J. Indian Inst. Sci.
A Multidisciplinary Reviews Journal
ISSN: 0970-4140 Coden-JIISAD

REVIEW ARTICLE

Role of Actin Cytoskeleton in E-cadherin-Based Cell–Cell Adhesion Assembly and Maintenance

Saad Rasool[†], *Anupriya M. Geethakumari*[†] *and Kabir H. Biswas*[*]

Abstract | The cellular cytoskeleton consisting of microtubules, intermediate filaments and the actin filaments is a dynamic structure providing shape and structural stability to cells. Particularly, the actin cytoskeleton formed by a combination of polymerized actin molecules and several other actin binding proteins including myosin is key to sensing and development of mechanical forces in cells. Given this and other features, the actin cytoskeleton has been implicated in a variety of cellular process including cellular motility and migration, cytokinesis, phagocytosis, cytoplasmic streaming, organelle transport, cellular transformation and metastasis, cellular metabolism, cell–matrix adhesion, and cell–cell adhesion. The latter is mediated by E-cadherin in the epithelial tissue and is fundamental to tissue morphogenesis and normal development. Here we discuss the role of the actin cytoskeleton in the assembly and maintenance of E-cadherin-based cell–cell adhesion through the formation of cellular appendages such as filopodia and lamellipodia and thus, impinging on one of the fundamental features of multicellular organisms.

Keywords: *Actomyosin, Adhesion, Cadherin, Cytoskeleton, Filopodia, Lamellipodia*

1 The Actin Cytoskeleton

Emerging from the assembly of monomeric globular actin molecules (G-actin) into double helical filaments (F-actin), in combination with various myosins (the acto-myosin complex), the actin cytoskeleton has been implicated in a wide variety of cellular process.[1–3] For instance, it is acutely critical for the physical movement of cells, i.e. cellular motility and migration, a process that is essential for many biological phenomena under normal processes, such as embryonic development, tissue formation as well as disease conditions such as cancer metastasis and wound repair, which is acutely dependent on the dynamic assembly and disassembly of the actin cytoskeleton.[4,5] Actin cytoskeleton is also required for cellular phenomena such as cytokinesis[6], the process by which a cell divides into two, phagocytosis[7], a process utilized by cells to engulf large particles such as during removal of pathogens, cytoskeletal streaming and organelle transport[8], processes that facilitate intracellular movement of cytoplasmic materials and organelles as well as cellular transformation and metastasis[9], processes that result in development and dissemination of cancer cells to different parts of the body. Furthermore, the actin cytoskeleton has also been implicated in regulating cellular metabolism through which cells convert external sources of energy to more useful forms enabling cellular processes[10] and cellular adhesion such as the cell-extracellular matrix adhesion or cell–cell adhesion through which cells interact with their microenvironment[11].

1.1 F-actin Assembly

To enable the above mentioned diverse set of functions, the assembly and disassembly of the actin cytoskeleton is required to be a continuous and dynamic process.[1,3] However, actin monomers (G-actin) do not assemble into filaments spontaneously. This is largely due to a weak inter-monomeric interactions in actin dimers or trimers as well as effective sequestering of the

[†] *Saad Rasool and Anupriya M. Geethakumari Equal contribution.*
[1] *College of Health and Life Sciences, Hamad Bin Khalifa University, Doha, Qatar.*
**kbiswas@hbku.edu.qa*

monomers by actin binding proteins such as profilin and β-thymosin.[12,13] However, this inhibition could be overcome by actin nucleators or nucleation complexes resulting in the formation of filamentous actin.[14,15] Till date, there are three major classes of actin nucleators described in the literature: (*i*) the Actin-Related Proteins ARP2 and ARP3 (Arp2/3) complex, which is regulated by ATP, nucleation promoting factors and actin[16,17]; (*ii*) formins, which assemble to allow binding of two actin monomers and accelerate elongation of the rapidly growing ends of actin filaments by both, attracting profilin-bound G-actin and antagonizing the depletion of growing ends through the capping protein (CP)[18,19]; (*iii*) and tandem actin-binding domain nucleators (e.g. Spire, cordon-bleu (Cobl), leiomodin (Lmod), JMY and adenomatous polyposis coli (APC)), which promote actin filament initiation through monomeric actin binding to other actin-binding proteins[20–23]. Thus, multiple signals can regulate the dynamic assembly and disassembly of actin filaments to enable actin cytoskeletal functions in live cells.

1.2 Acto-Myosin Complex

While the dynamic assembly and disassembly of the actin cytoskeleton alone may enable some of its functions, many of its functions described above invariably require the development of mechanical forces on actin filaments, and thus, in individual cells. This is achieved through its interaction with the ATP-dependent motor proteins, myosins, leading to the formation of the actomyosin complex.[24] The actomyosin complex is formed between a pair of actin filaments and an individual myosin molecule. In muscle cells, the actomyosin complex is assembled as sarcomeres consisting of long parallel filaments bundled together, and a sliding movement of the actin and myosin filaments against each other upon activation results in decrease in the length of the sarcomere, and ultimately results in muscle contractions.[25] In the non-muscle cells such as epithelial cells, myosin II is considered to play a central role in the development of contractile forces through its ATP-dependent motor function.[26] Furthermore, its unique ability to assemble into bipolar filaments with motor domains positioned at both ends of the filament, is considered essential for myosin II function when presented to actin filaments of opposite polarity. Furthermore, presence of multiple actin-binding sites allows myosin II to act as an actin cytoskeleton cross-linker.[27] It is important to realize that the development of mechanical forces in the acto-myosin complexes is regulated by different signaling pathways both at the cellular and the tissue level.[28] Typically, myosin II is activated through myosin light chain kinase-mediated phosphorylation of the myosin light chain that forms a complex with the dimeric myosin heavy chain. Upon activation, myosin II generates contractile forces within the actin filaments. Also, a load-dependent activation results in the polymerization of myosin II under high tension resulting in a greater number of bipolar filaments that enable the actin cytoskeleton to withstand the increased tension.[29]

1.3 F-actin-Based Cellular Appendages

While the acto-myosin complex can take several forms, many of its cellular functions such as cell migration require it to organize into unique subcellular domains in the cell membrane protrusions such as the lamellipodia, which are broad cell membrane protrusions, and the filopodia, which are finger-like cell membrane protrusions (Fig. 1).[30] Lamellipodia are primarily composed of Arp2/3 driven branched actin networks[31,32]. Arp2/3 is activated by the Rac subfamily of small GTPases such as Rac1[33] through the regulation of the WAVE regulatory complex.[34,35] Additionally, the heterodimeric CP, which accumulates close to lamellipodial edges, and actin disassembly factors contribute to the maintenance of lamellipodia[36–38].

