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Review

Structural organization of the tight junctions

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Abstract

Tight junctions are the most apical organelle of the apical junctional complex and are primarily involved in the regulation of paracellular permeability and membrane polarity. Extensive research in the past two decades has identified not only the individual molecules of the tight junctions but also their mutual interactions, which are the focus of the present review article. While a complete map of the interactions among the tight junction molecules is probably far from being complete, the available evidence already allows outlining the general molecular architecture of the tight junctions. Here, with the aim of gaining deeper mechanistic understanding of tight junction assembly, regulation and function, we have subdivided the known molecular interactions into four major clusters that are centered on cell surface, polarity, cytoskeletal and signaling molecules.

Keywords: Junction; Adhesion; Permeability; Polarity; Cytoskeleton

Contents

Abbreviations: aPKC, atypical protein kinase C; GAP, GTPase-activating protein; GST, glutathione-*S*-transferase; JAM, junctional adhesion molecule; LLGL1, lethal giant larvae-1; MAGI, membrane-associated guanylate kinase with inverted orientation; MAGUK, membrane-associated guanylate kinases; MDCK, Madin–Derby canine kidney; MUPP1, multi-PDZ domain protein-1; PI3K, phosphatidyl-inositol 3-kinase; PICK-1, protein interacting with protein C kinase-1; TGF-β, transforming growth factor-β; TJ, tight junctions; ZO, zonula occludens; ZONAB, ZO-1-associated nucleic acid binding

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1. Introduction

Tight junctions (TJ) are composed of several transmembrane and intracellular molecules. A detailed analysis of the individual molecules can be found in recent reviews [1–7], as well as in the accompanying articles by Chiba et al. [8] and Citi et al. [9]. In this review, we focus on the interactions between the TJ molecules. For each interaction, we will provide information on the methodology used for its discovery, the molecular determinants and the likely functional significance. We have subdivided all the interactions into four clusters that correspond to well-defined features of the TJ. In particular, in Section 2, we examine the interactions of the cell surface molecules, which ensure intercellular adhesion and regulate paracellular permeability. Then, in Section 3, we describe the interactions within the molecular system that determines the establishment of cell polarity and the localized assembly of the TJ. Subsequently, in Section 4, we analyze the interactions mediated by intracellular adapter proteins, which mostly connect the surface molecules with the actin-based cytoskeleton. Finally, in Section 5, we describe a cluster of interactions that link the TJ with signaling and trafficking molecules.

2. Cell surface molecules

The most extensively studied cell surface molecules of the TJ are occludin, as well as the members of the claudin and junctional adhesion molecule (JAM) families. Although these proteins have different structures and exert different functions, they display a similar organization, as they interact both with other cell surface proteins (on the extracellular side) and with many adapters (on the intracellular side), which in turn link the surface molecules to the actin cytoskeleton. The extracellular interactions involve binding to similar molecules either *in cis* (on the membrane of the same cell) or *in trans* (on the membrane of the contacting cells). Although these interactions are important in regulating paracellular permeability, they will not be discussed here, and the reader is referred to specific reviews [4,10], as well as to the accompanying articles by Blasig et al. [11] and Yu et al. [12]. A schematic picture of the interactions between representative membrane and adapter molecules is shown in Fig. 1.

2.1. Interactions of occludin

Occludin is a four-pass integral membrane protein that interacts with the actin cytoskeleton and with several intracellular proteins [13]. The interaction of occludin with the cytoskeleton requires adapters, such as the zonula occludens (ZO) proteins and cingulin, even though occludin also binds actin directly. In particular, occludin associates directly with ZO-1 [14], ZO-2 [15] and ZO-3 [16]. The association with ZO-1 and ZO-2 requires the carboxyl terminal portion of the domain E in the cytoplasmic tail of occludin, which encompasses residues 358-

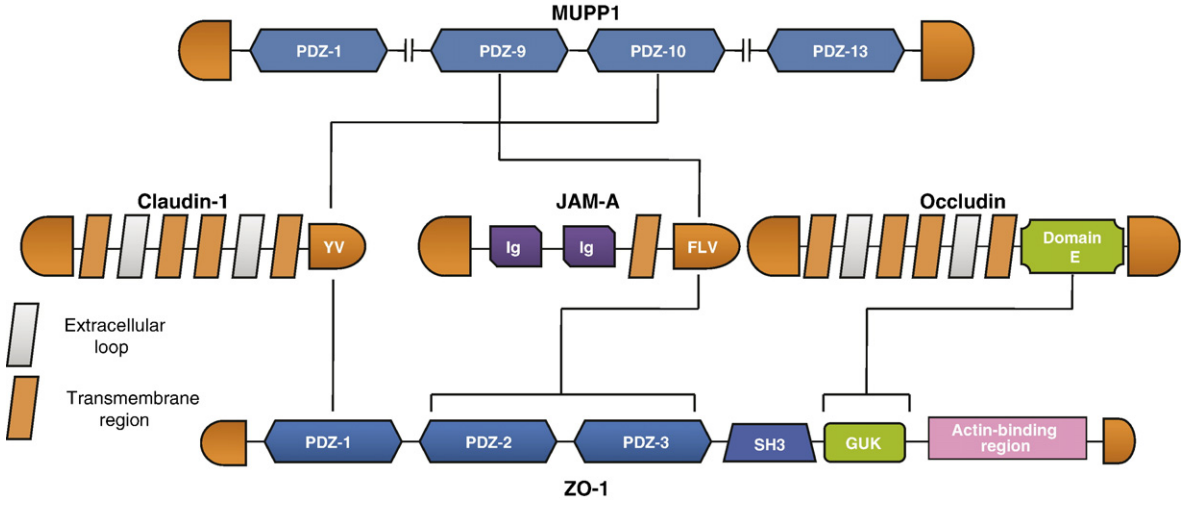

Fig. 1. Interactions between representative membrane and adapter molecules. The picture shows modular structure and molecular interactions of the cell surface proteins claudin-1, JAM-A and occludin, as well as their binding sites for the adapters MUPP1 and ZO-1. GUK, guanylate kinase.

504. As shown by *in vitro* binding assays, a recombinant protein corresponding to the domain E of occludin (fused to glutathione-*S*-transferase; GST) binds directly a recombinant construct that encompasses the guanylate kinase domain and the acidic region of ZO-1 [17]. In addition to the ZO proteins, another adapter that binds occludin is cingulin, which is an actin- and myosin-binding protein, as detailed in Section 4.2. The interaction between occludin and cingulin, which was detected in the *Xenopus* A6 epithelial cells, is direct and requires the carboxyl terminal region of occludin [18]. Occludin, however, also binds actin directly, thus providing an example of a direct link between a junctional cell surface protein and the cytoskeleton. Specifically, occludin interacts with F-actin *in vitro* and colocalizes with actin aggregates at the cell–cell borders in Madin–Derby canine kidney (MDCK) cells. In the co-sedimentation assay, in which polymerized actin filaments are pelleted at high-speed centrifugation, GST-occludin co-sediments with F-actin. In addition, occludin co-sediments with F-actin, when spun through glycerol, thus indicating that the interaction is not due to the mechanical trapping of occludin in the meshwork of actin filaments [19].

