

Review Article

The Dual Role of Zonula Occludens (ZO) Proteins

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ZO (zonula occludens) proteins are scaffolding proteins providing the structural basis for the assembly of multiprotein complexes at the cytoplasmic surface of intercellular junctions. In addition, they provide a link between the integral membrane proteins and the filamentous cytoskeleton. ZO proteins belong to the large family of membrane-associated guanylate kinase (MAGUK)-like proteins comprising a number of subfamilies based on domain content and sequence similarity. Besides their structural function at cell-cell contacts, ZO proteins appear to participate in the regulation of cell growth and proliferation. Detailed molecular studies have shown that ZO proteins exhibit conserved functional nuclear localization and nuclear export motifs within their amino acid sequence. Further, ZO proteins interact with dual residency proteins localizing to the plasma membrane and the nucleus. Although the nuclear targeting of ZO proteins has well been described, many questions concerning the biological significance of this process have remained open. This review focuses on the dual role of ZO proteins, being indispensable structural components at the junctional site and functioning in signal transduction pathways related to gene expression and cell behavior.

1. Introduction

Epithelial and endothelial cells attach to each other by various occluding junctions which rely on the function of specific proteins characterized by common structural features, that is, one or more transmembrane domains flanked by cytoplasmic and extracellular portions. Accumulating experimental evidence has suggested that protein-protein interactions at the cytoplasmic region(s) of such transmembrane junctional proteins modulate the extracellular action of the protein which accomplishes homophilic or heterophilic binding to extracellular domains of junctional proteins of neighbouring cells. In this way, a regulatable intercellular seal is created.

Zonula occludens (ZO) proteins, comprising ZO-1, -2, and -3, are peripheral proteins localizing at junctional sites. ZO proteins are scaffolding proteins recruiting various types of proteins to the cytoplasmic surface of the junction, thereby contributing to the so called “junctional plaque”. ZO proteins have originally been described to localize specifically to tight junctions (TJs) (*zonulae occludentes*) [1, 2]. However, this notion was quickly reevaluated, since these proteins were found to associate with the cadherin-based adherens

junctions (AJs) in cells lacking TJs [3]. Moreover, ZO proteins also associate with gap junctions (GJs) by directly interacting with connexins [4–6], which points towards a general role of ZO proteins in intercellular adhesion and communication.

ZO proteins carry some domains required for structural organization of intercellular junctions and additional domains capable of functioning in signal transduction pathways. The most prominent function of ZO proteins at the junctional site is the regulation of claudin polymerization in epithelial cells, which was demonstrated by use of a reverse genetic approach [7].

In recent years, intriguing evidence has accumulated suggesting that ZO proteins not only exert functions related to structural barrier mechanisms but are also involved in signal transduction and transcriptional modulation.

ZO proteins interact directly with most of the transmembrane proteins localizing at TJs, such as occludin, claudins, JAM (Junctional adhesion molecule), tricellulin, and CAR (coxsackievirus and adenovirus receptor) [8–14] (Figure 1).

Their association with AJs is accomplished through binding to alpha-catenin [14, 15] and members of the p120 catenin family [14–17] as well as to the nectin-interacting

