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The Biophysics of Regenerative Repair Suggests New Perspectives on Biological Causation

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Evolution exploits the physics of non-neural bioelectricity to implement anatomical homeostasis: a process in which embryonic patterning, remodeling, and regeneration achieve invariant anatomical outcomes despite external interventions. Linear "developmental pathways" are often inadequate explanations for dynamic large-scale pattern regulation, even when they accurately capture relationships between molecular components. Biophysical and computational aspects of collective cell activity toward a target morphology reveal interesting aspects of causation in biology. This is critical not only for unraveling evolutionary and developmental events, but also for the design of effective strategies for biomedical intervention. Bioelectrical controls of growth and form, including stochastic behavior in such circuits, highlight the need for the formulation of nuanced views of pathways, drivers of system-level outcomes, and modularity, borrowing from concepts in related disciplines such as cybernetics, control theory, computational neuroscience, and information theory. This approach has numerous practical implications for basic research and for applications in regenerative medicine and synthetic bioengineering.

1. Introduction

"A revolution can be neither initiated nor stopped."
– Napoleon Bonaparte

A central goal of biology is to explain the formation and dynamic remodeling of living structures. We seek to identify the "cause(s)" of various phenomena on different scales of temporal and spatial organization, and learn how they can be induced, prevented, or directed toward desired system-level outcomes. What does it mean to say that an event X (or intervention X) caused outcome Y? How do some processes lead to different outcomes from (macroscopically) the same starting conditions? Conversely, how do some morphogenetic mechanisms reach the same outcome from a wide range of initial configurations, despite perturbations? A mature understanding of causation is essential for transitioning basic knowledge into biomedical progress, which relies on identification of causes for disease conditions, and for the development of effective therapies that restore physiological or structural states. For example, the debate on the causal nature of mutations in carcinogenic dysregulation[1–4] is focused on the question of what actually induces cells to abandon the bodyplan and revert to a unicellular-like existence, and what intervention might be sufficient to induce a rescue.

Many important processes occur at multiple levels of size, scale, and organization. For example, limb regeneration requires growth that stops when correct size and morphology have been achieved. Anatomical "macrostates" can be implemented by a wide range of molecular and cellular arrangements and even time-series trajectories that all implement the same large-scale outcomes.[5–7] How does evolution exploit feedback loops between biophysical and genetic mechanisms to enable regeneration and development to reach the same anatomical end-state despite perturbations? The prevalence of large-scale pattern homeostasis suggests a perspective on causation and control that borrows established concepts in related disciplines such as cybernetics, control theory, and computational neuroscience, as well as new developments in information theory that help to rigorously identify and quantify tractable macrostates with maximal causal power.

Until fairly recently, bench biology research advanced via a classical definition of cause (e.g., the "necessary and sufficient" argument structure familiar to all developmental biology students) while largely ignoring the extensive literature in fields such as philosophy, physics, and engineering that have pointed out profound problems with respect to naïve models of causation that fail to capture essential aspects of physical and biological systems.[8–14] However, numerous fields of the life sciences are now facing a turning point that indicates the need for a paradigm shift toward a more mature understanding of causation in evolutionary, developmental, and biomedical contexts. This is largely due to five major developments: 1) the advent of novel technologies that provide an unprecedented amount of observational and experimental "Big Data" sets that reveal the incredibly tangled details underlying system-level outcomes in biological systems, 2) developments in network science and information theory that provide new mathematical approaches that extract rich control

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structures from biological data, 3) an increasing awareness of the presence and importance of variability, stochasticity, heterogeneity, and noise, 4) a pressing need to translate successes in molecular-level processes into biomedically important anatomical outcomes, and 5) a breaking down of the barriers between the study of life as it is (the zoology of existing model systems) and "life as it could be" (Artificial Life in silico and in vitro[15]). While technology is constantly improving in spatial and temporal resolution, providing ever more drill-down toward molecular-level events, researchers are tackling increasingly larger questions about control and dynamics of global patterning under a variety of perturbations.

It is now becoming clear that traditional concepts of causation need to be re-examined and redefined, to better match available data and the requirement of predictive control in regenerative medicine and synthetic biology. Traditional, linear notions of pathways, in which an upstream molecular signal induces downstream ones must be extended in two ways: in time (to encompass homeostatic setpoints guiding the future state of the system), and in scope (to account for causally effective physiological/biophysical spatially distributed states, as distinct from molecules). Expanding the understanding of causation is highly practical. For example, a focus on cell-level events (cell cycle control, differentiation) predicts that regenerative animals with large numbers of plastic, proliferative cells should be prone to cancer. In contrast, a focus on the control mechanisms that harness cell behavior toward specific organ-level outcomes predicts that regenerative animals, with robust patterning controls, should be resistant to carcinogenic defections of cells from the anatomical plan. The latter view in fact matches the data better[2,16,17]—regenerative and embryonic environments have been shown to be able to normalize even aggressive cancer cell types.[18–20]

Specific examples in developmental and regenerative biology have emerged at the interface between biology and physics, illustrating interesting new aspects of causation. It is impossible here to do justice to the rich literature on causation in biology, and philosophically inclined readers are invited to delve deeper.[11,14,21–35] I adopt a pragmatic view of causation: the best explanation of a system is whatever optimally facilitates effective, minimal-effort intervention strategies for rational modulation of biological structure and operation, without specific precommitments to the level at which the best explanation must be found.[11] The success criterion in regenerative biomedicine entails anatomical, not molecular, endpoints, and the effort to understand and control large-scale form is compatible with many philosophical perspectives, such as circular causality.[36] Box 1 discusses this in more detail, as well as the relationship between genetics and biophysics.

2. Left–Right Patterning: Far from Linear

To form the invariant left–right asymmetry of the vertebrate bodyplan, organs such as the heart and gut must develop differently on the right versus the left side. The textbook description of the transcriptional core of this process[37,38] involves a series of sequential gene activations occurring only on the left side: *Sonic hedgehog* (*Shh*) induces *Nodal*, which induces *Lefty*, which induces *Pitx2*, which controls the morphogenesis of the heart. This linear picture (**Figure 1**A) is correct on a short time scale; for example, experimental misexpression of *Shh* on the right side indeed results in bilateral *Nodal* expression, leading to right isomerism of the heart. The same is true for knockdown of *Shh* (which leads to an absence of *Nodal* expression on the left). As shown by loss- and gain-of-function studies, which comprise the classic standard of "necessary and sufficient," each of these components of the circuit is indeed regulated by the factor placed upstream of it in published depictions of this pathway (Figure 1B).

However, the linear pathway paradigm has a straightforward consequence: errors in the sidedness of expression of any node in the pathway should propagate downstream, causing errors in the expression of the next steps in the process. In any group of embryos, the incidence of incorrect expression of downstream genes should be as large as the incidence of incorrect expression of the upstream ones, or even larger if additional errors occur at subsequent steps. For example, if 50% of the animals in a cohort have incorrectly sided *Shh* expression, then at least 50% should acquire incorrect *Nodal*, *Pitx2*, etc. expression (and possibly more, if additional problems occur after the Shh signaling events). Surprisingly, this is not actually what occurs (Figure 1C): each subsequent step has fewer errors than the previous step,[39] suggesting that the classic linear pathway picture is importantly incomplete. Embryos recognize transcriptional deviations from the correct pattern and repair them over time (Figure 1D).

Of course, everyone is aware of the existence of redundancy and regulative development. For example, while embryos already distinguish their L from R sides by cleavage stages,[39–41] there are later steps, such as ciliary rotation during neurulation,[42,43] which feed into the process. But, it is still largely unclear how early embryos generate, store, and process information against which to compare developmental state so that course-corrections toward the proper target morphology can be made in cases where upstream cues disagree,[44] such as, for example, Hensen's node, which is instructed by asymmetrical cues from lateral tissues[45–47] but also possesses its own motile cilia. The existence of corrective pathways in embryogenesis and regeneration raises profound questions about the nearly ubiquitous stories our textbooks and "models" tell about the molecular explanations for specific events. In what sense is the "*Shh* → *Nodal* → *Pitx2*" cassette the explanation for how organs acquire laterality, if neither sufficiency nor necessity hold over realistic developmental timescales?

Like Escher's staircase (Figure 1E,F), the pathway model may be locally correct (properly describing the signaling interactions between individual gene products) but globally incorrect: knowledge of this pathway does not enable one to make quantitatively accurate predictions with respect to the complex final outcome (organ positioning incidences), which is the key property we require from a purported explanation of a biological process.

3. Pattern Homeostasis in Development and Regeneration: Setpoints as Causes

Regulative embryogenesis and regeneration exemplify the still poorly understood decision-making processes by which cells cooperate toward the dynamic maintenance and repair of complex 3D structures.[48] Salamanders regenerate entire limbs, stopping

Box 1
Causation and the relationship of genetics and physics on evolutionary and ontogenetic time scales

A relevant definition of causation arises from the perspective of engineering, isomorphic to Dennett's Intentional Stance in the philosophy of cognitive science: a mechanism or event is the *cause* of some outcome when it provides the most efficient way for experimenters, or the biological system itself, to induce it to occur, prevent it from occurring, or modify how it occurs. Efficiency is minimizing the amount of effort (energy, information) expended relative to complexity of outcome that results because of that intervention. There has been a rich debate in philosophy and science about the nature of causation,[6,8,12,13] and this is certainly not the only (or even an uncontroversial) definition of causation in general. However, it has several benefits.

