Manipulating the patterns of mechanical forces that shape multicellular tissues

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Abstract:
During embryonic development, spatial and temporal patterns of mechanical forces help to transform unstructured groups of cells into complex, functional tissue architectures. Here, we review emerging approaches to manipulate these patterns of forces to investigate the mechanical mechanisms that shape multicellular tissues, with a focus on recent experimental studies of epithelial tissue sheets in the embryo of the model organism *Drosophila melanogaster*.

Main text:

Introduction:
During development, the tissues, organs, and body of an embryo are shaped from an unstructured group of cells, a process called morphogenesis. Many morphogenetic events involve the bending, stretching, or flow of epithelial tissue sheets, and failure of these movements can be associated with birth defects. Epithelial tissues play key roles in shaping of the basic embryonic body plan (42) as well as more elaborate structures, such as the spatial ridges of gut villi and the branching patterns of lungs and mammary glands (96, 120). Transforming a simple tissue sheet into a complex, functional architecture requires generating mechanical forces in specific spatial and temporal patterns. Ultimately, to understand morphogenesis we must know the genes and molecules that pattern the embryo, the mechanical forces that build and shape tissues, and how mechanical cues feed back on the molecular control of cell behavior and fate (52, 67, 133, 138). Dissecting the mechanisms of morphogenesis has remained a significant challenge, in part due to the difficulty of manipulating patterns of mechanical forces within tissues inside developing embryos.

The actomyosin cytoskeleton plays a major role in generating the forces that shape tissues (48, 49, 65, 84). Non-muscle myosin II motor proteins (myosin) form processive assemblies that pull on anti-parallel actin filaments to generate contractile mechanical tension within cells. For example, during *Drosophila* development planar polarized localization patterns of myosin drive oriented cell rearrangements that narrow and elongate the embryonic body axis in a convergent extension movement (9, 10, 38, 57, 107, 132, 152). Contractile forces generated by myosin drive these cell rearrangements by promoting the contraction of cell-cell contacts that are disassembled as cells exchange neighbors (9, 10, 38, 64, 94, 106, 107, 132) (Fig. 1A, right panel). On the ventral surface of the *Drosophila* embryo, radially polarized actomyosin networks span the apical surface of presumptive mesoderm cells and drive apical constriction (24, 86, 87, 140). These cell shape changes generate a bend in the tissue and promote tissue invagination during gastrulation (77, 86, 87, 131) (Fig. 1B, right panel).

Morphogenetic processes are organized by molecules that control patterns of gene expression across tissues and regulate the patterns of mechanical forces generated by cells. Morphogens can provide long-range patterning of tissues by activating gene regulatory networks in a concentration dependent manner, which in turn control the cell signaling pathways that regulate the actomyosin machinery, for an excellent review see (42). These molecules can be expressed in patterns, often taking the forms of gradients, stripes, or spots. In *Drosophila,* spatiotemporal patterns of transcription factors expressed along the anterior-posterior (AP) and dorsal-ventral

(DV) body axes are required for specific patterns of actomyosin contractility. Myosin planar polarity in the germband requires the pair-rule transcription factors Eve and Runt (152), which are expressed in stripes along the anterior-posterior axis of the embryo and direct expression of Toll-family receptors in combinatorial patterns that provide spatial cues to cells (102) (Fig. 1A, left panel). Likewise, ventral furrow formation and mesoderm invagination require the transcription factors Twist and Snail, which are expressed in the ventral region of the embryo and are required for pulsed myosin-driven apical cell constriction (77, 86) (Fig. 1B, left panel). Although mesoderm invagination and body axis elongation are controlled by different upstream factors, actomyosin localization and activity in both cases are regulated by the Rho/Rho-kinase signaling pathway (64, 94, 121, 122, 140).

Mechanical forces generated by cells or transmitted by the microenvironment can also act as signals that influence cell behavior and fate. Mechanical factors influence myosin localization and dynamics in the *Drosophila* germband (38), ventral furrow (104), and wing imaginal disc (32). Moreover, forces have been reported to promote expression of the Twist transcription factor in the *Drosophila* embryo (27), to influence cell positioning and cell fate specification in a YAP-dependent process during formation of the mouse blastocyst (81), and to play a role in lateral inhibition mediated by compression that influences TAZ activity during zebrafish oogenesis (148).

