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Limb pattern, physical mechanisms and morphological evolution - an interview with Stuart A. Newman

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ABSTRACT Stuart A. Newman grew up in New York City. He received a Bachelor of Arts from Columbia University and obtained a Ph.D. in chemical physics from the University of Chicago in 1970. He did post-doctoral studies in several institutions and disciplines with a focus on theoretical and developmental biology. He had a rich experience interacting with people like Stuart Kauffman, Arthur Winfree, Brian Goodwin, and John W. Saunders, Jr. He was also exposed to many interesting experimental models of development. These early experiences fostered his interest in biological pattern formation. He joined the State University of New York at Albany as a junior faculty member when Saunders was still there. With his physical science background, Newman's approach to limb bud patterning was refreshing. In his major *Science* paper in 1979, he and H.L. Frisch proposed a model showing how reaction-diffusion can produce chemical standing waves to set up limb skeletal patterns. He then used limb bud micromass cultures for further development and testing of the model. Extending earlier ideas, he developed a comprehensive framework for the role of physical mechanisms (diffusion, differential adhesion, oscillation, dynamical multistability, reaction diffusion, mechano-chemical coupling, etc.) in morphogenesis. He also applied these mechanisms to understand the origin of multicellularity and evolution of novel body plans. Here Newman reflects on his intellectual growth, and shares with us his ideas on how pattern formation works, and how generic physical mechanisms interact with genetic mechanisms to achieve the evolution and development of animal forms.

KEY WORDS: *Systems biology, theoretical model, reaction-diffusion*

Stuart Newman was born in New York City (USA) in 1945. He received his Bachelor of Arts (B.A.) from Columbia University in 1965. Then he moved to the University of Chicago and completed a Ph.D. degree in chemical physics in 1970. Stuart A. Rice was his mentor. Late in his graduate student career, he attended a summer school in theoretical biology in Fort Collins, Colorado. This experience provided him with the opportunity to meet Brian Goodwin and Stuart Kauffman, pioneers of systems biology. His postdoctoral studies, carried out at several venues, led to interactions with many distinguished scientists and exposure to many interesting developmental systems and concepts. These included work in theoretical biology in University of Chicago from 1970 to 1971, and 1973, where he interacted with scientists such as Stuart Kauffman, Arthur Winfree and Leon Glass, among others. He was exposed to *Dictyostelium* development studies. He spent some time at the University of Sussex, in England (1971-72), and learned to do micro-manipulations on hydra under the guidance of Gerry Webster. He had frequent discussions with Jonathan Cooke who later developed the clock-and-wave model for somitogenesis, the evolutionary biologist John Maynard Smith, the chair of his host department, and the evolutionary geneticist Richard Lewontin, who was on sabbatical at Sussex during the same period. He spent a summer learning embryology at the Woods Hole Marine Biology Laboratory in 1973, where he studied with Eric Davidson, Gary Freeman, Joseph Gall, and Fotis Kafatos, among others. He also spent some time at the University of Pennsylvania, and gained experience in cell culture from chicken embryo tissue. These extraordinarily diverse experiences have fostered his appreciation of biological pattern formation and the roles of theory in this process.

In 1975, he started his faculty job at the State University of New York at Albany. Saunders, the pioneer in limb bud research, was also in that department at that time. In 1979, he moved to New York Medical College in Valhalla, New York, where he is currently a professor of Cell Biology and Anatomy.

Newman's research can be categorized in the following areas:

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cellular and molecular mechanisms of vertebrate limb development, physical mechanisms of morphogenesis, and mechanisms of morphological evolution. However, they all center around the theme of biological pattern formation. In 1979, he published a major paper in *Science*, proposing that the mechanism for patterning of the vertebrate limb skeleton is based on reaction-diffusion and self-organization. To test the model further, he carried out biochemical work to characterize the effect of growth factors and extracellular matrix molecules on the periodic patterning process of cartilage nodules in limb bud micromass cultures *in vitro*. He emphasized the role of physical mechanisms in pattern formation and morphogenesis. These physical mechanisms include diffusion, differential adhesion, oscillation, multi-stability, reaction-diffusion, mechano-chemical coupling, etc., which have been relatively neglected in comparison to molecular mechanisms. He developed models to explain the origin of multicellarity and evolution of novel body plans. Based on experiments in limb bud micromass cultures, he and his colleagues further developed a multi-scale stochastic model to describe and predict the patterning behavior of skeletal precursor cells. In addition, Newman has written commentaries about cultural and ethical aspects of biological research and its impact on society.

Newman is an original thinker. His way of thinking deserves more attention in the field of developmental biology. In the following, we asked Newman to reflect on the background of his education, intellectual growth, and how he develops his ideas and views on morphogenesis.

Would you please briefly introduce your educational background, and scientific career?

I am a developmental biologist, educated in the physical sciences, with a research focus on vertebrate limb development and a long-term theoretical interest in the evolution of developmental mechanisms.