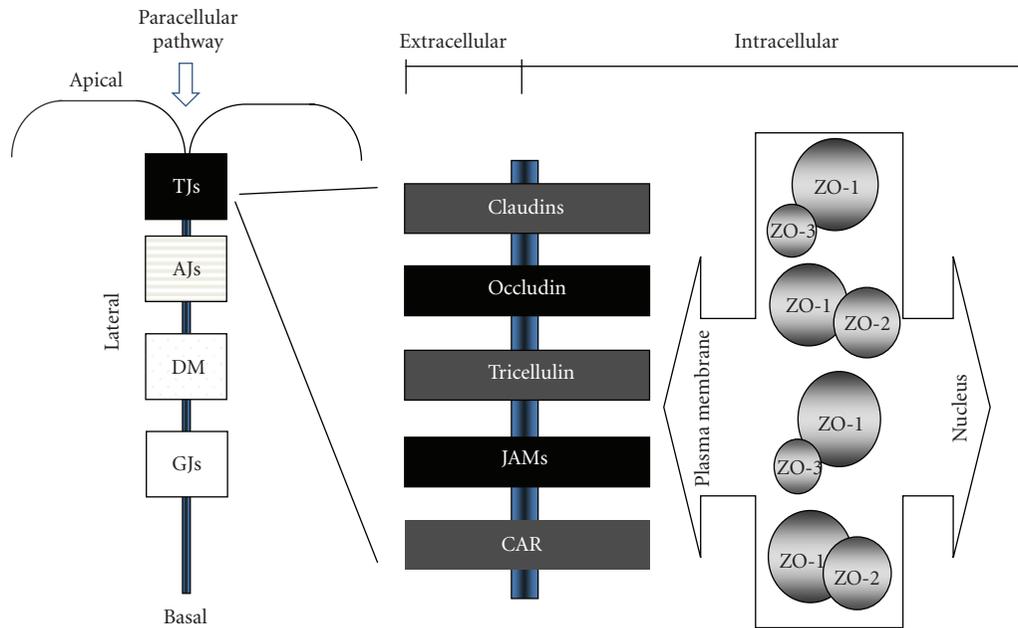


FIGURE 1: Localization of zonula occludens (ZO) proteins at tight junctions (TJs). Transmembrane components of TJs interact at least with one ZO protein. As mentioned in the text, only ZO-1 and ZO-2 localize to the nucleus, while nuclear targeting of ZO-3 has not been observed so far. Already described interactions of ZO proteins with other types of junctions is not included here. TJs: tight junctions; AJs: adherens junctions; DM: desmosomes; GJs: gap junctions; CAR: coxsackievirus and adenovirus receptor.

protein AF-6 (ALL-1 fusion partner at chromosome 6)/afadin [18]. This somehow reflects the canonical role of ZO proteins, which is to establish a link between the transmembrane components and the perijunctional cytoskeleton at cell-cell contacts [19–21]. In addition, ZO proteins interact with a series of cytoplasmic proteins, including adapters, signaling molecules and transcriptional regulators [22–27] suggesting a novel function of ZO proteins, far beyond their role as indispensable structural components at the junction site.

2. ZO Proteins Are MAGUK Proteins

ZO proteins belong to the large family of membrane-associated guanylate kinase (MAGUK) like proteins. The first MAGUK protein identified was the product of the *Drosophila* tumor suppressor gene lethal(1)discs-large (dlg). Loss-of-function mutations in *dlg* lead to the tumorous overgrowth of imaginal discs of *Drosophila* larvae [28, 29].

MAGUKs are scaffolding proteins which create and maintain multimolecular complexes at distinct subcellular sites, such as the cytoplasmic surface of the plasma membrane [30, 31]. By means of multiple protein binding domains, they bring together cell adhesion molecules, cytoskeletal proteins, receptors, ion channels and additional signaling components. Thereby, protein-protein interaction is accomplished by small modular domains, such as Src-homology3 (SH3) domains, phosphotyrosine-binding domains (PTB), or PDZ domains [30]. In spite of their similar domain structure, MAGUKs are distinctive enough to

be classified into seven subfamilies based on domain content and sequence similarity [32].

Junctional MAGUKs belong to the *dlg*-like and ZO-1-like subgroups of MAGUKs. They are characterized by one or more copies of PDZ (PSD-95/discs large/zonula occludens-1) domains, an SH3 (Src Homology 3) domain, and a region homologous to mammalian and yeast cytosolic guanylate kinase (GUK) (from N- to C-terminus). Similar to other MAGUKs, a number of highly variable regions are located between the conserved domains. The best studied variable region is the HOOK domain, a basic hinge region between the SH3 and GUK domain, involved in regulating ligand binding and oligomerization of MAGUKs [33, 34]

2.1. PDZ Domain. Although originally identified in metazoans, PDZ domains have been found to be spread through bacteria, fungi and plant lineages as well [35, 36]. PDZ domains (80–100 amino acid residues) play a key role in cellular signaling. They either form dimers or bind to C-terminal regions of integral membrane and intracellular proteins. PDZ is an acronym, combining the first letters of three proteins which were first discovered to share the domain: The post synaptic density protein (PSD95/SAP90), the *Drosophila* septate junction protein Discs-large (DlgA), and the epithelial tight junction protein zonula occludens-1 (ZO-1). PDZ domains are sometimes referred to as DHR (Dlg homologous region) or GLGF (glycine-leucine-glycine-phenylalanine) domains [36].

PDZ containing proteins are generally restricted to specific subcellular domains, such as regions of cell-cell contact in epithelial cells, the plasma membrane of red blood cells and lymphocytes, and synaptic and neuromuscular junctions [37]. The majority of PDZ containing proteins is associated with the plasma membrane enabling the creation of higher molecular structures [38]. Such structures are mainly involved in intracellular signaling, cell adhesion, ion transport, and the formation of the paracellular barriers.