In this review, we focus on new approaches for manipulating patterns of mechanical forces within tissues *in vivo*, with an emphasis on studies of epithelial tissue sheets in the embryo of the model organism *Drosophila melanogaster*. This research is rapidly accelerating our ability to dissect the complex mechanical and molecular mechanisms of morphogenesis.

Techniques to directly apply patterns of mechanical forces to tissues inside developing embryos: One approach to manipulating and studying the spatiotemporal patterns of forces in developing tissues is to directly apply mechanical loads. This can be achieved by externally confining, compressing, or indenting the embryo or by modifying interactions between the embryo and the surrounding environment (7, 20, 36, 95, 104). These techniques have provided valuable insight into the mechanisms by which tissues resist deformation and respond to mechanical forces. However, these approaches typically do not generate precise or localized patterns of forces characteristic of endogenous forces during morphogenesis. Alternative techniques with greater spatial resolution have also been used to apply loads at small length scales inside embryos by manipulating injected magnetic particles or ferrofluids (23, 27, 29, 89, 90, 117), cutting tissue structures by laser ablation (7, 37, 55, 105, 124, 130), applying pressure to tissues by locally aspirating with a micropipette (38), or by manipulating endogenous structures with optical traps (3). Many of these approaches have been used to measure tissue mechanical properties (16, 23, 29, 49, 117, 129), and some have also been used to study the effects of forces on tissue morphogenesis and development.

Laser ablation approaches to locally cut tissues have been widely used to study mechanical forces within tissues in developing embryos (55, 105, 124, 130). This approach has helped to elucidate the nature of forces that contribute to dorsal closure (55), germband extension (38, 107), and ventral furrow invagination (85) in the *Drosophila* embryo, cortical flows in the *C. elegans*

zygote (88), and gastrulation movements in zebrafish (7). Laser ablation has also been used to manipulate patterns of forces in developing embryos, revealing effects on gene expression (27, 130), myosin dynamics (38), cell division (17, 115, 143), and oriented cell rearrangement (151). While laser cutting disrupts the force patterns in a tissue, it cannot flexibly generate any desired pattern of force and can induce wound healing responses in the tissue (71).

Manipulation of magnetic nanoparticles has also been utilized to study how patterns of forces regulate expression of transcription factors in the *Drosophila* embryo. Magnetic field manipulations of magnetic nanoparticles were used to mimic forces exerted by the elongating germband on the neighboring stomodeal primordium to study the effects that mechanical forces have on gene regulation (27). This study suggested that forces regulate gene expression in the *Drosophila* embryo, where they promote expression of the Twist transcription factor (27). More recently, injected nanoparticles driven by arrays of small magnets were used to produce forces that mimic cell-scale patterns of apical contractions in mesodermal cells. These forces were shown to rescue mesoderm invagination defects in embryos mutant for the Snail transcription factor (89). While magnetic manipulation can provide localized control of forces and allows deep penetration into tissues, the presence of large magnetic objects within tissues may pose issues for long-term development. Moreover, the incorporation of particles inside tissues and the generation of magnetic fields to produce desired force patterns remain experimental challenges, limiting accessibility of these techniques for many labs.

Techniques to manipulate patterns of forces within tissues by harnessing the cell's own machinery: In an alternative approach, patterns of forces within tissues can be manipulated by harnessing the cells' own force-generating machinery. Cell and tissue movements during morphogenesis are largely driven by the cellular actomyosin cytoskeleton (48, 49, 65, 84), with distinct patterns of actomyosin activity associated with different tissue movements.

Developmental biologists can manipulate the genes that control morphogenesis, and the forces that drive it, with a powerful molecular genetics toolbox. This toolbox includes genetic mutants, RNAi-mediated knockdown, and transgenic over-expression or mis-expression. In *Drosophila* these approaches have been essential for determining the requirement of genes in development (99). For example, a combination of these approaches has helped to elucidate the regulation and function of myosin during epithelial tissue morphogenesis (24, 40, 62, 64, 70, 121, 140, 146). The Gal4-UAS system can restrict the expression of transgenes to specific time periods or locations in the embryo with tissue-specific Gal4 drivers (12), but expression patterns are restricted by available drivers and so cannot flexibly produce any desired pattern. Alternatively, small molecule inhibitors that target the cellular force-generating machinery can be micro-injected to specific regions of embryos. Yet, spatial patterns are constrained by injection and diffusion of the inhibitor. Moreover, micro-injection can be disruptive to the tissue, and the effects of the inhibitors often cannot be temporally controlled. The need for non-invasive tools for manipulating cell-generated patterns of forces with high spatial and temporal precision motivated the recent utilization of optogenetic (45, 68, 136), thermogenetic (50), and magnetogenetic (91) technologies in developing embryos. Here, we focus on studies using optogenetic technologies, which have recently been adapted for a wide variety of model organisms used in the study of development.

