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Finite Element Modeling of Embryonic Tissue Morphogenesis

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The intriguing shape changes that occur as embryos develop have been studied extensively from a biological perspective. However, from a mechanical standpoint, many questions remain concerning the source, magnitude, and timing of the driving forces. These forces originate within individual cells and cause sheets of cells to deform to produce organs and other essential structures. Finite element-based computer simulations make it possible to investigate which of the various possible driving forces actually operates to produce specific shape changes. They make it possible to investigate questions about the magnitude and duration of the driving forces, how variations in driving forces or initial geometries affect the shapes of the resulting structures, and what control mechanisms are present.

Although computer simulations of a variety of morphogenetic processes will be surveyed, this contribution will focus on a process called neurulation. During neurulation a sheet of cells called the neural plate rolls up to form the neural tube, a sealed tube which is the precursor of the spinal cord and brain. Finite element simulations show that the *sequence* of specific shape changes characteristic of neurulation can be produced by contraction of structural components called microfilaments and by elongation of a structure called the notochord, which underlies the neural plate. As the plate changes shape, the microfilaments act on a structure that has a new geometry and produce a different incremental, global effect than they did in the original configuration. Thus, the sequence of shape changes characteristic of neurulation is produced by mechanical interaction or feedback between the current geometry and the internal force generators.

Current finite element simulations provide an important step toward the development of "virtual embryos" in which virtual experiments might be carried out. They also make an important contribution to the development of numerical methods that overcome the significant technical challenges inherent in the modeling of embryo morphogenesis.

2.1 Introduction

The development of a fetus from a single cell, a fertilized egg, requires the successful completion of a sequence of elegant and highly specific processes (Alberts et al., 1989). These processes include cell proliferation, a repeated and controlled series of cell divisions necessary to produce the trillions of cells found in a normal fetus. Each mitosis requires the self-assembly, operation, and self-disassembly of complex biomechanical structures which are governed by finely tuned control systems. Cell differentiation, an intriguing process whereby cells become functionally different from each other, also occurs repeatedly to produce an embryo that has several hundred kinds of cells. Another critical process in embryogenesis is the occurrence of a succession of highly specific shape changes. These critical shape changes give rise to organs and other critical structures. Morphogenesis, literally the "the genesis of form," includes all three of these essential behaviors and the control mechanisms that govern them.

From species to species, striking similarities exist in these processes. These have led some to postulate a common evolutionary ancestry and others a commonality of design. From a practical standpoint, these similarities allow scientists to make qualified inferences about morphogenetic and physiological processes in humans based on studies of parallel processes in vertebrates ranging from apes and axolotls, species in which various interventions can be carried out more easily and legitimately.

The advent of realistic computer simulations provides another important means to investigate aspects of human development, including the mechanisms of normal development and the causes of malformation-type defects. Such simulations also provide a new focus, as suggested by Koehl (1990):

> 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.

The purpose of this work is to investigate ways in which computer simulations can be used to investigate the physics of morphogenesis, outline principles that must be followed in the formulation of simulations, review some recent simulations, and discuss reasons that simulations can be expected to become increasingly important to the field of morphogenesis.

2.2 Modeling of Morphogenesis

Perhaps the most important role of modeling at present is to test hypotheses about developmental processes. When the factors thought to be important to a certain process are incorporated into a soundly formulated computer model, the output of the model often shows whether or not the initial understanding is correct and complete. Initial simulations usually indicate that less is known about the physical problem than was originally thought. Computer modeling can also serve as a reliable adjudicator for distinguishing between what is a good idea and what is actually true. Simulations of neurulation, for example, have demonstrated clearly that not every plausible idea produces shape changes that match those that occur in real embryos. Fortunately, in simulation studies, the differences between the model behavior and the behavior of the real system often provide clues regarding the area of misunderstanding.

It is important to note that just because a simulation produces output that matches the corresponding real data does not guarantee that the model or input parameters are correct. This is especially true of phenomenological models, that is models that are not based exclusively on known physical properties and principles.

Computer models also allow sensitivity analyses to be carried out to investigate the effect of changing particular parameters or boundary conditions (Brodland and Clausi, 1994). Furthermore they provide an engine that can be used to solve so-called inverse problems. For example, in the neural plate, many structures that are known to generate forces in other contexts are present. To determine which structures actually act and produce the observed sequence of shape changes is a kind of inverse or, more specifically, parameter estimation problem (Beck, 1977).

Simulations provide a common point of focus for studies of embryo mechanics. They do this in part by forcing discoveries to be expressed in a quantitative fashion so they can be either incorporated into or used to test simulations. Koehl (1990) writes: "I stress the importance of formulating theories quantitatively, and of measuring the mechanical properties of embryonic tissues and the forces exerted during morphogenetic events." Simulations also require that relationships be established between commonly agreed-on quantities, so that one study might relate quantities A and B, another A and C, and another B, C, and D. Often, significant gaps in understanding are revealed simply by attempting to write the relationships (and interactions) between governing quantities in a form suitable for numerical modeling.

Computer simulations probably will soon be used to increase the confidence level of inferences between species. Consider, for example, a shape change such as neurulation, which occurs in both humans and axolotls. Although the subcellular structures that drive it appear to be common to both, the geometries of the two systems have significant differences. That differences in initial shape can profoundly affect subsequent shapes produced by a given set of forces and constraints is well known in mechanics (Brodland and Cohen, 1989). Suppose that computer simulations based on actual physical properties and known physical processes can be devised and verified in detail using axolotl embryos. Such verification might include comparisons over time between three-dimensional shapes produced in simulated and real embryos. It might also include similar comparisons with teratogen-treated and surgically altered embryos. Confidence can then be gained in the validity of the theoretical formulation, material properties, and other input parameters used, and in the software implementation. More confidence can then be realized when the same software is used to simulate similar aspects of normal or perturbed human embryogenesis, where the data is significantly more sparse.

It is reasonable to expect that subsequent generations of computer simulations will embrace a broader range of relevant phenomena. For example, mechanical, biochemical, and electrical behaviors and their interactions might be simulated. In addition, simulations will likely progress in terms of the smallest scale explicitly incorporated: from tissue to cell to subcellular to molecular and perhaps even to atomic level (Brodland, 1997). Although molecular level studies of entire embryos seem unlikely in the foreseeable future, molecular aspects of particular processes may be studied and subsequently embodied as constitutive-type equations that accurately describe in bulk or continuum terms the behaviors of large systems of molecules. Unlike present estimates of bulk behavior, these would be founded on and would accurately embody the molecular phenomena.

To date, most biomechanical modeling has focused on two morphogenetic processes: gastrulation and neurulation (Brodland, 1997). These have received attention because the shape changes associated with these processes could be observed and because they appeared to be reasonably straightforward mechanically. Both have turned out to be more intriguing and elegant than originally thought. Here, we will focus on the process of neurulation, since it is the process which to date has received the most attention and yielded the broadest insights.