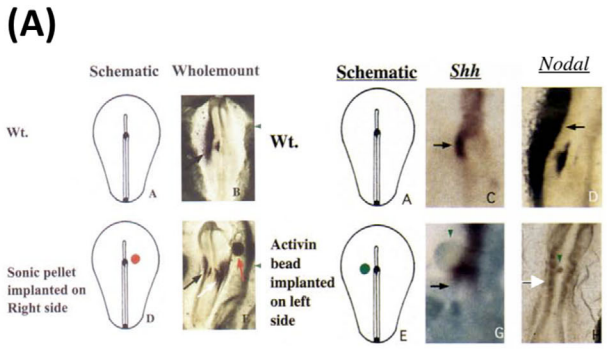
First, it is an empirical definition—instead of philosophical preconceptions about which level of description (subatomic, molecular, tissue, organism) must contain the causes, functional experiments evaluate interventions targeting the candidate causes; the claim of a specific cause can be revised when a new result shows that the same outcome can be achieved using a simpler manipulation. Second, it facilitates the consilience of evolutionary developmental biology and regenerative medicine. Causes are nodes in a functional network that has been optimized by the evolution for the efficient dynamic control of complex anatomy toward robust outcomes; biomedical strategies can exploit the discovery of efficient control nodes or subnetworks to induce system-level outcomes that are otherwise too hard or complex to micromanage. Modularity (simple events that serve as causes triggering complex downstream patterning outcomes) and homeostatic loops (in which setpoints are causes of dynamic behavior) greatly facilitate plasticity that is not only a selective advantage for organism survival but also enhance population evolvability. Novel environmental stimuli, or new mutations, are much less likely to produce catastrophic outcomes if complex behaviors of cells and cell networks—which re-establish anatomical structure and function—can be readily induced without needing many cooperating mutations (biochemical steps) to be concomitantly produced.

The examples of bioelectric components of pattern control provide interesting windows on aspects of causation, but this is not the only physical modality exploited by evolution. Biomechanical forces and biochemical gradients are also critical; **Figure 8** schematizes these three modalities as equal partners, all working together to constrain and enable aspects of morphogenetic control (Supporting Information). In any specific case, it is an empirical matter as to which modality serves as the controller. For example, in cell aggregation and sorting, biomechanics of adhesion is often the master driver[79]; in planarian regeneration, a bioelectric circuit functions upstream to control biochemical gradients that turn on head- or tail-specific cascades and morphogenesis.[56]

The focus in this perspective is on bioelectricity because recent data in this subfield clearly illustrate novel aspects of causation, which may be why evolution has especially exploited this type of biophysics not only for development but also for the incredible plasticity of brain function and behavior. It is likely, however, that biomechanics and biochemistry similarly offer the opportunity to expand our understanding biological computation and closed-loop control. Regardless of the type of modality that executes the top-level decision-making in a given case, a tight interplay of all three is critical for the implementation of pattern control.

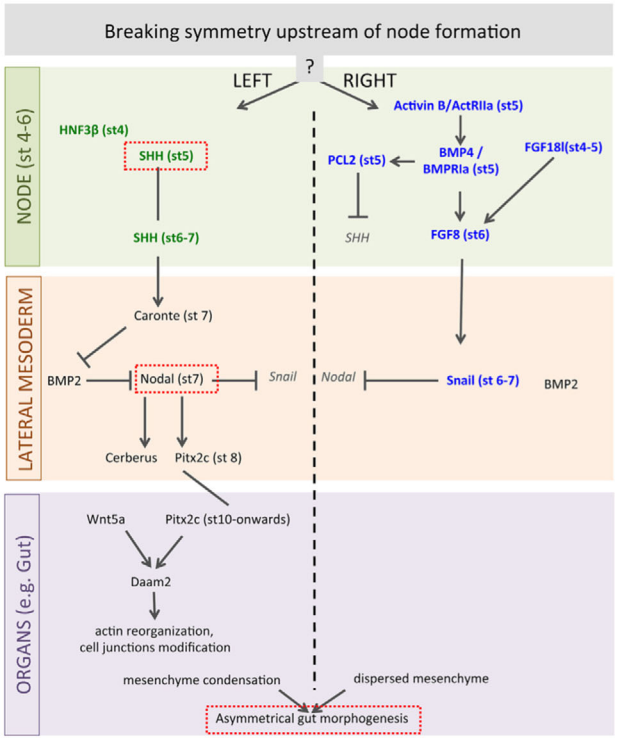
Bioelectric circuits are a medium well-known in neuroscience and computer engineering for storing memories and computation; but where do bioelectric prepatterns (such as the one in Figures **4**B and **5**A) come from? They are not directly encoded by the genome, any more than patterns of stress forces or biochemical gradients in tissue are laid out in the genome. A fitting metaphor is that of the electric activity (physiology) resulting when a set of electronic components is connected and energized. Any set of components will give rise to some emergent electrical pattern (akin to turing pattern self-organization from a homogenous substrate[80]), but if the components and their connectivity map has been subject to selection, it will give rise to electrical dynamics that are robust, process inputs into useful outputs, and in some cases can even implement memory (subsequent activity is modified by past experiences or input signals). The wiring diagram does not, directly, specify the computations that the device can implement, but the hardware is essential, and if it is good enough, the software layer may give the system a huge amount of adaptive plasticity that does not require physical re-wiring—a feature that increases fitness of the organism's development, self-repair, and behavior.

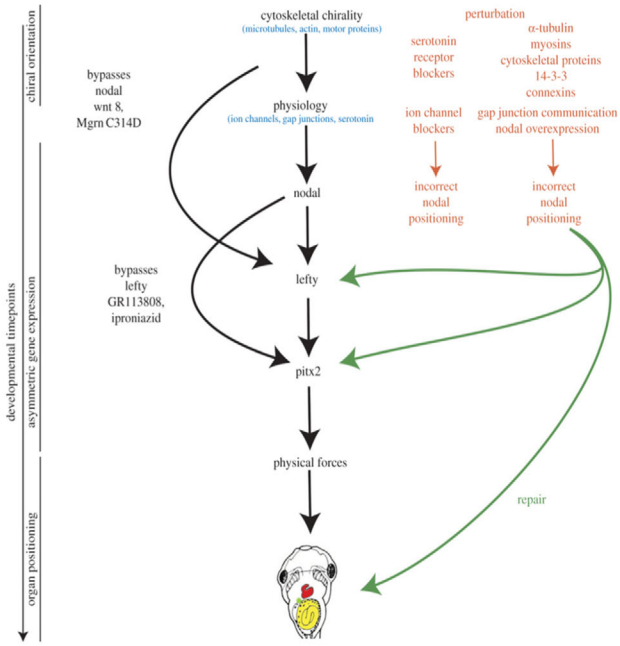
Ion channel genes can mutate, sometimes resulting in very different biophysical properties that in turn alter electric signaling among cell groups. However, bioelectric computational dynamics are a complex layer between the genome-specified hardware and the morphogenetic output. This provides significant buffering; for example, channel proteins can be replaced for one another as long as the bioelectric function is maintained, and the same kinds of homeostat and attractor dynamics can be maintained with significant changes of ion concentrations and protein diversity.[81] Moreover, the existence of brain-like dynamics in somatic tissue suggests the possibility of a kind of Baldwin effect, where beneficial patterning dynamics triggered at the physiological level eventually become canalized as transmissible (genetic) changes in ion channel genes that produce those same bioelectric prepatterns. Thus, bioelectric prepatterns, like others, are an excellent example of how selection forces acting on cellular hardware enables the genome to couple to, and exploit, the emergent self-organizing dynamics of flexible computational circuits.



(C)

	Linear Predicted	Actual
% incorrect *Shh*	40%	52%
% incorrect *Pitx-2*	45%	27%
% incorrect Organ *situs*	50%	14%





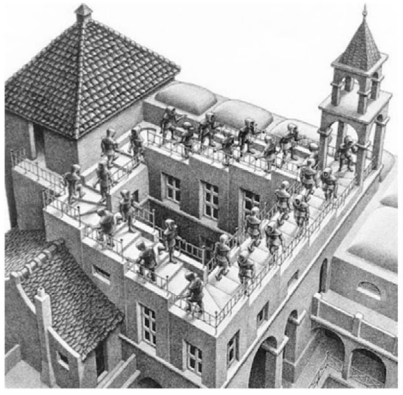


Figure 1. Left–right patterning: from sequential pathway to progressive repair. A) Gain- and loss-of-function experiments in the chick embryo use protein-soaked beads and viral misexpression to manipulate the presence of factors like Activin, Shh (Sonic hedgehog), and Nodal (cNR-1), while examining the expression patterns of downstream factors (purple stain, arrowheads). It was shown that blocking *Shh* on the left side prevents the normal domain of *Nodal* from being expressed subsequently (*Shh* is necessary for *Nodal*), while ectopic *Shh* introduced to the right side induces an ectopic domain of *Nodal* expression (*Shh* is sufficient for *Nodal* expression). Reproduced with permission.[37] Copyright 1995, Cell Press. B) These data have given rise to

precisely when a correct limb structure is complete.[49,50] Tails grafted to the flank of an amphibian slowly remodel into limbs, a structure more appropriate to the overall bodyplan; the cells at the tip of the transplanted tail turn into fingers, despite the fact that their local environment is a tail.[51]

Many processes in biology are not feedforward sets of invariant steps, but rather networks that exhibit tremendous plasticity. For example, as tadpoles metamorphose into frogs, the eyes, nostrils, and other organs need to move into different positions. It might be expected that every tadpole's organs need only to move in the same characteristic path to give rise to a standard frog face anatomy. Remarkably, however, tadpoles made to have highly abnormal faces nevertheless largely become normal frogs[52,53]: the craniofacial organs move in abnormal paths until a proper frog face morphology is achieved (**Figure 2**A,A'). This underscores the fact that genomes do not encode hardwired tissue movements but rather specify a computational system that flexibly implements context-dependent large-scale remodeling and can reach the species-specific target morphology despite drastic interventions and diverse starting conditions. Dynamical systems theory (via the notion of attractor dynamics) and control theory make clear that there is no fundamental teleological paradox here: these closed-loop systems work to reduce the error between the current morphogenetic state and an anatomical setpoint (Figure 2B) using a variety of genetic and biophysical processes to integrate cellular activity over large scales and propagate information about organ-level states across long distances in vivo. Work is ongoing to understand the molecular nature of the processes that measure the state, maintain the setpoint, and implement the means-ends process to achieve the target morphology.[44,48,54,55]

Homeostatic processes have long been studied in biology.[56] What needs to be understood next are the mechanisms that enable pursuit of setpoints that are not single-value physiological parameters, but rather complex anatomical states. Two other examples highlight the knowledge gaps in understanding pattern homeostasis from the perspective of molecular mechanisms alone, as contrasted with cybernetic perspectives that assess what the system measures and tries to achieve. The first demonstrates long-term storage of anatomical setpoints in one tissue to control growth and form of another. The second illustrates the fact that the setpoint is a macrostate compatible with several molecular mechanisms that can implement it.

