Integrating self-organization theory into an advanced course on morphogenesis at Moscow State University

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ABSTRACT **A lecture course on morphogenesis for fourth-year Moscow State University Specialist Diploma students specializing in embryology is described. The main goal of the course is to give the students an extensive theoretical background based on the tenets of the modern theory of Self-Organization and to show them how important this theory is for the proper understanding of developmental events. The corresponding mathematics are bound as tightly as possible to the actual morphogenetic processes. All of the lectures take the format of an active dialogue between the students and a tutor.**

Background Information

Scholarly Interests of the Author

I supervise the small Laboratory of Developmental Biophysics, affiliated with the Department of Embryology, at Moscow State University. The aim of the laboratory is to search for the basic physical laws of morphogenesis, assuming that the latter is, in its essence, a process of self-organization. As such a process, it should involve different kinds of feedback relations. Already several decades ago, we concluded that embryonic tissues are mechanically stressed, that the stresses exhibit regular patterns both in time and in embryonic space, and that remodeling of the stresses leads to extensive morphogenetic disturbances (Beloussov *et al.*, 1975, 1994). Together with Jay Mittenthal from the University of Illinois, we suggested that morphogenetic feedback can be to a great extent based upon a special kind of relationship between passive (that is, established prior to a given time moment or originating in another part of the embryo) and active (generated within a given part of the embryo and at a given time moment as a result of the activity of mechanochemical devices) mechanical stresses. We have hypothesized that the feedback is directed toward a hyperrestoration of an initial stress value. This suggestion has been tested in several kinds of experiments and by using a model approach. All of our studies today are based on self-organization theory (SOT). Meanwhile, we are well aware that the developmental applications of SOT depend not only upon the mechanics of development but also upon the chemokinetics, or on a combination of both. Therefore, in our lecture course we do not pay so much attention to developmental mechanics proper as to the general background that is common to different applications.

Representative Publications

Certainly, most recommended readings for my students are in Russian. However, for the international audience of this journal, I list below references to a few papers written in English that are more or less parallel to the Russian publications.

BELOUSSOV, L.V. (1989). Dynamical levels in developing systems. In *Dynamic structures in biology* (Eds. Goodwin, B., Sibatani, A., Webster, G.). Edinburgh University Press, Edinburgh, pp. 121-131.

This article describes briefly some elementary principles of SOT that are most relevant to developmental events, and shows how to formulate the concepts of competence, determination, differentiation and induction within the SOT framework.

BELOUSSOV, L.V., SAVELIEV, S.V., NAUMIDI, I.I. and NOVOSELOV, V.V. (1994). Mechanical stresses in embryonic tissues: patterns, morphogenetic role and involvement in regulatory feedback. *Int. Rev. Cytol.* 150: 1-34.

Here for the first time we tried to link morphogenesis, SOT, and mechanics, pointing to a possible involvement of feedback between passive and active stresses in driving morphogenesis forward.

BELOUSSOV, L.V. (1998). *The Dynamic Architecture of a Developing Organism. An Interdisciplinary Approach to the Development of Organisms.* Kluwer Academic Publishers, Dordrecht.

This book is the first comprehensive attempt to consider the development of organisms as a multilevel feedback-linked, self-organizing process. Accordingly, the data related to morphogenetic cell activities and to the development of entire organisms are reconsidered and reformulated in terms of theories of symmetry and self-organization, which are described in nonspecialist terms. The important role of biomechanical feedback is emphasized.

General Teaching Philosophy

My teaching philosophy is simple and even trivial. Its main idea is that each next course should be linked with the previous one and

