ZnUMBA Crosses the Border

ZnUMBA Crosses the Border

Jasmin Imran Alsous, Adam C. Martin

Department of Biology. Massachusetts Institute of Technology, MA 02142.

Breaks in tight junctions cause transient tissue leaks. In this issue of *Developmental Cell*, Stephenson et al. (2019) show that local RhoA activation and actomyosin contractions concentrate tight junction proteins at the breach, which repairs the leak.

Main Text

Epithelial tissues are sheets of closely adherent polarized cells that separate internal compartments within an organism and act as barriers to the external environment. Epithelia allow selective transport of ions and other solutes across their walls. Importantly, abnormal epithelial permeability is associated with several epithelial and endothelial organ pathologies, such as inflammatory bowel disease (Forster, 2008). In this issue of Developmental Cell, Stephenson et al. show how local breaches in the tissue of developing frog embryos are repaired through a dynamic RhoA GTPase-mediated damage control mechanism (Stephenson et al., 2019).

In vertebrates, tight junctions (TJs) ensure epithelial barrier function. Located at the apical-most end of the cells' lateral domain, TJs seal the cells' paracellular spaces through continuous rows or strands of transmembrane proteins that interact between cells. Claudins are the main constituent of TJs, forming charge- and size-selective pores at bicellular tight junctions (bTJs), but TJs also include tricellulin, found at tricellular junctions (tTJs). Other components of tight junctions include scaffolding proteins called zonula occludens (ZO-1 and 2) that connect the cytosolic domains of TJ molecules to the cells' actomyosin cytoskeleton (Anderson et al., 2009). Coupling between ZOs and the cells' contractile machinery is critical for TJ assembly, signal transduction, cell polarity, and maintenance of tissue barrier function (Fanning et al., 2009).

Molecules cross the TJ barrier through two distinct routes: the pore and the leak pathways (Shen et al., 2015). The pore pathway is charge- and size- selective and is governed by the electrostatic permeability of claudins; the leak pathway is non-selective and accommodates macromolecules. The two routes are regulated independently, yet the mechanisms that regulate flux through the leak pathway remain unclear (Stephenson et al., 2019). A key challenge has been a lack of techniques for visualizing when and where lapses in barrier function occur, and how long they last. Existing methods to detect barrier breaches provide time-delayed information and only at the level of the tissue. However, the leak pathway is dynamic, and passage through this route is thought to result from transient TJ breaks (Weber, 2012). Elucidating the mechanistic basis of epithelial barrier function and origins of tissue damage therefore requires observing TJs with high spatiotemporal resolution.

To solve this visualization problem, Stephenson et al. (2019) developed ZnUMBA -- short for Zinc-based Ultrasensitive Microscopic Barrier Assay. This method uses imaging of a cell-impermeable dye, FluoZin3's (FZ3), which dramatically increases in fluorescence when bound to zinc. By injecting *Xenopus* embryos with FZ3 and mounting them in zinc-containing media, the authors readily visualized local and short-lived breaches in epithelial barrier function when zinc encountered FZ3 and elevated its fluorescence. Critically, once the leak was repaired and barrier function reinstated, FZ3 levels fell to background levels in a few minutes. This new method provided a powerful tool to examine the dynamics of barrier function with high temporal and spatial resolution.

Using ZnUMBA, Stephenson et al. (2019) showed that junction stretching and the localized loss of TJ proteins, e.g. ZO-1 and occludin, preceded tissue leaks (Figure 1A, B). These visible breaks in TJ strands were followed by a burst of RhoA activation, called a RhoA flare (Figure 1C), and subsequent actomyosin-mediated contractions of the junction (Figure 1D). Finally, junction shortening was associated with recovery of a continuous band of tight junction proteins along cell-cell boundaries, indicative of breach repair (Figure 1E).

