The Way of the Cell

Molecules, Organisms and the Order of Life

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MORPHOGENESIS: WHERE FORM AND FUNCTION MEET

> "The main battle ground . . . is the problem of the relation of function to form. Is function the mechanical result of form, or is form merely the manifestation of function or activity? What is the essence of life, organization or activity?"
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> E. S. Russell (1)

> "There is a clear research programme for the study of biological morphologies as natural forms, as attractors in the space of morphogenetic field dynamics."
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> B. Goodwin (2)

DYNAMIC ARCHITECTURE OF EUKARYOTIC CELLS
FORCE AND COMPLIANCE
APICAL GROWTH
FIX ME AN AXIS
AMOEBOID CELLS: FORMS IN FLUX
ARRANGING A CILIATE
IN PURSUIT OF WHOLENESS

Biology began as an observational science, with its focus on the morphology of organisms and their parts; as far as the general public is concerned, the fascination of the living world still springs from the variety and beauty of its forms. And why not? We are visual creatures and know our world first and foremost by what we can see; were it not for those forms we could not recognize organisms or reflect upon their nature.

Scientists tend to view the subject from another angle. In the

that underlie the formal rules. To the visible frustration of investigators in this area, progress along that line continues to be stymied. Electron microscopy has not revealed anything that could correspond to a grid of positional values, biophysical approaches have not identified plausible gradients of mechanical or electrical parameters, and as yet we do not know in biochemical terms what ails the mutants in which the specification of pattern is perturbed. There is no shortage of ideas, some of which will be outlined in the following section, and optimists expect the crucial clue to pop up momentarily. But it may also turn out that we are in the position of the Mullah Nasruddin, who dropped a gold coin in the bazaar but insisted on searching for it under the lamp because the light was better there.

Perhaps we should prize the doubt, for the very intractability of the problem assures us that we are confronting a genuine mystery. One can argue that ciliates are unique creatures that have invented developmental mechanisms without parallels elsewhere. For myself, I suspect that ciliates are not extraordinary, except insofar as their elaborate cortical architecture parades a capacity for large-scale pattern formation that is universal but generally inconspicuous. After all, spatial order on the scale of micrometers to millimeters is visible in many cells, and we have already taken note of the widespread use of spatial markers in cell morphogenesis and continuity. When we do at last learn how these cells position their oral ciliature or contractile vacuole pores, the gains are likely to reach far beyond the humble organisms in which the discovery was made.

IN PURSUIT OF WHOLENESS

Morphogenesis illustrates at the cellular level what Warren Weaver meant when, fifty years ago, he identified the problems of organized complexity as biology's high frontier. A cell of *Tetrahymena*, say, with its particular morphology, anatomy and life cycle represents a pattern in space and time. Each such pattern coordinates the activities of innumerable molecules into a unified structure, reproduces itself periodically and persists in this manner indefinitely. From the examples discussed in the preceding sections it seems self-evident that the generation and perpetuation of such extended patterns cannot be understood solely in terms of the local, random, and scalar chemical events that are the stuff of molecular science, but depends on organizing principles that operate on the scale of cells and organisms three to five orders of magnitude larger. Curiously, this perception seems quite foreign to the majority of experimental biologists; its primary home is the still-emerging

field of complexity studies, inhabited by physicists, mathematicians, and computer mavens (20). Whether ideas spawned on those marches of experimental science can reveal principles of pattern formation and morphogenesis that have eluded us so far still remains to be seen. The musings that follow draw on the writings of many of the pioneers, particularly on those of Lionel Harrison and Brian Goodwin (21), whose influence pervades this section.

Consider for a moment how, as a matter of physical principle, regular forms and patterns arise in nature without the intervention of living organisms. There are not many. One way turns on structure: molecules come together into crystals according to rules inherent in their geometry and chemistry. In like manner, macromolecules assemble more or less spontaneously into ribosomes, viruses and membranes. Many of those who approach living organisms from the genetic and molecular perspective appear to believe that such molecular agglomeration can, in principle, account for the form of cells, even organisms. I have argued earlier (Chapter 5) that local interactions will not suffice because a cell is not a self-assembling structure, and a little reflection on the preceding examples of eukaryotic growth and morphogenesis should resolve any lingering doubts. The other purely physical sources of spatial order turn on kinetics: many dynamic physical systems organize themselves spontaneously into extended patterns whose basis resides in flow rather than structure. One such is the Bènard instability, the spontaneous emergence of discrete convection cells in a pan of oil uniformly heated from beneath. Others are a flame, a thunderstorm and a whirlpool. Chemists are particularly fond of the Beloussov-Zhabotinski reaction, a mixture of certain organic and inorganic chemicals that generates concentric rings of colored products that diffuse outward over a distance of centimeters. Biologists have never been persuaded that such purely physical and chemical processes are relevant to what goes on in an amoeba or an embryo, because we are so firmly wedded to the genetic program that informs living organisms, but is absent from nonliving ones. But dynamic patterns generated by physical and biological systems do have something in common that hinges on the concept of a *field*.

