Cell, Vol. 100, 79–88, January 7, 2000, Copyright ©2000 by Cell Press

Molecular "Vitalism"

Review

Marc Kirschner,* John Gerhart,† and Tim Mitchison*
*Department of Cell Biology
Harvard Medical School
Boston, Massachusetts 02114
†Department of Molecular and Cell Biology
University of California at Berkeley
Berkeley, California 94720

In the early nineteenth century, views on the nature of living organisms were broadly divided into two categories, chemical and vitalist. The former held that life was a consequence of complex, but ultimately knowable physicochemical processes, while the latter posited some nonnatural, perhaps unknowable, properties of living systems. Vitalism was progressively undermined by Wohler's synthesis of urea (1828) and by Pasteur's inability to demonstrate spontaneous generation (1862), as well as by Darwin's *Origin of Species* (1859) and Virchow's cell theory (1855). By the turn of the twentieth century the remarkable properties of living systems were more evident than ever, but vitalism was no longer invoked to explain them. The modern scientific quest for the chemical basis of life had begun in earnest. Although heredity was known as an important property of living organisms, investigations of the chemical basis of life concentrated as much on other attributes, such as metabolism and movement.

At the close of the twentieth century, genetics reigns triumphant as the central theme in biological thought. The sequence of the human genome is widely seen as the starting point for biological investigation in the next century, and the debate on the origin of life largely defines "alive" as equivalent to "accurately transmitting a genetic blueprint." We do not question the importance of genetics, nor dispute the role of DNA as the blueprint for all the components of living systems, but we think it worth asking to what extent the "postgenomic" view of modern biology would convince a nineteenth century vitalist that the nature of life was now understood. How close are we to understanding how a single cell functions or how an embryo develops? If the answer is not so close, will true understanding of living systems come from further annotating the database of genes, or must we explore the physicochemical nature of living systems? In this essay we discuss a few personal favorite examples, starting from macromolecular assembly and increasing in complexity and scale to patterning in vertebrate embryology. Our discussion illustrates the nature of biological organization and explores the potential chemical principles behind them. Although the units we consider, proteins, cells, and embryos are manifestly the products of genes, the mechanisms that promote their function are often far removed from sequence information. In a light-hearted, millennial vein we might call research into this kind of integrated cell and organismal physiology "molecular vitalism."

The Limitations of Machine Analogies in Biology

Analogies to machines are widely used in molecular biology to understand the nature of cellular processes. The DNA replication apparatus, spliceosome, nuclear pore, and ribosome all have substructures, moving parts, and integrated assemblies like conventional machines (*Cell*, 1998). Yet other biological systems, which might seem machine-like, on closer examination operate on very different principles. Even the simple signal-response of an allosteric enzyme is not machine-like when examined in detail. Allosteric proteins have intrinsically active and inactive conformations, which exist in some ratio in the absence of an external signal (Monod et al., 1965; Henry et al., 1997). Interconversion between the states is spontaneous, driven by thermal energy. Allosteric effectors bind preferentially to one of the states, perturbing the equilibrium, and leading to inhibition or activation. However, the allosteric effector does not directly change the chemistry or conformation of the protein appreciably, but merely stabilizes one of the two preexisting states—a case of state selection. Like macroscopic machines there is an input and an output, but unlike machines the intervening linkages are statistical and not mechanical. More complex "protein machines" like the ribosome can bias the statistics toward determined outcomes by hydrolyzing NTP, but the essential role of statistical thermodynamics in their levers and springs should not be forgotten.

Bacterial chemotaxis also appears superficially to be a simple signal-response machine, where an attractant or repellent is perceived by receptors on the bacterial surface to generate a signal that is converted to directed movement. We could imagine all sorts of linkages that would control a motor or a steering mechanism to guide the bacterium by chemical signals. In fact, bacterial chemotaxis is based on the modulation of random movement by ligand binding, resulting in a biased random walk. The specific path any bacterium takes is not directly informed by the binding of the ligand, nor does the individual bacterium at any moment sense a spatial gradient (Berg, 1988). This is quite different from any machine of human design!

Biological systems look even less like machines when one considers spatial organization. They can generate order from disorder and can arrive at functional states and responses over a range of starting points, sizes of components, and sizes of final product. As an example, consider the relationship between cell size and the size of the organism. In the 1940s Gerhard Fankhauser experimented with the effects of ploidy on newt development (Fankhauser, 1945). Polyploid embryos, generated by suppressing early cleavages, had fewer but larger cells. Cells in all tissues were affected, but the tissues of the organism and the adult itself remained the normal size. The consequence of ploidy was seen most clearly in well-defined structures, such the pronephric duct (the earliest kidney rudiment). Fankhauser found that the average number of epithelial cells forming the duct decreased with increased ploidy, while the duct size and wall thickness remained the same diameter! As shown in Figure 1, in pentaploid embryos there were just one to three cells straining to maintain a circular duct of dimensions that required three to five cells in diploid embryos and five to eight cells in haploid embryos. In

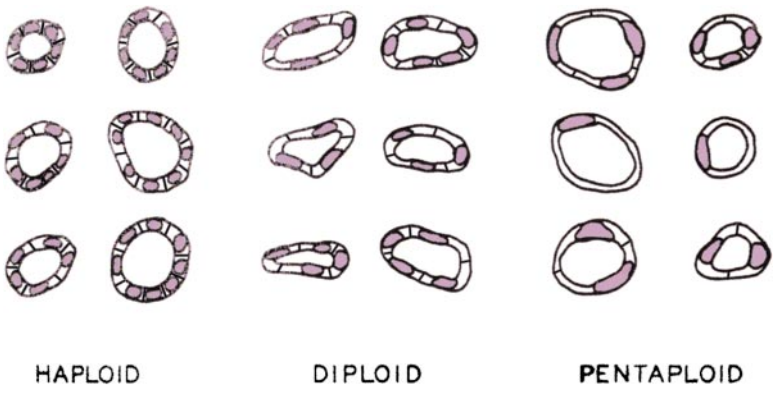

Figure 1. Cross Section of the Pronephric Ducts of Haploid, Diploid, and Pentaploid Larva of the Newt, *Triturus viridescens*, at a Stage in Development when the Hind Limb Buds Are First Apparent

Note that the normal size of the duct and the thickness of the duct is maintained despite the difference in size of the cells and the nuclei (colored purple). (Redrawn from Fankhauser, 1945.)

1945 Albert Einstein wrote Fankhauser, "Most peculiar, however, for me is the fact that in spite of the enlarged single cell the size of the animal is not correspondingly increased. It looks as if the importance of the cell as ruling element of the whole had been overestimated previously. What the real determinant of form and organization is seems quite obscure" (Fankhauser, 1972). Today we might not draw such a strong conclusion about the role of the cell, but we might be tempted to ask what cell biological properties can we draw upon to explain "the real determinant of form and organization"? What was the driving force for pronephric duct size that could operate despite changes in cell size?