2.3 The Process of Neurulation

The three-dimensional shape changes that are a critical part of the process of neurulation have intrigued researchers for millennia. Intensive research during the last 100 years (His, 1874; Roux, 1888; Lewis, 1947; Jacobson, 1978; Lee and Nagele, 1988; Schoenwolf and Smith, 1990; Clausi and Brodland, 1993; Brodland and Clausi, 1995) has identified the kinematics of the shape changes and revealed the morphology and mechanical properties of various structures in and around the neural plate which might

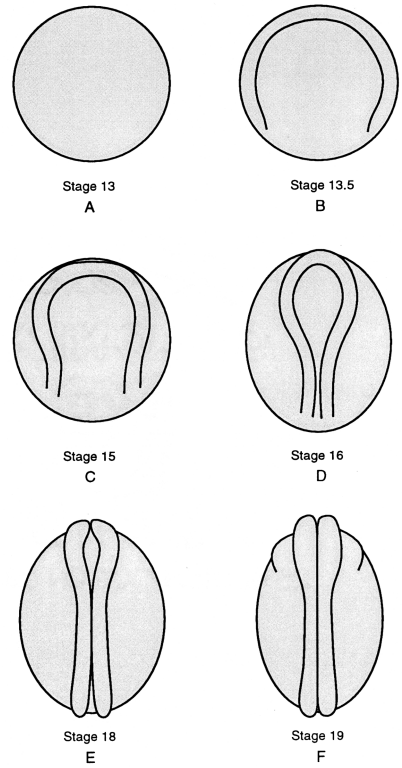

FIGURE 2.1 The process of neurulation. A: At the beginning of neurulation the embryo is a hollow ball. B: Neural ridges form. C: With time the ridges become more prominent and move toward the midline of the embryo. D-F: The ridges contact and fuse to form a sealed neural tube. After Brodland (1997).

drive these shape changes. Many theories have arisen to explain how various combinations of force generators, regulated by certain control mechanisms, might drive neurulation shape changes (Gordon, 1985). The advent of reliable computer simulations has made it possible to identify which of these theories makes mechanical sense and which does not (Clausi and Brodland, 1993; Brodland and Clausi, 1995).

Gross Shape Changes

During neurulation, a sheet of cells called the neural plate rolls up to form a sealed tube called the neural tube (Fig. 2.1). The cephalic end of this tube (the upper part of each figure) becomes the precursor of the brain, while the dorsal and caudal parts become the spinal cord. Figure 2.1 shows developmental Stages 13 through 19 of the axolotl (*Ambystoma mexicanum*). Neurulation in other vertebrates, especially amphibians, is very similar. Axolotl embryos are approximately 2.5 mm in diameter during neurulation.

At the onset of neurulation (Stage 13), the embryo is basically a hollow sphere with an outer layer comprised of a few thousand cells. The cells at the top of the embryo thicken and form a flat surface known as the neural plate. An indentation (neural groove) develops along the dorsal midline of the embryo. At the edge of the neural plate, ridges (neural folds or ridges) begin to appear (Stage 13.5). With time, the neural folds become more pronounced, rise, and bend toward each other. Meanwhile, in-plane motions cause the plate to change from a disk shape to a keyhole shape (Stages 13.5 to 16). The wide section of the keyhole occurs at the cephalic end of the embryo where the brain will subsequently form. The neural ridges along the narrow part of the keyhole shape meet and fuse along the midline of the embryo to form the neural tube (Stages 17 to 19). These shape changes are remarkably symmetrical.

If any part of the tube fails to close and fuse, a serious birth defect may result. Openings in the dorsal or caudal regions of the tube give rise to spina bifida, a potentially serious defect in which the spinal cord may not develop properly in the unfused area. Partial paralysis and other serious health problems may result. If the cephalic part of the tube fails to close, anencephaly results. Infants with this condition do not live. Neural tube defects are known to be caused by environmental, nutritional, and genetic factors (Mutchinick, 1990; Werler, 1993). However, it is not known whether these factors give rise to a mechanical defect through a common pathway, or whether they work through different mechanisms. Because the defect is first manifested as a mechanical abnormality, researchers have focused on the mechanics of the process, and especially the structures that drive it. A better understanding of the mechanics of neurulation is expected to provide a basis for steps directed toward its prevention.

Cellular Contributions to the Observed Shape Changes

The gross shape changes outlined above are caused by coordinated shape changes in the cells that make up the neural plate and by shape changes in underlying tissues, most notably the notochord. These shape changes have been measured in detail for the *Taricha torosa*, a newt whose patterns of neurulation are very similar to those of the axolotl. During the early stages of *Taricha torosa* neurulation (Stages 13 to 15), the neural plate cells change shape from cuboidal to columnar. They change from an approximate height of 58 μm and diameter of 18 μm to a height of 94 μm and diameter of 14 μm (Burnside, 1973). From Stages 15 to 19, the apical ends of the cells continue to constrict until the cells are approximately 145 μm tall and have a strongly tapered or "bottle" shape with a typical apical diameter of 6 μm.

When this shape change occurs in a coordinated way between adjacent cells, the plate they form is caused to bend more and more sharply. Cells in some regions become more strongly tapered than others, creating regions of high transverse curvature known as hinge points (Schoenwolf and Smith, 1990). Contraction of cell apices also produces the in-plane motions noted above.

2.4 Force-Generating Structures

A typical cell contains many different mechanical structures (Alberts et al., 1989). The primary structures that are known to play a mechanical role in neurulation and other morphogenetic shape changes are shown in Fig. 2.2. These include microfilaments, microtubules, intermediate filaments, the cytoplasm, the plasma membrane, the notochord, and cell adhesion.

Microfilaments

Microfilaments, with a diameter of 5 to 7 nm each, are a principal contractile component not only of neural plate cells, but of most embryonic cells. In the neural plate and many other epithelial cell sheets, microfilament bundles form a ring just inside the apical end of each cell (Fig. 2.2). As they contract, they have a purse-string effect, causing the apical end of the cell to narrow. Because the volume of the cell is constant, as the apical end narrows, the cell must become taller, wider at its basal end, or a combination of both.

