Muscle versus Snail: Muscle wins

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Epithelial–mesenchymal transitions (EMTs) are often governed by the transcription factor Snail and entail the loss of apical junctions from epithelial cells. In this issue, Weng and Wieschaus (2016. *J Cell Biol.* http://dx.doi.org/10.1083/jcb.201508056) report that actomyosin contractility can strengthen junctions to override Snail-dependent junctional disassembly and postpone EMT during *Drosophila melanogaster* gastrulation.

Common to both development and carcinoma progression, epithelial–mesenchymal transitions (EMTs) are driven by transcription factors such as Snail. Snail directs the loss of apical–basal polarity and intercellular junctions of epithelial cells. EMT often begins with apical constrictions, driven by a contractile actomyosin network, initiating the escape of epithelial cells from their native tissues. Actomyosin networks exert tension on and anchor apical junctions while contracting the apical surface (Martin and Goldstein, 2014). When a single cell undergoes EMT, apical constriction is coordinated with the loss of junctions, allowing the cell to leave the epithelium. However, in groups of constricting cells that undergo invagination before EMT, junctional disassembly and EMT are delayed, despite the presence of Snail. This raises an apparent paradox: how can cells disassemble intercellular junctions in a Snail-dependent manner while simultaneously relying on junctions to mechanically couple adjacent cells for these coordinated cell shape changes? In this issue, Weng and Wieschaus address this question by studying the relationship between the motor protein nonmuscle myosin II (myosin hereafter) and adherens junctions in Snail-expressing *Drosophila melanogaster* mesoderm cells.

The mesoderm in *Drosophila* embryos forms by invagination of hundreds of cells. Snail is expressed in presumptive mesoderm cells well in advance of tissue internalization to direct mesoderm fate specification, apical constriction, and invagination (Leptin, 1999; Martin et al., 2009). EMT is significantly delayed despite early expression of Snail and occurs only when invagination is complete (Fig. 1). Apical constriction of mesodermal cells drives tissue bending and the formation of a ventral furrow, during which adherens junctions are not only maintained but undergo a maturation process. Contractile pulses of actomyosin pull junctions inward, leading to incremental, ratchet-like decreases of the apical cell surface (Martin et al., 2009). Junctional integrity is crucial to mesoderm internalization, as in the absence of junctions, actomyosin activity rips the tissue apart (Martin et al., 2010).

Weng and Wieschaus (2016) tracked adherens junction remodeling and myosin activity in live embryos using quantitative live imaging. The majority of the spot adherens junctions found at this stage persisted and shifted their position within the lateral membrane to the apical edge within an ∼5-min period (Fig. 1). FRAP experiments revealed that junctional E-cadherin is highly dynamic during this apical shift, and a large fraction of the E-cadherin molecules within junctions is turned over. Junctions condense at the apical edge, increasing in E-cadherin density, which presumably strengthens cell–cell adhesion. Notably, the apical shift of junctions goes hand in hand with the recruitment of myosin from the basal to apical cortex.

Actomyosin contractility occurs in pulses in the mesoderm (Martin et al., 2009). Interestingly, the maturation of adherens junctions is also nonlinear and closely follows the pulsatile behavior of myosin, suggesting a link between tension and adherens junction maturation (Weng and Wieschaus, 2016). In support of such a mechanism, the authors show that loss of myosin either delays the repositioning of adherens junctions in the mesoderm or eliminates them altogether. Complementary experiments show that ectopic apical activation of myosin in ectoderm cells, which do not express Snail, enhances junctions and phenocopies the apical shift of junctions seen in the mesoderm. Collectively, these observations do not support a model in which lateral spot adherens junctions in mesoderm cells undergo Snail-dependent disassembly while apical junctions are reassembled de novo (Kölsch et al., 2007). In contrast, the new data suggest that junctions are repositioned and strengthened through engagement with an apical actomyosin network (Fig. 1).

