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# Endogenous Bioelectric Signaling Networks: Exploiting Voltage Gradients for Control of Growth and Form

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## Abstract

Living systems exhibit remarkable abilities to self-assemble, regenerate, and remodel complex shapes. How cellular networks construct and repair specific anatomical outcomes is an open question at the heart of the next-generation science of bioengineering. Developmental bioelectricity is an exciting emerging discipline that exploits endogenous bioelectric signaling among many cell types to regulate pattern formation. We provide a brief overview of this field, review recent data in which bioelectricity is used to control patterning in a range of model systems, and describe the molecular tools being used to probe the role of bioelectrics in the dynamic control of complex anatomy. We suggest that quantitative strategies recently developed to infer semantic content and information processing from ionic activity in the brain might provide important clues to cracking the bioelectric code. Gaining control of the mechanisms by which large-scale shape is regulated in vivo will drive transformative advances in bioengineering, regenerative medicine, and synthetic morphology, and could be used to therapeutically address birth defects, traumatic injury, and cancer.

### Keywords

bioelectricity; ion channel; gap junction; synthetic morphology; morphological computation; regeneration

## 1. INTRODUCTION

With sufficient understanding, it should be possible to control morphogenesis to drive the production of normal tissues or organs in order to address traumatic injury or degenerative disease, as well as to create entire novel biological constructs with desired functionality. A fundamental barrier to achieving this goal is complexity: Even if any cell type could be made from stem cells, how would we generate a functional hand or eye? Micromanaging the construction process at the lowest level is likely not feasible for such complex structures.

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We suggest that the path to these capabilities is through an understanding of the endogenous computational dynamics that allow living systems to create and drastically remodel complex three-dimensional (3D) anatomies.

Eggs reliably self-assemble into adults with many distinct tissues in precise geometric configurations. Crucially, the embryos of many species are not predetermined mosaics, but exhibit astonishing capabilities of self-repair, dynamic rescaling, reconfiguration, and functional plasticity (Figure 1). Embryos that are split or combined early in development revise their developmental program to generate complete, undamaged organisms. Many adult animals can regenerate complex organs after amputation or damage; for example, planarian flatworms can regenerate their entire body after almost any kind of amputation, and they continuously remodel their tissue to match the available cell number to its target morphology and correct proportions among all organs (1). Salamanders regenerate limbs, eyes, jaws, hearts, and brain; deer regenerate bone and nerves when replacing their antlers each year; and even human children can regenerate their fingertips completely (2). A key aspect of regeneration is that the process stops when a structure of the correct size and shape has been achieved, suggesting that organisms have implicit or explicit ways to encode and monitor anatomical target (or goal) states.

Some animals not only regenerate amputated parts but also actively remodel large portions of their bodies. Transplanted cockroach legs with the wrong number of segments undergo intercalation to restore proper leg segmentation (4). Tails ectopically grafted to the flank of an amphibian host will slowly remodel into limbs (5), and froglets will eventually establish a normal facial anatomy after drastic rearrangements of the tadpoles' craniofacial components are experimentally induced (6). We believe that one of the most powerful methods of controlling patterning could be achieved if we learn how to rewrite these target states, allowing the cells to build to specifications without micromanaging.

Remarkably, embryonic (7) and regenerative (8) environments can reprogram cancerous growth into normal anatomy, revealing the importance of context and patterning cues in regulating individual cell behavior. These data not only suggest that cancer may be a kind of disease of geometry (and thus that normalization of tumors is within the domain of bioengineering) but also point to the importance of global patterning information in the understanding of cell behavior.

Next-generation bioengineering must move beyond the direct assembly of cell types toward the control of the endogenous error-correcting morphogenetic networks and the programming of shape by specifying organs and their topological relationships (9). What all of the above examples have in common is a kind of shape homeostasis—the ability of systems to flexibly regulate cell-level events in order to achieve higher-level (organ-, tissue-, or whole organism–level) patterning states despite deviations from those states. A key issue for the future of biology and medicine is to find the appropriate theoretical paradigm with which to understand complex pattern regulation and derive quantitative models with predictive power that will enable rational modification of shape for engineering and biomedical applications. Global control systems are mediated by chemical gradients (10), physical forces (11), and bioelectrical signaling (12) (Supplemental Table

1; follow the Supplemental Material **link** from the Annual Reviews home page at http://www.annualreviews.org)—distinct and orthogonal layers of the morphogenetic field.

Here, bioelectricity (Figure 2) refers to endogenous electrical signaling via ion channels, pumps, and electrical synapses (gap junctions) at the plasma membrane (13). One of the earliest experimental approaches toward pattern regulation was Roux's (14) application of electric fields to developing eggs in 1892. Since then, solid functional data have implicated steady ion currents in embryogenesis and wound healing (15, 16). By tracking developmental currents and applying physiological-strength electric fields, investigators have shown that transepithelial electric fields regulate cell migration, orientation, and nerve growth (reviewed in Reference 17). However, recent advances and development of molecular-level techniques (18) have identified new aspects of bioelectricity that regulate individual cell function and help coordinate the embryogenesis and regenerative repair of complex structures.

In this review, we discuss advances in bioelectrical signaling from the perspective of integrating bottom-up (molecular mechanism focus) with top-down (information perspective) approaches to the control of biological pattern and function. We focus on endogenous bioelectric signaling (rather than on effects of applied fields) that implements morphological computation in vivo and explain how it can be exploited by bioengineering (19). Due to length constraints, we exclude transepithelial electric fields, the geomagnetic field, electromagnetic communication among cells via ultraweak photons, static charge effects in cell function, and subcellular organelle potentials.

Recent advances have revealed that such bioelectric dynamics are a novel control layer, the manipulation of which will enable much of the complexity to be off-loaded onto the inherent computational abilities of cells. Thus, we review data and techniques being developed in this exciting emerging field, focusing not only on cellular mechanisms but also on bioelectricity's role as a long-range integrator of instructive patterning cues within tissues. This review focuses on the instructive cues mediated by spatiotemporal patterns of voltage potentials across the membrane of nonneural cells and the roles they play in coordinating size control, organ identity, and axial patterning during regeneration, development, and cancer suppression. These examples will be of particular interest to bioengineers because they illustrate modular, top-down control of patterning (e.g., reprogramming a region of the gut into a complete eye), reprogramming patterns without genome editing (permanent change of planarian regenerative morphology to two-headed forms), overcoming genetic or pharmacological teratogenic influences to force normal brain pattern, or reprogramming tumors into normal tissue (20). Bioengineering and synthetic biology have not yet made extensive use of this modality; for this reason, we highlight the background information necessary to lower the barrier for these communities to enable them to begin to exploit the remarkable properties of developmental bioelectricity, and provide a forward-looking perspective on this emerging field.

# 2. BIOELECTRIC PATHWAYS: THE BASICS

## 2.1. Circuit Components: Building Blocks of Developmental Bioelectricity

Bioelectric circuits consist of several main components in vivo, including ion channel proteins, which passively segregate specific positive and negative charges across the membrane, and ion pumps, which use energy to transport ions against concentration gradients. Ion channels and pumps in the plasma membrane set the resting potential of the cell, the difference between the inside and the outside of the cell, which is measured in millivolts (mV). In general (Figure 3a), terminally differentiated, quiescent cells tend to be strongly polarized (bearing a more negative resting potential), whereas embryonic, stem, and tumor cells tend to be depolarized (closer to zero) (21).

Whereas these membrane proteins exchange ions with the milieu outside the cell surface, gap junctions are channels that allow direct cell-to-cell transfer of molecules, and therefore electrical signals, from the cytoplasm of one cell to a neighboring cell. Cells connect to each other via these electrical synapses (22), which facilitate the formation of bioelectrical networks that help shape the distribution of resting potential ($V_{mem}$) levels within large groups of cells and form isopotential cell fields within the body demarcated by gap junctional isolation zones (23). $V_{mem}$ gradients are defined as patterned spatial differences among cells' $V_{mem}$ values across anatomical distances. Bioelectrical signals are defined as temporal changes in such a pattern, which can trigger downstream patterning cascades.

## 2.2. The Source and Distribution of Bioelectrical Signals

The transmembrane resting potential, in concert with canonical biochemical signaling pathways, serves as an instructive cue for cellular behaviors that underlie morphogenesis, such as differentiation, proliferation, migration, cell morphology change, orientation, and transcriptional changes (Supplemental Table 2). Bioelectric signals (specific ion currents and the resulting changes in $V_{mem}$ pattern) occur in a wide variety of cell types from all developmental lineages, including stem cells, in both animal (24) and plant (25) cells. Supplemental Tables 3 and 4 list ion channels, pumps, and gap junction proteins that have been implicated by unbiased genetic screens as sources of bioelectric signals that are important in pattern regulation.

Cellular $V_{mem}$ is not static, but changes in time with the cell cycle (26) and longer period oscillations (27). Cell populations, even ones that appear homogeneous by molecular markers, often exhibit distinct subpopulations with respect to bioelectric gradients. For example, in induced pluripotent stem cells (iPSCs), potassium channels play a role in regulating proliferation (28) as they do in mesenchymal stem cells (MSCs) (29); whereas iPSCs are electrophysiologically homogeneous, MSCs are not. Cells in culture show distinct $V_{mem}$ patterns that are largely due to cell cycle differences, while in vivo a variety of signals impinge upon cells and cause spatial differences of bioelectrical states within cell populations (30).

### 2.3. Transduction Mechanisms: From $V_{mem}$ to the Nucleus

As in the brain, the most common way for cells to sense bioelectrical changes is by coupling voltage changes to the movement of small signaling molecules (Figure 3b-d). The most common one is calcium (31); a change in $V_{mem}$ leads to the opening of calcium channels and an influx of calcium ions into the cell. This mode of transduction has been implicated in control of growth-cone turning (32), eye patterning (33), and flatworm regeneration (34, 35) via voltage-gated calcium channels, which activate a plethora of well-known calcium sensors such as calcineurin. Another such molecule is serotonin, the levels of which change via the effects of voltage on the serotonin transporter (SERT) in the amplification of embryonic left-right asymmetry (36), metastatic induction (37), and neural pathfinding (38). The transporter SLC5A8 converts ion levels into the movements of butyrate, which in turn is an important regulator of histone deacetylases and thus of epigenetic chromatin state, as occurs in tumorigenesis in *Xenopus* (39) and in mammals (40). Additional mechanisms for transduction include voltage-sensitive phosphatases, which couple changes in $V_{mem}$ to the plethora of events regulated by phosphatase and tensin homolog (PTEN) phosphatases (41), and clustering of protein receptors in the membrane, as appears to occur for RAS signaling (42).