Filopodia, on the other hand, are usually long cellular appendages, which can grow up to 10 μm in length[39], and composed of parallel actin filaments that are compacted into dense bundles by actin cross-linkers[40]. Filopodia formation is induced by the Cdc42 Rho GTPase and are thought to extend by actin incorporation at their tips, and to retract either by rearward pulling of filaments into the cell body followed by their depolymerization[41] or by cofilin-mediated actin filament disassembly[42]. It is interesting to note that a number of proteins including VASP[43] and myosin-X[44] are enriched at the filopodial tip and that thought to regulate actin polymerization locally[45]. Overall, it is clear that these cellular appendages enable cells to physically probe their immediate microenvironment and respond to the cues, both biochemical as well as physical, present in the same.

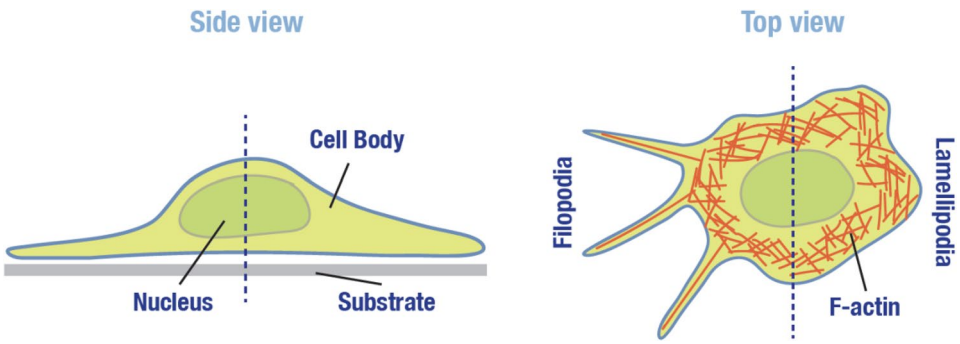

Figure 1: Schematic showing cellular cytoskeletal structures. Left panel: Side view of a cell adhering to a substrate. Right panel: Top view of an adherent cell showing actin cytoskeletal structures - filopodia and lamellipodia. F-actin is shown in red.

2 E-cadherin-Based Cell–Cell Adhesions

One of the significant roles of the actin cytoskeleton is in the regulation of E-cadherin adhesions (also known as adherens junction or zonula adherens) formed by the calcium (Ca^{2+})-dependent interaction of E-cadherin-β-catenin-α-catenin complexes between adjacent cells in the epithelial tissue.[46–48] Cell–cell adhesions are specialized cellular structures that link the actin cytoskeleton of adjacent cells, and regulate multiple cellular processes including cell proliferation and differentiation, mechanical tension sensing[49], cell polarity[50], and entry of cells into quiescent state upon mechanical stimulation[51]. At a larger scale, cell–cell adhesion plays a key role in processes such as the sorting of cells required for tissue formation[50] and contact inhibition of cell proliferation, a phenomenon in which cells stop dividing once they have formed adhesion with neighboring cells[52], and wound healing[53]. Located between the tight junctions and desmosomes in the epithelial tissue (and together forming the epithelial junctional complex that ensures selective and regulated diffusion to maintain homeostasis in organs and tissues[54]), E-cadherin adhesions are stable enough to maintain tissue cohesion, but also plastic enough to allow cell rearrangement during development[55]. Importantly, downregulation of E-cadherin gene expression, and thus, a loss of E-cadherin adhesion is a common feature of cancer originating from the epithelial tissue. Furthermore, mutations in E-cadherin that result in a loss of its function could result in cancer metastasis[56,57], suggesting a tumor suppressor activity of E-cadherin. We note that a recent report has indicated that certain cancer cells can utilize E-cadherin adhesion for their metastasis to distant organs[58].

2.1 Molecular Bases of E-cadherin Homodimerization

Mature E-cadherin is a multidomain single pass transmembrane protein consisting of an extracellular domain, a *trans*-membrane domain and intracellular domain. The extracellular domain enables homodimerization of E-cadherin, and is composed of a tandem repeat of 5 immunoglobulin-like domains (extracellular cadherin 1–5 or EC1-5)[59]. Four Ca^{2+} ion-binding sites are present interspersed between the five EC domains, each of which bind three Ca^{2+} ions. Binding of Ca^{2+} ions to these sites makes the E-cad-ECD rigid and is critical for its adhesive activity. Importantly, E-cadherin adhesion is primarily driven by the homodimerization of the E-cad-ECD domains from apposing cells. Also known as the homotypic *trans*-interaction, this relatively low affinity interaction is mediated by the EC1 domains. Biophysical studies have further revealed that the homodimerization goes through a high activation energy barrier for monomer to dimer conversion[60,61] with a slow monomer–dimer interconversion[62]. Initial encounter between monomers has been suggested to result in a weakly interacting X-dimer intermediate, which is based on the structure of the W2A mutant E-cadherin that crystalizes in this conformation[62,63]. Mutation in the X-dimer interface resulted in increased stability of E-cadherin molecules at junctions, adding support to an important role of the X-dimer intermediate in the dynamic assembly and disassembly of E-cadherin adhesions[64]. The initial X-dimer is followed by the formation of a stable dimer involving partial swapping of the N-terminal A* β-strand between the interacting monomers[62].

The swapped strands are anchored by the docking of a Trp (W2) into a pocket in EC1 domains while a positively charged N-terminal residue forms salt bridges in the dimer[62,65–67]. In agreement with structural data, a mutation of the Trp residue (W2A) results in a substantial decrease in the dimerization affinity of E-cadherin[62,63].

2.2 E-cadherin *cis* Interaction and Catch Bond

In addition to the *trans*-interaction described above, E-cad-ECD shows an interaction in *cis* i.e. between E-cadherin monomers arising from the same cell, mediated by residues present in the EC1 domain of one monomer and EC2 domain of the other monomer.[68,69] Computational as well as experimental results indicate that the *cis*-interaction, although weak as it cannot be detected in solution-based assays[70], can significantly aid in the assembly of E-cadherin adhesion through the formation of a crystal-like two-dimensional lattice of the *trans*-interacting E-cadherin dimers at the cell–cell interface.[71,72] Additionally, the X-dimer-mediated E-cadherin homodimerization has been shown to display a catch bond behavior wherein the lifetime of the E-cadherin interaction increases with the application of force with a peak at ~ 30 pN, which then decreases with additional increase in the applied force.[73] Interestingly, this behavior requires saturating Ca^{2+} ion concentration as a decrease in the same results in the loss in the catch bond behavior.[74] Thus, a combination of these extracellular domain-mediated interactions contribute to the assembly of stable E-cadherin adhesion.