Burnside (1971, 1973) correlated the degree of apical constriction of the cells to the cross-sectional diameter of the apical microfilaments. Her measurements also showed that the average bundle width increased from 0.17 μm (±0.005 μm) to 0.32 μm (±0.006 μm) between Stages 13 and 19. Calculations indicated that the total volume complement of microfilaments remains constant from Stage 13 to Stage 19. Thus, the individual microfilaments apparently intercalate as the bundle shortens. If the stress σ remains constant (based on current cross-sectional area), the force F_M produced by a microfilament bundle would be expected to follow a relationship of

$$F_M = \sigma A_0 L_0 / L, \tag{2.1}$$

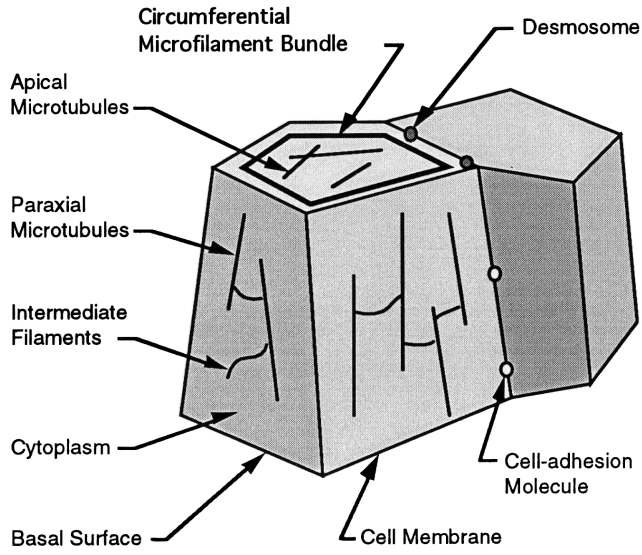

FIGURE 2.2 Structures known to be of mechanical importance to morphogenetic shape change. After Brodland (1997).

where A_0 is the initial cross-sectional area, L_0 is the initial length, and L is the current length.

What regulates microfilament contraction? Calcium ions are believed to activate microfilament contraction in a manner similar to smooth muscle (Alberts et al., 1989). Force in a microfilament bundle is assumed to be a function of its current cross-sectional area, which varies inversely with lengthening in order to maintain constant volume. Thus, as the microfilament bundle length decreases, its force magnitude increases. Microfilaments exist in adult epithelia and likely serve a variety of mechanical functions during the life of an animal (Ettensohn, 1985).

Microtubules

Microtubules (diameter of 24 nm and made of tubulin) are highly versatile organelles within the cell. At different times, they form the structures that produce chromosomal movements during mitosis, actively translocate particulate components of the cytoplasm, and maintain and change cell shape (Fawcett, 1981).

In neural plate cells, they occur as both apical mats and as paraxial elements that are oriented across the thickness of the neural plate (Fig. 2.2). The apical microtubules are randomly oriented in a plane parallel with and immediately below the apical cell surface (Burnside, 1971). These microtubules may affect cell shape by producing a constant outward force (Clausi and Brodland, 1993), thus opposing the action of microfilaments.

In contrast, paraxial microtubules may be partly responsible for cell elongation and for moving cytoplasm toward the basal end of cells to produce tapered or bottle-shaped cells. They might also push directly on the ends of the cell to produce elongation. In either case, it is apparent that intermediate filaments must provide lateral stability to the microtubules in order for them to buckle as they attempt to produce and carry these compressive loads (Brodland and Gordon, 1990).

Other Components in the Neural Plate

The material inside of the cell, exclusive of the nucleus, is called cytoplasm. The mechanical properties of cytoplasm in various biological systems have been measured by Hiramoto (1969a, b) and others. A meshwork of 10 nm diameter intermediate filaments runs through the cytoplasm. In addition to preventing the buckling of paraxial microtubules, they apparently give rise to the elastic component of cytoplasm.

Each cell is enclosed by a plasma membrane, which contains various channels that control entry of ions and other materials essential for regulating cytoskeletal components (Alberts et al., 1989). Structurally, this membrane is known to be weak. Thus, the plasma membrane is not considered an important mechanical component.

Cells have specialized structures for mechanically linking and communicating with adjacent cells. Desmosomes are button-like points of intercellular contact which provide strong connection sites for microfilaments (Burnside, 1971). Desmosomes act like rivets to transmit tensile and shearing forces within the neural plate.

Notochord

One of the key features of neurulation is elongation of the neural plate. This occurs in plates that have been excised from the rest of the embryo (Jacobson and Gordon, 1976). This elongation is driven largely by the notochord (Koehl, 1990). The notochord is a rod-like structure, approximately 80 μm in diameter, which is located immediately beneath the midline of the neural plate. Of the entire neural plate, the only cells that are attached at their basal ends are those above the notochord (Jacobson and Gordon, 1976). Notochord elongation also apparently causes the neural groove to form.

Cell–cell adhesions are important to certain kinds of morphogenetic movements (Nardi, 1981; Steinberg, 1996). However, their importance to neurulation is not clear.

Another mechanism that has been proposed as a driving force for neurulation is cortical tractoring (Jacobson et al., 1986). It postulates a flow of cytoplasm from the basal and lateral surfaces to the apical surface whereby membrane and adhesive structures would be carried and deposited where required. This phenomenon may also be referred to as cytoplasmic streaming. The authors argue that cortical tractoring explains thickening, invagination, and rolling of the neural plate. How such a process might be initiated at the right time and how it might be controlled is not clear. It may not be possible to evaluate the possible merits of this mechanism with certainty until computer simulations of the postulated subcellular cytoplasm movements are carried out.

Summary

A complete explanation for the shape changes that occur during neurulation has been elusive. Since neurulation occurs early in the development of an embryo with few cells and little if any cell specialization, it is likely that only a few mechanisms contribute to the shape changes. It seems unlikely that the behaviors of individual cells are somehow preprogrammed, although this was once a popular idea. Experiments have shown that neurulation and other morphogenetic processes are highly robust and synchronized and not easily perturbed by surgical and teratogenic interventions. The outcomes of these experiments strongly suggests that these morphogenetic movements are regulated by ongoing interactions between cells. In some cases, these interactions may be chemical or electrical. However, as we will show, mechanical interactions can be a powerful regulation device.

2.5 Simulations of Morphogenetic Processes

Computer simulations and other kinds of simulations play an important role in evaluating the mechanical validity of various theories about the forces that drive specific morphogenetic processes. Lewis (1947) constructed a physical model of the neural plate using brass plates hinged at their centers to a flexible spine, and tied to each of its neighbors by rubber bands. The lateral cell surfaces were represented by the plates, while the rubber bands acted as tension generators in the apical and basal ends. Different rubber band distributions produced different patterns of cell sheet folding. Practical considerations have limited the number of such physical simulation models. Fortunately, the rapid development of computers has made possible computer simulation models that are more accurate, realistic, and flexible than these early physical models.

Jacobson and Gordon (1976) constructed a mathematical model of "the formation of the neural plate based on different autonomous, preprogrammed schedules of shape changes for different regions of the neural ectoderm." In their model of in-plane motions, each cell had an autonomous program of shape change controlled by a cellular "clock" to synchronize the cell motion. Superposition showed that the coexistence of cell shrinkage and notochord extension produced normal in-plane shape changes. Using this model, they analyzed cell shrinkage without notochord extension and notochord extension without cell shrinkage (the latter test is not possible *in vivo*). That individual cells are preprogrammed to behave in such a manner is not a well-accepted notion. However, the model did illustrate that there are two separable aspects to the in-plane motions of the neurulation process. Jacobson (1980) subsequently addressed a number of related issues regarding computer simulations.