Several target genes, including *Hedgehog* and *Notch*, are known to be regulated by resting potentials (Supplemental Table 5). Although some of these are probably indirect effects, several promoters are sensitive to changes in $V_{mem}$; for example, depolarization upregulates cytomegalovirus (CMV) promoter activity by >90-fold (43). A recent study (44) using microarray analysis identified many genes that are induced by forced $V_{mem}$ change, regardless of the channel or ion used, that are shared among frog embryos, adult regenerating axolotl spinal cords, and human MSCs. A pathway analysis revealed that genetic responses to $V_{mem}$ change occur within pathways related to numerous organ systems and disease-relevant pathways. In some cases, $V_{mem}$ change also alters the expression of other ion channel genes (45), forming feedback loops that integrate bioelectric and transcriptional responses (Figure 4a).

### 2.4. How Cells Link into Networks

$V_{mem}$ regulation extends far beyond the state of single cells, as cellular resting potential is also linked to the $V_{mem}$ of proximal and distal cells. Bioelectric signaling is an ideal way for cells to coordinate their behaviors across anatomical distances. This idea was first suggested by Burr (46), who used voltage readings at remote locations of the body to detect transplanted or induced tumors as disturbances to large-scale morphogenetic fields. Such long-range signaling is mediated by ephaptic field effects (47), transepithelial potentials (48), tunneling nanotubes (49), transfer of ion channels via exosomes (50), and gap junctional connectivity implemented by connexin and innexin proteins (23), all of which occur in all cell types throughout the body but have been the most extensively studied in the central nervous system (CNS).

## 3. MODERN TOOLS

The study of bioelectricity has had a very rich history over the last century. Interested readers should consult Reference 16 for reviews from some of the main contributors to this field.

### 3.1. Implicating Bioelectric Components: Characterization

In the last decade, molecular tools that can measure and influence bioelectric patterns have been developed, as have tools that can be used to identify the molecular source and the downstream targets of specific bioelectrical events (51). Pharmacological screens are able to rapidly identify the type of electrogenic protein that could be the source of observed gradients, and systematic tiered screens (52) traversing a tree of progressively specific blocker drugs (followed by gene-specific validation) led to the identification of channel and pump proteins that are required for left-right patterning (53) and tail regeneration (54) in frog models. Unlike genetic screens, this approach overcomes the high redundancy among channel family members. More recently, high-throughput screens have been used to identify ion translocator-modifying compounds in assays for control of cancer and other stem cell behavior (55, 56). Ion channels and pumps are also frequently found within microarray or RNA sequencing data sets comparing different conditions; however, these proteins are often deprioritized for further analysis in favor of more typical targets, such as secreted proteins and growth factors, because of general unfamiliarity with the strategies for unraveling bioelectric signals' roles in patterning.

### 3.2. Visualizing Bioelectric Properties

The spatiotemporal distributions of ionic parameters cannot be inferred from expression data alone: Unlike messenger RNA (mRNA) and protein levels, bioelectric properties are ascertainable only in vivo and collapse immediately upon cell death or fixation. However, $V_{mem}$ patterns across tissues can be quantified using several approaches. For example, voltage gradients can be visualized in situ using fluorescent reporters of transmembrane potential (57) or nanoscale materials (58), which are suitable for use in any optically accessible region. Such methods are noninvasive and can report multiple $V_{mem}$ values across tissues and even within cell membrane domains (51). Other tools that can be used to characterize bioelectrical events include highly sensitive ion-selective extracellular electrode probes (59), which reveal ion flux at surfaces; dielectrophoretic analysis (60); and microelectrode arrays (61). New fluorescence chemistry approaches (62) are likely to revolutionize our ability to track physiological parameters in vivo by offering improved membrane targeting, calibration, brightness, and sensitivity. Significant opportunities exist for the development of specific, bright, ratiometric dyes that localize exclusively to the desired subcellular locale (e.g., plasma membrane or nucleus), and it will be particularly exciting to use multiple physiological dyes in fluorescence-activated cell sorting experiments to identify subpopulations of "pure" stem and other cell types that differ in key bioelectric properties (63).

### 3.3. Computational Modeling of Bioelectric Signals

One of the most promising recent developments in this field is the ability to use quantitative modeling to understand how patterns of voltage arise from the actions of multiple ion translocators and how patterns of resting potential evolve over time in collections of cells (Figure 4b). Quantitative modeling is essential because of the complex feedback loops and highly nonobvious behavior of coupled electrically active cells. For example, effects on $V_{mem}$ from electrode stimulation are very hard to predict, and emergent network properties such as stable attractors and "virtual electrodes" can arise within tissues (64). Some early research had already begun to integrate ion channels and gap junctions in physiological models (65), and quantitative models have started to do so in specific contexts, such as cancer (66), but most of the developmental bioelectricity analyses performed to date have been semiquantitative in describing the circuits that give rise to specific patterns with predicable $V_{mem}$ control properties (34). Recent research in this area includes cell-level models, including ones that revealed that "collective multicellular states show electrical coupling mechanisms that are not readily deduced from biochemical descriptions at the individual cell level" (67, p. 1; 68) and ones that showed how cells can maintain bistable memory states (69). Bioelectric circuits with memory are important because they illustrate how tissues can store stable state change purely at the level of physiology (undetectable by transcriptional or genomic approaches), and because they can be exploited as flip-flop circuit elements in synthetic bioengineering approaches.

Anewly developed comprehensive modeling environment, BETSE (70), facilitates quantitative experiments and modeling of physiology and biochemical signaling in an integrated virtual tissue. BETSE should significantly enhance efforts to model specific patterning events and gain a degree of predictive control in guided self-assembly applications in vitro. The integration of this and similar systems into existing platforms for multiscale tissue simulation environments (71), and in synthetic biology frameworks for the construction of programs (biological compilers) to be implemented in living tissues, is a goal for the future. Other recent models have tackled higher-level aspects of bioelectric signaling, exploring embedded electric circuit dynamics in the control of soft robot behavior and morphological computation (72), as well as neural network–like control of regenerative morphology (73).

### 3.4. Functional Tools: Reading and Writing Bioelectric States in Tissues

Perhaps the most important set of techniques involves the functional modification of $V_{mem}$ distributions to alter endogenous patterning and induce desired changes in growth or morphogenesis; this is accomplished by altering electrical connectivity (targeting gap junctions) or resting potential of specific cells (Figure 4c). Guided by the modeling platforms discussed above, this strategy can be used to test specific hypotheses based on the results of screens or physiomic profiling. Pharmacological manipulation of natively expressed channels or pumps (e.g., using channel-opening drugs) is the best approach in biomedical applications where gene therapy is not possible; for example, a mixture of small molecules that alter sodium flux was used to initiate the regeneration of a tail in an amphibian model of spinal cord and muscle regeneration (74). Chloride channels are particularly useful: Once locked in an open state by drugs like ivermectin, the amount of

chloride in the external milieu can be altered to set the resting potential to desired levels (75).

When possible, one should use gain-of-function strategies, such as the misexpression of well-characterized ion channels and pumps, to rationally alter $V_{mem}$ gradients in vivo. For example, the misexpression of hyperpolarizing and depolarizing channels was used to alter the endogenous bioelectrical patterns of the nascent brain, showing how the voltage states of cells in the local and even distant regions contribute to setting of brain size (76). Many well-characterized channels and pumps can be used to modulate the bioelectric state of specific cells; for example, misexpression of potassium channels in early frog embryos revealed the surprising reprogramming of many somatic areas into eyes (33). These can be introduced into cells by use of transfection, microinjection of mRNA or DNA vectors, or viral infection. It is also possible to use the misexpression of wild-type and mutant channels to unravel the mechanism of action. For example, a pore mutant can be employed to determine whether a channel's role is merely as scaffolding/binding or whether it is truly related to its ion translocation properties.

Furthermore, by changing the $V_{mem}$ to the same overall level using channels for different ions ($K^+$, $Na^+$, $Cl^-$), one can determine whether a particular ion's concentration is what matters or whether it is actually the $V_{mem}$ level that carries instructive information in the given system. A variety of connexin (gap junction protein) mutants exist that can be used to establish bioelectrical connections among cells with desired resistivity, selectivity, and directionality.

Recent advances in these reagents have mainly involved the availability of channels with diverse gating modalities, including ligand-gated channels sensitive to novel inert ligands (77) and optogenetic (light-gated) channels and pumps (78), as well as channels that are controlled by magnetic (79, 80), thermal (81), or acoustic (82) stimuli. Together with microfluidics to control delivery of channel drugs, these novel reagents offer a rich tool kit (Figure 4d) for spatial and temporal control of $V_{mem}$, and optogenetic tools are moving beyond CNS function (83) and toward control of stem cell differentiation (84, 85), tumor reprogramming (86), and induction of regenerative growth (87). They are also becoming incorporated into closed-loop systems (88) that continuously provide feedback and will ultimately be used for guided self-assembly applications for feedback-based control of $V_{mem}$ during patterning. In addition to in vitro systems for applying these modalities to tissue, in vivo applications are being developed in which ion channel–modulating stimuli are provided via wearable bioreactors, such as those that can be placed on an amputated limb for regenerative induction (89).

It is also important to determine how $V_{mem}$ changes are coupled to downstream transcriptional cascades in a given tissue. This is done through a suppression screen for transduction machinery. Given an assay in which a $V_{mem}$ change produces a specific cell or tissue outcome, each of the known transduction pathways can be inhibited in turn to determine which one prevents the $V_{mem}$ change from being sensed by cells. Known transduction mechanisms that allow cells to convert bioelectrical signals into gene expression changes include voltage-sensitive phosphatases (41), electrophoretic- and

$V_{mem}$-based gating of signaling molecules through gap junctions (90), voltage-regulated transporters of signaling molecules such as serotonin (36) and butyrate (91), and clustering of molecules such as RAS in the membrane (42).

## 4. CONTROL OF GROWTH AND FORM

### 4.1. Proliferation, Differentiation, and Migration

Bioelectric properties control a number of key aspects of cell behavior (Supplemental Table 1); for example, transmembrane voltage levels control the proliferation of a wide range of cell types (24), and $V_{mem}$ regulates differentiation in a range of stem/progenitor and iPSCs. Galvanotaxis, the movement of cells in response to an external electric field, has been known for almost a century (92), and endogenous electric fields are known to provide spatial cues for orientation, outgrowth, and migration for a broad range of cell types (93-95). An applied electric field is one of the simplest methods used to orient cells and induce their migration in a desired direction (19). For example, directed fibroblast migration has been observed in fields as low as 0.1 V/cm in 3D collagen gels but not in two-dimensional (2D) cultures, revealing the importance of context for cells' interpretation of electrical signals in their environment.

### 4.2. Effects on Networks: Gap Junctionally Coupled Cells

Cells in groups respond to and interpret bioelectrical signals differently than single cells. For example, migration in electric fields is different in collectives than in single cells (96). Bioelectric cues also provide spatially patterned signals to cells; for example, the differential activation of voltage-responsive transduction mechanisms on opposite sides of a cell allows bioelectric signals to regulate cell polarity (25, 97). Positional information can likewise be dictated by the voltage properties of cells and their neighbors (98). Studies of embryonic left-right patterning of the *Xenopus* embryo have revealed how bioelectrical processes link individual cell dynamics to axial patterning of the entire body plan: Cytoskeletal chirality within the fertilized egg drives the asymmetric distribution of ion transporter proteins in the early blastomeres, and the resulting gradient drives unidirectional (preneural) serotonin flow through cell fields, eventually triggering differential gene expression on the left versus right side of the body.

