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## Morphogenetic fields in embryogenesis, regeneration, and cancer: Non-local control of complex patterning

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### Abstract

Establishment of shape during embryonic development, and the maintenance of shape against injury or tumorigenesis, requires constant coordination of cell behaviors toward the patterning needs of the host organism. Molecular cell biology and genetics have made great strides in understanding the mechanisms that regulate cell function. However, generalized rational control of shape is still largely beyond our current capabilities. Significant instructive signals function at long range to provide positional information and other cues to regulate organism-wide systems properties like anatomical polarity and size control. Is complex morphogenesis best understood as the emergent property of local cell interactions, or as the outcome of a computational process that is guided by a physically-encoded map or template of the final goal state? Here I review recent data and molecular mechanisms relevant to morphogenetic fields: large-scale systems of physical properties that have been proposed to store patterning information during embryogenesis, regenerative repair, and cancer suppression that ultimately controls anatomy. Placing special emphasis on the role of endogenous bioelectric signals as an important component of the morphogenetic field, I speculate on novel approaches for the computational modeling and control of these fields with applications to synthetic biology, regenerative medicine, and evolutionary developmental biology.

### Keywords

morphogenesis; regeneration; development; cancer; embryogenesis; bioelectricity

## 2. Introduction and scope

“Thus, beyond all questions of quantity there lie questions of pattern, which are essential for the understanding of Nature.”

-- Alfred North Whitehead (1934)

### 2.1. A question of pattern

Embryonic development results when a single cell (the fertilized egg) reliably self-assembles a highly complex pattern appropriate to its species. This process is known as *morphogenesis* - the establishment and creation of 3-dimensional anatomy. During later life,

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multicellular creatures must maintain their pattern – an active process of *morphostasis* that works to maintain the whole while individual tissues age or are removed by traumatic injury. Some organisms replace large-scale structures during adulthood, illustrating the remarkable plasticity and dynamic control of shape by biological systems. For example, salamanders can regenerate eyes, limbs, lower jaws, hearts, and portions of the brain. The active process of maintaining a complex morphology may likewise be relevant to suppression of cancerous disorganization in favor of cellular activities that are aligned within the morphological needs of the host organism.

A true understanding of the signals underlying this process would enable rational control of growth and form, giving rise to regenerative medicine applications that correct damage done by birth defects, degenerative disease, cancer, traumatic injury, and even aging. Similarly, a mature understanding of the origin and regulation of shape, including its genetic and epigenetic aspects, would deepen our understanding of evolvability (Gilbert et al., 1996; Goodwin, 1994) and have untold benefits for the nascent field of synthetic biology - the bioengineering of functional artificial systems using principles and building blocks abstracted from the biological world (Davies, 2008).

Deciphering and learning to control shape is thus arguably *the* fundamental problem of biology and medicine. Modern molecular cell biology and genetics have made great strides in uncovering the mechanisms guiding cell behavior. However, major questions still remain about the ways in which the activities of individual cells are orchestrated and coordinated to result in large-scale pattern and its regulation (Belousov, 2010; Gilbert and Sarkar, 2000). This review will discuss classical and recent data bearing on the morphogenetic field as a construct that encapsulates key properties of instructive growth and patterning control. Particular focus is placed on endogenous bioelectrical signals as the physical embodiment of the morphogenetic field.

## 2.2. Defining “morphogenetic field”

The concept of “morphogenetic field” has a number of distinct definitions and a rich history (Belousov, 2001). For some, it is a descriptive tool not necessarily tied to first principles. For example, D’Arcy Thompson showed a myriad ways in which aspects of living systems often bear striking resemblances to patterns which are obtained as solutions to field equations in physics – potentials of static electricity, magnetism, etc. (Thompson and Whyte, 1942). The discovery of mathematical field-like structures that seem to recapitulate biological patterns (Levin, 1994; Pietak, 2009) does not address directly the question of whether or not those mechanisms are in fact used in biological morphogenesis. In contrast to such “metaphoric” fields, other models explicitly use physical and chemical principles best described by field equations to generate pattern (Brandts, 1993; Brandts and Trainor, 1990a, b; Tevlin and Trainor, 1985), and may describe specific physical or biochemical processes that actually pattern system in question (Briere and Goodwin, 1990; Goodwin, 1985; Goodwin and Pateromichelakis, 1979; Goodwin and Trainor, 1980; Hart et al., 1989).

“Field” denotes both informational and regional relationships (Weiss, 1939). The quintessential property of a field model is non-locality - the idea that the influences coming to bear on any point in the system are not localized to that point and that an understanding of those forces must include information existing at other, distant regions in the system. In a sense, the familiar “morphogen gradient” is already a field model, as it refers to changes of the prevalence of some substance across a spatial domain, as opposed to a single concentration level at some local spot. Cells *in vivo* are immersed in a number of interpenetrating sets of signals - gradients of chemicals, stresses/strains/pressures, and electric potential (Figure 1). It remains to be shown in each specific case of pattern formation whether a true field model best explains and facilitates the experimental control of

the morphogenetic event in question. In this review, I focus on the spatially distributed nature of instructive patterning signals, discussing the evidence from developmental, regenerative, and cancer biology *for nonlocal control of pattern formation*. Specifically, these data suggest the hypothesis that many diverse examples of pattern formation are best understood not as cell-level behaviors around any one locale but rather at higher levels of organization.

This way of looking at patterning is far from new. From the perspective of organicism, such fields have been invoked in various guises by Spemann, Weiss, and others (Burr and Northrop, 1935, 1939; Gurwitsch, 1944, 1991; Needham, 1963; Northrop and Burr, 1937; Weiss, 1939). More modern discussions can be found as well, although this is certainly not considered a mainstream subject among the molecular developmental biology community today (Belousov, 2001; Belousov et al., 1997; De Robertis et al., 1991; Gilbert et al., 1996; Goodwin, 1982; Goodwin, 1994; Martinez-Frias et al., 1998; Opitz, 1993). While Child was one of the first to propose a physical substratum for these fields - physiological gradients (Child, 1941b), recent data confirm that steady-state bioelectrical properties are likely an important component of this fascinating set of signals (Adams and Levin, 2012b; Levin, 2009, 2012). In addition to these, chemical gradients (De Robertis et al., 1991; Reversade and De Robertis, 2005; Schiffmann, 1991, 1994, 1997, 2008, 2011), shear flows (Boryskina et al., 2011), coherent photon fields (Fels, 2009; Popp, 2009), and gene expression profiles (Chang et al., 2002; Rinn et al., 2006) are additional candidates for mediators of field information during patterning. Nevertheless, formal morphogenetic field models, especially those incorporating specific mechanisms and making testable predictions, are not common. It is hoped that a discussion of modern and oft-forgotten classical data will spur the creation and testing of true field models of pattern formation that will actually be able to predict and explain some of the most remarkable feats of morphogenesis accomplished by biological systems.

### 3. What information do morphogenetic fields carry?

#### 3.1. Positional information

One important piece of information necessary to integrate cell activity into a system-level patterning program is positional information (Furusawa and Kaneko, 2003; Jaeger et al., 2008; Wolpert, 1971), enabling cells and tissues to discern their location relative to each other within a complex 3-dimensional structure. The traditional medium for fields of positional information is a chemical gradient of some morphogen molecule (Ashe and Briscoe, 2006; Gurdon et al., 1999; Lander, 2007; Teleman et al., 2001). In frog embryos for example, gradients of Wnt and BMP proteins form orthogonal Cartesian coordinates (Niehrs, 2010) that define the placement of organs along the anterior-posterior and dorso-ventral axes. It is not yet known whether this sort of mechanism, and the deformations of the coordinate system itself (Jaeger et al., 2008), could underlie the fact that simple coordinate system deformations can often transform specific animal shapes into those of related species (Lewis, 2008; Thompson and Whyte, 1942). Numerous mathematical (polar coordinate field) models have been proposed to explain epimorphic regulation – growth and pattern formation to repair a discontinuity in a global field of positional values, such as at a site of amputation (Bryant et al., 1981; French et al., 1976; Mittenthal, 1981a, b; Winfree, 1990). This kind of intercalary regeneration is observed in flatworms (Agata et al., 2003; Saito et al., 2003), insects and crustaceans (French, 1978; Mittenthal and Nuelle, 1988; Truby, 1986), and amphibians (Maden, 1980; Muneoka and Murad, 1987; Papageorgiou, 1984; Rollman-Dinsmore and Bryant, 1982; Sessions et al., 1989), as well as unicellular systems (Shi et al., 1991). This suggests a deep principle not inextricably tied to any specific signaling pathway (Ogawa and Miyake, 2011; Yoshida and Kaneko, 2009). Fewer models postulating a field of positional information have attempted to incorporate morphallactic

regulation (Brandts and Trainor, 1990b), and more work remains to determine whether such models prove useful to understand the reorganization of intact system such as changes in size and remodeling.

The relevance of such models in adult animals is consistent with the dynamic nature of morphostasis, in which shape must be maintained actively throughout life. If the adult rat bladder epithelium is removed from its normally associated bladder mesenchyme and placed in contact with embryonic mesenchyme, stratified squamous epithelium of the bladder converts to the secretory acinar epithelium of prostate –plasticity remains, and tissue interactions are required, even into adult life to maintain identity of some cells (Cunha et al., 1983). Even in the absence of large-scale regenerative events, there is recent evidence for a remarkable memory of positional information by adult human cells. The expression profile of Hox genes in adult human fibroblasts reveals that they encode their position along three anatomical axes (Chang et al., 2002; Rinn et al., 2006). The scale of gene expression differences among fibroblasts (a single cell type) taken from different locations of the body is on par with the levels of transcriptional differences seen among currently-accepted distinct cell types (Wang et al., 2009), showing the importance of position for determining cell state (even when measured just at the transcriptional level). Interestingly, such fibroblasts are now known to form a body-wide connected network (Langevin et al., 2004), and much may remain to be learned about the storage and processing of spatial coordinate information along such a network of linked cells. Thus, positional information plays a large role in the patterning behavior not only of single cells and tissues; examples of position-dependent remodeling will be discussed below in the context of deer antler damage and amphibian blastema transplant experiments. Even position along the left-right axis is remembered: when eyes are transplanted, the optic axon fibers penetrate the host's diencephalon on the side from which the eye was removed from the donor (Koo and Graziadei, 1995)!