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that the students should grasp during the first exposure the main ideas rather than the small details. In our department, the lecture course on morphogenesis is immediately preceded by that on molecular and cell biology (MCB). Both deal with the formation of structures. However, these two disciplines differ from each other by the important and often neglected criteria of the *characteristic spatial (linear) and time scales* of the studied processes. MCB is concerned roughly with spatial scales on the order of 10^{-9}–10^{-5} m and time scales on the order of 10^{-3}–10^{1} s, whereas morphogenetic processes are characterized by spatial scales on the order of 10^{-4}–10^{0} m and time scales on the order of 10^{2}–10^{4} s. Such a distinction is far from being purely phenomenological. A certain important barrier lies between the two scales that was first noticed by Joseph Frankel of the University of Iowa. By studying the structure of the so-called oral field in ciliates, which is composed of arrays of microtubules, Frankel noticed that up to a certain dimensional level or scale (which in this case was roughly 1 μm, but it may vary to some extent), the structure and the symmetry of the oral field components are "dependent only on the intrinsic properties of the building blocks" (Frankel, 1989, p. 143), while the larger blocks may acquire quite another type of symmetry, related to the entire body handedness rather than to the chirality of the microtubules themselves. This crucial dimensional level, which we propose to call "Frankel's barrier," is the upper limit of effectiveness of intermolecular forces, and hence constitutes a natural barrier between MCB and morphogenesis. Structures that are larger than Frankel's barrier are determined by what are essentially *macroscopic* forces, whose properties cannot be directly derived from the intermolecular forces proper. Such a situation is not a biological phenomenon only. We will return to this issue when we present the definition of autowaves given by Krinsky and Zhabotinsky (see below).

The students taking this course are assumed to be familiar with basic mathematical analysis techniques, but their calculation techniques do not need to be too refined. I often cite the French mathematician René Thom, who said that "catastrophe theory [which is a part of self-organization theory] is more about drawing pictures than it is about mathematics." The same may be said of SOT: a qualitative, rather than a quantitative, approach is preferred because it is in harmony with the principles of developmental biology.

General Features of the Lecture Course

This regular lecture course is addressed to fourth-year students in the Specialist Diploma program at Moscow State University (MSU) who are specializing in embryology (developmental biology). (By a system adopted by the Faculty of Biology, MSU, students who have completed their first year must select a field of specialization from one out of about 20 different departments; from this time on, their education becomes gradually more and more specialized). The main goal of this course is to make the students familiar with the extensive theoretical background provided by modern Self-Organization Theory (SOT) and to show how important this theory is for properly understanding developmental events. More precisely, we focus upon those developmental processes which *are associated with an ordered creation of specific structures and shapes,* that is, morphogenesis. Obviously, most developmental events belong to this category. In this lecture course, our objective is to regard morphogenesis as a shared fundamental problem, rather than to consider each different morphogenetic process separately, which is the usual educational practice.

Such a view of morphogenesis invites the students to assimilate some very basic scientific and philosophical concepts which are taught only rarely to biologists. Those are determinism, indeterminism, and stochasticity; different types of causality; the stratified structure of the world (and its subdivision into discrete structural-dynamical levels); the role of feedback mechanisms; and so on. From these abstract notions, we come immediately to very concrete developmental events, and we try to bind theory and empirical developmental biology as closely as possible.

Although the course, Morphogenesis, involves some math, I always tell the students that my purpose is not to make them professional mathematicians able to construct models. Rather, my main aims are to make them able to "understand the language of models," as they may have dealings with specialists in this field, and to teach them to formulate their own and other authors' empirical results within the framework of a strict system of concepts elaborated by SOT.

In other words, my main task is to expose the students to an elegant and universal system of concepts which are, as I strongly believe, ultimately indispensable for describing properly developmental phenomena and formulating new empirical tasks in this field. I recommend that the students not be restricted, in their future researches, to conclusions of the type "I discovered that A affects B." Such a conclusion is no more than the starting point for a real analysis. To achieve an adequate comprehension of a phenomenon under study, at least the following questions should be answered:

What are the characteristic times of the interacting processes?

To what extent are they stable (or, on the contrary, unstable)?

Are there any feedback loops within the system studied? If yes, are they positive, negative, or both?

Are the observed mutual interactions between the components of a dynamic or parametric nature?

These questions are especially important in relation to one of the most acute problems of the modern developmental biology, namely that of the relationship between genes and morphological structures. I try to demonstrate in my lectures that the only adequate solution to the problem is found within the SOT framework.

The lectures take the form of a continuous dialogue between the tutor and the students. Usually, a general mathematical task is formulated (for example, draw a "phase portrait" of a given nonlinear differential equation), and the students are invited to do so on the blackboard, helping one another if necessary. Or, alternatively, I may ask them to invent a tentative model image for a certain developmental process. As a rule, they like to do this and are very active.

