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From biomechanics to mechanobiology: Xenopus provides direct access to the physical principles that shape the embryo

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Abstract

Features of amphibian embryos that elucidated the genetics of vertebrate development enable study of the physics that shape morphogenesis and regulate development. Biophysical tools are revealing how genes control mechanical properties of the embryo. The same tools that describe and control mechanical properties are being turned to reveal how dynamic mechanical information and feedback regulate biological programs of development. In this review we outline efforts to explore the various roles of mechanical cues in guiding cilia biology, axonal pathfinding, goblet cell regeneration, epithelial-to-mesenchymal transitions in neural crest and mesenchymal-to-epithelial transitions in heart progenitors. These case studies, reveal the power of *Xenopus* experimental embryology to expose pathways integrating mechanical cues with programs of development, organogenesis, and regeneration.

Keywords

mechanosensors; mechanotransduction; force; modulus; strain; stress; tension; stiffness; elasticity; viscoelasticity; compliance

Introduction

During development, cells make critical decisions using biochemical and biomechanical cues from their microenvironment Biochemical cues are well documented but many

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novel roles of biomechanical cues are coming to light. In vitro studies show that biomechanical cues can direct cell differentiation, cell divison, and collective cellular behaviors [1,2]. Resurgent interest in the biomechanics on morphogenesis has resulted in the characterization of mechanical properties of embryoni tissues and the genetic and architectural contributions to those properties (see Box: Glossary for terminology). With these advances, the field of developmental mechanobiology is revealing how the dynamic and diverse microenvironments in the embryo contribute to developing tissues and organs.

The *Xenopus laevis* embryo is a unique model system for studying the mechanobiology of development for the following reasons: 1) embryos are available in large numbers, have a well-annotated genome, and can be manipulated by state-of-the-art gain- and loss-of-function technologies (Figure 1a, first row), 2) embryos are ~1.2 mm in diameter and can be easily freed from stiff vitelline membranes, allowing access to pharmaceutical treatments, and 3) embryos and organ primordia can be studied in a range of mechanical contexts. Precisely controlled stress and strain can be applied to intact embryos (Figure 1a; second row), organotypic explants that preserve tissue-tissue interactions (Figure 1a; third row), and even single cells (Figure 1a; fourth row) where the native tissue context is eliminated or reintrodued, as in an aggregate or surrounding cell implants. Challenging cells, tissues, and whole embryos from the same stock material allows detailed multi-scale studies on the contribu ion of molecular, junctional, and tissue level organization to the mechanical properties of the embryo during development. In this review, we will first summarize the types of mechanical cues and how to detect and manipulate them in the tissue, and then introduce several studies using *Xenopus* embryos to reveal how mechanical cues regulate developmental processes.

Cues in the mechanical microenvironment

In vitro studies on cultured cells have revealed a vast array of physical features that can be sensed by cells to regular their biology. We refer interested readers to several excellent reviews on this topis [2,3]. Physical features and mechanical properties are spatially and temporally patterned during development [1,4] [5]. Some features, such as stiffness, can be measured *in vivo* while others, such as nanotopology, lie beyond current methods. (Note: we briefly define several terms related to biomechanics and mechanobiology in the Box: Glossary). The case studies that are the focus of this review center on biomechanical material properties that can be measured directly.

How cells sense mechanical cues

Mechanosensors and mechanotransducers are cellular structures that allow cells to identify mechanical cues and to integrate responses to intracellular signaling pathways, respectively. Many types of mechanosensing systems have been identified in cultured cells and bacteria but only a few have been studied in embryos [4]. Studies on cell-matrix adhesion implicate mechanosensitive protein complexes in focal adhesions (FAs) where integrin receptors bind to the extracellular matrix [5]. Studies in *Xenopus* have identified a novel role for cadherin-11 in mediating adhesion between fibronectin and FAs and aiding in cell migration of neural crest cells [6,7]. In the developing embryo, cell-cell contacts are integral for

influencing cell behavior and distributing forces during collective epithelial migration [5]. Epithelial cells are mechanically coupled by several types of junctions: gap, adherens, tight, and desmosomes. These junctions link to the cytoskeleton as well as ion channels and are likely to regulate cell junction remodeling and collective migration through mechanosensing conditions between neighboring cells [3]. Adherens junctions have been well charactered in many different animal models, and their roles in mechanosensing have been verified in *Xenopus* embryos by an optogenetic tool that induces contact between E-cadherin, and α-catenin [8]. Junctions mediate cell signaling through the actomyosin network and well-characterized mechanosensing proteins such as talin, vinculin, and myosin-II, and recent findings suggest a role for Anillin in mediating tensile forces through vinculin recruitment [9]. Cell-cell signaling can also occur through channel proteins such as Pannexin-1. Under mechanical stress, ATP released through Pannexin-1 activates purinergic receptors in the neighboring cells to regulate cell-cell tension [10].

