Tricellular junctions: a hot corner of epithelial biology

Floris Bosveld, Zhimin Wang, Yohanns Bellaïche

Tricellular junctions: a hot corner of epithelial biology.

Floris Bosveld[1,2*], Zhimin Wang[1,2], Yohanns Bellaïche[1,2*]

1 : Institut Curie, PSL Research University, CNRS UMR 3215, INSERM U934, F-75248 Paris Cedex 05, France.

2 : Sorbonne Universités, UPMC Univ Paris 06, CNRS, CNRS UMR 3215, INSERM U934, F-75005, France

* Correspondence: floris.bosveld@curie.fr, yohanns.bellaiche@curie.fr

Declaration of interest: none

Abstract

As the result of an intricate interplay between mechanical and biochemical cues, coordinated cell dynamics are at the basis of tissue development, homeostasis and repair. Numerous studies have addressed the interplay between these two inputs and their impact on cellular dynamics. These studies largely focus on bicellular junctions (BCJs). Recent works have illuminated that tricellular junctions (TCJs), the junctions where 3 cells contact, play important roles in epithelial tissues beyond their well-known structural function in preserving epithelial barrier integrity. Indeed, TJCs have recently been implicated in the regulation of collective cell migration, division orientation, cell proliferation and cell mechanical properties. More generally, the TCJ distribution aligns with the cell shape and mechanical stress orientation within the tissue, while their positioning encapsulates the packing topology. Importantly, known regulators of growth signalling and of cell mechanical properties are also localized at TCJs. Therefore, TCJs emerge as spatial sites to sense and integrate biochemical and mechanical inputs to guide epithelial tissue dynamics.

Introduction

As selective permeability barriers epithelial cell sheets cover organs separating them from the external milieu. To ensure paracellular barrier function cells are tightly packed and form specialized adhesive contacts along their apical-basal axes, at both the bicellular junctions (BCJs) and at the tricellular junctions (TCJs). In vertebrate tissues, cell-cell adhesion is mediated by the apical tight junctions (TJs), basal-lateral adherens junctions (AJs) and the desmosomes, while in invertebrate tissues adhesion is mediated by the apical AJs and the basal-lateral septate junctions (SJs) (Figure 1a, b) (for detailed structural information on AJs, TJs, SJs and TCJs see reviews [1–6]). Thus, BCJs and TCJs are core structures of epithelial tissues. They have been extensively studied from a structural point of view and shown to be critical for tissue barrier functions in numerous tissues.

TCJ function and organization have been mainly studied at the level of TJ and SJ. The organization and structure of the tricellular vertebrate TJs and invertebrate SJs appear largely similar [3,5]. However, the apical-basal organization of the TJs and SJs is different and the TCJ molecular components do not share sequence homology, although they perform functionally analogous tasks (Figure 1a', b'). TCJ barrier integrity in vertebrate systems is mediated by the Tricellulin and the Angulin protein family [7–9], while in *Drosophila*, barrier integrity is provided by Gliotactin (Gli) and Anakonda (Aka) (also known as Bark beetle, Bark) [10,11,12••]. Numerous works have focused on the roles of BCJ mechanical properties, their formation and remodelling in promoting tissue dynamics and regulating epithelial packing (reviews [13–15]). Recent studies now show that epithelial tissue dynamics and mechanics can be understood, if the function of TCJs is taken into account. After a brief description of the TCJ structure in vertebrate and invertebrate systems, we will focus primarily on the emerging roles of TCJs in epithelial cell division, migration, cell mechanics and stem cell maintenance.

TCJ structure, protein composition and regulation

Vertebrate and invertebrate TCJ structure and formation are best characterized at the level of the TJ and SJ, respectively. In vertebrate and invertebrate model systems TCJ channels, or pores, are present along the apical-basal axis at the contact interfaces between three cells. They are formed by a series of stacked diaphragms, tricellular channel diaphragms (TCD) in invertebrates or a central sealing element (CSE) in vertebrates (Figure 1a,b) (reviewed in [5,6]). In vertebrate systems, the bicellular TJs form strands that attach to the central sealing element

[16], while in invertebrates the bicellular SJ strands connect to the diaphragm along the apical-basal axis forming a lateral limiting strand (LS) at the tricellular contact interface [17–19]. From a molecular point of view, in vertebrates, Tricellulin mediates the connection between the TJ strands and central sealing element via the Angulin proteins (Angulin1-3) [8,9,20]. In invertebrates the molecular link between the lateral limiting strands and the tricellular channel diaphragms has been proposed to be mediated by the Gli protein [10,21], while the connection between the tricellular channel diaphragms and Gli is mediated by Aka [12••]. Aka is a transmembrane domain with a large extracellular domain. Based on experimental data and computer simulations, it is proposed that the geometry of the TCJ facilitates the self-organization of the extracellular domains of Aka proteins from three adjacent cells into a tripartite septum in the TCJ lumen (Figure 1a')[12••].

To understand how TCJs are formed and remodeled, it is essential to determine how proteins are trafficked and targeted to the TCJs. Currently, the cellular and molecular mechanisms regulating protein targeting and localization to the TCJ are still not fully understood. However, the Auld lab has provided insight into this central question in *Drosophila* by analyzing how Gli levels are regulated at the TCJ. The localization and levels of Gli are regulated at the protein level by phosphorylation-dependent endocytosis and degradation facilitated by the SJ protein discs large (Dlg) [22–24] and the C-terminal Src kinase (Csk) [25••]. Moreover, Gli mRNA levels are regulated though a feedback mechanism that utilizes the bone morphogenetic protein (BMP) pathway to upregulate a microRNA, miR-184, which targets the 3'UTR of Gli for degradation [26].

Combined these studies show that TCJs are complex molecular structures, of which the molecular composition needs to be strictly controlled to ensure barrier integrity.

