Mechanoregulation of PDZ Proteins, An Emerging Function

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Chapter 15

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Abstract

Mechanical forces have emerged as essential regulators of cell organization, proliferation, migration, and polarity to regulate cellular and tissue homeostasis. Changes in forces or loss of the cellular response to them can result in abnormal embryonic development and diseases. Over the past two decades, many efforts have been put in deciphering the molecular mechanisms that convert forces into biochemical signals, allowing for the identification of many mechanotransducer proteins. Here we discuss how PDZ proteins are emerging as new mechanotransducer proteins by altering their conformations or localizations upon force loads, leading to the formation of macromolecular modules tethering the cell membrane to the actin cytoskeleton.

Key words PDZ proteins, Forces, Tight junctions, Adherens junctions, Actomyosin

1 A Brief Introduction on Mechanotransduction/Mechanoregulation in Biology

All the cells and tissues of the body are subject to external and internal forces. These forces can affect the shape and intracellular organization of cells, their proliferation, their migration, and their intercellular interactions. Forces influence the development of embryos [1] as well as cell functions and homeostasis in the adult [2]. Moreover, many disease states are characterized by changes in these forces and/or a loss of the normal cellular response to them [3]. Over the last decade, mechanotransduction has emerged as a key process in development and diseases. Mechanotransduction can be defined as a cellular event that converts a mechanical input such as fluid shear stress (blood vessels), stretch (lung, intestine), osmotic forces (urinary tract), mechanical load (bone, muscle) [4, 5] as well as the impact of the stiffness of the extracellular matrix (ECM) that surrounds most cells leading to a biochemical response [6]. In well-studied examples of mechanotransduction, proteins can undergo force-induced changes into conformations that lead to modification in affinity for binding partners or catalytic activity. The mechanical load triggers biochemical changes that can

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propagate via the activation of specific signaling pathways [7]. In a simple context, the mechanical load can accelerate the association or dissociation of protein–ligand bonds [8]. In the case of adhesion proteins, that exhibit catch-bond behavior, mechanical loads can produce changes in the conformation of the proteins that lead to high affinity for a binding partner [9–11].

Deciphering how fundamental physical principles are controlled by protein interactions is central to understand how forces are converted into biochemical signals. Elucidation of the specificity, selectivity, and regulatory mechanisms involved in protein–protein interactions can therefore provide important insights into many biological processes such as cell proliferation, cell migration, and cell polarity. Structural studies have revealed that mechanosensitive proteins with multiple domains and flexible interdomain interfaces can pass through multiple conformations [12, 13]. For example, a bent conformation can open up to a straighter arrangement of domains along the direction of the applied force. These changes in conformation result in modification of the affinity for the binding partners, exposure or hiding of different catalytic domains that will trigger differential intracellular signaling responses or even redistribution of the protein within the cell [14–16]. Among the proteins that mediate protein–protein interactions, there is the large PDZ (postsynaptic density 95/Disc large/Zonula occludens) family. Most of the PDZ proteins are multimodular scaffold proteins and often contain multiple PDZ domains which can interact with various binding partners and thereby assemble supramolecular signaling complexes [17].

As PDZ domains interact with motifs present in many proteins, understanding the regulatory mechanism of PDZ mediated interactions is important to gain insight into biological processes. So far, posttranslational modifications, autoinhibition, and allosteric interactions have been proposed to regulate PDZ-mediated interactions and thus intracellular signaling [18], but little is known about the impact of mechanical inputs on PDZ proteins. In this review, we will focus on the current knowledge on the mechanoregulation of PDZ proteins, focusing on few recent findings.

2 PDZ Proteins as Mechanotransducers

Biological mechanotransducers can be defined as a single protein or a protein complex that produce or enable a chemical signal in response to mechanical stimuli. These mechanotransducers can participate in mechanoreception and mechanotransmission as direct mechanosensitive structure, which respond by altering their conformation upon force loads, or as second line mechanotransducers. Among the mechanotransducer proteins studied so far, the PDZ protein family is emerging as a new pool of protein sensitive to

forces, but only few examples are known so far. In epithelial cells, the adhesion machineries at the cell–cell or cell–substrate interfaces are known to play an important role in mechanotransduction [10, 19]. These machineries are composed of several proteins, and many of them are part of the PDZ family [20]. Being integrated in a complex modular array tethering the cell membrane to the cell cytoskeleton, the adhesion scaffolding PDZ proteins might constitute an important link to transduce the mechanical signals into an appropriate cell response to maintain cellular homeostasis. We will thus focus on PDZ proteins from the adhesion machinery that have been recently shown to be regulated by forces in a direct or indirect manner (Fig. 1).

