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Biomechanical approaches to morphogenesis

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In order for morphological changes to occur in developing embryos, mechanical forces must be exerted. Therefore, if we are to unravel the mechanisms responsible for morphogenesis, it is crucial that we complement molecular studies of the control of morphogenesis with analyses of the physics of HOW morphogenetic processes occur. In this paper I outline the approaches used in biomechanical research and use the example of neurulation to illustrate how they are being employed to study morphogenesis. I stress the importance of formulating theories quantitatively, and of measuring the mechanical properties of embryonic tissues and the forces exerted during morphogenetic events.

Key words: biomechanics / forces / morphogenesis / neurulation / viscoelasticity

DURING morphogenesis embryonic tissues change shape and move with respect to each other. Forces must be exerted for these physical events to occur, and the mechanical properties of various components of the embryo determine how they deform when subjected to such loads. Therefore, as we try to unravel the mechanisms responsible for the genesis of form in developing embryos, it is crucial that we complement the popular molecular and biochemical approaches to the control of morphogenesis with nuts-and-bolts analyses of the physics of HOW morphogenetic processes occur.

Research on the mechanics of morphogenesis in animal embryos has recently been reviewed.[1] Although many theories have been proposed about the physical mechanisms driving morphogenetic processes in animals, most are in the form of plausibility arguments and only a handful have been expressed quantitatively. In spite of the many clever experiments that have been performed to isolate the roles played by different components of animal embryos in morphogenetic events, only a few studies have measured mechanical properties of developing embryos or forces generated by them.[2-8] One notable exception to this is the body of empirical work on the mechanics of cleaving eggs (recently reviewed in ref 9), and another is the research on mechanics of embryonic cells in culture (recently reviewed in ref 10). Quantitative approaches seem to have been more extensively applied in the study of plant morphogenesis (see reviews in refs 11-13) than in animal developmental biology.

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Biomechanics

Biomechanics is the application of basic principles of solid and fluid mechanics to study physical functions of organisms. Although many biomechanicians focus on humans and are involved in designing such things as crash helmets, running shoes, and artificial heart valves, some of us use engineering techniques to investigate the basic mechanical functioning of a wide range of creatures. Several books[14-20] and symposia[21,22] review various aspects of this branch of biomechanics, and a few recent symposia[23-25] cover the advances that have been made in biomechanics at the cellular level.

The purpose of the present paper is to outline the approaches used in biomechanical research and to illustrate how they are being applied to study morphogenesis. I will focus on the example of neurulation in vertebrate embryos. My intent is *not* to review the literature on neurulation, nor to evaluate the evidence relating to different theories about the possible mechanisms of driving neurulation (these issues are reviewed in refs 26, 27). Rather, my purpose is to provide examples of the types of information that biomechanical tools can contribute towards understanding morphogenetic mechanisms. In so doing, I want to stress the importance of *quantitative* theoretical and empirical studies, and to make a plea for the use of standard engineering measures of mechanical properties.

The biomechanical analysis of a process involves a variety of approaches: (1) qualitative description of

the phenomenon and qualitative statement of theories about the physical mechanisms underlying it; (2) qualitative experiments where components of the system hypothesized to be involved in the process are removed or altered, and the consequences to the process are observed; (3) *quantitative* description of the process, including (a) morphometric analysis of the structures involved and (b) kinematic analysis of their motions, as well as (c) measurement of the forces exerted during the process and (d) of the mechanical properties of the tissues subjected to the forces; (4) *quantitative* statement of theories of the mechanisms responsible for the phenomenon (i.e. mathematical models); and (5) empirical tests (both qualitative and quantitative) of the predictions of the models. Although I have presented these approaches as a list, I do not mean to imply that this list is a series of steps to be followed in order. Rather, there should be ongoing interplay between theory and empiricism, and between qualitative and quantitative approaches.

Biomechanics of neurulation

During the process of neurulation in vertebrate embryos, the neural plate (precursor of the central nervous system) thickens, elongates, narrows, and rolls into a tube (Figure 1). The details of these morphogenetic events, described in many textbooks and review articles (such as refs 1, 27-29) vary from species to species.

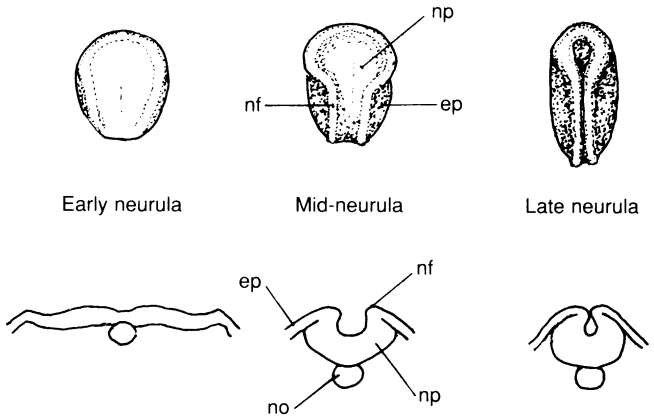

Figure 1. Diagrams of the process of neurulation in an amphibian embryo (based on descriptions and micrographs in 28, 30, 35). The top row shows a dorsal view of the embryo with its anterior end towards the top of the page (nf = neural fold). The bottom row shows transverse sections (at the region of the embryo where the spinal cord joins the brain) of the neural plate (np), epidermis (ep), and underlying notochord (no).