2.2. SH3 Domain. The Src homology 3 domain is a small protein domain of about 60 amino acid residues present in a large number of intracellular or membrane-associated proteins [39]. It was first identified as a conserved sequence in the viral adaptor protein c-Crk and the non-catalytic parts of enzymes such as phospholipase and several cytoplasmic tyrosine kinases [39]. SH3 domains are found in proteins that are involved in signaling pathways regulating the cytoskeleton, the Ras protein, and the Src kinase and many others. By binding to proline-rich ligands, these domains play critical roles in a wide variety of biological processes ranging from regulation of enzymes by intramolecular interactions, altering the subcellular localization of components of signaling pathways, and mediating the assembly of large multi-protein complexes [40].

2.3. GUK Domain. GUK (guanylate kinase) domains exhibit sequence similarity to guanylate kinase, which converts GMP to GDP using ATPase as a phosphate donor. Some members of the MAGUK family, such as p55-like and Lin-2-like MAGUKs, have intact GMP binding and ATP-binding sites, while ZO-1-like MAGUKs lack several residues predicted to bind GMP [34, 41]. Interestingly, using phylogenetic analyses and molecular modelling it was demonstrated that the MAGUK GUK domain originated from a catalytically active GK domain and gradually lost its enzymatic characteristics when new subfamilies emerged [32]. Several studies suggest that the GUK domain of MAGUK proteins has evolved as a protein-protein interaction domain. A number of vertebrate GUK domain binding partners, including the microtubule-associated protein MAP1A [42], the brain-enriched guanylate kinase-associated protein (BEGAIN) [43], and the kinesin-like protein GAKIN [44] have been identified so far. In addition, the MAGUK GUK domain was suggested to interact intramolecularly with the SH3 domain [41, 45].

3. Structural and Functional Properties of ZO Proteins

The molecular structure and functions of ZO proteins have extensively been described in a series of comprehensive reviews that appeared during the last couple of years [19, 22, 24, 27, 31, 46–48]. Therefore, only a short outline of major characteristics of ZO proteins is given here.

ZO proteins carry three PDZ domains, one SH3 domain, a GUK domain and a proline-rich region located either at the C-terminus (ZO-1, ZO-2) or between the second and third

PDZ domain (ZO-3) [31]. The variable domains, termed U(unique)1 to U6 are located between the core domains of the ZO proteins (U5 is also referred to as “HOOK” domain) [49]. The SH3-U5-GUK-U6 region of ZO-1 turned out to be of particular importance for TJ assembly and localization [49]. The U5 motif, which is found in several MAGUKs, though without sequence homology, is required for localization of ZO-1 to TJs in vivo and for binding of ZO-1 to occludin in vitro. In contrast, the U6 motif, a short sequence flanking the GUK domain, is unique to ZO proteins and was found to inhibit binding of ZO-1 to occludin in vitro [49]. Expression of a modified ZO-1, lacking U6, induces ectopic strands consisting of occludin and claudins but lacking most of the cytoplasmic plaque proteins [49]. As in other MAGUKs, the SH3 and GUK domain of ZO-1 form a hairpin loop through intramolecular interaction which could determine the position of the U5 and U6 motifs and thereby modulate the binding capacity of ZO-1 to occludin and to other proteins [49].

The first ZO protein identified was ZO-1, with a molecular mass of 220 kD [2], which was discovered as an antigen for a monoclonal antibody raised against a junction-enriched fraction from liver tissue. ZO-1 associates with ZO-2, a 160 kD protein [50, 51] and the 130 kD protein ZO-3 [52] through binding of their corresponding PDZ-2 domains [53].

Interaction of ZO-1 with the C-termini of claudins was found to be accomplished through PDZ-1 [10], while JAM (Junction adhesion molecule) and occludin are contacted by PDZ 2/3 [9] and a region localized at the SH3-hinge-GUK domain, respectively [13, 49] (Figure 2). As mentioned above, the unique motif located in this variable region (termed U5) seems to be indispensable for binding of ZO-1 to occludin [49]. Interaction with claudins via their first PDZ domain is a redundant function of all ZO proteins and thus appears to be crucial for the formation and function of TJs. Indeed, Umeda et al. [7] have demonstrated that ZO-1 and ZO-2 are indispensable for determining when and where claudins are polymerized.