One of the first computer models, by Odell et al. (1981), presented "a mechanical model for the morphogenetic folding of embryonic epithelia based on hypothesized mechanical properties of the cellular cytoskeleton." Cells were modeled as two-dimensional quadrilaterals with hypothesized properties and contraction behaviors at the apex. These models demonstrated a possible two-dimensional folding process for the neural plate. In these models, the cell body is not modeled as a continuum, but by truss elements connected to opposite nodes of the quadrilateral. A limitation, acknowledged by Odell et al., is that their neural plate cells should not remain in the same vertical plane during neurulation, as assumed in this model, but should undergo a migration process. Another issue is the ratio of cell height to the radius of the embryo (3:1 instead of the actual 20:1). The model does, however, dramatically illustrate the possibility of modeling plate bending and rolling under the action of apical constrictions.

A few years later, Weliky and Oster (1990) simulated coordinated cell rearrangement by accounting for the balance of forces between adjacent cells. The net force at cell junctions is the difference between the passive elastic inward forces of the microfilament bundles and the osmotic plus hydrostatic pressure of the outward forces. Cell protrusion is produced when the net force is not zero. The finite difference method (a technique related to the finite element method) is used, and viscous behavior is included. The cells are treated as two-dimensional polygons; however, cell sliding may require understanding of the interactions along the entire length of the cell. No mention of absolute force magnitudes nor cell mechanical properties is included. The authors recognize that the "model is a simplification of the actual cellular machinery responsible for the generation of these forces, but we believe it captures the essential mechanical forces." The paper offers insight to relative cell motion caused by unbalanced forces at cell interfaces and to the node rearrangements required to simulate this behavior.

More recent computer simulations have made use of the finite element method (FEM). These include plausibility tests of aspects of neurulation (Jacobson et al., 1986) and studies of the mechanics of individual cells (Cheng, 1987a, b). The basis, formulation, and principal conclusions obtained using detailed FEM simulations of neurulation are extensive and are, therefore, discussed in separate sections below.

In 1995, Davidson et al. used the FEM to investigate the onset of gastrulation, a morphogenetic event occurring prior to neurulation. Relatively few of the relevant mechanical properties had been measured mechanically. Thus, these simulations had a different objective than those of neurulation, where much is known about the relevant mechanical properties. During the invagination phase of gastrulation, a localized, circular dimple is formed in a hollow ball of cells. Five different hypotheses including apical constriction and cortical tractoring were considered, and the unknown mechanical properties were given different values. They concluded that all five hypotheses could create the observed morphological shape changes, if the values of the unknown properties are suitable. Thus, the study provides an important basis for the design of future experiments to measure the unknown properties and ultimately to determine which hypothesis or hypotheses might be correct.

Unfortunately, in their model, the mechanical properties of the tissue are assumed to be elastic, although viscous or viscoelastic properties seem more plausible. Also, standard aspect ratios are violated without explanation and no explanation is provided as to what kind of elements are used to model the thin layers. To maintain incompressibility of the cytoplasm, Davidson et al. compress the cell apex and simultaneously stretch the base using a comparable force. It is not guaranteed that the finite element

volume will remain constant. Since no biological explanation is stated for generating a basal force, basal stretching should only be a passive consequence of the apical constriction and volume constancy. Cell incompressibility is, apparently, ignored in the simulations of the other four hypotheses.

A number of significant technical challenges arise in the modeling of morphogenetic shape changes. During morphogenesis, large strains, deformations, and rotations occur. In addition, the material properties may be highly nonlinear. To model these properly requires the use of a carefully and suitably formulated updated Lagrangian approach. In addition, during morphogenesis, cells and tissues are typically incompressible. Thus, a Poisson's ratio of $\nu = 0.5$ must be used. Unless the element integrations and equations are formulated properly, the elements will suffer from either locking or hourglassing behaviors (Belytschko and Ong, 1984). Few, if any, commercial packages are capable of satisfying these criteria. In the sections that follow, we present a FEM model that addresses all of these key biological issues and discuss principal findings made using it.

2.6 Formulation of a Finite Element Model

What is the Finite Element Method (FEM)?

The label "finite element method" was first used in 1960, although mathematically similar techniques had been used since 1943 (Huebner and Thornton, 1982). Over the past three decades, coupled with the advent of high-speed digital computers, the finite element method (FEM) has become a well-established technique in a diverse range of engineering applications, including structural analysis, solid mechanics, heat transfer, mass transport, fluid mechanics, electromagnetics, vibration analysis, soil mechanics, and even acoustics. This method is especially useful to obtain approximate solutions to analytically intractable systems such as those that describe embryo morphogenesis.

A finite element formulation typically follows a canonical approach that is independent of the problem type (Zienkiewicz and Taylor, 1989, 1991). First, the continuum is subdivided into subregions of appropriate shape (discrete elements) interconnected by nodes. By increasing the number of elements in a regular fashion, a more accurate solution is obtained. The variation of the field variable (for example, displacement in the case of a mechanics problem) within each element is represented by shape functions. Next, the behavior of each element is formulated into a matrix system of linear algebraic equations. A global system of simultaneous equations is then constructed by assembling the matrices that represent each element. Assembly is possible because the value of the field variable is assumed to be the same at all points that share a node. In its most basic form, the resulting equations have the following form:

$$\mathbf{K}\mathbf{u} = \mathbf{f} \tag{2.2}$$

where K is the generalized global stiffness matrix, u is the generalized global displacement vector, and f is the generalized global force vector.

The equations are solved using standard numerical routines. The solution is not exact since a piecewise approximation between nodes is assumed. In the case of a transient problem, the solution is also piecewise over time. For a complete explanation of the FEM approach, see Zienkiewicz and Taylor (1989, 1991). A description of the FEM as applied to biological systems is presented by Brodland (1994).

Basic Criteria

To model morphogenetic shape changes, a FEM simulation must take into account complex temporal shape changes, large strain, displacement and rotation, physical property changes, creation of new cells by mitosis, sliding of cells past each other, viscous or viscoelastic material properties, material incompressibility and internal force generation. To accommodate these characteristics, custom software was written in the C programming language (Clausi, 1991).

A properly formulated updated Lagrangian formulation can accommodate these requirements. The time steps and element sizes should be sufficiently small so that if either is further reduced, the solution does not change; i.e., the result is not dependent on the temporal or geometric discretizations used.

Eight-noded isoparametric volume elements are used to represent the bulk properties of the cell cytoplasm. These finite elements do not have to necessarily model single cells, but can be used to model suitable groups of cells (Brodland and Clausi, 1994). In fact, without any loss of validity, the finite element boundaries do not have to correspond to any cell boundaries since the elements are assigned the bulk physical properties of the cell sheet. To model neighbor changes between cells is more difficult, but possible (Chen and Brodland, 1997).

Derivation of the elastic stiffness matrix for an isotropic, eight-noded isoparametric volume element is available in standard finite element texts (Zienkiewicz and Taylor, 1989). A viscoelastic version of this formulation is presented in Clausi (1991) and summarized in Brodland and Clausi (1994), and the simplified derivation for a purely viscous system is described in Brodland and Clausi (1995).