Remarkably, the setpoint of this anatomical homeostasis can be re-written. In deer antler regeneration, there is a phenomenon known as "trophic memory," in which ectopic tines will form at sites of prior years' injuries in an antler rack replacing one that has already been shed (Figure 2C,C'). Imagine trying to specify a molecular "pathway" for this—this phenomenon is fundamentally about representations of spatial structure, memory (storing that information for long periods of time), and directing cell activity (branching) of individual cells during the growth phase toward that specific antler pattern. These themes of re-writable pattern memory will come up again below in planarian axial patterning, where a transient stimulus can make permanent lines of genetically normal two-headed worms.

A classic example of flexible anatomical homeostasis illustrates cross-level control and the implementation of an anatomical goal state by diverse molecular mechanisms (analogous to a central concept in computer science, implementation independence). In newts, kidney tubules consist of a number of cells that communicate via a characterized mechanism to arrange into a structure with a lumen.[57] When polyploid animals are created, cell size is increased; remarkably, the lumen diameter stays the same (Figure 2D)—fewer cells are recruited to enable the large-scale structure to be constant. Amazingly, when cell size is increased further, each cell will bend around itself, now using cytoskeletal mechanisms instead of cell–cell interactions, to create the target morphology.[58,59] The system triggers diverse underlying molecular mechanisms as needed, to achieve a specific macrostate—a crucial insight for bioengineers seeking to implement desired structures, and for evolutionary developmental biologists seeking to understand the ontogenetic causes of major phase transitions changes in bodyplans.

However, closed-loop anatomical plasticity highlights an important distinct type of causation in which a counterfactual future state (anatomical setpoint) guides the behavior of the system. Top–down causation and teleology have been hotly debated in physiology,[12,13,22,23,25,26,30,60–63] as it has in neuroscience and cognitive science.[64] However, this perspective offers an important and practical strategy for bioengineers: re-writing the stored

a textbook model of embryonic left–right patterning in which a cascade of signaling proteins progressively regulate each other's expression in separate compartments on the L and R side of the body. For example, on the left side, *Shh* upregulates *Nodal* (which upregulates *Pitx2*), while on the right side this does not happen because right-sided *Activin* suppresses *Shh* expression. Reproduced with permission.[133] Copyright 2018, UPV/EHU Press. C) The model of a sequential pathway makes a clear prediction: errors in one step of the pathway should be propagated forward as incorrect expression of downstream genes, and thus the percentage of animals with abnormal sidedness of gene expression (and ultimately organ situs) should remain the same when examined at progressively later timepoints (or indeed, rise, if additional errors accumulate in the process). Functional experiments in chick and frog[39,109] has shown that this prediction is incorrect: the percentage of incorrect sidedness is reduced the further in development one looks (data shown are for proton pump inhibitors in chick embryos). D) Data using a variety of perturbations show that for many steps in the process, as-yet-unknown mechanisms can correct the sidedness of downstream genes despite errors in the expression of upstream genes that are supposed to instruct them. For example, overexpression of wild-type *Mgrn* results in high levels of incorrect *Nodal* expression, but correct *Lefty*, *Pitx2*, and organ laterality. Likewise, *Lefty* laterality can be normal in cases where *Nodal*, *Pitx2*, and organ *situs* are incorrectly positioned after frog embryo exposure to the serotonin receptor blocker Gr113808 or the monoamine oxidase inhibitor iproniazid. The identification of mechanisms that somehow detect abnormal sidedness of gene expression and institute corrections is one of the most exciting new vistas of the LR asymmetry field. Image at bottom shows the *situs solitus* outcome of normal frog embryo organ asymmetry (read heart, yellow gut, green gall bladder). Reproduced with permission.[39] Copyright 2016, the Authors published by the Royal Society. E) Like the well-known Penrose stairs in Escher's "Ascending and Descending",[134] the regulation of asymmetry looks simple and linear when examined at high resolution (focused on the interaction between any two steps in the pathway), but becomes a much different and integrated picture when the whole pathway is examined together (F). It is still unknown how the downstream genes determine correct sidedness of expression if the upstream elements are incorrectly establishing lateral tissue identity. M.C. Escher's "Ascending and Descending"

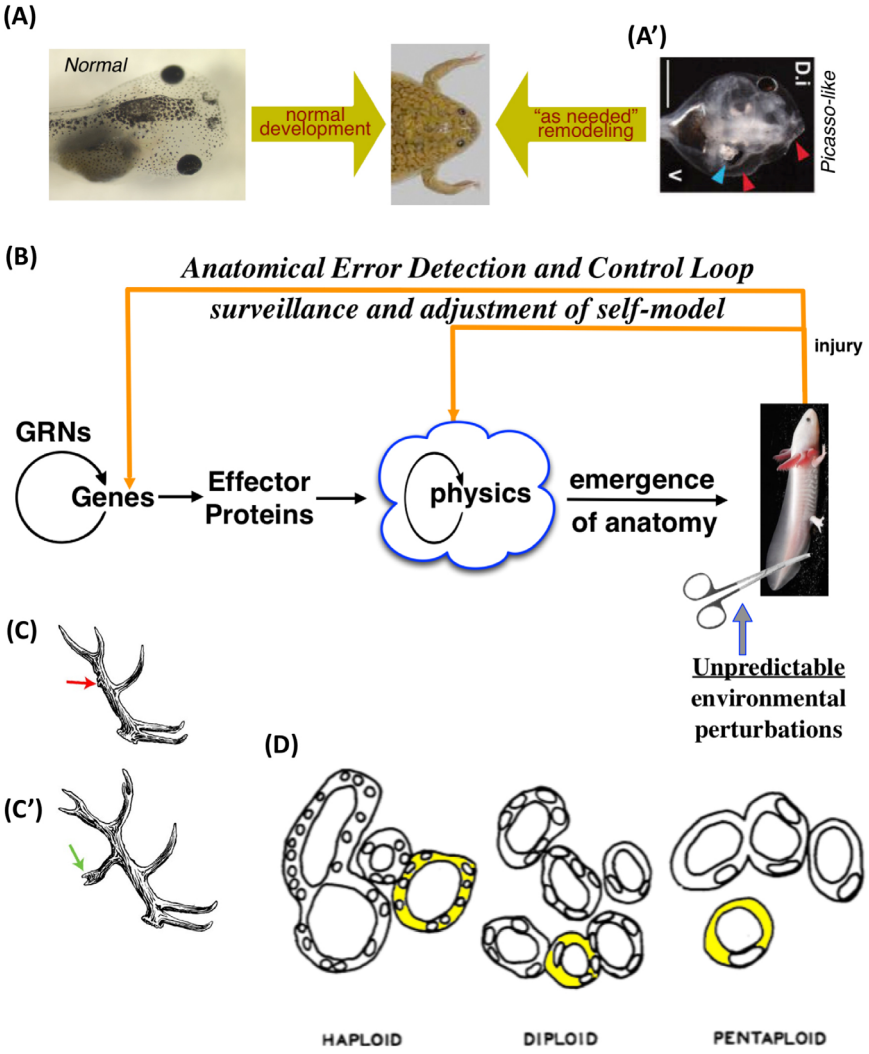


Figure 2. Pattern-homeostatic systems: invariant outcomes despite perturbation. A) Normal tadpoles rearrange their craniofacial organs to create a normal frog face (here is shown *Xenopus* laevis). Remarkably, "Picasso-like" tadpoles (A', made with eyes, nostrils, etc. in the wrong positions) also result in normal frogs, as the organs move in unnatural paths to end up in the "correct" frog face configuration despite starting from different locations.[52] B) This kind of remodeling, along with regeneration of limbs in animals such as salamanders, is a kind of pattern-homeostatic process: various perturbations to the anatomical integrity of the body (deviations from genome-default anatomy) trigger feedback processes of cell movement, proliferation, and apoptosis that work to reduce the error—the difference between the current anatomy and the target morphology (that anatomical state, which, when achieved, causes further growth and remodeling to cease). Reproduced with permission.[48] Copyright 2015, Oxford Academic Press. C) Work is only beginning to understand how living tissues store the setpoint (correct anatomical layout information) for these homeostatic feedback loops,[54] but classical data in deer antlers has shown that the setpoint can be re-written: in some species of deer (reviewed in ref. [65]), injury to one location in the branched pattern of bone causes ectopic tines to grow at this same location (C') in the next ≈5 years of growth, revealing that not only is the presence of injury remembered by the cells at the scalp and causes aberrant growth in subsequent years, but even the 3D location of the injury within the branches structure is remembered, and used to drive changes in cell branching behavior in the next year's bone morphogenesis. Reproduced with permission.[135] Copyright 1965, Wiley–Blackwell. D) Remarkably, the target morphology specified at macroscale can be implemented by diverse underlying molecular mechanisms. Cross sections of kidney tubules in the newt are normally made of ≈10 cells, which must communicate and interact with each other to form a tubule. However, when polyploid newts are made with very large cells, just *one* cell will bend around itself (using not cell–cell communication to accomplish tubulogenesis, but its own cytoskeleton to change shape) to accomplish the same anatomical macrostate: a tube with a given inner diameter. This demonstrates how diverse cell behaviors are harnessed under different conditions, not directly encountered by the animal in normal development, to reach the same invariant goal state. Adapted with permission.[58] Copyright 1945, Chicago University Press.