### 4.3. Developmental Models Reveal Endogenous Roles

We now consider some examples from developmental biology, which reveal how bioelectric signaling is used as an endogenous patterning cue that regulates the size, position, and identity of organ structures. Recurrent network activity (ion dynamics) controls large-scale morphogenesis, connectivity, and cell type (identity) during neurogenesis and network sculpting (99). Data on the endogenous roles of bioelectricity outside the CNS come from several sources, including the identification of ion channels and gap junctions as genes responsible for a number of human birth defects (channelopathies; Supplemental Tables 3 and 4). In addition, unbiased network analyses of transcriptional profiling data sets in development (100) and cancer (101) have pointed to ion channels as key functional nodes. Thus, upstream of endogenous bioelectrical signaling lies a set of ion channel and

pump proteins that establish resting potential and alter it in response to physiological, transcriptional, and mechanical signals.

Some of the earliest focused research to implicate endogenous bioelectrical gradients was on the establishment of left–right asymmetry, where differences in electric potential across the midline are responsible for direction of downstream asymmetric gene cascades (53, 102). More recent studies (13) have shown that bioelectric gradients set the size of regenerating zebrafish tails (103) and that ion transporters are involved in zebrafish fin regeneration (104) and eye development (105). In zebrafish fins, gap junctions and ion channels work together to control fin size (103, 106) via a calcium downstream target. A similar process occurs in the nascent brain in the frog embryo (76, 107). In the developing cardiac epithelium of zebrafish, Wnt is a target of electrical gradients that shape cellular networks (108).

In *Xenopus* embryogenesis, regionalization of the anterior field by patterns of hyperpolarized and depolarized cells specifies a prepattern for gene expression and subsequent anatomy of the face (109). Specific endogenous patterns of differential $V_{mem}$ in the naïve tissue preceded and controlled the position of eyes (33) and many components of the face (110), while experimental alterations of this native pattern produced predictable craniofacial defects. Bioelectric regulation of the embryonic face is likely to be highly relevant to human biomedicine, as several channels have now been implicated in craniofacial dysmorphias (Supplemental Table 3).

Such roles appear to be well conserved. The functions in left-right asymmetry have been found in *Ciona*, sea urchins, *Caenorhabditis elegans*, mouse, frog, and zebrafish. Other roles have been found in species ranging from *Drosophila*, in which the Kir2.1 channel drives aspects of the TGF-β patterning pathway in wing patterning (111), to mammals. Even wider (across kingdoms) conservation is observed in the role of proton pumps at the outer edge of pollen tube outgrowth in plant systems (25, 112, 113), which is also observed in the wound epithelium as a driver of outgrowth in regenerating appendages of vertebrates (54). These recent molecular studies support a very wide conservation of the roles of bioelectrics across the tree of life, as had been suggested from classical studies with models ranging from hydroids to bacteria and fungi to mammalian cells (114, 115).

### 4.4. Regulating Regenerative Response in Adult Organisms

Some animal models repair or rebuild numerous complex structures; for example, salamanders regenerate their limbs, spinal cords, eyes, jaws, hearts, and portions of the brain. One important component is bioelectric signaling. Perhaps the best-understood aspect involves the transepithelial electric field, which is a key detector of damage and guides migratory cell behavior in cornea, skin, and other types of wound healing (17, 116, 117). The role of resting potentials in regeneration has begun to be elucidated, and the link between $V_{mem}$ and transepithelial electric fields is becoming mechanistically clearer (118). Specific ion channels have been implicated in regeneration in *Planaria, Hydra*, and frog. Resting potentials are now known to be functional determinants of mammalian liver regeneration (119) and spinal cord regeneration in axolotl (120). In axolotl, the depolarization may interact with the microRNA system, as miR-125b is crucial for regeneration (121) and is known to regulate sodium channels (122). Overall, the data in this

field come from two general types of studies: (*a*) those investigating the roles of bioelectric signals in instructing the type of morphology that is regenerated and (*b*) those investigating ionic controls of a binary (modular) go/no-go decision for regeneration.

A series of recent studies explored the mechanisms by which electrogenic proteins expressed immediately after injury drive appendage regeneration in vertebrates. The *Xenopus* larva regenerates its tail, which is a highly patterned appendage that contains a spinal cord, muscle, peripheral innervation, vasculature, and connective tissues. Tadpoles undergo age-dependent decline of regenerative ability, but the nonregenerative (refractory) state in the *Xenopus* tail can be overcome by transgenes driving strong proton efflux (54) or by a cocktail that modulates sodium content (74). In either case, the downstream sequelae of the regeneration-specific physiological state are induction of regenerative genes (such as *Notch* and *BMP4*), a strong increase in cell proliferation at the wound, and extensive innervation toward the outgrowth. It is remarkable that the whole complex cascade of organ regeneration can be triggered by an extremely simple event: Proton pumping turns “on.” In the case of the sodium-based cocktail, exposure of only an hour was sufficient to initiate the whole regenerative process. Moreover, what formed was a normal tail of the right size, shape, and orientation—not a small tail or tumor—suggesting that what is encoded by $V_{mem}$ here is a “master regulator” or top network node signal. More recently (Figure 5a,b), a preliminary study showed that the same cocktail initiates leg regeneration after amputation (123), suggesting that this system interacts with positional information cues to specify a “build the structure that belongs here” signal and not a set of cues that micromanage the morphogenetic process. It is possible to repair damaged tissue using bioelectricity: Mutations in the neurogenesis gene *Notch* lead to severe forebrain and midbrain defects in tadpoles, but introducing hyperpolarizing channels such as Kv1.5 leads to an almost complete rescue of morphology and molecular markers (76). Thus, the control of bioelectric signals can be used to induce regeneration, alter the pattern of regeneration, or overcome defects via repair pathways, which could be very useful in a broad range of biomedical and synthetic bioengineering applications.

### 4.5. Altering Morphology via Bioelectric Signals

Some of the oldest functional data in this field revealed the remarkable power of bioelectrics to control large-scale shape (Figure 5c-h). For example, pioneering research by Marsh & Beams (124) in *Planaria* and annelids revealed that head–tail polarity could be experimentally reversed or duplicated by electric fields applied to a cut fragment. More recently, it has been possible to alter body plans by editing the endogenous resting potential gradients within tissue; for example, an implanted ectopic eye in the flank of a tadpole usually makes one nerve fiber, which often synapses onto the spinal cord [and confers vision (125)]. However, when the surrounding host tissue (not the eye itself) is depolarized, the eye is induced to drastically hyperinnervate the host (38). This happens without effects on the host’s native innervation, suggesting that neurons that “know” they are in the wrong location are more sensitive to the bioelectric topography of their neighbors in making growth decisions. Control of the bioelectric aspects of the neural microenvironment (126) is a promising approach for inducing connectivity of implants, whether transplanted organs or

hybrid bioengineered constructs, as well as for guiding neural connections to specific design goals within biobots.

Beyond the patterning of a single cell type within its tissue context, exciting data for future bioengineering applications come from gain-of-function studies investigating what shape changes are possible via the modulation of $V_{mem}$ (127, 128). For example, through modulation of $V_{mem}$ states in frog embryos, any location in the tadpole could be turned into eye tissue—in some cases, a complete eye with all of the normal tissues arranged in the proper morphology (33). Appropriate misexpression of ion channels was able to induce eyes anywhere, including in the gut, tail, and lateral plate mesoderm. Given that it was previously thought that only neurectoderm was competent to make an eye, these data suggest that bioelectric pathways may necessitate a revision to current lineage restriction maps, and could be a powerful way to control differentiation of iPSCs, embryonic stem cells, and somatic cells that need to be reprogrammed. Importantly, the whole eye was induced without having to specify the details of its construction (a desirable property for applications in regenerative medicine). By experimentally regulating the $V_{mem}$ at a planarian amputation site and the gap junctional communication among cells, one can coax worm fragments to produce zero-, two-, or four-headed (rhombus-shaped) worms (129). Other examples in which the modification of endogenous bioelectric prepatterns produce nonplanar body plans in *Planaria* and *Xenopus* morphospaces are discussed in Reference 130.

## 5. WHAT WE KNOW

### 5.1. Molecular Mechanisms of Bioelectric Signaling

Bioelectric signaling is conserved across the tree of life, illustrating common principles and the versatility of ionic controls. The study of how evolution utilized bioelectrics to control pattern regulation in these organisms has revealed a number of main themes; for example, specific bioelectric states induce organ-level patterning modules, resulting in the production of heteromorphoses (ectopic limbs, eyes, etc.) without the need to micromanage their construction. In addition, bioelectric cascades readily implement feedback loops (due to channels' and gap junctions' electrical gating), and bioelectric cues often trump competing biochemical signals, as occurs in human MSC differentiation assays (29), or genetic states, as observed in the ability to override the effects of mutations in *Notch* and *KRAS* genes in brain patterning and carcinogenesis, respectively (39, 76).

Neurotransmitters are emerging as important patterning regulators in addition to their CNS roles, for example, as revealed in developmental (131), neoplastic (75), and regenerative (132) roles of serotonergic signaling, and the teratogenic effects of neurotransmitter drugs like diazepam in limb (133) and cleft palate. The placement of neurotransmitter small molecules downstream of bioelectrical signaling is very highly conserved, even across independent origins of multicellularity. Not only was it exploited by the evolution of the brain for both ontogenesis of the nervous system (134) and adult behavior, but it also occurs in plants, which (like the frog embryo) use a voltage gradient produced by the electrogenic proton pump to move a serotonin-like morphogen (135). The sculpting of gradients of serotonin and the chromatin modifier butyrate by voltage gradients (via paracellular electrophoresis) and voltage-sensitive transporters represents a tractable strategy

for shaping signaling in vitro, especially given the many known roles of such effector molecules on differentiation, migration, and proliferation.

It is impossible to map bioelectric states onto specific channels or pumps in a one-to-one manner. The overall cellular $V_{mem}$ is a function of the distribution of several different species of major ions and is not reducible to the function of one channel or ion type. Moreover, resting potential patterns trigger specific anatomical outcomes regardless of the identity of the ion (33, 39, 51, 75). This is because many downstream modules (e.g., eye formation, metastatic conversion) are activated by transduction mechanisms that are sensitive to voltage level only, not to specific ions. Cell groups with very different genetic profiles of channels and pumps could be in very similar bioelectric (and ultimately anatomical) states, meaning that morphogenetic outcomes and bioelectrical control pathways must be thought of not as inextricably downstream of specific channel or pump gene products, but rather as resulting from a physiological state that can be achieved via any one of many different ion translocator proteins (136).