3 Force Regulated PDZ Proteins

3.1 ZO1

ZO1 is a tight junction scaffolding protein that belongs to the MAGUK (membrane associated guanylated kinase homolog) family. It is characterized by the occurrence of three PDZ, one SH3 and a GUK (guanylate kinase homologous domain) domains [21]. ZO1 is a cytoplasmic protein, that anchors actin filaments through its actin binding domain at its C terminal region [22, 23]. At the N-terminus, the PDZ1 and PDZ3 domains bind to the tight junction membrane associated proteins, Claudins and JAM-A, and to TAZ, a member of Hippo pathway, while the PDZ2 domain promotes heterodimerization between ZO1 and either ZO2 or ZO3 [22, 24–26]. A larger region encompassing the PDZ3, SH3, U5, and GUK domains (ZPSG-1) interacts with and recruits to junctions the transmembrane TJ protein occludin, but also DbpA/ZONAB (DNA binding protein A/ZO1 associated Nucleic Acid-binding protein) [27, 28]. The sequestration of DbpA by ZO1 and ZO2 at the junctions of confluent monolayers inhibits its nuclear activity that regulates gene expression and cell proliferation [27–30].

Recently, Spadaro et al. demonstrated that ZO1 stretches upon mechanical forces [31]. In 2011, Then et al. have already proposed an impact of membrane tension on the localization of ZO1 [32]. Under hyperosmotic stress that generates an increase in membrane tension, the actin cytoskeleton is reorganized with the appearance of a dense F-actin cortical ring. In this condition, ZO1 expression is increased, and it colocalizes with the newly formed actin ring [32]. As ZO1 is anchored to the actin filaments through its C-terminal region, this change in actin organization exerts direct pulling forces on the ZO1 protein. When present as a heterodimer with ZO2 within cells, ZO1 is in a stretched configuration that allows binding to DpbA. However, the depletion of ZO2 together with an inhibition of myosin contractility promotes a folding of the N-terminal and C-terminal end of ZO-1, and releases the