Qualitative theories and empirical evidence

Many theories of the mechanisms responsible for neurulation have been proposed, and the evidence consistent with or refuting each has been reviewed (for example, see refs 1, 26, 27, 29). The proposed mechanisms, which are not mutually exclusive, include: (1) cell elongation at right angles to the surface of the neural plate by increased adhesion or active extension; (2) shape changes (wedging) of neuroepithelial cells by apical constriction and/or basal expansion, or by basal migration of the nucleus (see chapter by Schoenwolf, this issue); (3) pushing on the neural plate by the adjacent lateral tissues; (4) cell rearrangement due to gradients in intercellular adhesion or by 'cortical tractoring' (*sensu* Jacobson *et al*[30]); and (5) buckling of the neural plate by elongation of the underlying notochord.

Kinematic and morphometric analyses

An important step in the biomechanical analysis of a process is the *quantitative* description of the structures involved and the motions and deformations they undergo. Although such morphometric and kinematic analyses do not address the forces driving the process, they provide crucial information that may (1) allow some hypotheses about mechanisms to be ruled out; (2) reveal that a proposed mechanism is not solely responsible for producing a morphogenetic change; (3) provide realistic values for parameters in quantitative models of proposed morphogenetic mechanisms; and (4) permit such models to be tested (i.e. provide numbers for rates of movements and magnitudes of changes in dimensions that can be compared with values calculated by the models).

Kinematic analyses are descriptions of the directions and rates of motions involved in a process. A number of studies have mapped the trajectories of individual cells during the process of neurulation (for example, see refs 30-33). Measurements on time-lapse movies have been used to calculate the speeds (up to 95 $\mu m\ h^{-1}$) of cell displacements as the neural plate changes shape.[32] Other studies that measured the *rates* of change of dimensions of the neural plate showed, for example, that the rate of mouse neural plate lengthening was exponential,[34] and that the width changes of the amphibian neural plate were not steady, but rather occurred in pulses every 18-24 min.[35] Some kinematic studies have correlated rates

of change of certain parts of the neural plate with particular morphogenetic events. For example, Jacobson found in both amphibians[35] and birds[28,36] that the rate of elongation of the midline of the neural plate is about ten times faster while the neural plate is rolling into a tube than it is before or after tube closure. He also noted that the rate of neural plate narrowing correlated with its rate of elongation.[35]

Morphometric analyses (i.e. quantitative descriptions of structures) can provide insights about morphogenetic events not available from qualitative descriptions. For example, measurements of the dimensions of the neural plate[35,37] and its cells[38] have revealed that volume does not increase during newt neurulation, thereby ruling out growth or overall swelling as a factor driving the morphogenetic changes. In contrast, morphometric analysis of chick neural plate revealed a 2.8-fold increase in volume during neurulation,[39] and measurements of blocks of quail cells grafted into chick neural plate demonstrated that growth could only account for half of the increase in neural plate length.[40]

The sizes and shapes of individual cells in developing neural plates have been measured. Some studies examine cell morphology using the frame of reference of the whole neural plate. For example, counts of wedge-shaped versus spindle-shaped cells in the chick neural plate revealed that the proportion of cells that are wedge-shaped at 'hinge points' (places where the neural plate bends) is greater than at positions between such hinges.[41] Measurements of cell dimensions at defined positions in the neural plate at various stages during neurulation[40-44] have shown that these cells become taller (i.e. lengthen at right angles to the plane of the neural plate such that the plate thickens) during neurulation in amphibians, birds, and mammals. More detailed morphometric examination in chick showed that the increases in cell height are greater in the region of the neural plate above the notochord than elsewhere.[27] Data on cell dimensions at defined positions in the neural plate has also been used to reveal that cell elongation alone can *not* account for all the narrowing of the neural plate (for example, see refs 37, 40). However, such data does not reveal the shape *changes* undergone by individual cells during neurulation, since cells translate with respect to fixed positions in the neural plate. A few morphometric studies have overcome this problem by using the frame of reference of individual cells rather than that of the whole neural plate.[37,45] For example, by following cell trajectories to determine where sections for morphometric analysis should be taken at various stages of neurulation, Burnside[45] determined that neural plate cells begin elongation and narrowing before becoming wedge-shaped.

