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Mechanical forces drive neuroblast morphogenesis and are required for epidermal closure

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ABSTRACT

Tissue morphogenesis requires myosin-dependent events such as cell shape changes and migration to be coordinated between cells within a tissue, and/or with cells from other tissues. However, few studies have investigated the simultaneous morphogenesis of multiple tissues *in vivo*. We found that during *Caenorhabditis elegans* ventral enclosure, when epidermal cells collectively migrate to cover the ventral surface of the embryo, the underlying neuroblasts (neuronal precursor cells) also undergo morphogenesis. We found that myosin accumulates as foci along the junction-free edges of the ventral epidermal cells to form a ring, whose closure is myosin-dependent. We also observed the accumulation of myosin foci and the adhesion junction proteins E-cadherin and α-catenin in the underlying neuroblasts. Myosin may help to reorganize a subset of neuroblasts into a rosette-like pattern, and decrease their surface area as the overlying epidermal cells constrict. Since myosin is required in the neuroblasts for ventral enclosure, we propose that mechanical forces in the neuroblasts influence constriction of the overlying epidermal cells. In support of this model, disrupting neuroblast cell division or altering their fate influences myosin localization in the overlying epidermal cells. The coordination of myosin-dependent events and forces between cells in different tissues could be a common theme for coordinating morphogenetic events during metazoan development.

1. Introduction

Epidermal morphogenesis is essential for metazoan development. However, the complexity of vertebrate tissues has made it challenging to study their development at the cellular level. Thus, many studies have been done '*ex vivo*', and it is not clear how these findings apply to tissues formed in their native environments (Heisenberg and Bellaïche, 2013). *Caenorhabditis elegans* epidermal morphogenesis is an ideal model to study tissue morphogenesis, since the epidermis is formed from a relatively small number of cells in comparison to other organisms. In addition, this nematode is amenable to microscopy and genetics, and previous studies have revealed the precise order and timing of events during embryonic development.

We study ventral enclosure, when ventral epidermal cells cover the belly of the embryo using cues from the underlying neuroblasts (neuronal precursor cells; Chisholm and Hardin, 2005). This process begins with the migration of two pairs of anterior-positioned ventral epidermal cells toward the ventral midline, followed by the migration of more posterior-positioned ventral epidermal cells (Chisholm and Hardin, 2005; Williams-Masson et al., 1997). As the posterior cells approach the ventral midline, they form a pocket that is lined by F-actin cables, which was hypothesized to close by myosin contractility (Williams-Masson et al., 1997). Ventral epidermal cell migration relies on the underlying neuroblasts, although it is not clear if they provide chemical and/or mechanical cues. Mutations in genes that affect neuroblast sorting and position, or their division and shape cause ventral enclosure phenotypes (Chin-Sang et al., 1999; Chisholm and Hardin, 2005; Fotopoulos et al., 2013; George et al., 1998; Ghenea et al., 2005; Giurumescu et al., 2012; Patel et al., 2008). In support of neuroblasts providing short-range cues, a recent study showed that a subset of epidermal cells relies on the formation of a neuroblast bridge to migrate successfully (Ikegami et al., 2012).

Non-muscle myosin contractility generates intracellular and extracellular forces *via* actin–myosin networks for several developmental events (Heisenberg and Bellaïche, 2013). Non-muscle myosin conformation and activity is stimulated by regulatory light chain (MRLC) phosphorylation by Rho-binding kinase (ROK; LET-502 in *C. elegans*; Matsumura, 2005; Wissmann et al., 1997), and is negatively regulated by myosin phosphatase (Matsumura and Hartshorne, 2008). ROK also can phosphorylate and inhibit the regulatory subunit of myosin phosphatase (MEL-11 in *C. elegans*;

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Wissmann et al., 1997) to promote myosin activity. Myosin and its regulators are required for multiple events throughout *C. elegans* embryogenesis, including polarity, cytokinesis, gastrulation and epidermal morphogenesis (Davies et al., 2014; Diogon et al., 2007; Gally et al., 2009; Jenkins et al., 2006; Motegi and Sugimoto, 2006; Piekny and Mains, 2002; Piekny et al., 2000, 2003; Pohl et al., 2012; Roh-Johnson et al., 2012; Shelton et al., 1999; Wissmann et al., 1997). The anterior cortex of the one-cell embryo is highly contractile in comparison to the posterior cortex, and corresponds with the enrichment of active myosin foci (Jenkins et al., 2006; Motegi and Sugimoto, 2006). During cytokinesis, an actin–myosin ring constricts the cell to form two daughters (Piekny et al., 2005). During *Drosophila* and *C. elegans* gastrulation, the ingression of mesodermal cells is mediated by their apical constrictions, which are regulated by a supracellular network of actin–myosin contractility (Martin et al., 2010; Pohl et al., 2012; Roh-Johnson et al., 2012). This pulsatile network is coupled to adhesion junctions to stably transmit forces into changes in cell shape (Heisenberg and Bellaïche, 2013). During *Drosophila* embryogenesis, morphogenesis of the dorsal epidermis requires the coordinated adhesion, extension and migration of epidermal cells toward the dorsal midline (Franke et al., 2005; Jacinto et al., 2002a). An actin–myosin cable forms along the edges of the dorsal-most row of epidermal cells and mechanically zippers the sheets together (Bloor and Kiehart, 2002; Jacinto et al., 2002b; Kiehart et al., 2000; Young et al., 1993). This organization of F-actin shares some striking similarities to the actin ring formed around the ventral pocket during *C. elegans* ventral enclosure. Dorsal closure also relies on the underlying amnioserosal cells, which are extraembryonic and epithelial in origin. These cells constrict apically to help pull the dorsal epithelial sheets together (Blanchard et al., 2010; Gorfinkiel et al., 2009; Solon et al., 2009). However, it is not clear if analogous changes occur in the neuroblasts to help regulate ventral enclosure, particularly since it is not known if these cells generate contractility or have adhesion junctions that could mediate changes in cell shape or position. Mechanical forces generated by changes in neuroblast organization or cell shape could help pull the overlying epidermal cells toward the ventral midline. Furthermore, the accumulation of myosin along the junction-free edges of epidermal cells could be triggered by mechanosensing changes in tension in the underlying tissue (Kee and Robinson, 2008).