Measurement and manipulation of mechanical cues in vivo

There have been many methods available, including sessile drop [11], laser ablation, strain mapping, and 3D stress mapping [12], but in this review we will focus on those used in the case studies discussed below, including: cantilever-based systems, microaspiration, and approaches to stretched or compressed organotypic explants.

Cantilever-based systems are some of the most common methods in measuring tissue mechanical properties. Both off-the-shelf and custom devices use cantilevers as force-transducers which are key for indenting or deforming small regions of exposed embryonic tissues (Figure 1a, second row). Contact forces and depth of indentation or strain with the tissue are recorded and can be used to calculate a modulus (see Box: Glossary). Commercial atomic force microscopy (AFM) systems are cantilever-based systems that were first adopted for in vitro studies of cell monolayers [13]. AFM can report mechanical properties from 1 to 5 μm of the surface. Additionally, custom cantilever-based systems can be used to measure forces produced by spreading explants, blastopore closure, and force convergent extension [14–16].

Microaspiration involves applying pressure to pull a tissue into a narrow channel (Figure 1a, second row). The modulus or compliance of a patch of cells on an embryo or aggregate can be calculated from the geometry of the channel, the pressure applied, and the distance the tissue moves into the channel [15]. In addition to being used to measure tissue stiffness non-destructively in different stages of embryos, aspiration can also apply defined strains to test the putative role of mechanical cues [15,17,18**,19**,20].

Compressive or tensile forces can be applied across a tissue, either within the intact embryo (e.g. Figure 1a, second row) or to explants. A uniaxial stress-relaxation has been extensively used to describe the elastic modulus of organotypic explants (first described in [21], most recently used in [22**]) (Figure 1b). Tensile testing, using various designs of tissue stretchers can also exert tension across explants ([23–25]). Explants can be either fully bonded to an elastic membrane (Figure 1a, third row) or suspended between two adherent mounts, and then responses are recorded as the tissue is stretched. The elastic modulus of

the tissue can also be calculated combined with a cantilever system. Such devices have been used in studying the role of mechanical stress in cell division, convergent extension, and numerous other studies.

Strain can be introduced in a purely mechanical form through inert mineral oil injections (see [35]). A moderate level of strain (between 10 and 20 %) can be achieved in one tissue by injecting a droplet of inert immiscible fluid into an adjacent location (Figure 1a, second row). Additionally, droplet volume can be precisely controlled with standard nano-injetion sstems and can be tracked with fluorescent dye. Stress and compliance can be calculated exactly from image analysis techniques such as strain mapping [54] and biophysical measurements obtained through micro-aspiration or AFM.

Developmental processes regulated by mechanical cues: examples in *Xenopus* embryos

There have been rapid advances in the field of mechanobiology of development, thanks to the ability to microsurgically expose tissues to biophysical testing and manipulation. Below, we discuss several recent studies using *Xenopus* embryos to show how embryos and organotypic tissues are regulated by mechanical cues from the microenvironment (summarized in Figure 1b).

Axon guidance

Pathfinding of dendrites and axons is well known to be instructed by biochemical cues [26]; however, in vitro studies have shown that neurite growth can be affected by tension and mechanical properties of the substrate [27,28]. The observation that the central nervous system is mechanically heterogeneous [29,30] suggests that mechanical cues also play a role in neuronal guidance *in vivo.*

This hypothesis is later verified using *Xenopus* retinal ganglion cells (RGC) [31]. Neurite outgrowth of cultured RGCs was found to respond to substrate stiffness, which depends on the stretch-activated ion channel Piezo1. After microsurgically exposing the RGCs in the brain, substrate stiffness along the track of RGC axon growth was measured by an AFM. By altering brain stiffness and Piezo1 activity, the study showed that RGC axons sense a stiffness gradient in the brain and grow towards softer tissue [31]. In a follow-up study, to better understand changes of brain tissue stiffness over time, the authors mapped stiffness and correlated axon growth in vivo [32**] and discovered that the stiffness gradient in the brain is established just prior to axon turning and requires a round of cell proliferation. These two studies provided in vivo evidence of axon guidance mediated by dynamically regulated mechanical cues, highlighting the effectiveness of the *Xenopus* embryo as a model system to study mechanobiology during neuronal development.