In addition to the adapters, occludin associates with enzymes that induce post-translational modifications of occludin (such as phosphorylation, dephosphorylation and ubiquitination) during TJ assembly and disassembly. Specifically, occludin is phosphorylated on either tyrosine or serine/threonine residues [20]. Phosphorylation of occludin on tyrosine involves the non-receptor kinase c-Yes and likely favors TJ maintenance. Actually, the complex between occludin and c-Yes dissociates not only upon treatment with the c-Yes inhibitor CGP77675, but also upon depletion of extracellular calcium, a treatment that mimics *in vitro* the disassembly of the junctions [21]. At variance, phosphorylation of occludin on serine/threonine involves casein kinase-2 [22,23] and atypical protein kinase C (aPKC). In particular, the zeta isoform of aPKC was shown to interact with the coiled-coil domain of occludin in the human intestinal T84 epithelial cells [23,24]. As described in Section 3.1, aPKC is a key component of the core polarity complex, thus suggesting that occludin phosphorylation on serine/threonine favors TJ assembly.

If occludin phosphorylation facilitates TJ assembly, then one can hypothesize the existence of control mechanisms that prevent excessive phosphorylation. Actually, protein phosphatase 2A, a holoenzyme with serine/threonine phosphatase activity, reduces the phosphorylation levels of occludin (as well as ZO-1, claudin-1 and aPKC). The phosphatase has a negative effect on TJ integrity, not only because stimuli that activate the phosphatase increase paracellular permeability (that is, disrupt preformed TJ), but also because expression of its catalytic subunit prevents the assembly of TJ *de novo*. The physiological importance of the interaction between the phosphatase and TJ proteins is reinforced by the observation that, upon calcium-induced junction assembly, the phosphatase is recruited to the cell membrane, where it can interact with its TJ targets [25]. In addition to the protein kinases, occludin interacts with lipid kinases. In particular, in human intestinal Caco-2 epithelial cells, occludin associates with p85, the regulatory subunit of phosphatidyl-inositol 3-kinase (PI3K). As the association of PI3K with occludin increases in response to oxidative stress, it may contribute to the PI3K-mediated disassembly of the TJ that is observed in this condition [26].

Besides protein phosphatase 2A and PI3K, two additional partners of occludin may contribute to TJ disassembly. First, occludin binds (and regulates the junctional localization of) the two subunits of the receptor for transforming growth factor-β (TGF-β), which plays a major role in TJ disassembly, as detailed in Section 3.7 [27]. Second, occludin binds the E3 ubiquitin-ligase Itch. The interaction requires the amino terminus of occludin and a multi-domain region of Itch, which encompasses four WW motifs. Itch ubiquitinates occludin, thus leading to its degradation in the proteasome [28]. Induced expression of Itch is also the likely mechanism whereby dibutyryl-cAMP causes TJ disassembly in Sertoli cells [29].

Finally, some TJ molecules (including occludin) interact with diverse connexins, which are the membrane components of the gap junctions. As the interactions between tight and gap junctions are reviewed by Hervé et al. [30], here we will just mention that occludin binds connexin-32. Using murine CHST8 hepatocytes transfected with human connexin-32, it was reported that connexin-32 and occludin expression are co-regulated [30]. The associations between tight (e.g., occludin, ZO-1 and ZO-3) and gap junction (e.g., connexin-32, -43 and -45) molecules suggest that connexins may assemble gap junction channels in proximity of the TJ, thus coupling cell–cell adhesion and communication.

2.2. Interactions of claudins

In addition to occludin and the recently described tricellulin [31], also claudins were discovered as cell surface components of the TJ [32]. Like occludin, also claudins associate with adapters and enzymes. Concerning the adapters, claudins interact with the ZO proteins. To test whether the interaction is PDZ-dependent, the first, second and third PDZ domains (PDZ-1, -2 and -3, respectively) of ZO-1 and ZO-2 have been recombinantly expressed (fused to a six-histidine tag) and used in binding assays with the cytoplasmic domain of claudin-1 (fused to GST). Only the PDZ-1 domains (of both ZO-1 and ZO-2) associate with claudin-1. Interestingly, deletion of the Tyr-Val sequence from the carboxyl terminus of claudin-1 abolishes the interaction with the PDZ-1 domains. Using the same assay, it has been shown that the PDZ-1 domain of ZO-1 directly binds the cytoplasmic tails of claudin-1 to -8, which all share the Tyr-Val residues in their carboxyl termini [33]. Another adapter that interacts with claudins is multi-PDZ domain protein-1 (MUPP1), which is exclusively concentrated at the epithelial TJ. MUPP1 contains thirteen PDZ domains, and the tenth domain (PDZ-10) is responsible for the interaction with the carboxyl terminus of claudin-1. Finally, claudin-1 interacts with the adapter PATJ, which is a regulator of cell polarity, as described in Section 3.4. The interaction is direct and requires the eighth PDZ domain of PATJ and the carboxyl terminus of claudin-1. Surprisingly, however, deleting the eight PDZ domain has little effect on PATJ localization [34]. As claudins are the building blocks of the TJ strands, all these findings support a model, in which the PDZ-binding residues of the claudins act as docking sites to attract PDZ-containing

proteins, such as ZO-1, ZO-2 (and possibly ZO-3), as well as MUPP1 and PATJ [35]. It should be noted, however, that, according to recent findings, ZO-1 and ZO-2 can determine the site of claudin polymerization [36].

Concerning the enzymes, claudins interact with protein kinases and matrix metalloproteinases. First, WNK4 is a kinase that binds and phosphorylates claudin-1 in MDCK II cells. Interestingly, gain-of-function mutations of WNK4, which enhance the ability of WNK4 to associate with and phosphorylate claudin-1, are linked to a rare form of hereditary hypertension that is characterized by increased chloride permeability [37]. Second, claudin-1 directly interacts with the matrix metalloproteinases MMP2 and MMP14, thus facilitating their recruitment to the junctional area of the cell surface, which in turn increases the MMP14-dependent activation of MMP2 [38].

2.3. Interactions of junctional adhesion molecules

Three members of the JAM family (JAM-A, -B and -C) share significant sequence homology, including conserved PDZ-binding motifs in the carboxyl terminus of their cytoplasmic tails. JAM-A, which is the prototype of the family, interacts with six adapters in a PDZ-dependent manner, as binding critically requires its carboxyl terminal PDZ-binding motif Phe-Leu-Val. First, JAM-A interacts with ZO-1. Full-length JAM-A (but not a deletion mutant lacking the PDZ-binding motif) coprecipitates with ZO-1 in the detergent-insoluble fraction of Caco-2 cells. In addition, either a GST fusion protein containing the cytoplasmic tail of JAM-A or a synthetic peptide corresponding to the carboxyl terminal residues of JAM-A bind *in vitro* transcribed and translated ZO-1 [39,40]. Second, JAM-A interacts with MUPP1, in a way that requires the ninth PDZ domain of MUPP1. Thus, MUPP1 is recruited to the epithelial TJ, by virtue of its (mutually non-exclusive) associations with claudin-1 and JAM-A (via the tenth and ninth PDZ domain, respectively) [35]. Third, JAM-A binds afadin, which is another PDZ domain-containing protein associated with both the tight and the adherens junctions. JAM-A lacking the PDZ-binding motif does not co-distribute with afadin and localizes exclusively at cell borders [40]. Fourth, JAM-A interacts with CASK, which (like the ZO proteins) belongs to the family of the membrane-associated guanylate kinases (MAGUK). However, in addition to the TJ, JAM-A and CASK also colocalize along the lateral surface of the plasma membrane [41]. Fifth, JAM-A binds protein interacting with protein C kinase-1 (PICK-1), a scaffold molecule localized at both tight and adherens junctions in epithelial cells. The interaction requires the PDZ domain of PICK-1 [42]. Finally, JAM-A binds the polarity protein Par-3, as discussed in Section 3.3 [43].

