CORE
Metadata, citation and similar papers at core.ac.uk
Provided by Elsevier - Publisher Connector

**Current Biology** Vol 24 No 15
R688

# Cell Biology: A Tense but Good Day for Actin at Cell–Cell Junctions

**Cells have evolved an elegant tuning mechanism to maintain tissue integrity, in which increasing mechanical tension stimulates actin assembly at cell–cell junctions. The mechanosensitive junctional protein α-catenin acts through vinculin and Ena/VASP proteins to reinforce the cell against mechanical stress.**

**Stephanie H. Nowotarski[1] and Mark Peifer[1,2,*]**

During embryonic development and tissue homeostasis, cells must balance the need to maintain tissue integrity, via cell–cell adhesive junctions, with the need to change shape and move, using the actomyosin cytoskeleton to generate force. In the good old days, the relationship between cell–cell adherens junctions (AJs) and the cytoskeleton was simple — the cadherin–catenin complex directly linked to actin filaments via α-catenin (Figure 1A). This provided a great way to build a stuffed animal, with cells glued together and made stiff. However, the last decade shook up this paradigm, revealing diverse ways of linking AJs and the cytoskeleton during the dynamic events of morphogenesis. Now a study in this issue of *Current Biology* by Leerberg *et al.* [1] reveals new complexity in this linkage process, providing evidence for a feedback loop that ensures the junctional–cytoskeletal linkage is maintained in the face of mechanical force.

Classic cadherin extracellular domains provide the adhesive interface joining cells to one another [2], but the adhesive force provided by single cadherin–cadherin interactions is quite small. To maintain effective adhesion, cadherins must be organized into multiprotein arrays. This is achieved in part by *trans*-interactions among cadherins, but is primarily maintained by interactions between cytoplasmic proteins that bind cadherin tails and the underlying actin cytoskeleton. In most epithelial cells, cadherins form an adhesive interface all along the lateral domain, but are organized into special adhesive complexes at the apical end, forming the AJ (or zonula adherens, ZA [2]). The cadherin–actin cytoskeleton relationship is a two-way street, with cadherin-based adhesion being essential for polarized apical assembly of a specialized actin array [3], and this actin array being essential for stabilizing cadherin-based junctions [4]. The link connecting cadherins and actin was thought to be direct, mediated by β-catenin and α-catenin (Figure 1A). However, work in 2005 cast doubt on this textbook view [5], stimulating a series of experiments revealing that there are multiple connectors (for examples, see [6,7]) employed at different times and places and, importantly for this discussion, under different force regimes.

These connections stabilize cell adhesion in a static epithelial sheet but are even more critical as cells change shape and move [8]. Nowhere is this more apparent than during the dramatic events of embryonic morphogenesis, during which actomyosin powers tissue rearrangements via the coordinated action of many individual cells. For example, apical constriction — in which an apical actomyosin network changes a columnar cell into a pyramid — drives critical events from mammalian neural tube closure to *Drosophila* mesoderm invagination [9]. More complex, planar-polarized, actomyosin-driven events drive another common developmental process — convergent elongation — which elongates the anterior–posterior body axis of many animals. Linking actin to AJs also plays an important role at the adhesive front during collective cell migration and embryonic wound healing. Even seemingly simple events, like responding to cell division, require remodeling of AJs and their cytoskeletal partners. In each event, force is exerted on AJs, and thus the connection must be mechanically secure. Eliminating the function of potential AJ–actomyosin crosslinkers, such as Canoe/Afadin, interferes with the completion of these morphogenetic events, disrupting development [7,10].