A number of techniques can be used to quantitatively describe the complicated shape and size changes that occur during morphogenesis. Morphological changes during newt neurulation have been depicted by the distortion through time of a grid of points, each of which represents the position of a particular cell.[32,37,46] Equations from fluid dynamics and continuum mechanics that are used to quantitatively describe the kinematics of plant development (reviewed in ref 12) might be applied to animal systems as well. Furthermore, a number of other techniques employed by evolutionary biologists to quantify shape and shape change might prove useful to developmental biologists (for example, see refs 47-50).

Although morphometric and kinematic studies provide information essential for the biomechanical analysis of morphogenetic events, they do not address the forces driving the morphological changes they describe.

Measurement of forces and mechanical properties in embryos

In order for morphological changes to occur in an embryo, forces must be exerted by some components of the animal; the deformations of the embryo that these forces produce depend on (1) the distribution of stresses these forces impose in the embryo, and (2) the mechanical properties of the cells and extracellular materials subjected to the stresses. Forces and mechanical properties of embryos can be studied empirically using techniques analogous to those used by engineers to analyze man-made structures and by biomechanicians to study macroscopic organisms.

Some background terminology

The literature on morphogenesis is over-flowing with what I will euphemistically call 'creative mechanics'. I believe that many of the problems arise when terms are used that have a variety of meanings in normal English parlance, but specific meanings in engineering. For example, to an engineer a 'force' is a vector equal to a mass times an acceleration; in contrast, developmental biologists use the term 'force' more

broadly to refer to structures and processes responsible for morphogenetic events. Another problem arises from the technical difficulty of measuring the forces in tiny embryos or cells: biologists come up with a variety of clever ways to assay mechanical behavior and then label their measures with terms like 'strength' (for example ref 3) that have specific but different meanings in engineering. Furthermore, the mechanical behaviors measured by these various assays are not comparable to each other. If the standard ways of defining and measuring mechanical properties used by engineers were also employed by biologists, the parameters measured in different studies could be more easily compared, and these empirical measures would be in a form that could be used in mathematical models (described below). Because of this confusion in the literature, I feel it is important to define a few terms before I discuss measurements of mechanical behavior in embryos.

A 'cookbook' for biologists of how to measure some basic mechanical properties of biological materials is presented by Koehl and Wainwright.[51] More detailed descriptions of these procedures can be found in texts on polymer mechanics (such as refs 52-56), and reviews of their application to biological systems can be found in biomechanics books (such as refs 14, 15, 17, 20, 21, 57).

When a *force* (= *load*) is applied to a structure, the response of that structure depends on its size and shape, and on the mechanical properties of the materials from which it is built. A *stress* is a force per cross-sectional area of material bearing that force. The type and magnitude of the stresses in a structure depend on where the load is applied and on the geometry of the structure (as described in engineering texts such as refs 58-60). (One of the steps in a number of the mathematical models that will be described below is to calculate the stress distributions in embryos when loads are applied by various mechanisms.) Materials can be subjected to *tensile stress* (tending to pull the molecules within the material further apart), *compressive stress* (tending to push the molecules of the material closer together), or *shear stress* (tending to slide the molecules of the material past each other) (Figure 2A). The stretch, shrinkage, or shear deformation of the material in response to a stress is expressed in non-dimensional form as *strain* (Figure 2B). For ease of discussion, I will refer to uniaxial tensile deformations in the paragraphs that follow, although similar descriptions could be presented for compression or shear.

The behavior of a spring when subjected to a force is a good analogy for the mechanical behavior of an elastic solid (Figure 3A). The force (expressed as stress, σ = force/cross-sectional area) with which an elastic solid pulls back at you when you stretch it is proportional to how far you have extended it (expressed as strain, ε) (Figure 2B):

$$\sigma = \varepsilon E$$

where E is the *elastic modulus*, or *stiffness* of the material. When you release such a material, it immediately springs back to its unstretched shape (i.e. it is *resilient*). (Techniques for quantifying resilience are discussed, for example, in ref 51). The slope of a graph of stress measured in a material as a function of strain applied is the elastic modulus (E) (Figure 2C). Many biological materials do not show the linear ('Hookean') stress-strain behavior just described, but rather have strain-dependent E's (i.e. the amount of strain already in a tissue determines its stiffness when subjected to further loading) (Figure 2C).

Many biological materials do not behave as simple elastic solids, but rather show some fluid-like properties as well. The behavior of a dashpot (a piston moving inside a cylinder, with a fluid between the cylinder and the piston, Figure 3B) is a good analogy for viscous mechanical behavior. When you pull on a dashpot, the force with which it resists your pull does *not* depend on how far you stretch it, but rather on the rate at which you stretch it:

$$\sigma = \mu \frac{d\varepsilon}{dt}$$

where μ is the viscosity (resistance to deformation) of the fluid. When you quit pulling on a dashpot, it remains extended (i.e. it has undergone *plastic*, or permanent, deformation).