2.3 E-cadherin Intracellular Domain and Mechanical Signaling

The homodimerization of E-cadherin at the extracellular side is coupled to the intracellular actin cytoskeleton through the *trans*-membrane domain, which is rich in Leu residues and may play a role in its clustering[75], and the intracellular domain. In contrast to the structure of the extracellular domain and the *trans*-membrane domain, the intracellular domain is unstructured in the absence of interacting partners. It directly interacts with the p120- and β-catenin adaptor proteins. However, it has been shown to interact with a large number of other intracellular proteins either through the p120- and β-catenin or by unknown mechanism, thus earning name as a cellular signaling hub.[76–78] Chief among these is the multidomain adaptor protein called α-catenin that is recruited to cadherin adhesions through β-catenin. Recent studies have pointed to the mechanosensory role of α-catenin in that it undergoes a force-dependent conformational change from a 'closed' to an 'open' structure.[79–81] This results in the accessibility of a cryptic binding site for vinculin, another mechanosensitive adaptor protein homologous to α-catenin, that can also bind F-actin.[82,83] Thus, an increase in the mechanical tension in the epithelial tissue results in an increased F-actin binding and strengthening of the cadherin adhesion, thus enabling cells to withstand such an increased tension.

3 Role of Actin Cytoskeleton in Cadherin Adhesion Assembly

E-cadherin at the cell–cell adhesion formed between epithelial cells is organized into micron scale clusters, which are flanked by actin–myosin II bundles located immediately next to the junctional plasma membranes (Fig. 2)[84,85]. These clusters have been further resolved into high density nanometer-scale clusters using super-resolution (3 dimensional-Stochastic Optical Reconstitution Microscopy; 3D-STORM) microscopy[86,87]. Importantly, the molecular density of E-cadherin at these nanoscale clusters and the size of the clusters are regulated by the extracellular (through the *cis*-interaction) and the intracellular domain (through the actin cytoskeleton), respectively.[86,87] Such clustering has also been observed through Fluorescence Correlation Spectroscopy (FCS) measurements in cells adhering to supported lipid bilayers in hybrid live cell-supported lipid bilayer experiments as evidenced by a significantly large reduction in the diffusion coefficient of E-cadherin.[48,88–93] However, a fundamental observation made with the super-resolution microscopy is the presence of the nanoscale clusters in the absence of extracellular domain interactions (either *trans* or *cis*) as well as in the extracellular domain deleted mutant, suggesting that these are the building blocks of E-cadherin adhesion and that the actin cytoskeleton can cause E-cadherin clustering on its own.[86] The latter agrees well with the observation of cadherin clusters in the single cell *Caenorhabditis elegans* embryos.[94]

While it could dimerize in solution, E-cadherin adhesion assembly is an active process requiring cellular energy based on the observation that a reduction in ATP levels through inhibition of cellular glycolytic pathway results in a loss in adhesion assembly[88]. Furthermore, it requires active myosin II generated tension since blebbistatin treatment of the cells results in a

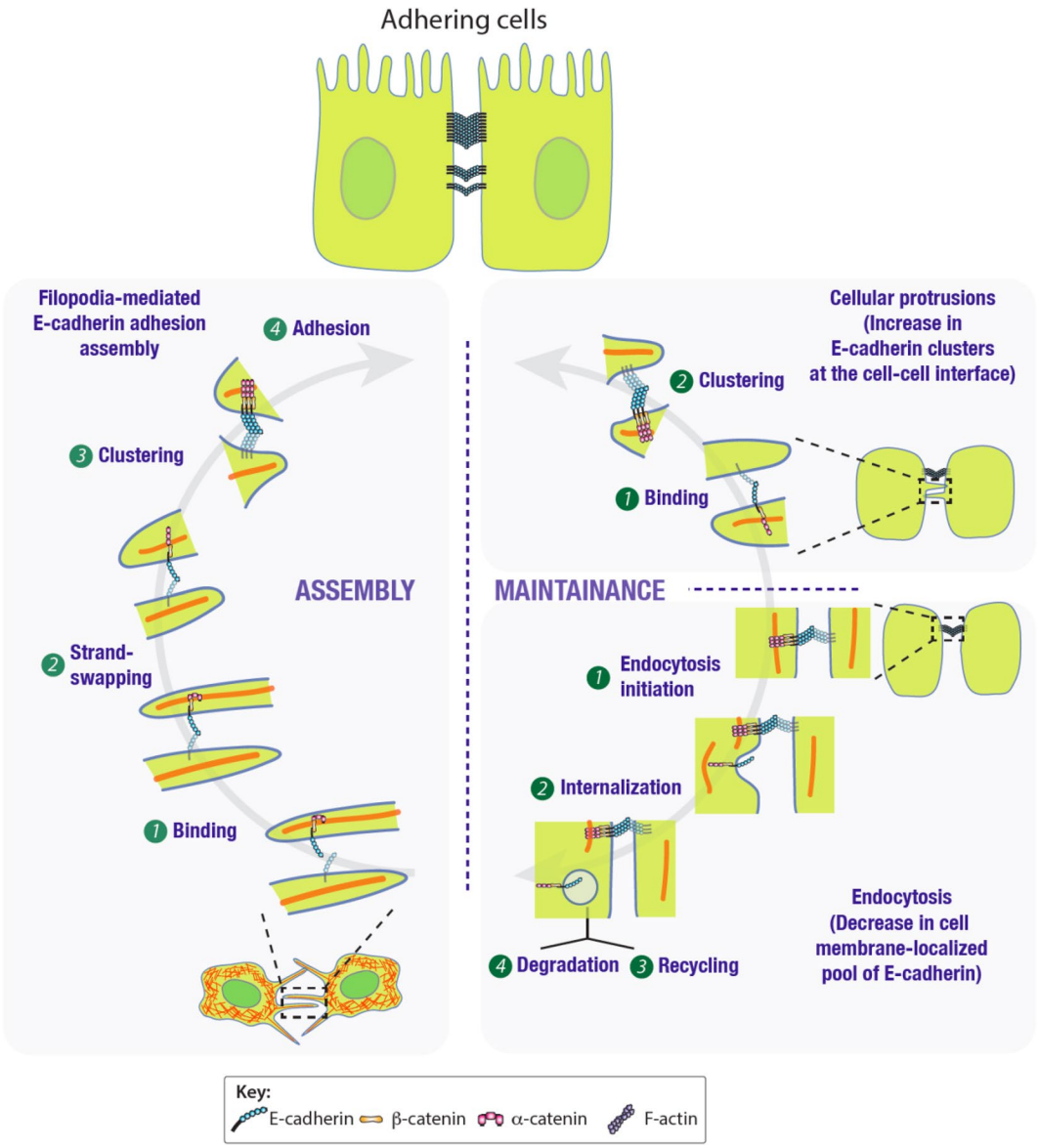

Figure 2: Schematic highlighting the role of actin cytoskeleton in the assembly and maintenance of cadherin adhesion. Top panel: Schematic of neighboring cells adhering through E-cadherin. Bottom panel: E-cadherin adhesion assembly begins with the initial interaction of E-cadherin monomers resulting in the formation of weakly bound X-dimers. Application of physical forces by the actin cytoskeleton (i.e. filopodia) leads to the maturation of the E-cadherin dimerization (i.e. strand swapping) which ultimately leads to the formation of an E-cadherin cluster through actin cytoskeletal-mediated physical movement of individual E-cadherin monomers during retraction of filopodia. Assembled E-cadherin adhesion is maintained by a combination of cytoskeletal activities such as filopodia formation and retraction and internalization of E-cadherin clusters through endocytosis.