Although these findings suggest that these intracellular interactions of JAM-A are instrumental (albeit to different degrees) in recruiting JAM-A to the junctions, it should be noted that also its extracellular and homophilic interactions might play a similar role [44]. More in general, there are many examples of associations between cell surface proteins. For instance, interactions between claudins lead to the formation of polymers within the TJ strands [45]. As already mentioned, the extracellular interactions between the cell surface molecules may represent the molecular basis for the barrier function of the TJ, which restricts and regulates paracellular permeability [4].

3. The polarity system

TJ are closely associated with cell polarity. On one side, TJ maintain the polarized subdivision of the plasma membrane into the apical and basolateral domains. On the other side, some polarity complexes determine the assembly and maintenance of the TJ in their proper location at the boundary between the apical and basolateral domains. The interplay between TJ and polarity is the focus of recent reviews [2,46], as well as the accompanying articles by Le Bivic et al. and Cereijido et al. Here, we concentrate on the molecular interactions within the polarity cluster and subdivide the cluster into different groups of interactions. First we describe a core complex that is linked to the small GTPase Cdc42. Then, we examine the interactions of the core complex with membrane proteins, regulatory mediators and the actin cytoskeleton. Finally, we make a brief mention to a group of interactions that links the core complex with the

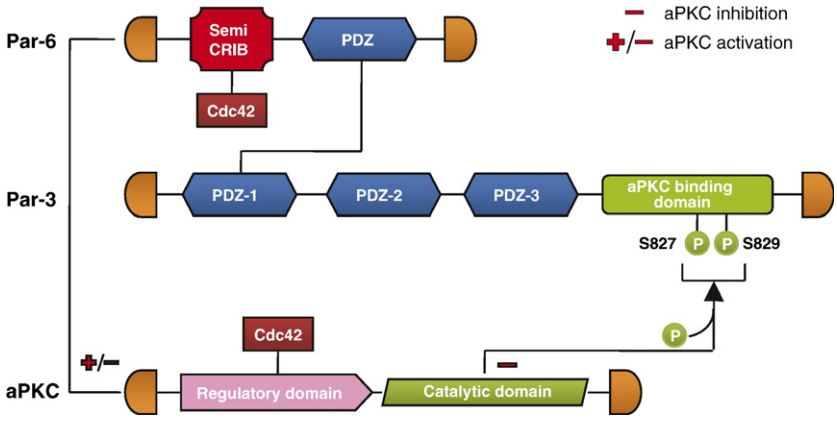

Fig. 2. Interactions between the members of the core polarity complex. The picture shows modular structure and molecular interactions of the core complex proteins Par-6, Par-3 and aPKC. Arrow indicates the serine residues of Par-3 that are phosphorylated upon interaction with aPKC. The plus/minus and minus symbols represent slight increase and decrease of aPKC kinase activity upon interaction with Par-6 and Par-3, respectively. The interaction of Par-6 and aPKC with Cdc42 is also shown.

molecular machinery responsible for the disassembly (rather than the assembly) of the TJ.

3.1. The core complex

Together with aPKC, the two adapters Par-6 and Par-3 form a highly conserved core complex within the polarity cluster. Specifically, the three isoforms of Par-6 (Par-6A, -6B and -6C) interact with Par-3 (which is also called ASIP). As shown in Fig. 2, Par-6 contains a highly conserved region of about 125 residues, which in turn comprises a short sequence of twenty-two residues and a PDZ domain. Part of the short sequence is called semi-CRIB domain, because it matches the amino terminal half of a consensus CRIB motif, an acronym for Cdc42 and Rac interaction binding. At variance, Par-3 contains three amino terminal PDZ domains. The PDZ domain of Par-6 is necessary for binding the amino terminus of Par-3. In particular, Par-6 binds the PDZ-1 domain (but not the PDZ-2 and -3 domains) of Par-3. In addition, recombinant fragments containing only the PDZ domain of Par-6 and the PDZ-1 domain of Par-3 retain the ability to bind each other, thus demonstrating that the interaction between Par-3 and Par-6 is solely PDZ-dependent. Importantly, transfection of MDCK cells with a construct corresponding to the amino terminus of Par-3 (which interacts with Par-6) disrupts cell–cell junctions with adjacent non-transfected cells. In contrast, transfection of the carboxyl terminus of Par-3 (which does not bind Par-6) has no effect, thus suggesting that the interaction between Par-3 and Par-6 is important for the assembly and the maintenance of the TJ [47,48].

Both Par-6 and Par-3 interact with the isoforms zeta and iota/lambda of aPKC. Specifically, Par-6 interacts with aPKC via a direct head-to-head association that involves the amino termini of both molecules. Experiments with GST fusion fragments of Par-6 demonstrate that the amino terminus of Par-6 (between residues 1 and 100) is necessary and sufficient for the interaction with aPKC. However, the mutual effects of the interaction are rather limited. On one side, no phosphorylation of Par-6 is detectable by kinase assay after the interaction with aPKC. On the other side, Par-6 induces just a modest increase in the kinase activity of aPKC [49]. At variance, the interaction of Par-3 with aPKC requires the carboxyl terminal region of Par-3 and the catalytic domain of aPKC. The aPKC-binding region of Par-3 includes two highly conserved residues (Ser827 and Ser829) that are phosphorylated by aPKC. In addition, the interaction of aPKC with Par-3 reduces the kinase activity of aPKC, thus suggesting that Par-3 holds aPKC in an inactive conformation. Importantly, aPKC colocalizes with Par-3 at the TJ of epithelial cells. Also, overexpression of Par-3 (but not a Par-3 deletion mutant lacking the aPKC-binding domain) accelerates TJ formation in MDCK cells, thus suggesting that also the interaction between Par-3 and aPKC favors the assembly of the TJ [50].

3.2. Interactions of the core complex with small G proteins

The core complex is linked to the general polarity system by virtue of its associations with Cdc42 (Fig. 3). In particular, both Par-6 and aPKC interact with Cdc42, while the exchange factor

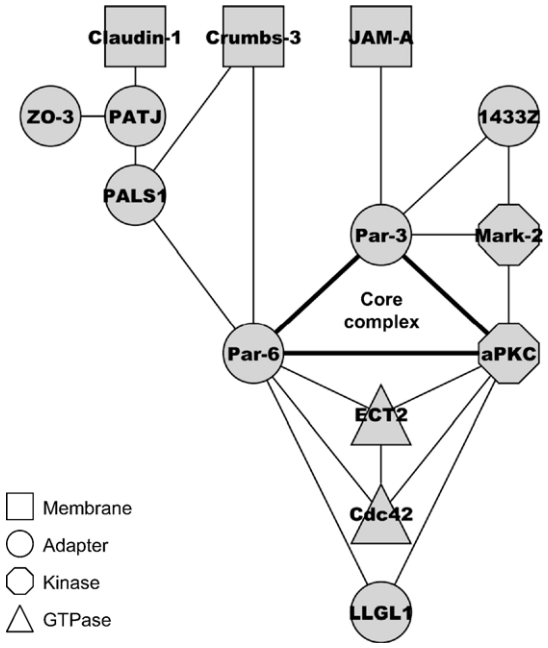

Fig. 3. Interactions within the polarity cluster. Part of the polarity cluster is represented as a network of proteins linked by protein interactions (solid lines). Thick lines highlight the interactions among the members of the core complex, while the shape of the nodes represents the functional category of the individual proteins.