setpoint (and letting un-modified cells build to that new specification), instead of attempting to micromanage (re-wire) individual cell interaction rules, hoping emergence of desired large-scale outcomes. The latter mainstream approach faces likely insurmountable limits due to the inverse problem—the difficulty of knowing how to modify micro-level rules to achieve emergent system-level outcomes.[65] In contrast, the top-down view has been exploited very successfully by control theory, cybernetics, computer science, and engineering of autonomous robotics, and could enable transformative advances in biomedicine. The most expedient path to the control of a complex system such as a regenerating limb could be to take advantage of its inherent

ability to process information to achieve specific goal states. This is well-appreciated in the field of cognitive neuroscience, where the operation of neural networks can sometimes be micromanaged by direct rewiring and activation, but in the general case is much more easily implemented by training (experiences), which leverages innate learning capacity and operational organization (not a new feature of neurons, but in fact an evolutionarily ancient aspect of early networks[66]).

4. Biophysical Macrostates as Causes

An important component of the morphogenetic control system is biophysical: instructive patterning information is encoded by bioelectric state dynamics distributed across tissues.[67] Importantly, while the next section focuses on illustrating important aspects of causation through the lens of examples taken from bioelectrical signaling, a similar account can no doubt be given of the rapidly developing body of work on physical forces such as fields of strain and mechanical tensions.[68–70] Box 1 discusses the relationships between bioelectricity and other control modalities such as biochemical and biomechanical signaling, all of which are critical for morphogenesis and its dynamic plasticity.

4.1. Non-Neural Bioelectricity as a Cellular Control Mechanism

The electrical gradient across all living cells' membranes (V_{mem}) is driven by the action of numerous ion channels and pumps. Importantly, this phenomenon scales easily, as cell sheets give rise to a trans-epithelial gradient,[71] and electrical synapses (gap junctions) enable cells to communicate electrically with their neighbors. The resulting bioelectric networks serve as a rich computational medium because both the gap junctions and the ion channels are themselves often voltage-sensitive,[72] readily implementing feedback loops (voltage-gated current conductances are equivalent to transistors—fundamental building blocks of logic circuits and decision-making machinery). The roles of such networks are well-known in the brain, where bioelectric hardware underlies behavioral software that integrates distributed sensors and effectors toward remembered global goals. It is increasingly apparent that neural networks evolutionarily appropriated these computational tricks from ancient cell types that were executing much the same evolutionarily advantageous functions, albeit directed toward the control of cellular behavior (anatomy) rather than organism-level behavior.[73] Functional experiments using targeted manipulation of ion flows during development, regeneration, and cancer,[67,74–76] have revealed that bioelectric states are instructive for control of scaling, morphogenesis, suppression of tumorigenic transformation, and axial patterning across taxa. These data complement and expand the conventional molecular approach to identifying causes of anatomical outcomes.

The relevant control parameter is physiological state, not the expression of specific channel genes (**Figure 3**A–C). Ion channels and gap junctions open and close post-translationally, which means that cells with exactly identical protein complements could be in very different bioelectric states based on the cell's history of physiological signaling. Likewise, the same voltage state can be achieved by numerous different channels (and transduced to downstream promoters and cell behaviors by a range of

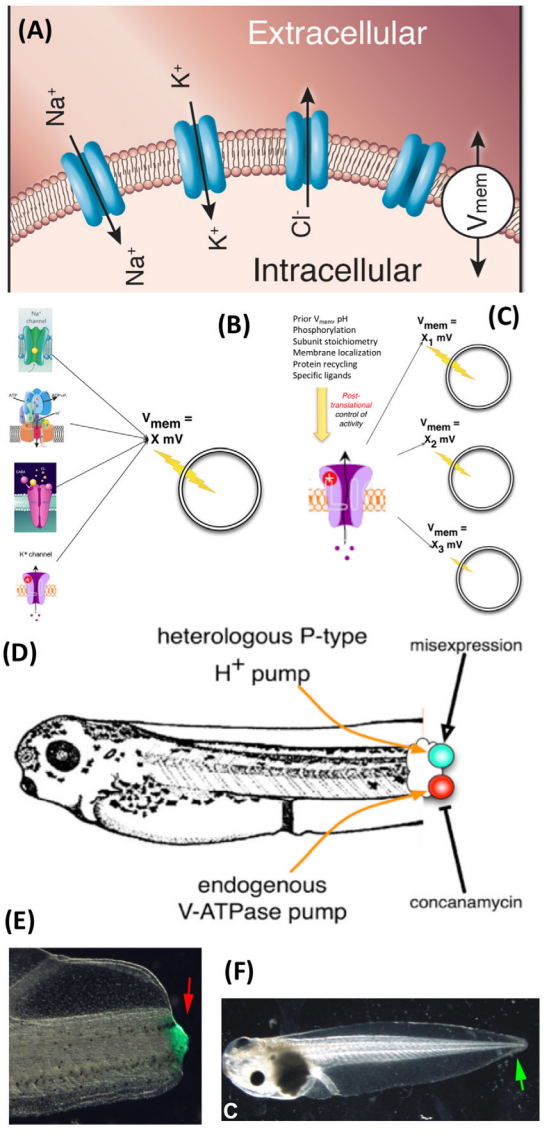


Figure 3. Bioelectric states do not map directly onto genetic states. A) A variety of ion channels and pumps in the cell's plasma membrane enable specific ions to pass down their electrochemical gradients, giving rise to an electric potential called V_{mem}. Vmem is a physiological state that is not reducible to the presence of any one (or more) ion channel proteins. B) Numerous ion channels affect V_{mem} and can establish the same V_{mem}. Thus, a cell's resting potential can be the same despite wide differences in what channels or pumps it expresses. C) Conversely, because many channels open and close post-translationally (due to signals like pH, phosphorylation, presence of calcium, or voltage itself), cells with exactly identical ion channel protein expression profiles can be in very different bioelectric states. Graphics in (A–C) are by Jeremy Guay of Peregrine Creative, used with permission. D) *Xenopus* tadpole tail regeneration normally requires the presence of an endogenous 13-subunit V-ATPase proton pump complex to establish the correct bioelectric gradient at the wound (green stain with fluorescent voltage dye as described in ref. [136]). E) When expression of this pump is inhibited, regeneration does not proceed; however, it can be rescued by expression of a completely heterologous single protein (the PMA1 P-type ATPase) from yeast[82] (F). These data show that the cause (kickstarting property) of regeneration in this system is not a specific protein(s) but a physiological state that can be implemented by a range of transcriptional conditions. The same is true of eye induction[137] and many other examples.

transduction mechanisms). Thus, tissue bioelectric state is a higher-order parameter not directly derivable from proteomic, transcriptomic, or genetic data, which affects the practical choice of technology with which to characterize causes of morphogenetic events.

4.2. Bioelectric Properties Causally Instruct Pattern Regulation

Although channelopathies (including many human syndromes[77–81]) reveal important endogenous roles of specific channel genes, this is just the tip of the iceberg. For example, the frog tadpole tail (Figure 3D–F) can regenerate using a 13-subunit ion pump complex. Knockdown renders the tail unable to regenerate, but it can be completely restored by the overexpression of a single heterologous pump protein from yeast that shares no structural or sequence homology with the native complex.[82] The driver for this process is a physiological state, not a genetic one, and the experimenter (and evolution) is free to modify the underlying transcriptional network (swap out channels) as long as the physiological state is achieved.[83,84] This slippage between the precise transcriptional profile (microstate) and the physiological profile (macrostate) has obvious implications for evolvability (smoothing the genetic fitness landscape for mechanisms controlled by bioelectrics) and impacts current research in two ways. First, loss-of-function screens targeting one channel at a time are missing a huge number of informative phenotypes because of physiological compensation and redundancy. Second, a given channel can be contributing to hyperpolarization, depolarization, or complex gating. For example, the recent interest in ion channels as oncogenes is an important advance,[85,86] but the focus on ion channel genes as simple targets for knockdown is limiting because a given channel protein might push cells toward, or away from, a tumorigenic state depending on context (ionic microenvironment profile and other regulatory states).