loss in adhesion assembly.[90,95] The initial interaction between two cells occurs when filopodia from opposing cells come in contact and initiate E-cadherin interaction and clustering[96,97], a phenomenon that is consistently observed in several model organisms such as nematodes, flies and mouse embryos[98–100]. This interaction is regulated by Cdc42[101,102] and formin-dependent actin polymerization[53,103]. Following E-cadherin interaction between two cells, an expansion of the contact region between cells takes place through the Rac1-induced and Arp2/3-dependent lamellipodial activity[104,105]. The cell response to cadherin activation is characterized by two main features, including the induction of lamellipodium protrusions, and the reorganization of

E-cadherin-β-catenin-α-catenin complexes and actin filaments in cadherin adhesions. The reorganization of the cytoskeletal machinery is tightly controlled by the activities of actin nucleating proteins, such as Arp2/3 complex and formin-1, as well as Rho GTPases.[106–110] Furthermore, recruitment of p120 protein to the membrane is also essential for these cell responses, thus, making it a major player in Rac1-dependent extension and maturation of cell adhesions.[111] Importantly, cells can sense and react to differences in the stiffness of the E-cadherin functionalized substrates by changing the distribution of traction stress and strain on the substrate.[112] These assays were performed using the polyacrylamide gels functionalized with the extra-cellular domain of E-cadherin and the elastic moduli ranged from 30 to 60 kPa to simulate the in vivo environment. The morphology and the dynamics of epithelial cells were found to change depending on the elastic moduli of the substrate. Furthermore, fluorescence and traction force microscopy of the cells revealed morphological changes corresponding to the stiffness of the substrates as well as the variation in the distribution of stress as the adhesion assembly proceeds.[112]

Further clarification on the role of filopodia in the assembly of E-cadherin adhesion came from experiments performed with E-cadherin functionalized supported lipid bilayers wherein epithelial cells extended filopodia on the bilayer and retraction of the same coincided with E-cadherin clustering on the bilayer (Fig. 2).[48,88–93] This was made possible by the simultaneous monitoring of filopodia through Reflection Interference Contrast Microscopy (RICM), which allows observation of objects close to the glass substrate, and epi-fluorescence imaging of the fluorescently labeled E-cadherin molecules on the bilayer. The role of filopodia was further confirmed by the loss of E-cadherin adhesion assembly by the treatment of cells with Cdc42 inhibitor which causes loss of filopodia formation.[88] Additionally, these reconstitution experiments revealed a dependence of the adhesion assembly on the diffusive mobility of E-cadherin on bilayers, i.e. cells readily assembled adhesion on low mobility, viscous bilayer while they failed to do so on high mobility, fluid bilayers.[88]

The hybrid live cell-supported lipid bilayer experiments also provided insights into the mechanism of α-catenin activation at E-cadherin adhesions.[48,88–93] While several lines of evidence including conformation-specific antibody staining[79], single molecule force measurement with either the isolated protein[83] or a complex of E-cadherin-β-catenin-α-catenin[113] and live Fluorescence Resonance Energy Transfer (FRET)-experiments[114] has indicated that α-catenin undergoes a force-dependent 'close' to 'open' conformational transition, which is important for high affinity F-actin binding[113]. However, the mechanism by which α-catenin is conformationally activated at the first place was not clear. The bilayer reconstitution experiments revealed that the filopodia retraction-mediated micron scale clustering of E-cadherin correlates well with α-catenin conformational activation as a reduction in the cluster size through fabrication of metal nano-grids, which act as barriers for free diffusion and movement of molecules on the bilayer,[88–93] resulted in a decreased α-catenin activation[80]. Furthermore, once activated, α-catenin stayed in the active conformation even after reduction in the cellular actomyosin tension achieved through treatment of cells with the ROCK kinase inhibitor, Y-27632.[80] This could be made possible either a post-translational modification of α-catenin e.g. phosphorylation of specific residues[115] or binding of α-catenin interacting proteins such as α-actinin[116]. These posit that perhaps α-catenin explores the 'open' conformation in the absence of interaction of F-actin and, thus, application of force,[81,117–120] and, thus, binding of F-actin to these α-catenin initiates E-cadherin clustering through application of mechanical force, especially on viscous bilayers,[88] which perhaps results in the activation of more α-catenin molecules as seen in the staining experiments. Additionally, binding of vinculin, which is seen to specifically associate with E-cadherin clusters at the cell periphery,[80] to the conformationally activated α-catenin molecules could further strengthen the interaction of the E-cadherin-β-catenin-α-catenin complex with the F-actin and thus efficient clustering leading to the formation of mature E-cadherin adhesion.

4 Actin Cytoskeletal Regulation of E-cadherin Adhesion Maintenance

Unlike many other types of transient interactions observed between cells such as interaction between immune cells, E-cadherin-mediated cell–cell adhesions are relatively long lived and therefore, require maintenance. It is a dynamic process and involves multiple mechanisms (Fig. 2). First, the *cis*-interaction plays a significant role in the stabilization of E-cadherin clusters at the cell–cell adhesions as mutation of the residues at the *cis*-interaction interface results in an increased dynamics of E-cadherin clusters[68,69]. Second,

radial actin bundles associated with E-cadherin clusters during the assembly are replaced by finer perijunctional actin belts at contact sites and by thick bundles at the contact edges through the action of actin regulators such as Rac1, Cdc42, Abl kinase, Arp2/3 and Cortactin[121], leading to the expansion and stabilization of cell–cell contact. Third, the F-actin-based cellular appendages continue to generate cadherin clusters in already adhering cells. For instance, with the help of thin-section electron microscopy and live cell imaging in adherent MDCK cells, Li et al., have shown that filopodia (microspikes) formed on the apical regions of the adjacent cells push the lateral membranes so as to keep cadherins in contact[122]. Using the mixed cultures of stable cells expressing E-cadherin-GFP and E-cadherin-mCherry, they have also shown that filopodia project cadherins into the adjacent cells, thereby repairing the cadherin adhesive junctions. The immunofluorescence results have shown that the actin assembly factors, Arp2/3, EVL, and CRMP-1 is required for actin protrusion[122]. The dysregulation of actin protrusive activity might lead to pathophysiological conditions such as cancer progression. Also, newer E-cadherin clusters formed on the lateral membrane of the cell can translocate up to join the apical clusters, thus stabilizing the adhesion.[123] Fourth, E-cadherin clusters at the adhesion could be endocytosed through both clathrin-dependent and -independent endocytosis as well as macropinocytosis.[124,125] Activation of Rac1 and Cdc42 and the actin-binding protein, IQGAP1, inhibits the E-cadherin endocytosis[124] while actin remodeling by RhoA promotes its endocytosis through Dia and AP2 adaptor proteins[126]. p120 catenin is also a regulator of clathrin-dependent cadherin internalization by binding to the cytosolic domain of E-cadherin.[127] p120 catenin is considered as an important regulator of E-cadherin trafficking by masking the docking sites for endocytic adaptor proteins and for proteasomal degradation by E3 ubiquitin ligase such as Hakai.[128,129] Internalized E-cadherin is either recycled to the plasma membrane through endocytic adaptor proteins such as Rab11[130] or directed to lysosome for degradation. Together, these highlight the role of actin cytoskeleton in the maintenance of E-cadherin adhesion.

