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VIEWPOINT

Afadin–nectin forces its way to the front

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Force transmission at cell–cell junctions critically regulates embryogenesis, tissue homeostasis, and diseases including cancer. The cadherin–catenin linkage has been considered the keystone of junctional force transmission, but new findings challenge this paradigm, arguing instead that the nectin–afadin linkage plays the more important role in mature junctions in the intestinal epithelium.

Cell–cell adhesion is a basic principle of multicellular organization, mediating essential steps in morphogenesis and structure, as well as adult tissue homeostasis and function (Campas et al., 2024; Harris and Tepass, 2010). Initial characterization of cell–cell adhesions used electron microscopy to reveal adjacent polarized epithelial cells with three discernable structures: tight junction at the top, closest to the apical domain, adherens junctions (AJ) further toward the basal surface, and desmosomes further still from the apical domain. Tight junction formation is driven by transmembrane junctional adhesion molecules, occludins and claudins that mediate homophilic binding across the intercellular space; AJs are formed by homophilic binding of cadherins with some help from nectins; and desmosomes are formed by desmosomal cadherins. Nectins, cadherins, and desmosomal cadherins all connect to cytoskeletal filaments in various ways, creating physical linkages that not only provide tissues with mechanical integrity but are also essential regulators of junction formation and signaling.

In recent years, mechanical forces have become an important focus of studies of AJ and especially of E-cadherin. Indeed, impairing function of this major AJ actor or its catenin partners affects AJ mechanical integrity required for embryo compaction at the morula stage, in which loosely attached individual cells become a coherent structure (Larue et al., 1994). The role of mechanical forces is apparent from the cell morphological changes and initial cell coordinated motions. Mechanical force generation is related to F-actin cytoskeleton organization and its association with myosin II to form actomyosin structures beneath the plasma membrane. But how forces from contractile actomyosin connect, transmit force, and then govern cell and tissue structure is still under intense investigation.

A scheme in which the cadherin extracellular domains mediate trans homophilic cell–cell adhesion, while its intracellular domain links sequentially to β-catenin, α-catenin, and finally to F-actin, has been reproduced in all cell biology textbooks for 30 years (Harris and Tepass, 2010). Vast amounts of data in multiple organisms support this model and demonstrate its biological importance (Harris and Tepass, 2010). However, this model has some weakness and was challenged by biochemical and structural studies showing that α-catenin could not bind simultaneously to β-cadherin and to F-actin (Yamada et al., 2005) but instead regulated actin polymerization separately from its role as a linker (Drees et al., 2005). Resolution of this paradox came in a series of studies showing directly that cadherins bear mechanical loads (Conway et al., 2013; Huveneers and de Rooij, 2013) and that α-catenin undergoes conformational transitions under tension that enable actin binding (Buckley et al., 2014). The E-cadherin–actin connection is further strengthened under mechanical loads when α-catenin unfurls to recruit vinculin, which provides a second link to F-actin (Huveneers and de Rooij, 2013). Additionally, α-catenin binds more strongly to actin filaments that are under tension, providing an additional level of mechanical regulation (Buckley et al., 2014; Huveneers and de Rooij, 2013). The concept of mechanical force transmission through the cadherin–catenin complex is thus firmly established.

But despite the strength of this paradigm, the cadherin–catenin complex is not the whole show. Indeed, AJs are in close proximity to nectin family receptors that also contribute to AJ formation. Nectins differ from E-cadherin by their Ca^{2+}-independent adhesion and connection to F-actin through afadin, a multi-domain adapter that binds additional partners (Mandai et al., 2015). In *Drosophila*, the afadin ortholog Canoe regulates linkage to the actin cytoskeleton at AJs during apical constriction, a process that requires force transmission between cells (Sawyer et al., 2009). Actin binding also appears to be important for afadin function in mammalian cells (Mandai et al., 2015). Moreover, afadin also binds α-catenin, creating a connection between the cadherin and nectin systems (Mandai

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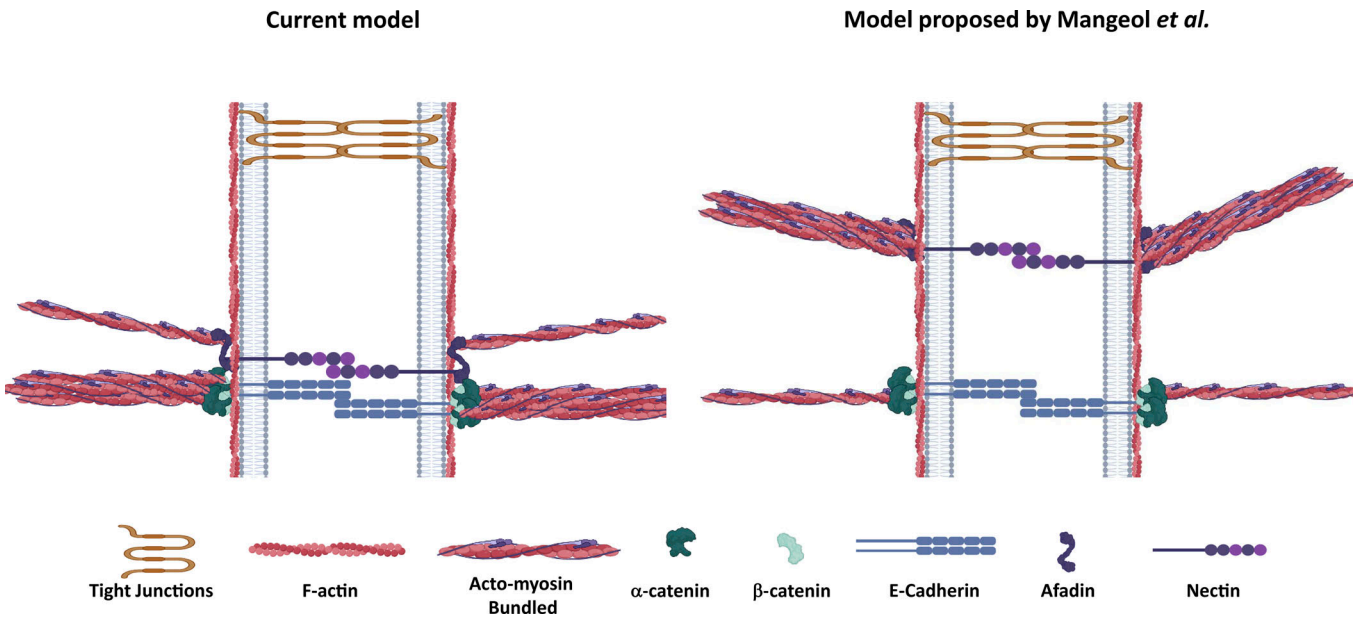

