Regulation of $\alpha$-catenin conformation at cadherin adhesions

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Abstract
Cells in our body utilize a variety of adaptor proteins for transmitting context specific signals that arise from the cellular microenvironment. Adaptor proteins lack enzymatic activity and typically perform their function by acting as scaffolds that bind other signaling proteins. While most adaptor proteins are functionally modulated by biochemical alterations such as phosphorylation, a subset of adaptor proteins are functionally modulated by a mechanical alteration in their structure that makes cryptic sites available for binding to downstream signaling proteins. $\alpha$-catenin is one such adaptor protein that localizes to cadherin-based cell adhesions by binding the membrane-localized cadherin-$\beta$-catenin complex at one side and the cytosolic F-actin on the other side. An increase in actomyosin tension is directly relayed to $\alpha$-catenin resulting in a change in its conformation making cryptic binding sites accessible to its interacting partners. Here, I describe an updated view of the mechanical regulation of $\alpha$-catenin in the context of cellular adhesion, including the role of cadherin clustering in its activation.

Keywords: $\alpha$-catenin, Actin, $\beta$-catenin, Cadherin, Conformation, Mechanical signaling, Phosphorylation

1. Introduction

Cells respond to microenvironmental signals by activating specific signal transduction pathways to produce an appropriate response (Pawson, 1995; Downward, 2001). These signal transduction pathways often consist of i) a receptor protein that senses the signal, ii) downstream proteins that relay the signal from the receptor protein and iii) transcription factors that regulate gene expression. Some of the downstream signal proteins function as scaffolds for other proteins and are referred to as adaptor proteins (Pawson and Scott, 1997; Flynn, 2001; Samelson, 2002; Mayer, 2008). These are structurally dynamic proteins and contain binding sites for other proteins that are biochemically regulated by the activity status of the particular signaling pathway. Thus, activation of a receptor, such tyrosine kinases, could lead to phosphorylation of an adaptor protein, which in turn, could bind other signalling proteins, thus allowing signal relay.

While this general principle of adaptor protein function in cellular signal transduction is applicable for a large number of signaling pathways, a new class of adaptor proteins has emerged that are directly regulated by mechanical forces in the cell. These are often part of mechanical signal transduction pathways, and are activated upon force-mediated structural change, which then allows them to bind other proteins. Such mechanical regulation of adaptor proteins could be understood in terms of their dynamics and allostery (Pruitt et al., 2014; Biswas et al., 2008; Saha et al., 2009; Biswas and Visweswariah, 2011; Biswas et al., 2015; Biswas, 2017). That is, proteins in solution could exist in a variety of possible conformations, including the active one, with a finite probability (conformational equilibrium). Application of mechanical force could act as an allosteric modulator and shift the conformational equilibrium of the protein towards the active conformation, much like ligand binding-induced allosteric regulation (Cooper and Dryden, 1984; Cui and Karplus, 2008; Fenton, 2008; Tsai et al., 2008; Tsai et al., 2009). This shift in the equilibrium increases the probability of accessibility of any cryptic site in the protein leading to an effective increase in the affinity of the protein for its binding partner.

Cell-cell and cell-matrix adhesions are key cellular structures that enable cells to sense their mechanical environment in a tissue via adaptor proteins such as $\alpha$-catenin (Kobiak and Fuchs, 2004; Dufour et al., 2013; Biswas and Zaidel-Bar, 2017) and talin (Yan et al., 2015). Specifically, cadherin-based cell-cell adhesions allow cells to sense the mechanical tension in the epithelial and other tissues. An increase in mechanical tension in the tissue such as when it is externally stretched results in an increase in the strength of the cell-cell adhesion (Ladoux et al., 2010; le Duc et al., 2010; Thomas...
et al., 2013). This has been proposed to occur by a force-dependent conformation change in α-catenin from a ‘closed’ to an ‘open’ structure (Yonemura et al., 2010). α-catenin is a multidomain protein, and associates with cadherin-based adhesion via binding to β-catenin on one side and F-actin on the other side. While α-catenin has emerged as the primary mediator of mechanical signal transduction from cadherin-based adhesions, it has also been reported to undergo β-catenin dependent nuclear translocation where it inhibits transcription of a set of genes by altering the nuclear actin organization (Daugherty et al., 2014) or serve as a tumor suppressor in basal-like breast cancer independent of E-cadherin (Piao et al., 2014; Sun et al., 2014). Here, I focus on the mechanism of epithelial α-catenin (αE-catenin; henceforth referred to as α-catenin)-mediated mechanical signal transduction at cadherin adhesions.