Conversely, ion channel, pump, and gap junction function can change posttranslationally (via gating), without requiring any change in the mRNA or protein levels of the ion translocator in question. Thus, cells can express the same channels and pumps but be in completely different bioelectric states depending on which channels are open. Unfortunately, this means that bioelectric state cannot be entirely deduced from mRNA or profiling: Because of posttranslational gating of ion transfer proteins, no amount of microarray or proteomic data can definitively reveal the bioelectric states of cells. Thus, the implication of ion channel roles in patterning is greatly masked in single-knockout studies due to the functional redundancy and robustness of physiological circuits. Fortunately, this means that bioengineers can use any electrogenic protein or pharmacological tool to obtain the desired $V_{mem}$ change at an appropriate place and time in their circuit. Mastery, however, cannot be achieved without a systems-level understanding, as the mechanisms of voltage-based control of anatomy are complicated by the fact that bioelectrical signaling is often inherently long range: Cells make decisions based on large-scale patterns of $V_{mem}$ and relative comparisons of polarization state with their neighbors, not only their own potential.

### 5.2. Overriding the Genome

One unexpected recent finding is that, in *Planaria*, briefly inhibiting gap junction–mediated communication after middle fragment amputation results in worms that develop heads at both ends (129, 137). What is remarkable is that weeks later, when these two-headed animals have their heads and tails amputated again (in water alone, with no further perturbation), the same two-headed phenotype results. This phenomenon (Figure 6a) occurs even after multiple rounds of subsequent amputations. Thus, a transient perturbation of physiological cell-to-cell communication stably changes the pattern to which the animal regenerates upon damage, despite a normal genomic sequence. The phenotype is stable across fission (this animal's most frequent reproductive mode), and thus could have significant implications for evolution. Chromatin modification mechanisms alone are not a sufficient explanation, because the ectopic heads are discarded at each generation of cutting. The entire animal regrows from a fragment of the gut, which somehow "knows" that it is

supposed to form two heads, not one, upon further cutting. This means that the information about basic anatomical polarity and body organization must be stored in a distributed form throughout the animal. Quantitative, fieldlike models of this circuit remain to be developed to understand precisely how information guiding specific shape outcomes is encoded in (represented by) bioelectric states among cells. A recent study of a different *Planaria* species (138) showed that perturbation of the bioelectric networks can induce the regeneration of heads belonging to other species on a fragment of a planarian with an unmodified genome.

Another example in which bioelectric and genetic state information diverge is in cancer (139). A metastatic phenotype can be induced in genetically normal melanocytes by depolarizing somatic cells (37, 75). Conversely, the formation of tumors by mutations in human oncogenes such as *p53* and *KRAS* can be suppressed, despite the strong presence of oncogenic proteins in the cells, by artificially preventing the depolarization that occurs during oncogenic transformation (Figure 6b,c) (39). An implication of these data is that the neoplastic state cannot always be predicted from examination of the genome, transcriptome, or proteome; whereas some ion channels' expression might be a useful disease marker (140), there will also be many cases in which the transcriptional profile reveals nothing (because of signaling via posttranslational gating of channel state), and drugs targeting one specific channel type (141) may have no effect (due to compensation and redundancy of channel types). If indeed cancer is augmented or induced by a depolarized bioelectric state (21, 142, 143), we will have to think less about individual ion channels as oncogenes (144) and more about the way in which many channels contribute to a bioelectrical oncostate.

It is clear that genomic editing is not the only path toward significant morphogenetic control; indeed, recent genetic screens in *Planaria* (145) and zebrafish (146) have found many mutants with patterning phenotypes, but none with the drastic body plan modifications observed in the bioelectric screens (130). The genome specifies the hardware (the available ion channels present in cells), but the resulting pattern outcome is a direct result of the software (the signaling dynamics driven by the electric circuits that run on the hardware).

## 6. WHAT WE NEED TO KNOW: FUTURE PROGRESS

Learning to direct the formation of complex synthetic tissues requires us to confront the basic question of developmental biology: Where does pattern come from? Diverse resting potentials across a tissue can arise from preexisting differences in ion channel transcription, but that is not the only way (136). Such regionalized patterns of $V_{mem}$ can also form de novo, in transcriptionally and proteomically identical cells, because cells coupled by gap junctions (electrical synapses) form a (slow) electrically excitable medium; this is a particularly interesting aspect because such media are known to have powerful computational capabilities (147). Positive feedback loops implemented by elements such as voltage-gated ion channels, which both set and respond to $V_{mem}$ changes, can drive spontaneous symmetry breaking and amplification of physiological noise (148). Understanding the origin of spatial anisotropy in the bioelectric state as well as gene expression in cells originally derived from the same fertilized egg is important not only for basic biology but also for efforts to guide and harness self-assembly properties in vitro.

### 6.1. Upstream of Bioelectric Signals and Downstream of Voltage Change

In order to effectively use bioelectricity in the aforementioned applications, it will be necessary to understand what kind of events and signals upstream of bioelectric signals lead to changes in specific ion fluxes, including factors like insulin-like growth factor 1 (IGF-1), bone morphogenetic protein (BMP), and insulin (149, 150). In addition, we have only begun to scratch the surface of cellular endpoints downstream of bioelectric change. Calcium is known to be an important target of resting potential shifts, and perhaps significant feedback loops remain to be discovered, given the rich regulation that may result from the nuclear translocation of the C-terminal domain of $Ca_v1.3$ by intracellular $Ca^{2+}$. The RAS signaling pathway could turn out to be central for the intersection of bioelectrics with canonical pathways, as it is involved in the regulation of a myriad of important cell and patterning events (151) and its activity is voltage sensitive (42). Functional links to RAS have already been observed in the ability of hyperpolarizing channels to prevent KRAS mutation–induced tumors (39). Signaling via the Hedgehog (152), BMP (111), and vascular endothelial growth factor (153) proteins is modulated by ion channel activity in cells, illustrating the functional cross talk between protein factors and biophysical states.

A system that is both upstream and downstream of bioelectrics is the use of physical forces by cells. For example, stretch-activated ion channels and piezo elements like Prestin (154) ensure that bioelectrical signals and tensile forces interact continuously. Given the increasingly widely recognized roles of mechanical forces in large-scale and cell-level patterning events, it will become imperative to understand the links between $V_{mem}$ and the mechanical properties of tissues (155). Electrical stimulation can alter biomechanical outcomes (156), mechanical force (157), and stiffness (158).

### 6.2. Reading and Writing Bioelectric States into Tissue

In order to fully exploit bioelectric signaling for applications in bioengineering and regenerative medicine contexts, we must first learn how to read and write specific bioelectric patterns across tissue. Current strategies include substrates with embedded patterns of ion channel drugs or $V_{mem}$-modifying nanoparticles (159), wearable bioreactors to control bioelectric aspects of the wound environment (89, 160), and new biosensors for ions like sodium (161) and other gap junction–permeable molecules. Perhaps the most versatile and powerful technology is the use of optogenetics—light-gated ion channels (162) that have recently been used to regulate stem cells (84, 85) and induce tail regeneration after amputation (87). Although a lot of patterning occurs on 2D surfaces such as epithelia (163), this type of control will ultimately have to be extended to 3D constructs by use of holograms or 3D-patterned light (164, 165).

The modification of bioelectric patterns in vivo is a goal for regenerative medicine applications targeting traumatic injury, cancer, and aging. One current effort in the community, backed by significant funding from industry and the US Defense Advanced Research Projects Agency (166), focuses on the use of state-of-the-art electrode technology to regulate body physiology via effects on the nervous system. Although electrodes are excellent at inducing spiking (CNS stimulation) and providing electric fields for guidance of migratory cell types for applications such as neural repair (94), they do not readily facilitate

the control of the steady resting potential of cells in spatial patterns. There is, however, another technology that is poised to make a significant impact in applied bioelectrics: a remarkably diverse set of ion channel and pump drugs, many of which have been approved for human use for other indications. Repurposing existing drugs for applications in developmental bioelectricity is likely to be an important part of the future use of these approaches for biomedicine.

Bioelectric components are a fascinating new addition to the synthetic biologist's tool kit; for example, bioelectric gradients provide a free vector toward points of damage in an energized epithelium for migratory cell types, which may explain why evolution exploits these signals from bacteria and fungi to human cells (167) and how bistable memory elements (168, 169), such as the newly discovered sodium-selective two-pore channel 3 (TPC3) (170), can be used in computational circuits. Bull et al. (171) used optical control of an excitable chemical medium to implement a classifier system, illustrating the possible applications of biological tissues with electric dynamics for unconventional computation. Others have expressed channels in nonexcitable cell lines in vitro, implementing synthetic bioelectric circuits with a slow timescale (172) and linking them into a tissue that exhibits the same dynamics predicted from in vivo bioelectric data (173). Ideally, this research could be merged with recent advances in modeling of soft-body robots implementing morphological computation (72).

### 6.3. Conceptual Open Questions

Perhaps the biggest knowledge gap and opportunity for progress concerns the relationship between patterned voltage gradients and anatomical outcomes. What spatiotemporal aspect of the pattern encodes size, shape, orientation, or organ identity? What do individual cells actually measure: absolute $V_{mem}$, differences between neighboring cells' $V_{mem}$s, or long-range properties? What is the fundamental unit of bioelectric specification? Is it a single cell, subcellular domains, or a multicellular network? Do cell networks map mainly spatial distributions of $V_{mem}$ to specific outcomes, does the encoding in the temporal pattern of $V_{mem}$ change, or do both mechanisms play a role? Why do bioelectric signals trigger correct repatterning of tissue with minimal information input in some contexts, allow respecification of local organ identity in other contexts, and function almost as a "paint by numbers" prepattern in yet others? One possibility, explored further below, is that somatic bioelectric networks are an information-processing system that shifts the configuration of bodies through morphospace by control of cell behaviors, much as the brain shifts bodies through 3D space by control of muscle cell activity.

## 7. CRACKING THE BIOELECTRIC CODE WITH HELP FROM COMPUTATIONAL NEUROSCIENCE

### 7.1. How Can Electrical States in the Brain Be Interpreted?

Interpreting the semantic content of an electrical state requires using novel methodologies. Some can be borrowed from neuroscience, which studies how cognitive processes such as perception, memory, attention, decision making, and learning arise from neuronal activity

and has developed powerful methodologies for the analysis and modeling of single neurons and the activity of ensembles of neurons.

Two popular methodologies to extract single-trial information from the activity of neuronal populations are information theory and decoding (174). Information theory measures such as mutual information (e.g., between input stimuli and neural activation) allow one to analyze the information content of spike trains of a neuron (e.g., whether a neuron carries information concerning the color or size of a visual stimulus) (175) or a population of neurons. For example, an analysis based on conditional mutual information revealed that specific prefrontal neurons in monkey prefrontal cortex carry information about the prospective action goal that is unconfounded by sensory characteristics (176). The decoding of neural activity is another powerful approach for understanding information processing in the brain. Decoding is typically cast as a mapping from neural data to a sensory or motor variable, such as the identity of a visually presented object or the reaching direction of a motor action. For example, it has long been known that a neuron's response in sensory areas (e.g., primary visual cortex, or V1) is best described as a function of a small set of features (e.g., motion orientation) that can be decoded, for example, with linear filters (177).