Like other metaphors imported from everyday life, a field is an elastic idea whose content depends on the speaker. I shall employ the term in a very general sense, to designate a territory that displays coordinated activity controlled by the differential distribution of some property or agent. The virtue of this abstract notion is that it lends itself to mathematical formulations that incorporate such features as continuity of field values at every point in space, smooth transitions and directional

change. Fields have the holistic quality that, given a global mathematical expression and a few local numerical values, it is often possible to reconstruct the field in its entirety. Furthermore, since the essence of a field resides in its mathematical description, one can examine the properties of a field without knowing anything about its physical nature. That is a great advantage, for the agents and properties whose distribution determines field behavior come in many forms. Fields of force (electrical, magnetic, or gravitational) are familiar, but fields of biological interest can also be sustained by a concentration gradient, or by a pattern of mechanical stress and strain. The fields most pertinent to morphogenesis and patterning are those generated by dynamic rather than static systems; the flame-like character of living things is more than a poetic simile.

Dynamic systems are characteristically maintained in a state remote from equilibrium by a continuous flow of energy. Given the right parameters, physical systems of this kind commonly undergo spatial self-organization, with concurrent enhancement of the energy throughput (when that heated pan of oil produces convection cells, the rate of heat transfer rises). Such patterns were designated "dissipative structures" by Ilya Prigogine, who regards them as one of the chief sources of order in the universe (20). Note that, like a living organism, a dissipative structure coordinates the random motions of innumerable particles over an extended territory, and may persist indefinitely so long as the supply of matter and energy lasts. The behavior of dynamic systems is typically non-linear. Over a certain range, an incremental input of energy or matter produces an incremental output, but at a particular threshold there is an abrupt change in behavior (Bénard's pan of oil or an excitable cell). Non-linearity is commonly a consequence of feedback interactions among coupled processes; their mathematical description calls for a sequence of coupled differential equations. To be sure, designating a growing hypha or a regenerating ciliate as a dynamic field does not in itself explain anything. But the label helps to focus the mind on the features that call for explanation, and it highlights parallels with the physical world that can be described with a common formalism. The fact that, in a growing number of instances, the field formalism rationalizes or predicts biological behavior, and sometimes allows one to compute the shape an organism ought to display, engenders confidence that there is more to this than formalism alone.

The general proposition that pattern formation and morphogenesis are directed by a dynamic physical field is far from novel. Embryologists have thought along these lines since the twenties, and an explicit hy-

pothesis was set out nearly fifty years ago by Alan Turing as a solution to a somewhat different question: How can spatial order arise from a prior state of disorder, as happens when an apparently homogenous egg turns into an embryo? In a paper boldly entitled "The chemical basis of morphogenesis," Turing (22) put forward two important ideas. One was the proposal that developmental events are called forth by specialized informational molecules, called morphogens, whose distribution in space supplies a prepattern for the subsequent location of biological structures. In contemporary idiom, the graded distribution of morphogens constitutes a field of positional information, a kind of map, that instructs individual cells in an early embryo concerning the developmental course that each should follow. The other, and entirely novel, idea was that a pattern of local concentration differences can arise spontaneously when two interacting substances diffuse at different rates. Contrary to intuition, which associates diffusion with smoothing out concentration differences, in systems that obey particular kinetic rules random fluctuations arising within a homogenous region will be amplified, generating stable local maxima and minima of morphogen concentration. Note that Turing's principle is grounded in physical chemistry; he worked out its biological implications in terms of multicellular embryos, but there is nothing to preclude its extension to single cells.