To assemble secure connections between AJs and actin, cells must first assemble actin at AJs. Like cadherins, F-actin localizes in apical circumferential rings along the AJ in cell culture and *in vivo*. This junctional actin is highly dynamic, with ~80% turning over with a half-life of 10–50 seconds. There are three well-characterized classes of actin assembly machines that are candidates for assembling junctional actin [11]. The Arp2/3 complex is activated by WASP family proteins and nucleates daughter filaments on the sides of existing filaments, thus promoting branched actin networks. Formins associate with F-actin barbed ends and facilitate rapid addition of profilin–actin, promoting linear actin networks. Lastly, Ena/VASP proteins (Ena/Mena/VASP/Evl) also aid barbed-end polymerization and facilitate actin bundling through their ability to tetramerize. Despite the fact that the junctional actin array is largely composed of linear actin filaments, previous work has implicated the WASP family members WAVE and WIRE, with actin nucleation at AJs occurring through a Rac–WAVE–Arp2/3 pathway [12,13], and the WIRE–N-WASP pathway appearing to play a role in reorganization. However, other studies have suggested possible roles for formins and Ena/VASP proteins in other cell types [14].

The dynamic nature of junctional–actin connections opens the possibility that feedback loops may exist that allow cells to respond to dynamic changes in force generation both within cells and between neighbors. FRET-based biosensors confirm that cadherins are under tension in epithelia and that the connection to actomyosin is essential for this state. However, for cells to respond to force, they must be able to sense tension. Studies of integrin-based focal adhesions provided paradigms: in focal adhesions, proteins like talin change conformation in response to tension, leading to increased recruitment of the actin-binding protein vinculin [15]. Similarly, recent work suggests both α-catenin and vinculin can act as force sensors at AJs. In the case of α-catenin, the a18-antibody recognizes an α-catenin epitope that is exposed only when AJs are under tension [16]. This led to the suggestion that tension induces a conformational change in α-catenin, exposing the epitope and the overlapping

CrossMark

vinculin-binding site, increasing vinculin recruitment (Figure 1B).

Cells thus have a mechanism built into AJs to sense tension. How do they respond? One danger faced by cells is that the dynamic forces involved in morphogenesis will exceed the resistance of junctional–actin connections and thus disrupt connections. This is exactly what one observes in situations when levels of putative actin crosslinkers, like α-catenin or Afadin/Canoe, are reduced. How then do cells react to tension to prevent junctional disruption? In the new study, Leerberg *et al.* [1] find that contractility supports and tunes actin assembly at AJs [1] (Figure 1B,C). In their polarized colon cell model, there is a rich perijunctional F-actin pool at AJs, and they find that both steady-state F-actin and actin assembly at the AJ is stimulated by myosin-based contractility. The authors thus hypothesized integral roles for both actin-binding proteins and proteins promoting actin polymerization. Based on its tension-dependent localization to AJs, they considered vinculin as a candidate modulator. Strikingly, they found that vinculin accumulated at AJs in a tension-dependent manner, via its interaction with α-catenin (Figure 1A). More importantly, vinculin was required for the increase in steady-state F-actin and new actin polymerization in response to myosin-based tension. They confirmed this by demonstrating that an α-catenin mutant unable to bind vinculin cannot support tension-dependent increases in actin assembly.

The tension-dependent increase in F-actin suggested that actin-nucleating/polymerizing proteins might be recruited by vinculin. The authors quickly ruled out Arp2/3 as the direct actin modulator and thus turned to Ena/VASP proteins [1]. They found that both Mena and VASP co-localized with vinculin at AJs and that vinculin was the dominant mechanism for their AJ recruitment (Figure 1A). They went on to reveal that Mena and VASP are necessary for vinculin's ability to regulate junctional actin (following simultaneous inactivation of Mena and VASP by forced recruitment to mitochondria). Furthermore, engineering vinculin-independent Mena/VASP recruitment to AJs was sufficient to render junctional actin assembly resistant to myosin inhibition, and thus making it independent of tension. Thus, Ena/VASP proteins appear to be the dominant players in tension-dependent actin regulation.

