Problems and prospects in morphogenesis

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Introduction

Towards the end of the last century there arose in Germany a strong reaction against the claims of Darwinists that they had succeeded in developing exact causal explanations of life in all its diverse shapes and forms, and that these were based on natural laws as mathematically precise as the law of gravitational attraction. The principles of variation and natural selection were presented as the logical basis from which the facts of biology flowed as the necessary effects of known causes. What physiologists such as His and Goette observed was that the historical reconstructions of Darwinism in terms of organic continuity, variation, and adaptation, far from settling the causal problems of biology, did not even *state* them because there was within the theory no account of how organisms are actually produced and reproduced. This was not simply because of the absence of any satisfactory theory of inheritance in Darwinism.

More fundamentally, it was the recognition that no historical theory of descent could explain the causes behind the distinctive manner in which organisms are made, via the process of development. Wilhelm Roux, the most celebrated pupil of Goette, was a cogent critic of the propositon that Darwin had found the real efficient causes of the complex forms of which the living world is made. His position was that despite the great amount of phylogenetic detail that can be organised coherently within the theorys of descent, there is a fundamental difference between simply following processes in their temporal course and understanding them causally. For Roux the real causes of biological processes were to be found in the mechanisms responsible for generating organisms before natural selection can act on them, to which study he gave the name 'Entwicklungsmechanik' (Development Mechanics). Without a developed theory of morphogenesis, he claimed, there can be no exact biological science because the whole of evolution depends upon this process of production and reproduction of organisms. Roux's theoretical manifestoes and his incisive experimental studies laid the foundations for a tradition of exact causal analysis in biology that has continued to this day.

The relevance of this little bit of history to the present state of play in the study of morphogenesis is that, as is its wont, history is repeating itself. Just about 100 years after Roux's initial publications on developmental mechanics, there is again emerging a movement to ground evolution causally in an exact understanding of developmental processes. Accompanying this, there is a reaction to the excessive claims of the neo-Darwinian 'evolutionary paradigm'[24] to provide causal explanations of morphogenesis. The particular form of this paradigm in relation to development is the notion that the generation of organisms is directed or caused by the information contained in the part of the organism that is believed to be fashioned by history, the genome. The objection to this is basically the same as Roux's critique of Darwinism's failure to provide a causal account of development but the criticism now takes place at the level of molecular rather than organismic phylogeny. The evolutionary paradigm presents the view that all aspects of biological processes are to be understood in terms of the histories of genomes (or replicators), and the sequence of molecules that these produce during the life histories of organisms. Once again, what this view fails to account for causally is the formation of organisms, those particular forms of spatiotemporal order out of which the living realm is fashioned. So morphogenesis has once again slipped through the net of historical description, and biology remains without an exact causal foundation.

The problem of morphogenesis

What is morphogenesis? It is the shape changes that developing organisms undergo during their life histories or during the process of regeneration. The capacity to generate complex morphologies and to regenerate these after loss of parts is shared by unicellular eukaryotic and multicellular organisms. A mature green alga such as *Acetabularia mediterranea* has such a large size and intricate form that the casual observer would assume that it is a multicellular plant, though it is a single giant cell. During its growth from a spherical zygote about 50 μm in size, the plant passes through a series of well-defined spatial forms to reach the adult morphology, with an overall length of 3–5 cm. This is very much larger than a hatched frog larva, which is only a few millimetres in length though the latter is much more complex in internal and external morphology, a complexity generated from a spherical zygote 1–2 mm in diameter.

