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Mechanics of blastopore closure during amphibian gastrulation

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ABSTRACT

Blastopore closure in the amphibian embryo involves large scale tissue reorganization driven by physical forces. These forces are tuned to generate sustained blastopore closure throughout the course of gastrulation. We describe the mechanics of blastopore closure at multiple scales and in different regions around the blastopore by characterizing large scale tissue deformations, cell level shape change and subcellular F-actin organization and by measuring tissue force production and structural stiffness of the blastopore during gastrulation. We find that the embryo generates a ramping magnitude of force until it reaches a peak force on the order of 0.5 μN. During this time course, the embryo also stiffens 1.5 fold. Strain rate mapping of the dorsal, ventral and lateral epithelial cells proximal to the blastopore reveals changing patterns of strain rate throughout closure. Cells dorsal to the blastopore, which are fated to become neural plate ectoderm, are polarized and have straight boundaries. In contrast, cells lateral and ventral to the blastopore are less polarized and have tortuous cell boundaries. The F-actin network is organized differently in each region with the highest percentage of alignment occurring in the lateral region. Interestingly F-actin was consistently oriented toward the blastopore lip in dorsal and lateral cells, but oriented parallel to the lip in ventral regions. Cell shape and F-actin alignment analyses reveal different local mechanical environments in regions around the blastopore, which was reflected by the strain rate maps.

Introduction

Morphogenesis relies on cell- and tissue-level control of mechanical properties and highly regulated, spatio-temporally controlled forces to dynamically reshape tissues (Heisenberg and Bellaiche, 2013; Holtfreter, 1943, 1944; Koehl, 1990; Lecuit et al., 2011). By tailoring force production to material properties the embryo is able to facilitate large scale tissue movements necessary to organize the three prospective germ layers during gastrulation (Davidson, 2011; Moore et al., 1995). Developmental programs regulate mechanical processes by regulating cell motility, cell shape, and cell adhesion through direct genetic control as well as through biochemical and mechanical signaling pathways (Beloussov et al., 1988; Discher et al., 2005; Orr et al., 2006). These programs coordinate cell behaviors and drive morphogenetic movements, but also encode cell responses to variations in both the local microenvironment as well as to larger scale environmental cues.

Tissue-scale mechanics are especially important during the large scale rearrangements of the germ layers during amphibian gastrulation (Keller et al., 2000). During gastrulation, large regions on the surface of the embryo involute and pass into the embryo while the remaining surface tissues expand to enclose the embryo. Gastrulation begins when a patch of epithelial cells known as bottle cells apically constrict and lengthen along their apical basal axis to create a local invagination that ultimately forms the anterior-most end of the future archenteron. Bottle cell constriction encircles the large yolky cells of the vegetal endoderm. Involution starts when dorsal marginal zone cells adjacent to the bottle cells and deepening groove move inward. The bending of the dorsal marginal zone as involution progresses forms the blastopore lip. Rather than being a static group of cells, the blastopore lip is a dynamic annular mass of cells, composed of both superficial epithelia and deep mesenchymal cells that transiently reside in the lip as they move into the embryo. The processes of bottle cell formation, blastopore lip formation, and involution all begin dorsally and spread laterally, and

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posterior-ventrally, to encircle the yolk plug formed from the large cells of the vegetal endoderm. As large scale tissue reorganization occurs during gastrulation, the blastopore progressively decreases in diameter and closes (blastopore closure; BC) at a constant rate (Fig. S1).

A number of cellular behaviors are thought to drive closure but the precise biomechanics of these processes and their relative contributions to the integrated tissue mechanics that drives closure remain unknown (Keller and Shook, 2008). On the dorsoanterior face of the embryo, presumptive notochordal and somitic mesoderm cells involute and undergo convergent extension. Mesoderm involution and convergent extension are thought to be driven by active mediolateral intercalation, which elongates the tissue and is proposed to generate an arc of tension that contributes to BC (Keller et al., 2003; Keller and Shook, 2008). In the posterior-ventral region, involuting marginal zone cells undergo convergent thickening which produces a thicker tissue further contributing to BC (Keller and Shook, 2008). The completion of blastopore closure signifies the end of gastrulation and is an early milestone of normal embryonic development. Defects during gastrulation, including delays or failure in BC, can lead to incorrect organization of the primary germ layers, lesions in organogenesis, and serious developmental defects.

Cell and tissue movements accompanying *Xenopus* gastrulation have been described previously (Ewald et al., 2002; Keller et al., 2003; Keller and Shook, 2008; Moosmann et al., 2013; Tyszka et al., 2005) but to understand the physical mechanics of BC requires quantitative measurement of spatial and temporal changes in cell and tissue rearrangement, cellular force generation, and tissue mechanical properties. Such quantitative studies require tools with which strain or strain rates can be measured after application of known forces or loads (Davidson and Keller, 2007).

