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Mechanical aspects of developmental biology: perspectives *On Growth and Form* in the (post)-genomic age

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Abstract
Simple experiments demonstrate that the development of an organism is both a genetic and a physical process. This statement is so obvious that it is seldom stated explicitly, and yet, there has been little progress toward integrating what should be complementary viewpoints. This paper focuses on the mechanical aspects of morphogenesis—highlighting those areas where mechanics and molecular genetics are converging toward a much-needed synthesis.

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1. Introduction

More than 90 years have passed since the publication of the first edition of d'Arcy Wentworth Thompson's classic tome *On Growth and Form* (Thompson 1917). Recent reprints are still easy to find (based on Thompson 1942) and this longevity speaks to the high quality of Thompson's writing and ideas. He advanced the notion that much of biological growth and form could be explained with simple physical principles and mathematical relationships—stating succinctly the form of an object is a 'diagram of forces'. Although his work has a strong appeal for scientists with a physical or mathematical bent, it frankly has had little impact on our present understanding of mechanics and mechanism in organismal development. The major impact of Thompson's tome has instead been inspirational. Even as non-molecular aspects of biology were swept aside by the molecular biology revolution, research into the physics and mathematics of development has never been completely dormant and is now beginning a resurgence.

In the current genomic—or perhaps post-genomic—age, the science of developmental biology is dominated by a gene-centric viewpoint. This is not surprising, nor unwarranted, given the huge success of molecular biology. A lot of experiments intuitively demonstrate that organismal development is a genetic process. Most obviously, mutations in single genes can have disastrous effects (Wieschaus 1996). On the other hand, simple experiments can also obviously and intuitively demonstrate that organismal development is a physical process. Any time-lapse movie of development conveys that sense, but movies involving mechanical disruptions are even more convincing. A pair of examples is shown in the sequence of still frames of figure 1 and the supplementary movies 1(*A*) and 1(*B*) (stacks.iop.org/PhysBio/5/015001). These are confocal, fluorescent images of living, GFP-labeled fruit fly (*Drosophila*) embryos. The embryos have just completed germ band extension (stage 9, some 4–5 h after fertilization (Foe *et al* 1993)) and the dorsal surface exhibits several morphologically distinct regions of tissue. When these regions are cut using a carefully controlled laser, three implications are immediately apparent: the tissues are under considerable mechanical stress; these stresses vary from tissue to tissue; and the stresses are locally anisotropic. Similar mechanical consequences are seen following laser-ablation of other embryonic tissues (Kiehart *et al* 2000, Hutson *et al* 2003, Supatto *et al* 2005). These simple examples illustrate the ample evidence that development is a genetic and a physical process, but integrating these perspectives has proved a daunting challenge.

In using a modern example, we do not want to imply that the physical nature of developmental biology is some recent discovery. Such experiments have a long history, and the

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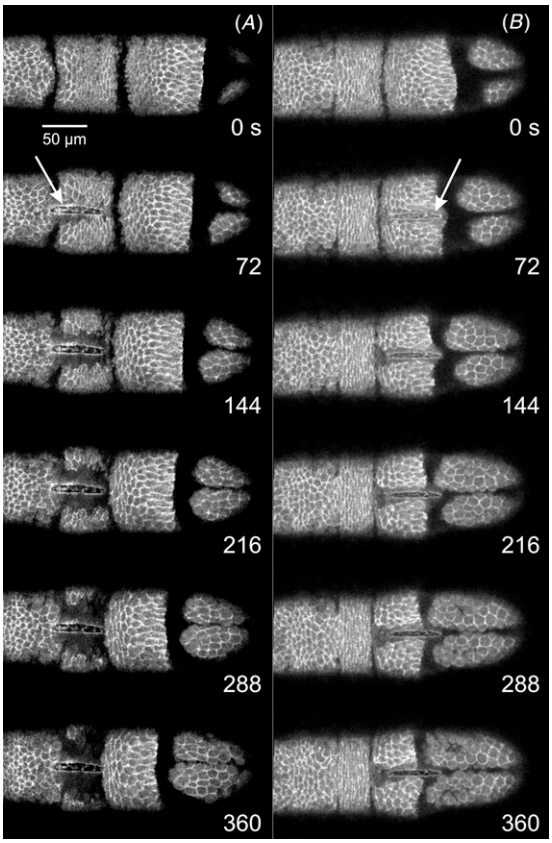

Figure 1. Laser microsurgery reveals stress patterns in embryonic tissues. Each panel is a series of confocal fluorescent images of a living, GFP-labeled (sGMCA) fruit fly embryo. This labeling outlines each cell via its cortical actin network. Between times $t = 40$ and 72 s, each embryo was subjected to a laser incision ($\sim$0.5 by 55 μm) The laser cut through both the embryo's vitelline membrane and its one-cell thick epithelium. In both panels, the incision to the vitelline membrane expands just slightly and halts expansion within a single image frame (dark areas with hyperfluorescent edges demarcated by white arrows). In contrast, the response of the epithelial layer is slower and depends strongly on where the tissue is cut—either along the dorsal midline of the (*A*) posterior or (*B*) anterior region of the presumptive amnioserosa. The wound in (*A*) gapes open laterally. The nearly identical wound in (*B*) barely expands. These are dorsal views of stage 9 embryos with the advancing germ band on the right (posterior). The scale bar is applicable to all images. Movies corresponding to each panel are available as supplementary material (stacks.iop.org/PhysBio/5/015001).

