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Epithelial machines that shape the embryo

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Summary

Embryonic form and the shape of many organs is the product of forces acting within and on epithelial sheets. Analysis of these processes requires consideration of the mechanical operation of these multicellular machines and an understanding of how epithelial sheets are integrated with surrounding tissues. From the diverse array of epithelial morphogenetic movements seen during embryogenesis we review examples of epithelial sheet bending, *Drosophila* ventral furrow formation and ascidian gastrulation and direct measurements of epithelial mechanics from *Xenopus laevis*. We present these examples as works-in-progress and highlight opportunities for future studies into both the direct consequence of force production and embryonic tissue mechanics and potential roles of signaling from biomechanical processes.

Keywords

biomechanics; cell and tissue mechanics; morphogenesis; gastrulation; neurulation; organogenesis; synthetic biology

Physical laws coordinate tissue assembly during development

Embryologists have long been attracted to the study of movements that shape multicellular organisms during early development and their efforts have been joined recently by cell biologists, bioengineers and physicists to elaborate the molecular, cellular and tissue-scale processes that drive these movements. Biophysical studies are revealing the mechanical properties of the cytoskeleton, how lamellipodia form and retract, and how focal adhesions are assembled and transmit force across the cell membrane. Combined with biochemistry, these studies reveal how cells dynamically regulate their material properties, generate force and sense the mechanical properties of their environment. There is a large gap, however, between our knowledge of the physicochemical properties of purified cytoskeletal preparations and individual cells and our understanding of how groups of cells integrate mechanically within tissues to carry out the tasks of morphogenesis. Recently, however, there has been increasing interest in adapting biophysical techniques for use with embryos and embryonic tissues. Here, we will review several engineering principles that govern the biomechanical behavior of epithelial tissues, provide examples from the recent literature on epithelial folding and bending, and outline important questions for future studies.

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Extending the language of the engineer to the anatomy of the embryo

We broadly define an epithelial sheet as a uniform array of cells physically connected to form a single cell layer, the physical connections between cells, and the repeated pattern of those connections (Figure 1A). This definition is not limited to cells joined by adherens junctions and includes tissues where cells may be only marginally polarized with apical and basolateral ends. Thus, the definition is broad enough to include vertebrate somites that are comprised of mesenchymal cells yet form tightly organized monolayers surrounding a myocoel, or early blastomeres within the gastrulating *C. elegans* worm that exhibit only the earliest manifestations of apical-basal polarity. Not all epithelia in early embryos are atypical; there are also many examples of mature epithelial sheets with familiar barrier, transport or secretory functions. Thus, comparative studies of diverse species and morphogenetic events allow the investigation of epithelial sheets from cell arrays that are barely polarized with little barrier function, to sheets with extraordinarily tight barriers rarely seen by physiologists.

Like any physical structure, epithelial sheets change shape in response to unbalanced mechanical stresses (see reviews on biomechanics of multicellular tissues [1, 2]). Unbalanced stresses may be produced by changes in force production or changes in the mechanical response of tissues to pre-existing applied stress. The mechanics of epithelia sheets can be divided into processes that generate shape change within-the-plane of the sheet or processes that produce shape change out-of-the-plane. Cells may change the shape of the epithelium in the plane by: i) moving from one region to another, ii) stretching or contracting to cover a larger or smaller area, iii) directed rearrangement, iv) the addition or subtraction of material (i.e. cells) to change the area covered by the sheet, and v) being divided or joined to another sheet. Thus, unbalanced stresses and the large-scale movements they generate are driven by a summation of cell movements and tissue mechanics.

In embryos, single mechanical processes or combinations of multiple processes integrated at the tissue-level are thought to drive a wide variety of epithelial morphogenetic movements (Figure 1B). Stresses that produce out-of-the-plane shape changes (e.g. changing the curvature of an epithelial sheet) do so by changing the dimension of one side of the sheet with respect to the other. Such processes require epithelial cells to do something different on one side of the sheet and can include: i) removal or addition of material, ii) enhanced adhesion, traction, or contractile forces at one end of the cell, and iii) assembly of unique multicellular structures at one end of the cell. Often, out-of-the-plane shape changes are the result of multiple stresses being combined (see discussions below). Furthermore, the effect of stresses can be modulated by the mechanics of surrounding tissues providing physical constraints or boundary conditions that redirect deformation and subsequent stresses into different directions (Figure 1C).