In addition to chemical and transcriptional gradients, it is now clear that bioelectric properties of cells provide positional cues also. It has long been known that individual cells respond to physiological-strength extracellular fields and recent genetic and biochemical experiments have begun to tease apart the mechanisms of this sensitivity at the cellular level (McCaig et al., 2005; Pu et al., 2007; Rajnecik et al., 2007; Yao et al., 2011). As far back as the 1930's however, it was already proposed that bioelectric properties form a field of positional information for migratory cell types and morphogenesis; these data were mostly derived from measurement and functional perturbation in amphibian experiments (Burr, 1932, 1941a, b; Burr and Bullock, 1941; Burr and Northrop, 1939; Burr and Sinnott, 1944; Northrop and Burr, 1937; Shi and Borgens, 1995).

The dynamics of storage of positional information in morphogenetic fields remains a major area of future investigation. In addition to understanding the mechanisms by which coordinates are *encoded* in varying physical/chemical properties of cells (whether directly or as a sort of stigmergy), it is crucial to also dissect the interpretation of these gradients by cells and multi-cellular structures as inputs to decision-making programs during morphogenesis. For example, the mapping of specific positional values to tissue outcomes implies a discrete code (French et al., 1976). Likewise, the time component (synchronization of growth and deformation) must be quantitatively explained, as has been done in phase-shift models that incorporate clocks (pace-maker cells) as well as maps (Goodwin and Cohen, 1969).

### 3.2. Subtle prepatterns

Another type of information that could be contained within the morphogenetic field is a prepatter – a scaffold that serves as a template (to some level of detail) for the shape being

assembled or repaired. With the discovery of the Hox code, it is well-accepted that gradients of Hox proteins specify a genetic prepattern for many areas, including the head (Hunt and Krumlauf, 1991), limb (Graham, 1994), and gut (Pitera et al., 1999). As presaged by Child (Child, 1941a), modern quantitative models of self-organization (Turing-type lateral inhibition/local activation systems) have been proposed (Meinhardt and Gierer, 1974; Schiffmann, 1997, 2001, 2004, 2005, 2006, 2011; Turing, 1990) to explain the origin and properties of the physiological pattern that usually precedes a spatially isomorphic anatomy. Recent data however have shown that true bioelectrical properties (voltage gradients) may also function as such templates of shape. Burr was one of the first to formulate an explicit model of a bioelectrical prepattern, finding that the ratios of two dimensions of cucurbit fruit were first predicted by voltage gradients measured in the embryo (Burr and Sinnott, 1944); similar experiments showed that electrical properties predicted subsequent developmental morphology of nervous system patterning during amphibian embryogenesis (Burr, 1932; Burr and Hovland, 1937).

Recent development of voltage-sensitive fluorescent dyes (Fig. 2A) allows direct, noninvasive visualization of transmembrane potential gradients in tissues *in vivo* (Adams and Levin, 2012b; Adams and Levin, 2012c; Oviedo et al., 2008). A landmark recent paper (Vandenberg et al., 2011) characterized, in real-time, the bioelectric properties of a highly dynamic morphogenetic event: the formation of the face in *Xenopus laevis* embryos. Using voltage-reporter dyes and time-lapse microscopy, a movie was made of the many dynamic changes occurring at this time in the distribution of cells with distinct transmembrane potentials. A single frame is shown in Fig. 2B, revealing a rich regionalization of voltage gradient that demarcates the interior of the neural tube, the future mouth, and thin bilateral crescents on the edge of the face (red arrowheads) that mark the position of the first pharyngeal pouch. Several of these bioelectrically-unique regions match the expression patterns of key genes that regulate differentiation and migration of tissues in the face. By misexpressing constructs encoding loss- and gain-of-function ion channel mutants to perturb pH and transmembrane potential in the embryonic face *in vivo*, it was shown that these gradients are natively driven by differences in the activity of the V-ATPase proton pump. Artificially perturbing the pattern of the voltage domains results in changes in the expression of important patterning genes such as *Sox9*, *Slug*, *Pax8*, *Mitf*, *Frizzled3*, and *Otx2* and thus produces the subsequent characteristic defects in the morphology of craniofacial structures. This quantitative spatio-temporal profiling of native physiology, combined with detailed characterization of anatomical and molecular-genetic perturbation of the boundaries of the hyperpolarization domains, is a superb example of physiology serving as a subtle prepattern for regions of gene expression, much as transcriptional states act as prepatterns for subsequent anatomy.

### 3.3. Epigenetic aspects of the morphogenetic field – beyond transcriptional networks

The storage of patterning information in physiological or biomechanical gradients highlights the importance of events that are epigenetic in Waddington's original sense of the word – not restricted to chromatin modifications but rather any physical information-bearing structures other than DNA. Modern biology's focus is largely on gene expression – high-resolution mapping of transcriptional networks is expected to contain all of the information needed to explain shape. However, recent work highlights alternative mechanisms that must be considered as participants in the morphogenetic field. The geometric shape of the substrate upon which cells reside has crucial implications for their future behavior (Chen et al., 1997, 1998; Huang and Ingber, 2000); this geometry is an ideal example of a signal that cannot be described by genetic or proteomic profiling alone. Additional physical properties that can serve similar functions include mechanical properties of tissues (Belousov, 2008; Belousov and Grabovsky, 2007; Belousov and Lakirev, 1991; Belousov et al., 2000;



Belousov et al., 1997; Brodland et al., 1994; Discher et al., 2005; Savic et al., 1986), ultraweak photon emission (Belousov, 2001; Popp, 2003), and bioelectrical gradients (Levin, 2007b, 2009, 2011a, 2012).

Physiological states of cells are crucial for determination of shape; it was recently shown that experimental control of transmembrane potentials can induce tail growth at non-regenerative stages in tadpoles (Adams et al., 2007; Tseng et al., 2010), reprogram the posterior-facing blastema in a fragment of the planarian flatworm to regenerate a head instead of a tail (Beane et al., 2011), reverse the left-right asymmetry of the internal organs in several species (Adams et al., 2006; Levin et al., 2002), and induce the formation of a complete eye in any part of the frog embryo, even in gut or mesodermal cells far away from the normally eye-competent anterior ectoderm (Pai et al., 2012). Crucially, such bioelectric determinants of morphology are completely invisible to modern molecular profiling techniques. Cells expressing precisely the same ion channels and pumps could be in very different physiological states, because ion transporter states are regulated post-translationally. Likewise, cells with very different genetic profiles could be in highly similar physiological states, because the same transmembrane potential can be established by the combined activity of numerous different ion translocators. Thus, transcriptional and protein-level profiling needs to be augmented with comprehensive quantitative physiomics; likewise, functional approaches must be altered to probe bioelectric controls because loss-of-function strategies targeting individual channel or pump genes often miss phenotypes due to compensation by numerous other ion translocator family members (Levin, 2012).

While important bioelectric events (including self-generation of physiological pattern among “excitable media” such as cell sheets) can take place independent of changes of transcription, of course such signaling is involved in numerous levels of feedback with traditional biochemical and transcriptional downstream steps. For example, rapid, transient gap junction-mediated flows of small molecules lead to permanent morphological change, as occurs in voltage-driven redistribution of serotonin molecules during left-right patterning in vertebrate embryos (Fukumoto et al., 2005) and the establishment of AWC neuron asymmetry in *C. elegans* development (Chuang et al., 2007), while temporary reversal of the pH or voltage gradient permanently resets the dorso-ventral polarity of the chick blastoderm (Stern and MacKenzie, 1983; Stern, 1987, 1991).

Remarkably, such changes in instructive physiological properties are “remembered” by tissue. In planaria, an amputated fragment always regenerates a head and tail at the appropriate ends. However, a brief (48-hour) isolation of cells from their neighbors via gap junction closure results in the formation of 2-headed worms. These worms will then continue to regenerate as 2-headed worms, even when cut in the absence of any other reagent! The transient, non-genotoxic perturbation of physiological networks active during regenerative repair permanently changed the pattern to which these animals regenerate after damage even in multiple rounds of amputation months after the initial treatment (Oviedo et al., 2010). The physiological network behavior becomes canalized into a long-term change of pattern, which is stable across the normal reproductive mode of this animal (fission + regeneration). Thus, a line of such 2-headed animals could be maintained, which would be identical in DNA sequence to the normal 1-headed worms and yet have radically different behavior and body-plan architecture. The evolutionary implications of this are apparent, and demonstrate that the biophysical, epigenetic aspects of patterning may play an important role in evolution, as selection operates on animal morphologies. Thus, it is likely that a full understanding of the morphogenetic field and its informational content will need to involve cracking the bioelectric code (the mapping between spatio-temporal ionic profile patterns and tissue morphology outcomes).

Bioelectric events have properties that make them ideal components for implementing the morphogenetic field, and indeed recent data has shown that their manipulation is a good entry-point into a molecular-level understanding of these mechanisms (Levin, 2007b, 2012). A next step in this field is the construction of specific dynamical systems models of patterning information stored in real-time physiological networks. Multidimensional spaces of many different bioelectric measurements will require concerted physiomics profiling efforts; such data may turn out to contain attractors that map to anatomical states, and may implement the “dynamically preformed morph” envisioned by Gurwitsch (Gurwitsch, 1944).

Are there any precedents for storing information in dynamic patterns of ion flow? Certainly the original computer memories stored bits as directions of current flow in tiny coils of wire, and a flip-flop circuit (the basis of modern computer memory) does the same. Much as the ion flows among electrically active cells are invisible to techniques focused on the material structure of cells and the mRNA/proteins expressed in them, the information content of electronic storage media is invisible to a description of the structural components of a computer memory system - energy flow patterns can store distinct bits among identical bi-stable units, whether they are implemented in cells (Gallaher et al., 2010; Gorostiza et al., 2007; Sachdeva et al., 2010) or transistor flipflop circuits. However, even closer to home, the importance of the nervous system in many aspects of morphogenetic regulation reminds us that cognitive science has a mature and well-developed history of investigating spatial maps encoded in the dynamics of electrically-active cells! The neurobehavioral community is quite comfortable with the storage of memory in neural networks, and techniques and results in this field should be combined with modern understanding of pattern formation. After all, both study information – spatial information processed in reorganization of geometry (morphogenesis), and temporal information remembered as patterns from the environment (learning and memory). Not surprisingly, ion translocators are involved in learning and memory storage (Daoudal and Debanne, 2003; Debanne et al., 2003; Pulver and Griffith, 2010), placing these molecules at an important focal point at the intersection of morphogenesis and cognition. Likewise, heart cells have been modeled as a neural-like network to explain memory effects relevant to remodeling (Krishnan et al., 2008; Sachdeva et al., 2010). While most somatic cells process voltage change signals much more slowly than do rapidly spiking neurons, it is tempting to speculate that the analogy may indicate a real, mechanistic relationship.