I was fortunate to receive my early education in the then premier New York City public school system. As a high school student, I was introduced to evolutionary theory (via the textbook *Life: an Introduction to Biology*, by Simpson, Pittendrigh and Tiffany, which had recently appeared), theoretical developmental biology (via a *Scientific American* article by S. Meryl Rose), and the social history of scientific discovery (via Lancelot Hogben's *Science for the Citizen*), in special interest courses presented by a group of remarkable teachers at Jamaica High School in Queens. I later learned that the evolutionary biologist Stephen Jay Gould had also attended this neighborhood school, a few years before me.

I also had the privilege of enrolling in the newly established Science Honors Program at Columbia University, a post-Sputnik institute for science-oriented New York area high school students. There I took a course in population genetics given by Richard Lewontin, then a young faculty member at the University of Rochester, who traveled to New York City for a semester's worth of weekends to lecture to a group of mostly wide-awake teenagers on early Saturday mornings. All of this occurred before I was 16 years old.

During that period I became fascinated by both the Linnaean taxonomic scheme and the periodic table of the elements. I imagined that, analogously to chemical theory's explanation of the latter, the former might some day yield to an analysis by which it could be generated from "first principles." In a way, my subsequent career has been motivated by this goal.

Fig. 1. Newman as a graduate student aged 24 (1969). *Photo by Jura Newman.*

I went on to study chemistry at Columbia, where I also benefited from wonderful teachers who were capable of imparting immense enthusiasm for theoretical analysis of complex problems – Harry B. Gray, Martin Karplus, S. Malcolm Miller and Raymond Disch, among others. I also worked for a summer at Rockefeller University, in the laboratory of Rollin D. Hotchkiss, one of the founding figures of molecular genetics, and absorbed biological concepts and practical knowledge from Professor Hotchkiss's patient explanations and those of his recently joined colleague, Alexander Tomasz. This early exposure to individuals who were at once at the top of their fields and exemplars of the most positive human qualities was to provide fortification against cynicism when this did not always hold true.

For graduate work I chose the University of Chicago's chemistry department, determined to work with Stuart A. Rice. I had attended a seminar he gave at Columbia, and though I could barely follow it, he impressed me enormously with his masterful excursions between theory and experiment. The fact that a world-class scientist shared my first and middle names (a trope that would, strangely, appear again in the course of my training), also appealed to my adolescent sense of destiny.

The Rice group was an ideal venue in which to pursue my doctorate, and chemistry the ideal subject. Stuart Rice was interested in so many different questions in chemistry and condensed matter physics that there was virtually no restriction on the prob-

lems one could work on in these broad areas. Unlike physics, where theory constituted a nearly autonomous scientific culture, and developmental biology, where, up until then, it had been entirely marginalized, chemistry was a field in which theory and experiment had existed in a productive dialectic for more than a century.

My early interest in biology had remained with me, although I had taken no formal courses in the subject as an undergraduate. Ilya Prigogine was a yearly visitor during the mid-1960s to the James Franck Institute, which housed our research group, and his periodic lecture series began to open new routes to crossing disciplinary boundaries between the physical and biological sciences. Members of our laboratory, including a senior graduate student, Leon Glass, who was later to become a renowned theoretical physiologist, were working on the theory of liquids, using dynamical models and methods of statistical mechanics. In my naïveté, I imagined that similar approaches would be applicable to the network of biochemical pathways I had seen on a wall-chart in Alex Tomasz's laboratory at Rockefeller, and undertook to formulate a "theory" of metabolism for my doctoral research. Stuart Rice, having little biology himself, dispatched me during the summer of 1969 to a summer school in theoretical biology in Fort Collins, Colorado, organized by James Danielli of unit-membrane fame, under the auspices of the American Institute of Biological Sciences. AIBS was an arm of NASA, the U.S. space agency, which that summer was flying high, quite literally. It was therefore a very favorable period in which to contemplate the general principles of life, here on Earth and perhaps elsewhere.

At Fort Collins, I met two pioneers in what is now called "systems biology," Brian Goodwin, with whom I was later to do a postdoctoral fellowship at the University of Sussex in England, and Stuart Kauffman, who was about to join the department of theoretical biology at the University of Chicago that fall. Stuart's new work on Boolean switching networks provided a template for the continuous element modeling for metabolism I had been struggling with, and I enlisted him to join my thesis committee. I now shared first and middle names with both my major research advisors.