Self-Assembly and Self-Organization

The basis of our understanding of supramolecular structure has been the doctrine of self-assembly. Self-assembly is an extension of the central dogma of molecular biology, bringing us from the realm of linear information to the realm of protein assemblies (Caspar and Klug, 1962; Oosawa and Asakura, 1975; Inoue, 1982). It is exemplified by a virus particle, which generates a single highly ordered (to atomic dimensions) structure that is "uniquely determined by size, number of components, geometry, and strength of interaction" (Gerhart and Kirschner, 1997). Typically systems of self-assembly reach equilibrium, a state of minimum free energy. Today a postgenomic view of self-assembly would extend this concept to a description of how each gene product functionally interacts with other gene products. These pair-wise interactions can be used to describe protein complexes and pathways of interaction. They could form the basis of our future understanding of higher level organization and information transfer in biological systems (Frederickson, 1998).

Self-organization is an extension of self-assembly, but employing several new chemical principles (Kirschner and Mitchison, 1986). In contrast to self-assembly, self-organization gives "structures under a wider set of condition; the rules tend to be more general and the structures more variable" (Gerhart and Kirschner, 1997). Self-organizing systems are characterized by reaching a steady state, where there is continuous energy consumption and gain and loss of material. In discussing examples of self-organization, we will focus on two of the most archetypal and unusual biological properties: (1) the capacity for unitary organization, also called polarization; and (2) the capacity to generate nearly regular biological structure when size and composition of components are altered, also called regulation. These properties are not what we would expect from mechanical processes, and no machines of human design evince such properties. They are a manifestation of complex yet robust chemical processes, some of which we are beginning to understand, some of which seem as remote as Fankauser's ploidy experiments.

Spontaneous Symmetry Breaking: Actin Comet Tails

In many biological systems the first step in generating spatial complexity is the breakdown of a symmetrical structure into a more organized asymmetric or polarized structure. van Oudenaarden and Theriot (1999) recently described a simple model of this process in the generation of polarized arrays of actin; their explanation for spontaneous symmetry breaking in this system provides new clues to principles of self-organization. *Listeria monocytogenes*, an intracellular pathogenic bacterium, hijacks the natural actin nucleation machinery of the cell and propels itself through the cytoplasm by triggering assembly of a polarized "comet tail" of actin. A single secreted protein of the bacterium, Act A, is sufficient to induce actin assembly. In their experiments Act A was purified and adsorbed *uniformly* on the surface of spherical polystyrene beads. When the beads were placed into a cellular extract, actin was initially polymerized in a symmetrical manner. With time actin assembly became asymmetric and the bead ultimately generated a single, completely polarized comet tail that propelled it through the extract (Figure 2).

Several questions arise. How is a symmetrical condition transformed into an asymmetric one? How do the individual actin filaments around the bead "communicate" to each other, so that actin is only significantly polymerized in one region and not another? Why is there always a single tail produced? van Oudenaarden and Theriot postulate that actin polymerizing against the bead exerts a force and the bead is continually buffeted by the actin pushing against the viscous nature of the surrounding fluid and the surrounding crosslinked actin filaments. Stochastic differences move the bead and these stochastic differences are amplified by the biophysical properties of actin assembly. As the bead moves, actin filaments on one side are inhibited in their polymerization, while actin filaments on the pushing side

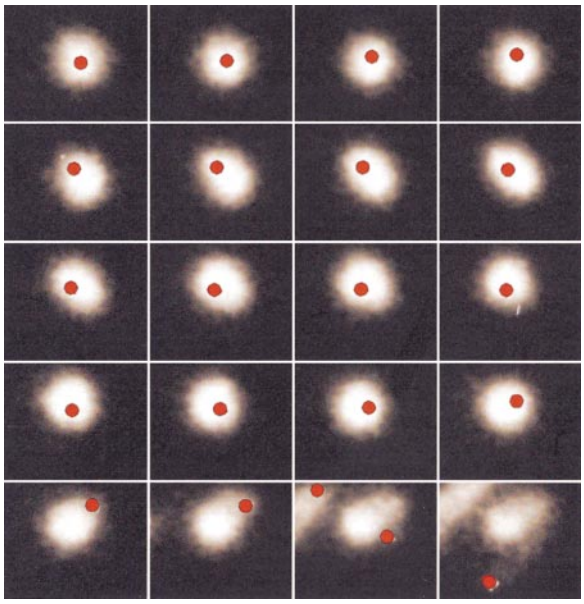

Figure 2. Actin Arrays around a 0.5 μm Carboxylated Polystyrene Bead Uniformly Coated with the *Listeria* Protein Act A

The beads were incubated in *Xenopus* egg extracts supplemented with tetramethylrhodamine labeled actin and visualized by fluorescence using a microscope and a CCD camera. Actin is white; the bead is pseudocolored red. Images were taken at 10 s intervals. Note the random oscillation of the bead within the actin cloud and the escape of the bead with a polarized tail in the bottom tier of frames. In the penultimate frame it is on the left being propelled out of the frame and is absent in the final frame. (Courtesy of Alexander van Oudenaarden and Julie Theriot, Stanford University.)

polymerize more readily. Symmetry once broken is exaggerated and the system becomes stable with a single tail.

This simple biophysical model achieves characteristics that, had they been observed in a cellular setting, might have been ascribed to external influences and complex regulation or at least to intrinsic asymmetry of the nucleating structure. Despite the absence of external forces or preorganization to break symmetry, a single polarized array self-organizes. It appears that amplification of a random inequality through mechanochemical coupling provided by the nondeformable bead is sufficient to generate spontaneous polarization. We see in this example that self-organization of an array of actin filaments is built on the self-assembly of individual filaments. In a dynamic system a variety of configurations of these filaments can be generated from stochastic differences at the molecular level. To achieve a unitary structure, albeit oriented at random, requires rapid off-rates and some large-scale force or structure that can serve to link the behaviors of the individual polymers. Actin assembly is made more dynamic by hydrolysis of ATP in the actin subunit. The formation of a single tail is an inevitable consequence of the kinetics of assembly, the response of assembly to compressive forces, and the existence of simple boundary conditions. Thus, self-organization can have a rather simple chemical and physical explanation.

In the *Listeria* system a weak initial bias is sufficient to entrain the polarized array in a specific direction. This conclusion could hold at a cellular level as well. In an example that might be mechanistically similar to *Listeria*, Borisy and coworkers showed that radially symmetrical fragments of motile cells could be induced to polarize and initiate persistent unidirectional locomotion by subjecting them to a weak external force from a jet of fluid (Verkhovsky et al., 1999). A more complex example is polarization of budding yeast. In this system the initial cue is usually provided by chemical "landmarks" in the cell wall or a gradient of mating pheromone, but in the absence of these cues the yeast cell will spontaneously self-polarize on a random axis (Drubin and Nelson, 1996).