5 Conclusions

The dynamic actin cytoskeleton, in conjunction with myosin proteins, regulates several aspects of E-cadherin-mediated cell–cell adhesion (Fig. 2). Starting with the assembly of E-cadherin adhesion, the F-actin-based cellular appendages such as filopodia and lamellipodia enable the initial interaction of cells that leads to the formation of E-cadherin clusters. This involves mechanical activation of α-catenin adaptor protein. These initial clusters then assemble into larger and more stable clusters leading to the assembly of mature adhesions. On the maintenance side, the actin cytoskeleton continues to play a key role by regulating processes such as E-cadherin endocytosis and extension of cellular appendages between adjacent cells. While we have briefly elaborated these processes in the current article, much remains to be explored in the future in relation to the role of the acto-myosin complex in the assembly and maintenance of E-cadherin adhesion. For instance, the mechano-regulation of E-cadherin clustering and endocytosis by cytoskeleton, the cytoskeletal alterations during loss of E-cadherin and the signaling mechanisms associated with E-cadherin assembly and maintenance are yet to be explored in detail. In this regard, novel technological advancements such as the use of synthetic substrates, single molecule assays and super-resolution microscopy is expected to provide further insights into the mechanisms of E-cadherin adhesion assembly and maintenance. For instance, a combination of the use of micropatterned membrane substrates and single molecule assays could potentially provide a dynamic picture of mechanical signal transduction at the molecular level and with high temporal resolution, details that are currently missing in the literature. Such insights are expected to help us understand the fundamental basis as well as evolution of E-cadherin adhesions, and thus, aid in our understanding of fundamental processes at the tissue level such as collective cell migration and at the organism level such as development and organogenesis.

Publisher's Note

Acknowledgements

This work is supported by an internal funding from the College of Health & Life Sciences, Hamad Bin Khalifa University, a member of the Qatar Foundation. A.M.G. and S.R. are supported by a postdoctoral fellowship and scholarship from the College of Health & Life Sciences, Hamad Bin Khalifa University, a member of the Qatar Foundation, respectively.

Author Contributions

K.H.B. conceived the article and prepared the figures. All authors contributed in writing the article.

Compliance with Ethical Standards

Conflict of Interest

The authors declare no conflict of interest.

Ethics Approval

Not applicable.

Consent to Participate

Not applicable.

Consent for Publication

The authors approve of this publication.

Received: 11 October 2020 Accepted: 19 November 2020
Published online: 9 January 2021

References

1. Skruber K, Read TA, Vitriol EA (2018) Reconsidering an active role for G-actin in cytoskeletal regulation. J Cell Sci 131
2. Oda T, Iwasa M, Aihara T, Maeda Y, Narita A (2009) The nature of the globular- to fibrous-actin transition. Nature 457:441–445
3. Blanchoin L, Boujemaa-Paterski R, Sykes C, Plastino J (2014) Actin dynamics, architecture, and mechanics in cell motility. Physiol Rev 94:235–263
4. Pollard TD, Borisy GG (2003) Cellular motility driven by assembly and disassembly of actin filaments. Cell 112:453–465
5. Yamaguchi H, Condeelis J (2007) Regulation of the actin cytoskeleton in cancer cell migration and invasion. Biochim Biophys Acta 1773:642–652
6. Dekraker C, Boucher E, Mandato CA (2018) Regulation and assembly of actomyosin contractile rings in cytokinesis and cell repair. Anat Rec (Hoboken) 301:2051–2066
7. May RC, Machesky LM (2001) Phagocytosis and the actin cytoskeleton. J Cell Sci 114:1061–1077
8. Rogers SL, Gelfand VI (2000) Membrane trafficking, organelle transport, and the cytoskeleton. Curr Opin Cell Biol 12:57–62
9. Button E, Shapland C, Lawson D (1995) Actin, its associated proteins and metastasis. Cell Motil Cytoskeleton 30:247–251
10. Fernie AR, Zhang Y, Sampathkumar A (2020) Cytoskeleton architecture regulates glycolysis coupling cellular metabolism to mechanical cues. Trends Biochem Sci 45:637–638
11. Parsons JT, Horwitz AR, Schwartz MA (2010) Cell adhesion: integrating cytoskeletal dynamics and cellular tension. Nat Rev Mol Cell Biol 11:633–643
12. Sept D, McCammon JA (2001) Thermodynamics and kinetics of actin filament nucleation. Biophys J 81:667–674
13. Xue B, Robinson RC (2013) Guardians of the actin monomer. Eur J Cell Biol 92:316–332
14. Quinlan ME, Kerkhoff E (2008) Actin nucleation: bacteria get in-Spired. Nat Cell Biol 10:13–15
15. Pollard TD, Cooper JA (2009) Actin, a central player in cell shape and movement. Science 326:1208–1212
16. Boczkowska M, Rebowski G, Kast DJ, Dominguez R (2014) Structural analysis of the transitional state of Arp2/3 complex activation by two actin-bound WCAs. Nat Commun 5:3308
17. Rotty JD, Wu C, Bear JE (2013) New insights into the regulation and cellular functions of the ARP2/3 complex. Nat Rev Mol Cell Biol 14:7–12
18. Pruyne D et al (2002) Role of formins in actin assembly: nucleation and barbed-end association. Science 297:612–615
19. Sagot I, Rodal AA, Moseley J, Goode BL, Pellman D (2002) An actin nucleation mechanism mediated by Bni1 and profilin. Nat Cell Biol 4:626–631
20. Quinlan ME, Heuser JE, Kerkhoff E, Mullins RD (2005) Drosophila Spire is an actin nucleation factor. Nature 433:382–388
21. Ahuja R et al (2007) Cordon-bleu is an actin nucleation factor and controls neuronal morphology. Cell 131:337–350
22. Chereau D et al (2008) Leiomodin is an actin filament nucleator in muscle cells. Science 320:239–243
23. Fowler VM, Dominguez R (2017) Tropomodulins and leiomodins: actin pointed end caps and nucleators in muscles. Biophys J 112:1742–1760
24. Green KJ, Getsios S, Troyanovsky S, Godsel LM (2010) Intercellular junction assembly, dynamics, and homeostasis. Cold Spring Harb Perspect Biol 2:a000125
25. Fujii T, Namba K (2017) Structure of actomyosin rigour complex at 52 A resolution and insights into the ATPase cycle mechanism. Nat Commun 8:13969
26. Vicente-Manzanares M, Ma X, Adelstein RS, Horwitz AR (2009) Non-muscle myosin II takes centre stage in cell adhesion and migration. Nat Rev Mol Cell Biol 10:778–790
27. Ladoux B, Mege RM (2017) Mechanobiology of collective cell behaviours. Nat Rev Mol Cell Biol 18:743–757
28. Munjal A, Lecuit T (2014) Actomyosin networks and tissue morphogenesis. Development 141:1789–1793