When I completed my thesis, I moved over to the theoretical biology department, which under the leadership of the neural network theorist Jack Cowan had recruited a number of individuals who were, or who were on their way to becoming, seminal figures in biological theory: Kauffman, as mentioned, the great conceptualizer of biological oscillations Arthur Winfree, term visitors such as Brian Goodwin and John Maynard Smith, and postdoctoral fellows such as Leon Glass, recently returned from Edinburgh where he had worked on pattern formation with Christopher Longuet-Higgins. Other university faculty with (genuinely active) cross appointments in theoretical biology included the ecologist Richard Levins, who conducted evening seminars in his home on the science of complex systems, Richard Lewontin, in whose Saturday class I had sat as a high school student, and the solid-state physicist Morrell Cohen, who, with his innovative experimentalist colleague Anthony Robertson, was analyzing *Dictyostelium discoideum* development as a set of physical problems. Cohen's graduate student Vidyanand Nanjundiah, now of the Indian Institute of Science in Bangalore, became a life-long friend and, like me, a physical science-trained developmental and evolutionary-developmental biologist. My retrospective image of myself in this luminous company is as something of a scientific Zelig[1].

A close institutional arrangement devised by Jack Cowan and John Maynard Smith permitted me to spend a portion of my theoretical biology postdoctoral experience at the University of Sussex, where I was hosted by Brian Goodwin who was exceptionally generous in sharing his perspectives and insights. The developmental biologist Jonathan Cooke, with whom I had constant discussions, was a postdoctoral fellow in the same group. Though primarily an experimentalist, he had a keen appreciation of the role of theory in pattern formation. A few years later, in collaboration with the mathematician Christopher Zeeman, he was to publish a classic of theoretical developmental biology, the clock-and-wavefront model for somitogenesis, which was verified experimentally more than two decades later.

Another member of the Sussex group was Gerry Webster, who had earlier published several important papers on pattern formation in hydra with Lewis Wolpert. From Gerry I learned how to perform microsurgical manipulations on this organism, which led to my earliest forays into experimental developmental biology. After returning to the United States, I spent the summer of 1973 participating in the Embryology course at the Woods Hole Marine

Fig. 2. Newman in India (1986). *Time out from a workshop at the North-Eastern Hill University, Shillong, on "The Living State".*

Note 1: Leonard Zelig is an innocuous character in a 1983 film directed by Woody Allen who is led by enigmatic circumstances into the world of celebrities and public figures.

Biological Laboratory, where I was introduced to newly invented methods for the molecular analysis of gene expression. This was followed by a second postdoctoral stint in the laboratory of the tissue biologist Howard Holtzer at the University of Pennsylvania, where I gained skills in preparing cell cultures from tissues of chicken embryos, which were to provide the main experimental system in my subsequent studies. Formal training thus completed, I moved on to a first faculty appointment. This was at the State University of New York at Albany (now the University at Albany), where the legendary limb developmental biologist John W. Saunders, Jr. had recently moved. He encouraged my appointment, having kindly remembered our meeting during a speaking visit he made to the University of Chicago several years before.

Early in your career, you published a remarkable conceptual paper in Science, entitled "Dynamics of skeletal pattern formation in developing chicken limb" (Newman and Frisch, 1979). Can you tell us what was in this study? How did the experience of doing that study shape your research direction? How has the paper impacted the field?

This paper, which was written in collaboration with my astonishingly insightful physicist colleague H. L. (Harry) Frisch at Albany, who sadly died in 2007, sought to provide an explanation for what I believed (and still believe) to be the most salient aspects of the developing vertebrate limb – the proximodistal increase in the number of elements and the quasi-periodic arrangement of elements in the zeugopod (i.e., radius and ulna, tibia and fibula) and autopod (fingers, toes). These are the features that would jump out at anyone who looked at this developmental phenomenon through the eyes of a physical scientist. Most limb developmental biologists at the time, however, perhaps coming out of an anatomical tradition, considered the existence of *differences* along the proximodistal, and more subtly, the anteroposterior and dorsoventral axes, more important than any repetitions or other regularities.

Our explanation, in the 1979 paper, of the general (what we have more recently termed “bare-bones”) limb pattern was based on a process capable of producing chemical “standing waves,” that is, repetitive stripe- and spot-like distributions of a molecule. The simplest such mechanism is the so-called Turing reaction-diffusion instability, in which positive and negative feedbacks in production of diffusible morphogens favor a nonuniform chemical pattern rather than the uniform distribution typically promoted by diffusion. In the 1979 paper we did not explicitly present a Turing system for the generation of the patterns of skeletal elements. Because knowledge of the molecular genetics of limb development was poorly characterized at the time, this would have been a more-or-less empty gesture. What we did instead was to *postulate* that a Turing mechanism underlies the formation of the molecular stripes corresponding to the primordia of the long bones of the limb, and to ask what form the skeleton would take under a set of changing, biologically realistic, conditions. These conditions included suppression of cartilage differentiation in an apical zone of the limb bud by a morphogen (separate from the reaction-diffusion morphogens) released by the apical ectodermal ridge (AER), with differentiation occurring in cells that lay just proximal to a threshold level of the suppressor. We also invoked Dennis Summerbell’s empirical finding that the proximodistal length of the undifferentiated apical zone declined progressively with development in the chicken limb, and asked what the consequence of this would be for the number of waves formed by a presumed reaction-diffusion mechanism.