above example is actually a modern twist on experiments from the late 19th century age of *Entwicklungsmechanik*—literally developmental mechanics—highlighted by the pioneering works of His (1874) and Roux (1888) in which embryonic tissues were partially ablated, dissected and transplanted to investigate their biophysical interactions. Nor do we wish to imply that mechanical stresses are the only physical aspect of development. They are simply the most obvious. The mechanical aspects alone have to be generalized to include fluid dynamics, differential cell adhesion and even the control of gene expression and differentiation through mechanical feedback. Beyond the mechanics, one must include pattern formation via reaction–diffusion or activator–inhibitor systems, and dynamical systems analysis of genetic regulatory networks. The latter two physical, yet non-mechanical, aspects of development have a readily apparent connection to molecular biology. Progress in these areas has been the subject of books and recent reviews (Meinhardt 1982, 2008, Goutsias and Lee 2007) and will not be elaborated upon here.

This paper will instead focus squarely on the mechanical aspects of development. Our main goals are to describe where the field of developmental mechanics is now, to highlight inroads toward integration of mechanics and genetics—when and where they exist—and to otherwise point out topics in development where molecular biology and physical biology are converging. We choose this focus because one cannot escape the fact that an 'acceptable explanation' in modern biology necessarily includes a molecular component. For developmental mechanics to have an appreciable impact on the wider biology community, a synthesis of the underlying physics and molecular genetics is indispensable.

2. Embryonic tissues as viscous fluids with differential adhesion

Before looking for routes toward this synthesis, it will be useful to briefly review the mechanical properties of embryonic tissues (see Forgacs and Newman (2005) for an accessible introduction). As with any tissues, those in an embryo are composed of cells and an extracellular matrix (ECM)—fabricated, modified and degraded by the cells. The ratio of cells to ECM varies strongly from ECM-dominated mesenchymal tissues to nearly ECM-free epitheloid tissues. True embryonic epithelia do have ECM in the form of a basement membrane; however, when compared to adult epithelia, this layer of ECM is much less developed. A similar statement holds for embryonic tissues in general, leading to the fact that embryonic tissues are often well-modeled mechanically as viscous fluids—particularly over the long time scales (minutes to hours) characteristic of morphogenetic movements. More generally, embryonic tissues are viscoelastic. The elastic properties become important under special circumstances, e.g. during out-of-plane bending of epithelia or on short time scales (seconds) when recoiling from laser incisions.

When embryonic tissues are modeled as viscous fluids, then analogs of two fluid properties are needed—viscosity and surface tension. The viscosity analog of cells and tissues includes contributions from remodeling the individual cellular cytoskeletons and from neighbor rearrangement in the pattern of cell–cell contacts. The surface tension analog includes contributions from the cell membrane, from the binding energy of adhesion molecules on the cell surface, and from the impact of this binding (via cellular signaling pathways) on the underlying cytoskeleton. Although both analogs are important for quantitative models, the viscosity only determines the rate at which events occur. The surface tension plays a much larger role in structure and form generation.

This role for a surface tension analog can be traced back to the work of Johannes Holtfreter. He found that when

two dissimilar embryonic tissues were placed in contact, one tissue would reproducibly spread over and engulf the other (Holtfreter 1939). Furthermore, when dissociated cells from these tissues were mixed, they would gradually sort themselves out (Holtfreter 2003). When sorting was complete, one cell type would again reproducibly engulf the other. This work was followed up by Steinberg who recognized that the tissue and cell behaviors were very similar to those of contacting or mixed immiscible fluids. Since the de-mixing of immiscible fluids is driven by differences in surface or interfacial energies/tensions, he proposed the differential adhesion hypothesis or DAH (Steinberg 1970): (1) the adhesive energy of a cell–cell or cell–medium interface depends on the type(s) of cell(s) that are in contact and (2) differences in these energies can drive sorting and rearrangement via a gradual approach to a stable energy minimum. See Steinberg (2003) for an excellent historical review of the experiments leading to the proposal and acceptance of this hypothesis. The DAH has become the *de facto* standard for explaining cell-sorting experiments. Its precepts have been incorporated into a wide variety of morphogenetic models from statistical, Monte Carlo-based Potts models (Glazier and Graner 1993) to deterministic finite element models (Brodland and Chen 2000).