Optogenetics: Over the last decade, light-sensitive protein domains have increasingly been utilized as genetically-encoded optogenetic tools for manipulating diverse cellular processes *in vitro and in vivo* because of their precision, controllability, and accessibility (1, 14, 18, 44, 46, 53, 56, 58, 59, 68, 74, 79, 112, 127, 144, 147, 150). These tools are activated by exposure to specific wavelengths of light and can achieve high spatial and temporal precision. They have been used to manipulate protein localization and activity, cell signaling, and gene expression at various stages during development. They offer precise, and in some cases reversible, perturbations that are difficult to achieve with other methods. The most common optogenetic tools are based on phytochromes (PHY), cryptochromes (CRY), Light Oxygen Voltage sensing (LOV), and Blue Light Using Flavin (BLUF) domains or their derivatives, which are largely derived from plants, bacteria, and algae. Many optogenetic tools are based on a light-dependent dimerization mechanism. For example, in CRY2-CIB1 tools blue light absorption by CRY2 induces a conformational change, leading to increased affinity for its partner CIB1 (68).

Some optogenetic tools are better suited for specific systems or questions. Despite success using optogenetic systems *in vivo*, there are limitations and constraints to be considered, including the degree of expression, dissociation time and reversibility, requirement of an external chromophore (e.g. some phytochromes) (14, 79), potential for self-oligomerization (e.g. cryptochromes) (15), degree of residual activity without light activation or 'leakiness', and level of subcellular control. General limitations of light-based methods include phototoxicity during long periods of activation and difficulty penetrating into deep sections of tissues. For excellent reviews of the growing collection of optogenetic tools, see (60, 108, 134). In general, optogenetic tools have the potential for fast (miliseconds to minutes) and local (micron or greater) control of intracellular processes, and so are well-suited to the study of development. Here, we highlight CRY2-derived and LOV-derived optogenetic tools, which have been most widely used in the interrogation of the forces that drive morphogenesis.

Optogenetic manipulation of molecules that directly regulate the contractile machinery: One strategy is to use optogenetic technologies to manipulate the molecules that directly regulate the cellular actomyosin machinery (Fig. 2B). Significant progress has been made in identifying many of these regulators and elucidating the mechanisms by which they influence myosin localization and/or activity, making them ideal candidates for optogenetics. The Rho/Rho-kinase signaling pathway plays a key role in regulating the patterns of myosin localization and activity in many cells and tissues (2, 109), including the planar polarized pattern of myosin in the elongating germband tissue (64, 69, 94, 121, 122) and the radial pattern of myosin in cells during mesoderm invagination (87, 140) in the *Drosophila* embryo.

Optogenetic tools to manipulate actin or myosin regulators have been widely used to modulate and study the behaviors of cultured cells *in vitro* (6, 68, 72, 79, 100, 101, 139, 142, 147). A LOV-derived TULIP tool was developed to recruit the RhoA guanine nucleotide exchange factor (GEF) LARG to the cell membrane, revealing that patterned RhoA activation is sufficient to induce formation of contractile rings during cytokinesis (127, 142). This photo-recruitable GEF tool was also used to modulate contractility in adherent cells, revealing a requirement for zyxin in stress

fiber mechanics (101). A cryptochrome-based CRY2-CIBN system was used to manipulate the catalytic DHPH domain of ARHGEF11, either recruiting it to the cell membrane to increase RhoA signaling and actomyosin contractility or sequestering it at the mitochondrial membrane to decrease ectopic contractility associated with expression of the tool (139). Optogenetically induced changes in contractility were associated with differences in the nuclear localization of the transcriptional regulator YAP, demonstrating optogenetic control over mechanotransduction in cultured MDCK-II cells (139). CRY2-CIBN-based tools have also served to manipulate GEFs during cytokinesis (72) and cell migration (6).