Self-Organization to a Steady State: The Mitotic Spindle

The mitotic spindle provides a more complex system in which to explore principles underlying the spontaneous self-organization of collections of proteins in the cytoplasm. The spindle is an ordered array of microtubules, motors, and chromosomes that assembles at the beginning of mitosis. Once all the chromosomes are attached and aligned, the spindle reaches a steady state, termed metaphase. The time-invariant average structure of the spindle at metaphase might suggest it had attained thermodynamic equilibrium like an assembled virus, but dynamic imaging quickly dispels this notion. In fact the chromosomes constantly move back and forth around their average position, and microtubules polymerize continually at certain locations while depolymerizing at others, generating rapid turnover of individual microtubules and directed movement of the microtubule lattice. These dynamics require continuous energy dissipation, notably from GTP hydrolysis coupled to tubulin polymerization and ATP hydrolysis coupled to force generation by molecular motors.

Steady-state thermodynamics can help us understand some of the implications of continuous energy dissipation by a self-organizing system like the spindle. The steady state resembles equilibrium in the sense that the system moves spontaneously downhill over some energy landscape to reach the steady state, where it comes to rest. If a steady state is perturbed, it will return to its preferred parameters. For example, if the length or organization of a spindle is perturbed by physical changes, drugs, or micromanipulation, and the perturbing agent is then removed, the spindle returns to normal over a few minutes (Inoue and Sato, 1967). This robustness provides error-correcting mechanisms that are thought to be important to ensure accurate chromosome segregation (Nicklas, 1997). Figure 3 shows an example of a spindle responding to, and recovering from, high pressure that depolymerizes microtubules. Prigogine, building on the work of Onsager, proved that a system comes to steady state when the rate of free energy dissipation is minimized, at least for a system not too far from thermodynamic equilibrium (Prigogine, 1955). The concept of the steady state as a thermodynamic minimum helps us understand the ultimate driving force behind self-organization, and may account in energetic terms for much of the robustness and pathway-independence of a process like spindle assembly.

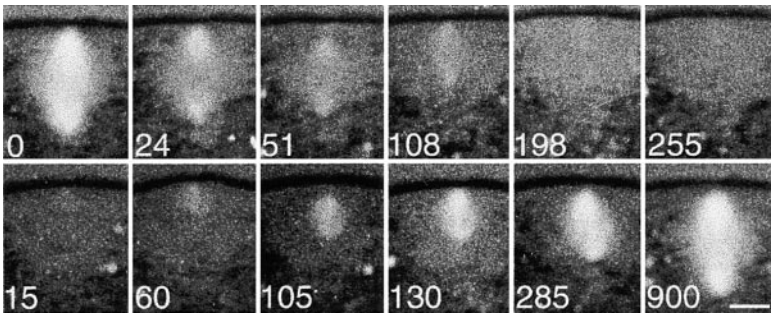

Figure 3. Response of the Meiotic Spindle in *Chaetopterus* Eggs to High Pressure, Imaged with Polarization Optics

In the upper series pressure of 3000 psi is applied causing microtubule depolymerization, and the spindle shrinks rapidly. In the lower series the pressure is released and the spindle recovers. Time elapsed from changing the pressure is shown in seconds; scale bar = 10 μm. Note the rapid changes in spindle length induced by perturbation of the tubulin-microtubule dynamic equilibrium with pressure. At lower pressures the spindle reaches steady state with a shorter length. (Reproduced, with permission, from Salmon, 1975.)

Two types of protein–protein interactions are thought to drive spindle assembly: tubulin–tubulin interactions and tubulin–motor protein interactions. Assembly driven by motors means that initially random microtubules can slide actively past each other to achieve correct positions. Such motor driven sorting is thought to be a major force in spindle assembly (Walczak et al., 1998). Tubulin–tubulin interactions in the microtubule lattice are coupled to GTP hydrolysis, which powers rapid microtubule turnover by drastically increasing the off-rate (Kirschner and Mitchison, 1986). This process, termed dynamic instability, accelerates the rate at which microtubules probe cellular space (Holy and Leibler, 1994), and destabilizes incorrect organizations relative to the correct ones (Kirschner and Mitchison, 1986). Motor-dependent assembly interactions and dynamic instability collaborate to make incorrect or partial assemblies dissipate energy faster than the correct one, and together provide a thermodynamic drive to the assembly of functionally correct structures.

This picture of self-organization to a thermodynamic minimum at steady state is likely applicable to many, perhaps all, cellular assemblies. Its relevance to other cytoskeletal arrays like the actin-based leading edge of migrating cells is obvious. Less obvious and perhaps more interesting is the relevance of steady-state thinking to dynamic membrane systems such as the Golgi apparatus. Like the spindle, the Golgi apparatus is an inherently steady-state structure in which continuous fluxes of material and energy are inherent to spatial organization. Proteins that use NTP hydrolysis to drive conformational cycles, such as arfs, rabs, NSFs, and dynamins, play central roles in Golgi organization (Rothman and Wieland, 1996). Energy dissipating fluxes through biochemical cycles of lipid modification may also play a central role in self-organization of membrane systems (Schmidt et al., 1999).

From Protein Assembly to Developmental Biology: *Stentor*

So far we have discussed examples of self-organization within a single cell that are not very far removed from self-assembly. How complex can self-organization be in a single cell? About a billion years ago, after the basic attributes of eukaryotes had emerged, one branch of the eukarya invented multicellularity and became the metazoa. But the unicellular organisms did not stop evolving, and today we can see among them examples of remarkably complex spatial organization. Unicellular organisms often assemble the equivalent of multiple different, specialized organs arranged in a specific body plan. Developmental phenomena resembling spatial gradients and induction have been observed in such organisms. Impressive examples of size-independent patterning and recovery from drastic perturbation attest to robust, self-organizing mechanisms for spatial patterning. To bridge between protein assemblies and metazoan embryonic developmental systems, we will consider what is known of self-organization mechanisms in a large ciliate, *Stentor coerulus*.

Stentor is a very large (up to 1 mm), trumpet-shaped, ciliated protozoan that lives in water with its pointed end (foot) typically attached to a substrate. It feeds by sweeping smaller organisms into a gullet through the action of rows of fused cilia in the oral apparatus at its broad end. Much of our knowledge of *Stentor* derives from the devotion of one man, Vance Tartar, who worked on them from 1950 to 1978 in an 8' × 10' shed named "Wits End" at the bottom of his garden on the Washington coast, and published a large monograph on their biology (Tartar, 1961; see also Frankel and Whiteley, 1993). Tartar was fascinated by the ability of *Stentor* to recover from surgical operations, which allowed him to test theories of how the spatial pattern of the organism developed. Conceptually these experiments resembled classic manipulations of multicellular embryos that led to such discoveries as Spemann's organizer, but they involved removing, rearranging, or transplanting pieces of a single cell (Figure 4). How it is possible for a single cell to survive being cut into fragments is a fascinating question in its own right.