What we obtained from this set of assumptions and biological boundary conditions was the generation of a proximodistally increasing number of parallel skeletal elements, in a fashion that was entirely dependent on the AER. In particular, removal of the AER was predicted to lead to terminal deletions, as found experimentally. We made no attempt to model differences across the anteroposterior and dorsoventral axes, but noted that gradients of molecules that acted as modulators of the basic pattern could determine such differences.

While evidence has accumulated for the validity of major aspects of this model over the past three decades (mainly through the efforts of my students and, most notably, the work of Takashi Miura and Kohei Shiota in Kyoto), the causal basis of the general form of the limb is still not a central concern for most limb developmental biologists. As a consequence, our 1979 paper and subsequent work in this vein by ourselves and a few others are rarely cited by the most active laboratories in this field. What has been perplexing to me is that our model (which has undergone a number of upgrades in mathematical and molecular detail and sophistication), and the phenomena it addresses, are not even discussed by way of dismissal.

In the mid-1980s a multinational group of investigators published a formally similar model for limb development, utilizing standing waves of progressively increasing number to model the proximodistal emergence of skeletal elements. In that version, the standing waves were mechanically compressed and expanded domains of mesenchyme rather than chemical concentrations. This provided a warrant, in the view of the authors, not to cite the 1979 *Science* article as a precedent. In any case, the mesenchyme

Fig. 3. Newman in New Zealand (1989). *Family excursion during period of Fogarty Senior International Fellowship at Monash University, Melbourne, Australia. Photo by Jura Newman.*

Fig. 4. Newman and colleagues at a workshop on "Phenotypic and Developmental Plasticity", Trivandrum, India (2007). *From left to right: Isaac Salazar-Ciudad, Gerd Müller, Stuart Newman and Vidyanand Nanjundiah.*

compression mechanism was eventually disconfirmed experimentally. Since then, as reaction-diffusion (and more generally, local autoactivation-lateral inhibition) mechanisms have gained credibility throughout the field of developmental biology, in systems as varied as patterning of feather and hair germs, coat colors, and glandular duct branching, the reaction-diffusion idea is occasionally mentioned in relation to limb development, but usually without much attention to the earlier work. Some investigators who have studiously avoided the idea over the last 30 years may now find it difficult to acknowledge its original source.

How did this experience shape the direction of my research? Despite the lack of interest from the immediate field, I saw no reason to shift my emphasis away from what I considered to be the most important aspect of the limb development problem. I was also gratified by the attention paid to our ideas by investigators and writers outside the limb field, including some with a deep understanding of the entire field of developmental biology, like Scott Gilbert. Beginning with early editions of his widely used textbook, Gilbert has provided a context for a broad appreciation of our model and related theoretical approaches.

"Reaction" and "diffusion," the formal concepts of the model, are much more complex than their counterparts in nonliving physicochemical systems. These phenomena, which do not lend themselves to analysis by widely used techniques such as *in situ* hybridization in whole mount embryos, are more easily studied in limb cell cultures than in the intact limb. Most of our experimental work has therefore focused on the *in vitro* system. While most physical scientists (including biological physicists) would probably be receptive to the idea that the spot and stripe patterns of precartilage condensation seen in culture arise from self-organizational processes akin to those that generate the developing limb skeleton, this is not the way most traditionally trained investigators in limb developmental biology see it, further distancing our work from that of many active limb groups. This has inevitably affected the way such work is reviewed by funding agencies. Still, it has been possible to obtain adequate research funding and thus to push this program forward. The National Science Foundation, and particularly Judith Plesset, its program director for Developmental Mechanisms during the time our concepts were taking form, have been very supportive.

Can you tell us the background of that paper? What observations or evidence shaped your line of thinking?

The genesis of the paper was my having perceived a quasi-periodic pattern in the limb, which struck me from observing museum and laboratory specimens. Had I been familiar with the late 19th century writings of the English biologist William Bateson, who pondered the significance of such repetitive organizational motifs, I would have seen this even earlier. Stuart Kauffman and his colleagues had been using reaction-diffusion models to understand patterns of compartment formation in the *Drosophila* embryo, and this gave me the motivation to bring the limb problem to Harry Frisch. It was Harry who "biologized" the problem by putting aside the impulse of a mathematical modeler to produce a rigorous (but inevitably oversimplified) Turing-type equation system, and to concentrate, rather, on the pattern selection rules determined by tissue size and shape, and other measurable system parameters. It was I (to the surprise of both of us), who came up with a set of mathematical functions that simultaneously satisfied the constraining relationships among the variables and parameters and produced limb-like skeletal patterns. Our collaboration, then, as is often the case with such cross-disciplinary endeavors, involved an element of role reversal.

We are all made of molecules. So in many instances, scientists knock out one gene after another to obtain clues on what molecules are involved. What do you think about this molecular analytical approach?