Ect-2 contributes additional linkages between these proteins. Specifically, the three isoforms of Par-6 associate with Cdc42 in its active (i.e., GTP-bound) state, as detected in binding assays [49]. The partial CRIB sequence of Par-6 is required for the interaction with active Cdc42. However, the critical residues that mediate the interaction are different from those found in classical CRIB domains. Actually, mutations of residues that are conserved in the CRIB domains (i.e., Ile133, Ser134, Pro136 and Phe139) do not abrogate the binding of Cdc42 to Par-6B. Thus, the interaction between GTP-bound Cdc42 and Par6 probably involves novel protein–protein recognition motifs [47]. In addition, also aPKC associates with GTP-bound Cdc42, via the regulatory domain of aPKC. Expression of activated Cdc42 results in the translocation of aPKC lambda from the nucleus to the cytosol, so that Cdc42 may then colocalize with aPKC at the plasma membrane. An additional effect of expressing activated Cdc42 is the loss of stress fibers, as does overexpression of either wild-type or activated aPKC [51].

Activation of Cdc42 requires the guanine nucleotide exchange factor Ect-2. However, besides activating Cdc42, Ect-2 also interacts with Par-6 and aPKC with important functional consequences. Actually, when co-expressed with Ect-2, Par-6 enhances the activity of both Ect-2 (with resulting accumulation of GTP-bound Cdc42) and aPKC. Also, overexpression of Par-6 alone stimulates Cdc42 and aPKC activity, albeit to a lesser extent than the co-expression of Par-6 and Ect-2. Thus, Ect-2, Par-6 and aPKC form another tripartite complex, which interacts physically and functionally with the core complex [52]. Finally, it is noteworthy that, in addition to Cdc42, the core complex interacts with other small GTPases. First, the three isoforms of Par-6

interact directly with GTP-bound Rac-1, via their semi-CRIB motif [49]. Second, Par-3 interacts indirectly with RhoA, as discussed in Section 3.6.

3.3. Interactions of the core complex with membrane proteins

During junction assembly, the core complex contributes to restricting the localization of Cdc42-dependent polarity stimuli to a well-defined region of the plasma membrane (i.e., the prospective junctional area) by virtue of associations with integral membrane proteins. These proteins belong to two immunoglobulin-like families of adhesion molecules, i.e., the JAM family (at the TJ) and the nectin family (at the adherens junctions). In particular, as mentioned in Section 2.3, JAM-A associates with Par-3. The interaction requires the carboxyl terminal PDZ-binding residues of JAM-A and the first PDZ domain of Par-3. The PDZ-dependent interaction of Par-3 with JAM-A is specific, as the interaction of Par-3 with occludin is very weak, while no interaction with claudins is detectable [43,53]. Thus, JAM-A may be regarded to as a membrane protein that acts as a docking site for the core complex, which is an essential step for the establishment of polarity and the formation of the TJ.

3.4. The core complex interacts with apical and basolateral polarity complexes

To coordinate membrane polarization in a more efficient manner, the core complex interacts with two conserved complexes that are localized to the apical and basolateral domains. The apical complex comprises homologs of the *Drosophila* Crumbs, Stardust and Inadl. The basolateral complex comprises homologs of the *Drosophila* Lethal Giant larvae, Discs lost and Discs large (Fig. 3).

Crumbs-3 is the human homolog of *Drosophila* Crumbs, which is an apical transmembrane protein crucial for epithelial morphogenesis in the fly embryo. In humans, Crumbs-3 is expressed in epithelial tissues and skeletal muscles. In addition, in intestinal epithelial cells, Crumbs-3 is localized to apical and subapical areas, which suggests that it may regulate polarity in humans as well. In MDCK cells, Crumbs-3 interacts directly with Par-6. The interaction requires the four last amino acids (Glu-Arg-Leu-Ile) of Crumbs-3 [54]. Furthermore, Crumbs-3 promotes the recruitment of Par-6 to the plasma membrane also indirectly, via the adapter PALS1 (or MPP5), which is the mammalian homolog of *Drosophila* Stardust. In MDCK cells, the interaction requires the amino terminus of PALS1 and the PDZ domain of Par-6 and is regulated by Cdc42-GTP [55]. Finally, besides interacting with Crumbs-3 and Par-6, PALS1 also interacts with the multi-PDZ domain protein PATJ, the mammalian homolog of *Drosophila* Inadl. The interaction requires a direct association between the L27N domain of PALS1 and the MAGUK-recruitment domain of PATJ [55]. Finally, to further stress the importance of the link between the apical complex and the TJ, it should also be mentioned that the carboxyl terminal residues of both ZO-3 (Thr-Asp-Leu) and claudin-1 (Tyr-Val) interact directly with the sixth and eighth PDZ domain of PATJ, respectively. Interestingly, a PATJ deletion mutant missing the sixth (but not the eighth) PDZ domain is localized away from the TJ, thus suggesting that the interaction with ZO-3 is important for the recruitment of PATJ (and its associated proteins) to the TJ [34].

Concerning the basolateral complex, the connections with the core complex are mediated by direct interactions of both aPKC and Par-6 with Lethal Giant larvae-1 (LLGL1). The PDZ domain of Par-6 binds directly the amino terminus of LLGL1 (which contains a WD40 interaction domain), whereas the association of the amino terminus of Par-6 with LLGL1 (observed *in vitro*) is probably indirect and mediated by aPKC. Furthermore, LLGL1 is a substrate for aPKC and contains five highly conserved and closely spaced serine residues that correspond to a consensus site for aPKC phosphorylation. Although the stable interaction of LLGL1 with aPKC is independent on these phosphorylation sites, the aPKC-dependent phosphorylation of LLGL1 is functionally important, because expression of a LLGL1 mutant lacking the five serine residues reduces cell polarization in response to cell wounding [56]. Finally, LLGL1 also interacts with myosin, thus providing a link between the polarity and cytoskeletal clusters [57].

3.5. Interactions of the core complex with signaling molecules

Signaling proteins, which are homologs of molecules that control polarization in the *Caenorhabditis elegans* embryo, also regulate the core polarity complex in mammalian epithelial cells (Fig. 3). Mark-2, which is the mammalian homolog of *C. elegans* PAR-1, binds and phosphorylates Par-3. Most of the effects of PAR-1-like molecules (including Mark-2) are mediated by the interaction of their phosphorylated substrates with 14-3-3 proteins. Actually, the Mark-2-induced phosphorylation of Par-3 (on two conserved serine residues) generates binding sites for the zeta isoform of protein 14-3-3, which is the mammalian homolog of *C. elegans* PAR-5 [58]. Thus, in epithelial cells, 14-3-3 binds Par-3 in a phosphorylation-dependent manner. The binding of 14-3-3 to Par-3 inhibits the formation of the core complex, by blocking both the self-association of Par-3 and the association of Par-3 with aPKC [59].

As Mark-2 exerts a negative effect on the assembly of the core complex, two control mechanisms limit the consequences of the Mark-2-dependent phosphorylation of Par-3. First, aPKC phosphorylates Mark-2, thus reducing both the kinase activity of Mark-2 and its localization to the plasma membrane [60]. Second, the aPKC-dependent phosphorylation of Mark-2 enhances the binding of 14-3-3 to Mark-2, which possibly leads to the dissociation of Mark-2 from the lateral membrane, thus again limiting its effects as inhibitor of polarity [61]. Lastly, as mentioned in Section 2.1, protein phosphatase 2A critically regulates activity and distribution of aPKC during TJ formation, thus providing an additional example whereby signaling pathways regulate the formation of the core complex [25].

