Crumbling under Pressure

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Crumbling under pressure

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In order for an organism to maintain its form, it must be able to withstand physical perturbation, including the pull of gravity. Recent work from Porazinski and colleagues suggests that mechanisms promoting tissue tension are critical to resist the earth's downward pull.

During the development of an organ or embryo, physical forces influence the final shape and form of tissues. Additionally, embryos must be able to withstand environmental perturbations, such as gravity. D'Arcy Thompson postulated "the forms as well as the actions of our bodies are entirely conditioned by the strength of gravity upon this globe" (Thompson, 1917). A new study in *Nature* demonstrates that, without the proper function of one gene, gravity can flatten an embryo (Porazinski et al., 2015).

A screen for genes required for medaka fish development identified a mutant (*hirami*), which mapped to the YAP transcription factor locus (Porazinski et al., 2015). These YAP mutants had improperly shaped or flattened embryos, and interestingly, embryo collapse correlated with orientation relative to the gravitational pull of earth. The authors hypothesized that embryo collapse could be due to reduced tension needed to counter gravity. Laser cutting and micropipette aspiration experiments demonstrated that YAP mutants lowered embryonic tissue tension.

Previous work showed that YAP functions in a mechanosensitive pathway and responds to increased tension, extracellular matrix stiffness, and cell spreading (Dupont et al., 2011; Rauskolb et al., 2014; Swift et al., 2013). These and other data suggest YAP may act in a positive feedback loop where tension stimulates YAP activation, and YAP responds by increasing tissue tension (Calvo et al., 2013). While much of our knowledge on YAP mechanosensitivity is from cell culture, an outstanding question is what cells or tissue types require YAP activity during embryogenesis and homeostasis? Interestingly, the authors note that YAP may function in a cell non-autonomous manner (Porazinski et al., 2015).

To determine which YAP transcription targets promote developmental tension, this study performed gene expression profiling of human retina pigmented epithelial (RPE) cells. Here, they identified ARHGAP18, a RhoA-specific GTPase activating protein (GAP), as having reduced expression following YAP-knockdown (Porazinski et al., 2015). Rho GAPs bind to activated GTPases, catalyze the hydrolysis of GTP, and inactivate the GTPase. Guanine nucleotide exchange factors (GEFs) stimulate the exchange of GDP-for-GTP to activate Rho GTPases. In the RPE culture system, ARHGAP18 knockdown lead to reduced non-muscle

Myosin-II activation, similar to YAP knockdown. However, embryonic ARHGAP18 mutants did not have a phenotype, which they hypothesize could be due to compensation by other Rho GAPs. Consistent with this hypothesis, knockdown of five other Rho GAPs give similar phenotypes to ARHGAP18 knockdown in RPE cells. Work in human cancer-associated fibroblasts, which require YAP function, also identified differential expression of several Rho GAPs and GEFs (Calvo et al., 2013). These combined data suggest that YAP functions to regulate Rho GTPase activity.

It is surprising and counterintuitive that the loss of a RhoA GAP, ARHGAP18, would lead to decreased Myosin-II activation and potentially decreased tension (Porazinski et al., 2015). In many cases, a loss of a RhoA GAP increases active RhoA and actomyosin contraction (Miller and Bement, 2009), which is the opposite result observed in medaka YAP mutants. So how would a GAP that inactivates RhoA promote tissue tension? One possibility is that a delicate balance of activation and inactivation of RhoA, mediated by GEFs and GAPs, respectively, is required to generate tissue tension or cellular contraction (Figure 1). An excellent example of balanced GTPase activation via GEFs/GAPs is observed during cytokinesis. While Ect2 GEF activates RhoA, MgcRacGAP can restrict or inhibit RhoA activity to organize actomyosin organization throughout cytokinetic furrow formation (Loria et al., 2012; Miller and Bement, 2009). Yet, it was recently shown that MgcRacGAP also promotes RhoA activation and Myosin-II accumulation during in *C. elegans* cytokinesis (Loria et al., 2012).