Abbreviated Course Content Outline

The course is begun by claiming that the problem of morphogenesis is wider than biology itself: one can speak about morphogenesis in chemistry, solid- and fluid-body physics, meteorology, cosmology, etc. Biological morphogenesis *per se* should be regarded as a part of an overall problem of the formation of structures out of a less-structured state. This encourages us to address the classical epistemological problem of a uniform (Laplacian) determinism. We discuss why this ideology cannot explain morphoge-

netic processes either in living or nonliving natural systems. Several remarkable attempts to go beyond this ideology are also introduced (including Driesch's theory of vitalism). Meanwhile, it is only SOT (which emerged from several different sources about the middle of the 20th century) which gives a broad enough conceptual framework for treating the problem of morphogenesis.

However, before addressing SOT directly, a brief excursion into symmetry theory is undertaken. This is a relatively easy, very beautiful and useful branch of mathematics for all biologists. We focus our attention on the processes that reduce symmetry (symmetry breaks) and analyze the classical Curie principle, which is related to the causality of these processes. This will bring us in a natural way toward the idea of instability, which is central to SOT.

After this intermezzo, we come

Fig. 1. The author with a group of students who have just successfully passed the final examination (2002).

directly to the basic concepts of SOT (dynamic and parametric stability/instability, dynamic and parametric regulation) taking as examples some simple differential equations that describe auto-catalytic and autoinhibitory processes. We start with first-order equations and then introduce second- and third-order equations. These latter will permit us to introduce *bifurcations* (the main elements of embryonic differentiation) that proceed in either a "soft" or a "hard" regime. Then, we return to embryology proper and give the definitions of competence, determination, differentiation, and induction in SOT terms, which are the only terms that permit us to do this in a really strict and unambiguous way.

Afterwards, we pass from one-dimensional to two-dimensional nonlinear differential equations and study the simple and elegant methods of zero isoclines, which allow us to create "phase portraits" of dynamic processes. We also discuss the important notion of robustness. We pay special attention to limit cycles and to three mutually coupled models related to auto-oscillations, relay oscillations, and a bistable trigger regime. We take as examples cell aggregation in slime molds, growth pulsations in hydroid polyps, and mesoderm segmentation in avian (chicken) and mammalian embryos to illustrate these models.

From deterministic limit cycles, we pass to states with increased stochasticity and chaotic states, suggesting that the organisms are probably living just on the verge of chaos. Out of several scenarios describing the passage from determinism to stochasticity, we choose one associated with the logistic equation. This equation is very important to biologists because it describes (among other events) S-shaped growth. We trace the increase in stochasticity and the appearance of fractal properties by gradually changing the parameter of this equation. We notice also a periodical appearance of "islands of stability" within chaos. We discuss briefly the phenomenon of self-criticality and different kinds of noise (including

"flicker noise"). Lastly, we come to the "strange attractor" as the most important example of dynamic chaos.

In the last part (but that most closely linked to embryology) of the lecture course, we come to *space-enfolded* nonlinear dynamic equations and corresponding models. *These are just what would be called morphogenetic fields*. We begin with the famous one-dimensional Kolmogorov-Petrovsky-Piskunov-Fisher equation, in which the diffusional term, which demands nonzero values of the second derivative, is first introduced. We emphasize the importance of the coexistence of a diffusional-like smoothing factor and a local "sharpening" factor for coherent morphogenesis. Then, we turn from the one-dimensional to two-dimensional equations, which permit us to model so-called autowaves (which, unlike "classical" waves, take their energy from an active medium). We use the definition of an autowave proposed by Krinsky and Zhabotinsky: "Autowaves exemplify a new type of dynamical process that generates a macroscopic linear scale from local interactions that individually possess no linear scale at all." This is the only definition that I ask the students to learn by heart.