## 4. Is the Target Morphology directly encoded?

### 4.1. Data to be explained

In what way might the information inherent in morphogenetic fields be encoded? To help focus this question it is useful to be reminded of some of the more remarkable aspects of morphological plasticity that must be explained by any mature theory of morphogenesis.

It has long been known that when one cell of a 2-cell embryo (of many species) is removed, the remaining cell gives rise to a complete embryo, not a half-embryo; size regulation is not only the province of embryos (Cooke, 1981): starved planarian flatworms shrink allometrically – as the cell number is reduced, they dynamically remodel all of their tissues to retain perfect mathematical proportion among the various organs (Oviedo et al., 2003). Cnidarian embryos establish appropriate final pattern despite tremendous variability of intermediate stages (Kraus, 2006).

In some species of deer, injuries made to the antler at a given spot not only produce a small bump as the bone heals, but also recur as larger ectopic growths in the same location in subsequent years' antler racks (Fig. 4A) (Bubenik and Pavlansky, 1965; Bubenik, 1990). Although this process (termed “trophic memory”, (Bubenik and Pavlansky, 1965; Bubenik

and Bubenik, 1990)) has not been quantitatively studied to determine exactly how much information is handled by this system (spatial precision with which injuries are mapped), its existence has several implications. First, the location of injuries at remote sites are communicated to the scalp cells a considerable distance away. Second, the cells at the growth zone in the scalp have a spatial memory that lasts at least several years. Finally, the behavior of those cells is altered in precisely the right way so that next year, when making growth decisions, an antler branching pattern is constructed that recapitulates the location of the original injury.

A salamander or lobster that loses its limb can regenerate it perfectly (Birbaum and Alvarado, 2008). Indeed some complex creatures (e.g., planaria) can regenerate the entire body (including their centralized brain) from a fragment of the original animal (Reddien and Sanchez Alvarado, 2004). Importantly however, large-scale morphostasis does not simply depend on recapitulating fixed developmental programs (Voskoboynik et al., 2007). For example, the tadpole face is quite different from that of a frog; during metamorphosis, a series of deformations must be executed and various organs and tissues displaced towards their appropriate locations. Remarkably, when developmental defects were induced in the tadpole (by manipulating the embryonic voltage gradients that guide craniofacial patterning), the process of metamorphosis was able to adjust accordingly (Vandenberg et al., 2012). Most organs were still placed into the right final positions, using movements quite unlike the normal events of metamorphosis, showing that what is encoded is not a hardwired set of tissue movements but rather a flexible, dynamic program that is able to recognize deviations, perform appropriate actions to minimize those deviations, and stop rearranging at the right time. Even the highly-mosaic *C. elegans* embryo can re-route cells through far-ranging movements (Schnabel et al., 2006) to counteract experimental perturbations.

This plasticity extends to adult forms of some species. Consider what happens when an amphibian tail is amputated. Blastema (undifferentiated, proliferating) cells arise at the site of injury (Butler and O'Brien, 1942) and the initial pattern formation is determined by the original position of the blastema within the donor's body: when transplanted onto the flank of a recipient animal, such a blastema graft first forms a tail. However, the host's morphogenetic fields exerts their influence, and slowly transforms the ectopic tail into a limb - the structure appropriate to the large-scale global context in which it is placed (Farinella-Ferruzza, 1953, 1956; Guyenot, 1927; Guyenot and Schotte, 1927).

The above-described regulative properties are still well beyond current capabilities of engineering and robotics. To help translate the data of biology towards synthetic systems with robustness and the capabilities of flexible self-repair, as well as spur new research to understand these phenomena using quantitative, state-of-the-art molecular approaches, it is important to consider the kind of algorithm that might need to be implemented to make use of dynamic patterning information.

#### 4.2. Direct encoding or emergence?

There are two major ways to look at the origin of form, regardless of the physical implementation of the morphogenetic field signals. The prevalent paradigm is that of emergence. Progress in the science of complexity (Kauffman, 1995; Mitchell, 2009) has revealed that when many subunits of a system interact according to simple specified rules, the outcome can be incredibly complex, difficult to predict, and have systems-level properties that are not directly specified by, or apparent in, the rules themselves (Fig. 3A,B). For example, the individual rules governing ant behavior are relatively simple and do not directly refer to any properties of the anthill that is eventually built when large numbers of ants carry out their individual instructions. As another example, consider a discrete cellular automaton model known as the "Conway's Game of Life" (Adamatzky, 2010; Hiatt, 1999;



Sapin et al., 2007): it is implemented on a checkerboard where each cell can be “alive” or “dead”. In each tick of the discrete clock, a cell converts to one or the other state depending purely on the number of its “live neighbors” (a set of 4 simple, deterministic rules). Running such a system (watching each successive generation as a new frame in a movie) reveals a staggering complexity of transforming shapes that send out traveling waves of “gliders” and roil with activity; indeed such a system is known to be computationally complete (Berlekamp et al., 2001), able to simulate all known algorithms if the gliders are interpreted as traveling signals. Importantly, the rules for the system refer only to local properties – counting the numbers of neighbors for each cell; they say nothing about the remarkable “spaceships”, “beehives”, self-reproducing structures, and other dynamic constructions that appear once this deceptively simple system is implemented. Similarly, it is largely assumed in the field today that the best path to understanding the generation of shape is by mechanistic dissection of the rules governing single-cell behavior. It is thus hoped by many that through systems biology (computational modeling), we will someday understand how the behaviors of cells add up to the dynamic construction and maintenance of a complex 3-dimensional morphology.

Emergent models are preferred because of their parsimony. While this is the methodological assumption behind most work in molecular developmental biology today, it is important to keep in mind that the sufficiency of emergence is an empirical claim – there is no intrinsically privileged level of explanation and it may turn out that shape is most effectively predicted and controlled by modeling at a higher level of organization. As an alternative strategy to emergence, it has previously been proposed that biological structures encode maps for their shape – a “target morphology” may be encoded in some form. This would not have to be at the resolution of individual cells (perhaps only the general layout of the bodyplan); however what makes target morphology models distinct from emergent models is the hypothesis that there are some measurable quantities contained in the living system that are directly isomorphic to the anatomy that is being constructed or maintained. In emergent models, there is no such process, the shape being assembled as the result of low-level rules and not by comparison to (or directives from) any informational structure that encodes a final shape. Thus, the target morphology hypothesis predicts that one does not have to explicitly model low-level interactions *in silico* in order to predict what shape will result from a given patterning state at time  $t$  – that some measurable quantity exists now that is predictive and functionally determinative of the next patterning states. The emergence hypothesis predicts that the only way to know what the pattern will be at time  $t+1$  is to explicitly model the interactions of some low-level component (e.g., single cells) and see what happens. Thus, these two models describe different ways of controlling the morphogenesis of complex systems and have very different implications for development of prediction and control strategies.

A “target morphology” is the shape, defined on multiple scales of size and levels of organization, which a biological system acquires during development, and maintains against cellular turnover (aging), stresses of life (remodeling and wound healing), and major injury (regeneration). Models involving the target morphology require a perspective, focused on information processing in cells and tissues, which emphasizes mechanisms common to the system-level patterning events that occur during embryonic development and regeneration, or fail to occur during neoplastic growth (more on this below). Target morphology models are eschewed in biology today, mainly because of a fear of teleology (Ruse, 1989; Teufel, 2011) harkening back to the early days of preformationism, and because the field has made such progress by focusing on the difficult problem of cellular-level controls. However, there are data that suggest that prepatter models should be considered.

One set of results that suggests a target morphology model is the trophic memory in deer antlers discussed above. If there is a target morphology for the rack shape encoded directly in some way, it is easy to see how changes of that shape can be long-lived. An injury to a specific place on one tine may induce a physical change in the map structure at the corresponding location (e.g., a change in a neural network storing the morphology), causing the extra tine to be recapitulated in subsequent years as the antlers grow and cells “consult” (are controlled by) the map. In contrast, an emergent model views the antler rack shape as the result of purely local decisions made by cells during their growth period. The question this system would have to solve is: how to modify the rules of cell growth to result in exactly the same rack shape plus one extra tine at the specified location? This is an excellent example of an inverse problem (Fig. 3C), and is in general computationally intractable – there is no way for the system to know how the cell behavior rule set is to be modified to result in the desired pattern. This seems to be a situation in which a map model would be preferable, and indeed *a priori*, the emergent model wrongly *predicts that such a phenomenon should not exist*. The key of course is that a useful target morphology model has to be mechanistic and have explanatory power: it has to make testable predictions and say something about the physical implementation of the map that is stored and the mechanisms by which cells and tissues interact with (are instructed by) that map during patterning. The development of molecular tools for the deer antler system (Price et al., 2005) may allow such models to be tested, and the properties of the map to be quantitatively defined. For example, what spatial resolution does the map have? Can it distinguish (remember and implement next year) the difference between damage sites that differ by 1 cm? 10 cm? etc.

Fortunately, the first molecular entry-point into this territory has recently been uncovered. As described above, planarian flatworms in which physiological communication is transiently inhibited among cells have a *permanent* re-specification of body shape, regenerating as 2-headed “Push-me-pull-you” shapes when cut without any further manipulation (Oviedo et al., 2010). This shows a unique example in which the shape *to which this worm regenerates its pattern upon damage* – the target morphology – can be specifically altered (Fig. 4B). These data suggest that the target morphology does indeed exist (and can be experimentally modified), since no “head organizer” remains when the 2-headed worm is cut into thirds – it appears that all regions of the animal have adopted the new shape to which the animal must repattern. On-going work is addressing the mechanisms by which physiological networks can store the information about anatomical head-tail polarity, and perhaps a quantitative model of bioelectrical storage of target morphology will result.

Importantly, this question is not just philosophy; it has real implications for strategies in regenerative medicine. Suppose a structure needed to be changed in a biomedical setting – e.g., fixing a birth defect or inducing remodeling of a damaged organ. How do we know what signals must be provided? If the shape is truly emergent, this may be an impossible problem in the general case, requiring direct bioengineering (which is unlikely to be feasible in the case of complex organs such as limbs, eyes, etc.) because the relationship between cell-level rules and final patterning outcome is simply too complex for any tractable model to be able to reverse. On the other hand, if a mapping exists between a known set of physical parameters and the final pattern that will be built by new growth, then it is of the highest importance to understand the mechanisms of information storage and encoding, so that the information in this structure can be changed, and thus induce the organism to remodel accordingly.