Decoding techniques have been used to study how neural networks encode spatial/topological maps—a topic that may have direct implications for understanding how somatic bioelectric circuits encode and process geometric maps corresponding to body organ layouts. One example is the study of transiently active cell assemblies (or sequences) in the hippocampus. The analysis of hippocampal activity often includes an encoding phase (i.e., obtaining tuning curves/place fields of single neurons from spike trains and the animal's position) and successively a decoding phase (i.e., reconstructing the animal's position from spike trains and the previously encoded tuning curves) (178). Interestingly, decoding permits one to assess the dynamics of covert neuronal variables in the rodent hippocampus when the rat is not actually moving (e.g., when it is sleeping). This technique has revealed that during sleep or wakeful rest, the rodent hippocampus covertly "replays" spatial trajectories that were recently experienced (also, to some extent, generalizing to never-experienced trajectories and planning novel trajectories)—and this sort of replay was proposed to be a key building block of navigational planning and memory consolidation (Figure 7a) (179-181). Intriguingly, perturbing internally generated sequences of neuronal activity (e.g., using optogenetics) permits the creation of false memories (182). Many techniques for extracting and analyzing semantic content in the brain of humans and other animals are currently in use (or under development) and could be employed in biology to extract the semantics of nonneural cells and cell networks. For example, natural images and videos have been efficiently decoded from patterns of functional magnetic resonance imaging activity in visual cortex (Figure 7b) (183). Recent advances in these techniques enable one to decode more complex semantic content such as linguistic stimuli from narratives (184).

Finally, there has been a recent trend toward the application of dimensionality-reduction techniques to multineuronal recordings, under the assumption that neuronal populations (that are intrinsically high-dimensional) may be parsimoniously represented by a smaller number of latent features, revealed by, for example, principal components analysis or its variants (185). These techniques then allow one to define a "neural state space" (i.e., a

low-dimensional representation of neuronal population activity) and to study how it evolves over time during a specific task.

Neuroscience also seeks to understand what brain processes (or computations) support cognitive functions such as memory, decision making, and goal-directed action. To answer this question, computational models spanning various levels of detail and complexity (e.g., from biophysically realistic neural networks to more abstract models; from single-neuron models to large neural networks) have often been useful, as they can provide detailed process models of neural computations and produce quantitative and empirically testable predictions (186). Computational models are especially useful for addressing integrative aspects of cognitive function, such as how decisions or goal-directed action or even consciousness (187-189) arises from the coordinated activity of several neurons, possibly across brain areas. Consider, for example, the case of a perceptual decision: a random-dot-motion direction discrimination, in which participants have to report whether dots in their visual scene are moving toward the left or right by doing a saccade either to the left or to the right (and the task can be made simpler or more difficult depending on the degree of coherence between the dot movements to the left or right). This perceptual decision is often conceptualized in terms of an “accumulation of evidence” in favor of the two alternatives, until one of the two reaches a critical threshold and the decision is settled. This framework is supported by neurophysiological evidence: The neuronal activity of neurons in monkey lateral intraparietal cortex (LIP; an area involved in the control of eye movements) may encode the “decision variable” for this task, which represents the accrual of evidence (as well as priors, etc.) used to produce the choice, and it may update the decision variable by accumulating evidence (for the left or right choice) from the neuronal activity of neurons in middle temporal area (MD; an area whose neurons are tuned for the direction of visual motion) and/or medial superior temporal area (MST; an area whose neurons are tuned for, e.g., rotation and other motion patterns in the optic flow) (190). This model exemplifies the fact that detailed mappings can be established between the activity of wide neural networks and mathematically specified functions (accumulation-to-bound) that realize complex cognitive functions (see Reference 191 for other examples).

### 7.2. The Somatic Computation Hypothesis

Our perspective is that evolution exploits the unique advantages of bioelectric signaling for computation, both in the brain for the control of movement and thought and outside the brain (somatic bioelectrics) for patterning and remodeling (Figure 8a-l). Neural-like computations in nonexcitable cells resemble nonspiking neurons. The shared origins are revealed in the reuse of a number of ion channels, such as Kir2.1, which patterns the face (109) and assists in executive function and abstract reasoning (192), and neurotransmitter transporters like SERT, which is involved in numerous aspects of cognition (the target of drugs like fluoxetine) as well as mediating left-right patterning (193) and metastatic conversion (75). Somatic cells can store information in their bioelectrical states (194), and because nonneural cells are able to participate in electrical communication with their neighbors via gap junctions (195), there may be no fundamental difference between electrically communicating somatic tissues and neural networks. The development of new technologies for tracking and modulating bioelectrical communication among actively patterning tissues

may reveal memory, decision making, and other functions formerly reserved for neural systems implemented in somatic tissues (196, 197), a finding that would have important implications not only for strategies to reprogram morphogenesis but also for the design of novel architectures for computer technology (198).

The existence of memory in nonneural tissues has been proposed for bone networks (196) and cardiac tissue, and is supported by numerous studies of cognitive processes in aneural organisms, including plants (reviewed in Reference 199), but the full implications of this concept have not yet been explored in detail or exploited for robotics applications. We suggest that the ability of biological systems to regulate cellular activity toward large-scale anatomical goal states can be modeled as a kind of memory and recall—casting morphogenetic remodeling and regeneration as a cybernetic system driven top down by information about its current shape and its target morphology. If true, this implies that memory and decision-making circuits could be implemented from many different somatic cell types, availing the synthetic biologist of the opportunity to integrate form and function within the same construct. We propose that collections of cells can remember target morphology, infer patterns from input stimuli, and make decisions based on previous and current input states—possibly using computations that are analogous to those used by brains (see, e.g., the above example of decision by “accumulation of evidence”). More specifically, we propose that such networks are endogenously implemented by bioelectrical signaling among all cells—pathways that can now readily be manipulated in synthetic settings. Testing and exploiting this idea will require (*a*) in silico mapping of quantitative neural models for logic and memory onto slow bioelectrical networks of nonexcitable cells to understand their control dynamics with respect to information and computation and (*b*) laboratory testing of pathways implicated in cognitive processes in patterning systems. The potential payoff is the construction of soft-body robots in which the components are not only structural but also computational, able to carry out information processing useful for pattern control and functional behavior.

This hypothesis suggests that conceptual frameworks for understanding cognition should be applicable to pattern regulation and that pharmacological and genetic perturbations designed to affect memory, learning, and integrated decision making should have specific effects on morphology at an organ-system (not just cell behavior) level. In addition, it suggests that complex pattern outcomes may be optimally attainable by a completely different strategy than is currently pursued by bioengineers. Specific patterns may best be attained by a top-down strategy of training or behavior shaping of tissue—analogous, in a sense, to the way neural networks can be trained and false memories can be created in hippocampal networks (see Section 7.1, above)—instead of building it bottom up with stem cell derivatives. A number of recent studies have demonstrated memory and learning in cultured neurons by providing reward and punishment to the cells for specific “behaviors” (physiological output states) (200-203). If our hypothesis is correct, there should be no major difference between neural cells and nonneural cells in ability to learn, and it should be possible to train cells in vitro for specific outcomes. If we can identify appropriately motivating learning signals (e.g., positive and negative reinforcement) and an “objective function” or high-level goal for learning, it may be possible to train tumors not to proliferate, organoids to acquire specific shapes, various cell populations to differentiate and undergo morphogenesis, and embryoids

to implement desired shape changes. Figure 8m,n shows a schematic of the device currently being built by the Levin group to test these ideas.

## 8. CONCLUSIONS

The emerging field of bioelectricity in pattern control is poised between the bottom-up approaches of molecular biology and top-down approaches of computational neuroscience, between emergence and control theory. Engineers are ideal contributors to this field because engineers, more than modern biologists, are comfortable with a rigorous utilization of the concept of goal states and systems that seek to implement them. Despite the exciting progress and data in this field, it is in its infancy. Many students of molecular biology and bioengineering are unaware of the data in this branch of developmental biophysics; integration of bioelectrics into large multiscale modeling projects, such as Physiome (204), remains to be done. Nevertheless, a number of the components of this field have already found applications in biomedicine. These applications include the use of physiological dyes as diagnostics (205), targeting of transepithelial electric fields for wound healing in the eye (206), electric bandages (207) and other applications of fields to wound healing, dissection of the roles of ion channels in syndromes such as fetal alcohol exposure (208), creation of electrically activated cells for healing response (209), and the screening of ion channel and pump drugs as cancer therapies (141, 210).

Ion channels and electrical synapses allow the genome to couple to the laws of computation, much as the discovery of cell adhesion proteins allowed the genome to exploit the physics of adhesion. Bioelectric signals are truly epigenetic, as it would be impossible to claim that genetics alone could explain cognition, memory, or neural function. Likewise, patterning (and, more importantly, the dynamic ability to repattern dynamically) is not a direct effect of DNA but of the activity of physical dynamics of bioelectric (and other) networks whose plasticity underlies much of what bioengineers seek to emulate.

One path forward for the development of deep, quantitative theory in this field is in the hypothesis that patterning information may be stored within nonneural bioelectric cell networks using the same (or closely related) molecular mechanisms and information-processing algorithms that underlie behavioral memory in the nervous system. This hypothesis is currently being tested in our laboratory. It is thus possible that the techniques such as those now used to extract “mental images” from electrical measurements of living human brains (183) may shed crucial light on the encoding of anatomical pattern in the electrical circuits of somatic cells; conversely, the cracking of the bioelectric code in development and regeneration may have important benefits for the understanding of the semantics of electric states in the brain.