Materials that have mechanical properties of both fluids and solids are termed *viscoelastic* materials. The mechanical responses of such materials, which depend on the rate, amount, and history of load application, are modeled mathematically as collections of springs and dashpots in series and/or parallel (for details, see ref 53). If a piece of material is deformed sinusoidally, and the time course of its deformation and force are measured, estimates of its time-dependent modulus and viscosity can be calculated (for example, as described in refs 61, 62).

Various biological materials show viscoelastic or other types of *time-dependent mechanical properties*. Although the time-dependent properties of embry-

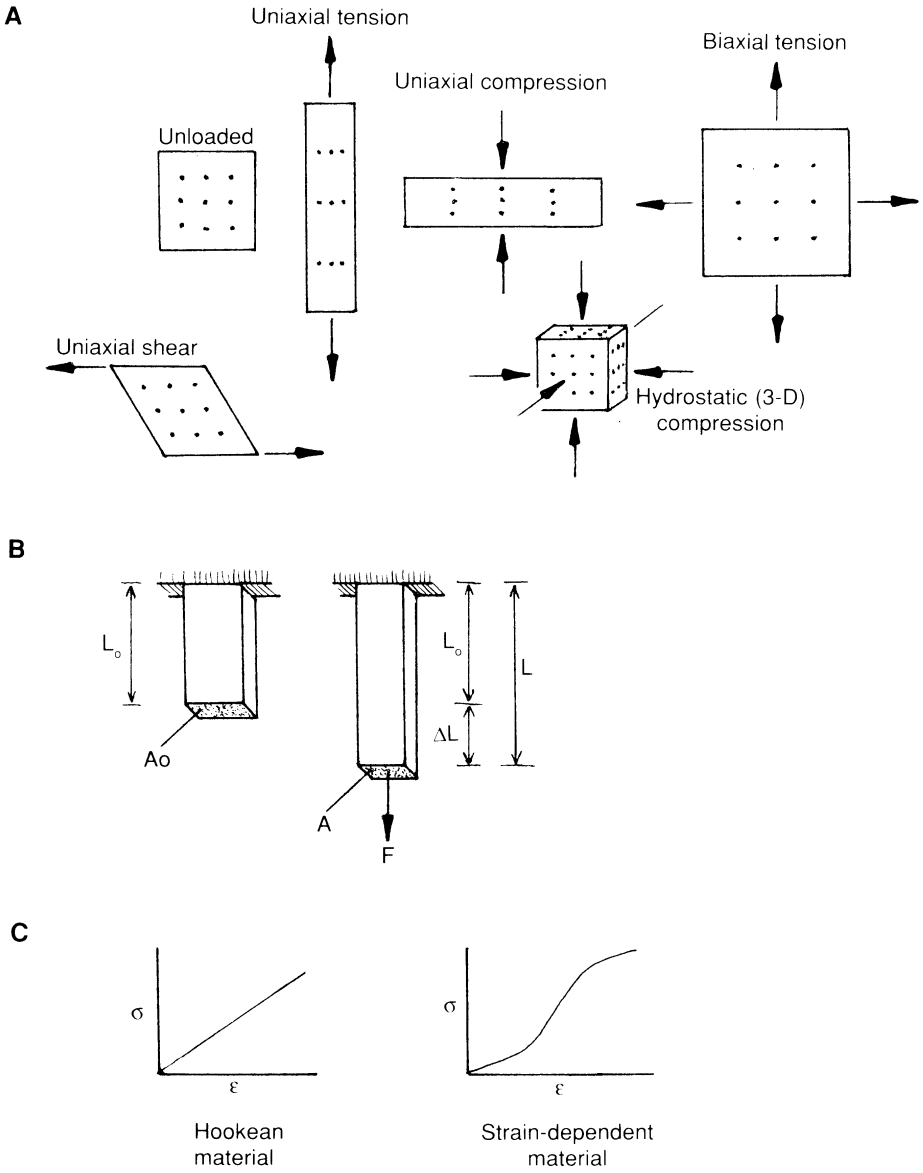

Figure 2. A. Diagrams of examples of the ways in which a block of material can be deformed. The material can be stretched (uniaxial tension), squashed (uniaxial compression), or sheared (uniaxial shear) along just one axis (with the other two axes free to lengthen or shorten). The material can also be deformed along more than one axis, such as being stretched along two axes simultaneously (biaxial tension) with the third axis free to shorten, or such as being squashed along all three axes simultaneously (hydrostatic compression) such that the volume of the specimen is decreased. B. If a strip of material of length L_o and cross-sectional area A_o is pulled by a force (F), the material will stretch by amount ΔL to a new length L. If the material maintains constant volume, its area will shrink to area A. If the material undergoes small deformations, stress (σ) is given by F/A_o and strain (ε) is given by $\Delta L/L_o$. However, if the material undergoes large deformations, stress may be expressed as 'true stress' ($\sigma_T = F/A$), and strain may be defined in terms of the length of the specimen at a given instant ('true strain', $\varepsilon_T = \int_{L_o}^{L} dL/L = \ln L/L_o$). Unfortunately, stress and strain are defined both ways in the biomechanics literature, and one must take care to note which definition is used before comparing results of different