TCJ formation during epithelial tissue remodeling and division

In order to preserve barrier function during homeostasis or morphogenesis, remodelling and *de novo* formation of BCJs and TCJs needs to be tightly coupled with local cellular dynamics such as cell elimination by extrusion, apoptosis, delamination [27] or fusion [28], cell addition upon division [29] or cell insertion [30], or changes in cell positions during cell-cell rearrangements [31]. Two studies have addressed how *de novo* TCJs are formed in the mouse ear skin epithelium [32,33••]. The mouse ear skin is a multi-layered epithelium composed of 1) an outer barrier, the stratum corneum (SC), 2) followed by the TJ barrier, the stratum

granulosum (SG), 3) the stratum spinosum (SS) and 4) the proliferative layer, the stratum basale (SB). Cells from the proliferative layer traverse though the stratum spinosum and stratum granulosum to finally reach the stratum corneum. Yokouchi et al. [33••] demonstrated that during this upward migration the cells form a specialized 3D shape filling structure resembling a Kelvin's tetrakaidecahedron (14-sided solid with six rectangular and eight hexagonal sides) and establish Tricellulin/Angulin-1 positive TCJs with the cells of the stratum granulosum to preserve barrier integrity when entering the stratum granulosum layer. The second study addressed how antigen-presenting dendritic cells of the skin, the Langerhans cells, located between the stratum spinosum and stratum basale layers, generate dendrites that penetrate the stratum granulosum to uptake antigens in the space between the stratum granulosum and stratum corneum layers and within the stratum corneum [32]. When a dendrite penetrates the TJ of the stratum granulosum layer, Tricellulin accumulates at the tricellular contact sites between two stratum granulosum cells and the dendritic cell to establish new TCJs, thus preserving barrier integrity.

In parallel to these studies on the remodelling of TCJ, the *de novo* TCJ formation in monolayered proliferative epithelia is currently emerging as an active and important field of research. *De novo* BCJ formation at the level of the AJs upon cell division within epithelial tissues has been extensively characterized. Combined, these studies demonstrate that the formation of a new BCJ relies on an intricate interplay between the dividing cell and its neighbouring cells [34,35,36••]. One recent study in *Xenopus* provides insights into TCJ formation at the level of the TJs during cell division and how this is coordinated with AJ formation [37••]. In cells of the *Xenopus* gastrula epithelium new TCJs are formed during the final stages of cytokinesis. Upon midbody formation and AJ establishment, the TCJ proteins Angulin-1 and Tricellulin are recruited to the nascent TCJs, which closely appose to the midbody to form two mature TCJs between each daughter cell and its two neighboring cells. This mode of cytokinesis results in the creation of a bicellular neighbor-neighbor interface, which has also been reported in the chick epiblast cells [35]. In many different epithelia and model systems, including *Xenopus*, *Drosophila*, *Hydra*, and the zebrafish cell division results in the formation of a daughter-daughter cell interface [38–44]. This raises the question of whether the creation of a daughter-daughter interface requires a different mode of TCJ establishment. Our recent findings show that within the *Drosophila* notum tissue, TCJ maturation at the level of the SJ initiates after the apical daughter-daughter AJ has been

formed (Wang et al., submitted). Upon AJ formation, the daughter cells and their neighboring cells remain connected to the midbody forming a 4-cell structure. This 4-cell structure resolves as the midbody undergoes a basal movement. This midbody basal movement is concomitant with the formation of a new SJ between the two daughter cells and the establishment of new TCJs between the two daughter cells and one neighboring cell.

TCJs as cell shape and stress orientation sensors: Cell division orientation

Cell division orientation contributes to cell fate specification, tissue organization and morphogenesis [29]. Within tissues the direction of the cell shape anisotropy generally aligns with the tissue stress axis, and, accordingly, divisions tend to be oriented along the global tissue stress axis [28,45–49]. Recently, it was shown that within the *Drosophila* pupal epithelium the TCJ distribution (TCJ bipolarity) aligns with the principal cell shape axis when cells are elongated, and with the direction of the mechanical stress within the tissue [50••]. Importantly, the conserved Dynein-associated protein Mud (NuMA in mammals), which controls the pulling forces on the astral microtubules to orient the spindle during mitosis [51], is recruited to the TCJs. Mud accumulation at TCJs occurs at the level of the SJs, in G2 interphase where it remains during mitosis. The TCJ localization of Mud is independent of the classical Gαi/Pins (LGN in mammals) regulators of Mud localization [51]. Instead, Mud TCJ localization depends on the TCJ protein Gli and on the SJ protein Dlg, which regulates Gli localization [22–24,50••]. While the cell shape anisotropy is lost upon mitotic rounding, the anisotropy of TCJ bipolarity remains relatively constant. Therefore, the TCJ distribution of Mud in the rounded mitotic cell encapsulates both the interphase cell geometry, and, possibly, the tissue stress axis ensuring division orientation along the cell shape and global tissue mechanical stress orientations (Figure 2). Importantly, a similar TCJ-based mechanism for division orientation was recently reported in the *Xenopus* animal cap cells [52]. In this context, it was shown that the loss of C-Cadherin abrogated spindle orientation relative to the TCJ distribution and it was proposed that C-Cadherin enrichment at the TCJs recruits the LGN/NuMA complex to orient the spindle.

Independently of the exact mechanisms of Mud TCJ recruitment, these studies establish that the TCJ distribution allows cell to “sense” the orientation of cell shape and mechanical stress anisotropy. As the TCJ distribution controls division orientation and TCJ bipolarity aligns both with the cell shape and tissue stress axes, the information encapsulated

by their position may further promote the dissipation of tissue stress and regulate epithelial cell packing during tissue development [41,46,53].

TCJs as tissue planar polarity cues

While the epithelial cells are polarized along their apical basal axis (apical-basal polarity), a conserved feature of epithelial tissues is that the cells are also polarized within the plane of the tissue (planar cell polarity, PCP), [54,55]. The *Drosophila* wing epithelium has served as a paradigm to study the mechanisms of PCP by the Frizzled (Fz) PCP pathway. Within the *Drosophila* wing, cells form polarized ridges at their cell apical membranes. In addition, each cell produces a distally pointed hair with the hair pedicle located at the peak of the ridge [56]. This planar polarized organization of the cells requires Frizzled PCP signalling and depends on their regular hexagonal packing during pupal stages [47,56,57]. Mutations in *Gli* disrupt the alignment of the hairs independently of classical Frizzled PCP signalling [58]. Gli localizes at the TCJs during early pupal wing development and translocates together with the SJ protein Coracle to a more apical position, where they form ribbon structures, which are the presumptive ridges, beneath the prehair base during later stages. Upon loss of Gli function, the formation of these ribbons was compromised and cells appear more disorganized. This study demonstrates that TCJs impact on PCP and possibly on cell packing.