3.6. Interactions of the core complex with actin regulators

Two sets of interactions provide a link between the polarity system and the actin-based cytoskeleton. First, Par-3 binds and inhibits the kinase LIMK-2. The interaction requires the

carboxyl terminus of Par-3 and the LIM domain of LIMK-2. In turn, LIMK-2 phosphorylates cofilin on Ser3 [62], thereby inhibiting the cofilin-dependent reorganization of actin [63]. Thus, by inhibiting LIMK-2, Par-3 relieves the inhibition of cofilin, thereby facilitating actin dynamics during cell polarization and TJ formation. Second, the carboxyl terminal region of Par-3 also binds the Rac exchange factor Tiam-1 [64]. In turn, Tiam-1 binds and activates Rac-1 [65]. The observation that the knockdown of Tiam-1 enhances TJ formation in cells lacking Par-3 suggests that, under normal conditions, Par-3 enables polarity establishment and TJ assembly by reducing Tiam-1 activity (and consequently Rac-1 activation).

3.7. Protein interactions in the disassembly of the TJ

Most of the interactions described in this section regulate TJ assembly during the establishment of cell polarity. At variance, an additional group of interactions regulates TJ disassembly, which may occur under both physiologic and pathologic conditions (e.g., the epithelial-to-mesenchymal transition in tumor cells). The pathway is regulated in response to TGF-β. Briefly, binding of TGF-β to its membrane receptor leads to RhoA degradation by activating a pathway that comprises the TGF-R1 and TGF-R2 subunits of the receptor, Par-6 and the E3 ubiquitin ligase Smurf-1. More specifically, in the absence of TGF-β, TGF-R1 and Par-6 form a complex at the TJ via the amino terminus of Par-6. However, in the presence of TGF-β, the TGF-β-induced assembly of the TGF-R1/TGF-R2 heterodimer brings TGF-R2 in proximity of Par-6 (bound to TGF-R1). In this way, TGF-R2 phosphorylates Par-6 on Ser345. Then, phosphorylated Par-6 interacts with Smurf-1, and in turn Smurf-1 ubiquitinates RhoA, thus leading to RhoA degradation in the proteasome and to TJ destabilization [66]. Finally, it should be mentioned that Smurf-1 also interacts with aPKC, even though the association is likely more important for the formation of cell protrusions in the context of polarized migration rather than for TJ disassembly [67].

4. Adapters and the cytoskeleton

TJ stabilization requires several interactions with the actin cytoskeleton. In particular, some adapters serve the specific purpose of linking the cell surface molecules (described in Section 2) with the actin filaments. We subdivide this cluster of interactions into three groups. We first describe the interactions of the three adapters ZO-1, -2 and -3 and then the interactions of two other adapters, namely cingulin and afadin. Finally, emerging evidence indicates that cell adhesion profoundly affects gene expression and cell proliferation. Thus, the adapters of the TJ may exert functions that go well beyond their structural role. Therefore, at the end of this section, we will make a brief mention to the interactions of ZO-1 and -2 with transcription factors and regulators of the cell cycle.

4.1. Interactions of the zonula occludens proteins

A central core of interactions within the adapter/cytoskeleton cluster is centered on the zonula occludens proteins ZO-1, ZO-2 and ZO-3. As represented schematically in Fig. 4, the ZO proteins interact with each other, as well as with actin and actin-associated proteins. Concerning the mutual interactions, it has been shown that ZO-1 binds both ZO-2 and ZO-3, but that ZO-2 and ZO-3 do not bind each other. Immunoprecipitation of protein constructs from transfected MDCK cells indicates that ZO-1 interacts with ZO-2 in the cell, and that the interaction requires the second PDZ domain (PDZ-2) of each interacting protein [19]. Similarly, ZO-1 interacts directly with ZO-3. Specifically, recombinant ZO-3 binds *in vitro* transcribed and translated ZO-1 (but not ZO-2), as detected by *in vitro* binding assay, and the interaction requires the amino terminal half of ZO-3. In addition, ZO-1 colocalizes with both ZO-2 and ZO-3 at the TJ of MDCK cells. Finally, in spite of the ability of ZO-1 and ZO-2 to bind each other, the presence of ZO-1 bound to a ZO-3 affinity column does not result in the retention of ZO-2, thus indicating that ZO-1 does not bridge ZO-2 with ZO-3 and that ZO-1 forms two distinct ZO-1/ZO-2 and ZO-1/ZO-3 complexes [16].

Concerning the interactions with the actin cytoskeleton, the three ZO proteins interact with actin either directly or indirectly via actin-associated proteins (including the adapters cingulin and afadin that are described in Section 4.2). The direct interaction of ZO-1 with actin requires the 220-amino acid long (and proline-rich) actin-binding region of ZO-1, as shown in pull-down assays from lysates of MDCK cells. Interestingly, the actin-binding region is required for the localization of ZO-1 not only to the junctions of contacting cells but also to the free edge of non-contacting cells before junction assembly. In the latter condition, the actin-rich pool of ZO-1 appears clustered with a punctuate pattern. Thus, the interaction between ZO-1 and F-actin likely plays different roles during the different steps of TJ assembly [68]. Finally, like ZO-1, also ZO-2 and ZO-3 colocalize with

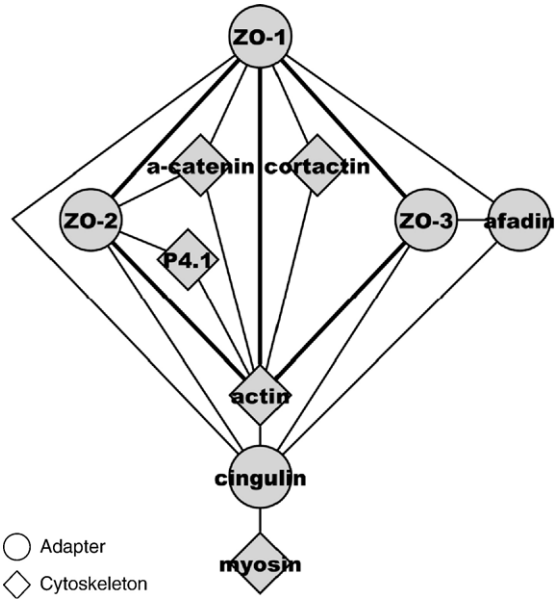

Fig. 4. Interactions within the adapter/cytoskeleton cluster. Part of the adapter/cytoskeleton cluster is represented as a network of protein interactions. Thick lines highlight the direct interactions of the ZO proteins with each other and with actin.

actin filaments at the cell–cell borders and interact directly with F-actin, as shown by *in vitro* binding and actin co-sedimentation assays [19].