Algae and frogs are but two of an immense diversity of organismic forms all of which are a result of morphogenesis. Making sense of this diversity requires the discovery of general morphogenetic principles. How is this to be done? The classical scientific procedure is to seek regularities of form in classes of organism and their parts as a prelude to seeking common generative mechanisms that

express law-like behaviour. This was actually the enterprise of the 'rational morphologists' of the late 18th and early 19th centuries such as Cuvier, Geoffroy St. Hilaire, Reichert, and Richard Owen, who addressed the question whether there are laws of form in biology. They demonstrated that there are indeed systematic morphological regularities over large groups of organisms, such as the limb structure of tetrapods and vertebrate skulls, which can therefore be understood in an abstract sense as transformations of one another. It was this theme that D'Arcy Thompson [21] developed in his theory of coordinate transformations applied to the adult morphology of a great variety of organisms. But he took the argument much further than this. He proposed an explicit mechanical theory of biological form that was an application of the physical principle of least action: that the shapes of organisms are minimal energy configurations, or shapes that minimize stresses, derivable from what are known as variational principles. And he provided much evidence for this from the shapes of simple organisms and of bones. Such principles are still used to explain aspects of morphogenesis [8], but there is not yet a general theory that links the dynamics of morphogenesis to the geometrical shapes generated by variational principles.

Generative principles of limb formation

A phenomenological theory of morphogenesis that was remarkably successful in unifying a diversity of observations on organisms across a large taxonomic range arose out of experimental studies on limb regeneration in amphibians and insects. There is an extensive classical literature on this problem based on studies of the types of limb structure regenerated when host limb stumps and donor limb tissue are grafted together in different combinations. One of the most striking findings was that if a composite is made by grafting a left limb (or limb blastema, depending on whether the experimental animal is an insect or an amphibian) onto a right stump, or vice-versa, two additional limbs of the same handedness as the stumps are produced at the junction between host and graft. Furthermore, these two supernumerary limbs always form in a well-defined location. There is no way in which a right stump and a left limb can be grafted together so as to match up, because of their mirror-symmetries. The supernumeraries form precisely where there is maximum mismatch between the limbs.

Why should this occur in organisms as different as newts and cockroaches? Here indeed is rule-like behaviour in morphogenesis. The phenomenon of spontaneously triplicated limbs had been remarked on many years before by W. Bateson [2], who also observed the characteristic alteration of handedness between the three structures and the relation to the handedness of the primary limb. This came to be known as Bateson's rule. But a formal explanation of the phenomenon came only with the publication by French, Bryant and Bryant [7] of a model that showed how such relational order arises from the operation of three simple rules describing the behaviour of the morphogenetic field. The first of these is a smoothing principle: where there are discontinuities in field values due to the grafting procedure, the field smooths out. The second states that, if smoothing can occur via two different paths (as always exist around a circle, such as the circumference where the limbs are joined), then the shorter path is followed. This is a principle of least effort. The third rule says that wherever a complete circle of field values (corresponding to a limb circumference) is produced by the operation of the first two rules, a limb will be produced whose handedness is determined by the circle of values, whether clockwise or anticlockwise. For obvious reasons, it became known as the clock-face model. It stimulated a great deal of research because it made a number of testable predictions. Some held up but others failed, the third rule in particular being violated [12]. It remains an elegant piece of abstract modelling that formalized very simply the behaviour of any field involving a Laplacian (the spatial smoothing operator) on cylindrical or conical domains with handedness (i.e. limbs, where the primary morphogenetic field is restricted to the surface tissue).

The focus of experimental work on limb regeneration is now at the molecular level, where the problem is the extremely difficult one of identifying which molecular species do what jobs in relation to the smoothing processes that influence adjacent cells states, the control of gene activity that is associated with cell differentiation, and the interactions within and between cells that result in changes of shape. There have been unexpected revelations concerning the effects of particular biochemicals on morphogenesis. An example is the discovery [11, 12] that exposure of regenerating or developing limbs of the amphibian or the chick to retinoic acid can dramatically alter morphology in characteristic ways, resulting in extensive duplications of the limb. A recent detailed study of this rather simple chemical in the chick limb bud [20] suggests that it functions as a shape-regulating factor in normal limb development. Thus a significant molecular component of the process appears to have been discovered, though its mode of action remains to be understood.