In some ways this approach is more illuminating when there is little or no change in the phenotype than when there is a large change. Phenotypes are produced by developmental systems, not individual genes acting alone. To make an analogy, you might be able to extract a proton from a nitrogen atom and turn it into a carbon atom. But examining the proton does not tell you why it has this effect. You need to know something about the systems it participates in to understand its role in different contexts. For atoms and their constituents, quantum mechanics is the appropriate theory. For development, our theories are still primitive. What allows an embryo that employs N-CAM in a hundred different contexts to develop essentially normally when N-CAM is knocked out? Some global organizing principles are at work, but we only have small hints of what they may be.

Over the years, you have promoted the concept of using generic, rather than genetic processes at the center for developmental biology. Is this in conflict with the specific molecular approach? How does one balance the molecular analytical approach and generic model type approach?

Generic properties and processes, understood as characteristics of relevant categories of materials, living or nonliving, are

inevitable determinants of organismal form and function. To take a simple case, *mass* is a generic property, and gravity acts on things according to how much mass they have, regardless of whether they are made up of one kind of atom in a lump of gold, or hundreds of thousands of different kinds of molecules in a frog embryo. From this example it can be seen that "generic" and "molecular" are not in opposition to each other, but are aspects of the same system. Specific properties are, of course, based on specific composition: a gram of mercury does not behave in all respects like a gram of gold. But to a gravitational field they look pretty much the same. Since developmental systems are entities on a larger spatial scale than molecules, understanding how they are reshaped over time must involve the generic physics of bulk materials, not just the interactions of specific molecules.

The earliest stage embryos are clusters of cells. It is well-known that cell clusters behave like droplets of a viscous liquid, and exhibit surface tension. So the default state of an early embryo is a sphere. Are all embryos spheres? Certainly not. But when we encounter one that is, understanding the generic properties of materials draws us away from seeking a specific genetic mechanism for constructing a spherical tissue mass. In contrast, we may be inclined to search for a special non-generic mechanism when we encounter a flattened or elongated embryo.

Incidentally, a cluster of cells will round up whether they are held together by N-cadherin, L-cadherin, N-CAM, or even an uncharacterized cell adhesion molecule. This is another aspect of generic mechanisms: they always involve molecules, but it doesn't always matter which ones.

To take another, more sophisticated, example, we now know from the remarkable work of Olivier Pourquié and his colleagues that vertebrate somitogenesis involves a molecular clock that ultimately leads to spatially periodic changes in tissue adhesion at somitic boundaries. Why does expression of certain genes oscillate in time? This is a question Brian Goodwin took up in the early 1960s and has been carried forward by other theorists, including Julian Lewis and Nick Monk. It can involve negative and positive feedbacks, time lags, etc. There is a mathematical theory of oscillations that has nothing to do with genes, but all indications are that gene expression oscillations are manifestations of the same generic processes described by that theory. An oscillatory regulatory molecule can perform functions that the same signal at a stationary concentration cannot. But it is the same molecule. The only thing that's different is that its interactions with other molecules are tuned in such a way that it is produced and broken down periodically with time. No gene is inherently oscillatory in its expression; in fact, recent work shows that gene products that oscillate during mouse somitogenesis are not entirely the same as those that oscillate during zebrafish somitogenesis. It would seem, then, that in order to understand oscillation-dependent pattern formation it is as important to enlist a mathematical colleague as it is to hit the microarrays.

It should be obvious from this that the biological systems within which generic mechanisms act are identical to the ones within which genetic mechanisms act. The point is, not everything that happens is due solely to genetic interactions. But while there is thus absolutely no conflict between the molecular analytical approach and consideration of generic mechanisms, one should not assume that all forms that originated as a result of generic processes continue, over evolution, to employ such processes in an exclusive fashion. To revisit an example mentioned previously, while many embryos may be spherical due to surface tension, others may have evolved zonae pellucida or cytoskeletal scaffolds to ensure they remain that way during key developmental stages when other forces are at work.

I began thinking about the relation between generic and genetic determinants of form in these precise terms during an extended visit to the Monash University (Australia) laboratory of my friend and colleague, the biophysical chemist Wayne Comper, in 1989. We intended to write a review about physical mechanisms of development and kept coming up with examples, such as the stripes of pair-rule gene expression in the *Drosophila* embryo, where it *looked* as if a physical or chemical-dynamic mechanism had been involved, but the genetic complexities suggested something different. We found that by "historicizing" developmental mechanisms you could have generic determinants acting early in evolution to establish morphological templates (and, in many cases, persisting to modern times), with genetic mechanisms continually arising to stabilize and reinforce the outcomes and, in some cases, apparently "taking over." The results of my work with Wayne were published as *'Generic' and genetic mechanisms of morphogenesis and pattern formation* (Newman and Comper, 1990).

You have developed the generic principle concept and apply these principles to explain Evo-Devo, evolutionary developmental biology, in your 2003 book in *"Origination of Organismal Form"*. Can you tell us more about this book?