Within developing embryos, optogenetic tools have been used to manipulate regulators of actomyosin force generation. In *Drosophila,* a CRY2-CIBN-based tool was used to disrupt actin through depletion of the phosphoinositide PI(4,5)P2 at the cell membrane (44, 56). Local optogenetic disruption of actomyosin-driven apical constriction in a small group of cells in the prospective mesoderm of the *Drosophila* embryo was sufficient to disrupt tissue invagination along the ventral midline of the embryo (44). To locally increase actomyosin contractility, a CRY2-CIBN-based tool was developed to recruit the DHPH catalytic domain of RhoGEF2 to the cell membrane in the epithelium of the early *Drosophila* embryo, triggering apical constriction and initiating ectopic tissue folding in the illuminated region (58) (Fig. 2B). The same tool was used to increase levels of myosin activity specifically at the basal side of presumptive mesoderm cells. This perturbation disrupted invagination, suggesting that relaxation of contractile forces on the basal side of cells is required for full tissue invagination (75).

Optogenetic manipulation of molecules that pattern the embryo: Another strategy to manipulate patterns of forces in tissues is to use optogenetic technologies to manipulate the upstream regulators that organize morphogenetic processes (Fig. 2A), such as the morphogens and transcription factors that direct development (42, 99, 103). For example, the requirement and function of molecules involved in myosin-driven body axis elongation in *Drosophila* have been elucidated over the past two decades (57, 69, 102, 152). Bicoid is localized in a gradient along the anterior-posterior (AP) axis and regulates expression of gap genes, which in turn regulate the striped expression pattern of pair-rule transcription factors (30, 41, 43, 128). These transcription factors regulate the striped expression of Toll-family receptors, which direct the planar polarized localization pattern of myosin (102) (Fig. 1A). The concentration and activity of upstream organizing molecules change in space and time to pattern the embryo, specify cell fates, and build tissues. Therefore, optogenetic manipulation of these upstream organizing molecules will have broad impacts on cell behavior and fate, and have the potential to orchestrate multiple processes required in complex morphogenetic movements, including patterns of actomyosin contractility.

In *Drosophila*, optogenetic control of Erk mitogen-activated protein kinase signaling was achieved using a LOV-derived light-switchable iLID membrane anchor paired with a light-recruited Ras activator, together called OptoSOS (59) (Fig. 2A). Global or local ectopic Erk activation in the embryo resulted in apical myosin localization and contractility (61). OptoSOS was used to dissect the spatial and temporal requirements for Erk in cell fate and tissue morphogenesis during *Drosophila* development, revealing that cells measure the cumulative load of Erk signaling to make an endoderm versus ectoderm fate decision (61). Spatial and temporal requirements for

the morphogen Bicoid were investigated with an optogenetic tool based on fusing the light-responsive CRY2 domain to the N-terminus of Bicoid, allowing light-controlled disruption of Bicoid transcriptional activity in the *Drosophila* embryo (53) (Fig. 2A). A CRY2 fusion to β-catenin allowed light-controlled aggregation of β-catenin and inhibition of canonical Wnt signaling to study patterning of the *Drosophila* embryonic epidermis (66). In zebrafish, LOV-domain based optogenetic tools have been developed and used to study non-canonical Wnt signaling (18) and Nodal signaling (112) during gastrulation.

Optogenetic manipulation of motor proteins, vesicles, and membraneless compartments: Beyond the manipulation of actomyosin regulators and upstream organizers of development, optogenetic tools have also been used to directly control cytoskeletal motor proteins and organelles within cultured cells. Direct recruitment of kinesin, dynein, or myosin-V motor proteins to selected cargos using a LOV-domain (8) or a CRY2-CIB1 (31) system was used to control organelle transport along cytoskeletal filaments (Fig. 2C). Optogenetic tools have also been used to control cellular trafficking processes by prompting intracellular vesicle aggregation (97) and to manipulate membraneless organelles that play numerous roles in cells, from facilitating biochemical reactions to sequestering proteins (11, 119). Optogenetic control over membraneless organelles has allowed quantitative study of their formation through liquid-liquid phase separation within cells (11, 119).

Magnetogenetic, thermogenetic, and electrical manipulation: A broad range of other physical modalities have been used to manipulate molecules that influence the cellular force generating machinery. Magnetic manipulation of functionalized magnetic nanoparticles can trigger biochemical events in cultured cells and developing embryos (91). This approach has allowed manipulation of microtubule nucleation (51), Rho-GTPase activity (35), and protein and organelle localization (80). A major limitation of magnetic manipulation is the difficulty of incorporating magnetic particles into embryos with high precision and minimal tissue disruption. New approaches are focusing on genetically-encoded molecules such as ferritin, although the magnetic properties of these systems may need to be optimized to ensure sufficient magnetic force magnitudes (91). Recently, thermogenetic approaches have been developed to locally inactivate fast-acting temperature-sensitive mutant proteins in *C. elegans* (50). These tools were used to manipulate and study the spatiotemporal requirements for myosin during cell division in *myosin-II(ts)* embryos (50). In addition, electric fields can influence cell behavior and migration and have been used to manipulate collective migration of cultured epithelial cell monolayers (22); for a comprehensive review see (78).