### 4.3. Morphogenetic modules: modeling the native software of pattern formation

An important aspect of the morphogenetic field and what information it encodes is the degree of *modularity*. Teratoma tumors have proper patterning at the tissue level – possessing hair, teeth, and other structures, what they lack is a proper 3-dimensional organization of those components relative to each other. Likewise, the Disorganization (*Ds*) mouse mutant (Crosby et al., 1992; de Michelena and Stachurska, 1993; Robin and Nadeau, 2001) exhibits a peculiar form of birth defect where numerous different coherent structures (including entire limbs, sense organs, genitals, tails, etc.) may be formed in ectopic locations. Each affected individual is different, and the spectrum of the syndrome (which affects an extremely wide range of structures) suggests that what is perturbed is a system of large-scale organization that places individual structures in a specific pattern relative to each other. An interesting phenotype was observed to result from disruption of the endogenous bioelectric state of frog embryos, in which overall form of the embryo was normal but internal histogenesis was drastically disrupted (Borgens and Shi, 1995), again revealing the experimental separability of large-scale vs. low-level organization.

Modularity is likewise readily apparent in the results of recent efforts to manipulate and control shape. For example, some bioelectric manipulations have a master-regulator property: a single signal is able to trigger complex, highly-orchestrated (and self-limiting) patterning cascades in the host. Induced changes of membrane potential have caused formation of entire tails (Adams et al., 2007; Tseng et al., 2010) eyes (Pai et al., 2012), and heads (Beane et al., 2011) in various model species. Crucially, the signal provided is a very simple one – these complex structures are formed not because we know how to assemble them or how to explain their morphogenesis but because certain stimuli activate downstream morphogenetic programs that the host organism already knows how to execute. Our manipulations of bioelectric state (Adams et al., 2007; Beane et al., 2011; Pai et al., 2012; Tseng et al., 2010) created not a tiny tail, a huge tail, a backwards tail, or a tumor – they created a tail of exactly the right shape, size, and orientation. This sort of modularity (Fig. 5) makes sense in light of the evolvability (Kashtan and Alon, 2005; Kirschner and Gerhart, 1998) of complex bodyplans (for which mutations must produce *coherent* changes in morphology).

Interestingly, the tendency of shape to be repaired appears to be a fundamental attractor or property of living systems, in contrast to the view of regeneration pathways as specially-evolved adaptations. For example, newts exhibit a highly specific transdifferentiation response to lens removal – a rather delicate surgery that is unlikely to occur in nature and thus serve as an evolutionary advantage for the appearance of this repair pathway (Henry and Tsonis, 2010). Organisms relying on the strategy of large numbers of offspring (where the investment in any one individual is not expected to be a strong driver of selective advantage) such as *Drosophila* nevertheless exhibit pathways for wound healing and self-repair (Belacortu and Paricio, 2011). Similarly, unicellular organisms can also regenerate, such as following removal of the flagellum (Lefebvre and Rosenbaum, 1986). Such intrinsic morphostatic mechanisms give great hope for regenerative medicine: we may discover these master regulator signals and induce reconstruction of complex organs and appendages long before we learn the much more difficult task of directly micromanaging (bioengineering) their construction from individual cell types.

A most natural way of describing this modularity, and capturing the fact that the physiological signal is much simpler than the anatomical structure that it induces, is through the notion of a “subroutine call” from the field of computer programming. The phenomenon of homeosis (transformation of one complex body part into another) induced by changes in just a few specific proteins (e.g., *HOX* gene levels) illustrates this: any complex set of actions can be encapsulated in such a way that a very simple trigger will induce the activity

without itself containing the information needed to carry it out. A number of other computational metaphors readily suggest themselves when thinking about pattern regulation. Consider the regeneration of an amputated newt limb, or the dynamic repair of craniofacial defects during amphibian development. One is tempted to describe the process as the behavior of a cybernetic system that 1) knows the shape  $S$  it is supposed to have, 2) can tell that its current shape  $S'$  differs from this target morphology, 3) can compute a kind of means-ends analysis to get from  $S$  to  $S'$ , and 4) performs a kind of self-surveillance to know when the desired shape has been restored. Note that information-based computational models fit very naturally with the notion of target morphology because a comparison template is necessary for such algorithms to know when and what morphogenetic change is needed.

Such computational models, using building blocks of information, shape descriptors, and message-passing (as opposed to models consisting of gene regulatory networks for example) can apply to most examples of the activity of morphogenetic fields. Whether they will turn out to be useful requires predictive, quantitative hypotheses about the exact physical implementation of each of these functions to be formulated and tested. The advantage of such models is that they are algorithmic and constructive, showing at each step the actions and information processing that needs to occur in a system that reproduces observed patterning behavior. Currently however, molecular mechanisms for “shape surveillance”, “determining a set of actions to transform one shape to another”, storage of shape information, etc. remain to be uncovered, if they exist. The motivation to formulate and test such models is high however, because current paradigms for molecular models of patterning largely comprise gene and protein interaction networks; while these stick closely to first principles of cell biology, by themselves they reveal only *necessary* components but are not *sufficient* to explain shape because such descriptions do not constrain geometry nor allow geometry to be predicted from the molecular pathway data.

## 5. Cancer - a disease of geometry?

### 5.1: Tumors as failures of morphostasis

The idea that cancer is a developmental disease is an old one (Baker et al., 2009; Potter, 2001, 2007; Rowlatt, 1994; Rubin, 1985; Tsonis, 1987); Needham and Waddington speculated that cancers represented an escape from the control of the morphogenetic field (Needham, 1936b; Needham, 1963; Waddington, 1935). On this view, tumors form when cells stop obeying the normal patterning cues of the body: “cancer as part of an inexorable process in which the organism falls behind in its ceaseless effort to maintain order” (Rubin, 1985). This view, focusing on the role of the cells’ microenvironment, has been defended recently (Sonnenschein and Soto, 1999; Soto and Sonnenschein, 2004) as an alternative to the mainstream gene-centered paradigm that sees irrevocable changes in DNA sequence or gene expression profile as a fundamental change driving tumor stem cells (Vaux, 2011). Important open questions focus on whether cancer is best understood as a cell-autonomous vs. environmental cue, modeled at the single cell level vs. as a fundamentally tissue/organ phenomenon, and whether genetic vs. epigenetic mechanisms play the biggest roles.

Understanding cancer as a reversible physiological state has significant medical implications because characterizing the impact of the cellular environment on neoplastic progression may impact prevention and detection strategies. Further, a mechanistic dissection of these pathways may give rise to strategies that reboot (Ingber, 2008) or normalize cancer, in contrast to current approaches that all seek to kill tumors and thus risk a compensatory proliferation response (Fan and Bergmann, 2008) by rogue cells that still remain. Certainly it is clear that context in the host plays an important role in the complex phenomenon of cancer, making it an interesting perspective from which to study the morphogenetic field

concept. The interplay between proper patterning and cancer suppression is retained throughout life; for example, if the endocrine gland is removed in *Dixippus*, regenerative capacity is lost, and spontaneous tumors begin to appear (Pflugfelder, 1938, 1939, 1950, 1954).

Biologists are beginning to explore the idea that cancer is not a genetic disease of specific loci but rather a kind of attractor in a multi-dimensional transcriptional space describing cell states (Dinicola et al., 2011): “The topology of the attractor is the ‘invisible hand’ driving the system functions into coherent behavioral states: they are self-organizing structures and can capture the gene expression profiles associated with cell fates” (Huang et al., 2009). Huang et al. also point out an interesting paradox: while many studies seek to “determine which gene is mutated to explain an incremental malignant trait, no one doubts that normal cells as distinct as a mature neuron vs. a blood or epithelial stem cell share the exactly same genome! No mutations are invoked to explain the remarkable phenotypes during cell lineages in development” (Huang et al., 2009). Tumor reversion (e.g., observed when cancer cells are placed in normal embryonic environments) contradicts irreversible, cell-autonomous genetically-deterministic models of the origin of cancer, and emphasizes the role of tissue structure (Bissell and Radisky, 2001; Bizzarri et al., 2011; Bizzarri et al., 2008; Ingber, 2008; Weaver and Gilbert, 2004). Thus while the dynamic physiological nature of cancer as a disorder of regulation is now a serious topic in mainstream molecular cell biology, the significance of large-scale morphogenetic cues (organization beyond the local tissue level) has not really been explored.

## 5.2: Morphogenetic field as tumor suppressor: importance of community

“Cancer is no more a disease of cells than a traffic jam is a disease of cars. A lifetime study of the internal combustion engine would not help anyone to understand our traffic problems” (Smithers, 1962). The hypothesis that cancer is fundamentally a phenomenon at the level of multicellular organization makes a number of predictions confirmed by experimental data. Cells in dispersed monolayer culture are several orders of magnitude more sensitive to chemical carcinogenesis than are organized tissues within an intact organism (Parodi and Brambilla, 1977), and placing normal primary mammalian cells in culture results in the appearance of cells with malignant potential. Newt regeneration blastemas exposed to carcinogenic chemicals or ultraviolet radiation produce ectopic limbs or lenses, not tumors (Breedis, 1952; Butler and Blum, 1955; Eguchi and Watanabe, 1973), demonstrating the ability of actively patterning tissues to suppress tumorigenesis and highlighting the possibility that cancer induction and large-scale patterning disorganization (ectopic organs) are different points on a single axis.

Consistent with this are data showing that tumorigenesis is promoted when cells are isolated from their neighbors (and thus from the morphogenetic guidance they would otherwise receive) by either gap-junctional inhibition (Mesnil et al., 2005; Mesnil et al., 1995) or by physical barriers. Implanting into connective tissue of the rat rectangles of inert plastic, metal foil, or glass coverslips induces sarcomas when the material is > 1 sq. cm. If the material is perforated, the incidence is reduced, and the effect is not recapitulated by powders of the same material (which actually increases surface area, ruling out chemical induction mechanisms) (Bischoff and Bryson, 1964; Oppenheimer et al., 1952, 1953a; Oppenheimer et al., 1953b).