2. Structural features of α-catenin

The key to understanding the mechanical signal transduction via α-catenin is the elucidation of its structure. Indeed, a number of structural studies on α-catenin have been reported in the literature that provides insights into the mechanism of mechanical regulation of α-catenin. The human α-catenin is a 906 amino acid long, 102 kDa protein and shares homology with vinculin, and thus the domains in α-catenin are sometimes referred to as vinculin homology domain 1, 2 and 3 (VH1, VH2 and VH3) (Nagafuchi et al., 1991; Dickinson et al., 2011; Choi et al., 2012; Hiroki, 2012). However, structural studies have revealed it to consist of four domains of helical bundles with an N-terminal β-catenin binding or dimerization domain, vinculin binding domain, a regulatory M domain and a C-terminal F-actin binding domain. The C-terminal of α-catenin contains the F-actin binding domain (Choi et al., 2012; Rangarajan and Izard, 2013). Following which is the vinculin binding domain (or site) and the regulatory M fragment or domain. The C-terminal of α-catenin forms a tripartite complex linking the adhesion receptor to the actin cytoskeleton. In fact, α-catenin binds to β-catenin with a high affinity ($K_d = 20$ nM), and this affinity is increased allosterically by the binding of the β-catenin to the intracellular domain of cadherin (Pokutta et al., 2014). However, the monomeric α-catenin in the tripartite complex has low affinity for F-actin and does not bind F-actin in solution (Drees et al., 2005; Yamada et al., 2005).

On the other hand, the cytosolic, dimeric form of α-catenin is known to bind F-actin with high affinity in solution, and alter actin dynamics by inhibiting actin polymerization or filament severing by cofilin, independent of the adhesion associated cadherin-β-catenin complex (Drees et al., 2005; Yamada et al., 2005; Benjamin et al., 2010; Hansen et al.,...
Structural studies have revealed an asymmetry in the dimerization of α-catenin, which explains the ability of the dimer to bind F-actin with a higher affinity than the monomer (Rangarajan and Izard, 2013). While it has been traditionally not thought to be consequential in cell adhesion and mechanical signal transduction, recent reports posit the dimeric α-catenin as an important player in cadherin adhesion. For instance, cells expressing a chimeric construct of the cadherin intracellular domain-β-catenin and α-catenin, which was designed to be constitutively monomeric, were found to form weaker adhesion in comparison to those which expressed the dimeric α-catenin (Bianchini et al., 2015). More recently, it has been shown that dimeric, but not the monomeric, α-catenin binds to phosphatidylinositol-3,4,5-trisphosphate-containing lipid membranes, and mutation of the residues important for this bind results in the formation of weaker adhesion (Wood et al., 2017). It is important to note that these properties of α-catenin cannot be generalized across different species since significant biochemical and biophysical differences have been reported between different species (Miller et al., 2013).