More broadly, to the extent that developmental bioelectricity data are erasing artificial distinctions between neural and nonneural cell types, the insights gained from computational neuroscience and cognitive science will become relevant to cell and developmental biology. It is possible that the most effective ways to understand high-order (anatomical-level) outcomes will involve not only bottom-up models of molecular pathways but also top-down models in which information and control theory concepts play central roles. In this way,

molecular bioelectricity may be revealing a mechanistic path toward understanding the intelligence exhibited by cell behavior and harnessing it for transformative advances in biomedicine and the information sciences (211, 212). As in computational psychiatry (213), we may be heading toward a future in which birth defects are understood and managed as circuit disorders, and morphogenesis in vitro is implemented by writing pattern memories into tissues as new memories are beginning to be optogenetically incepted into living brains (214). The parallel development of theory and techniques in this field will increasingly place the bioengineer at an exciting crossroads of several disciplines, including materials and information sciences. Working toward the arbitrary control of growth and form may avail bioengineers of the unique privilege of linking biology to some of the deepest insights from the fields of cognition and computer science—an extremely exciting prospect for both basic science and biomedicine.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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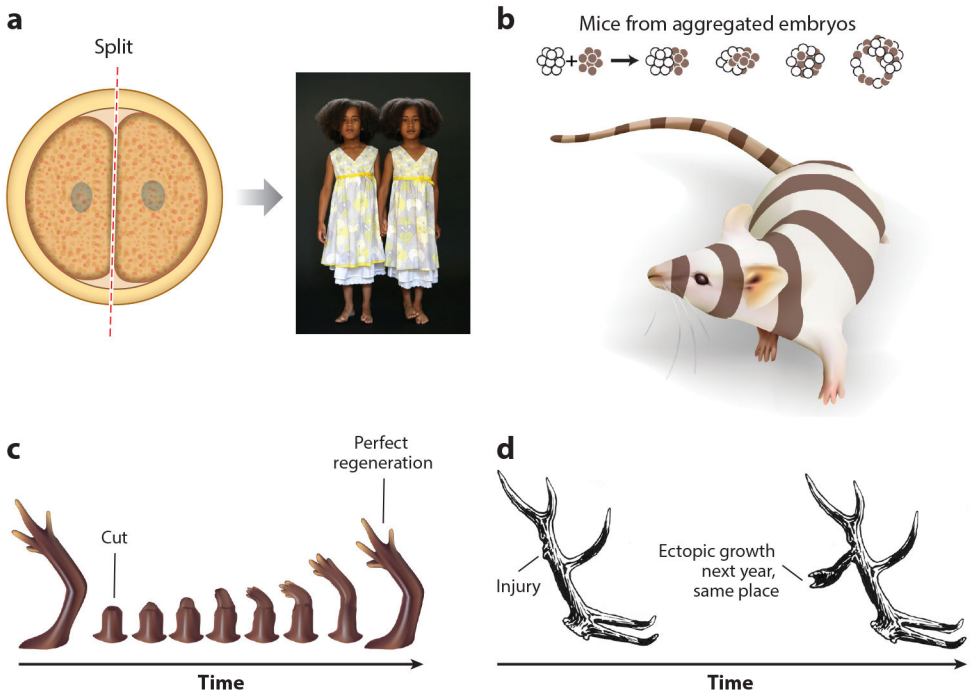


**Figure 1.**

Shape homeostasis in biological systems. (*a*) Early embryos give rise to two complete bodies when split in half. (*b*) Conversely, when mixed, embryos remodel to form a normal single animal. (*c*) Salamanders regenerate whole limbs when amputated. (*d*) Deer regenerate large amounts of bone and nerve during antler regrowth; the phenomenon of trophic memory in some species results in ectopic growths at sites of damage done in previous cycles to a structure that falls off completely and is rebuilt from scratch each year. Photograph of human twins in panel *a* reproduced with permission from Wikimedia Commons (Oudeschool; https://commons.wikimedia.org/wiki/File:Power20302.jpg; licensed under the Creative Commons Attribution 3.0 Unported license). Panels *b* and *c* drawn by Jeremy Guay, Peregrine Creative. Panel *d* reproduced with permission from Reference 3.

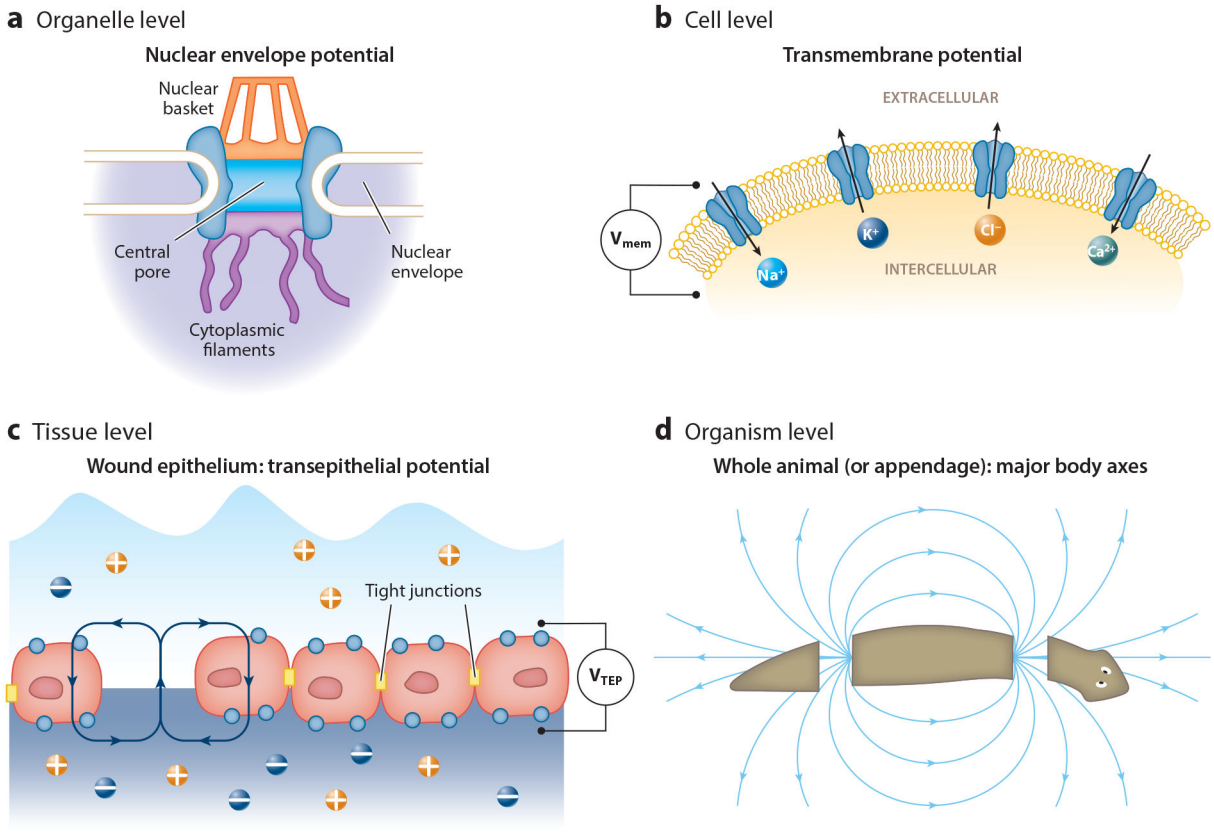


**Figure 2.**

Endogenous bioelectric properties. (*a*) Subcellular organelles (such as the nuclear envelope) maintain voltage potentials across their membranes. (*b*) Cell plasma membranes likewise maintain a $V_{mem}$ as a function of the ion channels and pumps in their membranes. (*c*) Cells organized into tissues drive a transepithelial potential, which gives rise to electric fields that provide a vector to points of damage. (*d*) Combinations of these local and long-range properties result in gradients across entire anatomical body axes. Abbreviation: $V_{TEP}$, transepithelial electrical potential. Modified with permission from Maria Lobikin.

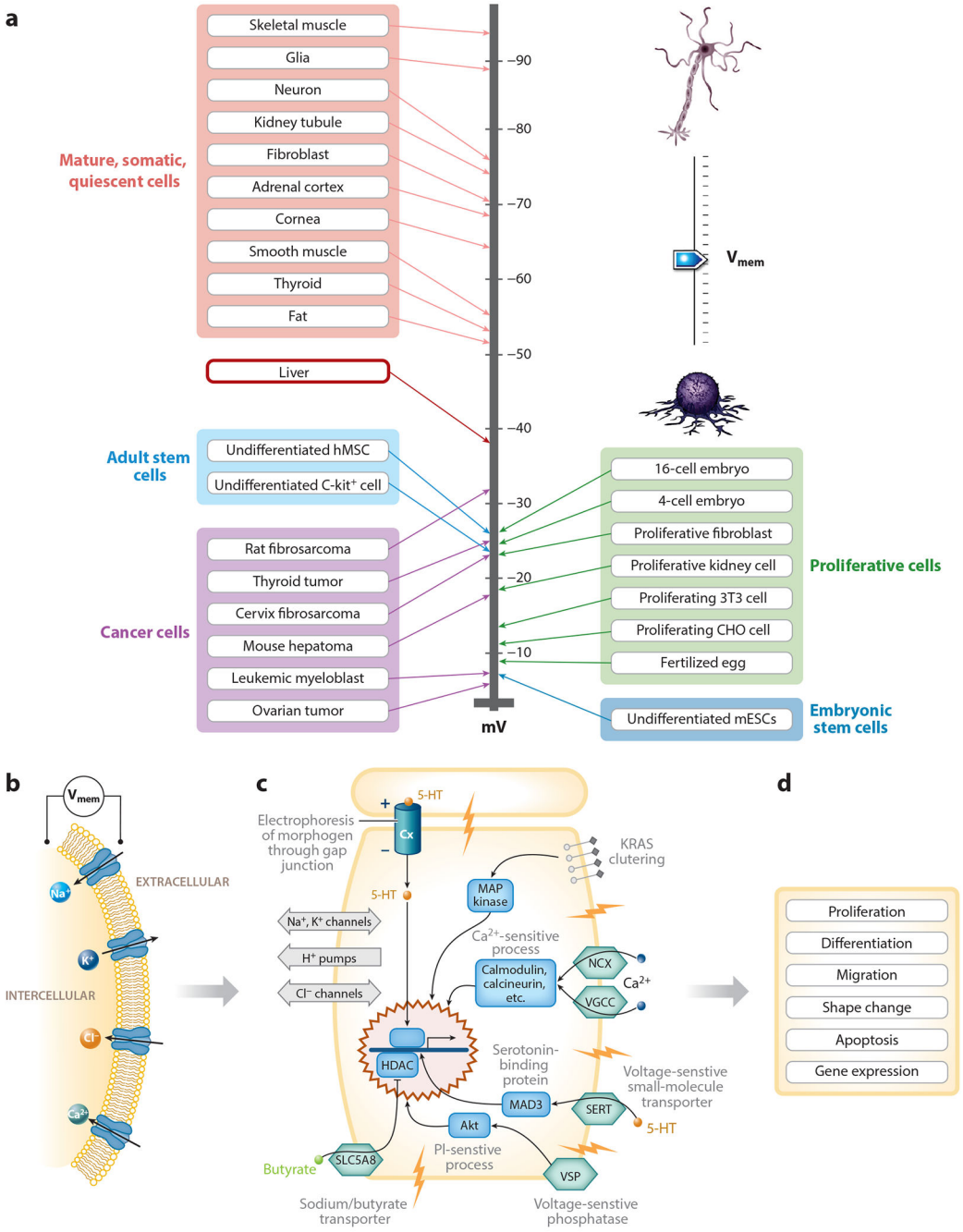


**Figure 3.**

Cell-level events in bioelectrical signaling. (*a*) The resting potential of terminally differentiated, quiescent cells tends to be hyperpolarized, whereas that of embryonic, stem, or tumor cells tends to be depolarized. This is a functional relationship, as artificial regulation of the $V_{mem}$ instructively sets cell proliferative capacity and plasticity. The resting potential of single cells is set by the function of ion channels and pumps in their membranes (*b*); changes in this voltage are transduced (*c*) by a set of membrane mechanisms (voltage-gated calcium channels, voltage-powered transporters of serotonin and butyrate, voltage-regulated phosphatases, and others) into second-messenger cascades that impinge on

transcription, thus regulating single-cell behaviors (*d*) such as proliferation, migration, cell shape, and programmed cell death. Abbreviations: 5-HT, 5-hydroxytryptamine, also known as serotonin; HDAC, histone deacetylase; hMSC, human mesenchymal stem cell; MAD3, Max-interacting transcriptional repressor; MAP kinase, mitogen-activated protein kinase; mESC, mouse embryonic stem cell. Panel *a* modified with permission from Reference 21 and drawn by Jeremy Guay, Peregrine Creative. Panel *b* modified with permission from Maria Lobikin. Panel *c* modified with permission from Reference 127.