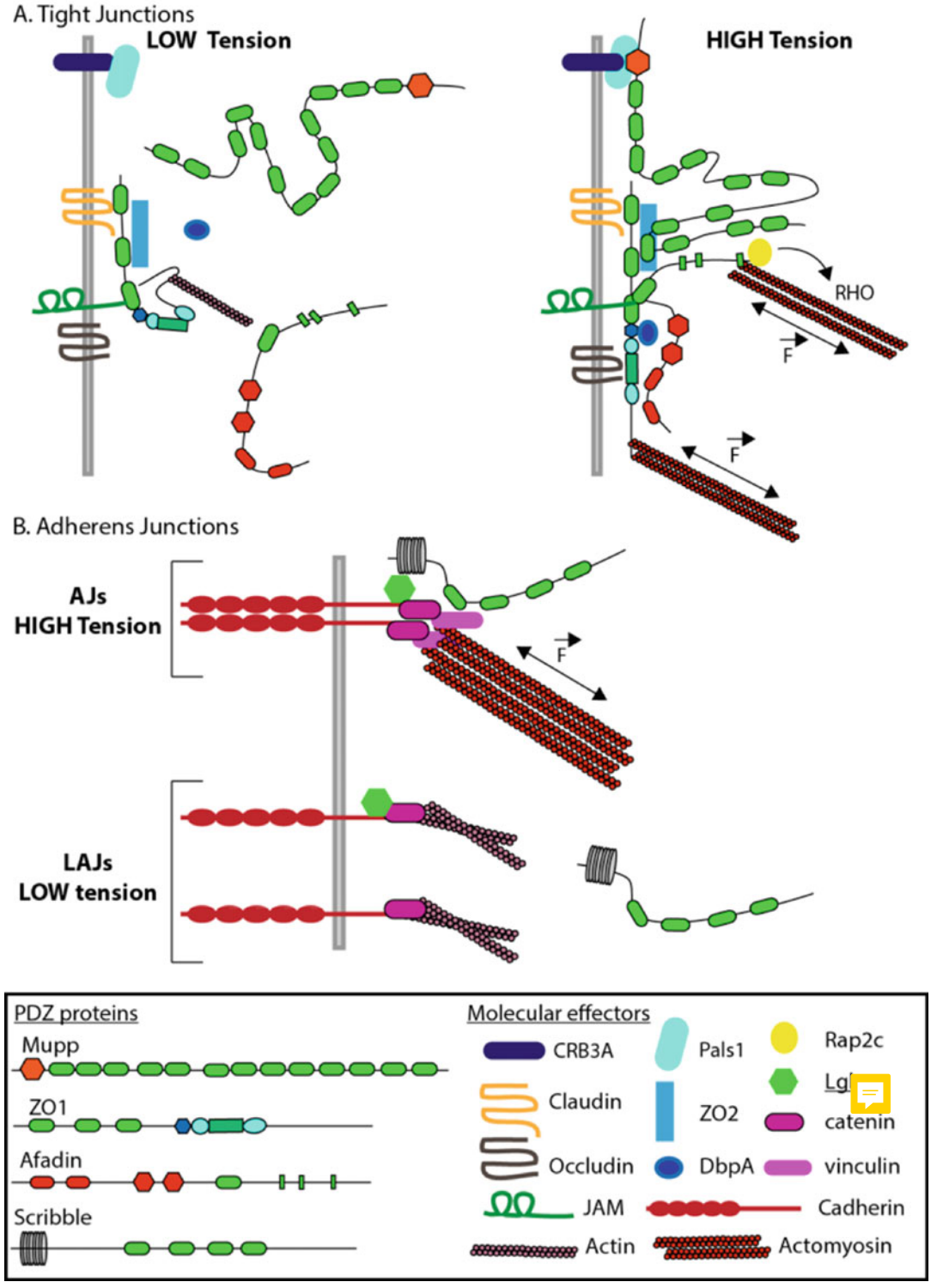

Fig. 1 Hypothetical role of the PDZ protein in the strengthening and stabilization of intercellular adhesions. (**a**) At the level of the tight junctions (TJs), under low tension the mechanotransducer ZO1 is in an inactive

interaction with DpbA and Occludin. By applying linearly increasing forces to purified full-length single ZO1 molecules with magnetic tweezers, Spadaro et al. could demonstrate that both the C-terminal region and the ZPSG-1 module of ZO1 that comprises the PDZ3, SH3, U5, and GUK domains unfold at forces ranging from 5 to 20 pN, releasing the autoinhibition interaction between ZPSG and C terminal domain of ZO1 [31]. ZPSG-1 domain is not only important for the junctional localization of ZO1 but also for its interaction with Occludin, DbpA, and thus for barrier formation and epithelial polarization [33–38]. Forces act as an allosteric effector by stretching ZO1 protein to promote the interaction of ZPSG-1 with its ligands, occludin and DbpA. While the stretched conformation of ZO1 is the active conformation, the folded conformation is the inactive form in which the ZPSG domain is autoinhibited. In its inactive form, junctional ZO1 remains anchored to the membrane through binding of its N-terminal domain with interactors such as TAZ or Claudins, while the C-terminal half is intramolecularly autoinhibited. The release of ZPSG-1 domains may also regulate the interaction of other proteins such as α-Catenin, Afadin, JAM-A, Vinculin and Shroom2 [39].

3.2 MUPP1

MUPP1 belongs to the family of multi-PDZ proteins and contains a L27 domain at its N-terminal region followed by 13 PDZ domains [40]. MUPP1 is a structural paralog of PATJ, and both share several binding partners such as PALS1, PAR-6, AMOT, Jeap, ZO3, Claudins, or Nectins [41]. Lanaspa et al. by the use of osmolarity changes demonstrated that both acute and chronic hyperosmolarity in inner medullary collecting duct 3 cells induce an increase in the expression of MUPP1, ZO1, and Afadin [42]. As MUPP1 expression increases, it localizes to the apical side of the membrane at the level of the Tight Junctions (TJs). To survive hyperosmotic stresses and to maintain the integrity of the cell sheet with efficient barrier functions, cells have to adapt through

