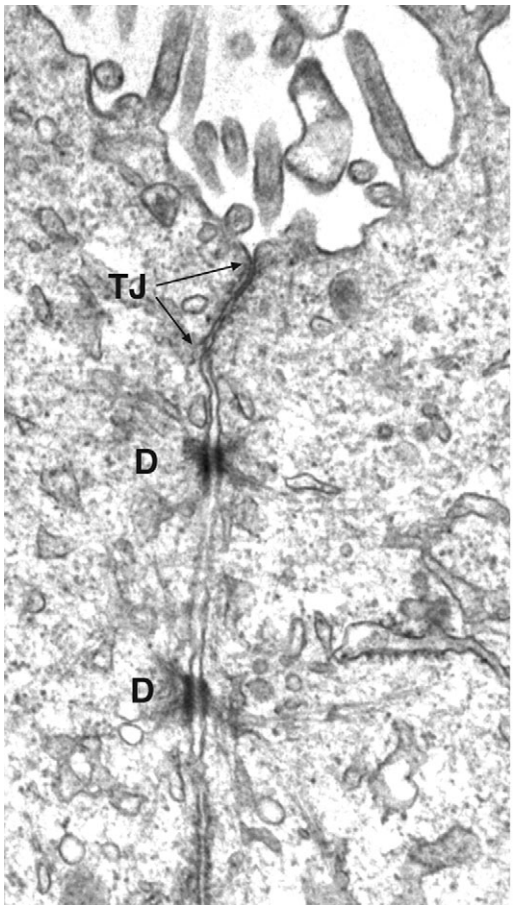

Fig. 1. Morphology of the epithelial junctional complex. Shown is an electron micrograph of an ultrathin section of Epon-embedded MDCK cells. The positions of tight junctions (TJ) and desmosomes (D) are indicated. Note, the close contacts between the neighbouring plasma membranes at the tight junctions. Reprinted from Huber et al. (Huber et al., 1998) with permission from Karger.

assembly and function. The membrane proteins can be classified into two classes: those with a single transmembrane domain (e.g. JAMs, Crb-3) and those with four transmembrane domains (e.g. occludin, tricellulin, claudins). Whereas both classes of proteins have adhesive properties and can function in various signalling processes, the polytopic proteins form the diffusion barrier and regulate paracellular permeability. Most of the transmembrane proteins contain PDZ-binding domains, which bind and recruit proteins containing PDZ (PSD95/DlgA/ZO-1 homology) domains – motifs found in many of the components of the cytoplasmic plaque, such as PAR3 and PAR6, Pals1 and PATJ, ZO-1, ZO-2 and ZO-3, the MAGIs and MUPP1. However, there are also non-PDZ-proteins among the scaffolding proteins, such as cingulin, a protein known to interact with junctional membrane proteins, actin and signalling proteins. Many of the scaffolding proteins contain multiple PDZ domains and other protein-protein interaction domains with which they bind to and regulate different types of signalling proteins, ranging from small and heterotrimeric GTP binding proteins (e.g. Rab13, $G_{\alpha 12}$) and guanine nucleotide exchange factors for Rho family GTPases (e.g. GEF-H1, Tuba) to protein phosphatases and kinases (e.g. atypical protein kinase C, protein phosphatase 2A) (for reviews, see Aijaz et al., 2006; Gonzalez-Mariscal et al., 2003; Macara, 2004; Shin et al., 2006; Tsukita et al., 2001).

Not all junctional components are exclusively associated with tight junctions. One important class of proteins recruited to them appear to shuttle between the junction and the nucleus, where they function in the regulation of gene expression. These are examples of proteins named NACos for proteins associated with the nucleus and adhesion complexes (Balda and Matter, 2003). Here, we review the tight-junction-associated NACos and how they participate in the regulation of gene expression.