PCP signaling has also been implicated in the directional migration of epithelial cell sheets or collective migrations. Collective cell migrations are essential for tissue morphogenesis and wound healing, and their dysregulation can lead to tumorigenesis (reviewed in [59]). Within the *Drosophila* ovary, the follicular epithelium covering the germ cells undergoes a stereotypic collective migration whereby the entire tissue crawls on the basement membrane and rotates around the germ cells to elongate the future egg [60–62]. This rotation is driven by whip-like actin protrusions that emanate from the basal TCJs [63••]. The direction of these protrusions is regulated by a basal planar signalling network involving the Fat2 proto-cadherin, the DLar receptor tyrosine phosphatase and the WAVE regulatory complex protein Abi, which are all enriched at the basal TCJs during early stages [63••,64–66]. It was proposed that the TCJ localization of Fat2 promotes the polarization of the actin cytoskeleton through a yet unknown mechanism to initiate the collective migration [63••]. Although compelling evidence indicates that Fat2 and DLar regulate follicle cell migration, the

interplay between these two signalling pathways remains elusive, since Fat2 and DLar do not directly interact [64,67].

Combined, these findings illustrate that TCJs act as signalling hubs for PCP signalling guiding cell polarity independently of the classical Frizzled PCP pathway. It will be interesting to explore whether TCJ-dependent PCP signalling exists in other tissues and contexts.

Mechanical regulation at TCJs

TCJs sustain the junctional tension generated by three or more BCJs during cell rearrangements [68–70]. The forces exerted on the TCJs are not constant, but continuously fluctuate due to the changes in BCJ length and tension associated with morphogenetic processes (reviewed in [31] and [71–78]). Accordingly, several studies have now revealed how the BCJ and TCJ mechanical properties are coordinated and provide feedback for each other.

BCJs are generally under tensile stress and are thus pulling on the TCJs [53]. As TCJs are mechanically coupled to the BCJs, TCJs are likely to respond to these forces generated along the BCJs. Indeed, several studies reported that changes in the BCJ tension or adhesion lead to changes in the molecular composition of TCJs. Increasing junctional tension by adding the drug calyculin A or ATP to embryonic tissues of *Xenopus* causes the accumulation of the F-actin binding protein Vinculin at TCJs. Upon wounding in cultured MDCK monolayers, an actomyosin purse string is formed, which participates in the closing of the wound. The tension generated by the constriction of this purse string induces the localization of α-Catenin in its stretched conformation and of Vinculin at the TCJs [79]. Similarly, increased BCJ tension after double knock-down of the ZO-1 and ZO-2 TJ proteins in MDCK monolayers increases the TCJ localization of Afadin, E-Cadherin, Vinculin and of α-Catenin in its stretched conformation [80]. Conversely, reducing BCJ tension by depleting the scaffolding protein Anillin results in an impaired junctional integrity and in a reduced amount of active MyosinII at the TCJs in gastrula-stage *Xenopus* embryos [81].

TCJs not only respond to changes in BCJ tension, but also feedback on the BCJ tension [82]. When Caco2 cells are grown at subconfluent conditions, the TCJ associated Tricellulin recruits the Cdc42 GEF Tuba, which activates Cdc42 to promote the assembly of an actomyosin meshwork at the TCJs as well as BCJs. Accordingly, the loss of the Tricellulin induced BCJ curving and changed tissue topology indicative of defects in BCJ tension [82]. Lastly, in the mammalian gut epithelial tissue, loss of EpCAM dependent cell-cell adhesion at BCJs results in

a hypercontractility at the TCJs [83••], due to the accumulation of myosins IIa and IIb at the TCJs. This increases the contractility along the apical-basal axis of the cells, which leads to the abnormal expansion of the cell apical domain and epithelial dysplasia (Figure 3a,b). Thus, the tight regulation of contractile forces at TCJs is essential to maintain tissue integrity and homeostasis [83••].

TCJs as regulators of stem cell homeostasis and tissue growth signaling

The role of TCJs as regulators of epithelial integrity is not restricted to the mammalian gut. Recently, Resnik-Docampo and colleagues showed that the age-associated loss of the barrier function in the *Drosophila* midgut is associated with an impaired TCJ organization and results in stem cell over-proliferation [84••]. They found that the loss of Gli at the TCJs of the midgut enterocytes promotes such age-associated barrier dysfunction by preventing terminal differentiation and increasing intestinal stem cell proliferation (Figure 4). The intestinal stem cell over-proliferation is due to the loss of Gli function in the differentiated enterocytes, which promotes Jun N-terminal kinase (JNK) signaling pathway in the intestinal stem cell. However, the link between TCJs and JNK signaling appears more complex and could be tissue dependent. Indeed, as opposed to the loss of Gli in the midgut, the over-expression of Gli in the *Drosophila* wing disc induces an increase in cell proliferation by upregulating the JNK pathway [24]. As Gli over-expression induces cell delamination and apoptosis, this increase in proliferation was proposed to reflect a compensatory mechanism necessary to buffer the cell loss. Since the loss of Gli function and the overexpression of Gli trigger JNK pathway activation, one can envision that both conditions cause tissue injury resulting in elevated JNK stress signaling and increased proliferation.

The role of TCJs in regulating growth signaling likely extends beyond the control of the JNK pathway. Indeed, several proteins that have been implicated in the Hippo growth control pathway are enriched at TCJs at the level of the AJs. The actin-associated proteins Zyxin and Enabled, which promote tissue growth through the Hippo pathway were found enriched at the TCJs in different *Drosophila* epithelial tissues [85–87]. Likewise, the Warts kinase, which phosphorylates the transcription factor Yorkie to inhibit growth, and its interaction partner, the actin-associated protein Ajuba, appear enriched at TCJs in the *Drosophila* wing disc epithelium [88,89]. Lastly, Hippo signalling activity at TCJs may be coordinated by Csk. Csk activity controls the levels of Gli at TCJs preventing Gli spreading in BCJs [25••]. Based on

genetic epistasis experiments, it was proposed that Csk regulates tissue growth upstream of Ajuba, Zyxin and Warts [90]. As described above, TCJs have been shown to play a conserved role in the regulation of cell division orientation [50,52]. It is therefore interesting to note that the Warts kinase phosphorylates Mud to promote its cortical localization and planar spindle orientation [91]. Thus, TCJs may integrate growth signalling and geometrical inputs to regulate division.