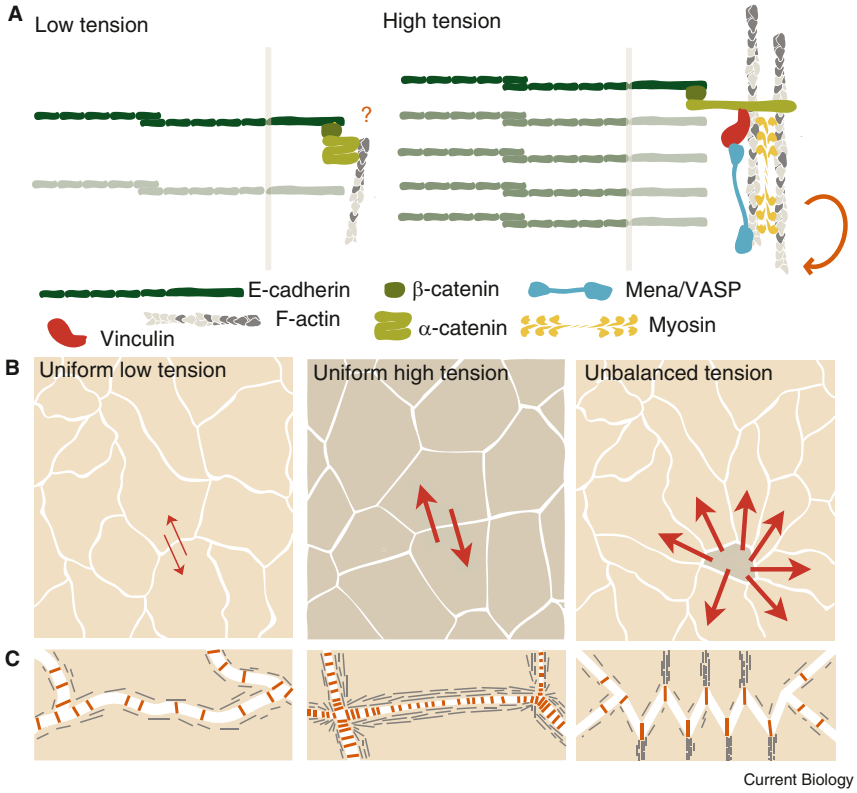


Figure 1. Model for tension feedback loop at adherens junctions.

(A) Under low tension, α-catenin is associated with β-catenin and E-cadherin in a closed conformation. In this state, the ability of α-catenin to bind actin is unclear. Under high tension, a conformational change allows α-catenin to recruit vinculin, which in turn binds Mena and VASP, promoting actin polymerization at barbed ends. This increased unbranched actin is favorable for myosin recruitment and action, thus generating more force, inciting a positive feedback loop between tension and actin polymerization. (B) Cell sheets experiencing uniform low, uniform high or unbalanced tension. Increased tension straightens cell borders. Red arrows indicate force exerted on neighbors. (C) Close-up diagram of cell borders under low, high or orthogonal F-actin-mediated tension (F-actin, gray lines). E-cadherin (red lines) accumulates at junctions under high tension and is enriched at tricellular junctions.

To cap off this work, the authors asked what role tension-dependent actin assembly plays in epithelial integrity, tying it back to potential roles in morphogenesis [1]. They found that Mena/VASP-dependent actin assembly is necessary for AJ stabilization of E-cadherin, reflecting the two-way feedback noted above between cadherin–catenins and the underlying actin. Finally, they used elegant laser surgery to cut AJs and directly measure junctional tension, finding that recruitment of Mena/VASP to AJs is both necessary and sufficient to support junctional contractile tension. Together, these data reveal a highly novel feedback mechanism, supporting a model in which α-catenin, when under tension, undergoes a conformational change and recruits vinculin (and perhaps actin directly) [1]. Vinculin, in turn, can both bind F-actin and recruit Mena/VASP to barbed ends. The resulting linear actin array at AJs provides a parallel actin network favorable for myosin, thus creating more tension and promoting more actin assembly (Figure 1A).