Fig. 1 (continued) conformation, and the second line mechanotransducers Afadin and Mupp1 are in the cytoplasm. The actin filaments are exerting low mechanical loads. Under high tension, generated by actomyosin bundles, ZO1 unfolds and unmasks several domains that leads to the binding with Occludin and JAM together with the recruitment of DbpA and Afadin. Afadin is then able to recruit Rap2c generating a positive feedback loop on RHO allowing for actin contractions and thus force generation. This increase in forces results in the recruitment of Mupp1 at the TJs, that will bind to ZO1, ZO2, and JAM but also potentially to the CRB3A/Pals1 polarity complex. The formation of this macromolecular complex that tethers transmembrane proteins (Claudins, Occludins, JAM) to the actin cytoskeleton is key for the strengthening and the stabilization of the TJs. (**b**) In the lateral domain of the cells, at the level of the lateral Adherens Junctions (LAJs), the actin filaments that binds to the E-cadherin–catenin complex, exert low tension and Scribble is in the cytoplasm. Above the lateral junctions, the E-cadherin–catenin complex is link to vinculin and binds to actomyosin bundles that are under high mechanical loads. SCRIBBLE is also enriched at the AJ level, and plays a key role in the stabilization of the AJs

reorganization of the actin cytoskeleton as mentioned previously but also through differential expression of proteins, that move to the junctions to counterbalance the changes in membrane tension. Depletion of MUPP1, results in a disruption of the TJ barrier, with a drop of transepithelial resistance of about 25% [42]. Molecularly, depletion of MUPP1, in hyperosmotic conditions, triggers a decreased expression and loss of membrane localization of Claudin4, a TJ protein [42]. These data clearly show that MUPP1 is important in the maintenance of the epithelial homeostasis and confirm previous study that pointed out a predominant role of MUPP1 in the disruption of both TJs barrier and apicobasal polarity [43–45]. A more recent study shows that MUPP1 stability and degradation in endothelial cells depends on the regulation of PDZRN3, an E3 ubiquitin ligase PDZ domain containing ring finger 3 protein [46]. The interactions between PDZRN3, MUPP1, PAR3, and aPKC regulate the stabilization of TJs in endothelial cells. Perturbation of PDZRN3 expression induces degradation of MUPP1 that correlates with destabilization of the actin cytoskeleton and disruption of the TJs. All these studies point toward a role of MUPP1 in the stabilization and maintenance of actin cytoskeleton and TJs in cells.

20 years ago, it was suggested that MUPP1 could change its conformation through the interaction of its PDZ10 domain with the 5-hydroxytryptamine type 2C receptors [47]. This interaction induces a conformational change in MUPP1 and triggers a clustering of the 5-hydroxytryptamine type 2C receptors at the cell membrane, triggering downstream signal transduction pathways. It is of interest that different PDZ domains of MUPP1 can bind to CADM1, a transmembrane cell adhesion protein, with different affinities. The PDZ2 of MUPP1, for example, presents the highest affinity for CADM1, but when this PDZ is absent, CADM1 can still interact with other PDZ domains of MUPP1. These data could point to a change in conformation of MUPP1 that would unmask the PDZ2 domain leading to a strong binding to CADM1 [48, 49]. In the case of CADM1 and maybe of other interactors of MUPP1, the interactions with multiple domains can be an alternative when some domains are either occupied by other ligands or hidden by an autoinhibitory conformation of MUPP1. These multi–low-affinity interactions might then serve as transient interaction contacts before MUPP1 reaches its stretched configuration. However, the unfolding/folding regulation of MUPP1 is still under debate as only indirect evidence has been obtained to date.