The study of the molecular biology of morphogenesis is essential to a complete understanding of the process, and the new recombinent DNA techniques applied to the spatial localization of specific gene activity during the development of *Drosophila* embryos is producing some extremely interesting results, about which more will be said later. However, there is clearly also a place for new techniques of investigation that can reveal large-scale coherent processes, and those with novel emphasis on the relevant types of course operating during morphogenesis. This multi-author review contains both of these, including descriptions of non-invasive techniques for imaging different aspects of embryos (NMR and magnetometry)

and emphases on physical mechanisms (e.g. electrical currents and ionic fluxes). What these lead towards is a new biophysics of morphogenesis, very much in the spirit of Roux' developmental mechanics. And this does seem to be a very promising direction in which to move, because the most serious deficiency in the study of morphogenesis is an understanding of the causal dynamics underlying the global symmetry-breaking processes whereby characteristic spatial order (microns to millimetres) emerges over characteristic developmental times (minutes to hours) in developing organisms. These are the processes that generate polarity in eggs and embryos, radially symmetric forms such as tentacles in hydroids, phyllotaxis in plants, bilateral symmetry, secondary fields with handedness (limbs, ears, etc.) in animals, and so on. This is the stuff of which biological form is made, and it is the least understood aspect of development. What are the physico-chemico-mechanical forces that cause these progressive transformations from simple to complex morphology in developing organisms? If there are biological universals, an exact science of the regularities that underlie morphogenesis, then this is where they are to be uncovered.

Metabolic and morphogenetic pathways

Let me make a point about the relationship between general morphogenetic principles and their particular expression in different species of organism by considering an analogy between metabolism and morphogenesis, which really goes back to ideas discussed by Goldschmidt and Waddington, two eminent explorers of developmental principles. What makes metabolic pathways *possible* is the differences in chemical potential between substrates and products. These thermodynamic relationships are measured, under conditions of constant temperature and pressure, primarily by free energy differences. There is no way in which a metabolic sequence can run up a free energy gradient. Like the proverbial river that provides us with such a rich source of metaphors, metabolism always run downhill, products having less free energy than substrates. A metabolic sequence is a series of such downward steps, from one or more (relatively) stable metabolite(s) to the next. The set of possible metabolic sequences is determined by these thermodynamic properties.

The rate at which different steps in a possible sequence occur is dependent not upon the free energy difference between substrates and products, but on the activation energy involved in converting one metabolite into another. In the real world of processes, rates are where the action is, and organisms control these by enzymes which reduce activation energies, and by ligands that secondarily affect an enzyme's ability to influence these energies. All of this is very familiar, and I apologize for the repetition of basic biological knowledge. However, what should be equally basic knowledge about morphogenesis is not so widely disseminated. In the metabolic case, there is a clear distinction between the laws of thermodynamics that make metabolic pathways possible, and gene products (enzymes) that alter rates of metabolic transformation. Consequently, gene products do not make metabolism possible; they stabilize particular expressions of the laws of thermodynamics in particular organisms by influencing specific transitions and by cross-linking rates in different pathways via control signals. The universal features of biochemistry are dependent upon basic chemistry and thermodynamics; the particulars that characterize different organisms arise from gene product specificities. Similarly gene products influence the various possible morphological transformations during development by facilitating the transition from one spatial pattern to another, defining a morphogenetic sequence. The problem of defining the exact causes of morphogenesis is then the spatial analogue of the thermodynamic problem. What is the set of possible spatial forms of developing embryos and what forces are responsible for their transition? This is the contemporary form of Roux's question about the exact causes of development, and its study defines the continuation of his research programme.

The first exact answer to this question was provided by the mathematical genius, Alan Turing[23], who showed that when the kinetics of particular kinds of metabolic reaction are combined with the diffusion of metabolites, then under certain parametric conditions spatial patterns of metabolites arise spontaneously. This was a completely counter-intuitive result, for diffusion is a dissipative process that tends to destroy non-uniform spatial order. However, the laws of physics and chemistry dictate that spontaneous symmetry-breaking can occur, resulting in periodic spatial patterns in the concentrations of metabolites. Such processes can in fact generate a whole series of periodic patterns of different wavelengths. These different patterns are referred to, in the linear theory of such spatio-temporal fields, as harmonics. These grow into stable solutions of the full non-linear reaction-diffusion equations.