ZO proteins associate with a series of peripheral junctional proteins thereby creating a complex intracellular network (Figure 2). Peripheral junctional proteins include actin- and myosin binding proteins, signalling molecules and transcriptional regulators. In addition, all ZO proteins interact directly with actin filaments either via their COOH terminal regions (ZO-1, ZO-2) or through a binding domain located in the N-terminal half (ZO-3) which emphasizes their role as cross linkers between TJ strands and the cytoskeleton [54–56]. The importance of the perijunctional actomyosin in ZO protein-mediated barrier formation was reported recently by Van Itallie et al. [21].

The indirect interaction of ZO proteins with the cytoskeleton involves several actin-binding proteins including cortactin [57], alpha-catenin [11], protein 4.1R [58], the Ras target AF6/afadin as well as the actin- and myosin-binding proteins cingulin [59] and Shroom [60].

Signalling proteins associating with ZO-proteins include the serin protein kinase ZAK (ZO-1 associated kinase-1) which binds to the SH3 domain of ZO-1 and phosphorylates

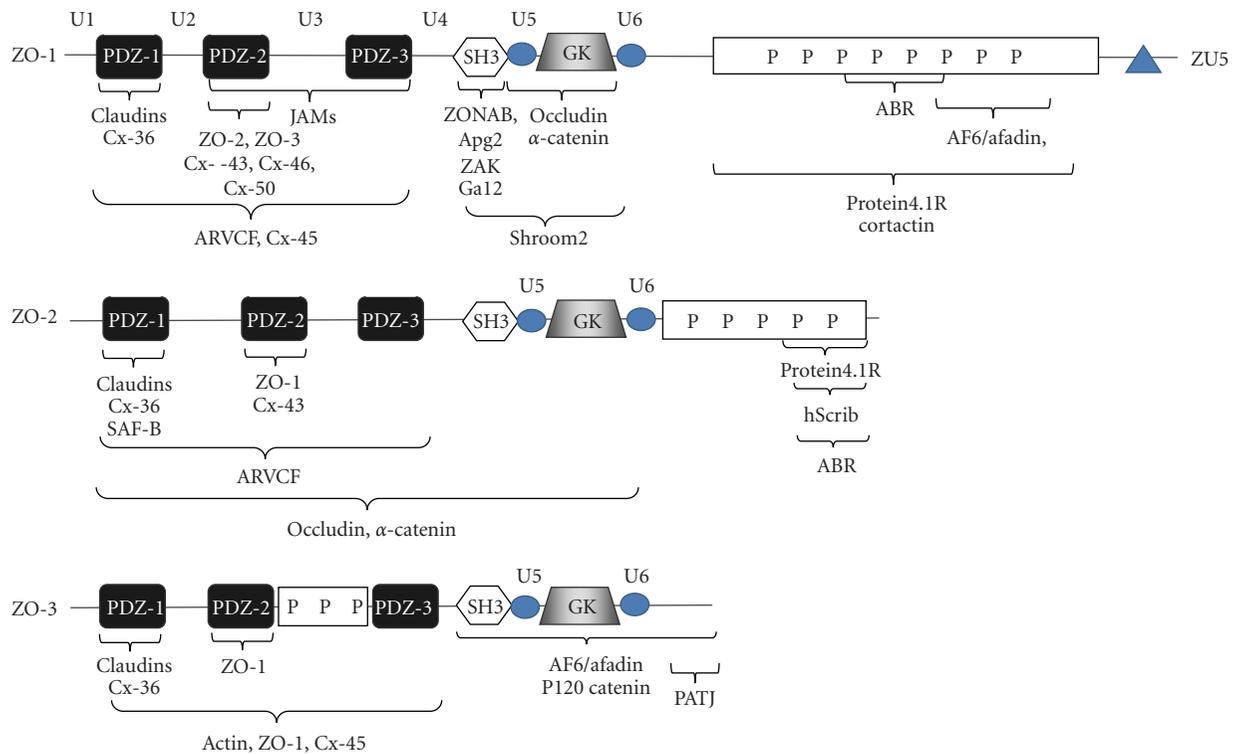


FIGURE 2: ZO proteins interact with transmembrane proteins and with peripheral cytoplasmic proteins at the junctional site. Details are mentioned in the text. Some proteins interacting with ZO proteins are not included in the figure since their association domain is less well described. These include CAR (coxsackievirus and adenovirus receptor), tricellulin, and cingulin. ABR: actin binding region; AF-6: ALL-1 fusion partner at chromosome 6; Apg-2: (ATP and peptide-binding protein in germ cells)-2; Cx: Connexin; Ga12: G protein α 12 subunit; GUK: Guanylate kinase-like domain; JAM: Junction adhesion molecule; hScrib: human Scribble; PATJ: Protein associated with tight junctions; P-P-P: Proline-rich region; PDZ: Pds95/discs large/zonula occludens-1 domain; SAF-B: Scaffold attachment factor-B; SH3: Src homology3 domain; U1-U6: Unique variable domains; ZAK: ZO-1 associated kinase; ZONAB: ZO-1 associated nucleic acid binding protein; ZU5: Domain present in ZO-1 and Unc5-like netrin receptors.