The general hypothesis, that a pattern of morphogen distribution guides biological development, is very much alive. It has proven directly applicable to the development of animal embryos, in which the major axes are blocked out by gradients of diffusible substances (proteins, as a rule) that instruct cells concerning their position in the embryo and direct them into the proper path of differentiation (23). Clear examples come from embryonic development in the fruit fly, *Drosophila*. Early in this process, a gradient of a protein called Bicoid arises by the localized translation of mRNA which had been deposited by the mother fly in the region destined to become the embryo's head. At this stage the embryo contains many nuclei, but these are not yet separated by cell membranes. The Bicoid protein activates the expression of certain genes involved in the establishment of the fly's segments, and nuclei respond differentially according to the concentration of Bicoid they encounter. Those at the high end of the gradient are induced to embark on the production of the head, lower Bicoid concentrations induce other anterior organs. Additional morphogens specify posterior structures and the differentiation of the dorso-ventral axis.

Can this particular model be applied directly to the specification of pattern and form in single cells? Probably not, for several reasons (but

see Harrison, 21, for an alternative view). First, a unicellular organism has no population of nuclei that can respond differentially to the local concentration of some instructive substance; very different mechanisms would be required for a morphogen to direct, say, localized exocytosis or the disposition of cilia. Second, the cytoplasm of most eukaryotic cells (including ciliates and fungal hyphae) is constantly stirred by streaming; local concentration differences of diffusible morphogens would soon be erased, unless they are confined somehow to the quiescent cortex. Finally, there is an awkward dichotomy between a map of morphogens that carry instructions and the separate interpretation of those instructions. Such a division of labor seems plausible on the scale of a fly embryo (half a millimeter in length), but not on that of an individual cell. Still, we should hold on to the general proposition that form and pattern on the cellular level are the expression of a field over which some agency acts in a coordinated manner. This agency might be electrical in nature, or a pattern of mechanical stress and strain, or something else altogether; and it need not be the same in all cells. Beyond laws that apply to dynamic fields generally, there may not be much unity beneath the quirky diversity of biological forms.

Fields are abstractions; to make them concrete, the general concept must be applied to particular cases by specifying the informational properties of the field, how it arises, and how its instructions are implemented. The most persuasive cases are those in which one can compute the pattern or form of an organism from a set of explicit premises. Once again, the fact that some formula or algorithm generates a biological shape does not guarantee that the mathematics capture the underlying physiology; but success does indicate a well-crafted hypothesis whose postulates can be verified or challenged by more empirical methods.

The placement of cortical organelles in *Tetrahymena* illustrates at once the powers and limitations of the field approach. In the model developed by Brandts and her colleagues (24), the central postulate is that the cell's surface can be represented as a smooth and continuous set of positional values arrayed around the circumference. Various organelles, such as the oral apparatus and contractile vacuole pores, correspond to particular values; the model does not specify whether these field values merely designate map positions or are instrumental in the construction of organelles. The field as a whole has a single value, designated an "energy," which is calculated by summing the energy density at all points. This energy is made up of two terms, one capturing the idea that there is an optimal gradient or spacing of cortical features, the other that changes in the magnitude or direction of the gradient cost energy.

The cell seeks configurations that minimize the overall field energy by adjusting the two terms. The output of the model consists of a set of patterns of oral apparatuses and contractile vacuole pores, that are allowed by the formulation and vary as a function of the cell's circumference. Doublets, singlets and transitional intermediates should exhibit different configurations.

This is an exceedingly abstruse model, unlikely to commend itself to either biochemists or physiologists. Yet it makes remarkably accurate predictions concerning the configurations of organelles to be expected in individual cells and populations. It predicts that reversion of right-handed doublet cells to the singlet state should follow a path different from that of left-handed doublets, and correctly forecasts the intercalation of a third oral apparatus between the other two. It also calls for certain configurations of contractile vacuole pores that were observed only after the model had anticipated their existence. This predictive power suggests that the model captures essential aspects of biological reality, and highlights the requirements that must be met by any system of real molecules specifying positions in real cellular space. Such a system may consist of two or more species of mobile molecules, diffusing Turing-fashion within the cortical layer and interacting in such a way that the positional interpretation reflects the ratio between the two species. But it seems more likely that the molecular basis of the positional field should be sought in physical parameters of the cortical layer itself. The game is afoot, and that the quarry continues to elude its pursuers should lend zest to the hunt.

