

Introduction

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Organisms are composed of cells, and cells are in turn made up of organelles, macromolecular complexes, proteins, polysaccharides, lipids and small molecules. The living world thus has a hierarchical organization as far as its composition and architecture are concerned (Harris, 1999). Causal interactions in biological systems, in contrast, move both up and down these scales. On the level of the individual organism, molecules are synthesized in a spatiotemporal fashion as result of, and resulting in, changes in cell number and state, and organismal geometry and topology. On the level of the organism type, or species, boundary conditions and compositional details are propagated across generations largely through the continuity of the genes. So while organisms are self-organizing systems, they are not entirely so. The multicomponent, multiscale, hybrid nature of living systems continues to provide novel challenges to theoretical and experimental biology.

Occupying an intermediate level of the organizational hierarchy, the cell provides a focal plane from which one can scale upward and downward. Because the cell is also the minimal unit of life, cooperation among such units is the driving principle of multicellular organization. The cell's central role was already recognized by those scientists who first came to understand that they constitute the bodies of all living things. This conviction is encapsulated in a legendary phrase of Raspail: “*Donnez-moi une vésicule organique douée de vitalité, et je vous rendrai le monde organisé*”¹ (Raspail, 1833).

Almost two centuries later Raspail's assertion remains a still-distant goal, appearing to confirm Kant's doubts that there would ever be a “Newton of the grass blade” (Kant,

¹ This phrase can be translated as: “Give me an organic vesicle endowed with life and I will give you back the whole of the organized world.”

1790). Multicellular systems have proven to be very complex. Whether cells are members of a community or components of a tissue, they are interacting continuously with one another and with their local environment. Cells live in an aqueous porous medium, where signaling molecules are subject to diffusion, transport and decay along the flow patterns generated by the movements of the cells and other structures. Cells undergo switches in type and thus in biosynthetic capability and behavior. Experimental work over the past three decades has established connections among these phenomena, and their molecular underpinnings and correlates. Some progress has been made in understanding multicellular developing systems at the theoretical level, that is, in the form of mathematical or computational models encompassing the dynamical behavior of cells and molecules at realistic spatiotemporal scales. This approach, though currently less developed than the experimental ones, is nonetheless essential for an understanding of morphogenesis and cellular pattern formation. This volume provides descriptions of recent research in this area using a wide range of systems and modeling strategies.

Constructing models is something of an art. Several models might be consistent with the data at hand; they might even yield the same mathematical or computational representation. The first role of a model is to test a verbal description arising from a biological hypothesis, using the language of mathematics which, unlike verbal reasoning, allows the outcome of complex nonlinear interactions to be precisely computed. If the model produces the incorrect predictions, then the biological hypothesis is incorrect. At a minimum, modeling can refine intuition. But a rigorously developed model coupled with experiments has the potential to accomplish far more.

The most realistic models of multicellular systems require the use of subcellular models, which can take into account biochemical kinetics and cytoskeletal mechanics. Such models are multiscale. When the subcellular and supercellular regimes are coupled these models can be used to investigate the large-scale, often visible, patterns seen in tissues.

Whether on one scale or many, then, modeling can serve two purposes. When the significant details of a developmental process are known, a mathematical model can be used as a surrogate for the living system, providing a way to carry out virtual experiments. These have the potential advantage of being more comprehensive and more rapidly performed than corresponding *in vivo* or *in vitro* tests. In such cases the model can provide a proof-of-principle that the known components are indeed sufficient to replicate important behaviors and that they interact as expected. Where important details of the developmental process are not known, modeling can serve as a tool for testing hypotheses and generating predictions. Increased understanding can arise from both approaches. Therefore we need a suite of models, each designed to address specific questions (Schnell *et al.*, 2007).

The Volume

This volume arises from the Ninth Biocomplexity Workshop held at Lake Monroe, Bloomington, Indiana in May 2006. This event was part of the Biocomplexity Institute workshop series organized by the Biocomplexity Institute and Indiana University School of Informatics. Biocomplexity 9 was titled “Multiscale modeling of multicellular systems: An interdisciplinary workshop.” Participants discussed current and future theoretical and experimental problems in the study of multicellular systems. Researchers were brought together from many disciplines, including experimental and theoretical developmental biology, applied mathematics, biophysics, engineering and computer science. In addition to containing contributions from most participants of Biocomplexity 9, the present volume has chapters contributed by several leaders in the field who were not in attendance.