Experiments have shown that during amphibian neurulation, tissue volume remains essentially constant, even when mitoses occur (Burnside and Jacobson, 1968; Keeton and Gould, 1986). To ensure incompressibility in the finite element formulation, Poisson's ratio (ν) should be set to 0.5. However, this causes some terms in the stiffness matrix to become singular. If ν is set to a value just below 0.5 and full integration is used, then all terms of the stiffness matrix can be determined. However, some terms are magnitudes larger than the other terms, and "locking" occurs. This is a spurious increase in stiffness caused by numerical difficulties. The problem can be overcome by reducing the order of the integration, but this leads to spurious zero-energy modes of deformation called "hourglass" modes. Proposed explanations and solutions for this phenomenon are presented in Belytschko and Ong (1984). In our formulation, reduced order integration is used and hourglassing is controlled using a technique devised by Liu et al. (1985). By using this approach, the incompressibility condition can be satisfied completely and without introducing other problems.

Internal force generation is another characteristic of morphogenetic systems. Truss elements are used to produce these forces. For example, the local net effect of the apical microfilaments and microtubules is determined using statistical mechanics, and truss elements that produce a mechanically equivalent effect are positioned around the apical perimeter of each volume element (whether it models a single cell or a group of cells).

Suitable formulations also allow individual cell behaviors and neighbor changes to be modeled (Chen and Brodland, 1997).

2.7 Simulations

The FEM formulation outlined above provides a methodology for studying a wide variety of morphological behavior. Simulations of neural plate shaping and fold formation, invagination, and pattern formation are presented here.

Neural Plate Shaping

To investigate the intriguing in-plane motions by which a circular neural plate is transformed into a keyhole shape, a flat plate model (Fig. 2.3) of one half of the neural plate (radius 1600 μm) is used. Patches of cells are modeled by volume finite elements, each initially 80 × 80 × 120 μm thick. Truss elements (identified with wider lines) are used to model the actions of constricting apical microfilaments. The region identified in Fig. 2.3C is elongated at a rate of 100 μm/h to model the elongation of the notochord. This agrees with the physical location of the notochord (Youn et al., 1980) and its rate and region of active elongation (Jacobson and Gordon, 1976).

Corresponding time-lapse photographs of the axolotl dorsal surface are shown in Figs. 2.3D through F. A regular grid was placed on the first figure and deformed manually to match observed cell motions. The deformation produced in the finite element simulation is quite similar to that observed in the time-

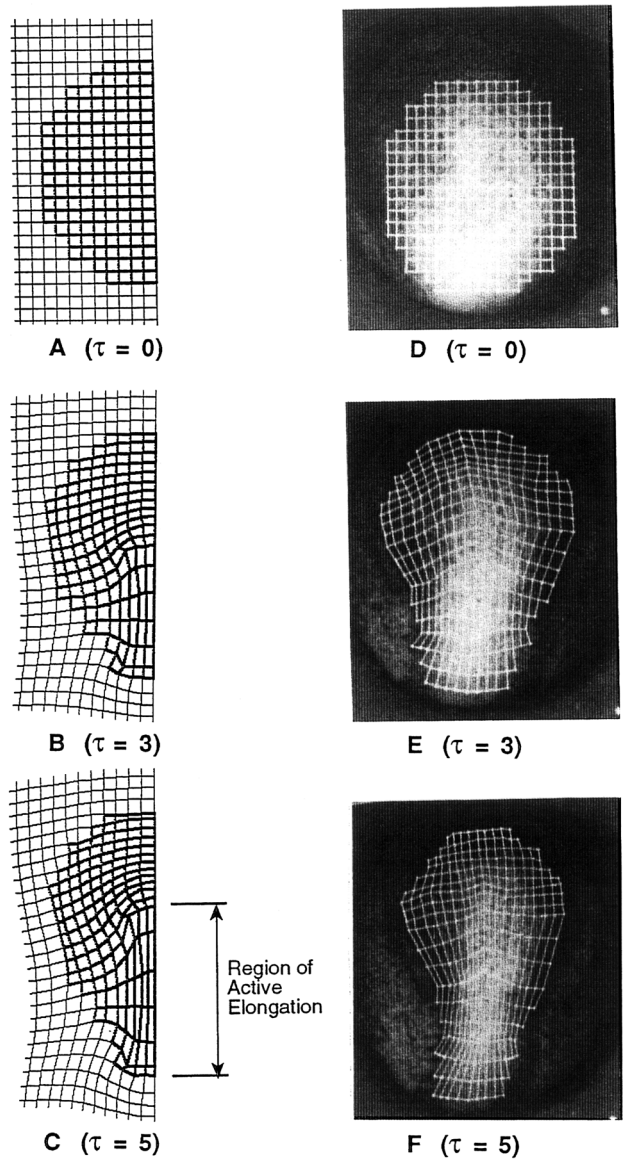

FIGURE 2.3 In-plane shape changes associated with neurulation. A-C: Finite element simulations driven by notochord elongation and microfilament contraction. D-F: Corresponding time-lapse photographs of axolotl embryo development. Cell motions were tracked manually and used to construct a tracking grid. After Clausi and Brodland (1993).

lapse sequence. Also, the patterns of thickness change parallel to those observed in *Taricha torosa* (Clausi and Brodland, 1993).

Neural Fold Formation and Tube Closure

To study the transverse or out-of-plane aspects of neural tube formation, a transverse strip from the left side of an embryo is modeled. By modifying the parameters, we can perform analyses that are not only impossible to perform *in vivo* but that allow precise investigation of the sensitivity of the process to variations in starting geometry or applied force.

For simulations of cross-sectional strips, the net apical constriction force is represented using the dimensionless parameter (Clausi and Brodland, 1993):

$$F_A = 2.3N_{MF}F_{MF} - N_{AMT}F_{MT}/\mu whθ \tag{2.3}$$

where N_{MF} and N_{AMT} represent, respectively, the total number of microfilaments and apical microtubules across the width of the strip, w and h are the initial cell sheet thickness and width, μ is the viscosity, and θ is an inverse time parameter given by

$$\theta = \tau/t. \tag{2.4}$$

The variable τ represents a dimensionless time and t the actual developmental time. If the microtubule force is assumed to be negligible, then the $N_{AMT}F_{MT}$ term can be assumed to be zero. For the simulations described in this section, $F_A = 0.10$.

In the reference case (Fig. 2.4), 20 finite elements, each 40 μm wide by 120 μm in the cephalocaudal direction by 120 μm tall, are connected side by side to model the transverse strip. Microfilaments are placed on the apical surface of the 10 elements closest to the midline of the embryo. To model the effects of the notochord, the cell sheet is stretched at a rate of 56 mm·θ. This rate is based on time-lapse images of axolotl neurulation taken in our laboratory.