### 3. Interacting partners of α-catenin

True to its classification as an adaptor protein, α-catenin interacts with a number of cellular proteins through binding sites that are spread all across its length (Vasioukhin and Fuchs, 2001; Kobielaek and Fuchs, 2004; Maiden and Hardin, 2011) (Fig. 1C). α-catenin localizes to the cadherin adhesion through its interaction with β-catenin, which in turn, interacts with the intracellular domains of cadherins. It is important to note that this interaction predates the emergence of cell adhesion proteins such as the classical cadherins since the α-catenin: β-catenin complex is required for the formation of a polarized epithelium, polarize secretion of proteins as well as morphogenesis in the multicellular, non-metazoan *Dictyostelium discoideum* (slime mold), which do not possess the classical cadherin genes in its genome (Dickinson et al., 2011). While α-catenin can exist either as a monomer or a dimer, it is only the monomeric form of α-catenin that interacts with β-catenin. The site for the high affinity, constitutive interaction with β-catenin lies in the N-terminal region of α-catenin. Importantly, the affinity of α-catenin:β-catenin interaction increases when β-catenin is bound to the cadherin intracellular domain (Koslov et al., 1997; Pokutta et al., 2014). However, the α-catenin:β-catenin interaction is insensitive to the binding of F-actin to the C-terminal of α-catenin (Pokutta et al., 2014).

Unlike the constitutive interaction with β-catenin, the monomeric α-catenin associated with cadherin-β-catenin complex interacts with F-actin with very low affinity (Buckley et al., 2014), and could not be detected in solution-based, biochemical assays (Drees et al., 2005; Yamada et al., 2005). This is due to the fact that the F-actin binding in the C-terminal F-actin binding domain is occluded in the ‘closed’, autoinhibited α-structure. Single molecule experiments have revealed that this interaction shows a catch-bond like behaviour and thus, application of force results in an increase in the life-time of the interaction (Buckley et al., 2014). Given the observation that cadherin molecules are under constitutive actomyosin-generated tension in live cells (Borghini et al., 2012), it is likely that α-catenin associated with the cadherin molecules are also under tension and are thus, likely to bind F-actin in live cells. Similarly to the F-actin binding domain, the vinculin-binding domain is also ‘cryptic’ or inaccessible in the ‘closed’, autoinhibited conformation of α-catenin (Ishiyama et al., 2013), thus it shows very low affinity for vinculin. Solution-based assays have revealed that vinculin binding is associated with large structural changes in α-catenin (Rangarajan and Izard, 2012). It is important to note that vinculin itself requires activation from an inactive conformation for it to be able to bind α-catenin, and the inhibitory tail domain of vinculin could displace α-catenin from the α-catenin-vinculin complex in solution (Choi et al., 2012; Rangarajan and Izard, 2012). *In vitro* single molecule force spectroscopy experiments have shown that application of force causes changes in the structure of α-catenin that opens up the cryptic vinculin binding site, leading to a large increase in the affinity of α-catenin for vinculin (Yao et al., 2014). Additionally, solution-based studies suggest that binding of F-actin to α-catenin can stabilize this interaction (Rangarajan and Izard, 2012).

Other cytoskeleton regulatory proteins that α-catenin has been shown to interact with are actin nucleator protein formin-1 (Kobielaek et al., 2004), F-actin cross-linking protein α-actinin (Knudsen et al., 1995; Nieset et al., 1997), F-actin binding protein afadin (Pokutta et al., 2002) through the central regulatory domains, F-actin binding protein EPLIN (epithelial protein lost in neoplasms) (Abe and Takeichi, 2008) and tight junction protein Zonula occludens-1 (ZO-1) (Itoh et al., 1997) through the C-terminal F-actin binding domain (Fig. 1C). Additionally, a biotinylation-based protein proximity assay has indicated a force-dependent interaction of α-catenin and myosin IIA suggesting a closer juxtaposition, if not a direct interaction, between the two proteins under force (Ueda et al., 2015). More recently, α-catenin has been implicated in the cell proliferation signaling through an interaction with Yap1 & 14-3-3 protein (Kim et al., 2011; Schlegelmilch et al., 2011; Silvis et al., 2011; Benham-Pyle et al., 2015). Considering its three-dimensional structure, it appears that α-catenin cannot interact with all these proteins simultaneously. Thus, it is likely that these interactions are either spatially or temporally regulated in the cell. Importantly, the binding of one could allosterically alter...
the binding of another protein. For instance, binding of α-catenin to the cadherin intracellular domain:β-catenin complex allosterically increases the affinity of E-cadherin-p120-catenin, another adaptor protein that found at the cadherin adhesion, interaction (Troyanovsky et al., 2011).