Neural crest cell migration

Xenopus neural crest (NC) has been used to investigate the role of biomechanical cues in the initiation of epithelial-to-mesenchymal transition (EMT) and collective migration. While the

molecular signals that pattern and guide NC cell migration are relatively well understood, the observation that these cells pause before initiating migration [33] suggests that they are waiting for additional cues from the microenvironment.

A recent stuy tested the substrate requirements for NC cell migration *in vitro* and then moved to identify mechanical changes in substrate stiffness *in vivo* [34**]. NC cells were found to wait for their mesoderm substrates to age and stiffen before they would initiate migration. Analysis of fibronectin ruled out cues from cell-ECM interactions; however, increasing mesoderm cell density and stiffness triggered NC migration and increased junctional protein expression. Mechanical perturbations of the embryos through ablation, myosin II inhibitors, and morpholinos were able to rule out the contribution of mesoderm contractility. Since mesoderm compaction relies on convergent extension of dorsal axial tissues, the authors perturbed the planar cell polarity (PCP) pathway to inhibit mesoderm stiffening, which could be restored through indentation via AFM. Furthermore, manipulation of the mechanosensors integrin, Vinculin, and Talin inhibited NC migration, suggesting essential roles of focal adhesions in sensing the mechanical properties of the adjacent mesoderm [34**].

Mesenchymal-to-epithelial transition (MET)

Heart progenitor cells (HPCs) begin collective migration as mesenchymal cells but end as epithelial cells. By tracking *Xenopus* mesenchymal HPCs and mapping the heart forming region using traction force microscopy, our recent work revealed a correlation between increased strain rates and the initiation of MET [35]. The ability of HPCs to sense stiffness of the adjacent endoderm was confirmed through fate-map targeted modulation of actomyosin contractility using constitutively active Rho or myosin II modulators. MET could be driven precociously or inhibited by modulating endoderm substrate stiffening. As with the previous cases, physically modulating local stiffness by microinjecting an oil droplet could also accelerate MET [35].

Organoids of stem cells are well known for their ability to initiate complex programs of morphogenesis and differentiation [36]. Our group recently discovered a role of biomechanical cues in driving MET and inducing goblet cell fates on the surface of *Xenopus* deep ectoderm organoids [19**]. Due to the rapid onset of MET in less than 5 hours, the *Xenopus* model allows quantitation of both tissue mechanics measured by microaspiration and cell behaviors recorded by live cell confocal microscopy. The nuclear localization of Yes-associated protein 1(YAP), a transcription factor correlated with mechanical changes, was found to coincide with cell contractility and apicobasal polarity. Like the study of HPCs, actomyosin contractility in ectoderm cells could be manipulated through both small molecules and gain-of-function mutant proteins to inhibit or drive precocious MET. Following MET, newly epithelialized cells undergo goblet cell differentiation, a step that was also sensitive to mechanical perturbation [19**]. In summary, our work established a role of mechanical cues in cardiogenesis and regeneration, but the identity of mechanosensors as well as the signaling pathways involved in triggering MET remains unresolved.

Ciliated cell differentiation

Directional flow driven by ciliated cells regulates diverse functions during embryonic development. As cilia are cell protrusions and generally considered a sensory organ of the cell, it comes as no surprise that cilia are suspected of sensing fluid flow, although the detailed mechanism remains to be determined [37]. Similar to mammals, *Xenopus* embryos have motile monocilia in the left-right organizer (LRO) and multiciliated cells in the skin [38,39]. To drive unidirectional fluid flow, the orientation of these cilia is based toward the posterior end of the cells [38,40]. The cilia polarity of multiciliated cell is established during gastrulation and refined by aligning themselves with the flow direction, forming a positive feedback loop [40]. The planar cell polarity (PCP) pathway is required for cilia polarization during gastrulation, but the mechanism remained obscure [41].

Following observation of strain patterns during early movements of gastrulation, a recent study hypothesized that cilia reorient in response to directional mechanical strains along the anteroposterior axis [17]. To test this hypothesis, the authors showed that microaspiration of embryos and organotypic explants could re-align cilia along the new direction of strain. In a follow-up study, the same group examined the role of early strain in the alignment of cilia in the LRO [18**]. The LRO is the organ that specifies left-right asymmetry and features long motile cilia at the center and short immotile cilia at the lateral region of the tissue [42]. Under directional mechanical strains, LRO cells responded similarly to ectodermal cells by polarizing the microtubule orientation and PCP protein distribution. Interestingly, mechanical strains not only instruct the direction of cilia beat but also regulate cilia length during differentiation in coordination with the transcription factor Foxjl [18**]. These studies highlight the significance of morphogenesis-induced mechanical strains as global cues to instruct cilia differentiation and polarity in ciliated tissue of different origin.