The DAH also provides the first opportunity to look at a synthesis between the physics and molecular biology of development. Although Townes and Holtfreter (1955) stated that 'at present, it would be futile to speculate further upon the possible subcellular factors that are engaged in cellular adhesiveness', it is now well accepted that a large part of cell adhesion in morphogenesis can be attributed to the cadherin family of proteins (reviewed in Gumbiner (2005) and Steinberg and McNutt (1999)). Two crucial *in vitro* experiments link the physical property of differential cell adhesion to cadherin expression. First, cells that were originally non-cohesive could be made cohesive by the genetically engineered expression of cadherins (Friedlander *et al* 1989). Later experiments found that the quantitative degree of cohesion—as measured via cell aggregate surface tension—was directly proportional to the number of cadherin molecules per cell (Foty and Steinberg 2005). Second, mixing two populations of these artificially cohesive cells resulted in de-mixing and engulfment of the more cohesive cells (expressing more cadherin) by the less cohesive cells (expressing less cadherin) (Steinberg and Takeichi 1994). The *in vivo* evidence of a linkage is more indirect. It hinges on (1) defects in normal development that occur when the normal expression/function of cadherins is disrupted—through either blocking antibodies (Bronner-Fraser *et al* 1992, Matsunaga *et al* 1988) or ectopic expression (Detrick *et al* 1990, Fujimori *et al* 1990)—and (2) the numerous examples in which morphogenetic events are preceded by cell-type-specific changes in cadherin-subtype expression (reviewed in Takeichi (1988))—where both the cadherin subtype and the degree of expression could play a role. The difficult step is showing that these developmentally regulated changes in cadherin expression lead to differences in cellular adhesion *in vivo*. The difficulty of *in vivo* measurements is a recurring theme and a major hindrance.

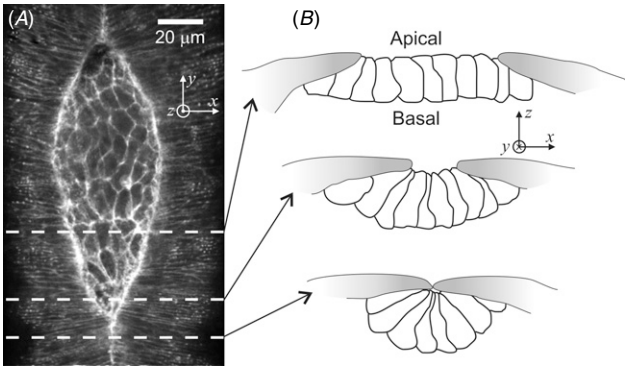

Figure 2. Apical constriction during *Drosophila* dorsal closure. (*A*) Confocal fluorescence image of a GFP-labeled (sGMCA) fruit fly embryo. Dorsal-side is up; anterior–posterior is along *y*; mediolateral is along *x*. The lens-shaped tissue is the amnioserosa and the surrounding elongated cells are the lateral epidermis. (*B*) Line drawings constructed from *xz*-scans along the indicated dashed lines. The shaded regions are lateral epidermis (individual cells not shown) and the unfilled cells are amnioserosa. Wedging begins at the anterior–posterior extremes of the amnioserosa and proceeds toward the middle.

Nonetheless, the relationship between cadherin expression and differential adhesion is one of the best links to date between developmental genetics and the physical determinants of morphogenesis.

3. Stereotypical morphogenetic events—apical constriction and convergent-extension

Cell sorting is an admittedly artificial system. In actual morphogenetic events, cells of various types start in a specific, non-random arrangement. From there, the cells undergo stereotypical neighbor exchanges and/or changes in shape. In the following, we examine two common developmental events—apical constriction and convergent-extension. We choose to focus on these two events for three reasons: (1) the cellular shape and neighbor changes of each are well documented; (2) the forces driving these changes have been explained by at least one hypothesis in a physical/mathematical model; and (3) the relevant genes and signaling pathways have been identified through mutations and genetic constructs that lead to morphological defects. To be complete, the causal chain from mutation to morphological defect must include the underlying forces; however, even for these well-studied events, there are few identifiable points of intersection between the physical and genetic perspectives.

3.1. Apical constriction

Apical constriction is the stereotypical wedging of epithelial cells. This wedging is accompanied by a local increase in the epithelial layer's curvature. Both processes are involved in numerous examples of morphogenesis from gastrulation to neurulation to dorsal closure. Each case involves coordinated shape changes in a subgroup of cells within a larger epithelium. For concreteness, consider the specific case of dorsal closure (figure 2). Here we have

two contacting epidermal layers—one surrounding the other—that lie in the xy-plane and are each one-cell thick (in z). The subset of cells that will undergo apical constriction is the central morphologically distinct layer known as the amnioserosa. As closure proceeds, the amnioserosa cells constrict their apical surfaces. The basal surfaces and the apical-basal cell thickness concurrently expand to maintain nearly constant cell volume. In xz-cross-section, the cells first become roughly trapezoidal and eventually wedge-shaped. The specific geometry of dorsal closure results in asymmetric constriction (more along x than y) that begins at the anterior–posterior (AP) ends of the amnioserosa and proceeds toward the middle. As constriction progresses, the epithelial curvature of the amnioserosa increases until opposing flanks of the surrounding cells (lateral epidermis) come into contact. Once these new contacts mature into stable adherens junctions and seal the epithelium, the amnioserosa cells begin programmed cell death (Abrams *et al* 1993). In other examples of apical constriction, the cells that wedge and invaginate either lose attachments in an epithelial-to-mesenchymal transition (gastrulation: Keller *et al* (2003)) or round up into a tube (neurulation: Colas and Schoenwolf (2001)).