In contrast, re-establishing appropriate interactions of human cancer cells with the host organism can reverse neoplastic behavior even in the presence of significant genetic damage (Barcellos-Hoff, 2001; Bissell et al., 2002). A number of authors stress the suppressive nature of signals from neighboring tissues (Baker et al., 2009; Potter, 2001, 2007; Soto and Sonnenschein, 2004). One of mechanisms recently implicated is the planar cell polarity



pathway – a set of protein components designed to coordinate cells over long distances (Gray et al., 2011). PCP has now been shown to function as a non-canonical tumor suppressor (Klezovitch et al., 2004; Lee and Vasioukhin, 2008). While the direct causal relationship between loss of PCP and tumor initiation in humans is not yet proven, it is clear that loss of polarity can be an initiating event in tumor formation in *Drosophila* (Wodarz and Nathke, 2007). Consistent with conserved mechanisms underlying coordination of long-range order in cancer and normal development, PCP is also involved in dynamic morphostasis: grafts of embryonic skin (after the planar polarity of hair becomes evident), when implanted into adults, realign their hair polarity to match that of the hosts (Devenport and Fuchs, 2008). PCP allows cells to align axes orthogonal to their apical-basal polarity with each other, and with major anatomical axes of the organism. It is interesting also to consider influences functioning at a higher level of organization than local tissue and specific inhibitory signals such as morphostats (Baker et al., 2010) – signals that pertain to the position and orientation of structures within the context of a larger morphology.

### 5.3: Positional information and cancer

In addition to simply being connected to normal neighbors, it appears that position within the host is an important factor for tumorigenesis, which is not a prediction of mutation models, but may imply differences in the strength or positioning of the morphogenetic field. For example, tumors grow on posterior regions of *Triturus* less readily than they do on anterior regions (Seilern-Aspang and Kratochwill, 1965), and numerous such differences are reviewed in (Auerbach and Auerbach, 1982). Disruption of normal topographical tissue relationships tends to induce cancer, which suggests a feedback model where the morphogenetic field can be altered by scrambled anatomy, or perhaps difficulty in cells' reading instructions at the borders of fields that are not supposed to be geometrically adjacent. For example, transplantation of rat testis to the spleen induces formation of interstitial cell tumors (Biskind and Biskind, 1945), and normal rat ovary tissue put into normal rat spleen results in malignant neoplasm (Biskind and Biskind, 1944). Likewise, implantation of mouse embryos into adults causes teratocarcinomas (Stevens, 1970), possibly due to an interference of the host and implanted morphogenetic field structures. While these observations have not yet been understood mechanistically, modern genetically-tractable model systems are beginning to provide contexts for their investigation. In mammalian breast cancer (Maffini et al., 2004) and frog melanoma-like transformation (Blackiston et al., 2011; Morokuma et al., 2008), clear roles for non-local (long-range) influence over carcinogenesis have been found and can now be dissected. This is clinically relevant, as seen in field effects in many different kinds of cancer in which surrogate sites are not necessarily adjacent to the main tumor (Kopelovich et al., 1999; Subramanian et al., 2009).

### 5.4: Normalization of cancer by developmental patterning

The morphogenetic field ought to be the most active and “accessible” during embryogenesis. It is thus not surprising that despite high malignancy and euploidy, tumor cells integrated into wild-type embryonic hosts become integrated as normal tissue (Astigiano et al., 2005; Illmensee and Mintz, 1976; Li et al., 2003; Mintz and Illmensee, 1975; Webb et al., 1984). Childhood neuroblastoma has a high rate of spontaneous regression (Brodeur, 2003; Nakagawara, 1998). Human metastatic melanoma cells injected into zebrafish embryos acquire a non-neoplastic phenotype, but form tumors when injected into zebrafish *after* organogenesis (Haldi et al., 2006; Lee et al., 2005). Likewise, implanted sarcoma progressed in 80% of adult rats but only in 6.4% of rat embryos. Similar data have been recently shown for chick and other kinds of embryos that are able to tame aggressive cancer cells when these are implanted (Hendrix et al., 2007; Kasemeier-Kulesa et al., 2008; Kulesa et al., 2006; Lee et al., 2005). These data are consistent with the morphogenetic field concept

because they indicate the power of active patterning cues to normalize cancer (over-ride genetic defects); they are less compatible with cell-level biochemical pathway cues, as embryos have high levels of many growth factors that could otherwise be expected to potentiate tumor growth.

### 5.5: Normalization of cancer by regeneration

Tumors have been described as wounds that do not heal – areas of disruption and cell growth without an appropriate patterning program that reaches a terminal goal state (Pierce and Speers, 1988; Riss et al., 2006). This analogy has been supported by profiling showing the molecular similarity of repair vs. carcinoma in renal tissue (Riss et al., 2006). What about wounds that not only heal but successfully rebuild a missing structure? It has been long known that regeneration and cancer are closely related (Brockes, 1998; Donaldson and Mason, 1975; Rose and Wallingford, 1948; Ruben et al., 1966; Tsonis, 1983; Wolsky, 1978). Highly-regenerative organisms are resistant to tumors (Brockes, 1998; Okamoto, 1997; Tsonis, 1983; Zilakos et al., 1996), and this inverse relationship between regeneration and cancer susceptibility (Breedis, 1952; Prehn, 1997) is more compatible with the importance of morphogenetic field guidance than a focus on cancer risk associated with the presence of highly-active, undifferentiated cells (Bizzarri et al., 2011). Mammalian liver regeneration can overcome cancer - early nodules initiated by carcinogens are remodeled to normal-appearing liver (Farber, 1984a, b). Additionally, of carcinogen-induced tumors, over 95% remodel into normal tissue by the highly-regenerative liver (Enomoto and Farber, 1982; Ogawa et al., 1980; Tatematsu et al., 1983). Amphibian limb regeneration can likewise normalize tumors (Needham, 1936a; Rose and Wallingford, 1948; Waddington, 1935). Thus, tumors may be wounds that do not pattern.

Remarkably, such influence is not necessarily local. Induction of *anterior* regeneration in planaria turns *posterior* infiltrating tumors into differentiated accessory organs such as the pharynx (Seilern-Aspang and Kratochwill, 1965), which suggests the presence of regulatory long-range signals that are initiated by large-scale regeneration. Modern molecular model systems are now available for the study of these still poorly-understood mechanisms: regeneration of the zebrafish tail prevented tumor formation from BRAF<sup>V600E</sup> mutation + p53 knockout (Richardson et al., 2011). It is likely that the normalization of tumors by active remodeling represents one of the most profound and exciting areas for future work in understanding morphogenetic fields and their interpretation by growing tissue.

### 5.6: Tumors, fields, boundaries, and selves

There are several hypotheses that could be framed to test these concepts, addressing the question of whether cancer was an intrinsic defect or a community effect. Why does a cell (or small group of cells) within a normal tissue initiate cancer? One possibility is that this is akin to asking why a certain group of atoms in a brick is the “center of gravity” – that is, there is nothing special about those cells at the local level but they are located at a *node* within (an altered?) morphogenetic field. Thus cancer could result from a failure of the host to impose or transmit necessary patterning information within a particular region; this class of models focuses on the spatial distribution of the field signals. Conversely, it is possible that tumor cells are those that stopped attending to the morphogenetic field cues (Donaldson and Mason, 1975; Lee and Vasioukhin, 2008; Tsonis, 1987), which is a class of models focused on the properties of the individual cells and their interaction with a field. Lastly, cancer could represent establishment of a local “subfield” – a fragmentation of the host’s field such that integration with the host bodyplan is lost. Anticipating recent discoveries of the importance of gap-junction cell:cell communication for planarian regenerative patterning (Nogi and Levin, 2005; Oviedo et al., 2010), in 1965 Seilern-Aspang described planarian experiments in which a carcinogen led to formation of many head teratomas with irregular

nerves and un-oriented eyes saying “the *cell-isolating* action of the carcinogen prevents formation of a single morphogenetic field and leads to the establishment of several separated fields of reduced dimensions” (Seilern-Aspang and Kratochwill, 1965).

One of the implications of such fragmented morphogenetic field models is a reduced scope of “self” – the view that a tumor is, in some practical sense, an independent organism (Vincent, 2012) with its own (primitive) morphogenetic field. Such a view is suggested by a number of findings. First, histological analysis indicates that tumors can indeed be regarded as complex tissues with a distinct internal organization (Clark, 1995; Dean, 1998). Tumors reproduce themselves via metastasis, and execute many adaptive strategies such as up-regulating multi-drug resistance proteins to preserve their homeostasis and existence as do organisms (Chabner and Roberts, 2005; Krishna and Mayer, 2000; Nooter and Herweijer, 1991). Much like organisms maintaining morphostasis, tumors maintain their identity during massive cell turnover during selection for founder cells resistant to chemotherapy drugs (Shah et al., 2007). Recent work describes the highly malignant brain tumor as an “opportunistic, self-organizing, and adaptive complex dynamic biosystem” (Deisboeck et al., 2001); proper characterization of the essential principles predictive of the properties of tumor invasion makes use of concepts such as least resistance, most permission, and highest attraction – these are systems-level, goal-directed elements that are very compatible with the conceptual modeling techniques suggested for computational approaches to morphogenetic fields discussed below in the context of whole organisms.

With respect to goal states, tumors of course pursue strategies quite at odds with those of their host. “Glioma cells are ill-equipped to participate in ion and amino acid homeostasis, those important altruistic tasks performed by their nonmalignant counterparts. Instead, gliomas are more concerned about their relentless growth and invasive migration” (Olsen and Sontheimer, 2004). Interestingly, cooperation occurs among the tumor cells that can be analyzed via the same mathematical tools that explain cooperation among somatic cells and members of societal groups (Axelrod et al., 2006; Bidard et al., 2008). While tumors typically lose heterologous gap-junctional communication to surrounding stroma, they often maintain good gap junctional connections among their own cells. Interestingly, gap-junctional connections have been proposed as a mechanism by which cells can recognize “self” (Guthrie et al., 1994).

The question of size control and field boundaries are central to developmental biology as well. During planarian regeneration, a regenerating head will inhibit the formation of heads elsewhere, but parts of the regenerating head do not inhibit the rest of that same head from forming. Future work must uncover the mechanisms that establish size and scope of morphogenetic fields, to understand how boundaries are established and altered during pattern formation and dysregulation, and what kinds of signals can be manipulated for desired outcomes in regenerative biomedicine settings.