Orientation of cell division

Cell division can play a critical role in the early developmental processes, and mechanical cues are highly correlated with the orientation and timing of cell division [43–45] However the molecular pathways that integrate cell division with external mechanical cues are unclear. A recent study suggested that mechanical forces deform cell shapes, but are not directly sensed by the mitotic spindle to orient cell division, rather acting as a cue for mitosis [46**]. Organotypic animal cap explants were cultured in a custom-designed stretch apparatus and subjected to uniaxial 19.67 ± 1.91% strain. Cell shapes elongated and cell division was significantly aligned along the axis of stretch, and the authors found that the position of tricellular junctions (TCJs) predicted cell division orientation better than other morphometric features. Simulation with a vertex-based model [47] showed that local cell-level stress aligns more with the orientation of TCJs than with global tissue-level stress. This phenomenon was disturbed by manipulating C-cadherin activity, implying that C-cadherin may play a role in recruiting the spindle orientation protein LGN to the TCJs Moreover, cell division rate was reduced by Myosin II knockdown but partially rescued by stretch, suggesting that mechanical cues are required to trigger mitosis [46**]. Computational modeling side-by-side with biomechanical manipulations suggest a new perspective on

the integration of mechanical cues with cadherins and TCJs during cell division; yet, the molecular pathways integrating mechanical cues with mitosis remain to be identified.

Conclusions and Future Perspectives

Rigorous mechanobiology studies in *Xenopus* test hypotheses suggested in mammalian systems regarding the regulation of stem cell specification [2], stem cell migration [2], early cell fate decision [36,48], and tissue patterning [49–51]. It is increasingly clear that mechanical cues in the microenvironment play pivotal roles in multiple morphogenetic and patterning events during embryogenesis.

Just as with genetics, mechanobiology can be investigated using the forward and reverse approaches: the former involves an unbiased screen to identify mechanical cues contributing to a certain process, while the latter is carried out by manipulating a mechanical property and looking for processes being affected. *Xenopus* embryos are highly suitable for both of the approaches. Organotypic explants from *Xenopus* embryos can be easily prepared and exposed to chemical and mechanical manipulations, allowing straightforward and detailed analyses similar to those obtained from stem cell cultures. Moreover, *Xenopus* embryos are more accessible to observation and manipulation compared to mammalian embryos, and therefore it is feasible and often very effective to verify hypotheses derived from in vitro studies on *Xenopus* embryos. In addition to the robust methods discussed above, new techniques allowing improved detection and finer spatiotemporal control of mechanical cues, such as the ferrofluid microdroplets and optogenetic tools [8,52,53] are being developed or adapted to use with *Xenopus* embryos, explants, and cells. The robust quantitative analysis of biomechanics and mechanobiology at multiple different scales during embryogenesis is unique to *Xenopus* and provides novel insights into the role of mechanical cues in guiding cell behaviors necessary for development and responsible for disease progression.

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Box 1.

Glossary

Biomechanics	Mechanical processes that directly shape living organisms. Genes and environment are involved in directing material properties.
Compliance	The ability to deform under an applied force.
Compression	The object is under compression when it experiences a negative strain.
Elastic Modulus	Material property that defines the elastic behavior under an applied stress.
Force	An interaction with a magnitude and direction that changes the motion of an object or deforms it.
Mechanobiology	The feedbacks from mechanical processes that guide biology.
Tension	The object is under tension when it experiences a positive strain.
Stiffness	The resistance to deformation under an applied force.
Strain	A dimensionless term to describe the deformation of an object caused by the force applied.
Stress	The amount of force that is applied to a unit area.
Viscoelasticity	Viscoelastic is a combination of viscous behaviors and elastic behaviors. Elastic materials completely recover from an applied stress (smaller than yield stress) and viscous materials slowly deform under an applied stress but do not return to original configurations.

Highlights

- Mechanical cues can be manipulated indentation, microaspiration, and stretch.
- Mechanical cues can pattern tissues and drive differentiation in many cell types.
- Accessibility and ease of manipulation make *Xenopus* embryos ideal for mechanobiology.