a region immediately C-terminal to this domain [61] and a G protein α subunit ($G\alpha_{12}$) which also associates directly with the SH3 domain of ZO-1 [62]. Interaction of p120 catenin with a C-terminal domain of ZO-3 provides an additional link to Rho signalling [17, 63]. Signalling at TJs has comprehensively been reviewed recently [64] and is not being dealt with further in this review.

Interaction of ZO-3 with the polarity protein PATJ (protein associated with tight junctions) was suggested to be important for the recruitment of PATJ and its associated proteins to tight junctions [65]. Finally, ZO-2 interacts directly with human Scribble (the human homologue of the *Drosophila* tumor suppressor Scribble) which was shown to be a substrate of high-risk human papillomavirus E6 oncoproteins for ubiquitin-mediated degradation [66].

The idea of a “dual function” of ZO proteins has emerged from observations that ZO proteins interact with proteins involved in cell cycle progression and transcriptional regulation [67]. The nuclear targeting of ZO proteins and their association with regulatory proteins participating in gene expression will be discussed in a following chapter.

4. Loss of Function Mutations of ZO Proteins

In order to gain more insight into the functional significance of ZO proteins in embryonic development and tissue differentiation, a series of loss-of-function mutations were analysed. Eph4 cells lacking ZO-1 show a retarded recruitment of claudins and occludin to TJs, and delayed barrier establishment [68]. Similar results were obtained by McNeil et al. [69] showing that knockdown of ZO-1 in MDCK cells retarded TJ formation by 3 hours. Interestingly, mature junctions seemed to be unaffected even in the persisting absence of ZO-1. Epithelial cells deficient in both ZO-1 and ZO-2 were well polarized but did not form TJs due to the lack of claudin polymerization [7].

Knockout of ZO-1 was shown to be lethal for mouse embryos around mid gestation [70]. No viable embryos lacking ZO-1 were found beyond E11.5. Disturbed yolk sac angiogenesis and delayed embryonic growth from E8.5 onwards were the most characteristic features of ZO-1 $^{-/-}$ mice. In addition, massive apoptosis in the notochord, in the neural tube area, and in the allantois at E9.5 was described. Interestingly, deficiency of ZO-1 did not exert any

effects on the localization of ZO-2/ZO-3 at junctional sites but did induce mislocalization of endothelial JAMs in the yolk sac, which might explain the disturbance of vascular development.

Results from ZO-2 targeting experiments are somehow controversial. Knockdown of ZO-2 using siRNA had no discernible effects on TJ structure or function in Eph48 cells, while in MDCK cells, the downregulation of ZO-2 yielded a distinct TJ-related phenotype [71]. This was reflected by changes in the gate function of TJs (i.e., increased paracellular permeability and low transepithelial electrical resistance) as well as alterations in the fence function of TJs as evidenced by a non-polarized distribution of E-cadherin. In addition, delayed arrival of ZO-1, occludin and E-cadherin at newly formed junctions following Ca^{++} -switch was observed.

Mouse embryos lacking ZO-2 die shortly after implantation due to an arrest in early gastrulation and disturbed mesodermal differentiation. In addition, decreased proliferation at embryonic day 6.5 (E6.5) and increased apoptosis at E7.5 together with elevated paracellular permeability of a low-molecular-weight tracer was observed in ZO-2^{-/-} embryos [72]. A critical role of ZO-2 for the blood-testis barrier was demonstrated recently by Xu et al. showing that a lack of ZO-2 caused a disturbed blood-testis barrier and lead to reduced fertility of chimeric mice [73].

Neither ZO-3 deficient cells nor mouse embryos lacking ZO-3 showed an apparent phenotype, suggesting that ZO-3 is not an indispensable component of TJs and might well be substituted by one of the other ZO proteins [72, 74].

5. Nuclear Shuttling of ZO Proteins

Besides their characteristic protein domains ZO proteins exhibit several nuclear localization (NLS) and nuclear export signals (NES) enabling them to shuttle between the cytoplasm and the nucleus [75–78].