New opportunities to study patterns of mechanical forces that emerge in populations of cells cultured *in vitro*: There are exciting new opportunities to study morphogenesis, and the forces that drive it, in cell populations that self-organize *in vitro*. Cell-cell interactions, cell-matrix interactions, biochemical cues, and mechanical cues can drive cultured cells to sort, form complex three-dimensional architectures, specify cell fates, and even generate organ-like and embryo-like structures that recapitulate aspects of tissue structure, composition, and function *in vivo* (21, 39, 76, 111, 113). Self-organizing *in vitro* systems can provide insights into the roles of mechanical forces in development, especially for organisms for which embryonic development occurs in

environments that have proven difficult to interrogate (118). In addition, *in vitro* systems offer precise control over environmental conditions and can be amenable to live imaging and direct mechanical measurements, providing new opportunities to dissect the roles of mechanical and biochemical factors in regulating cell behavior and fate within multicellular tissues.

In the 1950s, dissociated cells from amphibian embryos were shown to spontaneously organize into layered tissues that mimic embryonic tissue layers (137). More recently, cell sorting has been studied in cell lines (125) and in cells from zebrafish embryos (73, 82, 83, 116) and *Xenopus* embryos (98), revealing roles for cell-cell adhesion and cortical tension in driving sorting (Fig. 3A). Interactions between cells and the extracellular matrix (ECM) were shown to help support formation of functional alveolar structures by cultured primary mammary cells (4), and a recent study revealed a key role for cell-ECM interactions in the self-organization of mammary and prostate gland structures *in vitro* (19) (Fig. 3B).

A significant research effort over the last decade has revealed that stem cells and organ-specific progenitors can give rise to complex self-organized and self-patterned structures, called *organoids*, which mimic the structure and function of *in vivo* organs. Examples include intestinal organoids (114), optic cups (33) (Fig. 3D), brain-like organoids (34, 63, 93), and a wide range of other organoid systems (21, 111, 113). More recently, these approaches have been applied to mimic and study embryonic patterning and morphogenesis. Human and mouse embryonic stem cells self-organize into patterned germ layers and three-dimensional structures that mimic the early embryo (25, 26, 92, 110, 123, 145), opening new opportunities for studying mammalian development and morphogenesis (Fig. 3E). Remarkably, mouse embryonic stem cell cultures called *gastruloids* can undergo gastrulation-like and axis elongation events *in vitro* (5, 13) (Fig. 3E). *In vitro* models that recapitulate key developmental events offer unique opportunities to alter mechanical parameters of the environment and link them to changes in the cell and tissue behaviors that control tissue form and function (141, 149).

Combining self-organization with engineered cues to direct these processes may provide new pathways to engineer structures *in vitro* that better mimic *in vivo* tissues or even have novel, optimized characteristics. For example, engineered cell-cell interactions have recently been used to direct generation of mechanical stresses in tissue layers to drive folding into complex three-dimensional patterns (54) (Fig. 3C), and synthetic Notch receptor systems have been used to manipulate cell-cell interactions and engineer self-organized cell aggregates (135).

These *in vitro* systems provide promising new opportunities to study how mechanical and molecular factors work together to build and shape functional tissues and organs. Organoid and gastruloid systems clearly demonstrate that complex patterns of forces can be generated to drive morphogenetic events *in vitro*, but they have not yet been widely used for the study of mechanical mechanisms underlying morphogenesis. It will be exciting to combine these *in vitro* models of self-organizing cell populations with new tools and approaches for studying and manipulating the forces that drive morphogenesis.

Future perspectives:

Emerging techniques to manipulate patterns of mechanical forces within multicellular tissues are accelerating our ability to dissect how mechanical forces cooperate with biochemical signals to generate complex tissue structures during embryonic development. Combining these techniques with each other and with classical molecular genetics approaches is likely to provide to developmental biology a new toolbox of quantitative, precise perturbations. Future work will be required to calibrate the forces produced using these techniques, which is a necessary step in moving towards a quantitative, mechanistic understanding of morphogenesis. Theoretical and computational approaches for predicting tissue movements from patterns of forces will contribute to predictive models of specific morphogenetic events (28, 47, 126). Ultimately, increased understanding of the mechanisms that control tissue morphogenesis, combined with sophisticated tools to direct these processes, will contribute to efforts to predict and control tissue shape and structure *in vivo* and *in vitro*, with potential applications in tissue engineering as well as in fundamental biomedical research.