29. Shutova M, Yang C, Vasiliev JM, Svitkina T (2012) Functions of nonmuscle myosin II in assembly of the cellular contractile system. PLoS ONE 7:e40814
30. Lehtimaki J, Hakala M, Lappalainen P (2017) Actin filament structures in migrating cells. Handb Exp Pharmacol 235:123–152
31. Suraneni P et al (2012) The Arp2/3 complex is required for lamellipodia extension and directional fibroblast cell migration. J Cell Biol 197:239–251
32. Wu C et al (2012) Arp2/3 is critical for lamellipodia and response to extracellular matrix cues but is dispensable for chemotaxis. Cell 148:973–987
33. Steffen A et al (2013) Rac function is crucial for cell migration but is not required for spreading and focal adhesion formation. J Cell Sci 126:4572–4588
34. Steffen A, Koestler SA, Rottner K (2014) Requirements for and consequences of Rac-dependent protrusion. Eur J Cell Biol 93:184–193
35. Stradal TE, Scita G (2006) Protein complexes regulating Arp2/3-mediated actin assembly. Curr Opin Cell Biol 18:4–10
36. Mejillano MR et al (2004) Lamellipodial versus filopodial mode of the actin nanomachinery: pivotal role of the filament barbed end. Cell 118:363–373
37. Iwasa JH, Mullins RD (2007) Spatial and temporal relationships between actin-filament nucleation, capping, and disassembly. Curr Biol 17:395–406
38. Lai FP et al (2008) Arp2/3 complex interactions and actin network turnover in lamellipodia. EMBO J 27:982–992
39. Mellor H (2010) The role of formins in filopodia formation. Biochim Biophys Acta 1803:191–200
40. Jaiswal R et al (2013) The formin Daam1 and fascin directly collaborate to promote filopodia formation. Curr Biol 23:1373–1379
41. Mallavarapu A, Mitchison T (1999) Regulated actin cytoskeleton assembly at filopodium tips controls their extension and retraction. J Cell Biol 146:1097–1106
42. Breitsprecher D et al (2011) Cofilin cooperates with fascin to disassemble filopodial actin filaments. J Cell Sci 124:3305–3318
43. Svitkina TM et al (2003) Mechanism of filopodia initiation by reorganization of a dendritic network. J Cell Biol 160:409–421
44. Bohil AB, Robertson BW, Cheney RE (2006) Myosin-X is a molecular motor that functions in filopodia formation. Proc Natl Acad Sci U S A 103:12411–12416
45. Mattila PK, Lappalainen P (2008) Filopodia: molecular architecture and cellular functions. Nat Rev Mol Cell Biol 9:446–454
46. Yamada S, Nelson WJ (2007) Synapses: sites of cell recognition, adhesion, and functional specification. Annu Rev Biochem 76:267–294
47. Nelson WJ (2008) Regulation of cell-cell adhesion by the cadherin-catenin complex. Biochem Soc Trans 36:149–155
48. Biswas KH, Zaidel-Bar R (2017) Early events in the assembly of E-cadherin adhesions. Exp Cell Res 358:14–19
49. Malinova TS, Huveneers S (2018) Sensing of cytoskeletal forces by asymmetric adherens junctions. Trends Cell Biol 28:328–341
50. Maitre JL, Heisenberg CP (2013) Three functions of cadherins in cell adhesion. Curr Biol 23:R626-633
51. Benham-Pyle BW, Pruitt BL, Nelson WJ (2015) Cell adhesion. Mechanical strain induces E-cadherin-dependent Yap1 and beta-catenin activation to drive cell cycle entry. Science 348:1024–1027.
52. Mendonsa AM, Na TY, Gumbiner BM (2018) E-cadherin in contact inhibition and cancer. Oncogene 37:4769–4780
53. Rao MV, Zaidel-Bar R (2016) Formin-mediated actin polymerization at cell-cell junctions stabilizes E-cadherin and maintains monolayer integrity during wound repair. Mol Biol Cell 27:2844–2856
54. Campbell HK, Maiers JL, DeMali KA (2017) Interplay between tight junctions & adherens junctions. Exp Cell Res 358:39–44
55. Harris TJ, Tepass U (2010) Adherens junctions: from molecules to morphogenesis. Nat Rev Mol Cell Biol 11:502–514
56. Vasioukhin V (2012) Adherens junctions and cancer. Sub-Cellular Biochem 60:379–414
57. van Roy F (2014) Beyond E-cadherin: roles of other cadherin superfamily members in cancer. Nat Rev Cancer 14:121–134
58. Rodriguez FJ, Lewis-Tuffin LJ, Anastasiadis PZ (2012) E-cadherin's dark side: possible role in tumor progression. Biochim Biophys Acta 1826:23–31
59. Brasch J, Harrison OJ, Honig B, Shapiro L (2012) Thinking outside the cell: how cadherins drive adhesion. Trends Cell Biol 22:299–310
60. Haussinger D et al (2004) Proteolytic E-cadherin activation followed by solution NMR and X-ray crystallography. EMBO J 23:1699–1708
61. Li Y et al (2013) Mechanism of E-cadherin dimerization probed by NMR relaxation dispersion. Proc Natl Acad Sci USA 110:16462–16467
62. Harrison OJ et al (2010) Two-step adhesive binding by classical cadherins. Nat Struct Mol Biol 17:348–357
63. Pertz O et al (1999) A new crystal structure, Ca2+ dependence and mutational analysis reveal molecular details of E-cadherin homoassociation. EMBO J 18:1738–1747
64. Hong S, Troyanovsky RB, Troyanovsky SM (2011) Cadherin exits the junction by switching its adhesive bond. J Cell Biol 192:1073–1083
65. Boggon TJ et al (2002) C-cadherin ectodomain structure and implications for cell adhesion mechanisms. Science 296:1308–1313
66. Parisini E, Higgins JM, Liu JH, Brenner MB, Wang JH (2007) The crystal structure of human E-cadherin