The growing class of nucleo-junctional proteins

NACos are associated with all intercellular junctions and can also be found at sites of cell–extracellular-matrix adhesion, such as focal adhesions (Balda and Matter, 2003). The best and most intensively studied example is undoubtedly β-catenin, an adherens-junction-associated NACo that binds to cadherins and plays crucial roles in Wnt/Wingless signalling, development and cancer. Once stimulated, β-catenin accumulates in the nucleus owing to decreased degradation and binds to and activates the transcription factor LEF/TCF. At tight junctions, several NACos have been described. These seem to influence gene expression at different steps, including chromatin structure, transcription, RNA processing and, possibly, translation (Fig. 2).

ZO-1 and ZO-2: scaffolding proteins with multiple interaction partners

The discovery that the tight-junction-associated adaptor protein ZO-1 belongs to the same protein family as the *Drosophila* tumour suppressor Discs Large A (DlgA) first suggested that tight junctions might be involved in signalling mechanisms regulating cell proliferation and differentiation (Tsukita et al., 1993; Willott et al., 1993; Woods and Bryant, 1993). DlgA is a scaffolding protein that localises to *Drosophila* septate junctions and regulates epithelial proliferation; inactivation of DlgA results in excessive growth and, hence, large imaginal discs (Woods and Bryant, 1991). Several other tight junction components have since been found to belong to the same family of scaffolding proteins, and these generally have multiple junctional interaction partners (Aijaz et al., 2006). ZO-1 and ZO-2 have both been reported to localise to the nucleus in proliferating epithelial cells (Gottardi et al., 1996; Islas et al., 2002; Kausalya et al., 2004; Traweger et al., 2002). There are, however, some disagreements about the nuclear localisation of ZO-1 because not all investigators have been able to detect a nuclear pool (Balda and Matter, 2000; Reichert et al., 2000). Moreover, ZO-1 affects gene expression in a manner that does not require nuclear localisation (see below). What causes the differences is not known, but it is likely that the distribution of ZO-1 is affected by additional, unknown parameters. One possibility is that factors affecting association with other proteins and/or the localisation of interaction partners influence this. For example, a truncated form of ZO-2 that concentrates in the nucleus has been shown to stimulate nuclear accumulation of ZO-1 (Kausalya et al., 2004). ZO-1 and ZO-2 are unlikely to participate directly in gene regulation. Instead, they appear to do so indirectly, by regulating the localisation of transcription

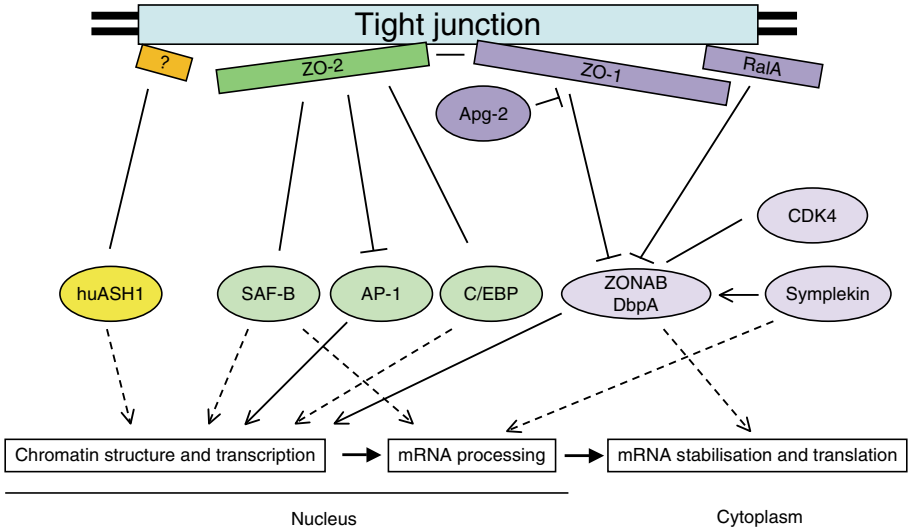

Fig. 2. Tight-junction-associated NACos and their interaction partners. If known, the functional consequences of interactions between the proteins are indicated. Arrows indicate processes that can be regulated by a particular protein. Continuous arrows indicate functional interactions that have been demonstrated to be influenced by tight junctions; dashed arrows indicate instances where it is not known whether junctional association is functionally relevant.

factors such as ZONAB/DbpA and AP-1 and other proteins that control gene expression.