3.3 PAR3

PAR3 is part of the Partitioning defective proteins and presents a CR1 oligomerization domain, 3 PDZ domains and aPKC binding domain. A recent study pointed out that cortical forces are responsible for the clustering of PAR3 on the cell cortex. In *C. elegans*, inhibition of actin contraction with blebbistatin or actin filament

polymerization causes a reduction of the PAR3 clusters on the cortex, demonstrating that higher cortical tension can drive PAR3 cluster formation at the cortex [50]. The actomyosin membrane associated network generates flows [51], which promote symmetry breaking along the anterior posterior axis through the advection of polarity components [52–54]. The polarized actomyosin contractions pull actin networks along the membrane toward the anterior region while triggering local disassembly and turnover via increased local tension, resulting in flow of material [55]. Based on the advective flow model proposed by Goerhing et al. [53] that predicts that diffusivity and turnover of cortical polarity proteins should be slow and stable enough to be passively transport by the advective cortical flow, Wang et al. [50] postulate a "clustering and stabilization" hypothesis for PAR3. By using fluorescence recovery after photobleaching, they demonstrate that the half-life of PAR3 and PKC are shorter before symmetry breaking. During the early to middle establishment phase, when the clusters are formed, the half-life increases. Molecularly, during this phase the activity of Cdc42 is reduced allowing PKC3 and PAR6 to associate with PAR3 clusters at the cortex and thus facilitates effective transport by advective cortical flows. During the maintenance phase, the half-lives of PAR3 and PKC are shorter, when the clusters disassemble, concomitant with an increased activity of Cdc42 that prevents PKC and PAR6 association with PAR3. As cortical tensions trigger a dynamic equilibrium between an unclustered and clustered form of PAR3 correlating with its association with PAR6 and PKC, it is tempting to speculate that PAR3 may undergo structural changes by mechanical stretching, which could relieve the amino-terminal CR1 domain from intramolecular inhibitions, thus promoting PAR3 oligomerization [56]. These mechanically induced conformational changes may directly activate the ability of the CR1 domain to facilitate its oligomerization, and proper localization in cells [56]. Furthermore, similar cortical flows are observed during cell division or cell migration, and then could, if proven, also explain the interaction between the different members of the PAR complex.

4 PDZ Proteins "Second Line" Mechanotransducers

In this part, we will describe how some PDZ proteins interact with proteins that are activated upon forces. These interactions may trigger a change in the conformation or in the localization of the "second line" mechanotransducers, thereby regulating different signal pathways.

4.1 PAR6

PAR6 is part of the Partitioning defective proteins in *C. elegans*, and encodes a protein with PKC binding domain (PB1), a semi-Cdc42/Rac1 interactive binding (CRIB) and a PDZ domain [57]. Recently a PDZ binding motif (PBM) was identified at its C-terminal region [58]. PAR6 in many species is involved in cell division, cell polarization, and cell migration, processes that are characterized by changes in cellular forces [59–61].

PAR6 function is able to segregate to the apical or leading edge of epithelial cells upon activation by Cdc42-GTP [57, 62]. Accumulating evidence indicates that Cdc42 responds to and is activated upon different mechanical loads such as hyperosmolarity, shear stress, and intercellular increased tension [63]. Upon exposure to mechanical loads, Cdc42 translocates from cytosol to the membrane and is concomitantly activated [64]. Activation of Cdc42 by exchange of GDP for GTP triggers actin polymerization and generation of tension [65, 66]. Binding of activated Cdc42GTP to the CRIB domain of PAR6 favors the interaction with CRUMBS over the interaction with PALS1 through a change in conformation of the CRIB-PDZ domains of PAR6 [67]. Upon Cdc42-GTP binding, a portion of the flexible CRIB motif folds into a stable conformation with the PDZ domain [68], triggering an increase affinity for the interaction with CRUMBS, and releasing the interaction with PALS1. The modular nature of PAR6 may allow the mutually exclusive interaction of CRUMBS and PALS1 with its PDZ domains, regulating the assembly and localization of different polarity complexes depending on the cellular context.

The localization of PAR6 to the plasma membrane depends on both the PDZ and the PBM domains, as both deletions caused a strong mislocalization of PAR6 to the cytosol. The PBM domain binds with different affinities to the PDZ1 and PDZ3 of PAR3, whose clustering is mediated by an increase in cortical tension as previously mentioned [50]. The weak but multivalent interaction of one PAR3 molecule with two PAR6 molecules might allow the assembly a large cluster of PAR complexes at the cell membrane. The formation of the large-scale cluster of PAR complexes can facilitate the actomyosin dependent advective transport of several PAR6 proteins during the establishment of polarity but also the formation of large clusters made with several proteins such as aPKC, PALS1, or CRUMBS that can serve different functions.