4. Phosphorylation-mediated regulation of α-catenin

While binding of interacting partners is key to the transduction of signaling via adaptor proteins, it is the post-translational modifications such as phosphorylation (in which a phosphate group is enzymatically added by protein kinases to serine, threonine or tyrosine amino acid residues) are “turn on” switches for adaptor proteins to bind their interacting partners (Jin and Pawson, 2012). Subsequently, dephosphorylation (in which the phosphate group is enzymatically removed by protein phosphatases) serves as the “turn off” switch. This fundamental mechanism of adaptor protein function appears to be conserved in the case of α-catenin as well. Several studies have indicated that α-catenin can be phosphorylated at the cadherin adhesion (Burks and Agazie, 2006; Ji et al., 2009), where a number of protein kinases and phosphatases are sequestered (Bertocchi et al., 2012). A recent detailed biochemical and cell biological analysis have revealed several conserved serine and threonine residues in the linker region between the regulatory M domain and the C-terminal F-actin domain to be phosphorylated in a dual kinase mechanism involving casein kinase 1 and 2 (Escobar et al., 2015). Importantly, mutational analysis of these residues revealed a role of phosphorylation in local structural changes, thus, implicating them in the mechanical regulation of α-catenin.

5. Mechanical regulation – multiple conformations of α-catenin

It is well known that epithelial cells sense mechanical signals from the cellular microenvironment by strengthening cadherin adhesions in response to increased mechanical tension in the tissue. In addition to the immediate increase in the strength of cadherin adhesions, increased mechanical tension in the tissue also causes changes in the cellular gene expression profile in the long term (Benham-Pyle et al., 2015). The key to this mode of mechanical regulation of cadherin adhesions is thought to be a conformational change in α-catenin (Yonemura et al., 2010), and not a change in the affinity between cadherin molecules (Barry et al., 2014). It is understood that an increase in mechanical tension on cadherin adhesion results in a conformational activation of α-catenin from a ‘closed’ to an ‘open’ conformation, which is present ‘sandwiched’ between β-catenin and F-actin (Yonemura et al., 2010; Ishiyama et al., 2013; Buckley et al., 2014; Yao et al., 2014; Kim et al., 2015). This, in turn, promotes interaction with F-actin and also enables recruitment of vinculin to the cadherin adhesions through binding to the available cryptic sites in α-catenin. The recruitment of vinculin further increases the interaction of F-actin with cadherin adhesions. This model of α-catenin-mediated mechanical regulation of cadherin adhesion is based on several independent observations. Among the very first indication of a force-dependent conformation change in α-catenin came from immunostaining of cells with a conformationally sensitive antibody, α18, which recognizes an epitope in the regulatory M domains (Yonemura et al., 2010). Under normal actomyosin tension, α-catenin localized to cadherin adhesions showed binding to α18. However, when cells were treated with pharmacological inhibitors that decrease intracellular acto-myosin tension, α-catenin assumes a ‘closed’ conformation due to inter-domain interaction in α-catenin, and the α18 epitope is stearically occluded leading to a loss in α18 binding. While the ‘open’ conformation has been deduced from biophysical and structural studies (Ishiyama et al., 2013), the ‘closed’ conformation has been directly observed in the crystal structure of the full-length α-catenin (Rangarajan and Izard, 2013), thus lending support to the two-state theory of α-catenin conformation. The force-dependent conformational change in α-catenin has been observed in vitro in two different single molecule force spectroscopy experiments. First, stretching of single α-catenin molecules resulted in a large increase in its affinity for the vinculin head domain, a construct of vinculin that does not contain the autoinhibitory tail region and, therefore, not autoinhibited (Yao et al., 2014). Second, it was shown that application of force on F-actin resulted in an increase in its binding lifetime with α-catenin leading to the proposal of a two-state model with differential affinity to describe the regulation of the α-catenin-F-actin interaction (Buckley et al., 2014).
Subsequent cell-based studies revealed further details of regulation of α-catenin conformation. An intra-molecular FRET-based conformational sensor construct, wherein a pair fluorescent proteins were introduced in α-catenin flanking the regulatory M-domain, showed a cadherin-adhesion dependent change in the FRET ratio and thus, α-catenin conformation (Kim et al., 2015). More importantly, substrate stretching-induced application of force on cadherin adhesion resulted in a further decrease in the FRET ratio perhaps resembling the force-induced unfolding of certain domains in the protein as seen in the single molecule force spectroscopy study (Yao et al., 2014), which recovered after the release of the force indicating the reversible nature of the α-catenin conformation. On the other hand, a hybrid live cell-supported lipid bilayer assay, wherein cells interact with the cadherin extracellular domain displayed on a synthetic, supported lipid bilayer and form adhesion (Biswas et al., 2015; Biswas et al., 2016; Biswas and Zaidel-Bar, 2017), showed that α-catenin is conformationally activated, as assessed by the binding of the α18 antibody (Yonemura et al.,