The elongation of epithelial tissue also relies on the coordination of myosin contractility between multiple cells to mediate changes in their organization during tissue morphogenesis (Blankenship et al., 2006; Harding et al., 2014; Heisenberg and Bellaïche, 2013). This process is accompanied by changes in cell–cell adhesion as cells undergo shape changes and form a circular rosette pattern with groups of cells sharing a common vertex (Blankenship et al., 2006; Heisenberg and Bellaïche, 2013). The cells then change shape again and organize into a different pattern such as columns or rows, or vice versa, as adhesion junctions expand between different neighbors (Blankenship et al., 2006; Heisenberg and Bellaïche, 2013). This process is conserved among metazoans and has also been shown to occur in mammalian neuroepithelial tissues and neural stem cell niches (Harding et al., 2014).

Non-muscle myosin contractility is controlled by the small GTPase RhoA, but it is not clear how RhoA is regulated to mediate specific developmental events. During cytokinesis, the highly conserved guanine nucleotide exchange factor (GEF) Ect2 generates active RhoA for contractile ring formation in *C. elegans*, *Drosophila* and mammalian cells (Davies et al., 2014; Kimura et al., 2000; Loria et al., 2012; Miki et al., 1993; Piekny and Mains, 2002; Piekny et al., 2005; Somers and Saint, 2003; Tatsumoto et al., 1999). During later stages of *Drosophila* embryogenesis, the Ect2 homologue, Pbl, and DRhoGEF2 (another RhoA GEF) regulate RhoA activity for the constriction of actomyosin cables during dorsal closure (Azevedo et al., 2011; Bloor and Kiehart, 2002; Jacinto et al., 2002b; Kiehart et al., 2000; Lu and Settleman, 1999; Young et al., 1993). In *C. elegans*, anterior–posterior polarity of the early embryo is controlled by ECT-2, which generates active myosin at the anterior cortex *via* RHO-1, and is countered by the GAP (GTPase activating protein) CYK-4 at the posterior cortex (Jenkins et al., 2006). During later stages of development, the migration of epidermal P cells, a subset of vulva precursor cells, is regulated primarily by the Rho GEF UNC-73/Trio *via* the Rho GTPases CED-10 and MIG-2, but zygotic ECT-2 also contributes to their migration *via* regulating the RhoA homologue, RHO-1 (Morita et al., 2005). In *Drosophila*, Pbl regulates cell migration during gastrulation to mediate mesoderm spreading over the ectoderm (Schumacher et al., 2004; Smallhorn et al., 2004; van Impel et al., 2009). Furthermore, in interphase cancer cells, Ect2 activates Rac for tumor cell migration (Fields and Justilien, 2010; Justilien and Fields, 2009). Therefore, studying myosin contractility during development has been complicated by the different functions of its upstream regulators. We recently found that *C. elegans rho-1* is required for ventral enclosure, supporting the idea that myosin contractility could be a crucial part of this process (Fotopoulos et al., 2013). However, we did not know how RHO-1 was regulated, or the cellular processes that myosin is involved in to mediate ventral enclosure. As described earlier, an obvious function for myosin would be to constrict the F-actin ring formed around the posterior epidermal cells, but myosin also could provide additional mechanical forces.

Here, we find that myosin is required for ventral enclosure, where it localizes as dynamic foci in the epidermal cells and neuroblasts. In epidermal cells, the foci form a ring around the ventral pocket that resembles the previously described F-actin ring (Williams-Masson et al., 1997). Myosin contractility is required for ventral enclosure, because disrupting myosin or its upstream regulator(s) causes ventral enclosure phenotypes. Furthermore, the accumulation of myosin foci in both the epidermal cells and neuroblasts is dependent on its upstream regulators, suggesting that they are contractile. Interestingly, using a tissue-specific rescue assay, we found that myosin is required in the neuroblasts for ventral enclosure. In support of this finding, blocking neuroblast cell division or altering neuroblast cell fate causes myosin foci to be more disorganized in the epidermal cells. Further, we found that a subset of neuroblasts undergoes changes in their organization, and the surface area of individual cells decreases as the overlying epidermal cells constrict. These data suggest that the neuroblasts undergo morphogenetic changes concomitantly with the overlying epidermal cells. The adhesion junction proteins α-catenin and E-cadherin localize to neuroblast cell boundaries, and disrupting myosin or E-cadherin slows the rate at which neuroblasts shrink. We propose a model where mechanical forces in both the epidermal cells and neuroblasts are coordinated for successful epidermal morphogenesis.

2. Results

2.1. Non-muscle myosin localizes as foci in the epidermal cells and neuroblasts during ventral enclosure

We wanted to determine if mechanical forces contribute to ventral enclosure. First, we characterized the localization of non-muscle myosin (hereafter myosin) during ventral enclosure by imaging embryos expressing GFP-tagged NMY-2 (myosin heavy chain), with GFP integrated immediately downstream of the *nmy-2* gene locus by CRISPR-mediated genome editing (*nmy-2(cp13*