Mechanical boundary conditions allow engineers and physicists to simplify problems of mechanics by abbreviating complex structures with simpler structures that have limited degrees of motion. For example, the fly embryo is confined to the space defined by a stiff extraembryonic chorion or vitelline membrane. The blastoderm may slide within the chorion but when active stresses within the blastoderm push it against the chorion the blastoderm cannot displace the chorion outward. In this special case, an engineer can reduce the complexity of the 3D structure of the chorion by simply restricting movement of the blastoderm; boundary conditions are mathematical statements that can indicate restrictions on movement or rotation in any direction. Thus, biomechanical analyses of embryos do not necessarily need to recreate the entire embryo but rather simulate parts whose movement or margins are restricted by explicit boundary conditions.

To understand how flat epithelial sheets are formed into 3D structures it is important to understand the principles of mechanical 'flexure' or 'bending'. A key concept from physics and mechanical engineering is that of a bending moment and how one is generated and how it affects a structure. A bending moment is produced by the action of a pair of forces, or force-couple, acting along the cross-section of a flat sheet, plate or beam (Figure 1D). Flexure of the sheet is determined by the manner with which the force-couplet is applied, the presence of any pre-existing stresses acting on the sheet, the architecture and material properties of the sheet, and the boundary constraints acting at the margins of the sheet. The mechanical properties of an epithelial sheet and the bending moment generated by a specific cellular process allows us to understand the separate contribution of cell biology to intrinsic cell shapes and their material properties, externally and internally generated stresses, and boundary constraints to the formation of 3D structures from flat epithelial sheets.

Mechanics couples cell biology to epithelial morphogenesis

How are mechanical processes regulated within epithelial sheets? Clearly, epithelial mechanics must depend on multiple cell biological processes as cells adopt an identity and self-assemble into a compact array; yet, we know very little about how these processes are integrated within cells and we know even less about how they regulate specific cell behaviors. We can draw upon extensive engineering studies of both natural and synthetic cellular structures to describe cell geometry and materials that comprise epithelial sheets (Figure 2A)[3]. Furthermore, many cell behaviors can be loosely categorized without addressing their molecular or biomechanical sources [4]: i) programmed cell shape changes such as cell division, cell packing, columnarization, shortening, wedging, formation of bottle cells or localized boundary shortening (Figure 2B), ii) cell movements such as cell mixing, directed in-plane rearrangement, ingression or intercalation, and iii) shifts in adhesion and topology such as sheet separation, sheet fusion, wound or gap sealing.

Descriptive analyses of cell shape changes, cell movements and shifts in adhesion or topology are the starting point for building biomechanical hypotheses but may not themselves indicate the cellular or mechanical mechanism of a particular tissue movement. Instead, these behaviors may be passive responses to external forces, or reflect other processes driving tissue movements. For instance, cell wedging may be due to compressive forces acting on the epithelial sheet causing it to bend; cell shortening may be due to tensional forces acting on the epithelial sheet and cell columnarization due to the application of compressive forces. The transition from columnar to wedge could be due to architectural features of the epithelial sheet such as how it is attached basally to neighboring tissues. With these caveats in mind we review two cases of epithelial morphogenesis. Readers are referred to an excellent classical review of comparative epithelial anatomy and morphogenesis [5] and reviews that incorporate more recent contributions from molecular genetic models [6–8]. Some of these movements have been the subject of genetic and biomechanical analyses (e.g. ventral furrow formation and dorsal closure) whereas most others have not.

Ventral furrow formation in *Drosophila*

Early development in *Drosophila* is rapid and involves a rich set of epithelial movements and folding events (for recent reviews of *Drosophila* epithelial morphogenesis see [9–12]). The process of gastrulation occurs soon after columnarization of the ventral surface of the fly embryo is complete and cells have assembled a cohesive junctional network at their apical ends [13–15]. Actomyosin contractions spread to neighboring cells in the ventral domain as multiple rounds of contraction drive more cells to narrow apically throughout the ventral midline [16, 17]; cells in the ventral furrow adopt a wedge shape as a furrow forms along the length of the embryo. As more cells contract their apical surface, the furrow deepens and lateral tissue moves toward the ventral midline. The narrowing of their apical