4.2 DLG

DLG is a MAGUK protein that presents an L27 domain, 3 PDZ domains, an SH3 domain, a HOOK domain and a Guanylate like domain. DLG is a member of the basolateral polarity complex SCRIBBLE, and is involved in processes such as cell division, cell migration and cell polarity [69] The N-terminal L27 domain interacts with the two L27 domains of calcium/calmodulin-dependent serine protein kinase (CASK) [70]. CASK is a membrane-associated guanylate kinase and a scaffolding protein, that has been

demonstrated to recruit or organize other proteins at the plasma membrane to coordinate signal transduction pathways within the cytoplasm and nucleus [71]. A study done in 2016, using a cell mechanical stretch device shows that CASK expression and localization to the basal membrane is needed for the inhibition of proliferation of cells under cyclic stretch [72]. The authors show that CASK interacts with ß1-integrin, however, the tension-driven interaction between these two proteins still has to be formally proven. Depletion of CASK results in aberrant proliferation of cells under mechanical stress demonstrating that CASK localization under tension is important for the mechanoregulation of the cell division [72]. In 2019, Porter et al. demonstrated that the direct interaction of CASK with DLG is required for normal cortical recruitment of NUMA, a key component of spindle orientation machinery [73]. Disruption of this interaction affects the integrity of epithelial architecture and results in misoriented cell division that give rise to multilumen cyst in 3D. Since the formation of 3D cysts generates an increase in tension at the cell–cell interface [74], it is tempting to speculate that CASK is recruited at the cell–cell interface in a force-dependent manner and then will recruit DLG allowing the formation of well polarized 3D cysts.

DLG interacts with the C terminal PDZ binding domain of CD97 and together these proteins are part of the adherens junctional signaling complex composed of E-cadherin and catenins [75]. This macromolecular complex is linked to the F actin cortex and is thus submitted to cellular forces. The localization of CD97 at the plasma membrane is actin dependent as blocking actin polymerization and elongation prevents its membrane localization. When present at the cell membrane CD97 strengthens the adherens junction (AJs) [76] while deletion of its PDZ binding domain results in the loss of cell–cell contacts [77] upon mechanical shear stress. Taken together these data show a role of CD97 in the mechanoregulation of cell–cell contacts. In a recent study, mechanical stimulation of epithelial cells applied by using shear stress or wound assay results in a rapid phosphorylation of Ser740 of CD97. This phosphorylation disrupts the binding of DLG1 to the PDZ binding domain of CD97, and correlates with a disorganization of the actin cytoskeleton. PKC contributes to the mechanical force induced cellular responses [78] and is a potential candidate to phosphorylate CD97 at S740. PKCα interacts via its PBM with PDZ3 domain of DLG1 [79]. Upon mechanical stress, PKCα might be recruited to the cell membrane via DLG1 and will then trigger phosphorylation of CD97 causing F-actin depolymerization, loss of cell–cell contact, and DLG1 detachment. This signaling pathway when activated induces depolymerization of actin and loss of cell–cell contacts, that will result in a relaxation of the tension at this particular cellular junction avoiding tissue breaking.

4.3 Afadin

Afadin is a filamentous actin binding protein with two Ras domains, a forkhead association domain, a dilute domain, a PDZ domain, and three proline-rich domains. Afadin is implicated in many cellular processes from cell survival, cell proliferation to cell migration and formation of the apical junctions in epithelial cells [80–83]. Afadin can interact both directly or indirectly with the actin cytoskeleton through several partners such as JAM-A, ZO1/ZO2, vinculin, and α-actinin [84–88]. Afadin is thus a strong candidate to be involved in mechanoregulated processes as many of its partners have been already linked to mechanotransduction. Interestingly, depletion of Afadin, JAM-A or double depletion of ZO1/ZO2 results in similar phenotypes with increased contractility triggered by activation of RhoA and phosphorylation of myosin-light chain [89–91]. In response to ZO1/ZO2 double depletion, Afadin is recruited to the cellular cell contacts, and inhibiting contractility perturbs the homogeneous localization of Afadin. Thus, myosin contractility is essential for maintaining Afadin uniform distribution along the zonula adherens (ZA). Removing Afadin in ZO1/ZO2 depleted cells specifically altered actomyosin architecture at the ZA of tricellular junctions where actin cables are anchored. This perturbation is accompanied by discontinuities in the E-cadherin, claudin, and occludin stainings [92]. Taken together, this data shows a synergy between ZO and Afadin depletions in disrupting tissue integrity under tension. Afadin may therefore act as a robust protein scaffold that maintains ZA architecture at tricellular junctions.