A remarkable outcome of the simulation was that a *sequence* of shape changes was produced. In addition, both general reshaping and distinctive, detailed characteristics were produced that closely matched the shape changes that occur in real embryos.

The mechanisms by which this sequence of shape changes is produced can be understood in mechanical terms. In the starting configuration, the apical microfilament forces are balanced between adjacent elements everywhere except at the junction between the neural plate and the nonneural ectoderm. The unresisted microfilaments in the cells near this junction contract and cause the edge of the neural plate to curl upward (Fig. 2.4B). The attached nonneural ectoderm is forced to bend the opposite way to maintain continuity at the junction. As the microfilaments contract, their force increases according to Eq. (2.1), and the ogee shape at the edge becomes increasingly sharp until the microfilaments have contracted their maximum amount (Fig. 2.4C).

As the microfilaments across the whole width of the neural plate continue to contract, the neural plate narrows and, because the volume of its cells remain constant, it thickens (Fig. 2.4D). As it thickens, the microfilament forces move further away from the neutral plane of the sheet, and produce an ever-increasing moment about the middle surface. This causes the thickened plate to roll up (Fig. 2.4E). Regions of higher curvature, called "hinges" (Schoenwolf and Smith, 1990), are produced because of the bending instability produced by Eq. (2.1) (Brodland and Clausi, 1994). The neural plate continues to roll up until it eventually forms a closed tube (Fig. 2.4F).

This simulation demonstrates that using only apical constriction and cephalocaudal elongation, the salient features of neural tube formation — ridge formation, plate narrowing and thickening, cell skewing, creating of hinge points, closure, and rounding — can be produced in the proper sequence. Only established biological characteristics of the neural plate cells are used. No cell preprogramming, cortical tractoring, or forced basal stretching are used to invoke the observed shape changes. One set of applied forces (apical microfilaments plus notochord elongation) produces an entire sequence of intriguing shape changes because the instantaneous effect of these forces changes as the geometry changes. Thus, a kind of mechanical feedback is produced between the current geometry and the applied forces. The neural plate can thus be considered to have a self-regulating mechanical control system.

How sensitive are the shape changes to details of the applied force? To answer this question, the microfilament forces are changed from Eq. (2.1) to a constant value; i.e.,

$$F = \sigma A_0 \tag{2.5}$$

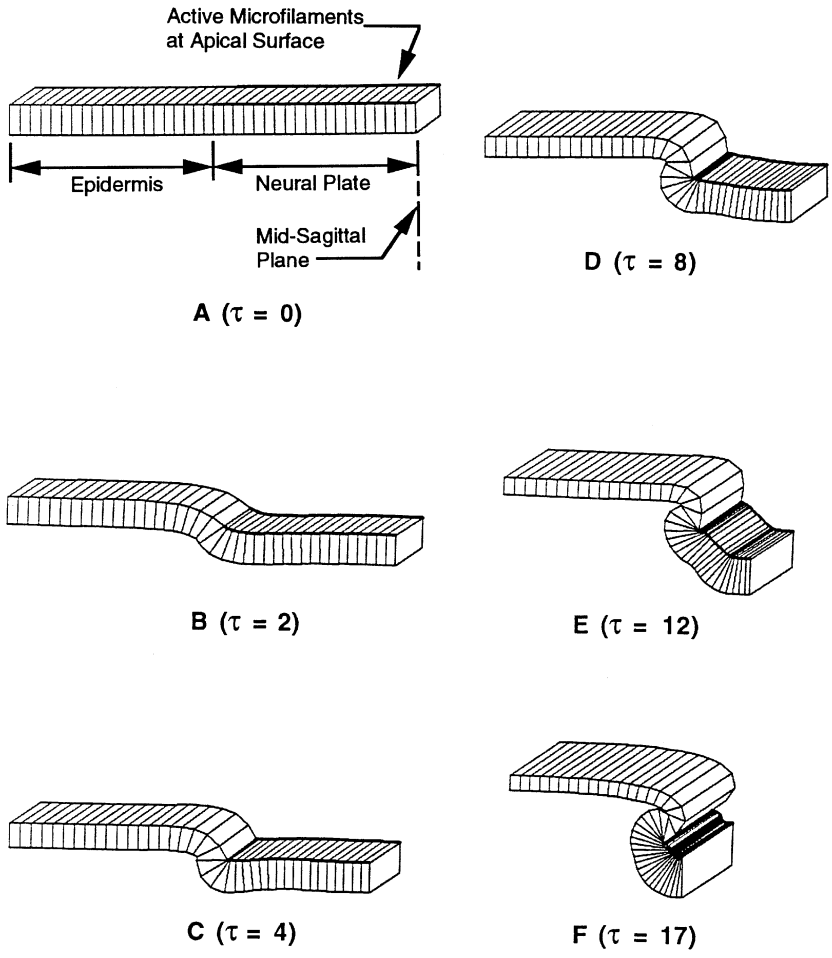

FIGURE 2.4 A "reference case" simulation of neurulation. The strip represents a symmetrical half of a transverse cross-section of an embryo. Microfilaments are placed on the neural plate part of the strip (the region adjacent to the midline). The shape changes in the strip are driven by axial elongation and microfilament contraction. After Clausi and Brodland (1993).

In this case (Fig. 2.5), no hinge points appear, and the plate curls in a more uniform way compared to the reference case (Fig. 2.4). The shape changes produced by this simulation do not match well with those that are typical of amphibian neurulation, and suggest that Eq. (2.1) provides a better description of microfilament forces during neurulation.

Is it possible that transverse forces produced by paraxial microtubules or cell-cell adhesions might cause an invagination or other neurulation-type movement to occur? These two driving mechanisms are mechanically equivalent (Brodland and Clausi, 1994). Figure 2.6 shows the results of a simulation in which transverse forces of dimensionless magnitude

$$F_T = \eta^d + N_{PMT}F_{MT}/\mu wh\theta = 0.04 \tag{2.6}$$

where η is the adhesion force per unit length are applied. Such forces thicken the neural plate, but do not produce an invagination or other neurulation-type shape change (Clausi and Brodland, 1993).