Weng and Wieschaus (2016) further demonstrated that junctional strengthening by myosin is able to counteract junctional disassembly driven by Snail. Strikingly, they found that ectopic expression of Snail is sufficient to down-regulate junctions in the ectoderm, an effect that can be blocked by activating myosin. Snail is generally thought to destabilize adherens junctions by repressing E-cadherin transcription in mammalian cells (Batlle et al., 2000; Cano et al., 2000). However, expression of E-cadherin in *Drosophila* by an exogenous ubiquitous promoter can support viability and normal morphogenesis in animals that lack endogenous E-cadherin. Moreover, in the absence of zygotic E-cadherin expression, which would be the target of Snail transcriptional regulation, maternally provided E-cadherin can fully support gastrulation. Thus, an unknown Snail target elicits disassembly of epithelial junctions, a mechanism now shown to be inhibited by the mechanosensitive interaction between actomyosin and adherens junctions. Snail was also shown to be responsible for building the normal apical actomyosin network in mesoderm cells (Martin et al., 2009).

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The Rockefeller University Press $30.00
J. Cell Biol. Vol. 212 No. 2 139–141
www.jcb.org/cgi/doi/10.1083/jcb.201512092

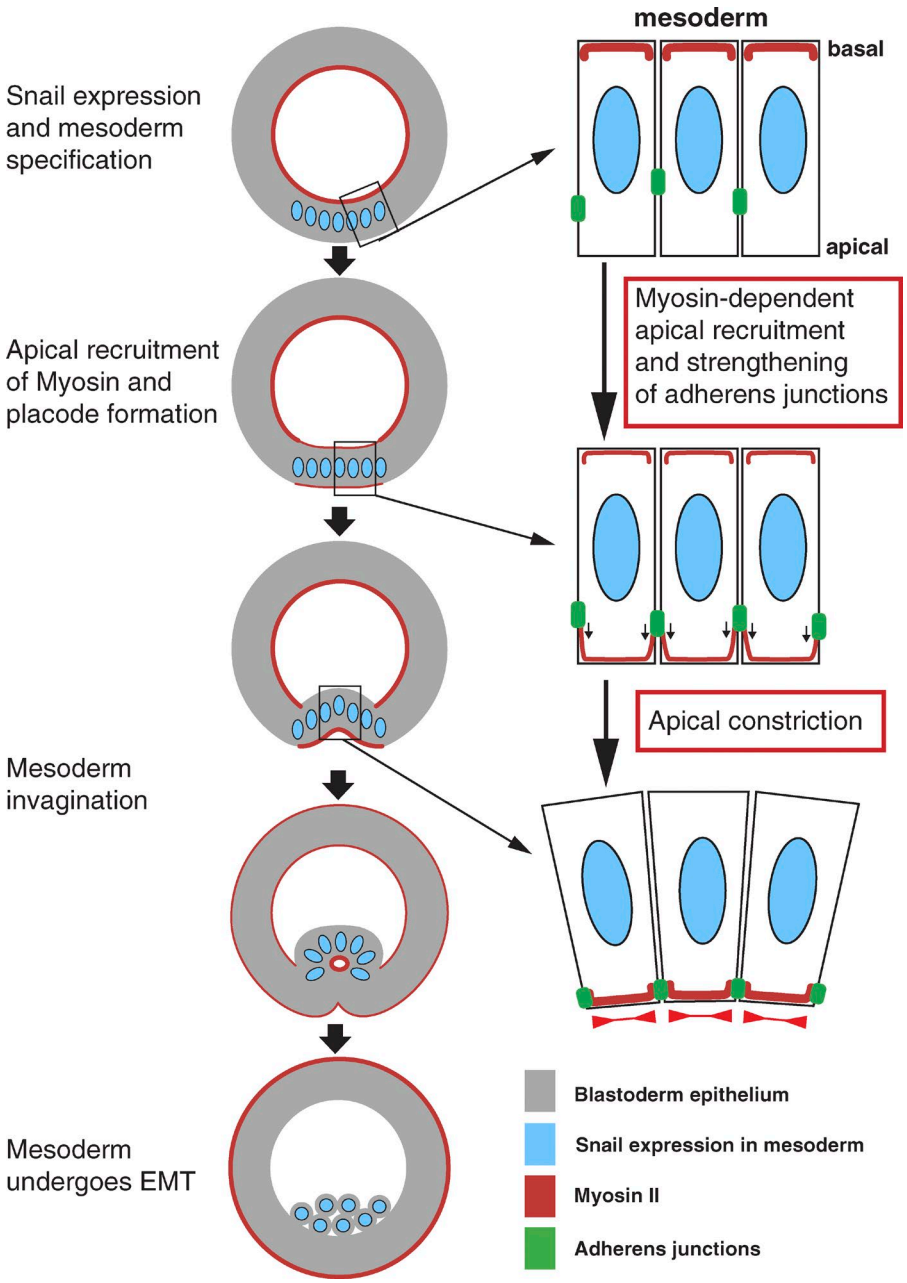

Figure 1. **Actomyosin-dependent shape changes in the *Drosophila* mesoderm.** The expression of Snail is regulated by the dorsoventral patterning machinery and contributes to the specification of the mesoderm on the ventral side of the *Drosophila* embryo. Mesoderm invagination is initiated by the recruitment of myosin to the apical cell pole, where it forms actomyosin networks. Actomyosin contractility recruits adherens junctions that have formed in the apico-lateral membrane to the apical edge and increases E-cadherin density to strengthen junctions. These junctions are required to maintain stable cell—cell contacts during apical constriction, allowing the coordinated inward movement of mesoderm cells during invagination.

Snail, therefore, must have at least two targets, one that elicits actomyosin network formation at the apical pole of mesoderm cells, which, in turn, counteracts the action of Snail targets that cause junctional disassembly. It will be exciting to identify the mechanisms downstream of Snail that either promote or prevent EMT and to investigate their conservation across animals and in human cancer.

