CLOSING THE GAP: TRICELLULIN/α-CATENIN INTERACTION MAINTAINS EPITHELIAL INTEGRITY AT VERTICES

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Tricellular junctions play a critical role in regulating epithelial barrier function. In this issue, Cho et al. (2022. J. Cell Biol. https://doi.org/10.1083/jcb.20209037) demonstrate a novel interaction between tricellulin and α-catenin, which connects tricellular junctions to the actomyosin cytoskeleton, thus supporting the epithelial barrier at cell vertices.

Epithelial tissues are made up of polarized cells connected to their neighbors. These connections, called cell–cell junctions, maintain tissue integrity and barrier function during tissue morphogenesis and homeostasis. In vertebrates, cell–cell junctions include tight junctions (TJs), which selectively regulate the permeability of small molecules and ions between cells, and adherence junctions (AJs), which physically adhere neighboring cells to each other.

While there has been extensive research about bicellular junctions (BCJs), much less is understood about the molecular makeup and regulation of tricellular junctions (TCJs), the vertices where three cells meet. Previous studies have shown that in the vertebrate epithelium, transmembrane angulin family proteins including angulin-1/LSR (lipolysis-stimulated lipoprotein receptor) localize specifically to tricellular TJs (tTJs) where they recruit another transmembrane tTJ-specific protein called tricellulin (1). Knockdown of either angulins or tricellulin affects paracellular permeability, indicating that tTJs contribute to epithelial barrier function (2). However, the mechanism by which tricellulin contributes to barrier function at TCJs remains unclear.

Notably, no homologs of tricellulin or the angulins have been identified in invertebrate epithelia. Instead, studies in Drosophila have discovered different proteins that regulate barrier function at TCJs in flies (2). Additionally, the transmembrane protein Sidekick is enriched at tricellular AJs in flies (3) and is important for anchoring the actomyosin cytoskeleton at TCJs (4). Although vertebrate homologs of Sidekick exist, potential roles for Sidekick at TCJs in vertebrate epithelia remain unclear. Thus, a key open question is: How is the actomyosin cytoskeleton anchored at TCJs in vertebrate epithelial tissues?

In this issue, Cho and colleagues (5) set out to discover the molecular mechanism that maintains junctional integrity at vertebrate TCJs. Previously, partial loss of tricellulin was shown to impair TJ organization and epithelial barrier function (1). The authors built upon those findings, utilizing CRISPR-Cas9 to generate a tricellulin knockout (KO) EPH4 epithelial cell line. The authors showed that tricellulin loss results in morphologically disrupted tTJs. Using a TJ marker (claudin-3) as a readout, they measured a significant increase in gaps at tTJs in tricellulin KO cells. Furthermore, they showed that barrier function is compromised when tricellulin is KO, demonstrating that paracellular permeability to both ions and macromolecules is impaired. Using a biotin tracer assay to investigate effects on local barrier function, they reported evidence of a tubular gap at vertices in tricellulin KO cells. Additionally, the authors confirmed that tricellulin KO does not affect angulin-1/LSR localization at tTJs; however, the enrichment of tricellulin at tTJs was lost in angulin-1/LSR KO cells, verifying that angulin-1/LSR is required for tricellulin recruitment to TCJs (6).

The authors then investigated how actomyosin contractility affects TCJs. Previous work proposed that the organization of actomyosin at TCJs might be important for generating a tightening force for sealing tTJs (7). Using a calcium switch assay, Cho et al. (5) demonstrated that during TJ formation, actin filaments form a crisscrossing meshwork at TCJs, and myosin II localizes on overlapping antiparallel actin filaments (Fig. 1). They then tested the functional role of actomyosin contractility at TCJs. First, they showed that cells treated with blebbistatin (a small molecule inhibitor of myosin II) exhibited diffuse tricellulin signal compared to controls. Second, to test the role of actomyosin contractility specifically at TCJs, the authors carried out a clever experiment where endogenous angulin-1/LSR was knocked out and replaced with angulin-1/LSR fused to the catalytic subunit of myosin phosphatase. In these cells where tension was decreased specifically at TCJs, there was a significant increase in disrupted vertices compared to controls, suggesting that actomyosin contractility is important for closing the gap at tTJs.

How are TJs physically connected to the actomyosin cytoskeleton? The authors...
began by investigating junctional proteins known to link other types of cell–cell junctions to the actin cytoskeleton. They found that the AJ proteins α-catenin and vinculin localized to TCJs in a tricellulin-dependent manner. Specifically, they showed that the tension-activated open conformation of α-catenin, which can recruit vinculin to strengthen the connection to the actin cytoskeleton (8), was enriched at TCJs; that vinculin KO cells have disrupted TCJs; and that tricellulin KO resulted in a reduction of α-catenin and vinculin at cell vertices.

Surprisingly, Cho et al. (5) demonstrated that α-catenin interacts directly with tricellulin. The authors made a strong case for this novel interaction using a combination of pulldown assays where they narrowed the binding regions using a series of deletion mutants, a binding assay with purified recombinant proteins, and a proximity ligation assay in cells. To further bolster their claim, the authors made use of a tricellulin mutant linked to heritable, non-syndromic deafness (9). This tricellulin C395X mutant lacks most of the C-terminal region due to a premature stop codon, and the authors showed it exhibited reduced binding to α-catenin. Strikingly, when expressed in tricellulin KO cells, the tricellulin C395X mutant failed to restore proper tTJ morphology, and the cells exhibited permeability defects, suggesting that the C-terminal region of tricellulin, which binds α-catenin, is important for tTJ formation and function in cells.

Collectively, these experiments represent an important step forward in our understanding of how TCJs interact with the actomyosin machinery. They also reveal an unexpected interaction between a classic AJ protein and a tTJ protein. To further support this novel role for α-catenin, the authors performed a nice experiment where they expressed a chimeric protein comprised of E-cadherin lacking its catenin-binding domain fused to α-catenin in α-catenin KO cells—such that there is no free α-catenin available to bind its partners. While this chimeric construct was able to rescue junctional defects along BCJs, it could not rescue defects at TCJs. This finding reinforces the conclusion that the pool of α-catenin can be split between two different complexes at cell–cell junctions: (1) the E-cadherin–α-catenin complex along BCJs and (2) the tricellulin–α-catenin complex at TCJs (Fig. 1).

The findings by Cho et al. reveal a novel mechanism underlying barrier function at TCJs. TCJs are known sites of increased tension that can threaten epithelial integrity. For example, these sites are exploited by pathogenic bacteria like *Shigella flexneri*, which causes bacterial dysentery (7). Additionally, loss of TCJ-specific proteins has been linked to diseases such as ulcerative colitis and cancer (10). The novel findings by Cho et al. provide a foundation for exciting future research questions. In particular, how are TCJs maintained when they are further challenged by mechanical force during morphogenesis or organ homeostasis? Are there situations when the gap at TCJs needs to be opened in a regulated manner, similar to patency in *Drosophila* (11), and is the tricellulin–α-catenin complex involved in that regulation? Additionally, the authors show that vasodilator-stimulated protein (VASP), which promotes actin filament assembly in a tension-sensitive manner at AJs (12), localizes to the crisscrossing actin meshwork during TCJ formation. This raises the question: Does VASP play a role in dynamic regulation of mature TCJs? These questions and others will be interesting extensions of the new paper by Cho et al., which represents a critical step forward in understanding how epithelial cells close the gap at TCJs.

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