ZONAB/DbpA: a ZO-1 binding transcription factor that regulates proliferation

ZONAB (ZO-1-associated nucleic-acid-binding protein) is a Y-box transcription factor that binds to ZO-1 (Balda and Matter, 2000). Y-box transcription factors are a small family of multifunctional proteins that can interact with DNA and RNA, and have been linked to the regulation of transcription, RNA stability, and translation (Matsumoto and Wolffe, 1998). ZONAB is the canine orthologue of human DbpA, an E2F1 target gene that is overexpressed in different types of carcinoma (Arakawa et al., 2004). Indeed, ZONAB/DbpA promotes proliferation of epithelial cells and represents the only known pathway by which ZO-1 regulates proliferation (Balda et al., 2003).

ZO-1 functions as an inhibitor of proliferation; this activity maps to its SH3 domain, which is the ZONAB-interaction domain. ZO-1 inhibits ZONAB/DbpA by sequestering it in the cytoplasm; ZONAB levels in the nucleus are high in proliferating cells when ZO-1 expression is low and decrease with cell confluence when ZO-1 levels increase, which results in cytoplasmic sequestration and recruitment of the transcription factor to junctions (Fig. 3) (Balda and Matter, 2000). ZONAB/DbpA stimulates transcription of genes encoding cell cycle regulators such as cyclin D1 and PCNA, which accounts for its stimulation of the G1/S phase transition. However, ZONAB/DbpA also forms complexes with the cell division kinase CDK4, which results in co-sequestration of the two proteins (Balda et al., 2003). Hence, regulation of CDK4 localisation is a second mechanism by which the ZO-1–ZONAB/DbpA pathway regulates entry into S-phase.

ZONAB also interacts with the small GTPase RalA, which localises along the lateral membrane, including tight junctions (Frankel et al., 2005). The interaction with RalA similarly results in inhibition of the transcription factor. However, the amount of active RalA is not affected by cell density and the interaction with ZONAB occurs only in dense cells. RalA is hence unlikely to regulate ZONAB localisation but rather its activation state. Ras signalling activates RalA and this thus represents a pathway by which Ras can impact on ZONAB/DbpA. In normal MDCK cells, however, RalA activation is Ras independent and its localisation to junctions and the lateral membrane occurs at high confluence in polarised cells. It is thus possible that this interaction is important for the regulation of cytoplasmic functions of ZONAB that operate in differentiated cells, such as regulation of RNA turnover and/or translation (Matsumoto and Wolffe, 1998).

Activation of ZONAB/DbpA is also regulated by the heat shock protein Apg-2, an Hsp110 subfamily member (Tsapara et al., 2006). Similarly to ZONAB/DbpA, Apg-2 binds to the SH3 domain of ZO-1, stimulating dissociation of the

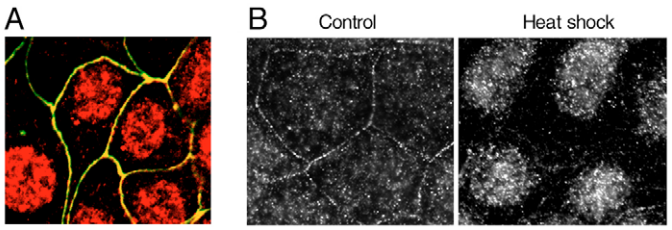

Fig. 3. Dual-localisation of ZONAB to the nucleus and epithelial junctions in proliferating (A) versus non-proliferating, confluent MDCK cells (B) fixed either directly or after heat shock. (A) The distributions of ZONAB (red) and ZO-1 (green) were visualised by double immunofluorescence; shown is a confocal section. (B) Epifluorescence images of ZONAB staining before and after heat shock [for experimental details, see Balda and Matter (Balda and Matter, 2000) and Tsapara et al. (Tsapara et al., 2006)].

transcription factor. In confluent MDCK cells at 37°C, Apg-2 is distributed throughout the cytoplasm and the nucleus; in response to heat shock, Apg-2 becomes recruited to intercellular junctions, which results in dissociation of ZONAB/DbpA from ZO-1, its translocation to the nucleus and, hence, activation of the transcription factor (Fig. 3B). Apg-2 thus links junctional signalling mechanisms to the cellular stress response.