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Figures and captions:

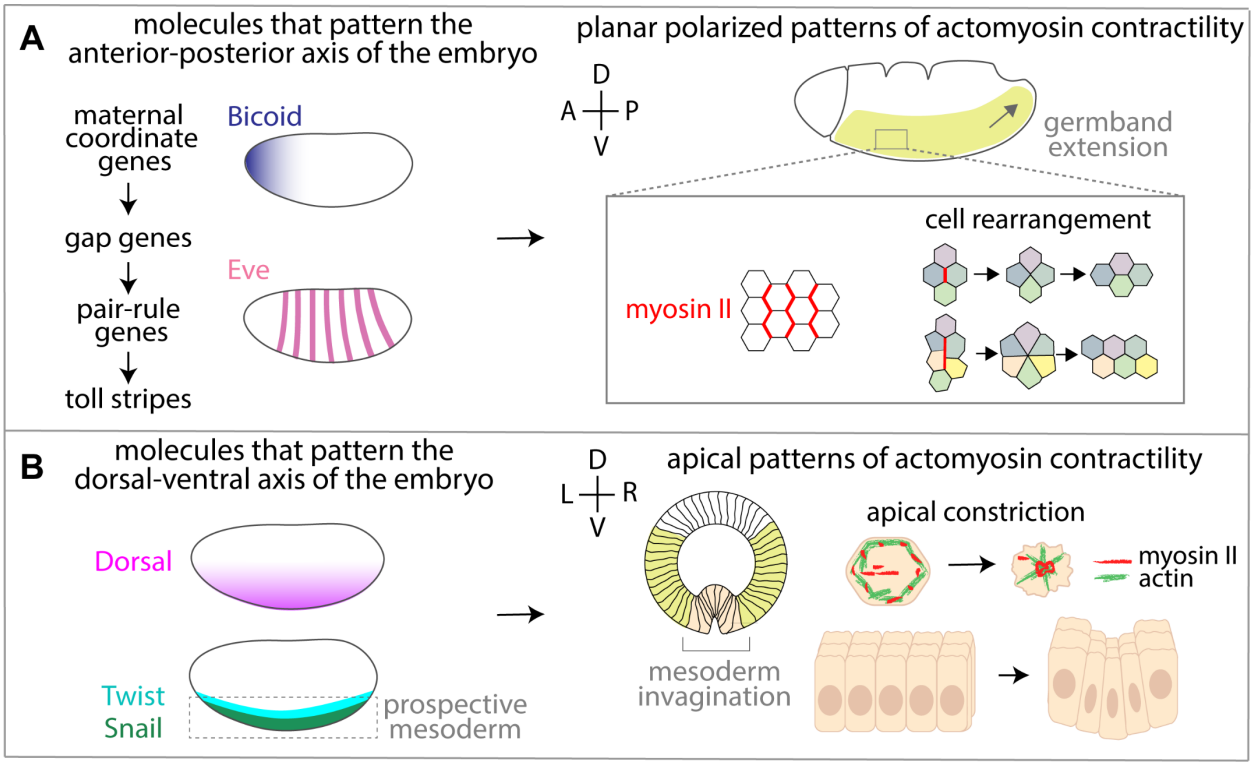

Figure 1. Regulators and patterns of cell-generated mechanical forces that shape the *Drosophila* embryo. Upstream molecules pattern the embryo and organize morphogenetic events, ultimately controlling the actomyosin-generated mechanical forces that shape tissues during morphogenesis and that can feed back on the molecular control of cell behavior and fate (42, 103). **A**. Molecules that pattern the anterior-posterior (AP) axis of the *Drosophila* embryo. Bicoid (blue) is localized in a gradient along the along the AP axis. Along with other maternal effect genes, Bicoid regulates the expression of gap genes, which in turn regulate the striped expression of pair-rule transcription factors such as Eve (pink). Pair-rule genes set up the expression of Toll-family receptor stripes and the planar polarized distribution of myosin in the germband tissue (102). Planar polarized myosin activity in the germband tissue is required for the oriented cell rearrangements that elongate the body axis. See (9, 10, 38, 107, 152). **B.** Dorsal-ventral patterning involves localization of the maternal transcription factor Dorsal (magenta) in a gradient, regulating expression of the transcription factors Twist (cyan) and Snail (green), which are required for the repeated pulses of actomyosin contractility at the apical cortex of the presumptive mesoderm cells that drive tissue bending and invagination. See (77, 85).