We have organized the volume by starting with the chapters dealing with general concepts of pattern formation, a focus of much mathematical and theoretical biology over the last three decades (Chapters 1 and 2). Chapters 3–5 deal with the process of gastrulation, the period in embryogenesis during which cell patterns and morphological complexity are first established from relatively simple multicellular systems. After gastrulation in vertebrates, the mesoderm lying along the dorsal side of the embryo to either side of the notochord gives rise to the serially repeated somites. In Chapters 6 and 7, the reader will find two contributions dealing with models of somitogenesis, an area that has experienced a productive confluence of theoretical and experimental work. In the five chapters that follow (Chapters 8–12), the development of specific structures and organs crucial to the formation of fully functional organisms, such as lungs, glands, limbs and teeth, are explored. This volume on theoretical approaches would be incomplete without the introduction of new methodologies. Chapters 13–17 illustrate important advances in theoretical approaches to develop more realistic models of multicellular systems.

In the middle of the last century, Alan Turing showed, using a simple mathematical model, that a system of chemical reactions with stable spatially uniform dynamics in the absence of diffusion, could be destabilized by diffusion so as to assume a stable spatially nonuniform configuration (Turing, 1952). The result was highly counterintuitive at the time, since diffusion generally evens out spatial heterogeneities. Turing suggested that the chemical pattern set up by the instability could serve as a prepattern for a cellular response. The plausibility of Turing’s “reaction–diffusion” model as a biological mechanism was reinforced when Gierer and Meinhardt presented a realistic reaction–diffusion system that undergoes the Turing instability and produces a pattern. This system comprises a pair of reacting chemicals labeled activator and inhibitor (Gierer and Meinhardt, 1972). The first activates the production of itself and the second chemical; the inhibitor in turn inhibits the growth of the autocatalytic activator. Pattern formation is possible if the activator in this system diffuses much more slowly than the inhibitor, and has a shorter half-life. This led to an important principle of pattern formation: activation at short range coupled with inhibition at long range.

This principle has proved to have general utility in developing systems even when the morphogenetic signals and means of their propagation are not literally soluble molecules and free diffusion. In [Chapter 1](#), Hans Meinhardt shows in an elegant fashion how simple activator–inhibitor systems can produce cell patterns and morphogenetic changes reminiscent of those observed in many areas of development and how evolution may have employed similar dynamics in different ways to generate different forms in different taxonomic groups.

The paper by David Umulis and coworkers ([Chapter 2](#)) shows that patterns can arise by different mechanisms. One of the major problems of pattern formation is discovering the mechanisms of localized production and transport that generate positional information. During the last 30 years, molecular biologists have focused on studying molecular components involved in signal transduction and gene expression in a number of model systems in developmental biology. Umulis *et al.* focus their attention on two patterns in the *Drosophila melanogaster* embryo which have been studied extensively by molecular developmental biologists: these are the anterior–posterior and dorsal–ventral patterning of the embryo. They show how molecular components are integrated in networks, and how these networks transduce the inputs they receive and produce the desired patterns of gene expression. Umulis *et al.* discuss a number of different aspects of robustness in *Drosophila* embryonic patterning and show how the models lead to new insights concerning scale-invariance in anterior–posterior patterning, the role of network topology and signature in the switching network used for control of the segment polarity genes, and the role of signaling via heterodimers in dorsal–ventral patterning.

The stunning successes of molecular biology in recent decades have mainly provided the ingredients for the complex mechanisms of morphogenesis. The challenge now facing us is to understand how these entities integrate in the correct manner so that the whole is greater than merely the sum of the parts. Biochemical dynamics and tissue mechanics must play key roles in this process. In [Chapter 3](#), Lance Davidson shows that there is no single molecular mechanism that controls morphogenesis, but rather there is a collection of cellular processes that work together to generate the architecture and modulate the forces responsible for changes in tissue form. Davidson summarizes the early development of the frog *Xenopus laevis* from a biomechanical perspective. He describes the cells, their behaviors and the unique microenvironments they traverse during gastrulation, demonstrating the important role of tissue mechanics in development.