The mouse ZONAB/DbpA orthologue, Yb-3, also co-localises with ZO-1 at gap junctions of oligodendrocytes, astrocytes and neurons (Ciolofan et al., 2006; Penes et al., 2005). Although the functional role of the association of this complex with gap junctions is not clear, these observations suggest that ZO-1–ZONAB/DbpA signalling might also be important in non-epithelial cell types.

Symplekin: a link to mRNA processing

Symplekin is one of the first tight-junction-associated NACos that was identified (Keon et al., 1996). It is present in the nucleus in all cells and can associate with tight junctions in epithelial cells. How junctional association is regulated is unknown because the presence of functional tight junctions is not sufficient for junctional recruitment (Kavanagh et al., 2006). Symplekin associates with the machinery that mediates polyadenylation in the nucleus and in the cytoplasm of oocytes, and it is thought to function as a scaffold that promotes assembly of the complexes that mediate polyadenylation (Barnard et al., 2004; Hofmann et al., 2002; Takagaki and Manley, 2000). The functional importance of this is illustrated by the observation that symplekin interacts with the transcription factor heat shock factor 1 (HSF1) and thereby promotes polyadenylation of Hsp70 mRNA in heat shocked cells (Xing et al., 2004). Moreover, symplekin is essential for the processing of the 3′ ends of histone mRNAs, which are not polyadenylated (Kolev and Steitz, 2005). Thus, it represents a mechanism by which tight junctions can influence gene expression by regulating mRNA processing and, thereby, stability. Nevertheless, whether, and if so how, tight junctions affect the role of symplekin in mRNA processing remains to be discovered.

The recently described interaction between ZONAB/DbpA and symplekin provides a functional link to tight junctions (Kavanagh et al., 2006). ZONAB/DbpA requires symplekin for its transcriptional activity; depletion of symplekin inhibits expression of the ZONAB/DbpA target gene cyclin D1 and proliferation of an intestinal adenocarcinoma cell line. Although likely, whether symplekin promotes cyclin D1 synthesis because of its role in polyadenylation is not known. Note that symplekin also interacts with ZONAB/DbpA in assays in which they inhibit transcription, which suggests that its role is not limited to promoting polyadenylation. Intriguingly, symplekin and ZONAB/DbpA have both been linked to the regulation of gene expression in response to heat shock (Tsapara et al., 2006; Xing et al., 2004). However, we do not know whether ZONAB/DbpA and the HSF1-symplekin complex function together in the regulation of Hsp70 expression.

ZO-2: a mediator of nuclear import and export?

ZO-2 associates with several proteins that have nuclear functions; some of these appear to shuttle themselves whereas others appear to be restricted to the nucleus. Some observations suggest that ZO-2 might actively promote nuclear import and export of other proteins. One such example is ARVCF, a NACo that associates with adherens junctions. ARVCF, a p120catenin family member, binds to the PDZ domains of ZO-2 (Kausalya et al., 2004). Nuclear localisation of ARVCF requires the PDZ-binding domain and can be mediated by the PDZ domains of ZO-2, which suggests that ZO-2 represents a mechanism by which ARVCR is imported into the nucleus.

A role of ZO-2 in trafficking might also be important for the regulation of two transcription factors. Both AP-1 and C/EBP interact with ZO-2 (Betanzos et al., 2004). Because both transcription factors associate with epithelial junctions and ZO-2 can be co-precipitated with the transcription factors from nuclear extracts, the interaction probably occurs at junctions as well as in the nucleus. ZO-2 overexpression reduces AP-1 activity, which suggests that ZO-2 functions as an inhibitor of AP-1. Since it contains functional nuclear export signals (Gonzalez-Mariscal et al., 2006; Jaramillo et al., 2004), ZO-2 might inhibit AP-1 by mediating its nuclear export. One would thus expect nuclear export of ZO-2 to be regulated; inhibiting it should lead to the observed nuclear accumulation and active AP-1 in proliferating epithelial cells. Because ZO-2 also promotes nuclear import of ARVCF, it is possible that ZO-2 functions as a transporter that shuttles numerous proteins in and out of the nucleus.

