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Evolution of Developmental Control Mechanisms

Independent migration of cell populations in the early gastrulation of the amphipod crustacean *Parhyale hawaiensis*

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ABSTRACT

Cells are the principal component of tissues and can drive morphogenesis through dynamic changes in structure and interaction. During gastrulation, the primary morphogenetic event of early development, cells change shape, exchange neighbors, and migrate long distances to establish cell layers that will form the tissues of the adult animal. Outside of *Drosophila,* little is known about how changes in cell behavior might drive gastrulation among arthropods. Here, we focus on three cell populations that form two aggregations during early gastrulation in the crustacean *Parhyale hawaiensis.* Using cytoskeletal markers and lineage tracing we observe bottle cells in anterior and visceral mesoderm precursors as gastrulation commences, and find that both Cytochalasin D, an inhibitor of actin polymerization, and ROCKOUT, an inhibitor of Rho-kinase activity, prevent gastrulation. Furthermore, by ablating specific cells, we show that each of the three populations acts independently during gastrulation, confirming previous hypotheses that cell behavior during *Parhyale* gastrulation relies on intrinsic signals instead of an inductive mechanism.

Introduction

How does morphogenesis occur? Among arthropods, research with the fruit fly *Drosophila melanogaster* has provided important clues about the molecular mechanisms regulating embryonic patterning and morphogenesis. Comparison with additional arthropod species contributes to our understanding of the evolution of early patterning, but relatively few comparative investigations focus on the cellular dynamics that drive morphogenesis. Gastrulation, which is a crucial morphogenetic event during early embryonic development among metazoans, has a long history of studies focused on cellular behavior, mechanics, and interaction (for review see Stern (2004)). During gastrulation, cells undergo dynamic changes toward the establishment of the embryonic germ layers that will give rise to the various systems of the adult. Cell behavior during gastrulation is an important and experimentally tractable manifestation of the molecular patterning that results in the morphology of an animal. Because cellular gastrulation strategies can vary widely from species to species, meaningful evolutionary comparisons can only be made through sampling a wide variety of taxa (Stern, 2004; Davidson, 2008).

Outside of *Drosophila*, little is known about how changes in cell behavior or cellular interactions drive gastrulation in arthropods. In the emerging model crustacean *Parhyale hawaiensis* (Amphipoda), gastrulation is multi-phasic and begins with the formation of two spatially and visually distinct cell populations, the rosette and the epithelial sheet (Gerberding et al., 2002; Browne et al., 2005; Price and Patel, 2008; Alwes et al., 2011; see Fig. 1). The rosette, which is comprised of germline and anterior and visceral mesoderm precursors, gastrulates underneath the ectodermal precursors of the epithelial sheet to form a multilayered germ disc that is a condensation of cells that will form the embryo proper. The somatic mesoderm and endoderm internalize later (Gerberding et al., 2002; Browne et al., 2005; Price and Patel, 2008; Alwes et al., 2011; see Fig. 1 and the following paragraph). Multiple phases of gastrulation are not unique to *Parhyale*. Most insects have temporally distinct internalization of mesoderm and endoderm (Roth, 2004), and there are examples among crustaceans and chelicerates where mesendoderm is internalized at different times (Gerberding and Patel 2004; Anderson, 1973). For example, the germline and mesendoderm precursors of another amphipod crustacean, *Orchestia cavimana*, form a cluster that is outlined by a sickle-shaped collection of ectoderm precursors. The presumptive germline initiates gastrulation by sinking into the yolk. A later phase of *Orchestia* gastrulation involves internalization of the mesendoderm and somatic mesoderm (Wolff and Scholtz, 2002; Scholtz and Wolff, 2002). Among chelicerates, canonical spider development features the internalization of

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mesendodermal cells that form the "primitive plate"(Anderson, 1973; Foelix, 1996). The primitive plate includes a smaller collection of mesenchymal cells, called the "cumulus," that break away from the main group of cells and migrate underneath the nascent germ disc. The continued internalization of mesendoderm occurs during and after cumulus migration.