These novel insights into a tension-generated feedback loop help us understand how cells resist force during the dynamic events of morphogenesis, and also raise many new questions. At the mechanistic level, it will be important to further probe aspects of the model, including the hypothesized conformational

change in α-catenin, the Mena/VASP-independent role of vinculin in actin filament alignment at AJs [1], and the effects of actin stabilization on the supramolecular organization of cadherin–catenin complexes. Pushing outward, it will be interesting to determine whether different cell types use different mechanisms to achieve the same end, while exploring levels of baseline tension on AJs and differing actin architectures in cells in different tissues and in different cultured cell lines (Figure 1B). The role of tricellular junctions is also a topic for further exploration. Furthermore, cells in tissues also need to contend with force generated at basal focal adhesions, and the balance between this force and the AJ forces will be important to consider. Finally, it will be exciting to take these new insights *in vivo*, exploring the roles of vinculin in morphogenesis and examining events where Ena/VASP proteins are already known to influence morphogenesis, such as dorsal closure in *Drosophila* [17], and investigating how cells accommodate differences in tension across tissues [18] (Figure 1C) or, in a planar-polarized way, within individual cells (for example, [19]).

## References

1. Leerberg, J.M., Gomez, G.A., Verma, S., Moussa, E.J., Wu, S.K., Priya, R., Hoffman, B.D., Grashoff, C., Schwartz, M.A., and Yap, A.S. (2014). Tension-sensitive actin assembly supports contractility at the epithelial zonula adherens. Curr. Biol. *24*, 1689–1699.
2. Meng, W., and Takeichi, M. (2009). Adherens junction: molecular architecture and regulation. Cold Spring Harb. Perspect. Biol. *1*, a002899.
3. Gumbiner, B., Stevenson, B., and Grimaldi, A. (1988). The role of the cell adhesion molecule uvomorulin in the formation and maintenance of the epithelial junction complex. J. Cell Biol. *107*, 1575–1587.
4. Quinlan, M.P., and Hyatt, J.L. (1999). Establishment of the circumferential actin filament network is a prerequisite for localization of the cadherin-catenin complex in epithelial cells. Cell Growth Differ. *10*, 839–854.
5. Yamada, S., Pokutta, S., Drees, F., Weis, W.I., and Nelson, W.J. (2005). Deconstructing the cadherin–catenin–actin complex. Cell *123*, 889–901.
6. Abe, K., and Takeichi, M. (2008). EPLIN mediates linkage of the cadherin catenin complex to F-actin and stabilizes the circumferential actin belt. Proc. Natl. Acad. Sci. USA *105*, 13–19.
7. Sawyer, J.K., Harris, N.J., Slep, K.C., Gaul, U., and Peifer, M. (2009). The Drosophila afadin homologue Canoe regulates linkage of the actin cytoskeleton to adherens junctions during apical constriction. J. Cell Biol. *186*, 57–73.
8. Harris, T.J., and Tepass, U. (2010). Adherens junctions: from molecules to morphogenesis. Nat. Rev. Mol. Cell Biol. *11*, 502–514.
9. Sawyer, J.M., Harrell, J.R., Shemer, G., Sullivan-Brown, J., Roh-Johnson, M., and Goldstein, B. (2010). Apical constriction: a cell shape change that can drive morphogenesis. Dev. Biol. *341*, 5–19.
10. Kwiatkowski, A.V., Maiden, S.L., Pokutta, S., Choi, H.J., Benjamin, J.M., Lynch, A.M., Nelson, W.J., Weis, W.I., and Hardin, J. (2010). In vitro and in vivo reconstitution of the cadherin-catenin-actin complex from Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA *107*, 14591–14596.
11. Chesarone, M.A., and Goode, B.L. (2009). Actin nucleation and elongation factors: mechanisms and interplay. Curr. Opin. Cell Biol. *21*, 28–37.
12. Verma, S., Han, S.P., Michael, M., Gomez, G.A., Yang, Z., Teasdale, R.D., Ratheesh, A., Kovacs, E.M., Ali, R.G., and Yap, A.S. (2012). A WAVE2-Arp2/3 actin nucleator apparatus supports junctional tension at the epithelial zonula adherens. Mol. Biol. Cell *23*, 4601–4610.
13. Tang, V.W., and Brieher, W.M. (2012). alpha-Actinin-4/FSGS1 is required for Arp2/3-dependent actin assembly at the adherens junction. J. Cell Biol. *196*, 115–130.
14. Michael, M., and Yap, A.S. (2013). The regulation and functional impact of actin assembly at cadherin cell-cell adhesions. Semin. Cell Dev. Biol. *24*, 298–307.
15. Maruthamuthu, V., Aratyn-Schaus, Y., and Gardel, M.L. (2010). Conserved F-actin dynamics and force transmission at cell adhesions. Curr. Opin. Cell Biol. *22*, 583–588.
16. Yonemura, S., Wada, Y., Watanabe, T., Nagafuchi, A., and Shibata, M. (2010). alpha-Catenin as a tension transducer that induces adherens junction development. Nat. Cell Biol. *12*, 533–542.
17. Gates, J., Mahaffey, J.P., Rogers, S.L., Emerson, M., Rogers, E.M., Sottile, S.L., Van Vactor, D., Gertler, F.B., and Peifer, M. (2007). Enabled plays key roles in embryonic epithelial morphogenesis in Drosophila. Development *134*, 2027–2039.
18. Martin, A.C., Gelbart, M., Fernandez-Gonzalez, R., Kaschube, M., and Wieschaus, E.F. (2010). Integration of contractile forces during tissue invagination. J. Cell Biol. *188*, 735–749.
19. Simoes Sde, M., Blankenship, J.T., Weitz, O., Farrell, D.L., Tamada, M., Fernandez-Gonzalez, R., and Zallen, J.A. (2010). Rho-kinase directs Bazooka/Par-3 planar polarity during Drosophila axis elongation. Dev. Cell *19*, 377–388.