Figure 1. **The current model (left panel) in which cadherin–catenin assembly forms the major connection to contractile actomyosin must be reconsidered in light of new data showing that in mature intestinal epithelial cells, the nectin–afadin complex links more strongly to actin and bears a higher proportion of the mechanical load (right panel).**

et al., 2015). Despite accumulating evidence of their functional importance (Mandai et al., 2015), the nectin–afadin complex remains the poor cousin in AJ investigations, likely due to a paucity of direct biochemical/structural evidence. However, a recent report (Mangeol et al., 2024) might provide the impetus to reconsider this view. Indeed, data from this study suggest a more complex, multi-component structure in which mechanical forces are transmitted between cells and across tissues through a variety of linkages, among which cadherin/catenin is not necessarily the most important one.

Mangeol et al. used STED (stimulated-emission-depletion) super-resolution imaging to resolve AJs in human intestinal tissue at a finer level than had been previously achieved (Mangeol et al., 2024). This approach showed that in fully mature junctions in vivo, the E-cadherin/β-catenin and nectin–afadin complexes form parallel belts with the cadherins 100–200 nm below the nectins. Unexpectedly, the nectin/afadin belt colocalized with the main F-actin bundles, whereas E-cadherin localized with a minor fraction. Further in vitro measurement of the elastic recoil after laser severing of actin bundles using the colon Caco-2 cell line as well as analysis of the shape of cell–cell junctions in co-cultures of wild-type and afadin knockout (KO) cells argued that the afadin–actin linkage was the major load-bearing structure (Fig. 1). Importantly, in vitro effects were only seen in the fully mature junctions that develop after extended times in culture. These data thus challenge the current cadherin-centric paradigm and call for additional studies to better understand the role of the nectin–afadin system at AJs.

These conclusions require confirmation from independent approaches. Comparative measurements using tension sensors (Conway et al., 2013) to measure forces across nectins and cadherins would go a long way toward establishing their relative contributions to force transmission. Use of culture conditions that allow full junction maturation will be essential. These studies will be complicated by regulatory effects of knockdown (KD) or KO of key players. For example, KO or KD of cadherins releases β-catenin to the nucleus (Harris and Tepass, 2010). KO or KD of nectins perturbs the kinetics of junction formation and apical–basal polarity and related signaling pathways (Mandai et al., 2015). Thus, careful analysis that integrates structural and regulatory effects will be required.

These questions are of high importance, as forces exerted at AJs are significant not only for tissue integrity but for regulation of signaling pathways that govern gene expression and cell fate in a multiplicity of biological settings (Campas et al., 2024; Harris and Tepass, 2010). Indeed, wide-ranging effects of deleting or mutating afadin and nectins on tissue architecture, cell fate, and developmental pathways in multiple organisms have been hard to explain without invoking mechanochemical functions (Mandai et al., 2013, 2015). Further, in addition to mechanical stresses transmitted across tissues that orchestrate morphogenetic motions, stresses and strains at the nanoscale level likely play regulatory roles, for example, through local membrane bending or tension. Super-resolution STED and atomic force microscope approaches will likely be needed to elucidate these types of effects.

Much remains to be done to understand the role of the nectin/afadin linkage, transmission and transduction of mechanical forces, and membrane deformations during embryogenesis. Initial studies on organoid models will provide significant clues to integrate and determine how mechanical force transmission and regulatory effects impact cell cycle (Perrais et al., 2007), metabolism (Bays et al., 2017), collective migration (Khalil and de Rooij, 2019), and other processes known to depend on mechanical forces at AJs. Beyond its relevance to embryonic development, this work will likely contribute to better define etiology and mechanisms for the human pathologies in which mechanical forces and parameters

play prominent roles (Mandai et al., 2015). Cancer and cardiovascular disorders (hypertension, atherosclerosis, aneurysms, cardiomyopathies) are the most obvious, which together with others (osteoporosis, organ fibrosis, emphysema) are the major causes of illness and death across the world.

This work calls for refocusing on nectin/afadin and its relationship with E-cadherin/β-catenin. It also reminds us that new methodologies can shake up dogmas that may be weaker than expected. As is often the case, Mark Twain said it best: "It ain't what you don't know that gets you into trouble. It's what you know for sure that just ain't so."

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