JAM-A, a component of the TJs, via its PDZ binding motif, can associate with signaling molecules such as scaffold PDZ proteins, ZO1/2, and Afadin, as well as with the guanine exchange factor PDZ-GEF2 [83, 93]. Monteiro et al. demonstrate that JAM-A interacts with Afadin, ZO2, and PDZ-GEF1 to activate the small GTPase Rap2c [89]. The activation of Rap2c controls the contraction of the apical cytoskeleton regulating the epithelial permeability to prevent cell injury. In this study, the authors also mention that Afadin is able to immunoprecipitate a doublet of JAM-A that might represent phosphorylated forms of JAM-A, although they did not investigate this phosphorylation but another team did. Scott at al show that mechanical stimulation can trigger phosphorylation of JAM-A [94]. Forces were applied by using fluid shear stress or by using magnetic tweezer. After applying forces, several biochemical analyses were performed that demonstrate that tension induces rapid phosphorylation of JAM-A S284. This phosphorylation of JAM-A induces activation of RhoA by PKCζ triggering cell stiffening by modifying actin cytoskeleton. These results clearly demonstrate that JAM-A is a direct transducer of mechanical forces. Interestingly, when JAM-A is localized at the TJs which are under high levels of tension [95] it is phosphorylated [96]. It is thus tempting to speculate that mechanical tension, above a certain

threshold might induce the phosphorylation of JAM-A. This phosphorylation will control the affinity for Afadin thereby controlling the contraction of apical cytoskeleton that allows for the maintenance of ZA and TJs.

4.4 SCRIBBLE

SCRIBBLE is a classical multimodular scaffold protein that contains 16 leucine-rich repeats (LRR) and four PDZ domains. N-terminal LRR domain can associate with LGL and is required for the association with the lateral cortex and the establishment of apicobasal polarity [97, 98]. The C-terminal PDZ domains of SCRIBBLE can interact with a diverse range of proteins such as β-Pix [99], Vangl2 [100], and LGN [101], allowing for cell polarization, cell migration [102], establishment of planar cell polarity, and cell division, respectively. In epithelial cells, SCRIBBLE is required for normal intercellular adhesion by stabilizing E-cadherin–p120 catenin at the plasma membrane [103]. 10 years ago, E-cadherin complex was tagged as a mechanosensor complex. Since then many studies using different approaches such as FRET sensor, magnetic tweezer, and cell stretching have confirmed that E-cadherin can sense changes in mechanical forces within an epithelial monolayer [104–106]. The role of SCRIBBLE in mechanotransduction has, however, not been investigated so far. Some hints based on the function of SCRIBBLE in the stabilization of E-cadherin point to a role in the mechanoregulation of AJs. E-cadherin receptors form adhesive clusters that are coupled to the contractile actomyosin cortex [107, 108]. At the cellular level, E-cadherin adhesion not only binds cells together but also mechanically couples the contractile modules of neighboring cells together to generate junctional tension [109]. This junctional tension has been mainly treated as homogeneous along the cell–cell interface. However, E-cadherin forms clusters of different sizes along the junctions between cells [108–110]. Interestingly, despite overall basolateral localization, SCRIBBLE is enriched at the ZAs, where it stabilizes E-cadherin–p120 catenin adhesions [111, 112]. At the level of the ZAs, clusters of stabilized E-cadherin are linked to large actomyosin bundles [113–115]. Below the AJs, at the lateral adherens junctions (LAJs), E-cadherin is coupled to less aligned actomyosin network. Contractile tension is thereby greater at the ZA than at the LAJ. SCRIBBLE could be enriched at the ZA in a tension-dependent manner, and thereby by stabilizing E-cadherin could be implicated in a feedback positive loop in the generation of forces at the ZAs. This tension driven localization of SCRIBBLE to E-cadherin can also take place during the orientation of the mitotic spindle to align epithelial cell division. In 2017, Hart et al., by applying uniaxial stretch on epithelial cells, showed that cell division aligned with the stretch axis [116]. The orientation of this cell division axis requires trans-engagement of E-cadherin adhesions and involves tension-dependent recruitment of LGN to