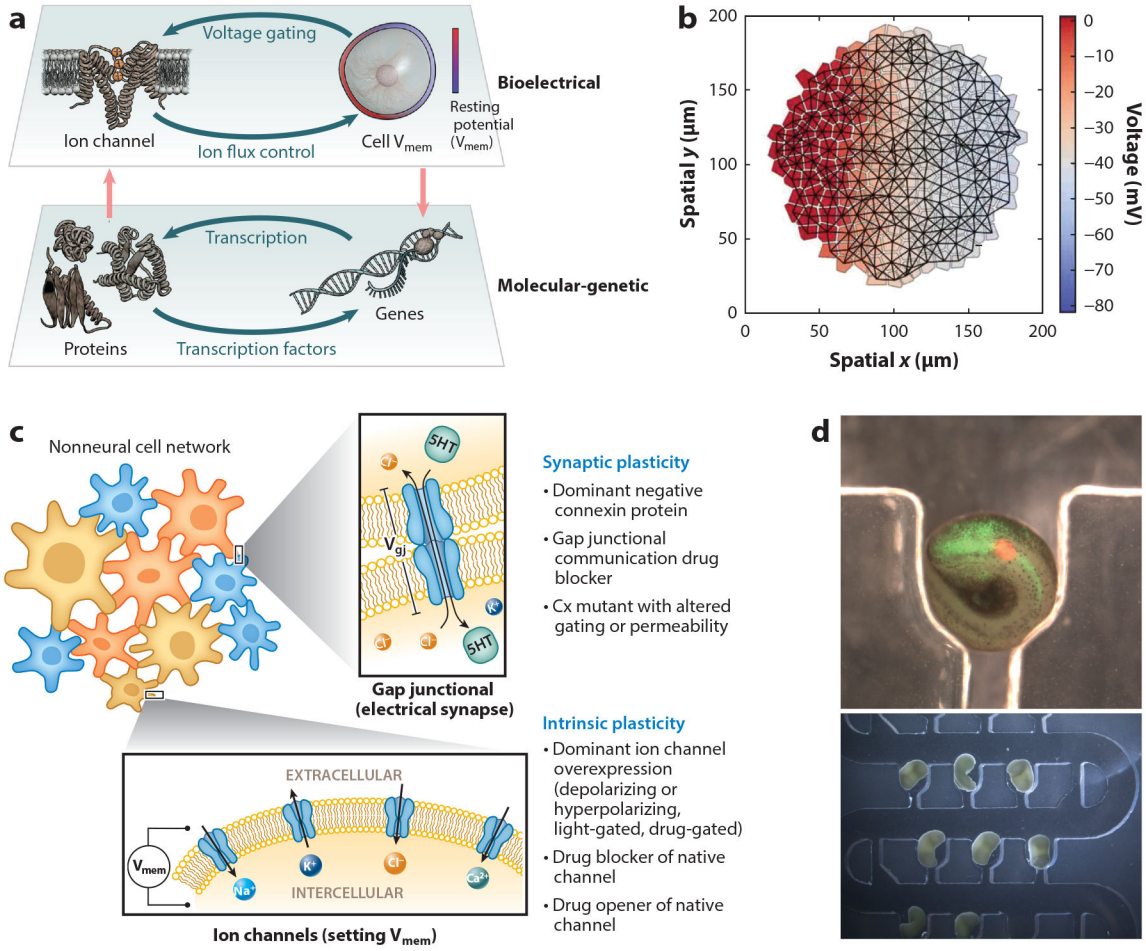


**Figure 4.**

Manipulation of the bioelectric control layer. (*a*) The feedback between ion channels' determination of $V_{mem}$ and their own sensitivity to $V_{mem}$ results in a layer of feedback that functions in parallel to canonical signaling via transcriptional circuits. These two layers are coupled but have their own intrinsic dynamics and play distinct roles in regulating patterning processes. (*b*) Simulation environments for physiological signaling are used to guide interventions in guided self-assembly of bioelectrical patterns. (*c*) Investigation of the functions of the bioelectric layer is performed via altering the network connectivity (via genetic or pharmacological change of gap junctions—synaptic plasticity) or by altering individual cellular activation levels (via genetic or pharmacological control of ion channels and pumps—intrinsic plasticity). (*d*) Optogenetic and microfluidic technologies are beginning to be developed to hold embryos (such as the frog embryo shown here) and apply patterned light that differentially triggers hyperpolarizing and depolarizing channels. Panels *a*, *b*, and *c* modified with permission from Alexis Pietak. Panel *d* reproduced with permission from Dany Spencer Adams, Jin Akagi, and Sebastien Uzel (83).

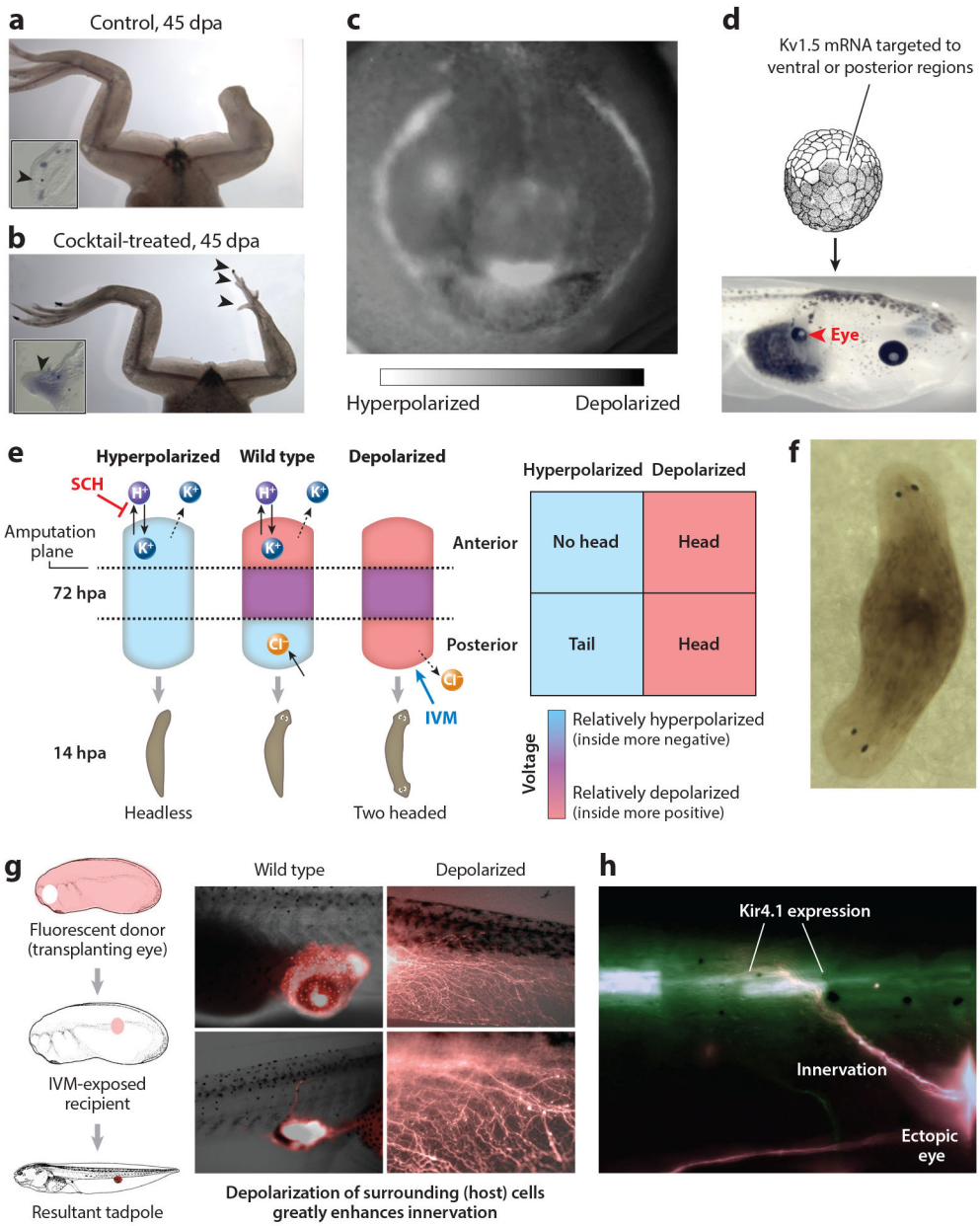


**Figure 5.**

Large-scale bioelectric patterns are instructive for shape. (*a*) Limb regeneration does not normally occur in froglets. (*Inset*) There is no blue stain for MSX1, a blastema marker, indicating that it is absent from the tissue sample. (*b*) A mixture of ionophores designed to specifically alter the bioelectric state of the blastema, after only 24 h of exposure, triggers the presence of an MSX1-positive blastema. (*Inset*) The growth of an entire limb (*arrowhead*). (*c*) Spatial distributions of resting potential revealed by voltage-sensitive fluorescent dyes, such as this image of a craniofacial voltage prepattern in *Xenopus*, determine downstream gene expression and anatomical outcomes. (*d*) Manipulation of these endogenous patterns by misexpression of ion channels can result in organ-level reprogramming, such as turning a portion of the gut into a complete eye. (*e*) Understanding

of the bioelectric circuit that controls, for example, anterior–posterior specification in a fragment of regenerating *Planaria* can be used to design drug cocktails that (*f*) alter the anatomical structure thus produced, such as inducing the posterior-facing blastema to build a secondary head in *Planaria*. (*g*) Depolarization of host tissues in the context of an eye transplant induces drastic overproliferation of nerve emerging from the implanted organ in comparison to a control host. (*h*) This technique can be used to pattern the ectopic nerve, inducing it to connect to specific regions by patterning the activation of ion channels in the surrounding tissue. Abbreviations: dpa, days postamputation; hpa, hours postamputation; IVM, ivermectin; mRNA, messenger RNA; SCH, SCH-28080. Panels *a* and *b* reproduced with permission from Reference 123. Panel *c* modified with permission from Reference 110. Panel *d* modified with permission from Reference 33. Panel *e* modified with permission from Reference 34. Panel *f* modified with permission from Reference 20. Panel *g* reproduced with permission from References 38 and 125 and from Douglas J. Blackiston. Panel *h* reproduced with permission from Douglas J. Blackiston.