Manli Chuai and Cornelis Weijer discuss the current understanding of the mechanisms underlying the initial phases of gastrulation, in particular the formation of the chick primitive streak ([Chapter 4](#)). The genetic basis of anterior–posterior axis development, germ layer and streak formation has been studied extensively. However, because of the small size of the embryo at these stages, little attention has been paid to the cell movement patterns associated with gastrulation. Chuai and Weijer review current experimental evidence of gastrulation movements and the possible cellular mechanisms underlying these processes. Cellular mechanisms involved in gastrulation

may include oriented cell division, cell–cell intercalation, chemotactic cell movement in response to attractive and repulsive signals and a combination of chemotaxis and “contact following.” Chuai and Weijer critically examine the experimental evidence in favor for and against these different mechanisms and outline open questions in gastrulation research. An important conclusion of their work is that mathematical models and computer simulations have a fundamental role to play in furthering our understanding of gastrulation, since many of the interactions between cell signaling and movement are dynamic and nonlinear.

In Chapter 5, Timothy Newman explores a mechanism based on planar cell polarity to explain coordinated cell movement lateral to the primitive streak during its formation. These complex cell movements were recently observed by the Weijer group (Cui *et al.*, 2005). Newman shows via computer simulations that planar cell polarity can generate large-scale cell movement resulting in two counter-rotating vortices, similar to those observed experimentally. The complexity of coordinated cell motion is modeled with a new computational method for studying multicellular systems, known as the Subcellular Element Model. This new methodology is a powerful tool for modeling intracellular mechanisms and adaptive cell shape changes. Newman also provides a brief review of grid-free modeling approaches. In this volume, the readers will find other methods for modeling morphogenesis at the cell level, such as the Cellular Potts Model (Chapters 7 and 11), finite element methods (Chapter 11), agent-based methods (Chapter 13) cellular automata (Chapter 14), and Monte Carlo simulations (Chapter 16). All these methods have helped us in understanding the important role of individual cells and their interactions in modeling morphogenesis.

In vertebrate embryos, the anterior–posterior axis segments into similar morphological units, known as somites, after the formation of the primitive streak. These segments constitute a prepattern for the formation of the vertebrae, ribs and other associated repetitive features of the body axis. The formation of the repeated somites is one of the areas of developmental biology in which an interplay between experimental and theoretical investigations has met with great success. The idea that temporal oscillations may underlie the spatial periodicity of somite organization was anticipated by the evolutionary morphologist William Bateson in the late 19th century (Bateson, 1894), first made part of a specific model by the experimentalist Jonathan Cooke and the mathematician Christopher Zeeman more than 80 years later (Cooke and Zeeman, 1976) and confirmed experimentally by the group led by Olivier Pourquié two decades after that (Palmeirim *et al.*, 1997). In Chapter 6, Baker *et al.* review and discuss a series of mathematical models motivated by newer experimental findings which account for different stages for somite formation. These models range from the creation of a genetic prepattern to the mechanisms involved in generating morphological somites. In his contribution, mentioned above, Hans Meinhardt proposes a reaction–diffusion model for somite formation. The paper by Baker *et al.* shows that the segmentation pattern can also arise from other mechanisms. As noted previously, this is a commonplace of mathematical biology—several models can produce the same results and make similar predictions. The challenge for theoreticians is to

suggest carefully designed experiments to distinguish between models, and the challenge for the experimentalists is to design ways of doing these experiments, which may help decide between alternative mechanisms.

In [Chapter 7](#), James Glazier and coworkers propose a model accounting for how cell determination and subsequent differentiation may translate into somite morphology. The model starts from an established prepattern of adhesive and repulsive molecules, which gives rise to the patterns of cell movement and morphological changes leading to segmentation. The simulations of Glazier *et al.* are implemented using the extended Cellular Potts Model. In this model cells are extended domains of pixels on a lattice. Cell interactions are described by an effective energy and fields of local concentrations of chemicals. The effective energy combines true energies, like cell–cell adhesion, and terms that mimic energies, e.g., the response of a cell to a chemotactic gradient.