Fig. 2 Mechanical regulation of α-catenin. A schematic representation of mechanical regulation of α-catenin showing some of its possible conformational states: 1. A closed, autoinhibited state in which the vinculin- and F-actin-binding domains cannot bind vinculin and F-actin, respectively; 2. An intermediate state that is partially open state with the F-actin binding domain binding F-actin; 3. An open state wherein both the vinculin- and F-actin-binding domains bind to vinculin and F-actin, respectively; 4. A partially open state in which the vinculin binding domain is bound to the vinculin head domain while the F-actin binding domain is free. Note that in all instances, α-catenin is bound to the E-cadherin intracellular domain-β-catenin complex and the direction of the cytoskeletal force applied is indicated with arrows.
2010), during the micron-scale clustering of cadherin molecules, a process that is regulated by α-catenin (Chen et al., 2015). Importantly, α-catenin persisted in the active conformation at the hybrid live cell-bilayer junctions even after a reduction in the actomyosin tension in the cell (Biswas et al., 2016), which is in contrast to the observation of loss of α18 staining upon reduction of actomyosin tension in cell-cell junctions in monolayer cell culture (Yonemura et al., 2010) or the increase in the FRET efficiency of the α-catenin conformational sensor (Kim et al., 2015). There are several differences between these studies such as the use of different cell lines which may possess different biochemical regulatory pathways or the physical organization of the adhesion including asymmetric force applied by the single cell on the hybrid live cell-bilayer adhesion vs. symmetric force applied by two apposing cells on the cell-cell adhesion between cells in a monolayer culture. However, the size of cadherin clusters formed by the cells in the two systems is perhaps the key reason for the difference in the α18 staining. That is, adhesion formed in the hybrid live cell-bilayer system contain large, micron scale clusters, a feature that is correlated with the levels of activated α-catenin (Biswas et al., 2016), and therefore, it is possible that the large clusters of cadherin sustain α-catenin in a conformational state that still binds the α18 antibody. It could also be due to a post-translational modification such as phosphorylation in the linker region between the regulatory M domain and the F-actin binding domain (Escobar et al., 2015) or binding of another interacting protein that binds to the central part of α-catenin such as α-actinin (Fig. 1C) (Zaidel-Bar, 2013).