ends coincides with the movement of nuclei toward the apical surface [18]. Once mesodermal precursors have moved into the embryo, apposing sheets of lateral epithelial cells reseal the epithelium at the new site of the ventral midline [19, 20]. Taking about 20 minutes to complete, ventral furrow formation is one of the fastest morphogenetic movements involving epithelia. Biomechanical analyses of this process has been largely limited to theoretical studies with the earliest studies testing the general plausibility or the capacity of hypothetical cell behaviors to drive epithelial folding [21]. More recent theoretical studies have attempted to extract stress patterns needed to shape theoretical tissue structures [22]. In these studies, mechanical models represent transverse cross-sections of gastrulating embryos and use boundary conditions to represent the full three-dimensional shape of the embryo. The architecture of the tissue is represented by stress-bearing structures such as struts or homogeneous materials. Structures at the anterior of the embryo, such as the cephalic fold, or processes at the posterior of the embryo, such as the mid-gut invagination, are omitted from the analysis and replaced by boundary conditions. In order to drive bending, these models impose apical-basal asymmetries in contractile structures but do not simulate how these structures may have formed or the mechanisms that trigger or signal the onset of contraction, nor do they incorporate details of the cellular structure or subcellular biology. A number of predictions from these models have emerged such as the need for apical-basal struts to prevent ventral cells from elongating when they apically contract [21] or the need for apical-basal directed stresses in the lateral epidermis to recreate tissue movements in the ventral midline [22].

Gastrulation in ascidian

Early development in ascidians involves few cells, stereotypical cell divisions, and invariant cell lineages that produce a notochord, bilateral muscle blocks, and a dorsal nerve cord. Prior to gastrulation, the ascidian embryo consists of a compact monolayer ball of cells. At these early stages of development, the epithelium is polarized by expression and localization of PAR family proteins but does not include adherens or tight junctions that define more mature epithelia at later stages [23]. Cell divisions establish cell identities and distinct cell behaviors in monolayered embryos by the 64-cell stage where ten endoderm-fated cells lie on the vegetal surface and are laterally surrounded by mesoderm-fated cells. Throughout early development, the basal surface of endoderm cells contact the basal surface of ectoderm-fated cells (Figure 2B) and during gastrulation these two layers fold coordinately as the endoderm moves into a deep pit within the embryo [24]. Folding and invagination are accompanied by a two-phase series of cell shape changes both within the mesoderm and ectoderm [25]. Apical contraction and apical-basal lengthening in the endoderm is coincident with apical expansion and apical-basal shortening of the laterally adjacent mesoderm and apposed ectodermal cells (Figure 2C). Shape changes occur with localization of differentially activated myosin at the circum-apical belts and around the basolateral surface of invaginating cells. The activity of myosin is required to position F-actin at both apical and basolateral cell surfaces throughout the embryo and when acutely perturbed can alter each phase with distinctive results (Figure 2D). Ablation experiments indicate that ectoderm cells produce a mechanical resistance in the first phase of invagination but then aid the deepening of the archenteron pit in the second phase [24, 25]. The modeling efforts of Sherrard and coworkers [25] can be understood in terms of boundary conditions that are used to simulate movements of cells within a transverse section of the embryo. Bending moments are induced by asymmetrically localized contractile elements representing specific cell surfaces where actomyosin accumulates. The plausibility of the two-phase model has been confirmed by this model and tested against experiments where tissues are ablated or actomyosin activity is altered by acute-acting drugs. Future studies will be needed to understand how contractile elements are asymmetrically assembled and how programs of individual cell mechanics are triggered and coordinated with neighboring cells.

Concluding remarks

The central role of biomechanics in morphogenesis has been recognized for more than 100 years yet we are only now making significant advances in understanding that role. Those advances have been largely driven by new imaging tools and fluorescent probes to measure deformation [26] and dynamics of morpho-mechanically active proteins within cells and tissues [8, 17]. By their nature, many of these advances have taken the form of descriptive studies and analysis of the effect of genetic lesions on cell behaviors. However, outside of studies in *Drosophila* there are very few studies seeking to connect molecular perturbations to macroscopic changes in epithelial mechanics. The model systems where molecular and cellular manipulations are simplest are some of the most challenging to measure absolute forces or material properties. In contrast, the model systems where tissue-scale forces and material properties can be directly measured are the most challenging to manipulate genetically. To overcome these challenges the field needs increased integration between cell biology, cell mechanics and biomechanical analyses of epithelial morphogenesis. We foresee several areas where advances are needed to address questions related to epithelial morphogenesis.