The present data show that actomyosin contractility supports junctional stability and strength, yet contractility also promotes junctional remodeling during tissue morphogenesis (Bertet et al., 2004; Fernandez-Gonzalez et al., 2009). How actomyosin can impact junctions in these two opposing ways remains unclear. Coordination between contractility and cadherin endocytosis may provide an answer to this apparent paradox. Cadherins organize in clusters, the size, number, and mobility of which depend on F-actin and myosin (Lecuit and Yap, 2015). Myosin activity can promote lateral clustering of cadherins, strengthening adhesion. However, the largest clusters are removed from junctions via dynamin-mediated endocytosis (Truong Quang et al., 2013), a process that has been linked to junctional disassembly during germband extension (Levayer et al., 2011).

One intriguing difference between the germband and the mesoderm is that the main contractile force in the germband results from multicellular actomyosin cables that associate parallel with junctions (Fernandez-Gonzalez et al., 2009; Rauzi et al., 2010). This arrangement may promote the formation of larger cadherin clusters that are consequently removed by endocytosis. In contrast, the main force generator in mesoderm cells are medial actomyosin networks that are connected to junctions via actin filaments oriented perpendicular to the junction (Martin et al., 2009). This topology may not elicit the same degree of cadherin clustering and therefore may not trigger endocytotic removal of junctional material. Junctional and medial actomyosin pools undergo rapid turnover (Munjal et al., 2015) and are linked

by myosin flows (Rauzi et al., 2010; Weng and Wieschaus, 2016). Changes in subcellular actomyosin organization could therefore direct junctional stability versus remodeling.

Weng and Wieschaus (2016) provide a compelling in vivo example for the prevalent notion that junctions are shaped by biomechanical feedback that integrates cadherin-based adhesion and actomyosin contractility (Lecuit and Yap, 2015). To better understand the role of tension in junctional organization and dynamics in vivo, it will be interesting to use junctional tension biosensors (Cai et al., 2014) to determine and compare mechanical forces sustained by junctions in different tissues during morphogenesis. Moreover, the application of optogenetic tools to modulate tension in vivo with high spatio-temporal precision (Guglielmi et al., 2015) may also allow assessment of the impact of tension on junctional composition, remodeling, and function.

Acknowledgments

We thank R. Fernandez-Gonzalez and D. Godt for critical reading of the manuscript.

The authors declare no competing financial interests.

Submitted: 31 December 2015
Accepted: 31 December 2015

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