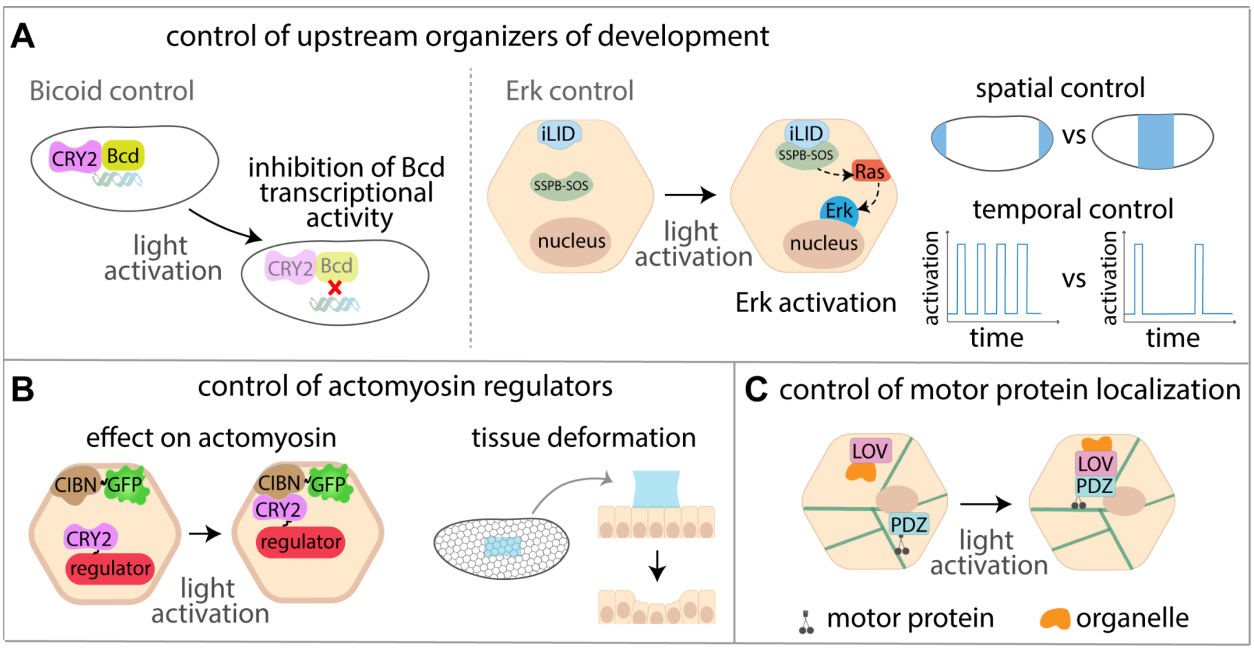

Figure 2. Optogenetic techniques to manipulate patterns of forces by harnessing the cell's own machinery. Light-responsive protein domains have been adapted as optogenetic tools to manipulate cellular processes (60, 108, 134). Optogenetics tools can be used to manipulate the cell's own machinery for generating forces. **A**. Optogenetic control of upstream organizers of development and morphogenesis. In the *Drosophila* embryo, Bicoid (Bcd) was fused to CRY2 for light-controlled inhibition of Bicoid transcriptional activity (53). An optogenetic system was used to study the spatial and temporal requirements of Erk signaling in cell fate specification and tissue morphogenesis during *Drosophila* development (59, 61). **B.** Optogenetic tools have been used to control regulators of actomyosin force-generating machinery. Recruitment of actomyosin regulators to the cell membrane can disrupt normal or generate ectopic cell shape changes and tissue folding (44, 58, 75). **C.** Optogenetics has also been used to directly control motor protein localization and control organelle transport in cultured cells (8, 31).