Cells within both spreading arrays of cultured epithelial cells [27, 28] and rearranging mesoderm cells in *Xenopus* explants [29] generate a range of traction forces against deformable substrates and can modulate levels of force over a short time span (Figure 3A and B). Such observations suggest that cells sense and respond to complex mechanical cues in their environment as they coordinate their efforts. However, direct tests of such sensing mechanisms and their responses during development have been hindered by the inability to carefully control the mechanical stimulus and to measure immediate cellular and signaling responses. Studies in *Drosophila* embryos have found myosin activation [30], cell shape change [31] and gene activation [32] can be stimulated by large ectopically applied stresses [32], however, a study applying physiological levels of stress in *Xenopus* embryonic tissues has found little evidence that mechanical stimulation alters the mechanical properties of tissues [33]. Whereas mechanics plays a clear role in driving epithelial movement, it remains unclear whether mechanotransduction, mechanosensing or other forms of mechanical feedback operate universally or whether feedback is only occasionally needed to fine tune otherwise robust programs of morphogenesis.

Experimental biomechanical platforms and theoretical models of epithelial mechanics are sorely needed to explore and test the physical principles of self-organization. First, theoretical models of morphogenesis are needed that are predictive and can identify experimentally testable features that would discriminate between different driving processes. The biomechanical theories of ventral furrow formation are some of the most mature in developmental biomechanics. However, even these models have significant gaps in their representation of cellular mechanics; for instance, these theories have not addressed potential roles for nuclear translocation or cellular mechanisms for cell shortening or how cells might locally reinforce their stiffness in the apical-basal direction. Since models do not incorporate molecular level cellular mechanics they have not delivered predictions that can be experimentally tested on that scale. For instance, detailed molecular models of epithelial cells are needed from which apical constriction or other bending-moment phenomena might emerge. Numerous genetic studies have identified roles for assembly and maintenance of adhesions at the lateral and apical junctional complexes, but these roles have not been comprehensively tested.

Synthetic experimental models of morphogenesis should also be used to test cellular mechanics of self-organization. Due to their small size or inaccessibility during development, many of the most interesting and clinically relevant biomechanical phenomena

are challenging to test experimentally. However, programs of morphogenesis could install within synthetic biology test-beds where the principles of self-organization can be experimentally dissected. Our group has succeeded in exogenously stimulating apical contractions within a biophysical apparatus that allows simultaneous measurements of compliance, force generation and 3D geometry (Figure 3C, D, and E). For instance, principles of self-organization can be observed in minimally patterned embryoid bodies [34] or *Xenopus* animal caps [35]. A range of approaches combining classical embryology, modern molecular biology, experimental biophysics and synthetically engineered tissues are being used to understand the naturally evolved machines of epithelial morphogenesis.

