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Cardiac morphogenesis: Crowding and tension resolved through social distancing

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Abstract

Organ maturation entails the reshaping of simple tissues into more complex structures critical for function. In a recent issue of Nature, Priya et al. (2020) show how tension heterogeneity between developing cardiomyocytes can coordinate the cell behaviors that remodel the architecture of the cardiac chamber wall.

Organogenesis often involves the conversion of a simple tissue into a more elaborate structure. In the embryonic heart, the ventricular myocardial wall transforms from a single layer of cells into a more complex configuration, consisting of an outer compact layer that encircles an inner layer of trabecular ridges. The mechanisms that allocate ventricular cardiomyocytes in order to create these distinct layers in appropriate proportions remain incompletely defined.

Due to its small size and amenability for high-resolution live imaging, the embryonic zebrafish heart offers unique opportunities to examine how cardiomyocytes dynamically organize into complex structures of the ventricular wall. Prior zebrafish studies have shown that individual cardiomyocytes delaminate from the ventricular wall into the lumen to create trabeculae (Staudt *et al.*, 2014; Liu *et al.*, 2010), whereas others remain within the outer layer. Utilizing a combination of transgenic tools that dynamically report on or alter the activity of developmental pathways, more recent studies have shed light on the signaling cues, including Notch and Neuregulin/Erbb2, that influence cardiomyocyte delamination (Han *et al.*, 2016). However, it is not yet clear how particular cardiomyocytes are selected to initiate the morphogenesis of the distinct layers of the ventricular wall.

In a recent issue of Nature, Priya et al. explore the role of biomechanical forces in directing individual cardiomyocytes to create trabeculae (Priya *et al.*, 2020). In an elegant series of studies, the authors show how local tension heterogeneity, due to proliferation-induced cellular crowding, instructs cardiomyocytes to delaminate into the ventricular lumen (Figure 1). Reminiscent of studies revealing a role for heterogeneous actomyosin tension in the

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transformation of an epithelial sheet into a multi-layered tissue (Miroshnikova *et al.*, 2018), the authors made the key observation that trabeculating cardiomyocytes display greater cortical actomyosin contractility and tension than non-trabeculating cardiomyocytes, as visualized by phosphorylated myosin and tested by nano-laser ablation. Notably, the authors discovered that increased cortical actomyosin contractility and tension are critical for the delamination of trabeculating cardiomyocytes, as constitutively active and dominant negative forms of *myosin light chain 9* (*myl9*) or *rhoa* can promote and inhibit delamination, respectively.

While investigating the cause of the tension heterogeneity in the ventricular myocardial wall, the authors noted that the initiation of trabeculation occurs during a period of increased cardiomyocyte proliferation. They therefore explored whether cellular crowding could be responsible for creating differential tension between individual cardiomyocytes. Indeed, they discovered that increasing or decreasing proliferation with vitamin D analogs or MEK/Erbb2 inhibitors, respectively, altered not only cellular crowding and cortical actomyosin contractility but also the number of delaminating cardiomyocytes. Altogether, these experiments revealed that tissue-level changes in the developing heart, such as cell proliferation and crowding, lead to heterogenous biomechanical changes in cortical actomyosin contractility that can initiate trabeculation (Figure 1B–D).

The authors then investigated the relationship between heterogenous biomechanical tension and other processes shown to regulate trabeculation such as cardiac function, Neuregulin/Erbb2 signaling and Notch signaling (Peshkovsky, Totong and Yelon, 2011; Liu *et al.*, 2010; Han *et al.*, 2016). They found that constitutively active *myl9* (*ca-myl9)* was able to rescue the delamination defects caused by disruption of blood flow or cardiac chamber contraction, thus placing myocardial cortical actomyosin tension downstream of these physiological processes. Coinciding with previous reports that the endocardium may mediate the role of cardiac function during trabeculation (Samsa *et al.*, 2015), the authors also found that myocardial cortical actomyosin tension is epistatic to *Nrg2a/Erbb2* signaling from the endocardium, which is mediated by hemodynamic flow and required for myocardial delamination (Liu *et al.*, 2010; Grego-Bessa *et al.*, 2007).