The indirect interaction between ZO-1 and actin involves cortactin and α-catenin, as well as cingulin and afadin. The interaction between the *Drosophila* homologs of cortactin and ZO-1 requires the SH3 domain of cortactin and the Pro-X-X-Pro motif in the proline-rich domain of ZO-1. In turn, the interaction of cortactin with actin requires the fourth of six tandem repeats and the amino terminal acidic region of cortactin. By virtue of these interactions, cortactin contributes to coupling the spatial organization of actin with junction assembly [69]. The indirect interaction of ZO-1 with actin also involves α-catenin, even though the latter is a component of the adherens junctions. Actually, in non-epithelial cells, ZO-1 interacts directly with α-catenin and actin, in a way that involves the amino and carboxyl terminal halves of ZO-1, respectively. In this way, ZO-1 links the cadherin/catenin complex with the actin cytoskeleton, at least in non-epithelial cells [70]. Interestingly, the structural requirements for the interaction of α-catenin with actin are similar (but not identical) to those of vinculin. In particular, although α-catenin shares sequence similarity with vinculin, α-catenin contains an additional stretch of forty-two residues that is carboxyl terminal with respect to the region of homology with vinculin. Deletion of these residues impairs the binding to actin (without affecting protein folding), thus suggesting that they may mediate the binding of α-catenin to actin [71].

The indirect interaction of ZO-2 with actin involves α-catenin and protein 4.1. Like ZO-1, also ZO-2 binds α-catenin directly via the amino terminal domain of ZO-2 [15]. At variance, protein 4.1R (the prototype of the protein 4.1 family) maintains the mechanical integrity of the membranes by binding cooperatively spectrin and actin (via a spectrin- and actin-binding domain). Two 4.1R isoforms of 135 and 150 kDa interact with ZO-2. The interaction requires the region encoded by exons 19–21 of 4.1R and the region between residues 1054 and 1118 of ZO-2 [72]. In addition, protein 4.1R colocalizes with ZO-2 at the TJ of MDCK cells. In turn, protein 4.1 interacts with actin via a eight residue motif (Leu-Lys-Lys-Asn-Phe-Met-Glu-Ser) within the spectrin- and actin-binding domain [73].

4.2. Interactions of cingulin and afadin

Cingulin is an intracellular component of the epithelial TJ. As cingulin contains distinct domains responsible for the interaction with other proteins, it represents an additional adapter that links the TJ to the cytoskeleton (Fig. 4). Specifically, besides binding actin and myosin, cingulin interacts with the ZO proteins and afadin. In particular, a conserved ZO-1 interaction motif encompassing residues 48–61 at the amino terminus of cingulin is required for the interaction of cingulin with ZO-1 *in vitro*. Also, the observation that the overexpression of *Xenopus* cingulin in the A6 epithelial cells affects the localization of endogenous ZO-1 further indicates that the interaction between cingulin and ZO-1 is functionally important [74]. In addition, like ZO-1, also ZO-2 immunoprecipitates of MDCK cells contain cingulin. Similarly, like ZO-1, also ZO-2 and ZO-3 interact *in vitro* with the amino terminal fragment of cingulin. However, the amino terminal fragment alone is not targeted to the TJ. Thus, sequences in the head region of cingulin are essential for targeting cingulin to the TJ (most likely because of the interactions with the ZO proteins), but not sufficient, and additional sequences in the rod region are important as well [75].

The interaction of cingulin with F-actin has been studied *in vitro*. Purified full-length cingulin co-sediments with F-actin under high-speed centrifugation and promotes sedimentation of F-actin under low-speed centrifugation, thus suggesting that cingulin is an actin cross-linking protein [76]. Cingulin, however, interacts not only with actin but also with myosin. The interaction with myosin may transduce the mechanical force (that is generated by the contraction of the peri-junctional actomyosin ring) to the TJ proteins, thus contributing to the regulation of paracellular permeability. Both the amino and the carboxyl terminal fragments of cingulin mediate the interaction with myosin *in vitro* [75]. Furthermore, cingulin also interacts with afadin, in a way that requires the amino terminal fragment of cingulin [75]. Finally, cingulin also interacts (and colocalizes at the junctions) with the oncoprotein GEF-H1. GEF-H1 is a guanine nucleotide exchange factor that activates RhoA [77]. The interaction between GEF-H1 and cingulin, which requires the Pleckstrin-homology domain of GEF-H1 and the rod domain of cingulin, results in the inhibition of GEF-H1, thus providing a molecular mechanism whereby TJ formation results in RhoA inactivation [78].

Besides associating with cingulin, afadin associates with ZO-1 [79] and ZO-3 [80]. Specifically, ZO-1 interacts with the Ras-binding domain of afadin, in a way that is inhibited by activated Ras. In cells lacking TJ, afadin accumulates with ZO-1 at the adherens junctions [79]. In addition, afadin binds α-catenin and nectin, thus contributing to the localization of the complex between nectin, afadin and ponsin in proximity of the adherens junctions. The afadin-binding site has been mapped to the 385–651 fragment of α-catenin, which contains two adjacent bundles of four helices. Interestingly, the relative orientation of the two bundles may switch from about 40° to approximately 90°, which likely correspond to closed and open conformations with reduced and enhanced ability to bind afadin, respectively [71]. Lastly, afadin binds Ras. Actually, afadin was discovered as a Ras-binding protein and partially purified by chromatography on a GST-Ras affinity column [81]. In turn, as it is well known, Ras interacts with Raf-1, thus establishing a link between junctions and the Ras signaling pathway [82].

4.3. Interactions of ZO-1 and ZO-2 with transcription factors and cell cycle regulators

As the interactions of ZO-1 and ZO-2 with transcription factors and regulators of the cell cycle have been reviewed in detail [83], here we will briefly summarize molecular determinants and functional significance of such interactions. Specifically, ZO-1 binds (via its SH3 domain) the Y-box transcription factor ZO-1-associated nucleic acid binding (ZONAB). As a consequence of their interaction, ZONAB and ZO-1 together regulate the promoter of erbB-2, which is a tyrosine kinase co-receptor important for epithelial differentiation. Interestingly, ZO-1 regulates

ZONAB activity by influencing the subcellular localization of the transcription factor. Specifically, in non-confluent cells, ZONAB is free to accumulate in the nucleus and to act as a repressor of the *erbB-2* gene. At variance, in confluent cells, ZONAB is sequestered at the TJ (because of its association with ZO-1) and can no longer act as a repressor in the nucleus. Thus, the discovery of the ZONAB-dependent regulation of erbB-2 expression has unveiled a mechanism whereby TJ modulate the expression of epithelial-specific differentiation genes [84]. In addition to ZO-1, ZONAB also associates with cell division kinase-4. Importantly, reduction of the nuclear levels of ZONAB (e.g., in confluent cells) results in reduced nuclear levels of cell division kinase-4 too. Thus, by sequestering ZONAB at the TJ, ZO-1 also prevents the nuclear accumulation of cell division kinase-4. In this way, TJ not only favor epithelial differentiation, but also reduce cell proliferation in a cell density-dependent manner [85]. Along the same line, ZONAB also interacts with RalA in a GTP-dependent manner. As the levels of the RalA-ZONAB complex increase as epithelial cells become dense, also the interaction of ZONAB with RalA (like the interaction of ZONAB with ZO-1) may relieve the transcriptional repression of ZONAB [86]. Finally, ZO-1 interacts with the heat-shock protein Apg-2. In particular, Apg-2 coprecipitates with ZO-1 and partially localizes to the intercellular junctions of MDKC cells. As Apg-2 competes with ZONAB for binding the SH3 domain of ZO-1, Apg-2 may favor the repressor activity of ZONAB [87].