Typical of ciliates, most of the spatially organized structures in *Stentor* are associated with a microtubule-rich cortex dominated by parallel rows of regularly spaced basal bodies running along the principle axis of the organism. Between these rows are pigmented stripes. The basal bodies are connected together within and between the rows by bundles of microtubules and other fibers in a quasi-regular geometric arrangement. The body plan of all ciliates is dominated by this structured cortex, giving the sense that their self-organization can be thought of as a problem in geometrical patterning of a two-dimensional sheet. *Stentor*'s body plan involves two developmental axes. An apical-basal (A-B) axis is defined by the basal foot and the apical oral apparatus.

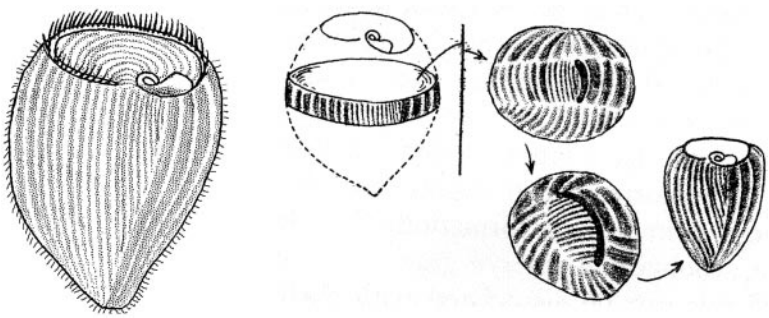

Figure 4. Dynamic Organization in the Large, Single-Celled Ciliate, *Stentor*

The left panel shows a normal *Stentor coeruleus*, typically 0.5–1 mm in length. Note the circumferential gradient in stripe spacing and the Locus of Stripe Contrast where the wide and narrow stripes abut. The LSC functions as an organizer during regeneration and normal cell division. The right panel diagrams a typical regeneration experiment performed by Tartar (1961). In this case a segment from the middle of the animal was excised. Within a day it reorganized to form a normal, smaller animal.

A circumferential axis is evident from the spiral geometry of the oral apparatus. It is also evident from, and perhaps defined by, a circumferential gradient in the width of the stripes. This continuous gradient generates a unique line where the widest and narrowest stripes abut, called the locus of stripe contrast (LSC).

Surgical manipulations revealed that the body plan of *Stentor* self-organizes by robust pathway- and size-independent mechanisms (Figure 4). Tiny cell fragments made by surgery can recover to form normally organized *Stentor* as small as 0.1% of the normal volume. These mini-*Stentor* gradually enlarge as they feed. Several conclusions emerged from surgical manipulations: To survive and recover, a *Stentor* fragment must contain some of the old cortex. A nucleus is also required for prolonged survival. Certain abnormal body plans produced by surgery, such as side-by-side conjoint twins, do not recover, but rather propagate as stable clones. During reestablishment of patterning after surgery, both the A-B and circumferential axes act as if they contain gradients of developmental potential. The LSC induces nearby cortex to assemble the new oral apparatus following normal cell division or surgery. This action of the LSC on nearby cortex formally resembles embryonic induction, a process whereby a group of cells (as opposed to a region of cytoplasm in *Stentor*) signals a responding cell population to generate the major structures of the vertebrate axial body plan. Interestingly, in *Stentor* an LSC active in signaling the adjacent cytoplasm can be formed surgically anywhere on the cortex at any point where broadly and narrowly spaced stripes are made to abut.

We know very little about how *Stentor*, or any other ciliate, self-organizes a specific body plan. How can we begin to think about such mechanisms? Extensive recovery of anucleate fragments implicates largely post-transcriptional mechanisms. The requirement for some cortex for recovery from surgery, and the stability of conjoint twins, might reflect mechanisms of basal body replication and insertion. New basal bodies form in association with old ones, and the position and geometry by which a new basal body is inserted into a row is governed by the old ones in the row (Beisson and Sonneborn, 1965). We do not know the exact mechanisms here, but some kind of structural templating seems to be operating (Aufderheide et al., 1999). One could imagine a gradient of a diffusible factor specifying A-B position in *Stentor*, though there is no direct evidence for such a factor. How the circumferential axis in *Stentor* is generated, and how the LSC works, are completely mysterious. Any proposed mechanism for self-organization of *Stentor*, or other ciliates, will have to account for size regulation. Ciliate morphogenesis may now be accessible at a molecular level in *Tetrahymena*, which has a well-developed genetic system with mutants having interesting abnormalities in global patterning (Frankel, 2000).

Multiple Strategies for Self-Organization: Eggs and Embryos

Metazoan multicellular development, as it has evolved in the past billion years, is an accomplishment in the informational realm, that is, of organizing cellular processes spatially and temporally. Most organization is achieved in the steps of development before cell types differentiate and begin their physiological functions. Since the organization of the multicellular adult animal is so much more complex than that of the single-celled egg from which it develops, embryonic development has long been thought to consist of numerous self-organizing processes. How is this self-organization achieved? The old view that the egg's cytoplasm possesses the equivalent complexity of the adult, in an invisible molecular miniaturized form, is clearly incorrect. And anyway, this proposal just pushes back to an earlier stage the question of how organization is established.

In embryonic axis formation, the initial organization of the embryo along anteroposterior and dorsoventral axes is established, much like the organization in *Stentor*, except in this case the organization is provisional and used in subsequent processes to build up greater complexity. A number of self-organizing processes operate at these early times. As expected for processes of initial organization, these, like the example of polarized actin growth off beads, have no or little dependence on prior organization. They can respond to such organization if it is present or can generate it stochastically, if not present. Since the initial symmetric arrangement of contents is lost when a new axis is formed, the process is a symmetry-breaking process.

There are many kinds of symmetry breaking. Some occur in oogenesis (e.g., *Drosophila*), some at fertilization (amphibians, ascidians), and some at multicellular stages (molluscs, birds, mammals). Some use the cytoskeleton, (amphibians, ascidians) some use internal singularities such as the aster (amphibians, fish), and some use the packing of cells in small groups (mouse, nematodes). The common theme in all of these is an amplifying process that starts from a small random departure from perfect symmetry, building that into a major

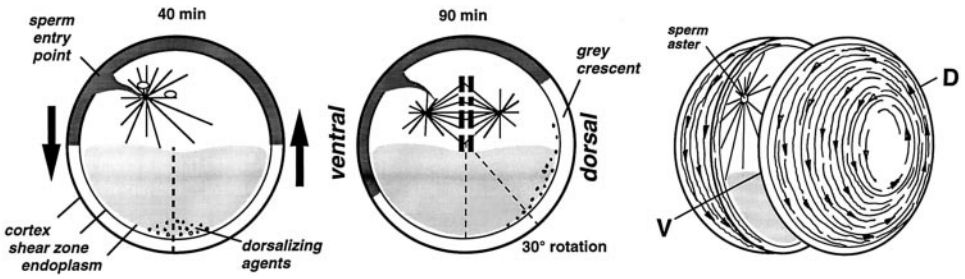

Figure 5. Cortical Rotation in the *Xenopus* Egg as an Example of a Self-Organizing Process