If these solutions are the initial components of form in organisms, then what gene products do is to influence the stability of different solutions and facilitate the transitions from one to another in transient sequence by affecting the parameters. But gene products do not cause the spatial patterns in the first place. These are a result of the laws of physics and chemistry as expressed in processes that are characteristic of living organisms, though not exclusive to them. For Turing waves can also occur in vitro, a celebrated example being the Beloussov-Zhabotinsky reaction[26]. This particular process reveals the additional property of wave propagation: the spatial periodicities move with a well-defined velocity, so that the whole pattern is dynamic. These systems can show different types of wave behaviour, spirals as well as concentric circles, and closed cycles of activity known as limit cycles.

So these fields have a rich spectrum of possible spatial and temporal patterns, defining a set of possible generators of biological form. Possible instances of such propagating waves in embryos have been described by Cooke and Zeeman[4] and by Saunders and Ho[17].

The biophysics of morphogenesis

Turing's achievement was remarkable, but it does not provide the solution to the problem of morphogenesis. The reason is that a spatial pattern in the concentration of metabolites within a developing organism does not itself explain the actual geometry of say, the tentacles on a hydroid, the leaves on a plant, or the limbs of an amphibian. Morphogenesis, as the name implies, is the generation of structures of specific shape, whereas spatial patterns of chemical concentration arise within some predefined geometry.

In order to get morphology, work has to be done in deforming cells or cell sheets into specific shapes, and growth must be localized to generate specific structures. There are various hypotheses about how spatial patterns of chemicals (called in this context 'morphogens') are converted into actual structures such as eyes or limbs, but these tend simply to invoke again genetic programs that switch on or off specific genes to do the job[27,28]. Now there is no doubt that such switching processes accompany morphogenesis, i.e. specific biochemical microenvironments in developing organisms influence the transcription states of genes. There are now beautiful demonstrations of such transcript patterns for a variety of genes in *Drosophila* embryos. What these studies are revealing is not simply the spatial patterns of gene activities in embryos that correspond to the mutant phenotypes observed when particular gene products are defective, but the much richer sequence of spatial states followed by the transcripts during development, only one of which correlates with the pattern deficiencies in the mutant[10,25]. We are thus able to visualize the actual sequence of harmonics through which gene activities develop. The picture that is now emerging from these studies is a series of global spatial patterns in the *Drosophila* embryo that proceed from simple to complex through a well-defined sequence of increasing wavenumber, the exact pattern varying for different gene transcripts.

This corresponds to the conclusions of classical embryological studies, that spatial complexity emerges gradually through progressively finer delineations of spatial detail as a global developmental field undergoes transformations in increasingly detailed spatial partitionings. This process can now be described as a sequence of solutions of the morphogenetic field whose superposition results in progressively finer spatial detail. However, the field is actually non-linear, so that initial superposition gives way to complex stable patterns.

This description does not solve the problem of the relationship between gene activity and the sequence of morphogenetic field solutions. How do gene products facilitate particular transitions, and stabilize a particular combination of wave solutions corresponding to the particular morphology of a species? In order to answer this, which is now the big question in morphogenesis, it is necessary to know more about the exact nature of morphogenetic fields and the types of influence that act upon them. We know that there is a coupling between the chemistry (or biochemistry) and the mechanics of development, because mechanical work is done during the process and gene products influence this, mutations giving abnormal morphology. In addition, there is a coupling to the electrical and ionic life of the cell, as demonstrated in recent years primarily by the work of Jaffe and his colleagues, described in this volume. The challenge is to put together these components, the electrical, the biochemical, and the mechanical, to define an integrated process that can account for both the spatial order and the hereditary stability of this order in different species. This is not simply the problem of morphogenesis, but of reproduction itself, the basis of organismic life-histories that is the stuff of biology. This would be the causal theory of the organism that Roux sought, without which there cannot be an exact biological science. So it is a problem that is not going to be easily solved.