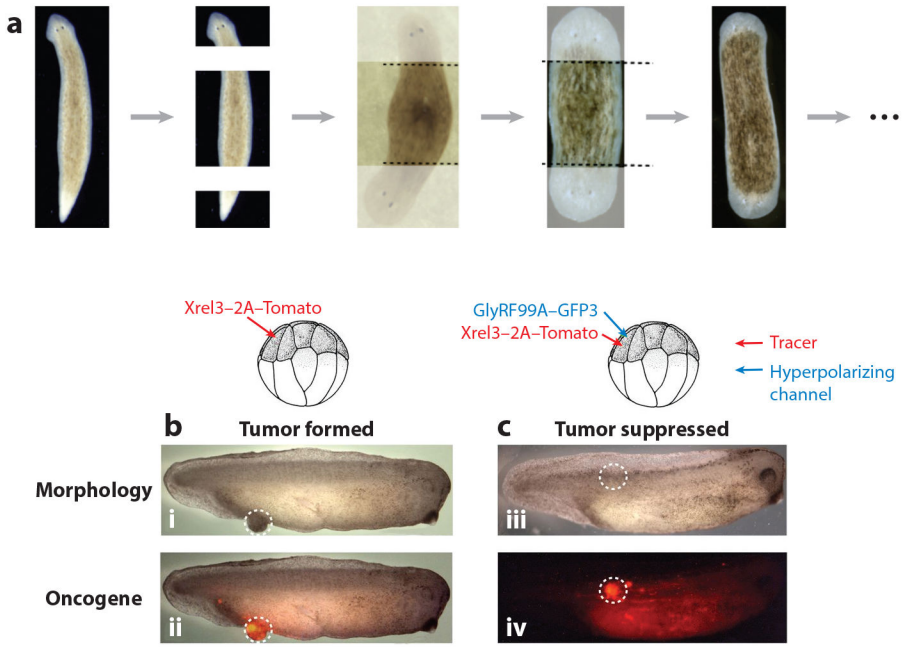


**Figure 6.**

Bioelectric states that can override genome defaults. (*a*) Brief pharmacological manipulation of connectivity within the bioelectric network that guides planarian regeneration results in two-headed forms that regenerate this new body plan in perpetuity: Long after the original reagent is gone, regeneration of a middle fragment in plain water reveals an altered pattern memory despite an unchanged genomic sequence and removal of ectopic tissue. (*b*) Oncogenes injected into frog embryos (with red fluorescent tracer) induce tumorous growths. (*c*) If their resting potential is forced into the normal state by coinjection with a GlyR chloride channel mutant, tumors do not form (*iii*) even though the oncogene is still present (*iv*). Panel *a* reproduced with permission from Reference 20. Panels *b* and *c* modified with permission from Reference 39.

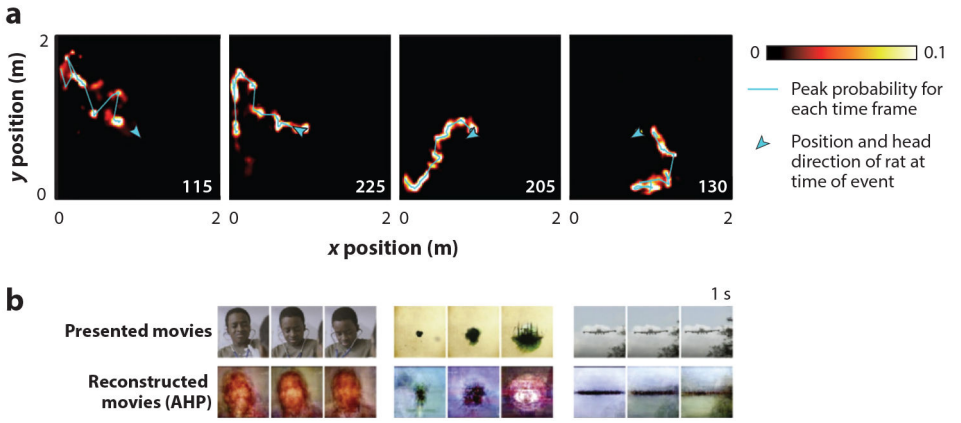


**Figure 7.**

Decoding content from neural data: two examples. (*a*) Place cell decoding: trajectory events (e.g., sequences of place cells forming a trajectory) in an open arena reconstructed from single-cell recordings in the rat hippocampus while the rat was at rest. These trajectory events may support navigational planning, in this example, potential plans toward the next goal site located at the center of the arena. Event duration (in milliseconds) is shown in the right corner. (*b*) Reconstructions of movies from functional magnetic resonance imaging blood oxygenation level–dependent signals in the occipitotemporal visual cortex of human subjects who watched movies. (*Top*) Frames of three movies presented to participants. (*Bottom*) The averaged high posterior (AHP) reconstruction of the same frames. Panel *a* modified with permission from Reference 179. Panel *b* modified with permission from Reference 183.

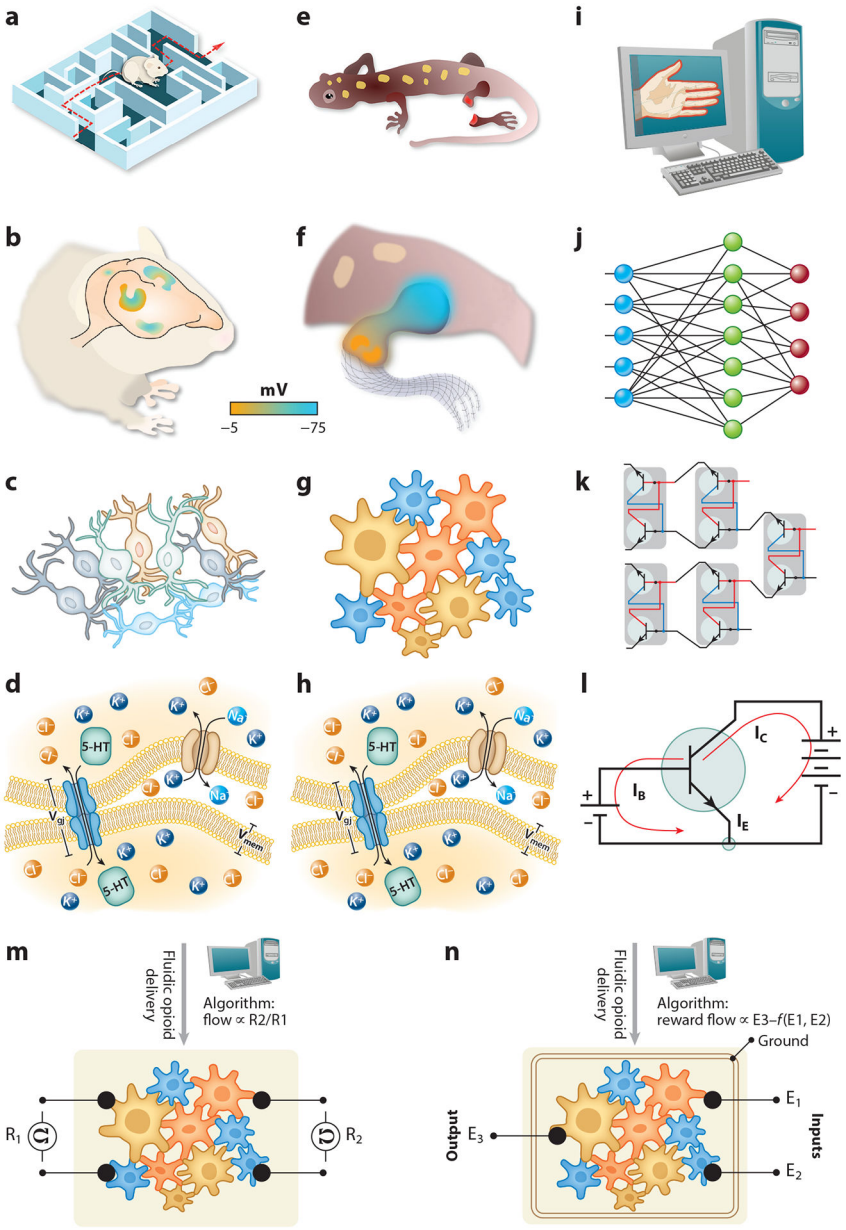


**Figure 8.**

Concepts from computational neuroscience applied to pattern regulation. Significant functional isomorphism exists between developmental bioelectricity and information processing in the brain and in artificial systems. Geometric memory (e.g., of a path through a maze) in the brain (*a*) is implemented by memory encoded as stable bioelectrical states (*b*), which are maintained by connectivity and electrical communication among brain cells (*c*). The electric states, in turn, are generated by ion channel and gap junctional proteins (*d*). Pattern memory (the shape that is regenerated after a salamander's limb is amputated and that serves as a stop condition for further growth) (*e*) is likewise implemented by information encoded in gradients of electrical potential in the tissue (*f*), which are maintained by $V_{mem}$ potentials of specific cells throughout the body (*g*) that, in turn, are

generated by ion channel and gap junctional proteins (*h*). In artificial cybernetic systems, specific patterns (*i*) can be remembered and processed by artificial neural net representations (*j*), which are built up from electrical circuits consisting of transistors (*k*); interestingly, gap junctions act much like transistors (*l*) because they regulate their permeability (current) on the basis of the voltage applied across them. A prediction and an implication of this view of bioelectric networks are that nonneural cells and tissues ought to be trainable for specific patterning topologies and computations. (*m*) In this example, the goal is to control the state of electric synapses (gap junctions) among specific regions, and force it to make one side of the network (e.g., the left half) well coupled while another side (the right half) stays uncoupled. Cells would be grown on a penetrating electrode array and assayed for gap junctional connectivity as a resistance measurement. The training paradigm would be a closed-loop system as follows: Every few seconds (over a period of days in culture), it would measure the coupling (resistance) of cells. To force the network to arrange its topology such that R2 is high and R1 is low (at first, they would be roughly equal), the system would mesofluidically deliver an amount of something the cells like (nutrients, opioids, endorphins, other addictive substances, etc.) proportional to the ratio R2/R1. We conjecture that, with time, the network would establish the needed connectivity pattern to maximize R2/R1 to optimize its receipt of the drug. (*n*) In this example, the goal is to train the tissue to perform a specific computation [an arbitrary function *f*(x) that maps inputs to outputs]. Over a period of days, the system applies stimuli to E1 and E2 and measures activity on E3. The reward in each cycle of the loop is proportional to the inverse of the error between E3 and the desired *f*(E1,E2). The tissue may learn to act as needed, revealing the ability to program physiological responses from the top down. Abbreviation: 5-HT, 5-hydroxytryptamine, also known as serotonin. Panels *a*, *b*, *e*, and *f* drawn by Alexis Pietak. Panels *c*, *d*, and *g–n* modified with permission from Alexis Pietak.