Bioelectric profile distinguishes proliferative, plastic cells (embryonic, cancer, and stem cells, which are depolarized) from mature, terminally differentiated, quiescent cells, which are hyperpolarized (**Figure** 4A). Indeed, cells can be artificially moved in either direction by the external control of V_{mem}.[87–90] This control extends beyond mere cell plasticity and regulates organ identity. For example, the developing face bioelectric prepattern (Figure 4B) shows where the eyes, mouth, and other craniofacial organs will later be located. This early distribution of V_{mem} states across the nascent anterior ectoderm sets the expression pattern of genes like *Frizzled* and other components of face patterning[91] and thus is an endogenous determinant of anatomy. If the pattern is artificially modulated (Figure 4B') by mRNA encoding dominant ion channels that modify the pattern, not only can organ morphogenesis be altered or prevented entirely, but indeed ectopic whole organs such as eyes can be induced in other locations such as in the gut endoderm (Figure 4B").[92] Experimental data thus demonstrate some bioelectric states to be both necessary for normal patterning and sufficient to induce it elsewhere. The data also reveal modularity meeting the criteria for potent causes: inducing a relatively simple bioelectric state kicks off a very rich, self-limiting downstream cascade of gene expression and morphogenesis. V_{mem}'s causal power can be directly compared to existing, well-accepted causes like the "master eye gene" *Pax6*: while shifts of bioelectric state can be used to induce eyes in gut, tail, spinal cord, etc., *Pax6* can only induce vertebrate eyes in the anterior neurectoderm. However, the situation is more complex than a channel protein producing a permissive range of V_{mem} (Figure 4C,D),[55] and often involves the circular causation of stable feedback loops such as that between V_{mem} and the gene *Notch*[83,93–95] in vertebrate development, and between V_{mem} and β-catenin signaling in planarian regeneration.[96,97]

4.3. Bioelectric Causes Are Powerful and Convenient Control Modalities

The *Xenopus* nascent brain is presaged in the anterior ectoderm by a distinct pattern of bioelectric states featuring two specific stripes of differential V_{mem} (**Figure** 5A,A'). Channel misexpression (Figure 5B) that altered the resting potential value of either region revealed that a correctly shaped and size-proportional brain requires the difference in voltage between the two compartments: neither voltage value is sufficient by itself if implemented homogenously; rather, it is the difference that sets the borders and anterior–posterior regionalization of the brain via control of downstream gene expression, proliferation, and apoptosis.[94,98] The understanding of this differential prepattern as a cause for correct downstream gene expression and morphogenesis predicted that brain defects induced by either genetic mutations or chemical teratogens could be functionally rescued by a "contrast enhancer" strategy that strengthened the V_{mem} differential pattern.

Indeed, amplifying this voltage difference proved successful in repairing birth defects (Figure 5C –C"), even ones induced by a dominant mutation of *Notch*—an important neurogenesis gene,[99] showing that physiological signals can sometimes override genome-default states (a theme also observed in recent experiments inducing genetically wild-type planaria to grow heads resembling those of other species[100,101]). Crucially, these approaches do not require genomic editing and do not rely on any one specific gene product: a wide range of ion channel proteins (and channel-modifying compounds, including those generated by commensal microbiota[102,103]) can be used by the experimenter to rationally alter the stable modes of the bioelectric network that drive downstream changes in gene expression and cell behavior. Remarkably, these modifications can be long-term stable: experiments in planaria have revealed that bioelectrical causes of specific morphogenetic outcomes can have consequences that propagate across multiple (albeit asexual) generations.

5. Bioelectric Flatworms: Permanent but Stochastic Re-Writing of Target Morphology

Where does a complex metazoan's anatomical specification come from—what are the causes of invariant multi-scale form? Clearly specific genetic material is required, but significant puzzles remain about the relationship between genome and anatomy that go far beyond epigenetic chromatin modifications. For example, some species of planaria reproduce largely by

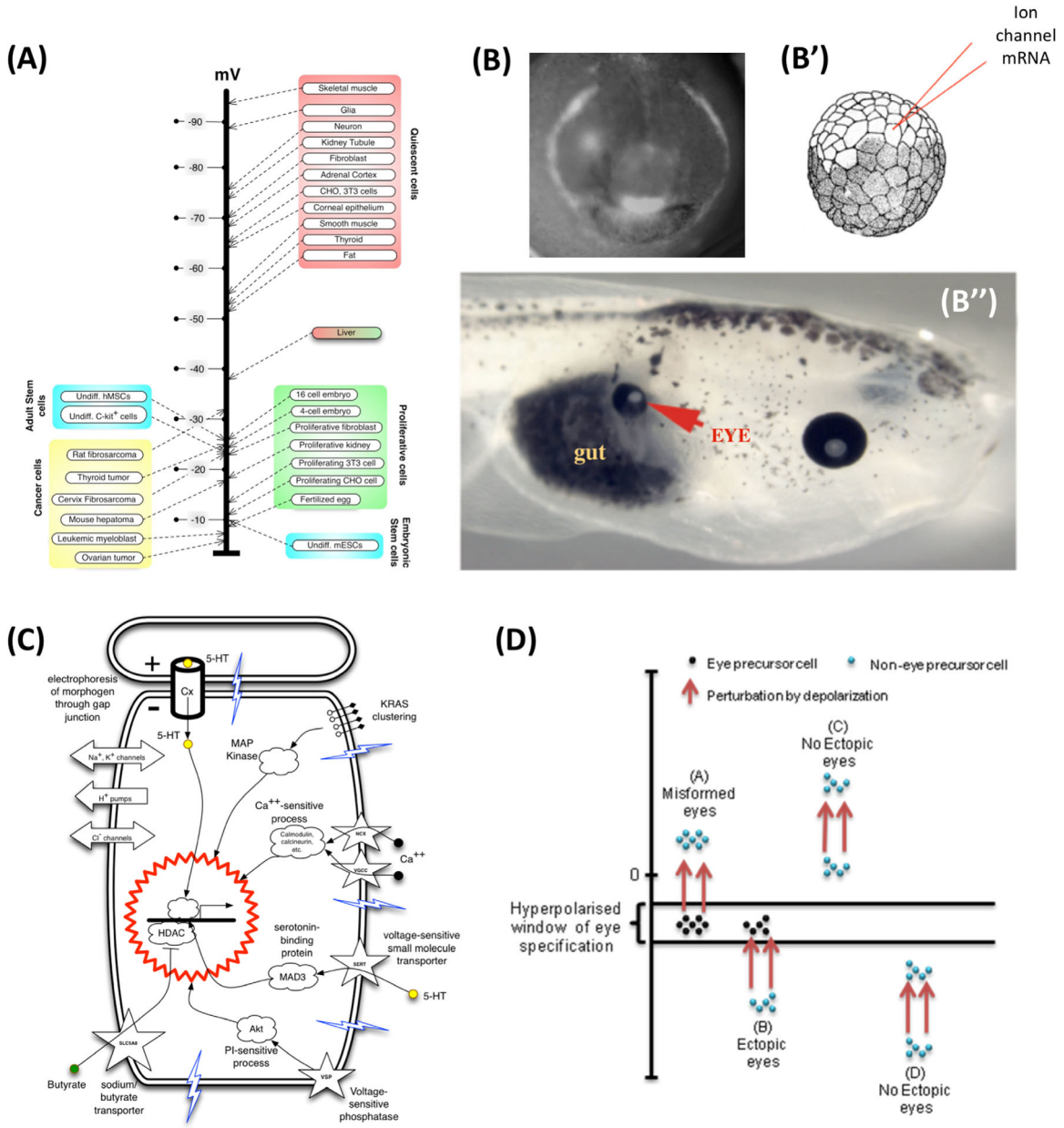


Figure 4. Bioelectric state control organogenesis. A) Single-cell mammalian V_{mem} data (modified after ref. [138]) show a conspicuous clustering: mature, terminally differentiated, quiescent cells tend to be strongly polarized (high negative V_{mem}) while plastic, highly proliferative cells (embryonic, tumor, stem) tend to be depolarized (V_{mem} closer to zero). Importantly, resting potential is not merely a readout of cell state but is instructive, as forced depolarization can induce proliferation in mature neurons[139] and prevent human stem cell differentiation,[90] while hyperpolarization can normalize tumor cells.[140] Adapted with permission.[138] Copyright 1986, Elsevier. B) Importantly, bioelectric states are not only functional at the single-state level. Endogenous bioelectrical prepatterns are seen, for example, in the nascent Xenopus embryonic face ectoderm, where voltage reporter dyes reveal regions of hyperpolarization (white signal) that indicate where the genes will come on—a process that ultimately produces the eyes, mouth, and various placodes. This pattern is instructive (a cause of normal craniofacial development) because modifying it by misexpression of any of a range of ion channels (B', frog embryo) to alter or remove specific organ domains results in aberrant gene expression and craniofacial morphogenesis,[91] while establishing ectopic domains of, for example, eye spots elsewhere in the body results in complete eyes produced well outside of the anterior neurectoderm,[137] such as in the gut (B", frog embryo)—an outcome not achievable by the "master" eye inducer protein Pax6.[141] C) Several mechanisms of transduction of changes in V_{mem} into downstream effectors are known, including electrophoresis of signaling molecules through gap-junctional paths, regulation of transporters like serotonin and butyrate (which trigger downstream receptors or block HDAC activity, respectively), clustering of KRAS receptors in the plasma membrane, changes in cortical cytoskeletal organization, and calcium influx. Reproduced with permission.[142] Copyright 2007, Cell Press. D) However, these transduction mechanisms are only informative on the single-cell level—they are mechanisms necessary for specific bioelectric induction events to occur. A tempting model driven by bioelectric data on eye induction is that of an eye-specific V_{mem} zone, outside of which neither endogenous nor ectopic eyes will form. Given the richness of the inner structure of a vertebrate eye, it is clear that a single parameter is not sufficient to dictate the multiple cell types and positions, and the spectrum of viable V_{mem} values does not give enough dynamic range to uniquely specify every type of organ. Reproduced with permission.[137] Copyright 2012, The Company of Biologists.