(Left panel) Cross section of the egg at 40 min after fertilization. The sperm has entered on the left and an aster has formed. Cortical rotation is about to begin. The egg cell has two movable parts: a thin rigid surface (cortex) and a rigid core (endoplasm). Dorsalizing agents, perhaps vesicles, are located at the vegetal pole (bottom). The egg is cylindrically symmetrical about a vertical axis, except for the aster. (Center panel) Cross section of the egg at 90 min after fertilization. Cortical rotation has stopped. Cleavage will begin at 100 min. The cortex has rotated 30° relative to the core, up on the right side, down on the left. Dorsalizing agents have moved as much as 120° up on the right. The egg is now bilaterally symmetric as the result of the off-axis movement of the two cylindrically symmetric units. (Right panel) The parallel polarized array of microtubules during rotation. The microtubule mat is about 4 μm thick and 4 μm deep from the egg surface. Arrowheads indicate the polarity of the microtubules, with plus ends in the direction of pointing. Microtubules in the egg can be oriented parallel to any plane that contains a line from the animal pole or top of the egg to the vegetal pole or bottom of the egg; a full 360° range of vertical orientations is possible. The single self-organized array is usually oriented in relation to the sperm aster in fertilized eggs. It will also self-organize in eggs without an aster (artificially activated). Microtubules are the tracks for movement of the cortex (cortical rotation), and cortical rotation orients the microtubules, a self-organizing process based on two reciprocal feedbacks. The dorsalizing agents then move on the singular array.

departure, which is the new axis. In most cases, the orientation of the new axis doesn't matter. What does matter is that there is one anterior–posterior axis (or one dorsal–ventral or left–right axis) and only one of each of these.

Symmetry breaking is exemplified by dorsoventral axis formation in the frog egg (Gerhart et al., 1989; Elinson and Holowacz, 1995). The unfertilized egg is cylindrically symmetric around its animal–vegetal axis, which is elaborated in oogenesis by the placement of some materials at the vegetal pole, others at the animal pole, and others in between, in layers. The sperm enters the egg randomly at one place in the animal half, and the embryo's definitive dorsoventral axis subsequently develops with its dorsal midline opposite the meridian of sperm entry. As the sperm can enter anywhere around the egg's circumference, the egg can develop dorsal, ventral, or lateral embryonic parts at any site, and normally makes the choice based on the sperm entry site or actually, on the aster formed internal to that that site. However, the sperm only provides a bias, because if the egg is activated in the absence of the sperm, it also establishes a dorsoventral axis. The dorsal midline of such a perfectly formed haploid embryo is unpredictable and not related to the activation site. However, if a bias is artificially imposed, the organization reflects that bias. This can be achieved if an artificially activated egg is tipped obliquely so that the denser vegetal cytoplasm moves slightly in a direction toward gravitational equilibrium, the dorsoventral axis is later formed reliably in relation to the direction of that slippage.

The molecular mechanism of symmetry breaking in this case again involves microtubules and motors. After fertilization, a thin mat of parallel polarized microtubules forms in the animal–vegetal direction just under the egg cortex (Figure 5). Materials initially located at the vegetal pole, move along the tubules toward their plus ends, 60°–120° to one side (Miller et al., 1999). That side becomes the dorsal side. The formation of this asymmetric array of microtubules illustrates the self-organizing nature of the cytoskeleton, which has played a prominent role in earlier examples. In the middle of the first cell cycle, microtubules start to polymerize in the cytoplasmic core and extend to the cortex of the egg, at first rather randomly oriented and short. The egg consists of only two mechanical units, the rigid spherical core and the rigid cortex shell. The cortex has motor proteins, probably kinesins, anchored to it, which can move on the microtubules toward the plus ends, using ATP energy. As the cortex can only move as a unit, it integrates all the kinesin forces exerted on the nearly random microtubules to move as a whole in whatever one direction is the vector sum. The movement of the cortex aligns materials of the cortex that feed back on microtubules, stabilizing them and enhancing their polymerization in that direction. With more tubules in one direction, movement in that direction is faster and more forceful, and more materials are aligned. The reciprocal positive feedback of polymerization and rotation on each other soon results in a parallel array of microtubules and a single direction of movement. Then vegetal materials, probably kinesin-coated vesicles, move along the aligned tubules toward one side. The second axis is generated as a systematic deformation of the first.

If the sperm aster is present, it biases the formation of the microtubule parallel array in relation to its location, and hence the embryonic dorsoventral axis is spatially related. In the artificially activated egg, it appears that a small random departure from perfect symmetry is amplified, and the microtubule array is oriented in relation to that. In the tipped artificially activated egg, materials in the cortex are probably aligned by gravity-driven movements, and this favors microtubule polymerization in that direction. Like the example of actin nucleation from a bead, a macroscopic rigid structure acts to integrate the behavior of microscopic elements. In both cases a singular result is achieved, which may or may not be randomly oriented, depending on the presence of biasing forces.

Embryonic Induction and Multipotential Competence of Cells

Although the initial axes of embryos are often generated without regard to other organization, subsequent steps are performed in relation to these axes. These steps increase embryonic organization and have extensive self-organizing properties, too. Sometimes they can be seen to operate as fully self-organizing, even at multicellular stages, as examples below will show. But generally their outcomes arise in relation to already established asymmetry. The major mechanism for increasing the organization of the embryo is induction, whereby an uncommitted and multipotential population is specified by signals coming from other tissues.

At multicellular stages, signal transduction (cell–cell communication) and individual cell competence take central places in the organizing events. By competence we mean that embryonic cells, in the period of patterning, can have two or more developmental paths open to them simultaneously, held in abeyance. This interconvertible multi-statedness is a key aspect of multicellular self-organization. Multipotent cells of a particular kind are contiguous in a large group, called an equivalence group, or competence group, or compartment. In the process of multicellular organization there has been a trade-off between the intracellular complexity that can be achieved in protists such as *Stentor* for a multicellular complexity, namely, a spatial arrangement of no longer equivalent cells. The big evolutionary achievement is competence, the cell's capacity to generate two or more states thanks to its special genetic regulatory and signaling circuitry, with conditions for a selection, and with the means for reciprocal suppression of unchosen options. The complexity of competence has only recently been appreciated, and reciprocally, the simplicity of the signals. This is a complete reversal of view from the early days of experiments on embryonic induction by Spemann and Mangold (1924), when it was assumed that induction involved the release of detailed instructive signals by an "organizer" to naive surrounding cells. The dependence of the cellular responses on local conditions underlies the mechanism of self-organization. This dependence on local conditions also increases the separation of the cellular phenotype from the pattern of gene expression, as the phenotype now depends additionally on circumstances. Nevertheless, gene expression provided the multipotent precursor, which is modified by the local conditions.