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References

1. Koehl MAR. Biomechanical approaches to morphogenesis. *Seminars in Developmental Biology*. 1990; 1:367–378.
2. Davidson LA. Embryo mechanics: balancing force production with elastic resistance during morphogenesis. *Curr Top Dev Biol*. 2011; 95:215–241. [PubMed: 21501753]
3. Gibson, LJ., et al. *Cellular Materials in Nature and Medicine*. Cambridge University Press; 2010.
4. Trinkaus, JP. *Cells into Organs: the forces that shape the embryo*. Prentice-Hall Inc.; 1984.
5. Fristrom D. The cellular basis of epithelial morphogenesis. A review. *Tissue and Cell*. 1988; 20(5): 645–690. [PubMed: 3068832]
6. Sawyer JM, et al. Apical constriction: a cell shape change that can drive morphogenesis. *Dev Biol*. 2010; 341:5–19. [PubMed: 19751720]
7. Lecuit T, Lenne PF. Cell surface mechanics and the control of cell shape, tissue patterns and morphogenesis. *Nat Rev Mol Cell Biol*. 2007; 8:633–644. [PubMed: 17643125]
8. Gorfinkiel N, Blanchard GB. Dynamics of actomyosin contractile activity during epithelial morphogenesis. *Curr Opin Cell Biol*. 2011; 23:531–539. [PubMed: 21764278]
9. Zhang H, et al. Tissue morphogenesis: how multiple cells cooperate to generate a tissue. *Curr Opin Cell Biol*. 2010; 22:575–582. [PubMed: 20822890]
10. Kasza KE, Zallen JA. Dynamics and regulation of contractile actin-myosin networks in morphogenesis. *Curr Opin Cell Biol*. 2011; 23:30–38. [PubMed: 21130639]
11. Lye CM, Sanson B. Tension and epithelial morphogenesis in *Drosophila* early embryos. *Curr Top Dev Biol*. 2011; 95:145–187. [PubMed: 21501751]
12. Lecuit T, et al. Force Generation, Transmission, and Integration During Cell and Tissue Morphogenesis. *Annu Rev Cell Dev Biol*. 2011; 27:15.11–15.28.
13. Sawyer JK, et al. The *Drosophila* afadin homologue Canoe regulates linkage of the actin cytoskeleton to adherens junctions during apical constriction. *J Cell Biol*. 2009; 186:57–73. [PubMed: 19596848]
14. Harris TJ, Peifer M. The positioning and segregation of apical cues during epithelial polarity establishment in *Drosophila*. *J Cell Biol*. 2005; 170:813–823. [PubMed: 16129788]
15. Kolsch V, et al. Control of *Drosophila* gastrulation by apical localization of adherens junctions and RhoGEF2. *Science*. 2007; 315:384–386. [PubMed: 17234948]
16. Martin AC, et al. Integration of contractile forces during tissue invagination. *J Cell Biol*. 2010; 188:735–749. [PubMed: 20194639]
17. Martin AC, et al. Pulsed contractions of an actin-myosin network drive apical constriction. *Nature*. 2009; 457:495–499. [PubMed: 19029882]

18. Sweeton D, et al. Gastrulation in *Drosophila*: the formation of the ventral furrow and posterior midgut invaginations. *Development*. 1991; 112(3):775–789. [PubMed: 1935689]
19. McMahon A, et al. Dynamic analyses of *Drosophila* gastrulation provide insights into collective cell migration. *Science*. 2008; 322:1546–1550. [PubMed: 19056986]
20. Supatto W, et al. Quantitative imaging of collective cell migration during *Drosophila* gastrulation: multiphoton microscopy and computational analysis. *Nat Protoc*. 2009; 4:1397–1412. [PubMed: 19745822]
21. Odell GM, et al. The mechanical basis of morphogenesis. *Developmental Biology*. 1981; 85:446–462. [PubMed: 7196351]
22. Brodland GW, et al. Video force microscopy reveals the mechanics of ventral furrow invagination in *Drosophila*. *Proc Natl Acad Sci U S A*. 2010; 107:22111–22116. [PubMed: 21127270]
23. Munro EM, Odell GM. Polarized basolateral cell motility underlies invagination and convergent extension of the ascidian notochord. *Development*. 2002; 129:13–24. [PubMed: 11782397]
24. Munro EM, Odell G. Morphogenetic pattern formation during ascidian notochord formation is regulative and highly robust. *Development*. 2002; 129:1–12. [PubMed: 11782396]
25. Sherrard K, et al. Sequential Activation of Apical and Basolateral Contractility Drives Ascidian Endoderm Invagination. *Curr Biol*. 2010; 20:1499–1510. [PubMed: 20691592]
26. Blanchard GB, et al. Tissue tectonics: morphogenetic strain rates, cell shape change and intercalation. *Nat Methods*. 2009; 6:458–464. [PubMed: 19412170]
27. Treppe X, et al. Physical forces during collective cell migration. *Nature Physics*. 2009; 5:426–430.
28. Saez A, et al. Is the mechanical activity of epithelial cells controlled by deformations or forces? *Biophys J*. 2005; 89:L52–54. [PubMed: 16214867]
29. Zhou J, et al. Macroscopic stiffening of embryonic tissues via microtubules, Rho-GEF, and assembly of contractile bundles of actomyosin. *Development*. 2010; 137:2785–2794. [PubMed: 20630946]
30. Fernandez-Gonzalez R, et al. Myosin II dynamics are regulated by tension in intercalating cells. *Dev Cell*. 2009; 17:736–743. [PubMed: 19879198]
31. Pouille PA, et al. Mechanical signals trigger Myosin II redistribution and mesoderm invagination in *Drosophila* embryos. *Sci Signal*. 2009; 2:ra16. [PubMed: 19366994]
32. Farge E. Mechanical induction of Twist in the *Drosophila* foregut/stomodaeal primordium. *Curr Biol*. 2003; 13:1365–1377. [PubMed: 12932320]
33. Von Dassow M, et al. Surprisingly simple mechanical behavior from a complex embryonic tissue. *PLoS One*. 2011; 5:e15359. [PubMed: 21203396]
34. Eiraku M, et al. Self-organizing optic-cup morphogenesis in three-dimensional culture. *Nature*. 2011; 472:51–56. [PubMed: 21475194]
35. Green JB, et al. Self-organization of vertebrate mesoderm based on simple boundary conditions. *Dev Dyn*. 2004; 231:576–581. [PubMed: 15376320]
36. Kim HY, Davidson LA. Punctuated actin contractions during convergent extension and their permissive regulation by the non-canonical Wnt-signaling pathway. *Journal of Cell Science*. 2011; 124:635–646. [PubMed: 21266466]