A first indication that a member of the ZO proteins localizes to the nucleus came from Gottardi et al., [79] showing that ZO-1 targets for the nucleus of subconfluent epithelial cells before maturation of TJs. Gonzalez-Mariscal and her group demonstrated that the nuclear localization of ZO-2 is particularly dependent on the state of cell cell contacts in epithelial monolayers [76]. Particularly in sparse cultures, ZO-2 was found to localize at the nucleus but gradually exits the nucleus in a leptomycin-sensitive way as soon as the monolayer reaches confluence. Transient nuclear expression of ZO-2 in cerebral endothelial cells and kidney epithelial cells was also observed by Traweger et al. [78]. ZO-2 was found to colocalize partially with the pre-mRNA splicing factor SC-35 and to coprecipitate with laminB1 and actin from nuclei of sparse cultures [76].

Following heat shock and chemical insult increased nuclear accumulation of ZO-2 was visible in epithelial and endothelial cells [78]. Further, nuclear localization could be induced by impairing cell-cell contacts by mechanical injury (wounding) [76]. This suggests that nuclear accumulation of ZO-2 is a general response of epithelial and endothelial cells to environmental or mechanical stress.

6. Nuclear Actions of ZO Proteins

Although the dual localization (cytoplasmic/nuclear) of ZO proteins is well documented, the biological significance of their nuclear targeting has long remained elusive. ZO proteins interact with nuclear proteins as well as with dual residency (cytoplasmic/nuclear) proteins (Figures 3(a) and 3(b)) suggested to be involved in cell growth and proliferation.

By using a yeast-based Two Hybrid screen and co-immunoprecipitation, it was shown that nuclear ZO-2 interacts directly with the hnRNP protein SAF-B (scaffold attachment factor-B) via its PDZ-1 domain [78]. SAF-B/HET (Hsp27-ERE-TATA-binding protein) is a component of chromatin and is expressed in all tissues investigated so far. Originally, SAF-B/HET has been described as one of the abundant nuclear proteins that function in chromatin structure by interacting with scaffold or matrix attachment DNA elements (S/MAR elements). In many cases, these elements co-map with boundaries of actively transcribed domains and have therefore been considered to exert regulatory effects on adjacent genes [80]. SAF-B/HET was shown to be highly concentrated in nuclear speckles colocalizing with SC-35 and to directly interact with various splicing factors and RNA polymerase II [81]. In addition, SAF-B/HET acts as a co-repressor of estrogen receptor alpha and SAF-B/HET levels were found to be inversely correlated with cell proliferation of breast cancer cells [82]. SAF-B/HET is a suppressor of the small heat shock protein 27 (hsp27) which enhances growth and proliferation of breast cancer cells and turned out to be a bad prognostic marker in certain subsets of breast cancer patients in breast carcinoma cells [83].

Further evidence to suggest direct involvement of ZO-2 in cell growth and proliferation came from Huerta et al. [84] demonstrating that ZO-2 modulates the expression of cyclin D1 (CD1) through interaction with the transcription factor Myc and subsequent binding to an enhancer box element of the CD1 promoter. In this way, ZO-2 downregulates transcription of CD1 and, in due course, suppresses proliferation of cultured epithelial cells. In addition, ZO-2 downregulates protein synthesis of CD-1 and increases its degradation at the proteasome complex [85].

Convincing experimental evidence to suggest the critical involvement of ZO proteins in epithelial cell proliferation came from Balda et al. [86, 87] and Sourisseau et al. [88] showing that ZO-1 interacts with the Y-box transcription factor ZONAB (ZO-1 associated nucleic acid binding protein), also referred to as DbpA/Msy3 (DNA binding protein/Mouse Y box protein3), which in turn binds to promoter regions of CD1 and PCNA (proliferating cell nuclear antigen), an eukaryotic DNA replication factor, thereby increasing their transcription. ZONAB is a dual residency protein localizing at TJs and/or in the nucleus, depending on the cells' proliferative state and on the amount of ZO-1 present at cell-cell contact sites. In confluent epithelial monolayers, ZONAB is bound to junctional ZO-1. If ZO-1 is absent from junctional sites, ZONAB shuttles to the nucleus while overexpression of junctional ZO-1 causes