The cell movements of apical constriction have been reproduced in several computational models. Early models explained the cell movements with plausible, but highly phenomenological rules. For example, Jacobson and Gordon (1976) modeled neurulation with spatially and temporally defined cellular 'shrinkage programs'. Each cell adopted one of the nine programs based on its initial location and then executed its programmed sequence of apical shrinkage steps. Coordinating all of these cellular programs required that each cell has its own synchronized cellular clock. Odell *et al* (1981) then proposed a much simpler set of rules by including the mechanical interactions between cells. They modeled cells largely as passive viscoelastic materials, but they added an active component in the form of a stretch-induced apical contraction. Thus, their model had just one phenomenological rule—if a cell's apical surface is stretched more than some critical amount, then the apical surface fires into a strong contraction. The action of one cell on its neighbors then leads to spreading waves of contraction. With slight variations in parameters, their model was able to mimic the cell movements of sea urchin invagination, neurulation and many other apical-constriction-type events. Regardless of complexity, both models posed the same quandary to biologists—given a set of plausible phenomenological rules, find the underlying molecular basis. Unfortunately, the sets of plausible rules are not unique. Worse yet, there is no immediately clear way to connect a mutation or genetic construct to a particular rule. The models have to go further.

The required direction is highlighted by two modeling efforts that evaluated the mechanical consequences of multiple hypothesized mechanisms. Davidson *et al* (1995) used a finite element model to evaluate five different hypotheses for the driving force(s) of sea urchin invagination. They found that all the five mechanisms were plausible, but each required the sea urchin's tissue layers to take on a specific and limited range of mechanical properties. Furthermore, each mechanism led to different spatial patterns of cell shape change. Similar mechanism-dependent patterns of shape change were noted by Clausi and Brodland (1993) in their evaluation of neurulation mechanisms. Thus, the models now provide at least two ways to test the proposed mechanisms: (1) measuring the actual pattern of cell shape changes, which is readily doable now with live-cell confocal imaging and (2) measuring the passive mechanical properties of the different tissue layers (Davidson *et al* 1999). Although such experiments could certainly winnow down the set of plausible mechanisms, they still do not provide a way to easily connect mutant to mechanism. Clausi and Brodland (1993) point the way here by using their model to evaluate the consequences of losing specific force-generating modules—a simulated version of a 'knock-out' experiment. The loss or modification of a mechanical property or phenomenological rule is thus translated into one or more observables, e.g. differences in the patterns of tissue stress or cell shape change. There is simply no way to connect these experimental observables to specific mechanisms without quantitative models.

From the molecular biology side, there is no shortage of genes known to be involved in apical constriction. *The Interactive Fly* (Brody 2008), an online compendium of *Drosophila* (fruit fly) developmental genetics, lists 9 different genes that affect gastrulation, and another 35 (plus one repeat) that impact cell movements in dorsal closure. Additional genes surely play a role (e.g. actin, α- and β-tubulin), but are not identified in mutant screens because they are lethal at very early points in development. The products of these genes can be grouped into four categories:

(1) components of the cytoskeleton, including cell adhesion proteins and myosin II,
(2) members of the Jun N(amino)-terminal kinase (JNK) signaling pathway,
(3) members of the decapentaplegic (Dpp) signaling pathway (Dpp is homologous to the transforming growth factor β or TGFβ family) and
(4) members of the small GTPase (e.g. Rho, Rac and Cdc42) signaling pathways.

The interplay between all of these gene products is the subject of several excellent reviews (Jacinto *et al* 2002, Harden 2002), and will suffice to note that this list of relevant genes points to highly regulated cytoskeletal remodeling. A similar list can be constructed for neurulation based on the identification of neural tube defects after homologous recombination or 'knock-out' experiments in mice (reviewed in Colas and Schoenwolf (2001)). Once again, many of the genes are cytoskeletal regulators.

For each gene in these lists, its role in apical constriction is almost always inferred from the biochemistry of homologous genes—not from differences in the patterns of stress or cell shape change predicted by computational models. The reason is fairly straightforward. The models that have made such predictions were based on the specific geometries of model organisms favored by morphologists, i.e. sea urchins and amphibians. The genetic screens were all conducted in organisms for which geneticists have a well-stocked toolbox, i.e. fruit flies (*Drosophila*), nematodes

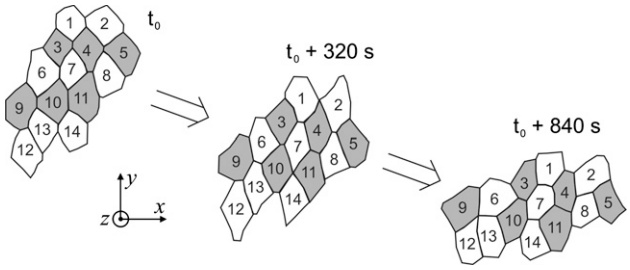

Figure 3. Convergent-extension during *Drosophila* germ band elongation. Line drawings were constructed from confocal images of an E-cadherin-GFP embryo. Just a small patch of germ band cells is shown and shading is added to highlight the patterns of cell neighbor exchanges. For example, cells 1 and 7 intercalate between cells 3 and 4. Overall, germ band cells converge and intercalate locally in the *y*-direction (dorsal–ventral) leading to an overall extension of the tissue in the *x*-direction (anterior–posterior).