studies. C. The stiffer a material, the greater the force with which it resists a given deformation. The slope of a stress (σ) versus strain (ε) curve is the elastic modulus (E) of the material. If the $\sigma - \varepsilon$ curve for a particular material is linear (as in the graph on the left), the material is said to be 'Hookean' and E is called the 'Young's modulus'. Many biological materials are not Hookean — their stiffness (E) depends on how far they have been strained. One example of such non-linear strain-dependent behavior is illustrated in the graph on the right. The slope of the tangent to the curve at a given strain is the E at that strain.

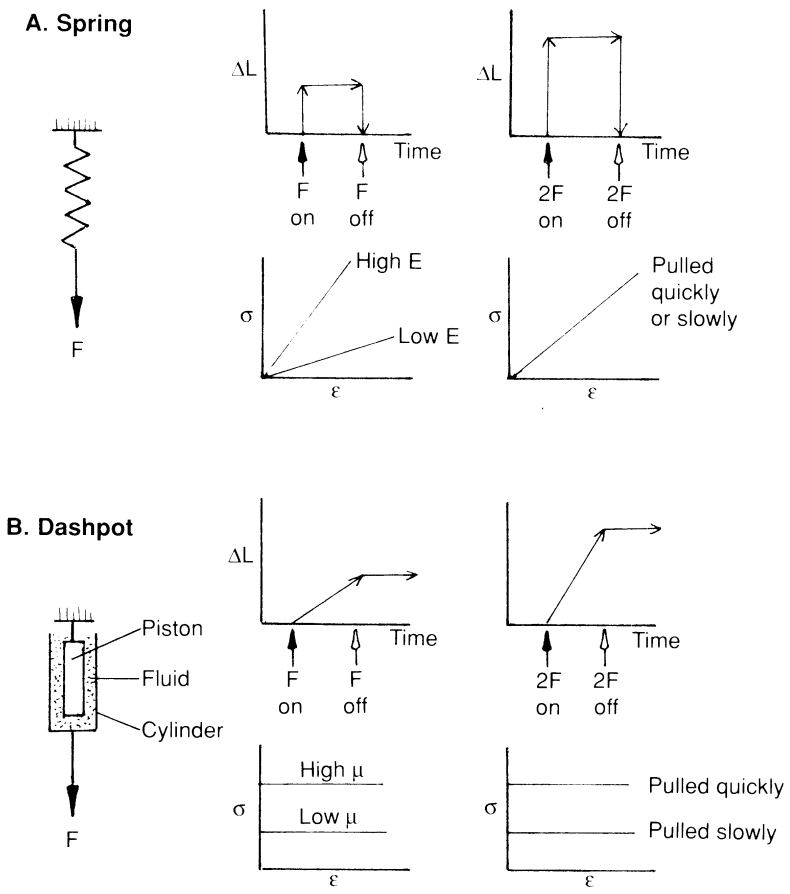

Figure 3. A. The mechanical behavior of a 'Hookean' (see Figure 2) elastic solid can be modeled by a spring. When a force (F) is applied to a spring, it instantaneously deforms by amount ΔL (see Figure 2B), and when the force is removed it instantaneously snaps back to its original length; the amount of deformation is proportional to the force applied (top two graphs). The slope of a graph of stress (σ) versus strain (ε) is the elastic modulus (E) of the spring; the stiffer the spring, the higher its E (lower left graph). The E of a spring is independent of the rate at which it is pulled (lower right graph). B. Linear ('Newtonian') fluid-like behavior can be modeled by a dashpot (a piston in a cylinder filled with a viscous fluid). When a force (F) is applied to the dashpot, it deforms at a rate (slope of the ΔL versus time graph) that is proportional to the magnitude of the force applied; when the force is removed, the dashpot remains extended (top two graphs). The higher the viscosity (μ) of the fluid in the dashpot, the greater its resistance to deformation, but this resistance is independent of how far the dashpot has been stretched (lower left graph). For a fluid of a given viscosity, the resistance to deformation depends on the strain *rate* (lower right graph).

onic tissues have not yet been characterized, we should be aware of some of the weird time-dependent properties shown by other biomaterials.