The Rho GTPases regulate spatial and temporal patterns of actin cytoskeleton activity in cells (Machacek et al. 2009). In the case of cell adhesion, active RhoA forms a stable zone at the apical ring of the zonula adherens in epithelial culture cells that promotes junctional contractility (Priya et al., 2015). The authors observed local RhoA activation that occurred in response to TJ breaks (Stephenson et al, 2019). RhoA flares repaired TJ breaches by concentrating junction proteins. Studies have shown that bicellular junctions can lengthen or shorten independently of neighboring borders (Choi et al., 2016), suggesting that local RhoA activation will specifically contract the breached junction. Consistent with this model, blocking RhoA-mediated myosin activation, with Rho-Kinase inhibitors, diminished ZO-1 levels at the leak sites. Furthermore, preventing junction repair resulted in repeated RhoA flares along the same junction, suggesting that junctions sense the breach and continually trigger RhoA flares until repaired. Notably, the observed kinetics of TJ breach and repair along junctions is consistent with the rapid transport of macromolecules across the paracellular space, suggesting that the dynamic breaking and annealing of claudin strands is a viable mechanism for leak pathway regulation.

The interplay between cell junctions and the cytoskeleton drives a variety of biological processes and is critical for maintaining tissue integrity during morphogenesis. This study illustrates the dynamic nature of subcellular signaling and its responsive to the status of junctions in developing *Xenopus* embryos. The findings of this study will likely apply to other junction systems as well. During morphogenesis of the *Drosophila* embryo, breaks between adherens junctions and the underlying actomyosin cytoskeleton result in local bursts of RhoA signals (i.e., Rho-Kinase), which mediate actomyosin reattachment to the adherens junction (Jodoin et al., 2015). Thus, a dynamic RhoA response could constitute a universal mechanism for repairing compromised cell junctions.

The work of Stephenson et al. (2019) opens the door to future exciting areas of research. While it is clear that breaks in TJs result in local RhoA activation, what initially senses the break and transmits the signal to RhoA is unknown. Furthermore, the authors showed that TJ repair elicits an unequal contribution from the affected cells. Namely, reinstating the barrier is associated with one cell inducing an actin-rich membrane protrusion that extends over the adjacent cell. The origin of this asymmetry is unclear. Lastly, the authors

showed that formin-mediated actin polymerization is dispensable for leak repair, suggesting that other actin nucleation factors and even other Rho GTPases may be involved in promoting this protrusion, as is observed in protrusions of migrating cells (Machacek et al., 2009). An appealing prospect for future work is to investigate whether several GTPases collaborate to promote TJ repair.

References

Forster, C. (2008). Tight junctions and the modulation of barrier function in disease. *Histochem. Cell. Biol.* 130(1): 55–70.

Stephenson, RE. et al. (2019). Rho flares repair local tight junction leaks. *Dev. Cell.*

Anderson, JM., Van Itallie, CM. (2009). Physiology and function of the tight junction. *Cold Spring Harb. Perspect. Biol.*1(2): a002584.

Fanning AS, Anderson JM. (2009). Zonula occludens-1 and -2 are cytosolic scaffolds that regulate the assembly of cellular junctions. *Ann. N. Y. Acad. Sci.* 1165:113-20.

Shen, L., Weber, CR., Raleigh, DR., Yu, D., Turner, JR. (2015). Tight Junction Pore and Leak Pathways: A Dynamic Duo. *Annu. Rev. Physiol.* 73: 283–309.

Weber, CR. (2012). Dynamic properties of the tight junction barrier. *Ann. N. Y. Acad. Sci.* 1257:77-84

Machacek, M. et al. (2009). Coordination of Rho GTPase activities during cell protrusion. *Nature.* 461(7260):99-103.

Priya R. et al. (2015). Feedback regulation through myosin II confers robustness on RhoA signaling at E-cadherin junctions. *Nat. Cell. Biol.* 17(10):1282-93.

Choi, W. et al. (2016). Remodeling the zonula adherens in response to tension and the role of afadin in this response. *J. Cell. Biol.* 213(2):243-60.

Jodoin JN. et al. (2015). Stable Force Balance between Epithelial Cells Arises from F-Actin Turnover. *Dev. Cell.* 35(6):685-97.

Figure Caption:

Figure 1. Transient Rho activation repairs compromised cell junctions. A-B) Junction stretching is associated with loss of TJ continuity and leaky barriers. C) Breaks in TJ strands are followed by a RhoA flare. D) Subsequent actin and myosin accumulation is associated with junction contraction, leading to E) TJ protein reinforcement and leak repair.