ZO-2 also binds to SAF-B (scaffold attachment factor B), a protein that seems to be restricted to the nucleus (Traweger et al., 2002). SAF-B binds to DNA motifs that mediate attachment to the nuclear matrix and is thought to participate in chromatin organisation and regulation of gene expression (Oesterreich et al., 1997; Renz and Fackelmayer, 1996). SAF-B also interacts with SR proteins that regulate mRNA splicing (Li et al., 2003; Nayler et al., 1998). The SAF-B-ZO-2 interaction occurs in the nucleus but it is not known whether or how ZO-2 binding affects SAF-B function. It is also possible that SAF-B functions as a nuclear anchor and, hence, regulates trafficking of ZO-2 complexes.

huASH1: a link to epigenetic regulation of gene expression

huASH1 belongs to the trithorax group of transcription factors and has been found to localise to tight junctions by immunofluorescence (Nakamura et al., 2000). It is the human orthologue of *Drosophila* ASH1 (absent, small or homeotic discs 1), a protein that helps maintain segment-specific homeotic gene expression. Because *Drosophila* ASH1 functions as a histone methyl-transferase, a catalytic activity thought to be important for its epigenetic function, huASH1 might be involved in regulating chromatin structure. However, it is not known how huASH1 becomes recruited to intercellular junctions or what the functional relevance of this process is.

Regulation of epithelial differentiation and morphogenesis

Although mechanisms by which tight junctions can impact on nuclear components and processes have been identified, we know little about the importance of these processes for epithelial morphogenesis and differentiation. In the case of ZO-1, various lines of evidence suggest that ZO-1 plays important roles in epithelial morphogenesis and differentiation, and, in

vertebrates, this seems to involve its role in the regulation of the transcription factor ZONAB/DbpA.

In *Drosophila*, the ZO-1 orthologue Tamou/Polychaetoid associates with adherens junctions and participates in the regulation of dorsal closure, epithelial migration, and cell fate determination in sensory organs (Chen et al., 1996; Takahisa et al., 1996; Wei and Ellis, 2001). Mutations in Tamou/Polychaetoid reduce the expression of extramacrochaetae, a repressor that inhibits proneuronal development. This results in formation of extra mechanosensory organs. More recently, ZO-1 has also been shown to regulate cell specification and rearrangement during tracheal morphogenesis (Jung et al., 2006). However, it is not known whether these different functions of ZO-1 are related and how ZO-1 regulates gene expression in *Drosophila*. A ZONAB/DbpA orthologue has not been identified in invertebrates.

In vertebrate epithelial cell lines, ZO-1 is not required for polarisation and junction formation in standard two-dimensional cultures, but a reduction or absence of ZO-1 expression slows down junction assembly in various epithelial cell types and increases the cell density in MDCK and MCF-10A cells (Aijaz et al., 2006; McNeil et al., 2006; Sourisseau et al., 2006; Umeda et al., 2004). By contrast, when cells are cultured in a three-dimensional matrix, ZO-1 is required for normal epithelial morphogenesis and polarisation (Sourisseau et al., 2006). Reduced ZO-1 expression results in activation of ZONAB/DbpA, and overexpression of the transcription factor is sufficient to induce a phenotype similar to that caused by ZO-1 depletion. Hence, regulation of ZONAB/DbpA is a pathway by which ZO-1 can control epithelial morphogenesis.

The reason for the difference in the functional consequences of ZO-1 depletion observed in two- and three-dimensional culture systems is not clear. However, such differences between culture systems have now been reported in RNAi experiments focusing on other junction-associated signalling proteins, such as the evolutionarily conserved polarity regulators (e.g. CRB3/Pals1/PATJ) (Lemmers et al., 2002; Lemmers et al., 2004; Shin et al., 2005; Straight et al., 2004). In a two-dimensional system, apical-basal orientation is not flexible and determined by the tissue culture surface; in three-dimensional cultures, cells lack such a rigid external cue and need to define their orientation in a relatively isotropic environment (Zegers et al., 2003). The absence of strong polarity cues makes three-dimensional culture systems more sensitive and physiologically relevant experimental models.