Figure 1. Anatomy and mechanics of epithelial sheet morphogenesis

A) Three axes of the epithelial sheets and the localization of distinct molecular complexes along the radial axis (Axis I) as well as along the two planar axes (Axis II and III). B) In-the-plane force-generating mechanisms can alter the geometry of an epithelial sheet. Asymmetries in force generation along the radial axis or constraining boundary conditions can cause out-of-the-plane changes in geometry, such as bending. C) Boundary conditions preventing or limiting the movement of an epithelial sheet can dramatically alter the final shape of the sheet after the application of a force; for instance, a fixed boundary can lead to sheet bending. D) Asymmetrical distribution of forces, either externally applied or internally generated, can also drive bending.

Figure 2. Cellular behaviors, molecular mechanism and a case of epithelial folding

A) A hexagonal 'unit-cell' in an epithelial sheet consists of discrete mechanical elements. In addition to the contents of the cell, the circum-apical junctions, apical cortex, lateral cortex and basal cortex (not shown) have specific geometries and material properties. These structural elements all contribute to the stiffness of the unit-cell. B) Either in response to internal or external stresses, cells may adopt a variety of shapes. Internally driven forces may produce counter-intuitive changes in cell shapes as stresses distributed throughout the sheet adjust to boundary conditions. C) Initial anatomy of ascidian embryo before gastrulation. Cells in the ectoderm, mesoderm and endoderm are arrayed in a single layer. Ectoderm and endoderm cells are in direct contact at their basal surfaces. Sherrard and colleagues suggest cells undergo a two-step program of apical constriction followed by basolateral contraction. For this program to move the endoderm into the embryo requires boundary conditions provided by a contractile ectodermal sheet. These differential programs of contractility can be seen in the relative abundance of F-actin and active non-muscle myosin II, and are thought to drive multiple phases of out-of-the-plane folding during gastrulation in the ascidian (C reproduced with permission from [25]).

Figure 3. Measuring physical mechanics of epithelial morphogenesis

A) Marginal zone explants from gastrulating *Xenopus laevis* embryos can be microsurgically isolated and placed onto force-reported polyacrylamide gels conjugated with fibronectin (PAG-FN). Cells in explants expressing membrane-targeted GFP (mem-GFP) bind fibronectin and displace red fluorescent beads embedded within the PAG-FN. B) Color-encoding shows regions of high traction force (arrows) on the mem-GFP image and can also be seen in a frequency histogram. C) Both the viscoelastic properties and the force-generating potential of an embryonic epithelial sheet can be recorded in a combination stimulator-microaspirator. A patch of the embryo can be drawn into a microchannel by reduced pressure in the channel. D) The full shape of the aspirated cells expressing mem-GFP and their 3D conformation within the channel may be recorded with a laser scanning confocal microscope. E) A kymograph of a transverse view of the microaspirated tissue reports deformation after a simple drop in pressure (ΔP - no stimulus) and after a brief electrical pulse is used to stimulate contraction (ΔP + stimulus). (A and B modified from [36]; C and E modified from [33]; D courtesy of M. von Dassow).