Like ZO-1, also ZO-2 interacts with transcription factors and regulators of the cell cycle. However, at variance with ZO-1, a pool of ZO-2 localizes to the nucleus (at least in non-contacting cells). As the nuclear localization suggests that ZO-2 itself might directly regulate gene expression, the interaction of ZO-2 (expressed as GST-fusion protein) with different transcription factors has been studied in detail. These studies have reported that ZO-2 interacts with Jun, Fos and CEBPG and that the interactions require the carboxyl terminal region of ZO-2. Surprisingly, however, the interactions do not occur only at the nucleus but also at the TJ. The association of ZO-2 with Jun/Fos at the TJ might sequester the Jun/Fos complex at the plasma membrane and thus lower its levels in the nucleus. As a net result, junctional ZO-2 might indirectly affect the activity of the Jun/Fos complex in the regulation of gene transcription. Although transfected ZO-2 down-regulates the expression levels of such factors, it does not affect aspecifically the global levels of gene transcription [88]. Furthermore, nuclear ZO-2 also interacts with the DNA-binding protein scaffold attachment factor-B, as shown by coprecipitation assays [89].

Finally, ZO-1 and ZO-2 interact with the p120-like catenin ARVC. Both interactions require the carboxyl terminal PDZ-binding motif of ARVC and the PDZ domains of ZO-1 and ZO-2. However, there are interesting differences between ZO-1 and ZO-2. On one side, ARVC and ZO-1 partially colocalize in the vicinity of the apical adhesion complex. In addition, ARVC, ZO-1 and E-cadherin are recruited as a complex to the sites of initial cell–cell contact. On the other side, ARVC colocalizes with ZO-2 in the nucleus. Thus, the selective interaction with either ZO-1 or ZO-2 may mediate the recruitment of ARVC to either the plasma membrane or the nucleus [90].

4.4. Other interactions of the ZO proteins

The ZO proteins interact with other signaling molecules and with some connexins. Specifically, the SH3 domain of ZO-1 binds the serine/threonine kinase ZAK, which appears to be associated with junctional complexes in MDCK cells. In turn, ZAK phosphorylates a region of ZO-1 that is carboxyl terminal with respect to the SH3 domain [91]. Furthermore, ZO-3 binds the p120 catenin, which (besides being a component of the adherens junctions) is implicated in the regulation of the cytoskeleton and cell motility. Notably, altered interactions between ZO-3 and p120 in cells expressing the amino-terminal half of ZO-3 affect RhoA activity, thus establishing a further link between the TJ and RhoA signaling [80].

Concerning the connexins, ZO-1 was shown to interact with connexin-43. The interaction requires the carboxyl terminus of connexin-43 and the second PDZ domain of ZO-1. Connexin-43 and ZO-1 colocalize at the gap junctions of transfected COS-7 cells, thus suggesting that ZO-1 is involved in the clustering and assembly of connexin-43-based gap junctional channels [92]. In addition, both ZO-1 and ZO-3 interact and colocalize with connexin-45. Again, the interactions require the PDZ domains of the ZO proteins and the carboxyl terminus of connexin-45. Mutation of the carboxyl terminal residues (Ser-Val-Trp-Ile) of connexin-45 abolishes the interactions with ZO-1 and ZO-3 [93].

5. Other adapters and signaling systems

An additional cluster of protein interactions is centered on a class of adapters named membrane-associated guanylate kinase with inverted orientation (MAGI). These interactions serve different functions. First, like the ZO proteins, they act as scaffolds that link cell surface molecules with the actin cytoskeleton. Second, they modulate cell signaling. Third, they interact with a system that regulates the recycling of the junctional cell surface molecules.

5.1. Interactions of MAGI-1 with cell surface molecules and the actin cytoskeleton

MAGI-1 is a membrane-associated protein expressed at the TJ, which encompasses a guanylate kinase domain, two WW domains and six PDZ domains. Like other PDZ proteins, also the PDZ domains of MAGI-1 interact with the carboxyl termini of other proteins, including three cell surface (and immunoglobulin-like) molecules (i.e., JAM-4, ESAM-1 and CXAR) and an actin-binding protein (i.e., α-actinin-4), as described below.

Like JAM-A, also JAM-4 belongs to the JAM family. The interaction between JAM-4 and MAGI-1 requires the carboxyl terminus of JAM-4 and two PDZ domains (the first and fourth) of MAGI-1. It is likely that the interaction provides the TJ with an additional adhesion system, which may regulate permeability in kidney glomeruli and the small intestine. Interestingly, JAM-4 does not bind ZO-1, whereas JAM-A does not bind MAGI-1 [94]. The biochemical basis of this specificity has been defined in detail. The carboxyl termini of PDZ-binding proteins are divided into two classes depending on whether they harbor a

consensus sequence for type I or type II PDZ domains. Type I PDZ domains (like the domains of MAGI-1) contain a critical histidine residue in the second α-helix and bind proteins ending with the consensus motif Ser/Thr-X-Φ_{COOH} (X, any residue; Φ, hydrophobic residue). At variance, type II PDZ domains (like the domains of ZO-1) contain a hydrophobic residue in the second α-helix and bind proteins ending with the consensus motif Phe/Tyr-X-Φ_{COOH} [95]. Thus, JAM-A, which ends in Phe-Leu-Val, binds ZO-1 (precisely, the type II PDZ-2 and PDZ-3 domains) [39,40], but not MAGI-1. In contrast, JAM-4, which ends in Thr-Leu-Val, binds MAGI-1 (precisely, the type I PDZ-1 and PDZ-4 domains), but not ZO-1 [94].

Endothelial cell-selective adhesion molecule-1 is another cell surface protein associated with the TJ. As the carboxyl tail of ESAM-1 ends with a consensus motif (Thr-Leu-Leu) for binding type I PDZ domains, it may anchor MAGI-1 to the endothelial TJ. Binding studies using a MAGI-1 fragment (which encompasses residues 789–876 and contains the PDZ-3 domain) further suggest that the interaction is PDZ-dependent [96]. In addition, the third cell surface molecule that binds MAGI-1 is Coxsackie and Adenovirus Receptor. This molecule plays a role not only in forming epithelial junctions, but also in modulating cell growth and, as the name indicates, in facilitating viral infection. Its carboxyl terminus, which ends with a consensus motif (Ser-Ile-Val) for binding type I PDZ domains, plays a role in cellular localization, cell–cell adhesion and cell growth, as well as in the interaction with MAGI-1. In addition to MAGI-1, the Coxsackie and Adenovirus Receptor also interacts with PICK-1, another PDZ protein which contains a single PDZ domain and forms homodimers [97].

As mentioned above, also MAGI proteins may link the cell surface molecules of the TJ with the actin cytoskeleton. Limited information, however, is available concerning the molecules involved. MAGI-1 binds actinin-4, the non-muscle isoform of the actin-binding protein α-actinin. The interaction requires the fifth PDZ domain of MAGI-1 and the cytoplasmic tail of actinin-4, whose carboxyl terminus ends with a consensus motif (Ser-Asp-Leu) for binding type I PDZ domains [98]. Interestingly, the expression levels of actinin-4 are increased at the edge of cells that migrate into a wound. In addition, it shuttles between cytoplasm and nucleus, thus suggesting that actinin-4 (possibly in association with MAGI-1) may regulate actin dynamics [99,100].