Neural induction and neural patterning are good examples of how multipotent cell populations achieve spatial organization and cell specification (see Weinstein and Hemmati-Brivanlou, 1999). Prospective ectoderm cells form neural plate if exposed to signals from the organizer. Each ectoderm cell has two options, epidermal or neural development. Each secretes BMP2, BMP4, and BMP7 signals and responds to these by repressing the neural and favoring the epidermal option. This is all part of the competence state. If there is no organizer signal, nothing stops the ongoing repression, and epidermis results. The organizer's signal is a BMP antagonist protein, such as Noggin, Chordin, Cerberus, or Follistatin, which interrupts intercellular BMP signaling

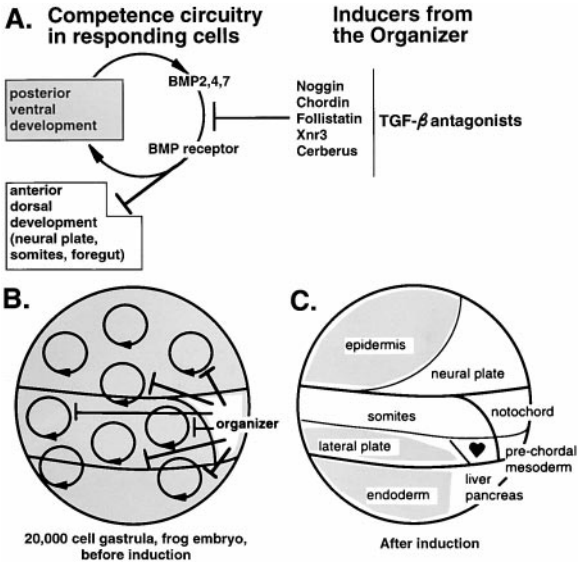

Figure 6. Competence and Induction as Variation and Selection

Here, variation means the generation of a variety of possible developmental options or states, the competence of the multipotential cell, and selection means a state selection based on intercellular induction. (A) The competence circuitry of the ectoderm is shown. Every cell has at least two options, to develop as epidermis (the posterior ventral option) or as neural tissue (the dorsal anterior option). Ectodermal cells secrete BMP2, -4, and -7 and Wnts and locally bind them by receptors. This intracellular signaling suppresses the neural option and affirms the epidermal option. Ectodermal cells near the organizer receive diffusable antagonists of BMPs (six kinds are listed). These are the organizer's inducers. They interrupt intercellular signaling, and the neural option is released. Cells farther away don't receive antagonists and so they keep affirming the epidermal option. (B) The early gastrula (20,000 cells) before the antagonists have acted. The ectoderm comprises the top half of the embryo, the mesoderm the middle belt, and the endoderm, the bottom third. The organizer is on the right, in the mesoderm. The blunt-headed lines indicate antagonists. The circles with arrowheads indicate the competence circuitry with local intercellular signaling preventing the release of dorsal anterior options. Although only the ectoderm was presented in (A), the mesoderm and endoderm also have comparable competence circuits with different options, and also have intercellular suppression of one option by BMPs. (C) After induction. If competent cells of the ectoderm, mesoderm, and endoderm are close to the organizer, they receive antagonists (inducers), and their dorsal anterior options are released (white areas). If too far away, they don't receive antagonists and continue to keep the ventral posterior options (gray areas). The kinds of tissues to develop from particular regions are named.

(Figure 6). The antagonist binds BMP (2, 4, or 7) and prevents its reception by ectodermal cells. The epidermal option is no longer sustained and the neural option is no longer repressed; the latter takes precedence. It is the default option, as it is one taken when all intercellular signaling fails. The antagonist carries no information of its own, and in fact, if ectoderm cells are disaggregated and reaggregated, they also start neural development because the BMPs are diluted. The antagonists are soluble inhibitors and are not bound to receptors of the ectoderm cells. Acting on a multipotent cell, the antagonists release a latent capacity for neural development. The cell relies entirely on its inherent competence for

this response and does not depend on other instructions. This is a prime example of the key role of competence in development, and a prime example of the role of a signaling center, the organizer, in giving orientation and scale to the domain of response of competent cells.

The organizer itself, or the node as it is called in amniote vertebrates, arises in cells uniquely receiving multiple signals. Any cell of the epiblast or animal hemisphere can become a member of the organizer population if it receives these signals, for example by being grafted into the right place. These might seem very special circumstances for its formation, but its formation has self-organizing properties. If the organizer or node is surgically removed at the start of gastrulation, a new organizer is formed from cells near the wound. If cells of the head-inducing and trunk-tail-inducing parts of the organizer are intermixed, the organizer reorganizes and functions. Any quadrant of the chick epiblast can generate an organizer when surgically removed from the whole, although only one of these would have done so in situ (Spratt and Haas, 1960). An inhibitor secreted by the first-arising organizer seems to block secondary organizer formation. Little is known of these means of self-organization and inhibition.

Because of large competence groups and the organizer, the pregastrula chordate embryo is capable of considerable regulation in the face of inflicted damage, to give normal development. When the embryo is split on the bilateral plane, and a lateral half is allowed to close the cut surface, this half develops as a well proportioned, half-size, bilaterally symmetrical tadpole. This can be explained by the existence of competent cells on the ventral side of the gastrula. When the lateral half closes after surgery, the organizer is then bordered by competent responsive cells on both sides, the healed side being bordered by what had been ventral cells. The organizer induces neural plate, heart, somites, and anterior gut on both sides, not just on one side.

With the advent of experimental embryological techniques in the beginning of the last century came an appreciation of the capacity of some types of embryos to survive many drastic manipulations. The embryonic fragments were said to regulate in reforming normal patterns and species capable of this kind of recovery were said to undergo “regulative development.” Part of the old misunderstanding of regulation, as even implied by the name, was the assumption that embryonic cells have a single path of development from a very early stage, and that regulation after surgery entails undoing that path and initiating another. Competence belies all this. The cells have a broad range of possibilities, all equally valid, and this state does not require undoing. If organizer signals arrive, they make one choice and if not they make another, no matter what the geometry of the cut and/or healed embryo. Presumably regulation was not evolutionarily selected for the embryo’s survival from surgery, but rather for two other reasons: first for developmental patterning to succeed in the face of the normal errors of cell number and cell position inherent in an embryo that undergoes extensive morphogenesis at gastrulation, and second, to tolerate mutational changes that underlie the selected phenotypic changes during evolution, thus facilitating evolution. In this sense cell competence contributes to the “evolvability” of developmental mechanisms (Kirschner and Gerhart, 1998).

Common Principles of Self-Organization?