Conclusions and future considerations

As discussed in this opinion, recent findings clearly demonstrate that TCJs have additional functions well beyond their previously known structural function in preserving epithelial barrier integrity. Rather than behaving as passive structures sitting at the corners of BCJs, TCJs are active structures that respond to changes in BCJ tension and feedback to regulate BCJ mechanical properties. Because, i) the TCJ distribution aligns with the cell geometric and stress patterns, and captures the cell topological information, ii) TCJs respond to changes in BCJ tension and can feedback on BCJ tension, iii) key regulators of cell mechanical properties and growth signalling are localized at TCJs, TCJs may act as signalling hotspots, of which spatial distributions allow cells to integrate mechanical and biochemical inputs to guide local cell dynamics, while preserving tissue barrier function and topology. Perhaps one of the biggest challenges to understand the specific contribution of TCJs versus BCJs on cellular dynamics will be to specifically perturb or modify protein functions at either of these junctions. Furthermore, the localization and activities of proteins at TCJs are regulated along the cell apical-basal axis. Dissecting the interplay between BCJs and TCJs along the apical-basal axis should provide exciting new avenues to better understand cellular dynamics and growth control during tissue development, homeostasis and repair.

Acknowledgements

We thank E. van Leen for contributing Figure 2, E. van Leen and F. di Pietro for comments on the manuscript. Research in the Bellaïche laboratory is supported by the European Research Council (ERC Advanced, TiMoprh, 340784), the Fondation ARC pour la Recherche sur le Cancer (SL220130607097), the Agence Nationale de la Recherche (ANR Labex DEEP; 11-LBX-0044, ANR-10-IDEX-0001-02), the Centre National de la Recherche Scientifique, the Institut National de la Santé et de la Recherche Médicale, and Institut CURIE and PSL Research University funding or grants.

References

1 Harris TJC, Tepass U: **Adherens junctions: from molecules to morphogenesis.** *Nat Rev Mol Cell Biol* 2010, **11**:502–14.

2 Campbell HK, Maiers JL, DeMali KA: **Interplay between tight junctions & adherens junctions.** *Exp Cell Res* 2017, **358**:39–44.

3 Izumi Y, Furuse M: **Molecular organization and function of invertebrate occluding junctions.** *Semin Cell Dev Biol* 2014, **36**:186–93.

4 Balda MS, Matter K: **Tight junctions as regulators of tissue remodelling.** *Curr Opin Cell Biol* 2016, **42**:94–101.

5 Furuse M, Izumi Y, Oda Y, Higashi T, Iwamoto N: **Molecular organization of tricellular tight junctions.** *Tissue barriers* 2014, **2**:e28960.

6 Higashi T, Miller AL: **Tricellular junctions: how to build junctions at the TRICkiest points of epithelial cells.** *Mol Biol Cell* 2017, **28**:2023–2034.

7 Ikenouchi J, Furuse M, Furuse K, Sasaki H, Tsukita S, Tsukita S: **Tricellulin constitutes a novel barrier at tricellular contacts of epithelial cells.** *J Cell Biol* 2005, **171**:939–45.

8 Masuda S, Oda Y, Sasaki H, Ikenouchi J, Higashi T, Akashi M, Nishi E, Furuse M: **LSR defines cell corners for tricellular tight junction formation in epithelial cells.** *J Cell Sci* 2011, **124**:548–55.

9 Higashi T, Tokuda S, Kitajiri S, Masuda S, Nakamura H, Oda Y, Furuse M: **Analysis of the “angulin” proteins LSR, ILDR1 and ILDR2--tricellulin recruitment, epithelial barrier function and implication in deafness pathogenesis.** *J Cell Sci* 2013, **126**:966–77.

10 Schulte J, Tepass U, Auld VJ: **Gliotactin, a novel marker of tricellular junctions, is necessary for septate junction development in Drosophila.** *J Cell Biol* 2003, **161**:991–1000.

11 Hildebrandt A, Pflanz R, Behr M, Tarp T, Riedel D, Schuh R: **Bark beetle controls epithelial morphogenesis by septate junction maturation in Drosophila.** *Dev Biol*

2015, **400**:237–47.

12•• Byri S, Misra T, Syed ZA, Bätz T, Shah J, Boril L, Glashauser J, Aegerter-Wilmsen T, Matzat T, Moussian B, et al.: **The Triple-Repeat Protein Anakonda Controls Epithelial Tricellular Junction Formation in Drosophila.** *Dev Cell* 2015, **33**:535–48.

This work provides a molecular explanation for how the tripartite septum is formed at tricellular junctions.

13 Heisenberg C-P, Bellaïche Y: **Forces in tissue morphogenesis and patterning.** *Cell* 2013, **153**:948–62.

14 Graner F, Riveline D: **"The Forms of Tissues, or Cell-aggregates": D'Arcy Thompson's influence and its limits.** *Development* 2017, **144**:4226–4237.

15 Ragkousi K, Gibson MC: **Cell division and the maintenance of epithelial order.** *J Cell Biol* 2014, **207**:181–8.

16 Staehelin LA: **Further observations on the fine structure of freeze-cleaved tight junctions.** *J Cell Sci* 1973, **13**:763–86.

17 Fristrom DK: **Septate junctions in imaginal disks of Drosophila: a model for the redistribution of septa during cell rearrangement.** *J Cell Biol* 1982, **94**:77–87.

18 Graf F, Noirot-Timothée C, Noirot C: **The specialization of septate junctions in regions of tricellular junctions. I. Smooth septate junctions (=continuous junctions).** *J Ultrastruct Res* 1982, **78**:136–51.

19 Noirot-Timothée C, Graf F, Noirot C: **The specialization of septate junctions in regions of tricellular junctions. II. Pleated septate junctions.** *J Ultrastruct Res* 1982, **78**:152–65.

20 Nayak G, Lee SI, Yousaf R, Edelmann SE, Trincot C, Van Itallie CM, Sinha GP, Rafeeq M, Jones SM, Belyantseva IA, et al.: **Tricellulin deficiency affects tight junction architecture and cochlear hair cells.** *J Clin Invest* 2013, **123**:4036–49.

21 Auld VJ, Fetter RD, Broadie K, Goodman CS: **Gliotactin, a novel transmembrane protein on peripheral glia, is required to form the blood-nerve barrier in Drosophila.**

Cell 1995, **81**:757–67.

22 Schulte J, Charish K, Que J, Ravn S, MacKinnon C, Auld VJ: **Gliotactin and Discs large form a protein complex at the tricellular junction of polarized epithelial cells in Drosophila.** *J Cell Sci* 2006, **119**:4391–401.