(1) *Viscoelastic strain-rate dependence.* Some materials (such as cnidarian mesoglea[63] and 'Silly Putty') are stiffer (have a higher E) if pulled quickly than if pulled slowly.

(2) *Viscoelastic creep and stress-relaxation.* Some materials (such as mesoglea[63]) creep (i.e. continue to extend when bearing a continuous stress) and show stress-relaxation (i.e. pull back with lower and lower force when held at a constant strain). Another characteristic of these materials is that they return slowly (partially or completely) towards resting shape when a load is removed rather than snapping back instantaneously. Techniques for measuring *time-dependent*

compliance (stretchiness) using creep tests, and *time-dependent modulus* (stiffness) from stress-relaxation tests, are described, for example, in ref 51.

(3) *Shear-thinning.* Some materials (such as slug mucus[64] and 'non-drip' paint) behave like an elastic solid until they are deformed beyond some critical strain, after which they flow like a viscous fluid.

(4) *Stress-softening.* Some materials (such as locust ovipositor cuticle[65] and spicule-reinforced connective tissues[66]) are less stiff the second time they are subjected to a given stress than they are the first time. When such materials are loaded in a pulsatile fashion, they can be extended much further than if loaded all at once.

The take-home message of this list of time-dependent behaviors is that biologists must be careful to assess mechanical properties on the time scale relevant to the morphogenetic process they are analyzing.

Qualitative assessment of stresses in neurulating embryos

Simple experiments of cutting an embryo can teach us a great deal about which components of the animal are being pushed or pulled. If a linear slit gapes open, the tissue that was slit had been under tension at right angles to the slit. If the slit does not gape, the tissue might be under compression, under no load, or under tension parallel to the slit. If a cut at right angles to the first one gapes, then the latter possibility is true. If tissue below the slit bulges or bursts out through the slit (for example, see ref 67), this suggests that the tissue below had been under compression. Examples of this sort of analysis applied to the process of neurulation[37,68,69] indicate that both the neural plate and the ectoderm immediately lateral to the neural folds are under tension in the embryo. Some investigators,[27] however, dismiss such results, claiming that they may represent active cell behaviors in response to wounding rather than passive mechanical events.

Quantitative mechanical measurements in embryos

Measurement of the forces generated by embryos and of the mechanical properties of their components is challenging for a number of reasons. One difficulty is the small size, and another is the complicated and changing geometry of the structures to be manipulated. A third problem to be overcome is the difficulty of separating effects due to passive time-dependent mechanical properties of embryonic tissues from those due to active behaviors of cells or to ontogenetic changes in their properties.

A number of clever techniques have been developed to measure very small forces exerted or resisted by single cells. These techniques (reviewed in refs 8, 70-73) range from letting cells pull on plastic membranes to deforming cells with glass needles, electronic strain gages, suction micropipettes, centrifuges, or tiny electromagnets. Unfortunately, a number of these methods do not yield standard engineering measures of mechanical properties, and hence cannot be readily compared with each other or used in mathematical models. Some of the theoretical treatments for converting various force measurements made on cells into basic mechanical parameters and the assumptions underlying these calculations are outlined in refs 70, 73.

The mechanical data available about neurulating embryos was gathered many years ago. Waddington[3] assayed breakability of various amphibian embryonic tissues and found a gradual 'strengthening' from the gastrula through neurula to tail-bud stages, especially of the ectoderm. Of particular interest to considerations of neurulation was his discovery that the lower convex surface of the neural groove was more easily broken than the upper concave surface. Unfortunately it is difficult to relate his assay to standard engineering measures of stiffness, strength, or toughness.

Selman[5,6] measured the forces exerted by the neural folds of newt and axolotl embryos as their neural plates rolled up (Figure 4). Although he could not convert measured forces to stresses or elastic moduli because he could not define which tissues were involved,[6] he did make some intriguing observations.

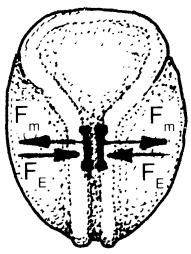

Figure 4. Diagram (based on information presented in refs 5, 6) of the dorsal surface of an amphibian embryo in the mid-neurula stage. Selman[5,6] measured force by placing a pair of electromagnetically-induced magnets (shown in black) against the neural folds and adjusting their force of mutual repulsion (F_M) until motion of the neural folds ceased, thereby balancing the closure force (F_E) exerted by the embryo.

(1) The force exerted by the neural folds increased with time, maximizing at 4.5×10^{-7} N for newts and at 11.0×10^{-7} N for the larger axolotls.