5.2. Interactions of MAGI proteins with signaling molecules

The three members of the MAGI family (MAGI-1, -2 and -3) also interact with the lipid phosphatase PTEN. As PTEN contains the carboxyl terminal PDZ-binding residues Thr-Lys-Val, also the interactions of PTEN with the MAGI proteins are likely PDZ-dependent. In general, the binding of the MAGI proteins to PTEN enhances the enzymatic activity of PTEN. Actually, PTEN converts the phosphatidyl-inositol PIP3 back to PIP2. In this way, PTEN counteracts the effect of PI3K (which catalyzes the production of PIP3) and indirectly inactivates the effectors of PIP3, including protein kinase Akt. Thus, MAGI proteins link the TJ with a major pathway that regulates growth and survival in normal cells. PTEN, however, is also a tumor suppressor. Some mutations in tumors (for instance, in glioblastoma and endometrial tumors) affect the carboxyl terminus of PTEN. In particular, mutations that either insert a premature stop codon or add residues after the stop codon alter the PDZ-binding motif and predictably abolish the interactions of PTEN with its PDZ partners, including the MAGI proteins. If the loss of such interactions results in reduced PTEN activity (and thus in enhanced Akt activity), then this cluster of junctional interactions may acquire relevance to neoplastic transformation as well. In this respect, it is noteworthy that the MAGI proteins are targets for viral oncoproteins.

More specifically, the isoform MAGI-1b interacts directly with PTEN via the carboxyl terminus of PTEN and the second PDZ domain of MAGI-1b [101]. Similarly, MAGI-2 localizes to the epithelial TJ and binds the carboxyl terminus of PTEN via the second PDZ domain. Expression of MAGI-2 reduces Akt activation, thus indicating that also MAGI-2 favors the activity of PTEN. Conversely, PTEN mutants that fail to bind MAGI-2 are impaired in their ability to prevent Akt activation. Nevertheless, these mutants only exert a partial effect, thus suggesting that these adapters merely optimize the activity of the associated enzyme [102]. In addition, also MAGI-3 localizes to the epithelial TJ and binds PTEN. Again, PTEN dephosphorylates PIP3 more efficiently when it is associated with MAGI-3 [102]. Finally, in addition to PTEN, MAGI-3 interacts with another phosphatase, the receptor tyrosine phosphatase PTPRB, which is involved in the control of cell–cell and cell–matrix adhesion. The interaction of PTPRB with MAGI-3 requires the carboxyl terminus of PTPRB and the third or fourth PDZ domain of MAGI-3. In this way, MAGI-3 may link PTPRB with its substrates at the plasma membrane [103]. Lastly, outside the TJ, PTEN interacts indirectly with β-catenin by binding MAGI-1b. In this way, PTEN is also recruited to the adherens junctions. As β-catenin also binds PI3K, the adherens junctions may thus function as a central point for regulating the PIP3 pool and its effectors. Thus, although devoid of enzymatic activity, MAGI proteins likely play a general role in organizing signaling complexes that control cell growth and differentiation [101].

5.3. Interactions of MAGI-1 with a system for the recycling of junctional proteins

Like other membrane proteins, also the cell surface components of the TJ undergo constitutive recycling from the membrane to the early endosomes and back to the membrane. It appears that the activation state of Cdc42 is critical for determining the fate of the endocytosed proteins. In particular, when Cdc-42 is inactive (i.e., GDP-bound), the endocytosed molecules remain in the early endosomes and are recycled to the membrane. In contrast, when Cdc42 is active (i.e., GTP-bound), the endocytosed molecules are transferred to the late endosomes and targeted to lysosomes for degradation. The molecule that negatively regulates the activation state of Cdc42 in the endosomes (and thus the fate of the endocytosed junctional molecules) is Rich-1, a GTPase-activating protein (GAP) for Cdc42 [104]. The importance of Rich-1 for TJ integrity is supported by the observation that silencing Rich-1 expression

disrupts TJ. Also, overexpressing a GAP-deficient mutant of Rich-1 induces a similar phenotype, thus stressing that the TJ-stabilizing effect of Rich-1 is attributable to the inactivation of Cdc42 [104].

Rich-1 comes in close contact with the TJ by virtue of its association with the scaffolding protein angiomotin. The BAR/coiled-coil regions of Rich-1 and angiomotin are necessary for such heterotypic binding. The BAR (an acronym for Bin/Amphiphysin/Rvs) domain is a widely conserved interaction domain [105]. In turn, angiomotin associates with three junctional proteins, MAGI-1, PATJ and MUPP1. First, the p130 isoform of angiomotin binds MAGI-1b. The interaction requires the amino terminal motif Pro-Pro-X-Tyr of angiomotin and the WW domain of MAGI-1 [106]. Second, angiomotin interacts with PATJ. The PDZ-binding motif at the carboxyl terminus of angiomotin (Asp-Tyr-Leu-Ile) binds PATJ. Third, it has been recently discovered that angiomotin also interacts with MUPP1. The interaction again requires the PDZ-binding motif of angiomotin and the PDZ-2 and -3 domains of MUPP1. Surprisingly, however, the absence of the PDZ-binding motif does not alter the subcellular distribution of angiomotin. Thus, it is likely that PATJ and MUPP1 may have redundant roles (with respect to MAGI-1) in determining the localization of angiomotin to the TJ [107]. Finally, it is worth mentioning that CD2AP and SH3K1 are two molecules that associate with Rich-1. CD2AP and SH3K1 bind each other directly and target cell surface molecules (e.g., growth factor receptors) for endocytosis. The ability of angiomotin to recruit Rich-1 may thus impact on the trafficking of TJ components via the association of Rich-1 with CD2AP and SH3K1.

6. Concluding remarks

The TJ exert two major functions, which are traditionally designated as ‘barrier’ and ‘fence’. While the term barrier refers to the control of paracellular permeability across lateral intercellular spaces, the term fence describes the emergence and maintenance of apico-basal polarity within the plasma membrane. Predictably, the individual features of the TJ molecules and their mutual interactions (that have been outlined in this review) represent the molecular basis for a mechanistic explanation of these complex functions.

Although our understanding is far from being complete, we have attempted here at drawing correlations between the different clusters of protein interactions and their possible role in TJ function. In general, the interactions that involve the adhesive surface molecules of the TJ (as described in Section 2) are primarily instrumental in building the paracellular barrier, as they seal the lateral intercellular spaces between adjacent cells. At variance, the interactions that involve the evolutionary conserved polarity complexes (as described in Section 3) are instrumental in setting up the polarity fence, as they determine the correct localization of the TJ at the apico-basal boundary and the ability of the TJ to maintain such polarization of the membrane. In addition to these two major protein clusters, emerging evidence indicates that, like other junctional systems of the cells (e.g., adherens junctions, desmosomes and focal adhesions), the actin-based cytoskeleton (as described in Section 4) plays a key role in reinforcing the adhesive complexes of the barrier and in translating polarity determinants of the fence into spatially defined responses. Finally, far from being a static system, the TJ undergo dynamic changes in response to various signaling pathways (as described in Section 5 and throughout the text).

Once the complete map of protein interactions will be available, we can expect to gain deeper understanding of TJ in all the steps of their lifespan, from the assembly (in response to coordinated polarity cues), to the stabilization (via dynamic linkages to adapter and cytoskeletal proteins), to the maintenance (via regulated membrane trafficking) and finally to the disassembly (for instance, via ubiquitination events). An additional major challenge will consist in defining how the molecular interactions among the TJ components translate stimuli from signaling mediators (for instance, phosphorylation events) into functional responses. In the end, when the TJ cartography will be complete, we foresee that the TJ, no matter how tight they may be, will no longer hold their molecular secrets so tightly.

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