Another hypothesis that has been suggested is that forces external to the neural plate might cause the plate to buckle and collapse to form a closed tube (Schoenwolf and Smith, 1990). To investigate this

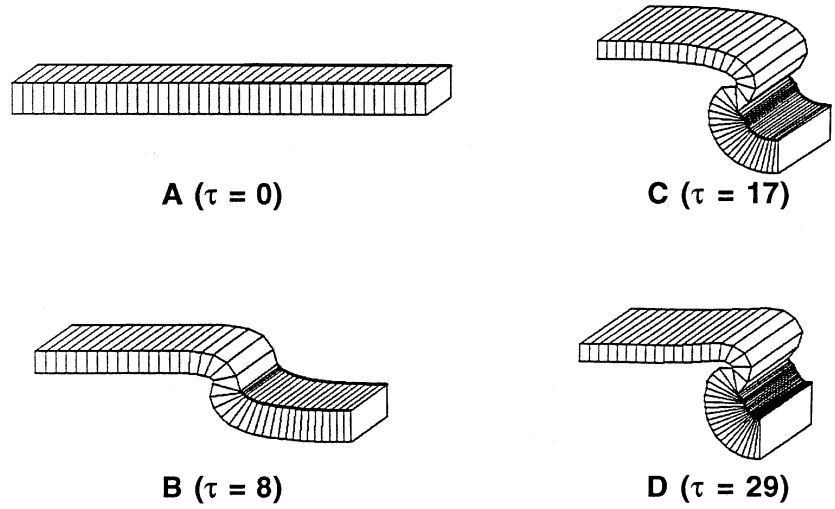

FIGURE 2.5 A simulation in which the microfilament force is constant. The driving forces and boundary conditions are identical to that shown in Fig. 4, except that the microfilament force is constant [Eq. (2.5)] rather than increasing as microfilament contraction [Eq. (2.1)]. After Brodland and Clausi (1995).

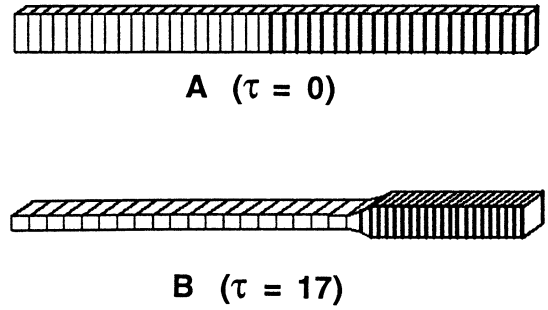

FIGURE 2.6 A simulation of the action of transverse forces from paraxial microtubule elongation or cell-cell adhesions. Microfilament forces are not acting. After Clausi and Brodland (1993).

hypothesis, the apical microfilament force from the reference case is removed, and a dimensionless, external force F_M of magnitude

$$F_M = F_{Medial}/\mu whe = 0.15 \tag{2.7}$$

is distributed uniformly between the nodes at the neural plate edge. The shape changes depicted in Fig. 2.7 are produced. This shape resembles the transverse section of a chick embryo treated to arrest microfilament contraction (Schoenwolf and Smith, 1990, Fig. 18). To date, a particular mechanism to generate such a force has not been identified.

The results of this simulation and the experiments of Schoenwolf and Smith (1990) do, however, suggest that there are redundant force systems at work. Thus, if the primary driving forces are weak or absent, a secondary system of forces may play a significant role. The presence of such redundant mechanisms may account, in part, for the robustness of the neurulation process.

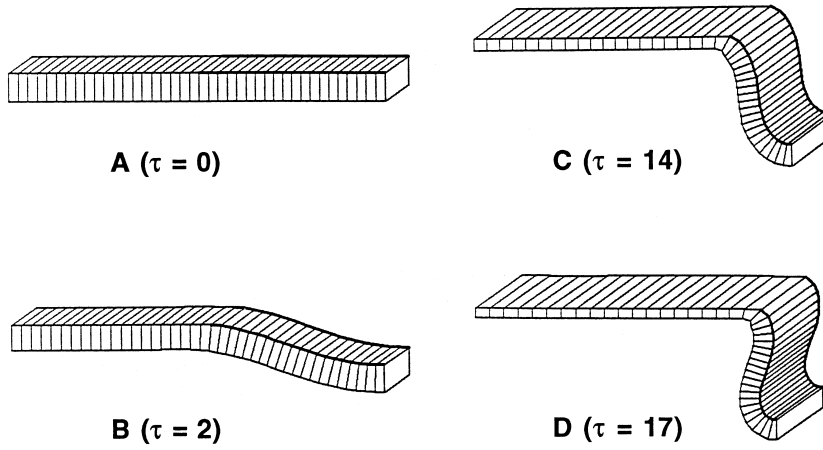

FIGURE 2.7 Extrinsic forces at the edge of the neural plate. The forces are directed toward the midline of the embryo, and there are no microfilament forces. After Brodland and Clausi (1995).

Invagination

Invagination is another fundamental morphogenetic process (Alberts et al., 1989). Here we model a symmetric invagination around a localized patch of cells (Brodland and Clausi, 1994). Due to the symmetry of the geometry and the loading, only one quarter of the plate needs to be modeled (Fig. 2.8). Each element in the top layer is initially $20 \times 20 \times 30$ μm. Microfilaments constrict the apical surface of the 5×5 patch of cells in the front right corner of the model.

Twenty time steps of $\Delta\tau = 0.2$ are used and steps $\tau = 0$, 2.0, and 4.0 are shown (Fig. 2.8). An invagination occurs by the same mechanism as the neural ridges are initially produced in neurulation. Additional simulations (not shown) have shown that any tissue below the epithelial cell sheet has little mechanical effect on formation of the invagination (Brodland and Clausi, 1994). Whereas Davidson et al. (1995) used basal expansion to generate an invagination, here we show that invaginations can be produced by apical constriction only, provided that the finite element engine is formulated appropriately so that cell volumes remain constant.

Pattern Formation

Pattern formation, "a process by which an initially homogeneous collection of cells develops heterogeneous features, is another critical embryonic process..." (Brodland and Clausi, 1994). It is known to be an important aspect of the creation of surface features, coloration, and internal structures. Both biochemical changes and mechanical events are involved in most pattern formation processes. For further examples and explanations for pattern formation, see Maini and Solursh (1991). Here, we demonstrate that pattern formation in epithelial sheets might be driven solely by microfilament contraction. In this case, the pattern results from the same basic mechanical instability as that which gives rise to hinges in the neural plate.

A simulation was created using a bilayer sheet of cells that are initially $20 \times 20 \times 60$ μm high (Fig. 2.9). Microfilaments described by Eq. (2.1) are applied to the top surface of the top layer. A perturbation (lateral displacement) of 1 μm is applied to the top left node. This causes the width of the top left cell to be increased slightly. According to Eq. (2.1), the force carried by the microfilament bundle along this top edge becomes slightly reduced and thus the cell continues to increase in width. This, in turn, allows adjacent cells to contract. As they contract, they exert an ever-increasing force. A propagating set of alternating imbalances thus arises and produces a sequence of alternating states (i.e., a pattern). This method of pattern formation is highly robust. Additional simulations (Brodland and Clausi, 1994) indicate that the pattern spacing is largely unaffected by cell size and the thickness and properties of the