E-cadherin. LGN can directly interact with the cytosolic tail of E-cadherin that localizes LGN at the cell–cell contacts [117]. Increase in tension does not trigger a polarized distribution of E-cadherin, while LGN and myosin IIA are polarized suggesting that an additional intermediate, LGN interacting protein might be involved in its polarized localization. This protein could be SCRIBBLE as other ones (DLG, Afadin, ERM proteins) were ruled out in this study. SCRIBBLE is indeed able to interact with LGN, and the interaction between LGN and E-cadherin is reduced in cells depleted for SCRIBBLE. The formation of this ternary complex is also important for proper cell division [101]. Taken together, all these data point to SCRIBBLE as a likely "second line" mechanotransducer to trigger signaling pathways downstream of the mechanosensitive E-cadherin units.

5 Concluding Remarks

In the last two decades, mechanical stimuli have emerged as essential regulators of several biological processes. Mechanotransduction has revealed a new layer of control of the interaction between proteins, and will potentially lead to global guiding principles for the organization of complex living systems. The convergent interests from physicists and biologists to understand the complexity of integrated systems have led to the development of new biophysical tools. These new technological advances such as stretching devices, optical/magnetic tweezers, or atomic force microscopy have enabled to measure and apply controlled forces on cells. Applying different forces on cells trigger several cellular responses and activate different signaling pathways that have led to the identification of an emerging mechanotransducing function for PDZ proteins. These mechanotransducers can participate in mechanoreception and mechanotransmission as direct mechanosensitive components or as second line mechanotransducers.

Pulling forces generated by the actomyosin network can stretch molecules exposing their cryptic binding sites or cryptic phosphorylation sites, triggering specific signaling pathways [5, 118]. In the case of ZO1, physical forces are responsible for the stretching of the molecule that is needed for its correct localization while allowing for the interaction with its different partners. This activated unfolded ZO1 protein is important for the maintenance of the TJs. The actomyosin network can thus directly by its contractions pull on proteins but also on the actin cytoskeleton, resulting in flow of material that leads to the formation of polarized clusters of proteins at the cell cortex that allow for adaptation of cell cytoskeleton [51, 119]. Under higher cortical tension PAR3 clusters and MUPP1 is recruited to the cell cortex. The tension-dependent clustering of PAR3 could promote its oligomerization at the cell

cortex where it could recruit PAR6 and aPKC, leading to the establishment of anteroposterior or apicobasal polarity. The recruitment of the PDZ protein MUPP1 to the TJs is key to maintaining the integrity of the TJ barrier during hyperosmotic stress. Finally, loading forces can regulate cell adhesion machineries [105, 106, 120]. The adhesive contacts can adapt to external forces, by modifying their binding to specific proteins, their structural conformations, their stability but also their links to the actin cytoskeleton. Some PDZ proteins such as SCRIBBLE, Afadin, or ZO1 can, upon loading forces, strengthen the cell adhesion machinery at the ZA and TJs localization respectively to preserve epithelial homeostasis by allowing for the proper polarization of the tissue and the tethering of the adhesions to the actin cytoskeleton. In these cases, the connections between adhesions are strengthened by assembly of new components, however in other cases the connections can be severed [121]. DLG is located at cell–cell contacts and upon forces generation will recruit aPKC at the ZA. The recruitment of aPKC by DLG at the ZA can then regulate the load of tension at the cell–cell contact by controlling the depolymerization of actin and disruption of cell–cell contact leading to a relaxation of the tissue when the forces are too elevated.

The active nature of cell adhesion machineries implies that the time from the mechanosensing to the mechanoresponse is slow and occurs within seconds to minutes allowing the occurrence of many different interactions between proteins [122–124]. In the case of PDZ scaffolds proteins, this could trigger specific interactions allowing for the cells to have a multimodal adaptive "repertoire" to properly adapt their responses to the change in forces.

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