23 Padash-Barmchi M, Charish K, Que J, Auld VJ: **Gliotactin and Discs large are co-regulated to maintain epithelial integrity.** *J Cell Sci* 2013, **126**:1134–43.

24 Padash-Barmchi M, Browne K, Sturgeon K, Jusiak B, Auld VJ: **Control of Gliotactin localization and levels by tyrosine phosphorylation and endocytosis is necessary for survival of polarized epithelia.** *J Cell Sci* 2010, **123**:4052–62.

25•• Samarasekera GDNG, Auld VJ: **C-terminal Src kinase (Csk) regulates the tricellular junction protein Gliotactin independent of Src.** *Mol Biol Cell* 2018, **29**:123–136.

This work demonstrates that Gliotactin levels at the tricellular junction are regulated by endocytosis in a Csk phosphorylation dependent manner.

26 Sharifkhodaei Z, Padash-Barmchi M, Gilbert MM, Samarasekera G, Fulga TA, Van Vactor D, Auld VJ: **The Drosophila tricellular junction protein Gliotactin regulates its own mRNA levels through BMP-mediated induction of miR-184.** *J Cell Sci* 2016, **129**:1477–89.

27 Gudipaty SA, Rosenblatt J: **Epithelial cell extrusion: Pathways and pathologies.** *Semin Cell Dev Biol* 2017, **67**:132–140.

28 Campinho P, Behrndt M, Ranft J, Risler T, Minc N, Heisenberg C-P: **Tension-oriented cell divisions limit anisotropic tissue tension in epithelial spreading during zebrafish epiboly.** *Nat Cell Biol* 2013, **15**:1405–14.

29 Panousopoulou E, Green JBA: **Spindle orientation processes in epithelial growth and organisation.** *Semin Cell Dev Biol* 2014, **34**:124–32.

30 Brooks ER, Wallingford JB: **Multiciliated cells.** *Curr Biol* 2014, **24**:R973-82.

31 Guirao B, Bellaïche Y: **Biomechanics of cell rearrangements in Drosophila.** *Curr Opin Cell Biol* 2017, **48**:113–124.

32 Kubo A, Nagao K, Yokouchi M, Sasaki H, Amagai M: **External antigen uptake by Langerhans cells with reorganization of epidermal tight junction barriers.** *J Exp Med* 2009, **206**:2937–46.

33•• Yokouchi M, Atsugi T, Logtestijn M van, Tanaka RJ, Kajimura M, Suematsu M, Furuse M, Amagai M, Kubo A: **Epidermal cell turnover across tight junctions based on Kelvin's tetrakaidecahedron cell shape.** *Elife* 2016, **5**.

This work shows how cells migrate from the basal to the apical layer of the mouse ear skin, while preserving barrier function thought the formation of a specialized 3D shape filling structure resembling a Kelvin's tetrakaidecahedron and of *de novo* tricellular and bicellular junctions.

34 Herszterg S, Pinheiro D, Bellaïche Y: **A multicellular view of cytokinesis in epithelial tissue.** *Trends Cell Biol* 2014, **24**:285–93.

35 Firmino J, Rocancourt D, Saadaoui M, Moreau C, Gros J: **Cell Division Drives Epithelial Cell Rearrangements during Gastrulation in Chick.** *Dev Cell* 2016, **36**:249–61.

36•• Pinheiro D, Hannezo E, Herszterg S, Bosveld F, Gaugue I, Balakireva M, Wang Z, Cristo I, Rigaud SU, Markova O, et al.: **Transmission of cytokinesis forces via E-cadherin dilution and actomyosin flows.** *Nature* 2017, **545**:103–107.

In this work, it is established that epithelial cytokinesis is associated with a mechanosensing mechanism signaling from the dividing cell to the neighboring cells, thereby ensuring correct *de novo* junction formation and the initial formation of the TCJ.

37•• Higashi T, Arnold TR, Stephenson RE, Dinshaw KM, Miller AL: **Maintenance of the Epithelial Barrier and Remodeling of Cell-Cell Junctions during Cytokinesis.** *Curr Biol* 2016, **26**:1829–42.

This study provides the first analyses of *de novo* tricellular junction formation during epithelial cell division.

38 Herszterg S, Leibfried A, Bosveld F, Martin C, Bellaiche Y: **Interplay between the dividing cell and its neighbors regulates adherens junction formation during**

cytokinesis in epithelial tissue. *Dev Cell* 2013, **24**:256–70.

39 Founounou N, Loyer N, Le Borgne R: **Septins regulate the contractility of the actomyosin ring to enable adherens junction remodeling during cytokinesis of epithelial cells.** *Dev Cell* 2013, **24**:242–55.

40 Guillot C, Lecuit T: **Adhesion disengagement uncouples intrinsic and extrinsic forces to drive cytokinesis in epithelial tissues.** *Dev Cell* 2013, **24**:227–41.

41 Gibson MC, Patel AB, Nagpal R, Perrimon N: **The emergence of geometric order in proliferating metazoan epithelia.** *Nature* 2006, **442**:1038–41.

42 Harrell JR, Goldstein B: **Internalization of multiple cells during C. elegans gastrulation depends on common cytoskeletal mechanisms but different cell polarity and cell fate regulators.** *Dev Biol* 2011, **350**:1–12.

43 Kieserman EK, Glotzer M, Wallingford JB: **Developmental regulation of central spindle assembly and cytokinesis during vertebrate embryogenesis.** *Curr Biol* 2008, **18**:116–23.

44 Olivier N, Luengo-Oroz MA, Duloquin L, Faure E, Savy T, Veilleux I, Solinas X, Débarre D, Bourgine P, Santos A, et al.: **Cell lineage reconstruction of early zebrafish embryos using label-free nonlinear microscopy.** *Science* 2010, **329**:967–71.

45 Guirao B, Rigaud SU, Bosveld F, Bailles A, Lopez-Gay J, Ishihara S, Sugimura K, Graner F, Bellaïche Y: **Unified quantitative characterization of epithelial tissue development.** *Elife* 2015, **4**:e08519.

46 Wyatt TPJ, Harris AR, Lam M, Cheng Q, Bellis J, Dimitracopoulos A, Kabla AJ, Charras GT, Baum B: **Emergence of homeostatic epithelial packing and stress dissipation through divisions oriented along the long cell axis.** *Proc Natl Acad Sci U S A* 2015, **112**:5726–31.