The chapter by Sharon Lubkin ([Chapter 8](#)) describes the physical forces responsible for the morphogenesis of branched ducts such as those found in glands and in the lung. Developmental biologists have experimentally uncovered a great deal of information concerning the genes and signaling pathways of branching morphogenesis. Lubkin illustrates how development must also take into account the physical aspects of change in tissue shape and form. Indeed, physics can be seen as the primary means by which alterations in molecular expression bring about such tissue changes ([Forgacs and Newman, 2005](#)). In her contribution, Lubkin reviews a collection of relatively simple and physically justifiable models for branching morphogenesis. The models presented have potentially measurable parameters which can be used to quantify the relative contributions of different mechanisms to morphogenesis. A challenge for experimentalists is to develop contexts and methods within which these novel models can be tested.

In [Chapter 9](#), Andras Czirok and coworkers review the patterning of the primary vascular plexus of warm blooded vertebrates. This is a process operating on various length scales. They show that the formation and rapid expansion of multicellular sprouts is a key mechanism by which endothelial cell clusters join to form an interconnected network. The work of Czirok *et al.* employs sophisticated microscopic methods to track cells and extracellular matrix fibers over an extended area of tissue. On the basis of these experimental observations, they propose a mathematical model of preferential attraction to elongated structures that can explain multicellular sprouting during vasculogenesis. This paper is another example of the benefits of theoretical frameworks for the comprehension of complex experimental results and *in vivo* reality.

Takashi Miura shows how mathematical models can help us understand the mechanism of branching morphogenesis, with emphasis on the lung ([Chapter 10](#)). With close attention to experimental findings, including those from his own laboratory, he reviews several models which make predictions concerning the *in vitro* systems. One of these models generates tree-like branching patterns by applying a set of simple rules iteratively. Models have been useful for understanding some aspects of branching morphogenesis, and they can be helpful for understanding the functional aspects of the bronchial tree. Miura explains how simple multiscale models can help in under-

standing both morphological and functional aspects of the bronchial tree. In addition, he shows how mathematical models can help developmental biologists with little experience in modeling gain new insights into the dynamics of pattern formation.

The development of the vertebrate limb has similarities to body axis segmentation in that a series of repetitive elements are formed, but also resembles branching morphogenesis in that more than one spatial dimension is needed to formally characterize the pattern. It is an apt developmental system for mathematical and computational modeling since it has been the subject of extensive experimental studies at the molecular and cellular level. In [Chapter 11](#), Stuart Newman and coworkers describe features of the developing limb itself, as well as a planar culture system that utilizes isolated mesenchymal cells of the embryonic limb to provide a simplified *in vitro* model for chondrogenic pattern formation. They present several different kinds of models for the various patterning processes, including a Turing-type continuum “reactor–diffusion” model that generates the well-known proximodistal order of appearance of skeletal elements, as well as a multiscale discrete stochastic model that reproduces several quantitative aspects of pattern formation *in vitro*. Since the full *in vitro* developmental process has both continuous and discrete aspects, they suggest that the most satisfactory model will have a hybrid nature.

In [Chapter 12](#), Isaac Salazar-Ciudad reviews still another developmental system that produces repeated elements—the dentition, or teeth, of vertebrates. As the author shows, moreover, this system brings into focus an important but neglected question in developmental pattern formation—the interplay between the released chemical signals termed morphogens and changing tissue geometry. It is commonly assumed that pattern formation proceeds in a “morphostatic” fashion, i.e., by the generation of spatiotemporal patterns of morphogens, followed by shape-changing tissue responses to these gradients. Salazar-Ciudad shows that this is not always the case: morphogen patterns can be dramatically affected when generated in concert with tissue rearrangements in three-dimensional-space in the form of complex developmental mechanisms that the author terms “morphodynamic.” Using computational models he shows that morphodynamic mechanisms can predict important topographic properties of tooth cusp formation. Equally important, such mechanisms must dictate more complex genotype–phenotype relationships over the course of evolution than simpler morphostatic mechanisms.