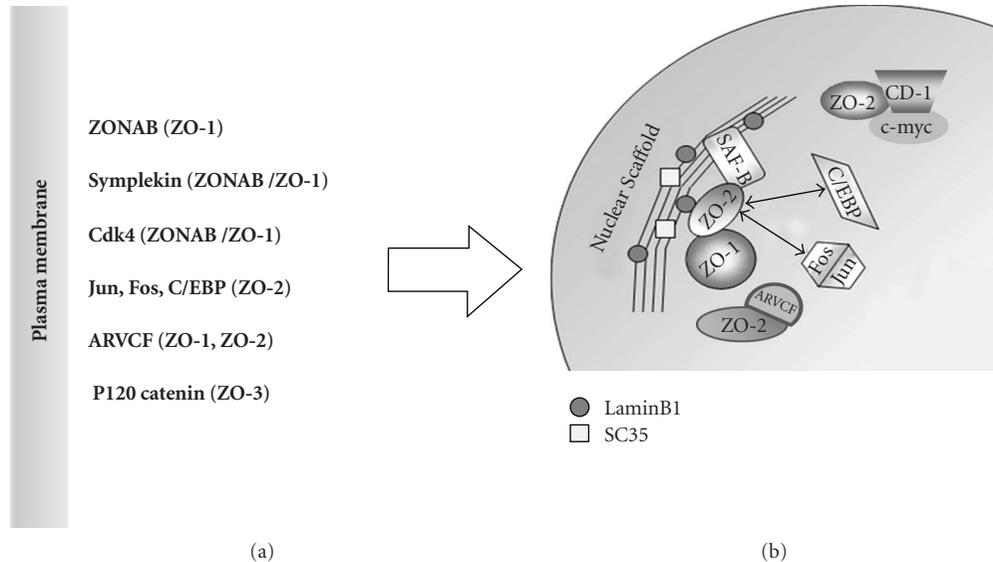


FIGURE 3: (a) Summary of “dual residency proteins” (locating at the plasma membrane and at the nucleus) associating with ZO-proteins at the junctional site. (b) Nuclear interactions of ZO proteins. ZONAB: ZO-1 associated nucleic acid binding protein; Cdk-4: Cyclin-dependent kinase-4; ARVCF: Armadillo repeat gene deleted in velo-cardio-facial syndrome; SC35: Splicing factor 35.

a redistribution of ZONAB from the nucleus to the cytoplasm. Another way, how ZONAB regulates epithelial cell proliferation and cell cycle progression is through association with the cyclin-dependent kinase-4 (Cdk4) which interacts and colocalizes with ZONAB at TJs. In this way, Cdk4 is prevented from entering the nucleus and participating in cell cycle progression [86].

The small heat shock protein Apg-2 was shown to regulate ZONAB function by competing for binding to the SH3 domain of ZO-1 [89].

In addition, ZONAB forms a stable complex with symplekin, a ubiquitous protein involved in mRNA processing [90]. Similar to ZONAB and Cdk4, symplekin is a dual residency protein which usually accumulates at cell nuclei but also associates with TJs in polarized epithelial cells [90]. Interaction of ZONAB with symplekin modulates the transcriptional activity of ZONAB as evidenced by alterations in CD1 expression [91].

A direct interaction of ZO-2 with Jun, Fos and C/EBP (CCAAT/enhancer binding protein) was found to occur not only in the nucleus but also at the plasma membrane [92]. Using reporter gene assays, ZO-2 was demonstrated to suppress gene transcription by binding to the AP-1 transcription factor protein complex [92].

Further, ZO-1 and ZO-2 interact with the dual residency protein ARVCF (Armadillo repeat gene deleted in velo-cardio-facial syndrome) [16], a member of the p120 catenin family localizing at cadherin-based cell-cell contacts in confluent epithelial cells. Upon disruption of cell-cell adhesion, ARVCF is partially translocated to the nucleus by associating with a PDZ domain of ZO-2. In addition, a C-terminal region of ZO-3 was shown to interact directly with p120 catenin, which regulates cadherin-based cell adhesion

and junctional stabilization through Rho signaling [63]. p120 catenin was found to undergo nucleo-cytoplasmic shuttling and to regulate gene expression by associating with the methylation-dependent transcriptional repressor Kaiso [93].

Experimentally induced nuclear accumulation of ZO-2 in cerebral endothelial cells led to the elevation of pyruvate kinase M2 (M2PK) protein levels [94]. Although experimental evidence is still missing, it cannot be excluded that this effect was elicited by the excessive targeting of nuclear ZO-2 to the estrogen receptor-binding domain of SAF-B. M2PK is an isozyme of pyruvate kinase particularly expressed in proliferating cells, such as embryonic stem cells and carcinoma cells. A recent report has demonstrated that M2PK interacts and cooperates with the POU domain transcription factor Oct-4 which is critically involved in maintaining the self-renewal capacity of embryonic stem cells [95]. Although there is a general consensus concerning the suppressive action of ZO-2 on cell proliferation, it appears that the ratio between the amount of ZO-2 localizing at TJs and the amount of nuclear ZO-2 adds an additional variable in the control of epithelial cell proliferation and differentiation.