From wave-like processes, we finally come to stationary dissipative structures. We discuss, of course, the famous Turing equation, which, in spite of having no direct parallels in biology, greatly affected the entire scientific community by increasing the interest in self-organizing processes. Then, we turn directly to models of biological morphogenesis, first to chemokinetic models (represented most clearly by those of Gierer and Meinhardt; see Meinhardt, 1982) and then to those that include mechanical components. I see the greatest merit of the work of the two above-mentioned authors in their introduction of the ideas of short-range activation and long-range inhibition, both of which seem to be universal and inevitable. However, by my view, models that include mechanical components are more realistic. Here, we

discuss the excellent models of floral morphogenesis suggested by the late Paul Green of Stanford University (Green, 1996) and the models of mesenchymal morphogenesis proposed by Harris, Oster, and Murray (e.g., Harris *et al.*, 1984). Afterward, we pass to the model of epithelial morphogenesis (Belintzev *et al.,* 1987). Its main advantage is in introducing feedback between local and global events, the first of which is exemplified by each single cell's tendency to polarize itself, while the second is exemplified by the overall tension of a layer that prevents the further polarization of its cells. As a result, an initially homogeneous cell layer is segregated, independently of scale, into domains of highly polarized and flattened cells. Then, if time permits, we further elaborate on this and related models, taking into consideration cell intercalation movements and the patterning of axial rudiments in vertebrate embryos. I also mention the hypothesis of the hyperrestoration of stress values, suggested in 1990 by Jay Mittenthal of the University of Illinois and myself. Within the past several decades, evidence has been collected by our research group that indicates that the driving forces of morphogenesis within large enough space–time domains fit this hypothesis (Beloussov, 1998).

Fig. 2. An example of a student's answer sheet for examination questions 13 and 14. *The annotations are as follows: ...Mesenchyme of the feather rudiments in birds; equal density; fluctuation adhesion, - peak and trough. Long range: stretching (dozens of micrometers); short range (adhesion); Gierer-Meinhardt model (reaction-diffusion). (Equations). Oster-Murray-Harris model: adhesion - stretching of a substrate by forces of elastic tension. Applications of a model: Geometry of a limb: differentiation of a skeleton depends upon the geometry of a cross section. No stable solutions in transition zones - hence joints. Levels of gastrulation, 1. biochemical parameters; 2. tensile fields; 3. cell polarization. Student: Ms. Nastya Koshelyeva.*

Exam Questions

1. Describe the basic concepts of symmetry theory. Discuss morphogenesis in terms of symmetry breaks and the Curie principle.

2. Describe the concepts of dynamic stability and instability using linear chemokinetic models as examples.

3. Describe the concepts of structural (parametrical) stability and instability using linear chemokinetic models as examples.

4. Please give definitions of competence, determination, induction, and differentiation in terms of models of "soft" and "hard" bifurcations based on third-order nonlinear differential equations.

5. Describe the D.S. Chernavsky trigger model of cell differentiation. Discuss variability during the transition period (as predicted by the model), its embryological and evolutionary significance.

6. Describe the concepts of limit cycles and orbital stability. Distinguish between robust and nonrobust limit cycles.

7. Discuss the importance of positive and negative feedback in developing systems. Include in your discussion equations with a small parameter, the concept of an attractor, and models of auto-oscillations and relay and trigger regimes. Choose examples from slime molds, hydroid polyps, and somitogenesis in the chicken embryo.

8. Discuss the transition from determinism to chaos, using the logistic

equation as an example. Describe fractal structures and their role in biological systems.

9. What is a "strange attractor," and why does it conflict with Laplacian determinism?

10. Describe self-organized criticality and define flicker noise.

11. What are space-enfolded systems. How are regular space structures created from noise. Use equations with a diffusion term. How did Krinsky and Zhabotinsky define an autowave.

12. Define the following: Stationary dissipative structures; Turing model; hysteresis; senior and junior modes.

13. Describe the Gierer-Meinhardt model of morphogenesis. Discuss the concepts of a short-range activation and long-range inhibition and the role of concentration gradients in embryonic patterning. Include arguments both pro and con.

14. Describe the role of mechanical stresses in organizing cell movements and in patterning embryonic tissues. Discuss the Oster-Murray-Harris model of mesenchymal morphogenesis as a mechanical analogue of the Turing model (Fig. 2).

15. Describe Belintzev and coauthors' model of epithelial morphogenesis. How is a cell layer patterned according to this model? What role do contact cell polarization and elastic tensions play. Give an example of a scale-invariance.

16. How are changes in a layer's geometry modeled? Use hydroid polyps as an example.

Textbooks for Assigned Readings

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BELINTZEV, B.N. (1991). *Fizicheskie Osnovy Biologicheskogo Formoobrazovanija* (*Physical Foundations of Biological Shape Formation*). Nauka, Moskva (in Russian).

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