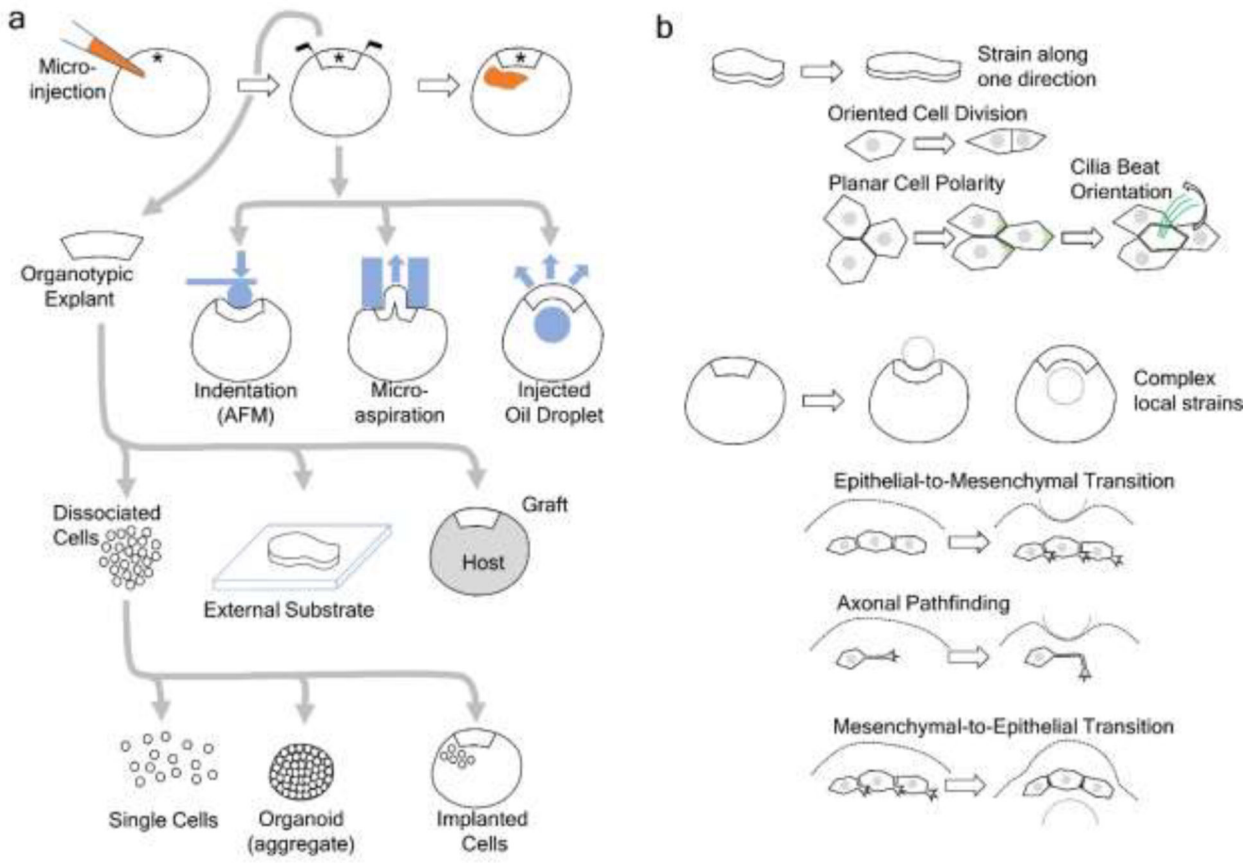

Figure 1. Diverse approaches to characterize and measure biomechanical properties in *Xenopus.*

a) Gain- or loss-of-function reagents (orange) can be injected into embryos at 1-cell or other early cleavage stages, and target tissues can be identified from 32- or gastrula stage fate maps (*). The whole embryo can be subjected to biomechanical manipulations (blue, device; blue arrows indicate force) to quantify mechanical properties or to apply specific deformations. Organotypic explants that preserve tissues in their native context can be isolated microsurgically. These explants an cultured on external substrates, which allow precise control of the microenvironment, or grafted into a host embryo (grey) where the mechanical microenvironment has been altered. Cell-cell and tissue-tissue interactions can be disrupted by dissociating the explant into single cells, which can be studied as-is by conventional in vitro methods or re-aggregate into an organoid. Cells may also be implanted into whole embryos to observe their reintegration into a native or perturbed microenvironment. b) Tools developed for biomechanical testing can also be used to apply defined strains or deformations to investigate the role of those mechanical cues in guiding cell behaviors. Organotypic explants can be subjected to defined strains to evaluate the role of anisotropic strain on cell division. Tissue strain, anologous to those occurring during epiboly, can specify the planar cell polarity and beat orientation of ciliated cells in both the epidermis and left-right-organizer. Manipulations of whole embryos with indentation or injection of oil droplets can instruct pathfinding in axons and modulate phenotypic

transitions between mesenchymal and epithelial cell types. See the main text for more details.