Large scale pattering at the subcellular, cellular, and organismal levels is not simply an extension of the principles of self-assembly. Self-assembly as a mechanism is very powerful and operates far beyond atomic dimensions to create structures of immense size and complexity like viruses and ribosomes. Although near perfect for their jobs, these structures are characterized by very limited capacity for size variation and error correction. Even though the components of the systems we have discussed in this essay are generated by self-assembly, the systems as a whole have very different properties. It is obviously risky to try to generalize between systems deliberately chosen from very different levels of biological organization, but we argue that three general features can be discerned: (1) a high off-rate that allows for energy-dependent exploration of an assembly landscape, and selection of a functional steady state; (2) two- or multi-statedness of the components of the system; and (3) induced collapse of the whole system into singular states.

High off-rates are most obvious for intracellular assemblies where energy dissipation at steady state maintains rapid dynamics. By contrast, in most self-assembling systems there is no need for high off-rates, since this merely reduces the efficiency of assembly. Self-assembling systems are not resilient and adaptable. They work best under a set of conditions but in general do not change the final state in response to changing conditions. The importance of high off-rates in self-organizing systems is apparent in the case of the polarized tails of actin. Here a high off-rate of actin from filaments is essential, so that mechanical compression can cause disassembly of the polymer on one side of the bead. In the mitotic spindle the high rate of microtubule depolymerization promoted by dynamic instability destabilizes assembly intermediates of the spindle relative to the metaphase steady state. Mechanical forces, generated by motor proteins, feed back on polymer stability also leading to destabilization of assembly intermediates and incorrect configurations. The role of high off-rates becomes less clear as complexity increases. In the case of *Stentor*, pattern reformation after surgery suggests that incorrect assembly states of the cortical cytoskeleton are less stable than correct ones, and that off-rates sufficient to explore new configurations must exist. We have no idea what design features promote differential stability and high off-rates but it is likely that they depend on the dynamic nature of the cytoskeletal components. Finally, in the multicellular systems exemplified by vertebrate gastrulation, the final spatial specialization of populations of cells is not so much a reflection of thermodynamic steady states but states of cell specification that are easily interconvertable. Energy dissipation might be required for the physical movements of cells or for dynamic signaling pathways that consume energy.

Two- or multi-statedness of components is again most obvious at the molecular level, but its applicability to cells is still clear. Two states of tubulin are generated by the binding of GTP and GDP, and their differential

behavior drives the dynamics that are central to spindle assembly, and possibly also to ciliate cortical patterning. Two- or multi-statedness of actin and motor proteins has formally similar origins and implications. In the vertebrate embryo the competency states for mesoderm, ectoderm, neuroectoderm, organizer, and endoderm can be thought of as alternative activity states of signaling and transcriptional pathways within the cell. These properties of competence place few demands on the accuracy in cell number and position or the sophistication of inducing stimuli. We have not had space to explore the cellular basis for this multi-statedness. Nevertheless, it is worth noting that competence is generated by signal transduction pathways, which themselves are characterized by components that exist in two or more functional states (e.g., G proteins, phosphorylated receptors, and kinases). The existence of multiple easily interconvertable states allows for systems that establish their functional configuration from among large numbers of available states by state selection. Numerous other examples exist of the biological strategy of selection from complex states, such as adaptive immunity, plasticity in the nervous system, and angiogenesis (see Gerhart and Kirschner, 1997).

Induced collapse embodies the idea that self-organizing systems are able to generate single and appropriate functional outcomes by coordinating the activities of many identical components over large distances. In the case of *Listeria* actin assembly and frog egg axiation, polymer polarity is linked to mechanical processes. In the former case the incompressible bead serves to link the responses of actin filaments on one side of the bead, while in the latter case the stiff egg cortex serves to link the mechanical responses of many randomly polymerized microtubules. In many developmental systems, such as the Notch/Delta pathway, a single outcome is assured by local self-activation and lateral inhibition. In all of these systems there is an amplifying process that acts on stochastic variation to generate a single outcome. Cell and tissue polarity can often be elicited, oriented, and scaled by weak outside influences. Hence, biasing forces can direct actin arrays, orient spindle asters, and determine the polarity of the axis of the embryo. Such systems are constructed to produce many possible states but by their own design always collapse to a singular state, though not to any particular singular state, where the orientation is induced by weak biases or in the absence of bias by stochastic variation. The importance of biasing forces acting on randomly generated states was considered long ago by Darwin, who suggested the various tropic movements in plants were modifications of random movements (Darwin, 1880; West-Eberhard, 1998).

Figure 7 emphasizes some of the major principles of information flow and functional adaptability in biological systems, as discussed in this essay.

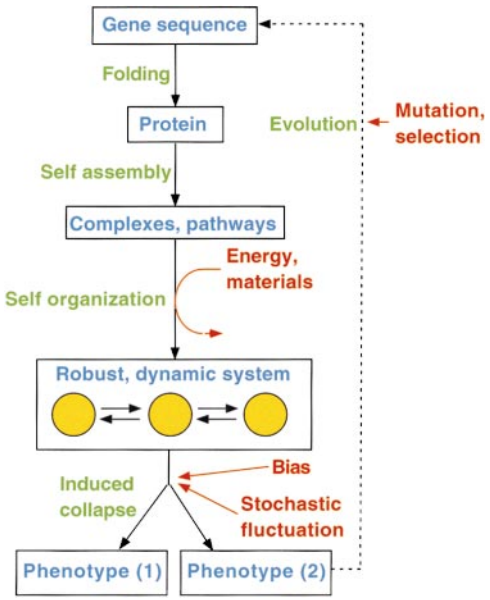

Figure 7. Information Flow in Biological Organization

This diagram illustrates information flow from the genotype (gene sequence) to the phenotype (structure, physiology). Folding and self-assembly limit the range of possible phenotypes that can be generated from the genotype. Self-organization uses two- or multi-stated components to generate robust, dynamic systems that can respond to external biases or amplify stochastic fluctuations and thus collapse into one of several potential functional states. By functional state we mean, for example, the vector of an actin comet tail or the differentiation state of an embryonic cell. The phenotype reflects this collapsed outcome. Natural selection acts on phenotypes, and together with mutation, feeds back on the genotype to produce evolutionary change. In this figure we have signified the components of a biological system with blue text, internal organization processes with green text, and external inputs to the organism as red text.

Whither Vitalism?

In the nineteenth century pattern formation, growth, physiological adaptability, and inheritance were considered properties of living organisms that seemed to separate them completely from the inanimate world. At the turn of the twenty-first century, we take one last wistful look at vitalism, only to underscore our need ultimately to move beyond the genomic analysis of protein and RNA components of the cell (which will soon become a thing of the past) and to turn to an investigation of the "vitalistic" properties of molecular, cellular, and organismal function. Such an opportunity is now possible because of the great advances in genetics and in molecular and cell biology during the past century. As it is now clear that gene products function in multiple pathways and the pathways themselves are interconnected in networks, it is obvious that there are many more possible outcomes than there are genes. The genotype, however deeply we analyze it, cannot be predictive of the actual phenotype, but can only provide knowledge of the universe of possible phenotypes. Biological systems have evolved to restrict these phenotypes, and in self-organizing systems the phenotype might depend as much on external conditions and random events as the genome-encoded structure of the molecular components (Figure 7). Yet out of such a potentially nondeterminist world, the organism has fashioned a very stable physiology and embryology. It is this robustness that suggested "vital forces", and it is this robustness that we wish ultimately to understand in terms of chemistry. We will have such an opportunity in this new century.