Tumorigenesis

The types of nuclear processes affected by tight junctions and the effects of ZO-1 depletion on epithelial morphogenesis suggest that the processes described here might also play a role in epithelial transformation and tumorigenesis. Tight junctions generally function as suppressors of pathways that stimulate proliferation (Matter et al., 2005), and changes in the activity of the ZO-2-interacting transcription factor AP-1 have been associated with many cancers. Hence, it is likely that the junction-associated signalling pathways discussed here are deregulated in cancer cells.

Several reports support the possibility that changes in the expression and/or activity of junctional scaffolding proteins occur during tumorigenesis. ZO-1 as well as ZO-2 expression levels are deregulated in different types of cancer, and low ZO-1 expression in breast cancer tissues has been correlated with a poor prognosis (Chlenski et al., 2000; Chlenski et al., 1999; Hoover et al., 1998; Kleeff et al., 2001; Martin et al., 2004; Morita et al., 2004; Resnick et al., 2005; Takai et al., 2005). Moreover, ZO-2 and other junctional scaffolding proteins are bound to and inactivated by viral oncogenes (Glaunsinger et al., 2001; Latorre et al., 2005). The ZO-1–ZONAB/DbpA pathway can be deregulated at different steps in different cancers, and ZONAB/DbpA and Apg-2 are both upregulated in hepatocellular carcinomas. Because Apg-2 activates ZONAB/DbpA signalling, both of these observations suggest stimulation of this proliferation-promoting pathway during tumorigenesis (Arakawa et al., 2004; Gotoh et al., 2004; Hayashi et al., 2002).

Conclusions and Perspectives

NACos that possess nuclear functions but associate with adhesion complexes have now been linked to the regulation of many steps of gene expression, ranging from transcriptional regulation and chromatin structure to mRNA processing, and might also impact on mRNA stability and translation. In several cases, however, the functional relevance of the junctional association is still unclear. Similarly, the role of these pathways in tissue and organ development and homeostasis will need to be investigated.

Tight junctions contain a range of other transmembrane and submembrane proteins, and many of them interact with proteins discussed above. ZO-1, for example, forms complexes not only with ZO-2, ZO-3, ARVCF, Apg-2 and ZONAB/DbpA but also with various junctional membrane proteins (e.g. JAM-A, occludin and claudins) as well as cingulin and other cytoskeleton-associated proteins. These might therefore indirectly affect signalling functions mediated by ZO-1. GEF-H1, occludin and cingulin have indeed been associated with the regulation of signal transduction pathways as well as proliferation and gene expression (Aijaz et al., 2005; Guillemot and Citi, 2006; Guillemot et al., 2004; Yu et al., 2005). However, the mechanisms involved and how they affect signalling by tight-junction-associated NACos remain to be determined. Similarly, several proteins in tight junctions function as receptors for viruses and other pathogens (Sousa et al., 2005). It will thus be important to determine how engagement of these proteins with pathogenic ligands alters the junctional signalling mechanisms.

Because many of the nucleo-junctional interactions linked to tight junctions and other adhesive structures have only recently been discovered, we know little about whether they cooperate with each other and other signalling cascades. Tight junctions have been linked to various signal transduction mechanisms, including suppression of Raf-1 signalling, modulation of TGFβ-induced EMT, modulation of signalling by phosphatidylinositols and RhoA, as well as the evolutionarily conserved signalling mechanisms linked to Par3/Par6 and Crb3 (for reviews, see Bose and Wrana, 2006; Macara, 2004; Matter et al., 2005; Shin et al., 2006). The contribution of tight-junction-associated NACos to the processes regulated by these pathways remains to be determined, however.

Many observations suggest that tight-junction-associated signalling pathways are deregulated in cancer cells. However,

whether these changes are a cause or consequence of transformation is not known. Perhaps more importantly, we need to determine whether these pathways can be targeted to develop new cancer therapies.

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