Tricellular junctions

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What are tricellular junctions?

The three-dimensional organization of epithelial cell sheets, which cover all organs, provide a selectively permeable barrier, separating the internal milieu from the external environment. This cellular organization is accomplished by specialized cell-cell adhesion complexes that are present along the lateral cell membranes, at both tricellular junctions (TCJs), the points within epithelial tissues where at least three epithelial cells contact, and bicellular junctions (BCJs), the junctions connecting the TCJs (see Figure 1 for septate, tight and adherens junction organization). Combined the TCJs and BCJs define the cellular geometry and the epithelial packing.

Why are tricellular junctions relevant?

Disruption of TCJ structure due to the loss of TCJ components or proteins that regulate TCJ homeostasis impairs epithelial barrier function and monolayer organization maintenance causing dysplasia. Aberrant TCJ function has been linked to several human genetic diseases such as the inflammatory bowel disease ulcerative colitis (loss of Tricellulin, also known as Marveld2), congenital tufting enteropathy (loss of EpCAM), deafness (loss of Angulin-2/ILDR1 or Tricellulin), and cancer progression and malignancy (loss of Angulin-1/LSR or Tricellulin). In addition, several pathogenic bacteria can utilize TCJs, as sites to enter and spread to neighbouring cells (*Shigella flexneri*), to release toxins into the cells (*Clostridium difficile*), or as passageways to invade other tissues (group A *Streptococcus* and enteropathogenic *Escherichia coli*). Furthermore, in recent years, studies in both invertebrate and vertebrate model systems have illuminated additional key functions of TCJs in the regulation of cell division orientation, cytokinesis, cell packing, cell-cell rearrangements, planar cell polarity, collective cell migration, stem cell proliferation and cell mechanical properties.

How to build a tricellular junction?

To date only few proteins have been identified that are specifically localized at the TCJ and that are required for invertebrate tricellular septate junction (tSJ) or vertebrate tricellular tight junction (tTJ) formation (Figure 2). Proteins thus far identified at the tTJ are Tricellulin, Angulin-1/LSR, Angulin-2/ILDR1 and Angulin-3/ILDR2, while Anakonda (Aka or Bark), Gliotactin (Gli) and the M6 protein are specifically localized at the tSJ in *Drosophila melanogaster*. The geometry of the TCJ is proposed to be defined by the interaction of proteins from three adjacent cells via their extracellular domains, Angulin-2/ILDR1 and Aka for vertebrate and invertebrate TCJ respectively. More recently, Gli and Aka were shown to form a protein complex with Discs large (Dlg) and Scribble (Scrib) to regulate tSJ formation and positioning along the lateral cell edge.

The formation of the tricellular adherens junction (tAJ) is poorly studied. Only the *Drosophila* Sidekick (Sdk) protein has been identified as a component of the tAJ. Sdk is a homophilic adhesion molecule. Via its interaction with Canoe (Cno) and *Drosophila* ZO-1 Polychaetoid (Pyd), it connects the actin cortices of two neighbouring cells at the level of the tAJ. Sdk can also form a complex with β-Catenin (β-Cat) and Myosin II (MyoII).

Are tricellular junctions tension and geometry sensors?

TCJs must bear the pulling forces produced by three BCJs. Furthermore, because junctional tension continuously fluctuates, it is tempting to speculate that TCJs constitute spatial locations within epithelia that can sense changes in BCJ tension, adapt their mechanical properties and in turn respond by regulating the mechanical properties of the BCJ. Indeed, recent evidence points towards such mechanical role of Sdk at tAJ. The germband extension during *Drosophila* embryogenesis coincides with cell-cell rearrangements, which rely on the oscillatory cycles of E-Cad and MyoII accumulation at the tAJ. Sdk was shown to be critical for such cell-cell rearrangements. The protein level of Sdk at the tAJ is modulated by junctional tension, since Sdk level increases upon increasing junctional tension. In turn, Sdk regulates the BCJ tension as Sdk loss of function reduces BCJ tension. By regulating the mechanical properties at the tAJ, Sdk, E-Cad and MyoII facilitate the junction shrinkage, exchange and lengthening during cell-cell rearrangements, and thus influence the geometry and topology of both the cells and the tissue. In addition to Sdk, Afadin, MyoII, Vinculin, E-Cad, Ajuba, Shroom, Steppke, and α- and β-Cat are localized at the tAJ in a BCJ tension dependent manner in different contexts.

In addition, a mechanical role has also been demonstrated at vertebrate tTJs. In Caco2 cells grown under subconfluent culture conditions Tricellulin promotes the accumulation of the Cdc42 GEF Tuba at the tTJ. In turn, Tuba activates Cdc42 to stimulate the assembly of an actomyosin meshwork both at the TCJ and BCJ, thereby regulating BCJ curvature and tissue topology. Thus, in response to changes in junction tension, TCJ-dependent signalling can provide feedback signalling, regulating the BCJ tension.

TCJs may also act as sensors of cell geometry and mechanical stress anisotropy, as within the tissue the TCJ distribution tends to align with the cell long axis and with the global direction of tissue mechanical stress,. When *Drosophila* cells round up during mitosis the distribution of the TCJs confers a memory of the interphase cell shape. By recruiting the spindle guidance protein Mud (NuMA in mammals) to the TCJs, this ensures that cells divide according to the interphase cell geometry and the main tissue mechanical stress orientation. A similar mechanism for TCJs acting as cell shape sensors regulating division orientation was recently identified in *Xenopus laevis*. Because TCJs also control the cell geometry and topology by facilitating cell-cell rearrangements, we hypothesize that cells may utilize TCJs as spatial landmarks to control their geometry and topology within the tissue during tissue development.

Do tricellular junctions constitute signalling hubs?