The chapter by Michael Meyer-Hermann ([Chapter 13](#)) starts with an overview of mathematical methods in biology to model multicellular systems, paying special attention to the current agent-based methods, their strengths and limitations. By developing a new agent-based method, he shows that the construction of a physically well-defined modeling architecture for dynamic cellular systems is essential in order to gain predictive power. Only when the parameters of the model are observable quantities does the model acquire the potential to be falsified, which is a prerequisite of any scientific approach. The novel method developed by Meyer-Herman is an agent-based model for cell mechanics based on geometrical representations known as Delaunay triangulations and Voronoi tessellations. The methodology combines physically realistic cell

mechanics with a reasonable computational load. He illustrates the power of the new method with two examples, avascular tumor growth and genesis of lymphoid tissue in cell-flow equilibrium.

Cells can be modeled as discrete entities distributed on a two-dimensional artificial grid. This type of simulation is called a cellular automaton, completed by assigning a set of rules governing the behavior of cells. In [Chapter 14](#), Haralambos Hatzikirou and Andreas Deutsch use cellular automata to understand the interplay of moving cells in the typical heterogeneous environment of multicellular systems. This is of great importance as cells move in a complex extracellular matrix composed of fibrillar structures, collagen matrices and other cells, which can affect the cell response to external signals. They introduce a special subtype of automaton, known as a lattice-gas cellular automaton, which has been widely used as a discrete model of fluid dynamics ([Wolf-Gladrow, 2000](#)). The extension of this automaton for investigation of cell–cell interactions and cell–environment interactions enables the observation of the macroscopic evolution of the cell population and estimation of cell dispersion speed under different environments.

Modeling approaches have strengths and weaknesses. Cellular automata are computationally efficient, and allow a wide range of cell behaviors to be implemented; however, they are also strictly defined on a grid, which may lead to artifacts, and do not easily lend themselves to analytic calculation. In [Chapter 15](#), Ramon Grima addresses this and other issues. Multicellular systems are complex and can be studied at different scales. Grima discusses how mathematical models can be constructed at different spatial scales so as to provide insight into the fundamental biological processes central to cellular pattern formation. He concludes that the simultaneous theoretical and numerical analysis of models of the same biological system at different spatial scales provides a better understanding than a single-scale model.

In [Chapter 16](#), Elijah Flenner and coworkers use a combination of experiment, theory and modeling to relate measured tissue-level biophysical quantities to subcellular parameters. Their work concentrates on the morphogenetic process of tissue fragment fusion, a phenomenon seen in many episodes of organogenesis, by following the coalescence of two contiguous multicellular aggregates. The time evolution of this process can be described accurately by the theory of viscous liquids. They study fusion by Monte Carlo simulations and a Cellular Particle Dynamics model equivalent to the Subcellular Element Model described in T. Newman's chapter. The multidisciplinary approach of combining experiment, theory and modeling provides a general and versatile way to study multiscale problems in living systems.

Nicola Bellomo and Guido Forni look at the problem of developing a general mathematical theory to model multicellular systems ([Chapter 17](#)). They use the mathematical kinetic theory for living particles to describe complex multicellular systems dealing with cell expansions, cell death and immune surveillance. Kinetic modeling describes the statistical evolution of large systems of interacting particles (e.g., cells) whose microscopic state includes *activity*, a variable related to the expression of biological function. The modeling is developed at the cellular scale, as an intermediate

between the subcellular and macroscopic scales. Bellomo and Forni apply their new theory to investigate competition between neoplastic and immune cells. This work is important in that it illustrates that while theoreticians strive to include more realistic biology in their models, they must also not fail to neglect the development of mathematical theory to underpin and justify their modeling and computational approaches.

The contributions collected in this volume show how major questions in developmental systems can be addressed through a multidisciplinary effort, with particular focus on the importance of mathematical and computational biology. The biology of multicellular systems is now among the most active areas in all of science, relating not only to embryonic development, the focus of most of these contributions, but also reparative medicine, cancer biology and immunology. The unprecedented growth of these fields and the complexity of their experimental findings require new and innovative theoretical frameworks for their successful comprehension and application. This book is intended to serve as a comprehensive review of the current state-of-the-art in the subject.

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