(*Caenorhabditis elegans*) and mice. Although the genetic toolbox for amphibians and sea urchins is not empty, models that target *Drosophila* or *C. elegans* are likely to make more rapid progress toward a mechanics/genetics synthesis. Unfortunately, neither organism is a vertebrate, but perhaps zebrafish will fill that gap.

Despite all of these hurdles, there are a few places where the mechanics and genetics of apical constriction have intersected. The first is a cellular-automata based model of neurulation (Kerszberg and Changeux 1998) that explicitly incorporates soluble morphogen signaling (based on TGFβ family proteins BMP-2 and BMP-4), contact-mediated Notch/Delta signaling and strict genetic control of differential adhesion. As an automaton, each cell makes decisions as to its mechanical properties based on its signaling inputs. Unfortunately, the subsequent mechanical interactions are not handled explicitly as forces, but only as additional phenomenological rules. With a more explicit handling of the mechanics, such multi-level models could effectively translate molecular signaling defects into morphological defects. The second are laser-microsurgery experiments that match the observed, dynamic morphology of specific mutants with the morphology induced by targeted laser incisions (Hutson *et al* 2003). Such experiments allow one to assign mechanical consequences to the mutation, but only so far as the mechanical consequences of the incisions themselves are understood. Such experiments could be much more powerful if combined with the sorts of hypothesis-testing models discussed above.

3.2. Convergent-extension

Convergent-extension is the stereotypical rearrangement of cell contacts that leads to an extended body axis (reviewed in Keller *et al* (2000)). As shown in figure 3, individual cells intercalate between neighbors. This intercalation has a preferred direction, i.e. convergence, which leads to an overall extension of the cell sheet in a perpendicular direction. In some cases, intercalation-driven cell rearrangement is supplemented by the formation and resolution of multicellular rosette structures (Blankenship *et al* 2006). Examples of convergent-extension can be found in nearly all metazoans—in both epitheloid and mesenchymal tissues—with the most well-studied examples in fruit fly (Irvine and Wieschaus 1994) and frog *Xenopus laevis* (Elul *et al* 1997, Keller and Hardin 1987, Keller and Shih 1995).

Zajac *et al* (2000, 2003) proposed a model for convergent-extension based on anisotropic differential adhesion. This model uses the Extended Potts Model and incorporates two novel terms for each cell in the system Hamiltonian. The first is a term that defines a target moment-of-inertia for each cell. This term introduces a tendency for cell elongation. The second is a modification of the normal differential adhesion term so that it is anisotropic—the adhesion energy being more favorable when the point of cell–cell contact is farthest from a line parallel to the cell's long axis and passing through its center of mass. The next result of such anisotropic adhesion is to favor cell arrangements where the long cell borders are parallel to the cells' long axes. With these energy terms, simulations of initially random cells show cell elongation, alignment and intercalation leading to tissue extension. Moreover, they do so without any sort of pre-pattern.

Interestingly, genetic studies provide an independent, but indirect, evidence for anisotropic adhesion. Mutational analyses of germ band extension in *Drosophila* (Adler 2002, Strutt 2003) and of mesenchymal cell intercalation in *Xenopus* (Keller *et al* 2000) reveal important roles for genes of the planar cell polarity (PCP) pathway (reviewed in Mlodzik (2002) and Klein and Mlodzik (2005)). Cells in an epithelium always have an apical-basal polarity, but the PCP pathway can induce a second polarity (i.e. anisotropy) within the tissue plane. Furthermore, localization studies in *Drosophila* have shown that certain cell surface proteins are distributed anisotropically (Zallen and Wieschaus 2004, Blankenship *et al* 2006). The anterior–posterior borders of intercalating cells are enriched in non-muscle myosin II. The complementary dorsal–ventral borders are enriched for Bazooka/PAR-3, a scaffolding protein found in adherens junctions.

Although these localization studies confirm that the intercalating cells are anisotropic, they do not prove that this anisotropy extends to cellular adhesion. We again face the problem of needing difficult *in vivo* measurements. Furthermore, the observed anisotropy does not exactly match that suggested by the models. During germ band extension, the intercalating cells are not much elongated in any particular direction. The actual anisotropy appears to be genetically encoded through the *even-skipped* and *runt* pair-rule genes (Zallen and Wieschaus 2004). Although fly embryos appear to use pre-patterns for specifying this anisotropy, the models show that such pre-patterns are not strictly necessary. Perhaps this will turn out to be an example where genetics canalized a physical process (Waddington 1962, Newman and Bhat 2008). Nonetheless, the modeling and genetic studies of convergent-extension provide a fascinating example in which cell mechanics and molecular biology are converging.