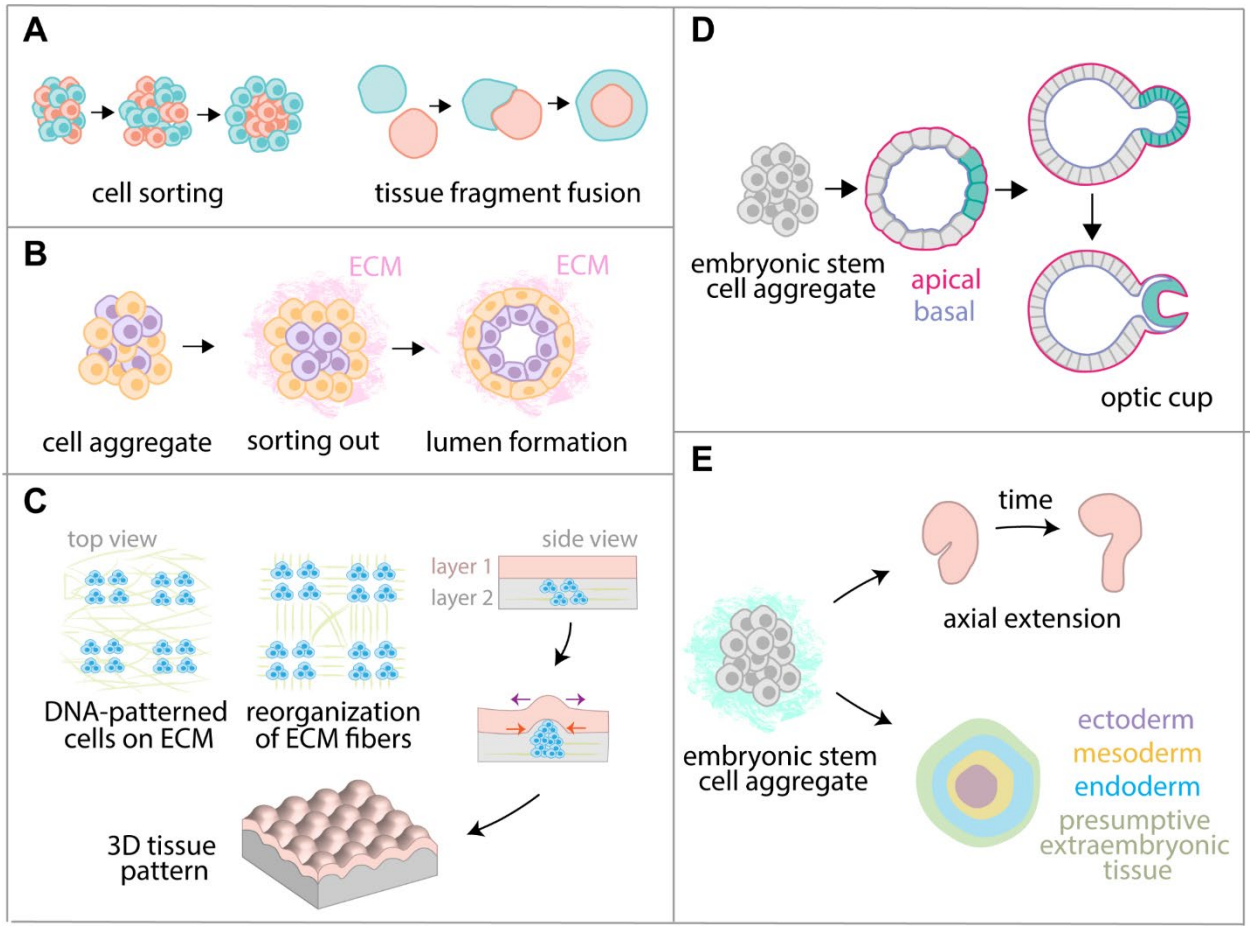

Figure 3. Opportunities to study patterns of forces within self-organizing populations of cells cultured *in vitro*. Patterns of mechanical forces arising in populations of cells cultured *in vitro* provide new opportunities to study and manipulate forces within tissues under controlled conditions. **A.** Cells self-sort based on cell-cell interactions, arising from differences in adhesion or cortical tension (39). **B**. Cells organize into functional structures similar to *in vivo* lumen architectures based on cell specific interactions with the extracellular matrix (ECM) (19). **C.** Cell-cell and cell-matrix interactions can be engineered to direct tissue morphogenesis (54). **D.** Aggregates of embryonic stem cells can self-organize into tissue structures called *organoids* that resemble adult organs, as illustrated by the example of optic cup formation *in vitro* (33). **E.** Aggregates of embryonic stem cells can self-organize into structures called *gastruloids* that mimic features of developing embryos, including defined germ layers when cultured on micropatterned surfaces (25, 26, 123) or axis elongation (5).