### 5.7: Bioelectric signals and cancer

The view that cancer is a developmental disorder predicts that molecular mechanisms known to be important mediators of the morphogenetic field would be involved in tumorigenesis. Indeed, there is mounting evidence that the bioelectric cues that establish normal pattern can go awry and result in cancerous growth. Ion channels, pumps, and gap junctions are now recognized as oncogenes (Becchetti, 2011), predictive markers (Prevarskaya et al., 2010), and an important set of targets for new cancer drugs (Arcangeli et al., 2009). For example, manipulation of membrane  $H^+$  flux can confer a neoplastic phenotype upon cells (Perona and Serrano, 1988), and voltage-gated sodium channels potentiate breast cancer metastasis (Fraser et al., 2005). Metastatic potential correlates with voltage-gated inward sodium current and it has been suggested that some sodium channels

may be oncofetal genes, encoding signals that are active during the rapid and autonomous growth of tumors and embryos (Brackenbury and Djamgoz, 2006; Diss et al., 2005; Fraser et al., 2005; Onganer and Djamgoz, 2005; Onganer et al., 2005).

Importantly, ion translocators are generally treated as single proteins responsible for a specific cell behavior (metastasis, hyperproliferation, etc.) – a cell-level view that neglects their role as mediators of large-scale patterning cues (Blackiston et al., 2009; Kunzelmann, 2005). Future work remains to fully understand the role of ion flow as part of the patterning influence that normally suppresses neoplastic transformation, and the storage of information in *physiological* networks that is misprocessed in cancer (Rubin, 1990, 1992).

## 6. Organizational level and scale properties of morphogenetic guidance

### 6.1: At what level of organization is pattern best understood?

A major question concerns the correct level at which most efficiently to describe patterning systems and the manipulations that bring about morphogenetic change. For example, field theories and positional information models that normally are thought to describe multicellular cell fields have also been proposed at the level of single cells, such as ciliates (Brandts and Trainor, 1990a; Frankel, 1974, 1992) and *Acetabularia* (Hammerling, 1953; Rommelaere and Hiernaux, 1975). A pre-existing mouth from a ciliate, transplanted to another cell using microsurgery, is capable of inducing formation of an ectopic mouth (Tartar, 1956), just as occurs during organizer signaling in metazoan development. While only multi-cellular systems of positional cues are considered in this review, it is possible that fundamental aspects of positional guidance can work at many different scales of size and do not require multi-cellular interactions.

Indeed, the distinction between cell morphology and tissue morphology may be a false dichotomy and the same structure may be specified regardless how the material is partitioned into cells (Marshall, 2011). For example, in the pronephric duct of polyploidy salamanders, cell size can increase without increase in diameter of the duct, so that the number of cells in cross-section can go down from the normal 8 to even just one, which will still fold over to create the appropriate lumen (Fankhauser, 1945). Pattern is primary and multi-cellularity isn't crucial. Similarly, when cytokinesis in polychaete worms is prevented, mitosis continues (syncytium) but the massive single cell still took on an asymmetric bilobed appearance with tufts of cilia in the right place and it looked remarkably like a normal trochophore larva (Lillie, 1902). Going even further, functional studies have shown that it is possible to dissociate change in organs systems and the organism as a whole: remarkably, experimental collapse of the transneural bioelectric gradient during amphibian development resulted in a severe disaggregation and disruption of histogenesis of internal organs including brain and spinal cord despite overall normal external development of the embryo (Borgens and Shi, 1995).

Taken together, such data suggest that the fundamental unit of morphogenesis may not be the single cell; it likewise argues that a multi-cellular GRN governing differentiation fate is not necessarily the appropriate basic unit in terms of which large-scale structure is to be understood.

### 6.2: Functional data suggesting a view beyond the single cell level

The current paradigm focuses on cell-level activity (proliferation, differentiation, migration), but might tissue- or organ-level systems properties be the right basal concepts with which to explain adaptive shape repair, anatomical polarity, and size control? At the level of pathways, stem cells and cancer cells share many similarities (Dreesen and Brivanlou, 2007; Reya et al., 2001; White and Zon, 2008); patterning influence is needed to push them

towards a coherent, developmental program vs. cancerous proliferation. Similarly, anatomical context is crucial to the fate of stem cells and needs to be taken into account when designing molecular strategies for driving stem cells towards specific behaviors. For example, transduction with a cocktail of transcription factors sufficient to induce an eye from a group of multipotent progenitor cells, but it only does so when implanted into a host, not *in vitro* (Vicgian et al., 2009). The morphogenetic field concept is most compatible with a top-down view, focused on information flow (*what do cells need to know in order to build or repair a structure? in what form is encoded the final morphology of any given organ or bodyplan?*), as distinct from the more popular bottom-up molecularly-focused approach (*what does protein X bind to? which genes does transcription factor Y activate or repress?*).

The difference between these approaches has practical implications. For example, a focus on cell cycle checkpoints and TGF- $\beta$  signals leads to the prediction that cancer and regenerative potential should go together: animals with ready access to plastic, highly proliferative cells should be prone to neoplasia, and long-lived humans would be forever barred from powerful regenerative pathways because of the need to suppress cancer. Conversely, a morphogenetic field model would suggest that regeneration and cancer should be *inversely* related, as robust patterning pathways necessary for regeneration would also keep cells within a coherent patterning plan and away from tumorigenesis.

In fact, the most highly regenerative animals tend to have the lowest incidence of cancer (Brookes, 1998; Rose and Wallingford, 1948; Ruben et al., 1966; Tsonis, 1983). Moreover, If a tumor is induced on the limb of a salamander and the limb is amputated through the tumor, the remaining cancer tissue becomes part of the newly regenerating limb (Brookes, 1998; Donaldson and Mason, 1975; Rose and Wallingford, 1948; Ruben et al., 1966; Tsonis, 1983; Wolsky, 1978)! This readily illustrates the profound relationship between cancer and regeneration and the importance of dissecting systems-level concepts (“exerting strong patterning control at the level of a whole appendage”) for what is often thought of as a cellular- or gene-level process. It also suggests a highly optimistic view of the potential for regenerative pattern control in human medicine.

### 6.3: Neural and other long-range signaling by the morphogenetic field

Is complex morphogenesis best understood as the result of purely local cell interactions, or do significant instructive signals function at long range? The hypothesis of the morphogenetic field suggests that information be processed and communicated at significant distances across the organism during patterning. Interestingly, evidence from embryogenesis, regeneration, and cancer suggests that there is much to investigate beyond the cellular events ongoing at the site of morphogenesis itself. For example, in salamanders, even a small cut in the hand causes the entire limb to regress back to shoulder level (not drop off or undergo necrosis, as might be expected from a simple trophic influence, but actually ‘remodel’) if the limb is denervated (Carlson, 1977).

Cancers can be detected by their disruption of large-scale bioelectrical properties of the host (at locations far away from the tumor) (Burr, 1941a); this works for transplanted tumors as well, and is consistent with a key role for the bioelectrical component of the morphogenetic field and the view that tumors are a disruption of host-field-cell interactions. Similarly, interruption of cell:cell communication via ions and other small molecules (gap junctional isolation) is known to be a tumor-promoting agent (Loewenstein, 1969, 1979, 1980; Loewenstein and Kanno, 1966; Mesnil et al., 2005; Rose et al., 1993; Yamasaki et al., 1995); for example, Connexin32-deficient mice have a 25-fold increased incidence of spontaneous liver tumors (Temme et al., 1997). Gap junction-mediated, bioelectrically-controlled cell:cell communication is also a critical system by which the large-scale left-



right asymmetry of the heart and visceral organs is determined during embryogenesis (Chuang et al., 2007; Fukumoto et al., 2005).

One of the most interesting and least-well understood mediators of long-range influence is the central nervous system. It has long been known that innervation is required for limb regeneration (Goss, 1969; Rose, 1948; Singer et al., 1967), and recent molecular evidence has uncovered genes responsible for the acquired nerve-dependence of amphibian limbs (Kumar et al., 2007). Classical work suggested that the CNS indeed carries important aspects of morphogenetic fields (Becker, 1961), but the information content of neutrally-mediated signals remains to be probed in mechanistic detail.

In some invertebrates, the presence or absence of the optic ganglion determines whether an eye or an antenna-like organ is regenerated (Polezhaev, 1972). Importantly, a modern molecularly-tractable model system has now been developed in which these signals can be analyzed. In planaria, the integrity of the ventral nerve cord is actually required to specify appropriate fate for a regeneration blastema: if the VNC is cut in a gap junction-inhibited worm, an ectopic head can result (Oviedo et al., 2010). The pattern of regenerating tails is markedly different from the normal pattern if spinal cord contiguity is interrupted by a laser pulse at points far away from the tail amputation in *Xenopus* tadpoles (Mondia et al., 2011) or surgical perturbations of the brain/spinal cord (Hauser, 1969; Jurand et al., 1954). Moreover, the shape alterations are different when the spinal cord is targeted at different positions along the anterior-posterior axis, and two individual spots of interruption produce a phenotype distinct from that resulting from either spot alone.

An amphibian flank wound will generate a limb if a nerve is deviated to it (Stocum, 1991), and antler shape is likewise affected by functional innervation (Suttie and Fennessy, 1985). Remarkably, innervation is important not only for the active regeneration of an amputated structure (Bryant et al., 1971; Maden, 1978; Yntema, 1959), but also for dynamic morphostasis of normal form and resistance to neoplastic transformation. For example, tongue papillae buds become disorganized when their innervation is perturbed (Sollars et al., 2002). Victims of paralysis acquire prostate cancer less frequently than control individuals (Frisbie, 2001). Tumors are readily induced by denervation in salivary organ and alimentary canal in cockroach (Scharrer, 1953; Scharrer and Lochhead, 1950); similarly, tumors are chemically induced more easily in denervated rabbit ears as compared with contralateral controls (Pawlowski and Weddell, 1967). Such results are predicted by models in which nervous system components transmit long-range morphogenetic field cues (Becker, 1974; Burr, 1941b, 1944; Burr and Northrop, 1939), but contradicts the prediction of local “neural-derived growth factor” models of tumor growth (Cannata et al., 2001; Kumar et al., 2007), illustrating once again the linkage between cancer and regeneration by mechanisms focused on morphostasis, and suggesting the importance of neutrally-mediated signals for this system.

## 7. Conclusions and summary

### 7.1: Open questions and next steps

Taken together, recent and classical data suggest that morphogenesis and morphostasis are core concepts unifying three major areas of study – development, regeneration, and cancer. Many questions remain about the physical mechanisms by which prepatterns or templates may be stored in tissue, and the pathways through which cells and tissues interact with morphogenetic fields. Major themes applicable to future work include:

- Is patterning best understood as an emergent property, or through a prepattern map of the final structure (template), or both?