(2) Closure movements of the neural tube were made intermittently in steps of about 13 μm; if these motions were prevented by the magnets (Figure 4), forces were measured to occur intermittently. (As mentioned above, Jacobson[35] also measured pulsatile narrowing of the neural plate. Such pulsatile loading suggests that we should look for stress-softening behavior in the tissues being deformed during neural tube closure.)

(3) When force against the neural folds was held constant, the folds moved apart between the pulses of force exertion. Does this reflect passive creep behavior, or a gradual reduction of the active force generation by the embryo?

(4) The embryo showed two behaviors similar to those of an elastic solid: (a) there was a linear relationship between force applied to the folds by the magnets and the distance the folds were pulled apart, and (b) when the force on the folds was removed, the folds returned to their former unloaded position in a few seconds.

Although the literature on the mechanics of neurulation has been rich with ideas, the *quantitative empirical* assessment of forces and mechanical properties necessary to test these ideas have not yet been made. As we look to future empirical work on the mechanics of neurulation, I would like to reiterate several points discussed in earlier reviews. (1) The best test of any theory about the mechanics of a morphogenetic process is *in situ* measurement of stresses and deformations.[74] (2) Values for cell mechanical properties measured in different ways may not be comparable.[70] We should strive to use standard engineering parameters whenever possible. (3) It is important to carefully formulate the biological theory being tested, and to let the *biology* direct selection of the appropriate mechanical tests to conduct.[51,73]

Mathematical modeling

A powerful way to formulate a mechanical theory so that it can be tested is to express it quantitatively with a mathematical model. Although a number of mechanisms have been postulated to drive neurulation, only a few of these ideas have been formulated mathematically.

The development of mathematical models of morphogenetic processes can serve several purposes. (1) One important role for models is to simplify complex problems, abstracting the essential elements of what is known about a system. Stating a theory quantitatively can clarify which parameters of a system need to be measured, and can suggest new experiments to test the theory. In turn, results of such experiments can lead to rejection or modification of the theory. The interplay of modeling and empirical work is discussed in more detail by Jacobson[75] and by Koehl.[76] (2) Models allow us to explore what *could* happen if defined mechanisms operate and components of the system have defined properties. Models also permit us to conduct 'experiments' to explore the consequences of manipulating the system in ways that may or may not be possible empirically. (3) Quantitative models are especially important in studies of the physical behavior of organisms that are much smaller than humans. Our intuitions (based on our experience of the physical world) cannot be trusted when we consider creatures as small as embryos, for whom inertial forces are negligible and viscosity is very important (for example, see refs 77-81). Mathematical models are a powerful tool for making us see the non-intuitive physical behaviors of small creatures. (4) Many ideas that seem perfectly sensible when stated qualitatively turn out not to work when examined quantitatively.

One example of the latter role of modeling is provided by investigations of the theory that elongation of the notochord causes the overlying sheet of neural plate cells to buckle and roll into a tube (much as a sheet of rubber buckles and rolls when stretched along a line).[26,34-37,82] Jacobson *et al*[30] explored this idea quantitatively using a finite element model (see, for example, ref 83 for descriptions of finite element models, which are extremely useful in simulating systems like embryos with complex geometries). Their calculations indicate that buckling and rolling only occurs if the epithelial sheet is unrealistically thin. However, their calculations assumed a flat neural plate showing Hookean elastic behavior. The observation of 'hinge points'[41] in the neural plate calls the former assumption into question. Although the latter assumption may not be too bad given the data of Selman,[6] empirical measures are needed for the mechanical parameters used in these calculations before we can reject the theory of neural plate buckling. In any case, this model suggests that notochord elongation *alone* is not sufficient to roll the neural tube; this is not inconsistent with the observation[30] that the presence of the notochord enhances tube rolling, which can also occur without the notochord.

Types of models

Some models are phenomenological descriptions of a system, whereas other models assume the mechanisms by which the components of the system operate and build up from these an overall prediction of how the system behaves. These two approaches are complementary: phenomenology helps organize observations so that mechanistic laws can be formulated, which in turn can explain the phenomenological rules, and both approaches can stimulate new experiments and can focus our attention to the important parameters to be measured.[76,84]

Phenomenological models of neurulation

Jacobson and Gordon[37,85] modeled the amphibian neural plate as an array of units of constant volume whose apical areas shrink at defined rates. The spatial distribution of shrinking rates used was based on the data of Burnside and Jacobson.[32] This model showed that apical shrinking alone does not give rise to the keyhole shape of the neural plate. In another model, they elongated (by an empirically determined amount) the region of the neural plate just above the notochord, and again found that the model plate did not change its shape like the neural plate. However, when they combined both cell apical shrinking and supranotochordal elongation, the model plate reshaped realistically, indicating that together these two processes are necessary and sufficient to shape the neural plate. Note that these phenomenological models can explore the consequences of shrinkage and elongation without having to specify the mechanism(s) that bring about that shrinkage or elongation.