Nevertheless the foundations for such a theory do now appear to be emerging from a combination of developments, experimental and theoretical. Cells do mechanical work through the activities of the cytoskeleton, whose state is modulated by a variety of ions (particularly Ca^{2+} and H^{+}) and metabolites. Its functional continuity with the plasma membrane and both the cell wall in plants and the extracellular matrix via membrane proteins in animals extends this integrating structure outwards[1] while the continuity with the nuclear membrane and the nuclear cytoskeleton, although still points of dispute, suggest a structural foundation throughout the whole cell. So the dynamics of the cytoskeleton and its extracellular extensions suggest a possible basis for a morphogenetic field theory that integrates electrical, biochemical, and mechanical activities into a spatio-temporal pattern generator.

In addition there is the extensive evidence for specific patterns of cell adhesion in animal embryos that result in differential forces whose effect is to transform one spatial arrangement of cells into another, depending upon the exact pattern of interactions of the cells and constraints on their movements. Thus cells in an aggregate can sort out into layers[18,19], cell sheets can bend and buckle in particular ways, discs can transform into limbs[13] and a variety of shapes can result from specific temporal and spatial patterns of cell adhesion molecules on their surfaces[6]. In plants, cells are constrained by their walls so that forces causing morphogenetic changes arise in a different way, resulting from the strains occurring as cells divide in particular planes determined by previously established stress patterns. Green[9] has produced a con-

vincing analysis of the full range of leaf phyllotaxis patterns produced by the apical meristem as a result of such stresses and strains in a morphogenetic field. The challenge now is to integrate these components of morphogenesis into a theory that contains the relevant elements of Turing's theory with a mechanical treatment of geometrical deformations.

An indication of how this might be achieved was provided by Odell et al.[16] in a description of the first major morphogenetic event in animal embryos, gastrulation. Since then a mechanochemical theory of morphogenesis has been developed which describes how cell deformations and movements can arise from the interactions of the cytoskeleton with calcium, and of cells with one another and the extracellular matrix[14,15]. This work has been extended to apical morphogenesis in plant cells[3,8]. These mechanochemical fields have the requisite dynamic properties of spontaneous symmetry-breaking and pattern formation as in Turing reaction-diffusion fields, but they are accompanied by geometrical changes due to the mechanical behaviour of the cytoskeleton, the extracellular matrix, and in the case of plants, the effects on cell wall deformation and growth. A variety of experimental studies connecting calcium and ionic fluxes to symmetry-breaking and axis formation in many different embryos, plant and animal, support the validity of the cytoskeleton-ion membrane system as a primary pattern generator. Also the cytoskeleton is a highly conserved structure throughout the eukaryotes, unicellular and multicellular, and it is these organisms rather than the prokaryotes that have complex morphogenesis. The protein constituents of the cytoskeleton are as stable in their primary structure over evolutionary periods as the homeobox sequences found in what appear to be fundamental regulatory gene products, suggesting an equally basic role.

What is now required is an integration of the elegant and fascinating results of gene transcript studies, revealing dynamic spatial patterns of gene activities in developing organisms, with a biophysical theory of the mechanics of pattern generation and transformation. How do gene products stabilize or transform these patterns; and how do the microenvironments resulting from these systematic differences of spatial state affect gene activities? Answers to these questions would provide a causal theory of morphogenesis of the type that Roux envisaged in his Entwicklungsmechanik. At the same time the historical or evolutionary aspects of organisms that Roux rejected on the basis of a sufficient theory of morphogenesis would be reconciled with the exact, universal properties of organisms as systems with a distinctive type of spatio-temporal organization that is most clearly seen in their morphologies.

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