[1]Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-3280, USA. [2]Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA.
*E-mail: peifer@unc.edu

http://dx.doi.org/10.1016/j.cub.2014.06.063

# Neural Circuits: Interacting Interneurons Regulate Fear Learning

**A recent study has found that, during associative fear learning, different sensory stimuli activate subsets of inhibitory interneurons in distinct ways to dynamically regulate glutamatergic neural activity and behavioral memory formation.**

**Takaaki Ozawa and Joshua P. Johansen**

Aversive experiences are powerful triggers for memory formation and adaptively change our behavior. For some individuals, however, aversive learning becomes excessive leading to anxiety disorders such as post-traumatic stress disorder, which has a lifetime risk of 7–8% in US citizens and even higher (14–16%) in soldiers with combat experience [1]. Auditory fear conditioning is a powerful model for investigating the neural circuits of aversive learning and possibly for understanding pathological anxiety disorders: during auditory fear conditioning, animals learn that an auditory tone (conditioned stimulus, CS) predicts the occurrence of an aversive outcome such as a mild electrical shock (unconditioned stimulus, US) [2–6] (Figure 1A). Excitatory glutamatergic neurons in a brain region called the amygdala are known to store fear memories and contact other regions to produce fear responses. There are, however, other cell types within the amygdala, the γ-aminobutyric acid (GABA)ergic interneurons, which can inhibit neural communication locally and modulate the function of glutamatergic neurons.

It was not clear from previous research how the coordinated activity of these different intermixed cell populations in the amygdala participated in fear memory formation. Wolff *et al.* [7] addressed this question by taking advantage of a combination of techniques including optogenetics, *in vivo* recordings of single cell electrical activity, and behavioral

CrossMark