For Brian Goodwin and Lionel Harrison, the object of study is growth and regeneration of apical structures in the giant unicellular marine alga *Acetabularia* (Fig. 7.1f). Briefly, when the umbrella-shaped cap is lopped off the stem forms a tip, elongates and eventually regenerates the cap; in the process it also puts out successive whorls of hairs that have no known function and soon fall off. Goodwin, Trainor and their associates set out to devise a mathematically explicit model grounded in established cell physics that specifically predicts this sequence of events; and while that goal has been achieved only in part, a very good start has been made (25). They begin by considering the stem as a closed vessel consisting of three apposed elements: a stiff and strong wall, a fluid-filled vacuole that exerts hydrostatic pressure (turgor) upon the wall, and a thin shell of cytoplasm sandwiched between the vacuole and the wall. Pattern is generated within the cytoplasmic layer thanks to reciprocal interactions between the concentration of free calcium ions and the mechanical state (stress and strain) of the cortical cytoskeleton.

The emerging pattern is transmitted to the wall, perhaps by the intervention of ion pumps in the plasma membrane, and this localizes the wall's expansion in compliance with the force of turgor. All this and much more was incorporated into a set of coupled differential equations, more than twenty of them, which define a morphogenetic field within the apical region. The physical nature of this field is very different from that envisaged by Turing, but their mathematical properties turn out to be similar.

What makes this abstract model a serious contribution to the science of form is its capacity to generate realistic shapes from a nearly uniform initial state. Computer simulations begin with the apex as a low, featureless dome. As the program goes through its paces the dome puts forth a tip that advances and then flattens, just as the real tip does. The apical calcium concentration peaks at the apex, then turns into an annulus that breaks spontaneously into a series of peaks that have the symmetry of a whorl of lateral hairs. Harrison's laboratory demonstrated earlier that calcium ions are involved in hair production, and that calcium accumulates at the site of hair emergence. All this suggests that the lateral hairs do not represent a functional structure, but emerge as a consequence of system dynamics. It has not been possible to model the emergence of the hairs themselves, or of the reproductive cap, partly for technical reasons and partly because even this very complex model contains far fewer terms than the living alga employs.

Note that this model does more than describe shapes; it generates them thanks to its internal dynamics, just as the living cell must do. One might expect this gratifying outcome to be critically dependent on the numerical values assigned to the many parameters that must be specified, but that is not the case. The model is robust, in the sense that it "works" over a broad range of parameter values, generating a family of forms and sequential transformations. The biological implication is that the morphology of a regenerating tip is both stable and probable, a form that will emerge naturally in diverse cellular systems. Indeed, apical extension is a widespread mode of growth. *Acetabularia* itself is one of a large order of related algae, the *Dasycladales*, whose fossil remains go back to the Cambrian era 500 million years ago. Goodwin does not doubt that these algae are related historically, by descent from a common ancestor, but the fact that they display variations on a common morphological theme is explained, not by their common ancestry but by their shared physiological dynamics. "From this perspective, the *Dasycladales* constitute a natural group not because of their history but because of the way their basic structure is generated" (25).

To put it in technical lingo, they constitute a discrete basin of attraction in morphospace. Goodwin also sees a conflict between such "laws of form," rooted in systems dynamics, and the common understanding that natural selection has shaped organisms over time; but here I must part company from him. We shall revert to this subject in Chapter 9.

Let me conclude this section by returning briefly to a somewhat simpler example of cellular morphogenesis, apical extension in fungal hyphae, which was discussed in some detail above. It will be recalled that the tip of a growing hypha contains a prominent vesicular structure known as the Spitzenkörper, or apical body; observations suggest that this body plays an important but ill-defined role in the passage of secretory vesicles to the site of exocytosis at the extreme tip. Salomon Bartnicki-Garcia and his colleagues (9) succeeded in modeling hyphal extension on the premises that the Spitzenkörper is the immediate source of secretory vesicles which are discharged at random at a rate N, and that this body is endowed with directional mobility, traveling at a rate V. Vesicles that reach the surface are incorporated, causing it to enlarge; and since vesicles shot out in the direction of travel will reach the edge soonest, the surface will preferentially expand ahead of the Spitzenkörper. Now, this is surely a brutally simplified description of hyphal extension, but it could be expressed in an extremely simple equation with N and V as the sole variables. This plots out as a curve, dubbed a hyphoid, that is instantly recognizable as that of a fungal hypha in cross-section.