4. Mechanical feedback on differentiation and gene expression

Thus far, we have focused on the forward pathway for synthesizing cellular mechanics and molecular genetics, i.e.

where the genetic program of development determines the local mechanical properties, which in turn determine the morphogenetic stresses that shape the embryo. Recent evidence points out that one also has to consider mechanical feedback on gene expression and even cell differentiation. Two laser-microsurgery experiments demonstrate that such feedback must exist. First, repeated incisions at one canthus of the amnioserosa during dorsal closure led to the acceleration of closure at the opposite canthus—a site more than 100 μm or ten-cell diameters away (Hutson *et al* 2003, Peralta *et al* 2007). This feedback effect is well documented, but the analysis stopped short of identifying the molecular details. Second, laser ablation of early fly embryos can modulate the subsequent expression of *twist*, a transcriptional regulator of dorsoventral polarization (Supatto *et al* 2005). This modulation occurs in cells that are more than 100 μm distant from the ablation site, but are nonetheless mechanically impacted via strain relaxation. Other non-laser perturbations have also shown that *twist* expression can be mechanically induced (Farge 2003, Brouzes and Farge 2004). Further strong evidence for mechanical feedback in development comes from cell culture studies that vary the mechanical properties of the growth substrate. The adhesive nature and stiffness of the substrate can modulate whether cells spread and divide, round up and die, or differentiate (reviewed in Ingber (2006)). Perhaps the most striking observation is that stem cells can be induced to differentiate into either bone or fat by controlling the substrate mechanical properties (McBeath *et al* 2004). Work on mechanical feedback is growing rapidly and will likely have a large impact on the eventual synthesis of mechanics and molecular biology in development.

5. Summary and outlook—reverse engineering morphogenesis

There is no better analogy for the way scientists are tackling developmental biology than reverse engineering. "An apt quote describes one of the many reasons companies engage in this practice: 'reverse engineering often is done because the documentation of a particular device has been lost (or was never written)' (Wikipedia 2008)". Scientists face the daunting task of writing the documentation for the development of living organisms. For the last 50 years or so, biologists have tackled this task by cataloging genes and identifying how they affect the development. Based on their biochemistry and how one gene impacts the effects of others, these genes have been organized into pathways. In the last decade, as biologists found more and more evidence for crosstalk, these pathways have become networks—giving rise to systems biology. All well and good, but perhaps the largest (and most overlooked) source of crosstalk in developmental biology is continuum mechanics. If the full documentation of development is ever to be written, scientists working from the gene-side and the mechanics-side must join forces.

How can we foster the collaboration that is needed? To find out, we posed a question to several of our developmental biologist colleagues: why has research into the physical aspects of development had limited impact on the developmental biology mainstream? Two common themes were present in nearly all their responses: (1) they have little doubt that the physical phenomena are important; but (2) very little in their training prepared them to think about these topics (and they have too much on their plate to learn what they need now). Those with a historical bent noted that biology used to have a much larger physical-biology side; however, the molecular biology revolution captured nearly an entire generation of biologists. In doing so, it broke the physical-biology training stream. This training stream can be repaired, but physicists, engineers and mathematicians will need to become deeply engaged in the training of biologists—not just to capture a few students for deeply interdisciplinary research, but also to cultivate a biology mainstream that is ready to accept what are currently foreign physical concepts.

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Glossary

Amnioserosa. A one-cell thick embryonic epithelium that covers most of the dorsal surface of fruit fly (*Drosophila*) embryos in the latter half of embryogenesis.

Apical constriction. A type of morphogenetic event in which a subset of cells in an epithelium contract their apical surfaces (the ones facing the medium or lumen) to adopt a wedge-shaped morphology which leads to a local bending or invagination of the tissue.

Canthus. Area at the anterior or posterior end of the amnioserosa where the two flanks of the lateral epidermis zip together.

Cell sorting. The ability of heterotypic mixtures of cells to gradually separate into distinct homotypic domains analogous to phase separation of immiscible fluids.

Convergent-extension. A type of morphogenetic event in which cells (epithelial or mesenchymal) move toward and intercalate between one another along one axis (convergence) which leads to an overall extension of the tissue along a perpendicular axis.

Differential adhesion hypothesis (DAH). Proposal of Steinberg (1970) that different cell types bind to one another with distinct homotypic and heterotypic cohesive/adhesive energies and that these energies determine the results of cell sorting and tissue engulfment experiments.

References

Abrams J M, White K, Fessler L I and Steller H 1993 Programmed cell death during *Drosophila* embryogenesis *Development* **117** 29–43

Adler P N 2002 Planar signaling and morphogenesis in *Drosophila* *Dev. Cell* **2** 525–35

Blankenship J T, Backovic S T, Sanny J S P, Weitz O and Zallen J A 2006 Multicellular rosette formation links planar cell polarity to tissue morphogenesis *Dev. Cell* **11** 459–70

Brodland G W and Chen H H 2000 The mechanics of heterotypic cell aggregates: insights from computer simulations *Trans. ASME, J. Biomech. Eng.* **122** 402–7

Brody T B 2008 The Interactive Fly. http://www.sdbonline.org/fly/aimain/8organ.htm

Bronner-Fraser M, Wolf J and Murray B 1992 Effects of antibodies against N-cadherin and NCAM on the cranial neural crest and neural tube *Dev. Biol.* **153** 291–301