While the direct mechanism(s) of RhoA activation downstream of MgcRacGAP are still to be determined, these data show that GAPs can be as important as GEFs in regulating Rho GTPase activity. These counterintuitive results raise several fundamental questions, including how would ARHGAP18 or other GAPs organize contractility at the cellular level and could GAPs play a role in transmitting tension across the tissue? To generate high tissue tension, cells must be mechanically coupled through cell junctions. One possibility is that the loss of a GAP may increase cellular tension, but higher tension may disrupt cell adhesion and cause cells to become uncoupled, leading to lower tissue tension. Another possibility is that YAP/ARHGAP18 may be responsible for differentiation of cells or tissues that upregulate tension. For example, modulating RhoA activity can promote stem cell differentiation towards specific cell fates (McBeath et al., 2004). Future experiments, including the role of cell adhesion molecules or YAP-mediated differentiation, will be critical to understanding the etiology of this complicated ARHGAP18 tension phenotype.

Where might ARHGAP18 signal to regulate RhoA? Work in *Drosophila* imaginal disc development shows Moesin, an ERM protein that links the apical membrane to the cell cortex, recruits ARHGAP18 to the apical cortex of epithelial cells (Neisch et al., 2013). Overexpression of apically anchored ARHGAP18 can stimulate cell proliferation, an intriguing result considering YAP medaka mutants may have less proliferation and increased apoptosis (Porazinski et al., 2015). *Drosophila* ARHGAP18, through an unknown mechanism, also promoted Rac1

activation, which suggests cross-talk of the Rho and Rac pathways (Neisch et al., 2013).

The exciting and surprising results of Porazinski and colleagues demonstrate that YAP, potentially via Rho GAPs, is required to generate tension necessary to counteract gravitational pull and promote organismal form. Yet, their work highlights how little we know about YAP function during development. It is unclear what embryonic cells require YAP to generate tension. Also, we do not understand how the loss of YAP influences differentiation as well as the balance between proliferation and cell death, all of which could contribute to the generation of tissue tension. Future work will be critical to determine how these factors establish precise spatiotemporal organization of tissue tension needed for embryonic organization or structure.

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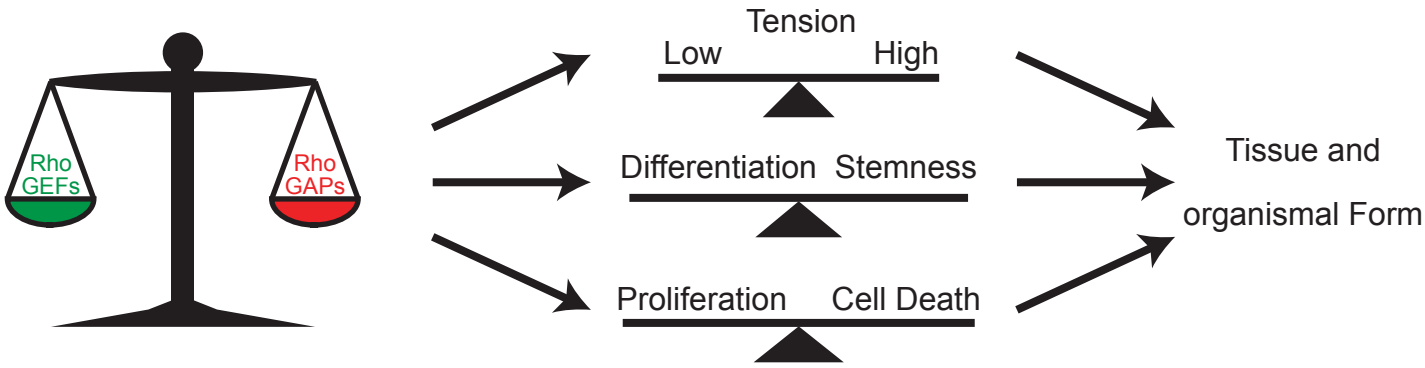
Figure 1 Legend:

Balancing Rho GTPase activation is critical for establishing tissue form. Rho GTPase activity is controlled by a balance of activation via Guanine nucleotide exchange factors (GEFs) and inactivation via GTPase activating proteins (GAPs). Rho activity can regulate the state of cellular tension, differentiation, and proliferation/cell death. The combination of these factors directs cellular and tissue tension, and ultimately, organismal form.


Rho
GEFs
Rho
GAPs
Tension
Low
High
Differentiation
Stemness
Proliferation
Cell Death
Tissue and
organismal Form