Acknowledgments

We thank Julie Theriot and Ted Salmon for providing figures and Anneli Mynttinen of the Einstein Papers Project of Boston University for researching the Einstein quotation. We also thank the National Institute of General Medical Sciences for support.

References

Aufderheide, K.J., Rotolo, T.C., and Grimes, G.W. (1999). Analyses of inverted ciliary rows in Paramecium. Combined light and electron microscopic observations. Eur. J. Protistol. *35*, 81–91.

Beisson, J., and Sonneborn, T.M. (1965). Cytoplasmic inheritance of the organization of the cell cotrtex in Paramecium aurelia. Proc. Natl. Acad. Sci. USA *53*, 275–282.

Berg, H. (1988). A physicist looks at bacterial chemotaxis. Cold Spring Harbor Symp. Quant. Biol. *53*, 1–9.

Cell (1998). Review issue: Macromolecular Machines, Vol. *92*, No. 3, pp. 291–390.

Caspar, D.L.D., and Klug, A. (1962). Physical principles in the construction of regular viruses. Cold Spring Harbor Symp. Quant. Biol. *27*, 1–24.

Darwin, C. (1880). The power of movement in plants (London: John Murray).

Drubin, D., and Nelson, W. (1996). Origins of cell polarity. Cell *84*, 335–344.

Elinson, R., and Holowacz, T. (1995). Specifying the dorsoanterior axis in frogs: 70 years since Spemann and Mangold. Curr. Top. Dev. Biol. *30*, 235–285.

Fankhauser, G. (1945). Maintenance of normal structure in heteroploid salamander larvae, through compensation of changes in cell size by adustment in cell number and cell shape. J. Exp. Zool. *100*, 445–455.

Fankhauser, G. (1972). Memories of great embryologists. Amer. Scientist *60*, 46–55.

Frankel, J. (2000). Cell biology of Tetrahymena thermophila. Methods Cell Biol. *62*, 27–125.

Frankel, J., and Whiteley, A.H. (1993). Vance Tartar: a unique biologist. J. Eukaryotic Microbiol. *40*, 1–9.

Frederickson, R. (1998). Macromolecular matchmaking: advances in two-hybrid and related technologies. Curr. Opin. Biotechnol. *9*, 90–96.

Gerhart, J., and Kirschner, M. (1997). Cells, Embryos, and Evolution (Boston, MA: Blackwell Science).

Gerhart, J., Danilchik, M., Doniak, T., Roberts, S., Rowning, B., and Stewart, R. (1989). Cortical rotation of the Xenopus egg: consequences for the anteroposterior pattern of embryonic dorsal development. Development (Suppl.) *107*, 37–51.

Henry, E.R., Jones, C.M., Hofrichter, J., and Eaton, W.A. (1997). Can a two-state MWC allosteric model explain hemoglobin kinetics? Biochemistry *36*, 6511–6528.

Holy, T.E., and Leibler, S. (1994). Dynamic instability of microtubules as an efficient way to search in space. Proc. Natl. Acad. Sci. USA *91*, 5682–5685.

Inoue, S. (1982). The role of self-assembly in the generation of biological form. In Developmental Order: Its Origin and Regulation, S. Subtelny and P.B. Green, eds. (New York: A.R. Liss), pp. 35–76.

Inoue, S., and Sato, H. (1967). Cell motility by labile association of molecules. The nature of mitotic spindle fibers and their role in chromosome movement. J. Gen. Physiol. (Suppl.) *50*, 259–292.

Kirschner, M., and Gerhart, J. (1998). Evolvability. Proc. Natl. Acad. Sci. USA *95*, 8420–8427.

Kirschner, M. and Mitchison, T. (1986). Beyond self-assembly: from microtubules to morphogenesis. Cell *45*, 329–342.

Miller, J., Rowning, B., Lalrabell, C.A., Yang-Snyder, J.A., Bates, R.L., and Moon, R.T. (1999). Establishment of the dorsal-ventral axis in Xenopus embryos coincides with the dorsal enrichment of dishevelled that is dependent on cortical rotation. J. Cell Biol. *146*, 427–437.

Monod, J., Wyman, J., et al. (1965). On the nature of allosteric transitions: a plausible model. J. Mol. Biol. *12*, 88.

Nicklas, R.B. (1997). How cells get the right chromosomes. Science *275*, 632–637.

Oosawa, F., and Asakura, S. (1975). Thermodynamics of the Polymerization of Protein (London: Academic Press).

Prigogine, I. (1955). Introduction to the Thermodynamics of Irreversible Processes (Springfield, IL: Thomas).

Rothman, J.E., and Wieland, F.T. (1996). Protein sorting by transport vesicles. Science *272*, 227–234.

Salmon, E.D. (1975). Pressure-induced depolymerization of spindle microtubules. I. Changes in birefringence and spindle length. J Cell Biol. *65*, 603–614.

Schmidt, A., Wolde, M., Thiele, C., Fest, W., Kratzin, H., Podtelejnikov, A.V., Witke, W., Huttner, W.B., and Soling, H.D. (1999). Endophilin I mediates synaptic vesicle formation by transfer of arachidonate to lysophosphatidic acid. Nature *401*, 133–141.

Spemann, H., and Mangold, H. (1924). Über Induktion von Embryonalanlagen durch Implantation artfremder Organisatoren. Roux' Arch. Entwicklungsmech. *100*, 599–638.

Spratt, N., and Haas, H. (1960). Integrative mechanisms in development of the early chick blastoderm. I. Regulative potentiality of separated parts. J. Exp. Zool. *145*, 97–138.

Tartar, V. (1961). The Biology of Stentor (Elmsford, NY: Pergamon Press).

van Oudenaarden, A., and Theriot, J. (1999). Cooperative symmetry-breaking by actin filament polymerization in a model for cell motility. Nat. Cell Biol. *1*, 493–499.

Verkhovsky, A.B., Svitkina, T.M., and Borisy, G.G. (1999). Self-polarization and directional motility of cytoplasm. Curr. Biol. *9*, 11–20.

Walczak, C., Vernos, I., Mitchison, T.J., Karsenti, E., and Heald, R. (1998). A model for the proposed roles of different microtubule-based motor proteins in establishing spindle biplority. Curr. Biol. *8*, 903–913.

Weinstein, D.C., and Hemmati.-Brivanlou, A. (1999). Neural induction. Annu. Rev. Cell Dev. Biol. *15*, 411–433.

West-Eberhard, M.J. (1998). Evolution in the light of developmental and cell biology, and vice versa. Proc. Natl. Acad. Sci. USA *95*, 8417–8419.