This book arose from discussions I had with my colleague Gerd Müller, of the University of Vienna and the Konrad Lorenz Institute for Evolution and Cognition Research (KLI). Gerd had been thinking for some time about developmental plasticity as a mechanism of evolutionary innovation. Based on the confluence of our ideas, we had begun working in 1999 on a paper, eventually published in *J. Exp. Zool. B. Mol. Dev. Evol.* the following year, on evolutionary developmental biology (Newman and Müller, 2000). Our theme was an elaboration of our common conviction that generic physical processes and other unprogrammed, inherent tissue properties (collectively, "epigenetic determinants"), were likely to have established the morphological motifs of metazoan body plans and organ forms early in evolution, due to the inevitable morphological plasticity and biochemical excitability of cell aggregates. While much genetic evolution followed this initial burst of origination and innovation, we suggested that it was mostly of a reinforcing, stabilizing nature. (See my example, above, on spherical cell aggregates. The paper contained many more illustrations of this principle).

This proposal inverts the neo-Darwinian paradigm, which holds that large-scale differences between organismal forms (e.g., at the level of phyla) accumulated over vast amounts of time as a result of incremental changes due to genes of small effect. Our view, instead, was that large-scale morphological differences arose rapidly due to epigenetic effects (in the broad sense, defined above), in organisms that were initially genetically very similar, and that genetic change followed, rather than caused, such diversification.

Although this analysis contained echoes of the ideas of scientists active in the early part of the twentieth century, such as William Bateson, J. Mark Baldwin, D'Arcy W. Thompson, C.H. Waddington,

Fig. 5. Newman at home in New York, 2007. *Photo by Jura Newman.*

I.I. Schmalhausen and Leo Berg, it was formulated entirely in terms of contemporary molecular-developmental and evolutionary biology. In our work-in-progress, we had been drawing on research from many different fields – among them paleontology and comparative anatomy, developmental genetics, tissue morphogenesis and pattern formation and dynamical systems theory. Under the auspices of the KLI (Gerd is its director), we organized a workshop in October, 1999, to which we invited about 15 investigators who had broken new ground in the areas we were concerned with. We were hoping to educate ourselves, and perhaps also to recruit the disparate group of participants to our emerging viewpoint. The first goal was definitely accomplished, the second somewhat less so, although we are still trying. In any case, the early volume in the Vienna Series in Theoretical Biology, with a mixture of topics in developmental and evolutionary biology that had probably never before appeared between the same covers, has stimulated a lot of discussion.

An interesting bit of lore about *"Origination of Organismal Form"* is the annoying enlistment of it to the cause of the "Intelligent Design" movement. As I said earlier, it takes a position that is in conflict with certain tenets of neo-Darwinism. This immediately drew the attention of all manner of creationists, who mined the book for passages that could be portrayed as calling naturalistic evolution, rather than the standard account of it, into question. They struck gold with our statement in the book's introduction that the origination of forms, as opposed to the transformation of existing ones, remained an unsolved problem. Needless to say, in the scores of citations of the book by creationists there is no mention of the focus, by us and other contributors to the volume, on the organizational effects on tissue masses of the physics and chemical dynamics of condensed materials.

You have recently published a book on *"Biological Physics of the Developing Embryo"*. Can you tell us about the theme of this book?

The physicist Gabor Forgacs (University of Missouri-Columbia) is one of my longest-standing colleagues. We met at Albany when we were both collaborating with Harry Frisch on entirely different subjects; Gabor's having nothing whatever to do with biology. Several years after we went our separate ways, I contacted Gabor again to help us interpret a set of morphogenetic phenomena my graduate student Dorothy Frenz and I had observed in cell-free extracellular matrices *in vitro* (Newman *et al.,* 1985). This initiated a more than two-decades collaboration, during which time Gabor was bitten by the biology bug and has become an important biological physicist.

Picking up on my earlier work with Wayne Comper, Gabor and I decided in the late 1990s to see if we could write a developmental biology text from first principles, that is, saying something useful about all the major stages and transitions during embryogenesis – blastula formation, cell-type switching, gastrulation, neurulation, somitogenesis, vasculogenesis and so on, using "generic" physical and chemical-dynamic processes and concepts. I think we were fairly successful in this, having had a wealth of excellent research to draw on from a recent profusion of studies in systems and computational biology. I am convinced that the sequencing of the human, mouse and other genomes spurred a new era in developmental biology as much by what it didn't deliver as what it did. The field seemed to be holding its breath in anticipation of the vaunted developmental "programs" and "blueprints" that were promised to appear once genomes were fully sequenced. When these failed to turn up, previously unfashionable mathematical and physical approaches to morphogenesis and pattern formation began to attract new interest. Of course, the rise of high-speed computers and increasingly sophisticated mathematical methods for handing complex systems helped this new trend along. Theoretical agendas in biology that were first broached in the mid-twentieth century, associated with the names Ludwig von Bertalanffy and Nicolas Rashevsky, were unrealizable without substantial technical progress in hardware, software, and concepts of dynamical systems that occurred after that period.