47 Aigouy B, Farhadifar R, Staple DB, Sagner A, Röper J-C, Jülicher F, Eaton S: **Cell flow reorients the axis of planar polarity in the wing epithelium of Drosophila.** *Cell* 2010, **142**:773–86.

48 Legoff L, Rouault H, Lecuit T: **A global pattern of mechanical stress polarizes cell divisions and cell shape in the growing Drosophila wing disc.** *Development* 2013, **140**:4051–9.

49 Nestor-Bergmann A, Goddard G, Woolner S, Jensen OE: **Relating cell shape and mechanical stress in a spatially disordered epithelium using a vertex-based model.** *Math Med Biol* 2017, doi:10.1093/imammb/dqx008.

50•• Bosveld F, Markova O, Guirao B, Martin C, Wang Z, Pierre A, Balakireva M, Gaugue I, Ainslie A, Christophorou N, et al.: **Epithelial tricellular junctions act as interphase cell shape sensors to orient mitosis.** *Nature* 2016, **530**:495–8.

This article demonstrates that the tricellular junction distribution aligns with the direction of cell shape in interphase cells and regulates cell division orientation upon mitotic rounding. This cell shape "memory" is provided by the localization of Mud at the tricellular junctions, thereby this work provides an explanation for the century old Hertwig rule (1884): cells tend to divide along their cell long axis.

51 di Pietro F, Echard A, Morin X: **Regulation of mitotic spindle orientation: an integrated view.** *EMBO Rep* 2016, **17**:1106–30.

52 Nestor-Bergmann A, Stooke-Vaughan GA, Goddard GK, Starborg T, Jensen OE, Woolner S: **Decoupling the roles of cell shape and mechanical stress in orienting and cueing epithelial mitosis**. *bioRxiv* 2017, doi:10.1101/177592.

53 Farhadifar R, Röper J-C, Aigouy B, Eaton S, Jülicher F: **The influence of cell mechanics, cell-cell interactions, and proliferation on epithelial packing.** *Curr Biol* 2007, **17**:2095–104.

54 Hale R, Strutt D: **Conservation of Planar Polarity Pathway Function Across the Animal Kingdom.** *Annu Rev Genet* 2015, **49**:529–51.

55 Campanale JP, Sun TY, Montell DJ: **Development and dynamics of cell polarity at a glance.** *J Cell Sci* 2017, **130**:1201–1207.

56 Doyle K, Hogan J, Lester M, Collier S: **The Frizzled Planar Cell Polarity signaling pathway controls Drosophila wing topography.** *Dev Biol* 2008, **317**:354–67.

57 Classen A-K, Anderson KI, Marois E, Eaton S: **Hexagonal packing of Drosophila wing epithelial cells by the planar cell polarity pathway.** *Dev Cell* 2005, **9**:805–17.

58 Venema DR, Zeev-Ben-Mordehai T, Auld VJ: **Transient apical polarization of Gliotactin and Coracle is required for parallel alignment of wing hairs in Drosophila**. *Dev Biol* 2004, **275**:301–314.

59 Ladoux B, Mège R-M: **Mechanobiology of collective cell behaviours.** *Nat Rev Mol Cell Biol* 2017, **18**:743–757.

60 Haigo SL, Bilder D: **Global tissue revolutions in a morphogenetic movement controlling elongation.** *Science* 2011, **331**:1071–4.

61 Crest J, Diz-Muñoz A, Chen D-Y, Fletcher DA, Bilder D: **Organ sculpting by patterned extracellular matrix stiffness.** *Elife* 2017, **6**.

62 Cetera M, Horne-Badovinac S: **Round and round gets you somewhere: collective cell migration and planar polarity in elongating Drosophila egg chambers.** *Curr Opin Genet Dev* 2015, **32**:10–5.

63•• Squarr AJ, Brinkmann K, Chen B, Steinbacher T, Ebnet K, Rosen MK, Bogdan S: **Fat2 acts through the WAVE regulatory complex to drive collective cell migration during tissue rotation.** *J Cell Biol* 2016, **212**:591–603.

In this study, the authors identified that the formation of whip-like actin protrusions at the basal tricellular junctions. The Fat-2 dependent actin protrusions drive the collective migration of the follicle cells in the *Drosophila* ovary.

64 Barlan K, Cetera M, Horne-Badovinac S: **Fat2 and Lar Define a Basally Localized Planar Signaling System Controlling Collective Cell Migration.** *Dev Cell* 2017, **40**:467–477.e5.

65 Bateman J, Reddy RS, Saito H, Van Vactor D: **The receptor tyrosine phosphatase Dlar and integrins organize actin filaments in the Drosophila follicular epithelium.** *Curr Biol* 2001, **11**:1317–27.

66 Viktorinová I, König T, Schlichting K, Dahmann C: **The cadherin Fat2 is required for planar cell polarity in the Drosophila ovary.** *Development* 2009, **136**:4123–32.

67 Horne-Badovinac S: **Fat-like cadherins in cell migration-leading from both the front and the back.** *Curr Opin Cell Biol* 2017, **48**:26–32.

68 Blankenship JT, Backovic ST, Sanny JSP, Weitz O, Zallen JA: **Multicellular rosette formation links planar cell polarity to tissue morphogenesis.** *Dev Cell* 2006, **11**:459–70.

69 Fernandez-Gonzalez R, Simoes S de M, Röper J-C, Eaton S, Zallen JA: **Myosin II dynamics are regulated by tension in intercalating cells.** *Dev Cell* 2009, **17**:736–43.

70 Trichas G, Smith AM, White N, Wilkins V, Watanabe T, Moore A, Joyce B, Sugnaseelan J, Rodriguez TA, Kay D, et al.: **Multi-cellular rosettes in the mouse visceral endoderm facilitate the ordered migration of anterior visceral endoderm cells.** *PLoS Biol* 2012, **10**:e1001256.

71 Martin AC, Kaschube M, Wieschaus EF: **Pulsed contractions of an actin-myosin network drive apical constriction.** *Nature* 2009, **457**:495–9.

72 Rauzi M, Verant P, Lecuit T, Lenne P-F: **Nature and anisotropy of cortical forces orienting Drosophila tissue morphogenesis.** *Nat Cell Biol* 2008, **10**:1401–10.