Because of an 8-cell fate map and extensive lineage tracing, the cellular origin and composition of the *Parhyale* rosette and epithelial sheet are well understood (Gerberding et al., 2002; Alwes et al., 2011). The first three *Parhyale* cleavages are total. The third cleavage is highly unequal and results in an 8-cell embryo with four micromeres and four macromeres. Based on the position of the polar bodies, the animal hemisphere is associated with the yolkier macromeres while the vegetal hemisphere is associated with the less yolky micromeres. The embryo then transitions to asynchronous and asymmetrical cleavages that result in repositioning the yolk to the center of the embryo. Cells after this stage are at the periphery of the egg and are of approximately the same size due to accelerated cleavage of the macromeres (Browne et al., 2005; Alwes et al., 2011). At this point, approximately 12 h post-fertilization (hpf) at 26 °C, the rosette and the epithelial sheet begin to form. The rosette is a cluster of roughly 12–16 cells comprising descendants from the sister 8-cell blastomeres **Mav** and **g**, and the epithelial sheet is a cluster of roughly 50 cells made of descendants from the blastomeres **El, Er,** and **Ep** (Gerberding et al., 2002; Browne et al., 2005; Alwes et al., 2011; See Figs. 1 and 2). The rosette and the epithelial sheet develop on opposing sides of the egg, with the rosette developing on the vegetal half and marking the future anterior end of the embryo (Browne et al., 2005). Prior to formation of the germ disc, the rosette and epithelial sheet are easily distinguished with brightfield microscopy and/or lineage tracing. After germ disc formation, the two populations are discernible in vivo using lineage tracing with fluorescent dyes and/or mRNA constructs (Gerberding et al., 2002). Descendants of the blastomeres **ml, mr,** and **en** give rise to the left, right somatic mesoderm and endoderm, respectively. These precursors ingress and remain under the surface after germ disc condensation has already begun (Gerberding et al., 2002; Browne et al., 2005; Alwes et al., 2011; Fig. 1).

Lineage tracing has led to current hypotheses that the *Parhyale* rosette internalizes through ingression or invagination combined with epiboly of the epithelial sheet (Gerberding et al., 2002; Browne et al., 2005; Price and Patel, 2008). While similar descriptive studies implicate ingression and invagination as mechanisms for cell internalization in additional crustacean species, there are, however, also species that primarily gastrulate through delamination or oriented cell division (Gerberding and Patel, 2004). Treatment of *Parhyale* embryos with an inhibitor of zygotic transcription did not prevent rosette internalization, but did affect normal germ disc formation. This suggests that the inward migration of rosette cells proceeds independently of the epithelial sheet, but that normal epiboly of ectoderm precursors relies on zygotic transcription-dependent signaling through cell–cell contact with the rosette (Alwes et al., 2011). Reliance on cell-to-cell contact during gastrulation would be consistent with some other crustaceans and chelicerates. Hertzler et al. (1994) cultured blastomeres isolated from 2-, 4-, 8-, and 16-cell *Sicyonia ingentis* (shrimp) embryos and found that the mesendodermal D blastomere undergoes gastrulation regardless of its cell–cell contacts provided there are enough cells to form an archenteron. Without the D blastomere, the other blastomeres never progress beyond blastulae. Not only does this indicate that cell fates are determined early, but also suggests that the D blastomere may induce neighboring cells to form portions of the archenteron (Hertzler et al., 1994). Interestingly, cells in the cumulus of the spider *Parasteatoda tepidariorium* have been shown to signal with the