Brouzes E and Farge E 2004 Interplay of mechanical deformation and patterned gene expression in developing embryos *Curr. Opin. Genet. Dev.* **14** 367–74

Clausi D A and Brodland G W 1993 Mechanical evaluation of theories of neurulation using computer simulations *Development* **118** 1013–23

Colas J F and Schoenwolf G C 2001 Towards a cellular and molecular understanding of neurulation *Dev. Dyn.* **221** 117–45

Davidson L A, Koehl M A R, Keller R and Oster G F 1995 How do sea-urchins invaginate—using biomechanics to distinguish between mechanisms of primary invagination *Development* **121** 2005–18

Davidson L A, Oster G F, Keller R E and Koehl M A R 1999 Measurements of mechanical properties of the blastula wall reveal which hypothesized mechanisms of primary invagination are physically plausible in the sea urchin *Strongylocentrotus purpuratus Dev. Biol.* **209** 221–38

Detrick R, Dickey D and Kintner C 1990 The effects of N-cadherin misexpression on morphogenesis in *Xenopus* embryos *Neuron* **4** 493–506

Elul T, Koehl M A R and Keller R 1997 Cellular mechanism underlying neural convergent extension in *Xenopus laevis* embryos *Dev. Biol.* **191** 243–58

Farge E 2003 Mechanical induction of twist in the *Drosophila* foregut/stomodeal primordium *Curr. Biol.* **13** 1365–77

Foe V E, Odell G M and Edgar B A 1993 Mitosis and morphogenesis in the *Drosophila* embryo *The Development of Drosophila melanogaster* ed M Bate and A Martinez-Arias (New York: Cold Spring Harbor Laboratory Press)

Forgacs G and Newman S A 2005 *Biological Physics of the Developing Embryo* (New York: Cambridge University Press)

Foty R A and Steinberg M S 2005 The differential adhesion hypothesis: a direct evaluation *Dev. Biol.* **278**

Friedlander D R, Mege R M, Cunningham B A and Edelman G M 1989 Cell sorting-out is modulated by both the specificity and amount of different cell adhesion molecules (CAMs) expressed on cell surfaces *Proc. Natl Acad. Sci. USA* **86** 7043–7

Fujimori T, Miyatani S and Takeichi M 1990 Ectopic expression of N-cadherin perturbs histogenesis in *Xenopus* embryos *Development* **110** 97–104

Glazier J A and Graner F 1993 Simulation of the differential adhesion driven rearrangement of biological cells *Phys. Rev.* E **47** 2128–54

Goutsias J and Lee N H 2007 Computational and experimental approaches for modeling gene regulatory networks *Curr. Pharm. Des.* **13** 1415–36

Gumbiner B M 2005 Regulation of cadherin-mediated adhesion in morphogenesis *Nature Rev. Mol. Cell Biol.* **6** 622–34

Harden N 2002 Signaling pathways directing the movement and fusion of epithelial sheets: lessons from dorsal closure in Drosophila *Differentiation* **70** 181–203

His W 1874 *Unsere Körperform und das physiologische Problem ihrer Enstehung, Briefe an einen befreundeten Naturforscher* (Leipzig: F.C.W. Vogel)

Holtfreter J 1939 Gewebeaffinität: Ein Mitel der embryonalen Formbildung *Arch. Exp. Zellforsch. Besonders Gewebezücht* **23** 169–209 Revised and reprinted in English 1964 in *Foundations of Experimental Embryology* ed B H Willier J M Oppenheimer (Englewood Cliffs, NJ: Prentice-Hall)

Holtfreter J 1944 Experimental studies on the development of the pronephros *Rev. Can. Biol.* **3** 220–50

Hutson M S, Tokutake Y, Chang M S, Bloor J W, Venakides S, Kiehart D P and Edwards G S 2003 Forces for morphogenesis investigated with laser microsurgery and quantitative modeling *Science* **300** 145–9

Ingber D E 2006 Mechanical control of tissue morphogenesis during embryological development *Int. J. Dev. Biol.* **50** 255–66

Irvine K D and Wieschaus E 1994 Cell intercalation during *Drosophila* germband extension and its regulation by pair-rule segmentation genes *Development* **120** 827–41

Jacinto A, Woolner S and Martin P 2002 Dynamic analysis of dorsal closure in *Drosophila*: from genetics to cell biology *Dev. Cell* **3** 9–19

Jacobson A G and Gordon R 1976 Changes in shape of developing vertebrate nervous-system analyzed experimentally, mathematically and by computer simulation *J. Exp. Zool.* **197** 191–246

Keller R, Davidson L, Edlund A, Elul T, Ezin M, Shook D and Skoglund P 2000 Mechanisms of convergence and extension by cell intercalation *Phil. Trans. R. Soc.* B **355** 897–922

Keller R, Davidson L A and Shook D R 2003 How we are shaped: the biomechanics of gastrulation *Differentiation* **71** 171–205

Keller R and Hardin J 1987 Cell behaviour during active cell rearrangement: evidence and speculations *J. Cell Sci.* Suppl. **8** 369–93