Complementing the tension heterogeneity that the authors observed within the ventricular wall, recent studies have revealed that Notch signaling is activated in cardiomyocytes that are adjacent to delaminating cardiomyocytes (Han *et al.*, 2016; Jiménez-Amilburu *et al.*, 2016), and appears to regulate lateral inhibition mechanisms that distinguish the fates of outer compact and inner trabeculating cardiomyocytes (Han *et al.*, 2016). Intriguingly, the authors found that the *ca-myl9* rescue of trabeculation defects due to inhibited *nrg2a*/*erbb2* signaling or decreased cardiac function also rescued differential activation of Notch signaling in the myocardium. Notch signaling appears adjacent to delaminating cardiomyocytes in these rescue experiments, indicating that delamination itself can trigger Notch activity in neighboring cells. Additionally, Notch activation was also shown to contribute to cortical actomyosin heterogeneity by repressing myosin activity and filamentous actin enrichment in outer compact cardiomyocytes. Consistent with these findings, the authors found that *ca-myl9* is unable to drive delamination in cells where Notch signaling is ectopically activated. These results reveal a complex interplay between Notch

signaling and cortical actomyosin contraction, suggesting a role for biomechanical processes in lateral inhibition mechanisms that regulate cell fate and behavior (Figure 1C, D).

Tissue morphogenesis is regulated at several different levels, including tissue-scale events, biomechanical cellular processes, and intracellular signaling, all of which are often studied in isolation. In this multidimensional study, the authors elegantly connect tissue growth via proliferation, biomechanical changes in local cortical actomyosin tension, and activation of signaling pathways that drive cell fate (Figure 1). Looking forward, these studies unveil several intriguing avenues worthy of future investigation, including how Notch signaling inhibits cortical actomyosin contractility and, conversely, how cortical actomyosin contractility regulates Notch signaling, as well as how cortical actomyosin contractility interacts with other relevant signaling pathways, such as the BMP pathway (Grego-Bessa *et al.*, 2007; Han *et al.*, 2016). Overall, these findings contribute to a greater understanding of how biomechanical forces intersect with biochemical cues to regulate the fundamental process of multilayered epithelial morphogenesis (Maître *et al.*, 2016). In the future, the integration of biomechanics, cell behavior and signaling is likely to stimulate new strategies for promoting the organization of distinct human pluripotent stem cell derived cell types into functional tissue organoids.

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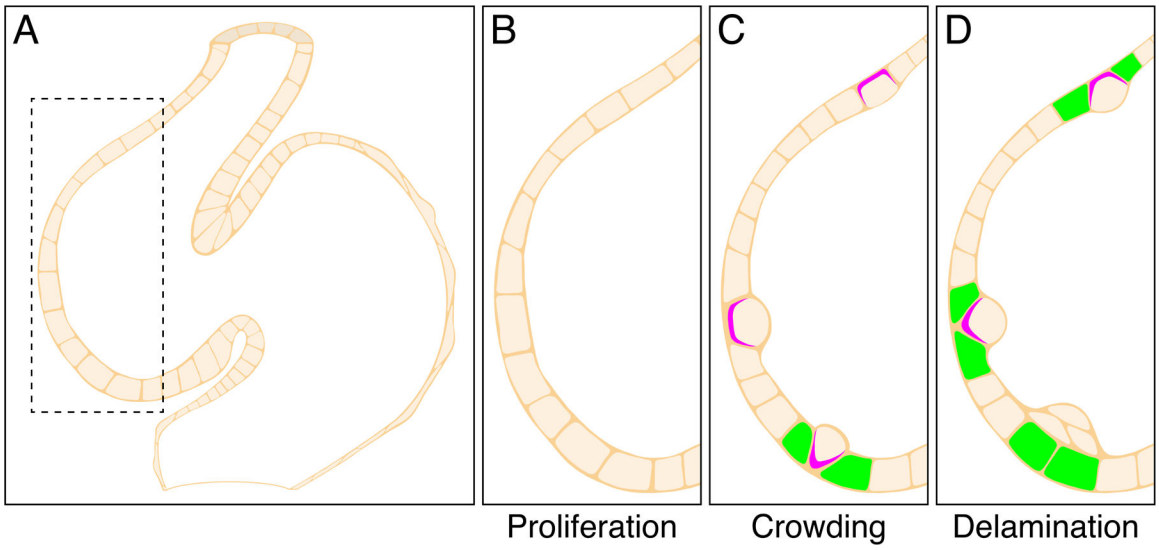

Figure 1. Tension heterogeneity drives cardiomyocyte delamination during cardiac trabeculation.

(A) In the embryonic zebrafish heart, the ventricular myocardial wall is initially a single layer but transforms into a complex multi-layered structure through a series of morphogenetic events (B-D). (B) Cardiomyocyte proliferation creates (C) cellular crowding and heterogeneous cortical actomyosin tension (purple) in the ventricular wall, which in turn drives (D) cardiomyocyte delamination. Delamination triggers Notch pathway activation (green) in neighboring cardiomyocytes, preventing their delamination and regulating the allocation of cardiomyocytes between outer compact and inner trabeculating (blue) layers. Boxed area is highlighted in B-D. Purple – enriched localization of phosphorylated myosin. Green – Notch signaling. Blue – trabeculating cardiomyocytes