- What is the right level at which to understand pattern control networks and modularity – cells, subcellular structures, multicellular groups, organs?
- If target morphology is a useful concept, what molecular mechanisms might underlie the storage of patterning information and templates, and allow cells to read and modify this information?
- How can constructive, predictive, algorithmic (Levin, 2011b; Lobo et al., 2012) or generative (Belousov, 2008; Belousov and Grabovsky, 2007) models be formulated that utilize cellular and genetic pathways to implement systems-level computations that compare shapes and orchestrate anatomical polarity and remodeling towards a specific goal state?

It is no coincidence that many of the papers describing some of the most profound data cited in this review are quite old. Indeed, many of the most important questions of pattern formation have yet to be addressed, because the currently-available functional and bioinformatics techniques are not ideal for asking these questions. However, with the development of tractable model systems such as planaria and *Xenopus* in which state-of-the-art molecular genetics and quantitative biophysics can be brought to bear on these problems, a number of specific areas of high relevance to morphogenetic fields can now be tackled:

- What information is encoded in the CNS that is used for pattern control and cancer suppression? Does information from a wound site go “backwards” along the CNS to be processed remotely, or does all influence pass from the CNS towards injury points?
- What genetic mechanism is affected in the *Disorganization* mouse that results in the scrambling of the relative positioning of organs and appendages?
- How can we best crack the bioelectric code, to understand the quantitative mapping between bioelectric states and specific developmental modules (tissue/organ outcomes)?
- What would cancer look like, in a unicellular organism such as a ciliate, which has field-encoded complex patterning information expressed on the scale of a single cell (Brandts, 1993; Frankel, 1991; Frankel, 1992, 2000; Grimes et al., 1980; Jerka-Dziadosz et al., 1995)?

## 7.2: A speculative outlook on modeling information storage in morphogenetic fields

It is imperative that we identify and quantitatively model the information-processing and computational activities of patterning systems to gain control of molecular mechanisms by which morphogenetic information orchestrates low-level (cell) behaviors towards the patterning needs of the host. In what kinds of patterning systems is it best to consider top-down causation instead of just modeling low-level rules? What criteria (degree of predictive control in functional experiments? parsimony of model?) are to be used to decide among top-down and bottom-up models? This has important implications beyond philosophy and basic developmental biology. Our choice of strategies for regenerative medicine and bioengineering depends crucially on finding the easiest path towards gaining rational control over complex biological shapes and understanding the still mysterious link between rapid growth of cancer vs. regenerative repair.

In formulating algorithmic models of patterning, it is natural to ask what the system needs to know, and what information is being processed, to guide its activity. Such work is often the role of neural networks, and it is likely that sheets of cells or whole organs that are in electrical communication could perhaps support similar processes. Such computational tissues would be ideal media in which to store and manipulate the information used by

morphogenetic fields. Recent techniques of molecular bioelectricity (Adams and Levin, 2012a; Levin, 2012), combined with tools like optogenetics (Berndt et al., 2009; Fenno et al., 2011; Schultheis et al., 2011), in which electrical properties of cells are controlled by light pulses with exquisite spatio-temporal specificity, should allow the testing of this hypothesis by “reading” and “writing” physiological information to and from complex patterning structures *in vivo*. These technologies could represent an exciting new canvas on which to implement cybernetic, bioengineered devices.

The field faces major questions that may require fundamental shifts in our methodology, both experimental and theoretical. The morphogenetic field concept will be tested and refined as part of these efforts, as an exciting convergence of technologies is finally allowing the mechanistic testing of hypotheses put forward by profound thinkers many years before these concepts could be properly tested. The implications of fundamental advances over shape control will have transformative impact on many areas of biomedicine, as well as our understanding of evolution, cognition, and the possibilities inherent in synthetic biology.

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## 11. Glossary

<b>gap junctional communication</b>	Direct cell:cell transfer of small signaling molecules (ions and metabolites, generally < 1 kDa) among adjacent cells through aqueous channels made of connexin, innexin, or pannexin protein hexamers docking from each side of the cell junction (Goldberg et al., 2004; Levin, 2007a; Simon and Goodenough, 1998; Wong et al., 2008)
<b>morphogenetic field</b>	The mediator of pattern formation and remodeling can be viewed as a “morphogenetic field” (Belousov, 2001; Belousov et al., 1997; De Robertis et al., 1991; Martinez-Frias et al., 1998; Opitz, 1986) – the sum total of local and long-range patterning signals that impinge upon cells and bear instructive information that orchestrates cell behavior into the maintenance and formation of complex 3-dimensional structures (Schnabel et al., 2006)
<b>organicism</b>	A perspective that stresses the organizational aspects, rather than the physical composition, of biological objects as a path to understanding and predictive control (Gilbert and Sarkar, 2000, Noble, 2010)

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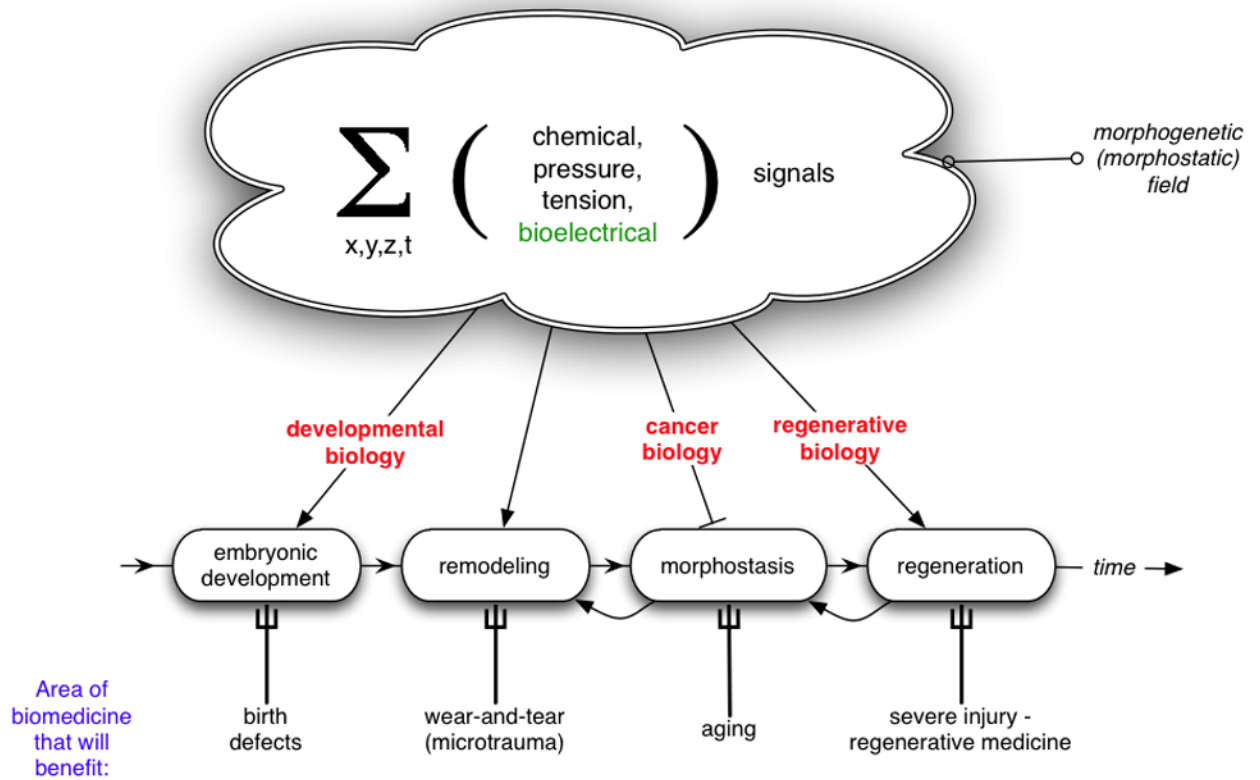
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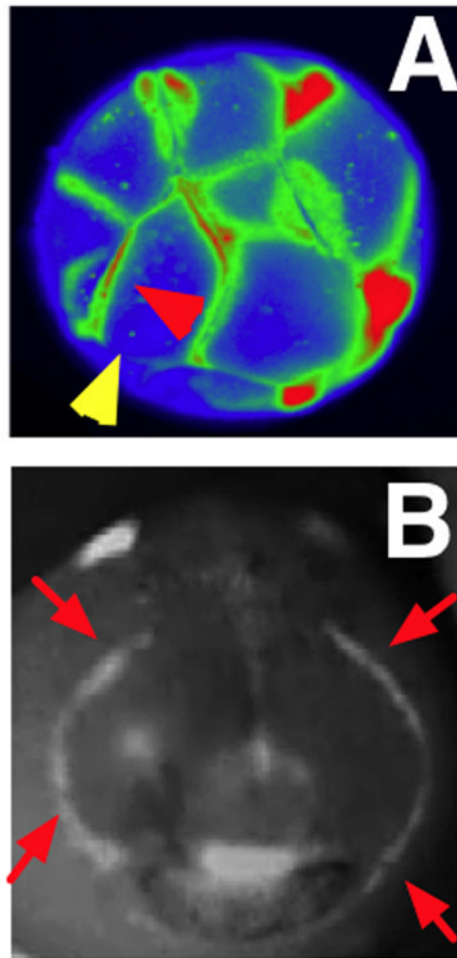
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**Figure 1. The morphogenetic field in development, regeneration, and neoplasm and its applications to medicine**

The morphogenetic field can be defined as the sum, integrated over 1 temporal and 3 spatial dimensions, of all non-local patterning signals impinging on cells and cell groups in an organism. Functionally, long-range signals (such as planar polarity of proteins on cell surfaces, standing waves of gene expression, voltage potential, and tensile forces, and chemical morphogen gradients) carry information about both the existing and the future pattern of the organism. This allows the initial development of complex form from a single fertilized egg cell, as well as the subsequent maintenance of form in adulthood against trauma and individual cell loss. Errors in various aspects of the establishment and interpretation of these fields result in failures to maintain systems-level properties of anatomical shape, manifesting as birth defects, cancer, aging, and failure to regenerate after injury. Thus, almost every area of biomedicine is impacted by our knowledge of how cells interact with and within this set of complex signals.