Keller R and Shih J 1995 Cell and tissue behavior during convergence and extension of the embryonic axial mesoderm in the frog *Xenopus laevis Interplay of Genetic and Physical Processes in the Development of Biological Form* ed D Beysens, G Forgacs and F Gaill (New York: World Scientific)

Kerszberg M and Changeux J P 1998 A simple molecular model of neurulation *Bioessays* **20** 758–70

Kiehart D P, Galbraith C G, Edwards K A, Rickoll W L and Montague R A 2000 Multiple forces contribute to cell sheet morphogenesis for dorsal closure in Drosophila *J. Cell Biol.* **149** 471–90

Klein T J and Mlodzik M 2005 Planar cell polarization: an emerging model points in the right direction *Ann. Rev. Cell Dev. Biol.* **21** 155–76

Matsunaga M, Hatta K and Takeichi M 1988 Role of N-cadherin cell-adhesion molecules in the histogenesis of neural retina *Neuron* **1** 289–95

McBeath R, Pirone D M, Nelson C M, Bhadriraju K and Chen C S 2004 Cell shape, cytoskeletal tension and RhoA regulate stem cell lineage commitment *Dev. Cell* **6** 483–95

Meinhardt H 1982 *Models of Biological Pattern Formation* (New York: Academic)

Meinhardt H 2008 Models of biological pattern formation: from elementary steps to the organization of embryonic axes *Curr. Top. Dev. Biol.* **81** 1–63

Mlodzik M 2002 Planar cell polarization: do the same mechanisms regulate *Drosophila* tissue polarity and vertebrate gastrulation? *Trends Genet.* **18** 564–71

Newman S A and Bhat R 2008 Dynamical patterning modules: a 'pattern language' for development and evolution of multicellular form *Int. J. Dev. Biol.* at press

Odell G M, Oster G, Alberch P and Burnside B 1981 The mechanical basis of morphogenesis: I. Epithelial folding and invagination *Dev. Biol.* **85** 446–62

Peralta X G, Toyama Y, Tokutake Y, Hutson M S, Venakides S, Kiehart D P and Edwards G S 2007 Upregulation of forces and morphogenic asymmetries in dorsal closure during *Drosophila* development *Biophys. J.* **92** 2583–96

Roux W 1888 Beiträge zur entwickelungsmechanik des embryo. Über die künstliche hervorbringung halber embryonen durch zerstörung einer der beiden ersten furchungskugeln, sowie über die nachentwickelung der fehlenden köperhälfte *Virchows Arch. Pathol. Anat. Physiol. Klin. Med.* **114** 113–53 and 289–91

Steinberg M S 1970 Does differential adhesion govern self-assembly processes in histogenesis? Equilibrium configurations and the emergence of a hierarchy among populations of embryonic cells *J. Exp. Zool.* **173** 395–434

Steinberg M S 2003 Cell adhesive interactions and tissue self-organization *Origination of Organismal Form: Beyond the Gene in Developmental and Evolutionary Biology* ed G B Müller and S A Newman (Cambridge, MA: MIT Press)

Steinberg M S and McNutt P M 1999 Cadherins and their connections: adhesion junctions have broader functions *Curr. Opin. Cell Biol.* **11** 554–60

Steinberg M S and Takeichi M 1994 Experimental specification of cell sorting, tissue spreading, and specific spatial patterning by quantitative differences in cadherin expression *Proc. Natl Acad. Sci. USA* **91** 206–9

Strutt D I 2003 Frizzled signalling and cell polarisation in *Drosophila* and vertebrates *Development* **130** 4501–13

Supatto W, Debarre D, Moulia B, Brouzes E, Martin J L, Farge E and Beaurepaire E 2005 *In vivo* modulation of morphogenetic movements in *Drosophila* embryos with femtosecond laser pulses *Proc. Natl Acad. Sci. USA* **102** 1047–52

Takeichi M 1988 The cadherins: cell–cell adhesion molecules controlling animal morphogenesis *Development* **102** 639–55

Thompson D A 1917 *On Growth and Form* (Cambridge: Cambridge University Press)

Thompson D A 1942 *On Growth and Form* (Cambridge: Cambridge University Press)

Townes P L and Holtfreter J 1955 Directed movements and selective adhesion of embryonic amphibian cells *J. Exp. Zool.* **128** 53–120

Waddington C H 1962 *New Patterns in Genetics and Development* (New York: Columbia University Press)

Wieschaus E 1996 From molecular patterns to morphogenesis—the lessons from studies on the fruit fly *Drosophila* (Nobel lecture) *Angew. Chem. Int. Edn Engl.* **35** 2189–94

Wikipedia 2008 Reverse Engineering. http://en.wikipedia.org/wiki/Reverse_engineering

Zajac M, Jones G L and Glazier J A 2000 Model of convergent extension in animal morphogenesis *Phys. Rev. Lett.* **85** 2022–5

Zajac M, Jones G L and Glazier J A 2003 Simulating convergent extension by way of anisotropic differential adhesion *J. Theor. Biol.* **222** 247–59

Zallen J A and Wieschaus E 2004 Patterned gene expression directs bipolar planar polarity in Drosophila *Dev. Cell* **6** 343–55