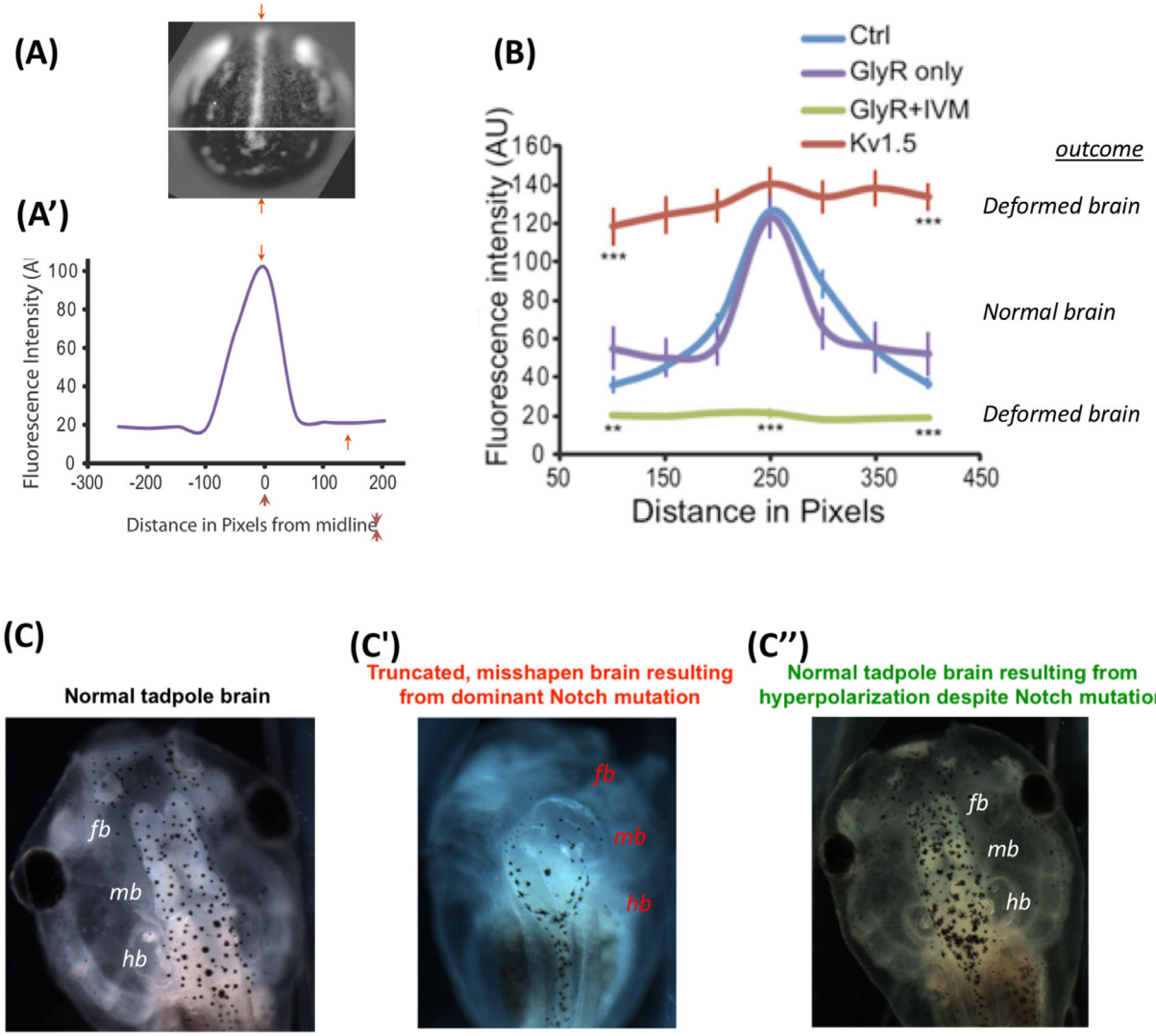


Figure 5. Bioelectric encoding of brain size and shape by a V_{mem} differential border. A) The Xenopus embryonic brain exhibits a very specific pattern of bioelectric states prior to brain development: hyperpolarization inside the region that will become the brain, and depolarization outside of it (bioelectric dye signal is quantified in A'). B) This unique contrasting pattern between the inside domain and the lateral domains, quantified here across the ectoderm (blue line, embryo midline is at 250) can be perturbed in two ways: drive the outside regions to hyperpolarized state (by expression of Kv1.5 potassium channel, red line), or drive the internal region to depolarized state (using the chloride channel GlyR + GlyR opener drug Ivermectin [IVM], green line). Either scenario gives rise to highly abnormal brains[94]—correct size and shape of the brain depends not on a single V_{mem} value, but the difference between two regions. This theme, of a bioelectric distribution across an epithelium being responsible for the transcriptional and anatomical outcome, is also seen in the vertebrate face[91] and the planarian bodyplan.[96,97] C) The wild-type tadpole brain has a characteristic size, and separate forebrain (fb), midbrain (mb), and hindbrain (hb) compartments. This pattern is abolished by teratogens and mutations of genes like the important neurogenesis gene Notch (C'). Indeed, artificially enforcing the bioelectric difference via misexpression of an HCN2 channel, which works like a contrast enhancer to strengthen differences at the edge eroded by teratogenic influences, rescues brain patterning (C''), gene expression, and behavioral function.[99] Reproduced with permission.[94] Copyright 2015, Elsevier.

fission and regeneration. This results in somatic inheritance, where any mutation that does not kill a stem cell is propagated into the next generation. Planarian genomes are extremely messy and individual animals are mixoploid,[104,105] having different numbers of chromosomes. Despite hundreds of millions of years of scrambling the genome, their anatomy is extremely robust—they are champion regenerators producing the same perfect body each time they are cut.

When planaria are bisected, one wound re-grows a head, while the other re-grows a tail—radically different anatomical fates despite the fact that the cells on either side of the cut plane were adjacent neighbors before the cut and had the same positional information. The head/tail decision cannot be driven purely by local factors but must be the result of processes that inform the wound cells of the state of the rest of the fragment. The search for the system that mediates this long-range transfer of instructive information implicated bioelectrical communication: interference with this system results in the production of two-headed or two-tailed animals (anterior–posterior mirroring) (**Figure 6**A,B). A striking outcome was observed when two-headed animals were

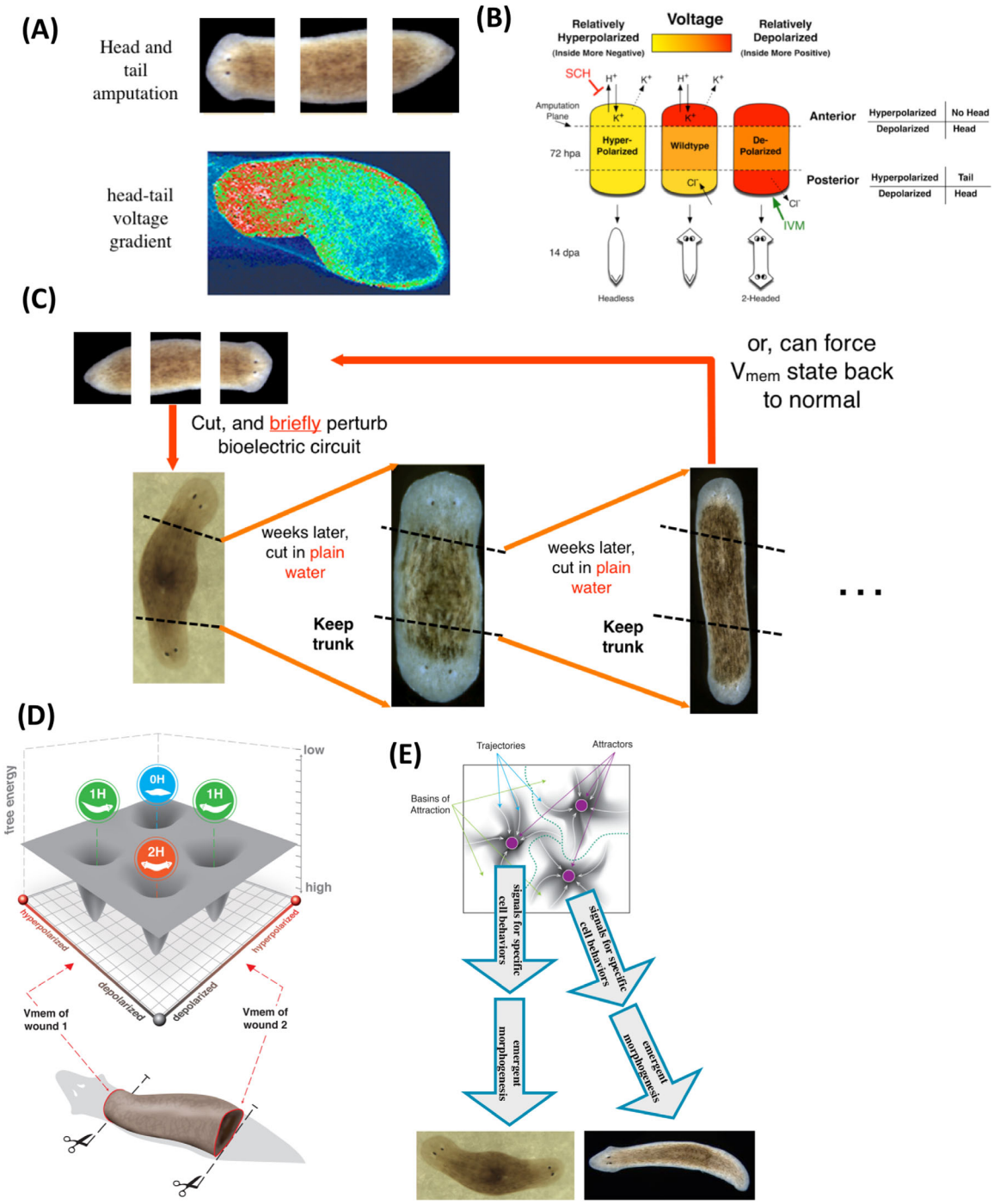


Figure 6. Bioelectric control of planarian regenerative patterning. A) Amputated planarian fragments display a bioelectric gradient (image obtained by Taisaku Nogi). B) Functional experiments to manipulate V_{mem} in the planarian *Dugesia japonica* show that re-setting this gradient with RNAi or drugs targeting specific ion channels can result in worms with one, two, or no heads. Reproduced with permission.[96] Copyright 2015, Elsevier. C) Remarkably, two-headed worms produced via these methods are permanently re-specified: middle fragments continue to generate two-headed worms in subsequent cuts in Poland Spring water, demonstrating that the pattern to which this animal regenerates can be re-written by brief physiological stimuli not involving transgenics or genome editing. Two-headed worms can be reset back to single-headedness by a different bioelectric modulation. Reproduced with permission.[143] Copyright 2014, Wiley–Blackwell. D) Computational modeling is currently underway to map out the state space of the bioelectric circuits controlling planarian regeneration to understand the long-term stable attractor states corresponding to one, two, and zero-head conditions. Drawn by Jeremy Guay of Peregrine Creative. E) One possibility is that these attractors represent stable pattern memories in a neural-network-like tissue collective, in which distinct bioelectric states redistribute morphogens and trigger gene expression to result in specific anatomical outcomes. Drawn by Jeremy Guay of Peregrine Creative.