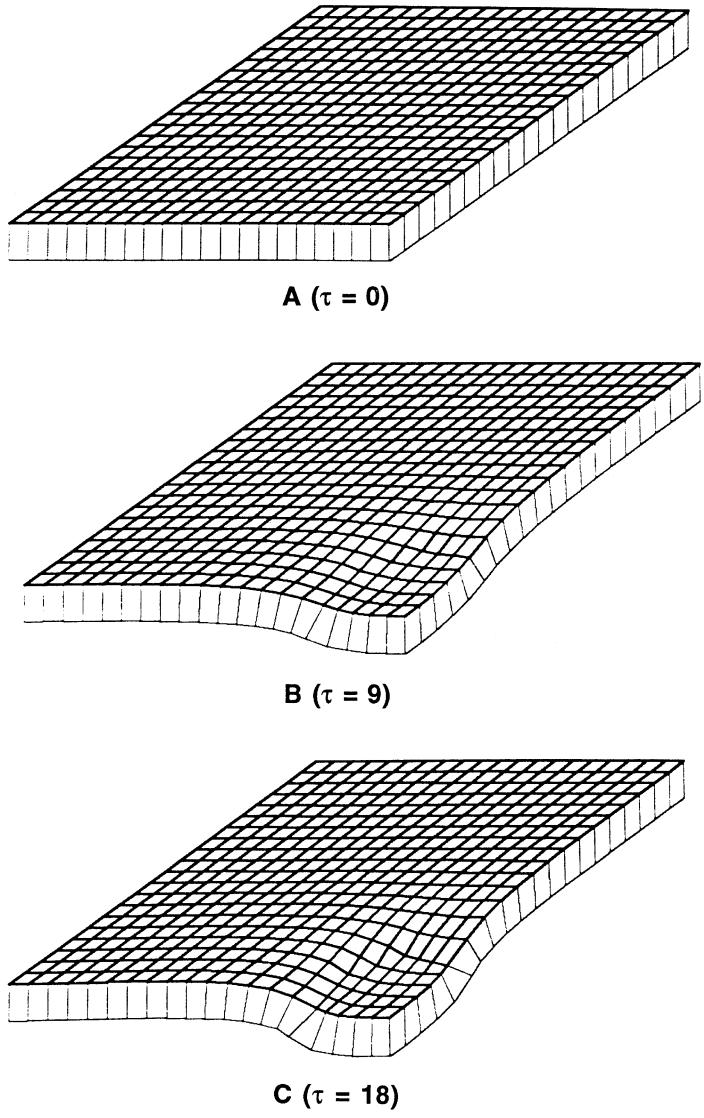

FIGURE 2.8 Simulation of an invagination. Due to bilateral symmetry, one quarter of the total tissue is shown. Microfilaments act only on the apical surface of the 5×5 cell region at the front right corner.

underlying layers. The primary outcome of this simulation is to show that purely mechanical systems can produce regular patterns.

2.8 Conclusions

A number of important principles for the modeling of biological systems have become apparent:

1. Models must be firmly anchored on established biological data. This means that properties must be derived from known morphologies and properties of cytoskeletal components and other force-generating structures. In addition, reference and progressive geometries predicted by simulations must be compared with similar data from live or fixed embryos.

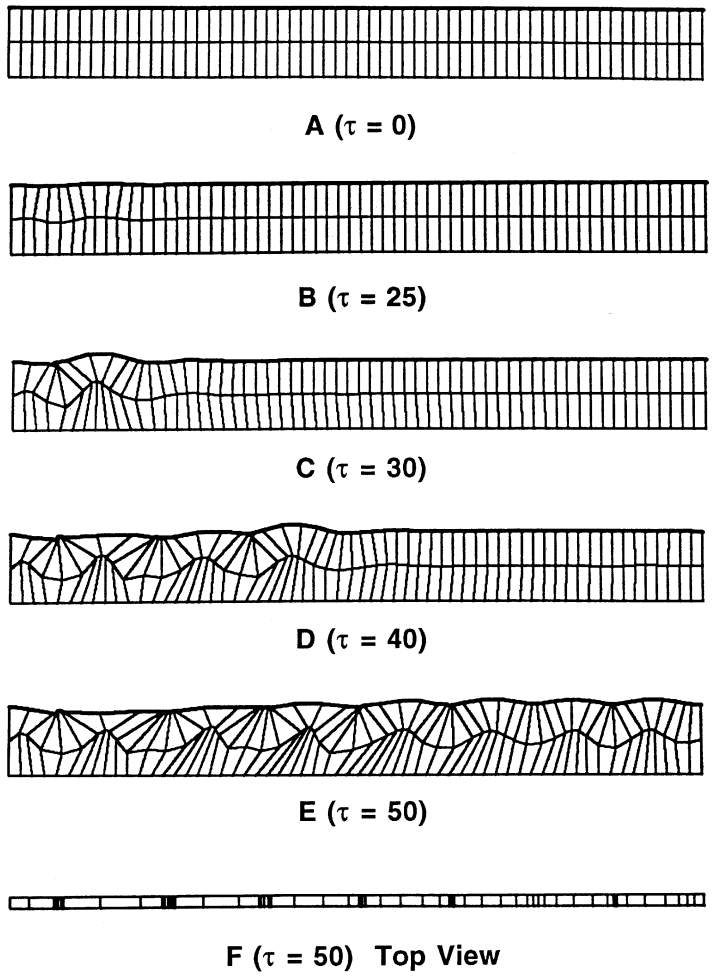

FIGURE 2.9 Pattern formation produced solely by microfilament contraction. A mechanical instability develops because the microfilaments are described by Eq. (2.1). The phenomenon is related to "hinge" formation during neurulation. After Brodland and Clausi (1994).

2. Models must have a sound mechanical basis. In studies of morphogenetic shape changes, this requires that numerical formulations must properly accommodate large strains, deflections and rotations, nonlinear material properties, and the various kinds of coupling that can occur between these.
3. Finite element models can be formulated to satisfy all of the mechanical criteria and are sufficiently general that all relevant biological data such as initial geometries and mechanical properties can be incorporated.

Much can be learned from suitably formulated finite element-based computer simulations. In particular, computer simulations provide a powerful means to evaluate hypotheses about the forces that produce specific morphogenetic shape changes such as neurulation. Because a large number of possible force-generating structures exist, it is not surprising that numerous theories have arisen. Although drug studies can provide some important information, they are often not conclusive. This is in part because standard teratogenic drugs are believed to have a variety of unknown side effects.

Simulations of neurulation have demonstrated that many popular and apparently plausible theories about neurulation are mechanically unsound. In some cases, the difficulties are apparent only after

boundary conditions and other physical constraints are applied. These simulations showed that the shapes that are produced are highly sensitive to the active forces and to the starting geometries. The presence of redundant sets of forces has also been supported by the simulations.

Computer simulations have also revealed the presence of elegant mechanical control systems. During neurulation, for example, the effect of microfilament contraction changes as the geometry of the plate changes. Since the shape changes are caused by these same microfilaments, a mechanical control system is seen to be at work. That mechanical control system causes a distinct sequence of shape changes to be produced. Mechanical control and feedback are also apparent during pattern formation.

Simulations of this kind form an important step toward the development of "virtual embryos" in which extensive virtual experiments might be carried out. It also makes an important contribution toward the development of numerical methods that overcome the technical challenges inherent in modeling embryos that are made of incompressible materials that undergo large strains, deformations, and rotations.

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