In our textbook we tried to stay away from the notion that any one kind of physical model can explain all or most of development. Where the subject matter calls for it, we introduce the reader to viscoelasticity, adhesive forces, oscillations and dynamical multistability, reaction-diffusion mechanisms, fractals, percolation, electrical potential, mechanical excitability, among other concepts and phenomena.

Because it is more difficult to measure the physical parameters or test generic principles, sometime they are criticized as "only theoretical" or "non-precise science". Can you tell us the evidence that these models are indeed operating *in vivo*? What caution should be taken for those who adopt this approach?

Certainly it is difficult to make these measurements, but scientists are inexhaustibly clever and keep coming up with new ways of measuring oscillations in gene regulation and signaling, morphogen transport, viscoelasticity of tissues, and so on. (We discuss a number of such examples in *"Biological Physics of the Developing Embryo"* and more have appeared since.) It is, of course, similarly difficult to measure rates of gene expression and absolute levels of transcription factors, which are is the bread-and-butter of "a-theoretical" developmental biology. I heard a talk by Leroy Hood not long ago in which he showed that levels of mRNA abundance in a number of standard systems, and directions of change in these levels under various treatments, were frequently uncorrelated with

the levels and directions of change in the cognate proteins. It seems to me that this raises questions about the reliability of the reigning experimental paradigm in developmental biology, which depends to an enormous extent on data from whole-mount *in situ* hybridization and microarray analysis. Experimental difficulties and ambiguities are endemic in all areas of this field. We can only do our best with the techniques and concepts we have. But it would be unwise, in my opinion, to question the existence or relevance of physical processes in development just because they are difficult to measure.

What is the driving force for the patterns in the biological world? As you know, the non-biological world can also form patterns as discussed in Hazen's review on geological patterns (Hazen, 2009 in this issue). So is it just the inevitable consequence of physical-chemical interactions upon which evolution selects? Or, is it driven by some forces unknown to us?

In my view, patterns in the biological and physical world have similar causes, but there are important differences. As mentioned above, aggregates of cells exhibit surface tension, so they tend to round up – a simple pattern – because physical law determines that surface tension tends to a minimum, consistent with other constraints. If two tissues have different surface tensions (or cohesivities, which have the same physical basis), they will not mix, and a boundary will form between them, as with oil and water. In fact, the tissue with the lower cohesivity will engulf the more cohesive one, again as with physical liquids. This set of phenomena – differential adhesion – has been studied for many years by Malcolm Steinberg and his colleagues, and has been shown to account for arrangements of cell populations during some developmental processes, such as localization of *Drosophila* oocytes and pancreatic islet cells. Are tissues like liquids in all respects? Clearly not. Their constituent "molecules" are cells, and unlike the molecules of nonliving liquids they have a complex, energy-consuming, reactive world within them. The differential adhesion hypothesis does not attempt to explain things at the intracellular scale, but it is simply unscientific to ignore what it says about tissue and cell patterning at the macroscopic scale of the embryo.

Nonequilibrium physical mechanisms similar to the ones that generate mineral formations, ripples of sand on the beach, and waves on water and clouds are also relevant to developmental pattern formation. The big difference is that no nonliving system has nearly as many components and possibilities for their interaction as a living one. And while chemistry successfully describes and predicts the outcomes of highly precise interactions among *submicroscopic* atoms and molecules, the interactions that occur among nonliving *mesoscopic* entities (things on the same spatial scale as cells and embryos) are typically much less exact and regular. Studying abstract systems, mathematicians like Henri Poincaré and Alan Turing characterized conditions for the precise interactions among idealized physical components that would produce periodic behavior in time or space, but the real world of nonliving matter contained few examples of such phenomena.

We now know that the dynamical oscillations and reaction-diffusion-like processes studied by these theorists underlie somitogenesis and a variety of "spacing patterns" in embryos. But as with the liquid phase-separation mechanism mentioned above, the interacting entities are quite different from the simplest ones that are capable of exhibiting these behaviors. That being said, the patterns form for the same reasons.