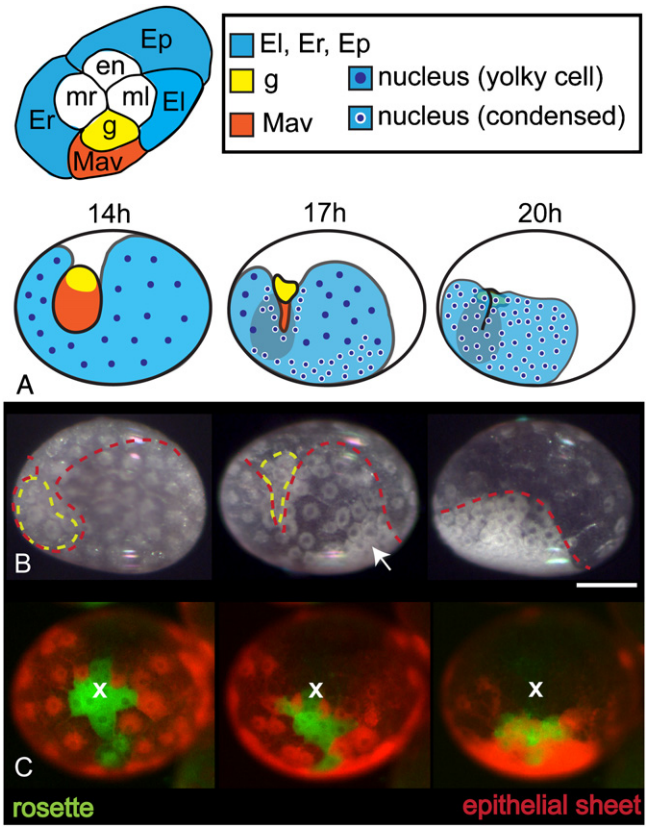

Fig. 1. Two embryos and a schematic show a vegetal view of the first phase of gastrulation. (A) The *Parhyale* fate map at the 8-cell stage (top) and a schematic of the rosette and the epithelial sheet during rosette internalization (bottom). Blastomeres that give rise to the rosette are the anterior and visceral mesoderm (Mav, orange) and the germline (g, yellow). Blastomeres that give rise to the epithelial sheet are the left, right, and posterior ectoderm (El, Er, and Ep, respectively; blue). Colors correspond to Price and Patel (2008). The left and right somatic mesoderm (ml and mr) and the endoderm (en) are not colored and are left in white. (B) Brightfield images of a single embryo at 14 hpf, 17 hpf, and 20 hpf of development at 26 °C. Dashed lines estimate areas covered by the rosette (yellow) and the epithelial sheet (red). Area outside the dashed line corresponds to white area in (A). Arrow at 17 hpf indicates condensing and migrating epithelial sheet cells, some of these cells originated on the animal half of the embryo. By 20 hpf, the rosette is no longer visible underneath the condensed epithelial sheet cells. (C) The rosette and epithelial sheet move to one side of the embryo during internalization. Stills were taken from a timelapse video of embryos embedded in agarose and filmed at room temperature (~22 °C). Images are cropped to focus on a single embryo. Blastomeres were microinjected at the 8-cell stage to label the rosette (FITC, green) and the epithelial sheet (TRITC, red). Stills were chosen to match staging of brightfield images; total elapsed time from left to right image is 8 h. Although some natural variability in egg shape does occur, the rosette looks different than in B because the embryo is rotated slightly and pressed against the glass to centralize and focus on the rosette. Dark unlabeled area corresponds to the white area in (A) and the area outside the dashed lines in (B). X indicates the approximate center of the rosette before internalization. Scale bar is 100 μm.

morphogen *decapentaplegic* to overlying ectoderm precursors (Akiyama-Oda and Oda, 2003; *Parasteatoda* was reclassified from *Achaearanea* by Saaristo (2006)). This signal is thought to help define the dorso-ventral and anterior–posterior axes of the developing embryo (Akiyama-Oda and Oda, 2006). Furthermore, removal of the cumulus in the spider *Agelena labrynthica* results in radialized embryos, and transplantation of cumulus cells to a different area of the germ disc induces twinning (Holm, 1952).

In this study, we investigate cell-shape change in the cell types that comprise the *Parhyale* rosette, treat gastrulating embryos with pharmacological inhibitors of the actin cytoskeleton and the actin regulator Rho-kinase, and ablate portions of the rosette and the epithelial sheet immediately prior to and during gastrulation. Cell-shape changes and the actin cytoskeleton are key components of cell