On a previous occasion, I have presented a critical analysis of this hypothesis and of the evidence that is beginning to weigh in its favor (26). Here we will only note that this is once again a field theory: the advancing Spitzenkörper spraying vesicles in all directions generates a field in which exocytosis takes place in a coordinated and predictable manner. The model developed by Bartnicki-Garcia looks much simpler than Goodwin's because most of the physiological complexity of hyphal growth is subsumed under the collective variables V and N. This drastic summation allows one to formulate a model that is comprehensible and experimentally testable; and the reports from the laboratory suggest that it captures something fundamental to the way hyphae grow and shape themselves. Incidentally, the proposal that calcium influx localizes the apex represents yet another instance of a spatially extended field, one that has not yet been expressed in mathematical language.

When the field concept is applied to apical growth in fungal hyphae, it loses something of the precision that it brings to the specification of map coordinates in a ciliate. But that is not altogether detrimental, for

the spatial organization of a whole eukaryotic cell is not likely to be dictated in a straightforward manner by a single master gradient. The major features of hyphal organization probably stem secondarily from the vectorial extension of the cytoskeleton, with its attendant traffic in vesicles and localized exocytosis. These establish the polarized organization of the plasma membrane and localize the origins of subsidiary gradients, including gradients of cytosolic pH, of membrane transport proteins, of wall mechanics and signal-carrying molecules. One thus arrives at the notion of a hypha as an extended matrix of multiple interwoven gradients, all of which are ultimately consequences of vectorial tip growth, and many of which also feed back upon tip extension. As in the case of parallel processing by informational networks, redundancy is built into the system. Multiple interactions may prevail over rigid hierarchy, and there may be no one indispensable vector; linear causality then dissolves into a web.

Biologists are apt to be uncomfortable with field theories, and understandably so. As matters stand there is still something half-baked about their application to biological organization, and in any event you cannot isolate a field, clone it or patent it. But it seems that we need some such idea, if only to rationalize the paradoxical relationship between morphology and genes. Everyone knows that the forms of cells and organisms are quite strictly inherited, and can be altered by mutation with specific and reproducible effects. Yet forms cannot be explicitly engraved in the genome; they are remote implications of the genetic instructions, each arising by the collaboration of numerous gene products distributed in space (Chapters 5, 6). I take the position, argued compellingly and in detail by Goodwin and others (27), that a spatially extended dynamic field generated by the cell as a whole is an obligatory intermediate between genes and form. Its function is to organize gene action in space: the morphogenetic field is the agency that defines the pathways of molecular transport and positioning, and ultimately localizes the forces and compliances that shape the cell. Fields remain hypothetical, and their physical nature a subject for speculation and research; but it seems to me self-evident that morphogenetic fields must revolve around the organization of the cytoskeleton. The particular field (or more likely, fields) that guides morphogenesis need not be the same in all organisms, but organisms related by descent will surely share field dynamics, just as they share gene sequences and molecular architecture. With each generation, the morphogenetic field is recreated afresh. The reason that forms are nevertheless faithfully transmitted is that each cell carries two kinds of heritable information: the linear sort, written in

nucleotide sequences, and the three-dimensional sort embodied in the spatial architecture of the cell as a whole. Genes specify macromolecular functions, and collectively determine the kinetic and thermodynamic parameters of the morphogenetic field. The structural markers and cortical domains that are turning up in the molecular descriptions of cell morphogenesis are part of the mechanisms that define field boundaries, and fields defined by vectorial physiological process ultimately shape the cell. I realize, of course, that this point of view is thoroughly out of fashion; Jan Sapp (28) recently referred to those who hold it as "cytoplasmic heretics." But there is really nothing radical about it, or even novel; it's largely common sense, and therefore questions about the existence and nature of morphogenetic fields ought to rank high on the research agenda.

What, then, of the relationship of form to function: are cell forms shaped by the predictable workings of a physical system, or selected gene by gene for superior function? Both, surely, and unlike Goodwin I see no necessary conflict between these two viewpoints. Systems behavior determines the organism's form, which is in most cases plainly adaptive. There may well be morphological features that are not the result of direct selection, such as those whorls of lateral hairs upon a stem of *Acetabularia*, or the wing patterns of the desmid algae. But the fields that apparently guide morphogenesis should themselves be products of variation and selection. Most, though perhaps not all, of the variation that affects field dynamics occurs initially at the gene level; and most, though possibly not all, of the winnowing that modifies and transforms morphological patterns actually judges whole organisms by their functional performance. And when one reflects upon fungal hyphae forging across an agar plate, or a hungry amoeba trapping its prey with ponderous dexterity, form and function become indistinguishable.