To understand how the strain rate patterns relate to the global mechanics of blastopore closure we have developed a method to measure force production and material properties of the tissues surrounding the blastopore in vivo and used quantitative image analysis to map mechanical strain rates of dorsal and ventral tissues surrounding the blastopore during gastrulation. To complement our tissue-scale analysis of biomechanics, we collected high resolution confocal images to characterize shape and cytoskeletal orientation of cells surrounding the blastopore lip, and use latrunculin B to evaluate the role of F-actin in the mechanics of blastopore closure. By combining a biomechanical analysis of gastrulation including strain rates, tissue force production and stiffness with descriptions of cell shapes and apical F-actin cytoskeleton we aim to separate the contribution of active from passive tissue shape changes to blastopore closure.

Results

Changing patterns of radial strain rate from mid- to late gastrulation

To understand the location and direction in which cellular forces are being generated, we analyzed mechanical strain rates in tissues surrounding the blastopore using digital image correlation on time-lapse sequences (Fig. 1A, G, M; Supplementary Video S1). Strain rate is a scale- and geometry-free measure of tissue deformation over time that can be used to identify potential sources of force production or regions where mechanical properties change (see Methods for a definition of strain (Blanchard et al., 2009; Davidson et al., 2009)). In contrast to simple deformation or trajectory maps, strain rate maps can indicate where tissues are expanding or contracting in radial and circumferential directions by comparing the displacement of multiple pixels together and calculating whether the distance between them is larger or smaller than in previous time frames. To calculate strain rate we estimate a displacement field or mathematical transform needed to align the two sequential images (Arganda-Carreras et al., 2006). The displacement field produced from this analysis consists of an array of two-dimensional (2D) vectors that bring each pixel in the first image into alignment with the second image. Displacement fields can be visualized by superimposing a subset of these vectors onto the original time lapse images (Fig. 1B, H and N). Spatial gradients of these displacement vectors produce strain rate tensors which can be displayed as maps that reveal local variations in strain rate (Fig. 1C–F, I–L, O–R). In principle, displacement and strain measured between images collected at different times represent the near-instantaneous velocity and the strain rate over a time interval. To recast the strain rates from image-coordinates onto embryonic axes we used a geometric transformation to calculate strains perpendicular to the blastopore (e.g. radial strain) and strain parallel to the blastopore lip (e.g. circumferential strain) for each stage (see Methods). During early gastrulation, after dorsal lip formation, nearly all tissues surrounding the blastopore are expanding with the greatest expansive strain rate appearing dorsally (Fig. 1E). As gastrulation progresses, that radial strain rate at the dorsal lip becomes contractile at Stage 11 (Fig. 1K) then expansive again by Stage 12.5 (Fig. 1Q). Fig. 1 represents strain and displacement results of a single embryo to clearly illustrate our analyses. Typical magnitudes and directions of changes in median strains around the embryo at Stages 10, 11 and 12.5 are consistent amongst 4 other embryos and are summarized in Fig. S2.

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During mid-gastrulation involution spreads from the dorsoanterior lip progressively reaching the ventral-posterior region of the blastopore by late-gastrulation and the surface contractility we observe correlates with these large scale involution movements. For instance, radial strain dips in posterior-ventral regions of the embryo as prospective posterior mesoderm and endoderm initiate involution (Fig. 1Q).

Force of blastopore closure

Since strain is a product of the application of force against a mechanical structure, we measured both the force of blastopore closure and the mechanical resistance of the blastopore lip within the intact embryo. To quantify tissue force production we used flexible cantilever beams constructed from aramide-polymer fibers. Aramide fibers allow the construction of 5–30 μm diameter cantilevers that can be fashioned into force transducers that are sensitive to nano-Newton (10^{-9} kg m/s^2) scale forces. To measure the force produced during blastopore closure we fabricated a dual-cantilever device consisting of two fibers mounted on a single manipulator. The cantilevers were mounted so their tips were initially separated by ~300 μm, approximately the diameter of the blastopore. We inserted the cantilever tips into opposite sides of the blastopore at Stage 10.5 (Fig. 2A and B). As time progressed, the cantilevers deflected under the force of the closing blastopore and we recorded a "ramp-like" linear increase in force leading to a plateau phase approximately 4 h into BC (mid- to late-gastrula, Stage 12; Fig. 2C; $n=3$). At the plateau phase, the mechanical resistance of the cantilever stalls progress of blastopore closure between Stages 12 and 12.5, suggesting the force reported by the cantilever at the plateau phase is the peak force generated during closure. The peak force ranged from 300 nN to 500 nN for the embryos tested. We also measured force production along the mediolateral axis of the blastopore. Neither the ramp-like increase nor the peak-force differed significantly from forces measured from probes placed parallel to the dorso-ventral axis (peak forces of 330, 380 and 440 nN in 3 embryos; Fig. 2C) suggesting closure forces are integrated from multiple cellular sources and distributed equally around the

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