In addition to sensing and responding to mechanical cues, TCJs have also been implicated in polarity, growth and proliferation signalling. First, recent evidence indicates that their spatial distribution may allow for the regulation of tissue planar cell polarity. Indeed, a planar cell polarity signalling module involving the proto-cadherin Fat2, the receptor tyrosine phosphatase DLar and the WAVE regulatory complex protein Abi is present at the TCJs of the *Drosophila* follicular epithelium, where it promotes the formation of whip-like actin protrusions that underlie the collective migration of this epithelium. Second, impaired Gli function at TCJ triggers JNK pathway signalling which increases proliferation. Last, several key constituent proteins of the mechano-sensitive Hippo growth signalling pathway, the Warts kinase and its interactor Ajuba, and the actin binding proteins Zyxin and Enabled are present at TCJs. How a putative TCJ-dependent Hippo signalling would contribute to growth control and how this local signalling is integrated with signalling emanating from the BCJ remains to be established.

What's next?

TCJs are emerging as key spatial sites in epithelial tissues that integrate biochemical and mechanical signalling to control local cell dynamics while maintaining tissue barrier function. Our understanding of how TCJs sense, interpret and transduce signalling input will vastly dependent on defining how TCJs are assembled and remodelled during tissue development or homeostasis,

and how TCJ remodelling is coordinated with the local cellular dynamics in 3D. Currently, studies have begun to address these questions and several key components of the TCJ have been identified. Yet, our knowledge of the dynamics of the tricellular cortex structure and mechanical properties still remains sparse.

Where can I find out more?

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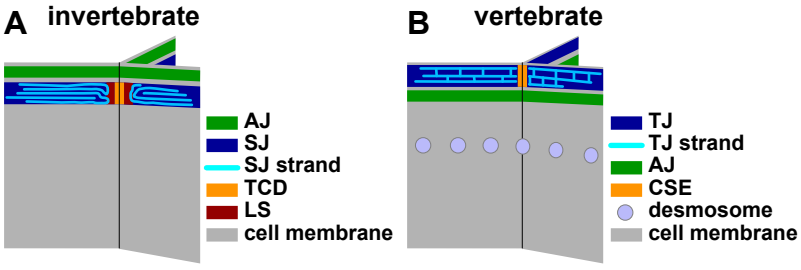
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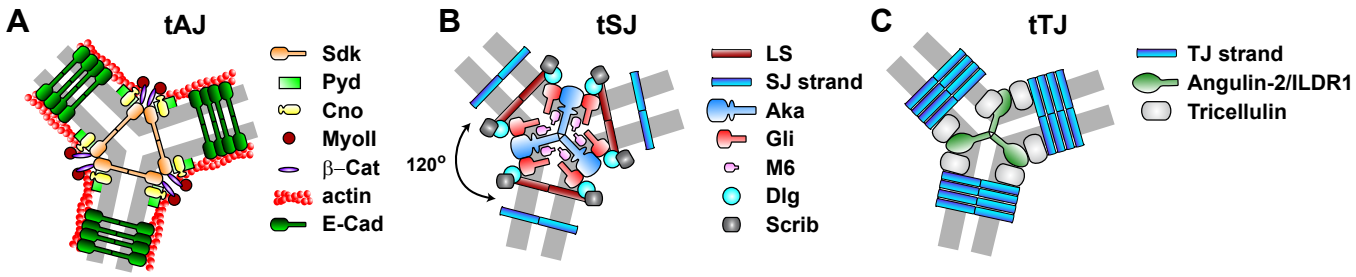
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Figure 1. Invertebrate and vertebrate TCJ adhesion organization.
(A-B) Schemes depicting the invertebrate and vertebrate TCJ organization along the lateral cell cortex. In invertebrate tissues the adherens junction (AJ) is located above the zonula occludens; the septate junction (SJ). In vertebrate tissues, the functionally equivalent tight junction (TJ) is located above the AJ. In both model systems TCJ channels are present along the lateral contact interfaces between three cells. At the level of the zonula occludens the TCJ channel in invertebrates is composed by a series of stacked diaphragms, tricellular channel diaphragms (TCD) or a central sealing element (CSE) in vertebrates. In invertebrates the bicellular SJ strands connect to the TCD along the lateral axis forming a lateral limiting strand (LS). In vertebrates TJs form strands that attach to the CSE. Vertebrates exhibit an additional adhesion structure the desmosome, where the plakophilin PKP3 is localized.

Figure 2. Tricellular adherens, septate and tight junction protein composition.
(A-C) Diagrams showing the protein composition at the *Drosophila* tAJ and tSJ, and the vertebrate tTJ. (A) At the *Drosophila* tAJ, Sdk proteins from two adjacent cells may form homophilic dimers in *cis* and in *trans*. Sdk can be found in a complex with β-Cat and MyoII and its interaction with Cno and Pyd facilitates interaction with the actin cortex. In turn the actin cortex is linked to E-Cad clusters at the BCJ. (B) More basally, at the tSJ, the scaffolding proteins Dlg and Scrib recruit Aka and Gli to the membrane. At the membrane Aka recruits Gli, and Aka proteins from three adjacent cells self-organize into a tripartite septum ensuring the 120° geometry of the junctions. The Dlg/Scrib/Aka/Gli complex in turn interacts with the LS of the SJs. Transmembrane glycoprotein M6 is necessary for Gli localization, yet its role in tSJ assembly is unknown. (C) At the tTJ in mammalian cells, 3D protein modelling suggests that Angulin-2/ILDR1 proteins from three adjacent cells can form a homo-trimer through their Ig-like domains. Via their intracellular domains the Angulin family of proteins (Angulin-1/LSR, Angulin-2/ILDR1, Angulin-3/ILDR2) are thought to interact with Tricellulin, which connects to the TJ.