73 Bosveld F, Bonnet I, Guirao B, Tlili S, Wang Z, Petitalot A, Marchand R, Bardet P-L, Marcq P, Graner F, et al.: **Mechanical Control of Morphogenesis by Fat/Dachsous/Four-Jointed Planar Cell Polarity Pathway**. *Science (80-)* 2012, **336**:724–727.

74 Collinet C, Rauzi M, Lenne P-F, Lecuit T: **Local and tissue-scale forces drive oriented junction growth during tissue extension.** *Nat Cell Biol* 2015, **17**:1247–58.

75 Yu JC, Fernandez-Gonzalez R: **Local mechanical forces promote polarized junctional assembly and axis elongation in Drosophila.** *Elife* 2016, **5**.

76 Hara Y, Shagirov M, Toyama Y: **Cell Boundary Elongation by Non-autonomous Contractility in Cell Oscillation.** *Curr Biol* 2016, **26**:2388–96.

77 Bardet P-L, Guirao B, Paoletti C, Serman F, Léopold V, Bosveld F, Goya Y, Mirouse V, Graner F, Bellaïche Y: **PTEN controls junction lengthening and stability during cell**

rearrangement in epithelial tissue. *Dev Cell* 2013, **25**:534–46.

78 Curran S, Strandkvist C, Bathmann J, de Gennes M, Kabla A, Salbreux G, Baum B: **Myosin II Controls Junction Fluctuations to Guide Epithelial Tissue Ordering.** *Dev Cell* 2017, **43**:480–492.e6.

79 Yonemura S, Wada Y, Watanabe T, Nagafuchi A, Shibata M: **alpha-Catenin as a tension transducer that induces adherens junction development.** *Nat Cell Biol* 2010, **12**:533–42.

80 Choi W, Acharya BR, Peyret G, Fardin M-A, Mège R-M, Ladoux B, Yap AS, Fanning AS, Peifer M: **Remodeling the zonula adherens in response to tension and the role of afadin in this response.** *J Cell Biol* 2016, **213**:243–60.

81 Reyes CC, Jin M, Breznau EB, Espino R, Delgado-Gonzalo R, Goryachev AB, Miller AL: **Anillin regulates cell-cell junction integrity by organizing junctional accumulation of Rho-GTP and actomyosin.** *Curr Biol* 2014, **24**:1263–70.

82 Oda Y, Otani T, Ikenouchi J, Furuse M: **Tricellulin regulates junctional tension of epithelial cells at tricellular contacts through Cdc42.** *J Cell Sci* 2014, **127**:4201–12.

83•• Salomon J, Gaston C, Magescas J, Duvauchelle B, Canioni D, Sengmanivong L, Mayeux A, Michaux G, Campeotto F, Lemale J, et al.: **Contractile forces at tricellular contacts modulate epithelial organization and monolayer integrity.** *Nat Commun* 2017, **8**:13998.

In this work, it is established that EpCAM-dependent cell-cell adhesion maintains contractile force at the epithelial TCJs in the mammalian gut.

84•• Resnik-Docampo M, Koehler CL, Clark RI, Schinaman JM, Sauer V, Wong DM, Lewis S, D'Alterio C, Walker DW, Jones DL: **Tricellular junctions regulate intestinal stem cell behaviour to maintain homeostasis.** *Nat Cell Biol* 2017, **19**:52–59.

This work shows that epithelial tricellular junction integrity is essential for gut tissue homeostasis, thus preventing premature aging and dysplasia.

85 Rauskolb C, Pan G, Reddy BVVG, Oh H, Irvine KD: **Zyxin links fat signaling to the hippo**

pathway. *PLoS Biol* 2011, **9**:e1000624.

86 Grevengoed EE, Loureiro JJ, Jesse TL, Peifer M: **Abelson kinase regulates epithelial morphogenesis in Drosophila.** *J Cell Biol* 2001, **155**:1185–98.

87 Gaspar P, Holder M V, Aerne BL, Janody F, Tapon N: **Zyxin antagonizes the FERM protein expanded to couple F-actin and Yorkie-dependent organ growth.** *Curr Biol* 2015, **25**:679–89.

88 Rauskolb C, Sun S, Sun G, Pan Y, Irvine KD: **Cytoskeletal tension inhibits Hippo signaling through an Ajuba-Warts complex.** *Cell* 2014, **158**:143–156.

89 Sun S, Reddy BVVG, Irvine KD: **Localization of Hippo signalling complexes and Warts activation in vivo**. *Nat Commun* 2015, **6**:8402.

90 Kwon HJ, Waghmare I, Verghese S, Singh A, Singh A, Kango-Singh M: **Drosophila C-terminal Src kinase regulates growth via the Hippo signaling pathway.** *Dev Biol* 2015, **397**:67–76.

91 Dewey EB, Sanchez D, Johnston CA: **Warts phosphorylates mud to promote Pins-mediated mitotic spindle orientation in Drosophila, Independent of Yorkie**. *Curr Biol* 2015, **25**:2751–2762.

Figure 1. Cell-cell adhesion structures in *Drosophila* and mammals.

(**a**, **b**) Schematics showing the organization of the invertebrate and vertebrate cell-cell adhesion sites along the apical-basal axis (a,b), and cross sections of the TCJ at the level of the septate junction (SJ) and tight junction (TJ) (a',b'). In invertebrate tissues, adhesion is mediated by the apical adherens junctions (AJs) and the septate junctions (SJs), while, in vertebrate tissues, cell-cell adhesion is mediated by the tight junctions (TJs), AJs and desmosomes (for reviews see [1–6]). In *Drosophila* the AJs are located above the SJs, while the functionally equivalent structures in vertebrates, the TJs, are located above the AJs. In both invertebrates and vertebrates, at the level of the SJ or TJ, respectively, TCJ channels or pores are present along the apical-basal axis formed by a series of stacked diaphragms, tricellular channel diaphragms (TCD) in invertebrates or a central sealing element (CSE) in vertebrates (a,b) (reviewed in [5,6]). In vertebrate systems, the bicellular TJs form strands that attach to the central sealing element [16], while in invertebrates the bicellular SJ strands connect to the diaphragm along the apical-basal axis forming a lateral limiting strand (LS) at the tricellular contact interface [17–19]. At the dihedral angles of 120° at the *Drosophila* TCJ the Aka proteins from three cells form a trimer and recruit Gli [10,12••]. In turn, Gli interacts with the lateral limiting strand (LS) connecting the SJs with the TCDs to form a mature TCJ and to provide a protective barrier (a'). In mammalian cells, the Angulin family of proteins Angulin-1, (also known as lipolysis-stimulated lipoprotein receptor), Angulin-2 (also known as immunoglobulin like domain-containing receptor) and Angulin-3 (also known as immunoglobulin like domain-containing receptor 2, C1orf32 or LISCH-like) likely interact though their extracellular Ig-like domains with the CSE, while their intracellular domains can interact with Tricellulin which in turn connects to the TJ strands to form a mature TCJ and provide barrier function (b') (reviewed in [5,6] and [7–9,20]).