domains 1 and 2, and comparison with other cadherins in the context of adhesion mechanism. J Mol Biol 373:401–411
67. Shapiro L et al (1995) Structural basis of cell-cell adhesion by cadherins. Nature 374:327–337
68. Harrison OJ et al (2011) The extracellular architecture of adherens junctions revealed by crystal structures of type I cadherins. Structure 19:244–256
69. Bunse S et al (2013) Role of N-cadherin cis and trans interfaces in the dynamics of adherens junctions in living cells. PLoS ONE 8:e81517
70. Haussinger D et al (2002) Calcium-dependent homoassociation of E-cadherin by NMR spectroscopy: changes in mobility, conformation and mapping of contact regions. J Mol Biol 324:823–839
71. Wu Y et al (2010) Cooperativity between trans and cis interactions in cadherin-mediated junction formation. Proc Natl Acad Sci U S A 107:17592–17597
72. Wu Y, Vendome J, Shapiro L, Ben-Shaul A, Honig B (2011) Transforming binding affinities from three dimensions to two with application to cadherin clustering. Nature 475:510–513
73. Borghi N et al (2012) E-cadherin is under constitutive actomyosin-generated tension that is increased at cell-cell contacts upon externally applied stretch. Proc Natl Acad Sci USA 109:12568–12573
74. Rakshit S, Zhang Y, Manibog K, Shafraz O, Sivasankar S (2012) Ideal, catch, and slip bonds in cadherin adhesion. Proc Natl Acad Sci USA 109:18815–18820
75. Huber O, Kemler R, Langosch D (1999) Mutations affecting transmembrane segment interactions impair adhesiveness of E-cadherin. J Cell Sci 112(Pt 23):4415–4423
76. McEwen AE, Escobar DE, Gottardi CJ (2012) Signaling from the adherens junction. Sub-cellular biochemistry 60:171–196
77. Guo Z et al. (2014) E-cadherin interactome complexity and robustness resolved by quantitative proteomics. Sci Signal 7:rs7.
78. Zaidel-Bar R (2013) Cadherin adhesome at a glance. J Cell Sci 126:373–378
79. Yonemura S, Wada Y, Watanabe T, Nagafuchi A, Shibata M (2010) alpha-Catenin as a tension transducer that induces adherens junction development. Nat Cell Biol 12:533–542
80. Biswas KH, Hartman KL, Zaidel-Bar R, Groves JT (2016) Sustained alpha-catenin activation at E-cadherin junctions in the absence of mechanical force. Biophys J 111:1044–1052
81. Biswas KH (2018) Regulation of α-catenin conformation at cadherin adhesions. J Biomech Sci Engg 13:17–00699
82. le Duc Q et al (2010) Vinculin potentiates E-cadherin mechanosensing and is recruited to actin-anchored sites within adherens junctions in a myosin II-dependent manner. J. Cell Biol. 189:1107–1115
83. Yao M et al (2014) Force-dependent conformational switch of alpha-catenin controls vinculin binding. Nat Commun 5:4525
84. Hirokawa N, Tilney LG (1982) Interactions between actin filaments and between actin filaments and membranes in quick-frozen and deeply etched hair cells of the chick ear. J Cell Biol 95:249–261
85. Yonemura S (2011) Cadherin-actin interactions at adherens junctions. Curr Opin Cell Biol 23:515–522
86. Wu Y, Kanchanawong P, Zaidel-Bar R (2015) Actin-delimited adhesion-independent clustering of e-cadherin forms the nanoscale building blocks of adherens junctions. Dev Cell 32:139–154
87. Truong Quang BA, Mani M, Markova O, Lecuit T, Lenne PF (2013) Principles of E-cadherin supramolecular organization in vivo. Curr Biol 23:2197–2207
88. Biswas KH et al (2015) E-cadherin junction formation involves an active kinetic nucleation process. Proc Natl Acad Sci USA 112:10932–10937
89. Biswas KH, Groves JT (2019) Hybrid Live Cell-Supported Membrane Interfaces for Signaling Studies. Annu Rev Biophys 48:537–562
90. Biswas KH (2020) Molecular mobility-mediated regulation of E-cadherin adhesion. Trends Biochem Sci 45:163–173
91. Biswas KH, Zhongwen C, Dubey AK, Oh D, Groves JT (2018) Multicomponent supported membrane microarray for monitoring spatially resolved cellular signaling reactions. Adv Biosyst 2:1800015
92. Biswas KH, Groves JT (2018) in Physics of Biological Membranes (eds Patricia Bassereau & Pierre Sens) 537–560 (Springer International Publishing, 2018).
93. Biswas KH, Cho NJ, Groves JT (2018) Fabrication of multicomponent, spatially segregated DNA and protein functionalized supported membrane microarray. Langmuir 34:9781–9788
94. Padmanabhan A, Ong HT, Zaidel-Bar R (2016) Non-junctional E-Cadherin Clusters Regulate the Actomyosin Cortex in the *C. elegans* Zygote. Current Biology
95. Liu Z et al (2010) Mechanical tugging force regulates the size of cell-cell junctions. Proc Natl Acad Sci USA 107:9944–9949
96. McNeill H, Ryan TA, Smith SJ, Nelson WJ (1993) Spatial and temporal dissection of immediate and early events following cadherin-mediated epithelial cell adhesion. J Cell Biol 120:1217–1226
97. Vasioukhin V, Bauer C, Yin M, Fuchs E (2000) Directed actin polymerization is the driving force for epithelial cell-cell adhesion. Cell 100:209–219
98. Raich WB, Agbunag C, Hardin J (1999) Rapid epithelial-sheet sealing in the Caenorhabditis elegans embryo requires cadherin-dependent filopodial priming. Curr Biol 9:1139–1146
99. Tanaka-Matakatsu M, Uemura T, Oda H, Takeichi M, Hayashi S (1996) Cadherin-mediated cell adhesion and cell motility in Drosophila trachea regulated