While the nuclear targeting of ZO-1 has well been described, its nuclear functions have remained unclear. Based on all experimental evidence so far, it may be concluded that the nuclear localization of ZO-1 is inversely related to the extent and/or maturity of cell-cell contacts [79].

7. A Role for ZO Proteins in Tumor Growth

Some proteins of the MAGUK family behave as tumor suppressors. The homology of ZO proteins with the *Drosophila*

tumour suppressor protein *dlgA* provided a first clue towards a yet undefined role of ZO proteins in epithelial cancer development and/or progression. Dlg proteins with mutations in the PDZ and SH3 domains cause neoplastic overgrowth of larval imaginal disc epithelial cells [28]. The *Drosophila* orthologue of ZO-1, Tamou/Polychaetoid, has been implicated in a signaling pathway which activates the expression of the helix-loop-helix repressor gene *emc* (extramacrochaetae). Mutations in Tamou/Polychaetoid reduce the expression of *emc*, which causes enlargement of a proneural cell cluster resulting in extra mechanosensory organs in the fly [96].

ZO proteins are delocalized or down-regulated in several types of carcinomas [97]. Further, ZO-1 mutants encoding only the N terminus including the PDZ domains of ZO-1 appeared delocalized in the cytoplasm of MDCK cells and induced a dramatic loss of the epithelial phenotype of MDCK accompanied by repression of epithelial and induction of mesenchymal marker genes [98]. Similar results were obtained by Wittchen et al showing that expression of exogenous mutated ZO-3 constructs carrying only the amino terminal half of ZO-3 led to a significant delay in the formation of TJs and AJs and to mislocalization of ZO proteins and occludin [99].

Epithelial cancers are often related to mutations in the small GTPase Ras, which mediates cellular signal transduction regulating cell growth and proliferation [100]. The peripheral PDZ domain protein AF-6/afadin localizes at AJ and TJs by directly associating with nectin and JAM [9, 101]. AF-6 was shown to be targeted by Ras and the Ras-like GTPase Rap-1 [102, 103]. In polarized epithelial cells, AF-6/afadin colocalizes with ZO-1 at TJs, and ZO-1 interacts with the Ras-binding domain of AF-6/afadin. Overexpression of activated Ras in Rat1 cells resulted in the perturbation of cell-cell contacts, demonstrating a critical interplay of Ras, AF-6/afadin and ZO-1 at intercellular junctions [104].

Junctional proteins carrying PDZ domains were found to be targeted and degraded by various tumorigenic viruses [105]. Thereby; PDZ binding motifs located in the viral protein are used to interact with PDZ domains of the targeted junctional protein. For example, a conserved 4-amino-acid PDZ binding motif is present at the carboxy termini of high-risk human papillomavirus (HPV) E6 proteins [106]. Similarly, adenovirus (Ad) type 9 causing exclusively estrogen-dependent mammary tumors in experimental animals contains a PDZ domain-binding motif required to induce both cellular transformation in vitro and tumorigenesis in vivo. The PDZ domain of Ad9 mediates interaction with Dlg1, PATJ and ZO-2 which promotes disruption and loss of cell polarity of the infected cells [107].

8. Concluding Remarks

Due to their homology with the tumor suppressor protein *dlgA*, zonula occludens proteins have been considered to play a role in cell growth and proliferation. Since the discovery of the first TJ-associated MAGUK, ZO-1, the importance

of peripheral junctional proteins has become increasingly recognized. It was considered a major breakthrough when it was demonstrated that ZO proteins not only exert a scaffolding function at the junctional site but are also involved in intracellular signaling and gene expression. The nuclear targeting of ZO-1 and ZO-2 has focused much interest on the nuclear function of ZO proteins. To date, convincing experimental evidence suggests that ZO proteins are capable of associating with regulatory molecules (adapter proteins, signaling molecules, growth factors) thereby modulating the cell's progress through the cell cycle. Now, future work must concentrate on several aspects, including (i) the elucidation of extra- and intracellular signals which trigger the nuclear targeting of ZO proteins, (ii) the identification of additional genes the expression of which is up- or downregulated by the nuclear presence of ZO proteins, and (iii) the role of ZO proteins in virus-induced cancer.

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