Hilfer and Hilfer[84] provide another example of a phenomenological model that has been applied to neural tube morphogenesis. This model was used to explore (1) the shapes that could be generated from epithelial sheets when assigned various patterns of cell shape change, and (2) the locations where cell shape changes should be expected when particular patterns of sheet folding occur. Like the Jacobson and Gordon model,[37,85] this model permitted 'experiments' to be performed that would not be possible in the living embryo, such as separating out the effects of changes in cell height versus width versus volume independently.

Mechanistic models

One mechanistic model that has been formulated for neurulation expresses quantitatively the theory that apical contraction of neural plate cells causes the flattening, thickening, and subsequent rolling of the plate.[80] The mechanical properties of each cell are modeled by an array of springs and dashpots, with a ring of filaments at the cell's apical end that contracts if stretched beyond a critical length. The model includes both mechanical and chemical connections between the cells. Two-dimensional simulations showed that a circle of cells following these local rules can generate a globally coherent morphogenetic infolding. By juggling parameters (such as the modulii and viscosities of the cells) appropriately, Odell *et al*[80] created a set of local conditions that led to a flattening and subsequent rolling into a tube reminiscent of neural plate behavior. Although they do not make clear whether any of the values for the parameters used in their simulations were based on measurements of embryos, their model provides a clear 'shopping list' of parameters that should be measured. It would be interesting to use measured values for these parameters in their model to calculate the forces exerted at the 'neural folds' of the model. One test of their theory would be comparison of these predicted values with those measured by Selman.[5,6]

Another mechanistic model formulated for neurulation, the 'cortical tractor' model, also explores the global consequences for the embryo of local rules followed by cells.[30] This model assumes that the cortical cytoplasm of epithelial cells flows (as during the crawling of solitary cells[86]) from the basal to the apical ends of the cells, carrying with it adhesion molecules that can build up or be resorbed when the 'tractoring' cytoplasm reaches the apical ends of the cells. The model shows that if neighboring cells tractor at different rates, basal elongation of the faster cells occurs and shear forces are generated that deform the epithelial sheet. If the right spatial distribution of tractoring rates is assigned to the cells in the model, deformations of the epithelial sheet occur that look like neurulation. Furthermore, conducting 'experiments' with the model (such as requiring neural plate cells overlying the notochord to be fixed, and letting the lateral boundaries of the neural plate elongate) lead to the generation of even more realistic shapes. One appealing aspect of the cortical tractor model is that it provides a mechanism by which epithelial cells can change neighbors (which kinematic studies have revealed they do) without breaking the apical seals between cells. If parameters measured on living embryos are used in this model, will

the model predict forces at the neural folds that are of the order of those measured by Selman[5,6] as well as rates of neural plate reshaping that mimic those measured in kinematic studies?

Both the apical contraction and the cortical tractor models, which are not mutually exclusive, result in apical narrowing of neural plate cells and in the thickening, flattening, and rolling of the plate. Hence, they show that the cell properties postulated in each model *could* produce neurulation. Neither model, however, demonstrates that these mechanisms *do* cause neurulation.

Another group of mechanistic models explores the shape changes that can be produced by differential adhesion between neighboring cells. Although one such simulation can produce invagination by a ring of cells,[87] another fails to produce notoplate elongation.[26] As with the other mechanistic models mentioned above, empirical measures of the necessary parameters for embryonic tissues are not available yet.

Physical modeling

Physical (i.e. 'string-and-sealing-wax') models can serve many of the same functions as mathematical models, allowing us to explore what *could* happen if defined mechanisms operate and if components of the system have particular properties. The properties of the pieces of a physical model are analogous to the assumptions of a mathematical model. Although physical models have been used to illustrate ideas about mechanical aspects of morphogenesis (for example, see refs 35, 41, 68, 88), developmental biologists have not tended to use them as *quantitative* tools (for examples of quantitative physical models, see refs 18, 66, 89).

Conclusions

Waddington's[3] assessment of the study of morphogenesis in 1943 is still true today:

"Recent years have seen considerable advances in our knowledge of chemical interactions between different parts of developing embryos, and of the metabolic processes by which the stimulating evocators are released . . . On the other hand, the forces which actually bring about the changes in shape which are perhaps the salient feature of early development have remained almost unstudied."

The proximal causes for morphogenesis are mechanical forces, and yet they remain poorly understood. The plausibility arguments of the past need to be formulated quantitatively, and these theories need to be tested with empirical mechanical measurements on embryos. Using the techniques of biomechanics to unravel the physical mechanisms of morphogenesis can provide a powerful set of tools to complement the more popular molecular and biochemical approaches used to investigate the regulation of the genesis of form.

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