The idea that α-catenin samples multiple conformations during a force cycle in the cellular context as alluded above (Fig. 2) is reinforced by the observation that the intramolecular interactions (Li et al., 2015) in the auto-inhibited ‘closed’ conformation of α-catenin are not as strong as seen in other mechanically regulated adaptor proteins. For instance, the vinculin head domain can bind to the vinculin binding site in α-catenin in solution suggesting that the biochemical interaction between α-catenin and vinculin head domain could overcome the interdomain interactions in α-catenin in the absence of any physical force (Choi et al., 2012; Ishiyama et al., 2013). In contrast, full-length vinculin, which also undergoes a similar ‘closed’ to ‘open’ conformational activation (Izard et al., 2004; Chen et al., 2006; Ishiyama et al., 2013), cannot do so suggesting that the inhibitory C-terminal tail domain of vinculin has a stronger interaction with the head domain (solution $K_d$ value of 0.3-1 nM compared to the α-catenin-vinculin $K_d$ value of 80-100 nM) (Johnson and Craig, 1994; Bakolitsa et al., 2004; Choi et al., 2012; Peng et al., 2012; Yao et al., 2014). In addition to these in vitro assays, such differences between α-catenin and vinculin has also been seen in cells in the hybrid live cell-supported lipid bilayer assay (Biswas et al., 2016). First, vinculin specifically associated with the peripheral cadherin clusters that localized with F-actin and not with the central cadherin clusters that did not localize with F-actin. Second, while α-catenin persisted in the open conformation upon reduction of cellular acto-myosin tension, vinculin did not localize to the cadherin clusters. Thus, the mechanically tensioned interaction of α-catenin and F-actin at the peripheral cadherin clusters allows α-catenin to activate vinculin, which is not possible in the absence of mechanical tension (Choi et al., 2012; Peng et al., 2012; Yao et al., 2014). More importantly, a recent single molecule force spectroscopy study has shown the existence of multiple states of α-catenin (Maki et al., 2016). That is, application of force on α-catenin resulted in its activation as well as mechanical stabilization. This is further reinforced by vinculin binding, leading to the transition of α-catenin to an even more mechanically stable conformational state.

Based on the points presented above, I propose the following mechano-biochemical cycle that α-catenin likely undergoes at the cadherin adhesions. α-catenin bound to the cadherin intracellular domain-β-catenin complex largely stays in the ‘closed’, autoinhibited state in which neither vinculin nor F-actin can bind to their respective sites in the protein (state 1; Fig. 2). A small fraction of these proteins may explore a conformation that is amenable for F-actin binding and thus, the complex is poised for application of cytoskeletal force (state 2). The conversion of α-catenin to the ‘open’, activated conformational state (state 3; Fig. 2) is, however, dependent on the application of cytoskeletal force. That is, application of mechanical tension increases the lifetime of the α-catenin-F-actin interaction resulting in the opening of α-catenin and accessibility of the cryptic vinculin binding site, to which vinculin can bind. This conformational state of α-catenin is also amenable for biochemical modifications such as phosphorylation or binding of other interacting partners such as α-actinin. Upon release of mechanical force, F-actin could dissociate from its binding site in α-catenin (state 4; Fig. 2). Subsequently, the loss of cytoskeletal force also leads to the dissociation of vinculin from α-catenin since vinculin itself requires mechanical activation. However, vinculin binding site may persist in the accessible state for a prolonged period of time, and proteins such as the isolated vinculin or the α18 antibody could still bind. This conformational state of α-catenin is essentially ready to bind F-actin in case mechanical tension rises at the adhesion. α-catenin may finally return to its ‘closed’ state after removal of biochemical modifications that it has undergone during the activation process and in the absence of binding of its interacting partners.
6. Conclusion

To conclude, α-catenin is a versatile mechanical adaptor protein that assumes different conformations under different mechanical states of cadherin adhesions. In addition to modulation by biochemical changes such as phosphorylation, α-catenin conformation is directly regulated by the application of mechanical forces. Moreover, while α-catenin assumes a certain conformation during the process of cadherin clustering and adhesion formation, it may convert to another, mechanically more stable conformation under increased tension in the tissue. It is important to realize that while the role of these different conformations of α-catenin has been appreciated in the context of mechanical signaling at cadherin adhesions, their cellular implications in other contexts are yet to be elucidated. For instance, it remains to be seen how mechanical activation of α-catenin or which conformations of α-catenin impinges on the long term signaling arising from cadherin adhesion through the YAP/TAZ pathway, which couples to α-catenin through binding of the 14-3-3 adaptor protein to its regulatory M domain (Kim et al., 2011; Schlegelmilch et al., 2011; Silvis et al., 2011; Benham-Pyle et al., 2015).

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