by the transcription factor Escargot. Development 122:3697–3705
100. Fierro-Gonzalez JC, White MD, Silva JC, Plachta N (2013) Cadherin-dependent filopodia control preimplantation embryo compaction. Nat Cell Biol 15:1424–1433
101. Kuroda S et al (1997) Regulation of cell-cell adhesion of MDCK cells by Cdc42 and Rac1 small GTPases. Biochem Biophys Res Commun 240:430–435
102. Kim SH, Li Z, Sacks DB (2000) E-cadherin-mediated cell-cell attachment activates Cdc42. J Biol Chem 275:36999–37005
103. Gauvin TJ, Young LE, Higgs HN (2015) The formin FMNL3 assembles plasma membrane protrusions that participate in cell-cell adhesion. Mol Biol Cell 26:467–477
104. Yamada S, Nelson WJ (2007) Localized zones of Rho and Rac activities drive initiation and expansion of epithelial cell-cell adhesion. J Cell Biol 178:517–527
105. Yamazaki D, Oikawa T, Takenawa T (2007) Rac-WAVE-mediated actin reorganization is required for organization and maintenance of cell-cell adhesion. J Cell Sci 120:86–100
106. Kobielak A, Pasolli HA, Fuchs E (2004) Mammalian formin-1 participates in adherens junctions and polymerization of linear actin cables. Nat Cell Biol 6:21–30
107. Kovacs EM, Goodwin M, Ali RG, Paterson AD, Yap AS (2002) Cadherin-directed actin assembly: E-cadherin physically associates with the Arp2/3 complex to direct actin assembly in nascent adhesive contacts. Curr Biol 12:379–382
108. Verma S et al (2004) Arp2/3 activity is necessary for efficient formation of E-cadherin adhesive contacts. J Biol Chem 279:34062–34070
109. Braga VM, Machesky LM, Hall A, Hotchin NA (1997) The small GTPases Rho and Rac are required for the establishment of cadherin-dependent cell-cell contacts. J Cell Biol 137:1421–1431
110. Braga VM, Del Maschio A, Machesky L, Dejana E (1999) Regulation of cadherin function by Rho and Rac: modulation by junction maturation and cellular context. Mol Biol Cell 10:9–22
111. Gavard J et al (2004) Lamellipodium extension and cadherin adhesion: two cell responses to cadherin activation relying on distinct signalling pathways. J Cell Sci 117:257–270
112. Collins C, Denisin AK, Pruitt BL, Nelson WJ (2017) Changes in E-cadherin rigidity sensing regulate cell adhesion. Proc Natl Acad Sci USA 114:E5835–E5844
113. Buckley CD et al (2014) Cell adhesion. The minimal cadherin-catenin complex binds to actin filaments under force. Science 346:1254211
114. Kim TJ et al (2015) Dynamic visualization of alpha-catenin reveals rapid, reversible conformation switching between tension states. Curr Biol 25:218–224
115. Escobar DJ et al (2015) alpha-Catenin phosphorylation promotes intercellular adhesion through a dual-kinase mechanism. J Cell Sci 128:1150–1165
116. Nieset JE et al (1997) Characterization of the interactions of alpha-catenin with alpha-actinin and beta-catenin/plakoglobin. J Cell Sci 110(Pt 8):1013–1022
117. Biswas KH (2017) Allosteric regulation of proteins. Resonance 22:37–50
118. Biswas KH, Badireddy S, Rajendran A, Anand GS, Visweswariah SS (2015) Cyclic nucleotide binding and structural changes in the isolated GAF domain of Anabaena adenylyl cyclase, CyaB2. PeerJ 3:e882
119. Biswas KH, Visweswariah SS (2011) Distinct allostery induced in the cyclic GMP-binding, cyclic GMP-specific phosphodiesterase (PDE5) by cyclic GMP, sildenafil, and metal ions. J Biol Chem 286:8545–8554
120. Biswas KH, Sopory S, Visweswariah SS (2008) The GAF domain of the cGMP-binding, cGMP-specific phosphodiesterase (PDE5) is a sensor and a sink for cGMP. Biochemistry 47:3534–3543
121. Cavey M, Lecuit T (2009) Molecular bases of cell-cell junctions stability and dynamics. Cold Spring Harb Perspect Biol 1:a002998
122. Li JXH, Tang VW, Brieher WM (2020) Actin protrusions push at apical junctions to maintain E-cadherin adhesion. Proc Natl Acad Sci U S A 117:432–438
123. Cao J, Schnittler H (2019) Putting VE-cadherin into JAIL for junction remodeling. J. Cell Sci. 132.
124. Izumi G et al (2004) Endocytosis of E-cadherin regulated by Rac and Cdc42 small G proteins through IQGAP1 and actin filaments. J Cell Biol 166:237–248
125. Ivanov AI, Nusrat A, Parkos CA (2004) Endocytosis of epithelial apical junctional proteins by a clathrin-mediated pathway into a unique storage compartment. Mol Biol Cell 15:176–188
126. Levayer R, Pelissier-Monier A, Lecuit T (2011) Spatial regulation of Dia and Myosin-II by RhoGEF2 controls initiation of E-cadherin endocytosis during epithelial morphogenesis. Nat Cell Biol 13:529–540
127. Bulgakova NA, Brown NH (2016) Drosophila p120-catenin is crucial for endocytosis of the dynamic E-cadherin-Bazooka complex. J Cell Sci 129:477–482
128. Venhuizen JH, Jacobs FJC, Span PN, Zegers MM (2020) P120 and E-cadherin: double-edged swords in tumor metastasis. Semin Cancer Biol 60:107–120
129. Hartsock A, Nelson WJ (2012) Competitive regulation of E-cadherin juxtamembrane domain degradation by p120-catenin binding and Hakai-mediated ubiquitination. PLoS ONE 7:e37476
130. Cadwell CM, Su W, Kowalczyk AP (2016) Cadherin tales: Regulation of cadherin function by endocytic membrane trafficking. Traffic 17:1262–1271

Saad Rasool graduated with a Bachelor of Science degree in Biological Sciences from the Carnegie Mellon University, Qatar, in May 2018. Following his graduation, he worked as a Research Specialist in the Translational Research Department of Sidra Medicine, where his research focused on identifying the genetic signatures of inflammatory bowel diseases and on understanding the changes in gene expression of tumor initiating Cells. Since January 2020, he has been a Master of Science student in the Genomics and Precision Medicine program at the College of Health and Life Sciences, Hamad Bin Khalifa University (Qatar), and is working on developing peptide-based strategies for modulating protein function with a focus on cell–cell adhesion.

Anupriya M. Geethakumari is a postdoctoral fellow at College of Health and Life Sciences, Hamad Bin Khalifa University. She received her PhD degree in Biotechnology from Rajiv Gandhi Centre for Biotechnology, University of Kerala, in 2018. She is interested in understanding mechanisms of cell signaling.

Kabir H Biswas is an Assistant Professor at the College of Health & Life Sciences, Hamad Bin Khalifa University, Doha, Qatar. He obtained his Master of Science and PhD degrees in 2007 and 2011, respectively, as a part of the Integrated PhD program at the Indian Institute of Science (IISc), Bangalore, India, and was awarded the Mrs. C V Hanumantha Rao Medal (2012) for the research work embodied in his PhD thesis. He then moved to Singapore in the end of 2011 as a Research Fellow, first at the Mechanobiology Institute (MBI), National University of Singapore, and later at the Nanyang Technological University (NTU). He has made significant contributions to the field of engineering precision biointerfaces for understanding cellular adhesion and signaling. He was awarded the Yamaguchi Medal (2017) by the Asian-Pacific Association for Biomechanics for his research work in the field of cell–cell adhesion. Dr. Biswas is primarily interested in developing biosensors and biointerface to enable fundamental understanding and diagnostics and therapeutic intervention in the realm of human health and disease.