re-cut in normal Poland Spring water (no further interventions or reagents): the bipolar animal body plan, induced by a brief change in their bioelectrical circuit, continued to regenerate as two-headed in perpetuity (Figure 6C). Without editing the genomic sequence, the target morphology (pattern to which the animals regenerate upon damage) can be permanently altered to a different anatomical bodyplan that is stable across the animals' normal reproductive mode (and can be shifted back, by shifting the bioelectric circuit back via targeting the proton/potassium exchanger).[96,97,106] Thus, the bioelectric circuit (and its downstream morphogen gradients) serves as an important causal input into patterning decisions—a kind of extra-genomic memory, akin to the cytoskeletal non-genetic cortical inheritance described in ciliates.[107,108] This memory is implemented by attractors in the state space of the electrochemical circuits that control organ-level patterning decisions (Figure 6D,E).

There is, however, an additional twist. When treated with inhibitors of bioelectrical synapses, about 30% of the animals became shifted to the two-headed state. The rest were one-headed, considered to have escaped the drug treatment (**Figure 7**A–D). However, though these animals had wild-type molecular marker expression, stem cell distributions, and anatomy, when re-cut they again formed one-head and two-headed animals at a 3:1 ratio.[106] The decision is stochastic, occurring randomly (but at the same ratio) in a cohort of animals living in the same petri dish, on each future cut (Figure 7E). Indeed, two pieces of one animal can have different anatomical fates—every fragment flips a (biased) coin to decide whether it will shift to a permanently two-headed target morphology or remain in the de-stabilized "cryptic" state, where an anatomically normal body belies a permanently altered regenerative outcome. The decision to become one of these distinct two lines of worms is stochastic at the level of the fragment, but coordinated at the level of individual cells (all of the cells of a given fragment agree and build a coherent head or tail, not a speckled hybrid structure). The same type of organ-level randomization despite cell-level concordance exists in bioelectric control of left–right patterning in embryogenesis[109] and in bioelectric/neurotransmitter control of melanoma transformation[110] in vertebrates.

Interestingly, in wild-type and two-headed worms, the bioelectric pattern reflected the current anatomy that it induced. However, the difference between wild-type, two-headed, and cryptic (destabilized) animals is the steady-state bioelectric pattern.[106] In cryptic animals, the abnormal bioelectric pattern did not match the current one-headed, normal anatomy. It is, in this case, a latent memory, which is not currently apparent but will become activated and determine patterning after injury occurs. Thus, there is a temporal distance (which can last weeks or longer) between the cause and the effect that it will induce. Moreover, this is one illustration of the fact that the bioelectric pattern is not an epiphenomenon reflecting current tissue state, but an information structure that will determine (drive the outcome of) future regenerative events. We are currently modeling the state space of bioelectric circuits upstream of morphogen gradients in planaria to identify "edge of chaos" effects in which very similar starting conditions can drive fragments to end up in highly distinct regions of the planarian anatomical morphospace.[100,111–114]

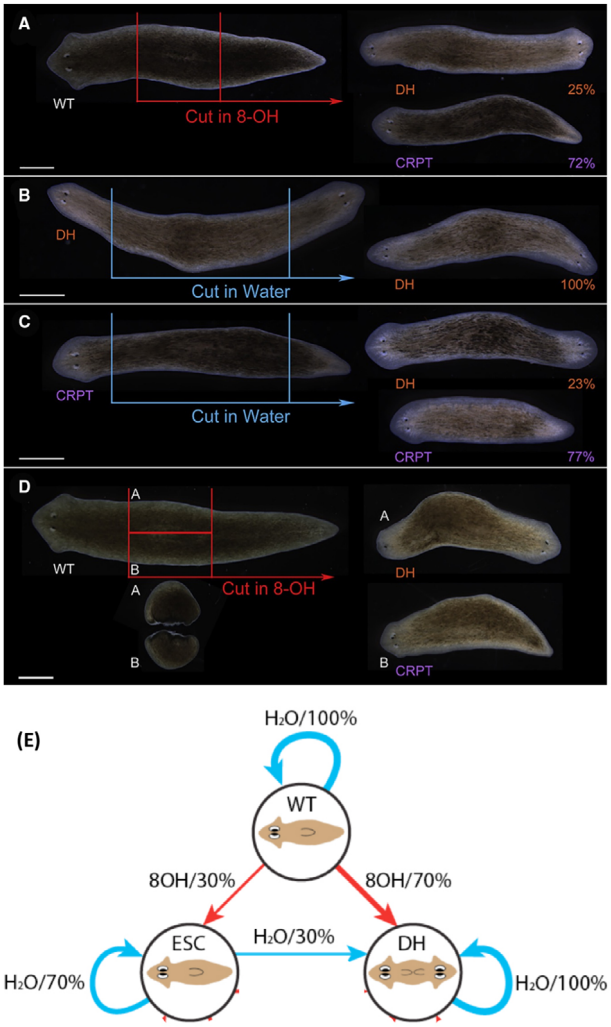


Figure 7. Stochastic outcomes in planarian patterning. A) A cohort of planarian fragments (*Dugesia japonica*) treated with octanol (8-OH, a gap junctional blocker) in the same dish give rise to normal-seeming worms and two-headed worms in an ≈1:3 ratio. B) Two-headed (double-head, DH) worms cut in water always result in two-headed worms. C) The one-headed worms resulting from this experiment are in fact not normal, because when cut, they do not show the 100% one-headed outcomes of truly wild-type animals, but rather exhibit the same 1:3 pattern of two-headed animals to destabilized (cryptic, CRPT) animals. D) Two pieces cut from the same worm can have different anatomical outcomes, illustrating that the stochastic decision is made independently by each piece, not by the parent. E) A schematic state diagram showing that planaria exist in three possible states (wild-type, two-head, and cryptic) and can transition between them.

Taken together, these phenomena reveal novel aspects of causation in pattern control. First, long-term anatomy can stably diverge from the genome-default pattern and can be shifted by transient changes in physiological circuit states. Second, the exact same starting state (fragments of a single parent worm, treated together) results in stochastic, not deterministic, outcomes among fragments. Finally, the system offers two very distinct levels of organization and size scale at which to understand its behavior: the emergent collective makes a random decision, on which