Figure 2. TCJ distribution controls cell division orientation.

During G2 phase Mud (red) localizes at TCJs and remains associated with TCJs during mitosis, where it regulates the pulling forces on the astral microtubules to orient the mitotic spindle [50••]. The TCJ distribution (TCJ bipolarity, red bars) aligns on average with the orientation of cell shape (blue bars) and the stress direction (black bars). Upon mitosis cells round up and the cell shape anisotropy is lost, while the TCJ bipolarity is maintained. Thus, the Mud TCJ

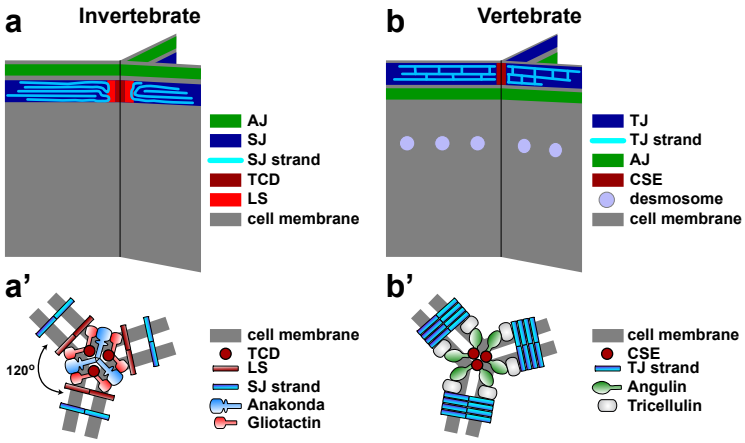
distribution controls the division orientation along the interphase cell shape and tissue stress directions.

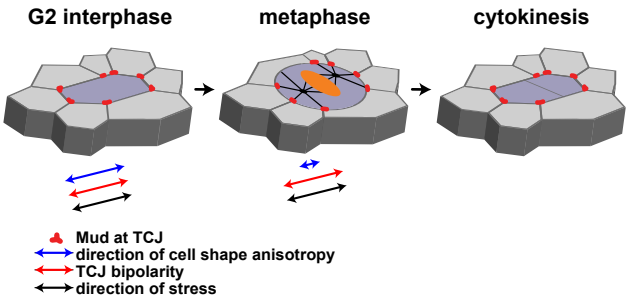
Figure 3. Increased contractility at TCJs promotes apical cell area increase and dysplasia.

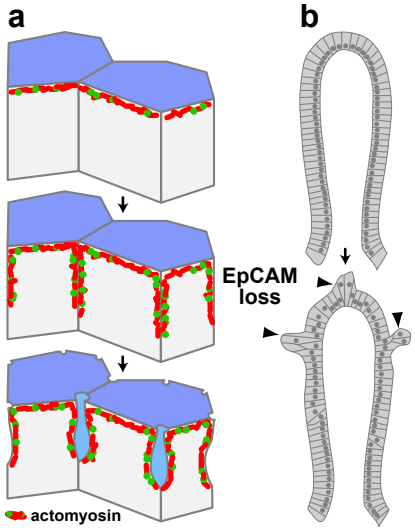
(**a**, **b**) Schematic showing the effect of the loss of EpCAM mediated cell-cell adhesion in the mammalian gut epithelium on the cellular level (a) and the tissue level (b). Upon EpCAM loss of function actomyosin accumulates at the TCJs resulting in an increase in contractility along the apical-basal cell axis at TCJs (a) [83••]. As a result the apical cell area (blue area) increases (b), triggering epithelial dysplasia and the formation of tufts in the gut epithelium (b, arrowheads).

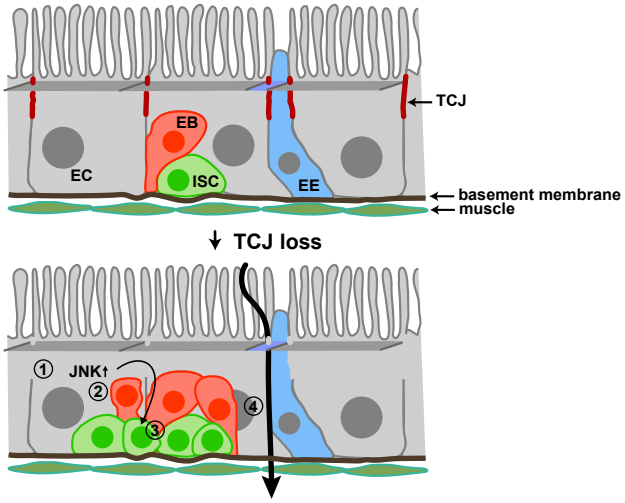
Figure 4. TCJ prevent intestinal dysplasia and premature aging.

Schematic of the *Drosophila* midgut. Intestinal stem cell (ISC), Enteroblast (EB), Enteroendocrine (EE), Enterocyte (EC). Upon loss of TJC integrity (1) the JNK pathway is upregulated in the ECs (2) [84••]. In turn JNK signaling promotes ISCs to proliferate, while terminal differentiation of EBs is prevented resulting in intestinal dysplasia (3). Finally, the paracellular barrier is compromised and flies eventually die prematurely (4).


a
Invertebrate
AJ
SJ
SJ strand
TCD
LS
cell membrane
b
Vertebrate
TJ
TJ strand
AJ
CSE
desmosome
cell membrane
a'
120°
cell membrane
TCD
LS
SJ strand
Anakonda
Gliotactin
b'
cell membrane
CSE
TJ strand
Angulin
Tricellulin


G2 interphase
metaphase
cytokinesis
Mud at TCJ
direction of cell shape anisotropy
TCJ bipolarity
direction of stress


a
b
EpCAM
loss
actomyosin


TCJ
EB
EC
ISC
EE
basement membrane
muscle
TCJ loss
① JNK↑
②
③
④