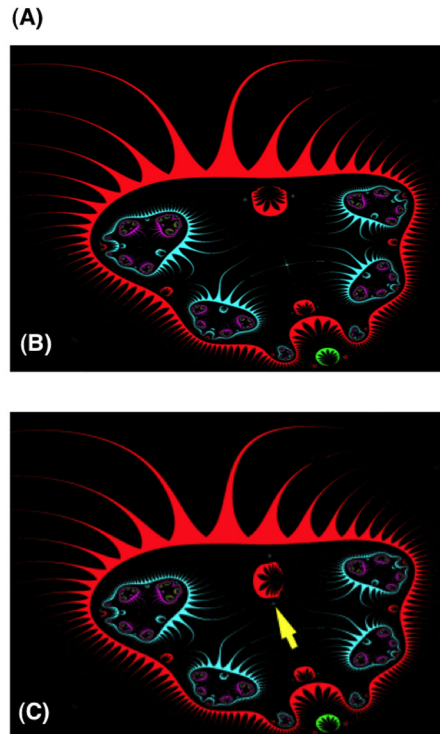
**Figure 2. Voltage gradients in vivo**

**A:** Fluorescent voltage reporter dyes allow characterization of physiological gradients in vivo, such as this image of a 16-cell frog embryo that simultaneously reveals cells' transmembrane potential levels (blue = hyperpolarized, red = depolarized) in vivo, as well as domains of distinct  $V_{\text{mem}}$  around a single blastomere's surface (compare the side indicated by the yellow arrowhead with the one indicated by the red arrowhead). Provided courtesy of Dany Adams.

**B:** Isopotential cell fields can also demarcate subtle prepatterns existing in tissues, such as the hyperpolarized domains (red arrowheads) that presage the expression of regulatory genes such as *Frizzled* during frog embryo craniofacial development; these patterns of transmembrane potential are not merely readouts of cell state but are functional determinants of gene expression and anatomy (Vandenberg et al., 2011).



$$Z_t = 2.71^{(Z_{t-1} \cdot \tan(Z_{t-1}))} + Z_{t-1}^{4.5} + Z_{t-1}^{5.5}$$

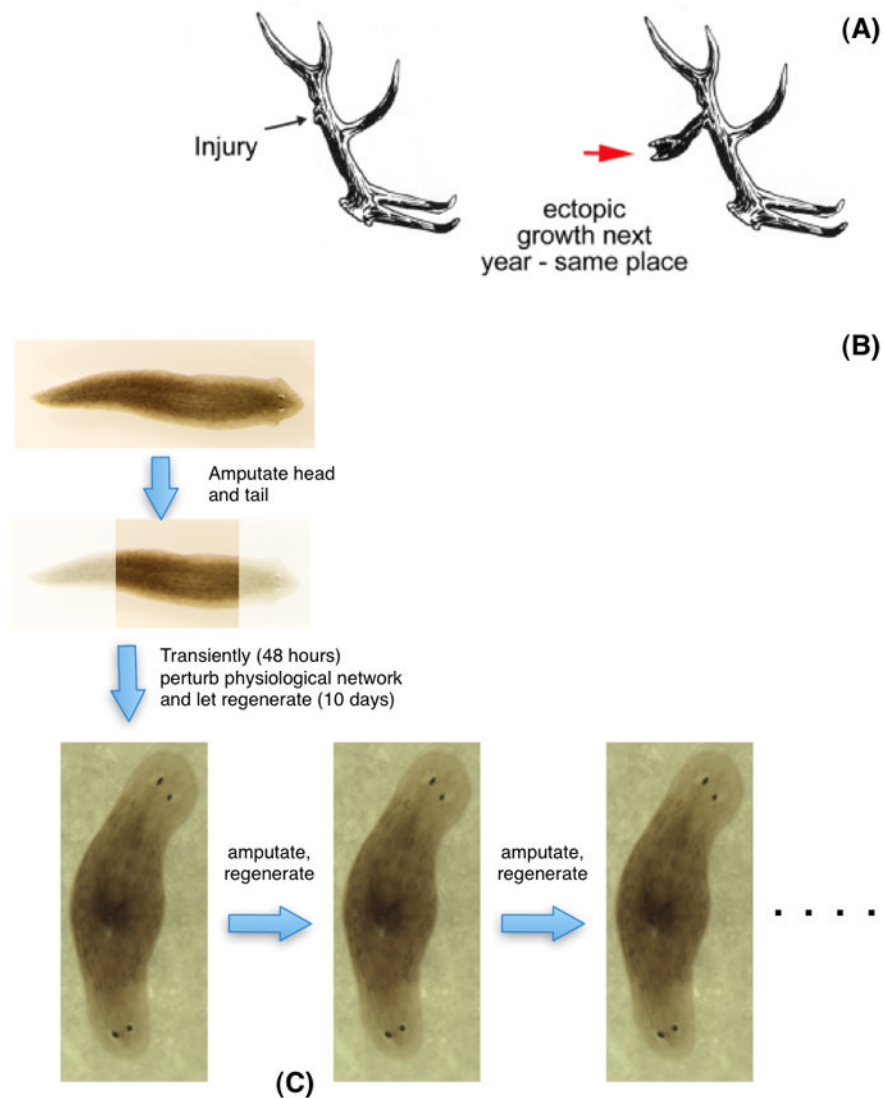


**Figure 3. Emergence of complex morphology from simple low-level rules**

**A:** A short function can be defined over a complex variable  $Z$ ; this function is iterated – applied repeatedly to each result of the previous iteration (Levin, 1994; Mojica et al., 2009; Pickover, 1986).

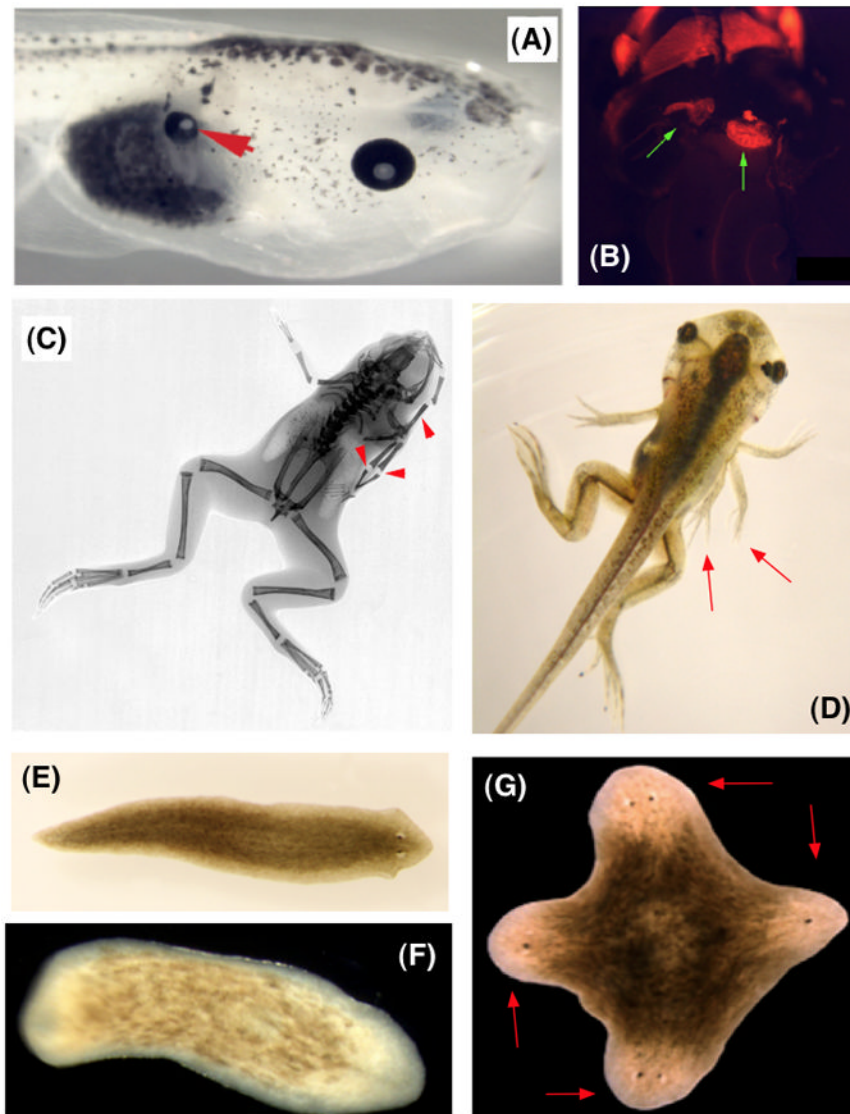
**B:** A Julia set pattern can be created by iterating such a function for each point in the plane:  $Z_0 = X + Yi$  for coordinates of each point  $(X, Y)$ . Each point is then assigned a color based on how fast the absolute value of  $Z$  exceeds a threshold upon iterated application of the function to the initial  $Z$ . This extremely simple algorithm gives rise to a complex morphology, illustrating how spatial complexity can emerge from a simple set of low-level rules without being directly specified or encoded anywhere in those rules.

**C:** While it is easy to produce an image corresponding to a set of rules ( $A \rightarrow B$ ), the inverse problem is much harder. In general, it is impossible to know how to modify the generative rules ( $A$ ) to give rise to a desired pattern – for example, a modified version of  $B$  where one element is rotated  $90^\circ$  (yellow arrow).



**Figure 4. Alteration of target morphology**

(A) In the red deer *Cervus elaphus*, an experimental incision in one location induces a slight hypertrophy in the first year but results in a supernumerary (ectopic) tine at that same location in the next year. Image modified (with permission) after Fig. 22 of (Bubenik, 1966). In planaria, the anterior-posterior polarity (head vs. tail) during fragment regeneration can be perturbed by manipulating the flow of ions among cells. When communication is reduced for 48 hours, a fragment will regenerate into a 2-headed form in 7 days (B). Cuts made over months following this treatment, in plain water (no exposure to any perturbation) result in the regeneration of 2-headed worms (C), demonstrating that information present in a dynamic physiological network can be canalized or remembered so that the shape to which the animal regenerates in further rounds of damage (target morphology) is directly and (permanently?) altered without modification of DNA sequence. Planarian images in B courtesy of Junji Morokuma.



**Figure 5. Modular alteration of pattern by biophysical modulation**

Changing the pattern encoded in physiological networks results in coherent, modular alterations of form *in vivo*. Gradients of resting transmembrane potential were artificially altered by misexpressing mRNA encoding specific ion channels (in frog embryos) or by pharmacologically manipulating native ion translocator proteins (in planaria). The results in *Xenopus laevis* embryos include induction of: whole ectopic eyes on the gut (A, red arrow), a complete beating ectopic heart (B, green arrow), and well-formed ectopic limbs with normal bone structure (C,D, red arrows). In regenerating planarian flatworms (normal morphology in E), such modulation can be used to control the anatomical polarity and overall body-plan, including no-head worms (F) and 4-headed worms (G, red arrowheads indicate the heads), all of which are viable.