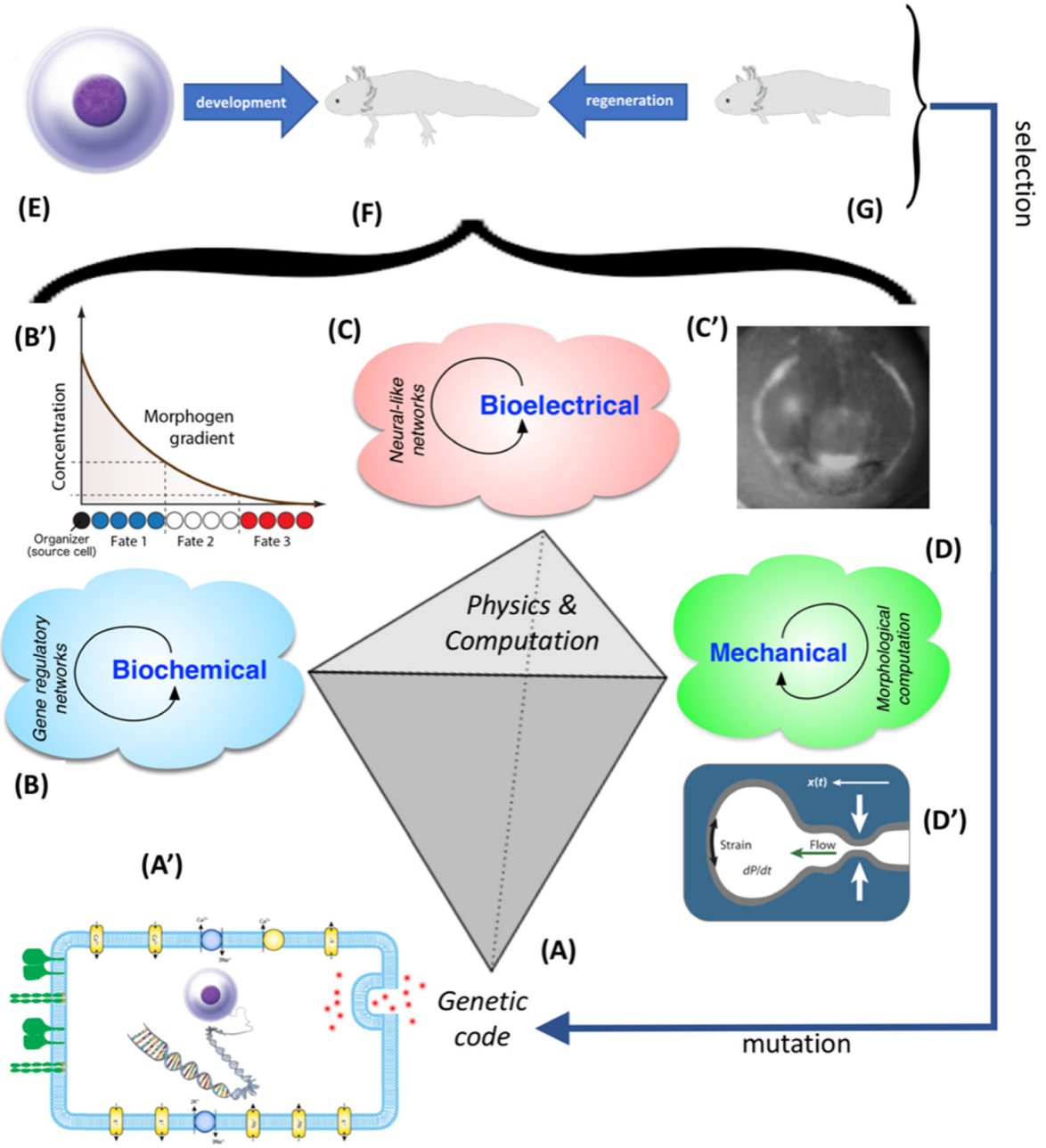


Figure 8. A schematic of anatomical homeostasis in the context of evolution: from genetics to physiological computation. The relationship between the genome and the primary biochemical and biophysical modalities that control form and function can be schematized as a tetrahedron supported by one vertex (A). The bottom vertex represents the genome, which encodes the hardware provided to each cell (A'): the ion channels (yellow), secreted factors (red), and adhesion molecules (green) that allow it to exploit rich and useful aspects of physics. The base of the tetrahedron represents three modalities, which interact with each other to form the physiological "software" that drives patterning decisions in ontogenetic time. This includes: biochemical signaling that contains gene-regulatory networks (B) and forms chemical morphogen gradients (B'), bioelectrical signaling that enables long-range neural-like computational networks (C) that set up prepatterns of resting potential such as the one that sets up the face (C'), and biomechanical signaling (D) that enables morphological computation via tensile properties of materials and strain fields (D'). Together, the interaction of cells that process information via chemical messengers, biomechanical forces, and bioelectrical signaling enables large-scale pattern homeostasis that drives eggs (E) to self-assemble invariant anatomies such as the axolotl shown in (F), and to re-establish these complex outcomes when they are artificially deviated by injury (such as the amputated limbs and eyes shown in (G)). On evolutionary timescales, the self-repairing bodies formed by these processes face selection pressures, which change the population frequencies of variants of genes encoding the biophysical hardware. A critical task of the post-genomic age is to better understand how the modular and robust physiological layers smooth the fitness landscape by implementing self-organization and information-processing capacities that lie between the genotype and the phenotype. Panels A',E were created by Jeremy Guay of Peregrine creative. (B') Reproduced with permission.[144] Copyright 2017, Japanese Society of Developmental Biologists. (C') Reproduced with permission.[145] Copyright 2011, American Association for Anatomy. (F,G) Reproduced with permission.[146] Copyright 2019, Springer Nature. (D') Reproduced with permission.[147] Copyright 2012, Annual Reviews.

every subunit agrees. Thus, the cause of head or tail outcome in a middle fragment of a worm is not local but depends on physiological experiences of the parent worm during prior regenerative instances and on events that occurred at considerable distance from the blastema. Controlling or predicting the outcome requires an understanding of causes that include the lability of pattern memory and of the stochasticity of group decision-making.

6. Evolving Concepts: The Future of Finding and Exploiting Biological Causes

Causation in biology is an extremely exciting frontier, driving the intersection of evolutionary-, developmental-, cancer-, and neurobiology with deep ideas from computer science, cybernetics, and cognitive science.[115,116] Understanding causes of anatomical change is critical not only for understanding evolutionary change and development but for advances in biomedicine and the creation of novel synthetic living machines.[117] An immediate societal impact of this question arises during litigation on teratogenesis potentially caused by environmental chemicals and pharmaceuticals. Often, large class-action legal cases turn on the question of what exactly caused specific embryonic defects in an individual and what might cause them in a population. Critically, such discussions are often stymied by the desire to assign a single, reliable cause to a complex and stochastic fetal phenotype. Policy and pharmacological regulations would be revolutionized by a more nuanced, multifactorial notion of causation that replaced a binary expectation of cause and effect with the one that took into account the differential ability of some embryos to resist teratogens and the factors (like nervous system activity[118]) that contributed to that capability.

The ability of complex biosystems to make decisions at different scales of organization opens the possibility of taking advantage of their causal structure. Imagine trying to get a rat to perform a circus trick. The mainstream approach involves direct micromanagement at the micro level: controlling all of the relevant neurons in the brain to achieve the need movements—an extremely difficult task. Another strategy, discovered by prescientific human populations, is to train the rat: positive and negative reinforcements can motivate the system toward a desired goal state. This offloads the computational complexity onto the system itself. This is a much more tractable task; even collections of neural cells in a dish can be trained to operate an airplane using positive and negative reinforcements,[119] and it is tempting to speculate about the biomedical outcomes that could be achieved if we understood the inputs that motivate cell groups toward system-level outcomes.

A contrast to pre-existing commitments to specific levels of explanation is a level-agnostic, pragmatic approach focused on control. The amount of effort that needs to be exerted by the experimenter toward achieving a specific outcome is a quantitative, objective metric of the efficiency of a particular approach to a problem and of the appropriateness of a given level of analysis for identifying causes. Recent advances in information theory[8,9,120] confirm that the most salient causes in a system are not always found at the lowest level of organization (in this field, often thought to be subcellular biochemistry). Interestingly, this applies not only to the experimenter, but also to the biological system itself: the widespread prevalence of modularity[121–123] reveals that evolution discovered a very efficient way to control system-level outcomes: rewriting large-scale homeostatic setpoints and letting the system regulate to them, instead of micromanaging all of the myriad low-level details to achieve the desired outcome in the face of novel circumstances.

Many of the key examples illustrating the need for novel conceptions of pattern homeostasis are many decades old—trophic memory in deer antlers, tail to limb remodeling in amphibia, cancer normalization in mammals, and kidney tubule development in polyploid newts. They have not been investigated with modern tools because the field has largely focused on those phenomena that are best understood via classical notions of causality (linear pathways, or at best networks). The field is moving in the right direction, however, via sophisticated analyses of dynamical systems portraits of developmental contexts.[124–127] Ideas from cybernetics and cognitive science to truly exploit large-scale causes are only beginning to be incorporated,[44,48,54,116] but will be increasingly more apropos as physical forces and bioelectric mechanisms are integrated with biochemical and genetic information.

Importantly, while conceptual advances are beginning to tackle complex states as biological causes—expanding upward and outward, technology continues to drill downward, seeking ever-smaller-scale controls. Single-molecule manipulation and single-cell RNAseq are leading to an unprecedented accumulation of big data. These datasets come with a risk. What would have happened if nineteenth century physicists thought they actually had a hope of tracking each individual molecule of a gas? We would have missed out on thermodynamics, and deep truths of statistical mechanics (entropy, etc.). Might we be delaying the development of higher-level laws—e.g., a Boyle's Law of biology—because the community has the feeling that large-scale laws are not necessary because soon we will really be able to track every microstate?

7. Conclusions and Outlook

A focus on higher levels of causation makes a number of predictions for future work. For example, instead of synthetic biology approaches to directly alter cellular control circuits (hardware), could organ-level rules be implemented in vitro (synthetic morphology) or in vivo by rewriting the biophysical setpoints to which the system builds? It will be crucial to expand such data beyond planaria, in which transiently editing the stable bioelectric memory determines the structures that will form after future injury. Moreover, the conservation of neural network functions from pre-neural developmental tissue activities suggests that morphogenetic outcomes could be achieved via training, not direct rewiring. Could positive (e.g., nutrient pulses or opioid delivery) and negative (e.g., stressors) reinforcements be an efficient way to motivate a biological system to alter target morphology? Our lab is currently testing these ideas by building closed-loop environmental controls that attempt to guide morphogenetic behavior by exploiting their large-scale homeostatic capabilities.

When dealing with complex systems governed by feedback loops, circular causality, and large-scale setpoints, the control structures (causes) are not obvious and hard to determine; they are often distant, both spatially and temporally, from the event

in question. It is likely that machine learning (AI) will be a critical part of the future of this field—not as an endpoint (via black box prediction), but as the first step of a profound understanding, by helping to identify richer kinds of causes as new testable hypotheses. The development of artificial intelligence platforms—the next generation of a bioinformatics of shape—could help identify potent interventions that enable control of form, and thus reveal the causal structure of complex biological systems that can then be investigated mechanistically.[128–130] Conversely, a more nuanced understanding of causation is itself critical for the development of novel machine learning strategies that extract actionable intelligence from the ever-increasing deluge of data.[129–131]

Efforts to provide machine learning platforms with the ability to identify causes in perturbational biological data may well turn out to be a critical enabling step to general artificial intelligence, for which identifying causes in complex scenarios is a prerequisite. In this way, as well as through the efforts to make self-repairing robust robotics,[132] the search for a mature understanding of biological causation goes well beyond the life sciences. At stake will be not only advances in biomedicine and bioengineering, but also aspects of engineering and robotics. Thus, taming developmental causation is much more than a philosophical issue—it offers the promise of widespread impact in science and technology.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The author declares no conflict of interest.

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bioelectricity, causation, development, embryos, pathways, regeneration

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