BOX 1

RESEARCH LANDMARKS

Year	Significance	Reference
1977	Isolation of pure population of precartilage cells from avian limb bud	Newman, 1977
1979	Proposal of reaction-diffusion model for vertebrate limb skeletal pattern formation	Newman and Frisch, 1979
1985	Description of Matrix-Driven Translocation	Newman *et al.*, 1985
1989	Evidence for role of fibronectin in mediating precartilage condensation	Frenz *et al.,* 1989a,b
1990	Proposal that multiple physical processes underlie generation of animal form	Newman and Comper, 1990
1991	Proposal and evidence for TGF-β as key morphogen in skeletal pattern formation	Leonard *et al.*, 1991
1994-2000	Proposal of physical and epigenetic basis for evolutionary origination of body plans	Newman, 1994; Newman and Müller, 2000
2002	Evidence for role of FGFs in lateral inhibition of condensation during skeletal pattern formation	Moftah *et al.*, 2002
2003	Multi-authored consideration of physico-epigenetic evo-devo perspective	Müller and Newman, 2003
2003	Description of varieties of pattern formation mechanisms and their evolutionary consequences	Salazar-Ciudad *et al.*, 2003
2005	Textbook presentation of "physicalist" framework for developmental biology	Forgacs and Newman, 2005
2004-2007	Mathematical and computational analyses of local autoactivation-lateral inhibition (LALI) mechanisms for chondrogenic pattern *in vivo* and *in vitro*	Hentschel *et al.* 2004; Christley *et al.*, 2007
2008-2009	Proposal of the concept of "dynamical patterning modules" and the associated "pattern language" for evolution and development of animal form	Newman and Bhat, 2008, 2009

Why do biological systems exhibit these patterns? Looking at things through the lens of physical science raises the surprising possibility that there may be no particular reason – not optimization of a function, not benefit to survival or reproduction. Patterns may exist because they are inevitable, given enough molecular complexity and richness of interactions. Once such "novelties" emerge they may find some use, and thus help establish new evolutionary lineages. But this "function-follows-form" scenario is quite different from the standard neo-Darwinian picture of large phenotypic differences emerging gradually due to small adaptive genetic changes, with marginally different populations progressively superseding one another over very long periods of time.

Scientists now have genomic databases, microarrays and bio-informatic tools for a more systematic approach to a biological problem. How would these new disciplines affect our approach to pattern formation? Do you think it will help to mend the gap between the molecular and model approaches? What kind of progress can we expect in 5 years, 10 years, 20 years?

My feeling is that bioinformatic tools will ultimately confirm the utility of theoretical and modeling approaches. This is happening already. After a number of false starts, whereby a series of "-omics" have quickly succeeded one another and have proved equally inadequate to capturing the holistic reality of physiological and particularly developmental systems, a more sophisticated multiscale, dynamical systems-based paradigm has begun to take hold. New biological journals with terms like "systems," "computational," and "physical" in their titles have joined more venerable theoretical and mathematical ones, while mainstream high-profile venues like *Nature*, *Science* and *PNAS* have become much more

receptive to model-based approaches. I would guess that two decades from now the conceptual framework of the genetic-determinist developmental biology of the last quarter of the 20th century will seem like just so much celestial epicycles and phlogiston[2].

Do you think the study of biological pattern formation is pure basic research or it can also have practical use?

I think anything that is true about the natural world has both pure scientific and practical implications. I didn't used to think so. When I moved from the physical science fields of my training into developmental biology during the era of the Vietnam and "Cold" wars, I was consciously leaving behind the disciplines that had given us nuclear bombs, ballistic missiles and napalm, and entering the arcane world of mosaicism vs. equipotency and the "epigenetic landscape." Who would have guessed that in 30 years time half the developmental biologists would be involved in commercial ventures and some would even be endorsing the creation of headless clones for spare body parts?

What is your motto in doing science? If a young scientist is fascinated by biological patterns and contemplating whether he/she wants to enter this field, what advice would you give?

I think it is essential to scrutinize one's preconceptions every step of the way. These will inevitably include the received notions that "everyone believes" but which hold science back, sometimes for more than a generation. Taking this seriously will require giving respectful attention to views, if carefully argued, that may seem at first like fringe notions, as systems biology did in the 1950s and 60s and the role of phenotypic plasticity in evolution did throughout the 20th century.

With regard to pattern formation, it is important to approach these questions at the right level or scale, though what this is may not always be obvious. What is certain, however, is that confining oneself exclusively to the level of "genes communicating with genes via gene products" will not provide solutions to these difficult problems.

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Note 2: "Epicycles" were hypothesized complexities of motion introduced by early astronomers to account for planetary orbits under the assumption of a geocentric system. "Phlogiston" was a substance hypothesized by 17th century chemists to be released from combustible bodies as they burned; this idea was discredited by the discovery that burning involves the uptake of a substance (oxygen) rather than the release of one.

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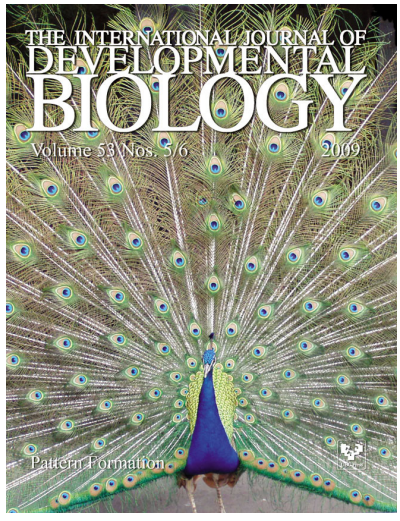

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