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The body electric 2.0: recent advances in developmental bioelectricity for regenerative and synthetic bioengineering

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Breakthroughs in biomedicine and synthetic bioengineering require predictive, rational control over anatomical structure and function. Recent successes in manipulating cellular and molecular hardware have not been matched by progress in understanding the patterning software implemented during embryogenesis and regeneration. A fundamental capability gap is driving desired changes in growth and form to address birth defects and traumatic injury. Here we review new tools, results, and conceptual advances in an exciting emerging field: endogenous non-neural bioelectric signaling, which enables cellular collectives to make global decisions and implement large-scale pattern homeostasis. Spatially distributed electric circuits regulate gene expression, organ morphogenesis, and body-wide axial patterning. Developmental bioelectricity facilitates the interface to organ-level modular control points that direct patterning *in vivo*. Cracking the bioelectric code will enable transformative progress in bioengineering and regenerative medicine.

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Introduction

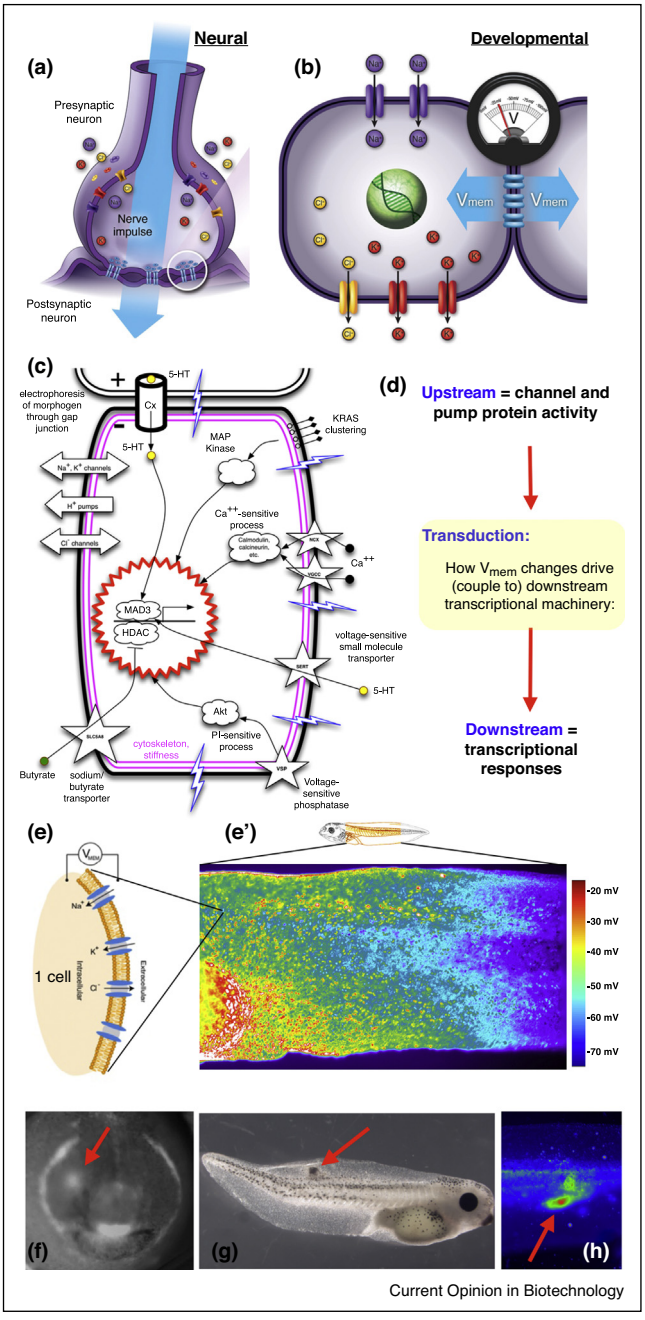
Bioengineers have become proficient at manipulating biological hardware — the molecules of life — with ever-higher resolution into biochemical pathways and subcellular events. Our ability to control large-scale outcomes — to repair or create functional anatomies to spec — lags far behind. This is a crucial capability gap: the rational control of form and function [1] *in vivo* is key for treating birth defects and cancer biology, as well as for applications in regenerative medicine and synthetic bioengineering. We look to computer science and how it bridged the gap between low-level physical processes and large-scale outcomes, driving a revolution in information technology that impacts every aspect of modern life. Computer scientists understand and exploit the causal efficacy of control policies above the level of their electronic medium. Working at the level of information (transcending the need to re-wire physical circuits for different outcomes) and exploiting modularity enabled limitless possibilities of rational design of technology. Here, we argue that this same journey awaits biology: complementing bottom-up molecular approaches with top-down strategies that exploit the computations, not only the mechanisms, of living tissue. One pathway toward mastery of the algorithms of life is exploiting the same ancient mechanisms that evolution optimized as neural systems (brains): bioelectrical networks across cell fields that integrate information and mediate morphological decision-making [2].

How do cellular collectives make decisions about organ-scale patterns during regulative embryogenesis and regeneration? Here we describe the most recent advances in an important, emerging field: developmental bioelectricity. Endogenous bioelectric signaling among many cell types (not just neurons) is an ancient, well-conserved system for morphological computation — enabling cells to coordinate their activity toward the morphogenetic needs of the organism. Bioelectric circuits regulate gene expression and cell behavior, maintaining spatial patterns of resting potential as prepatterns and morphological setpoints used in pattern homeostasis (Figure 1). While biochemistry is routinely exploited in the synthetic construction of computational circuits, we focus on progress in bioelectrics, a voltage-mediated communication and control system poised to offer uniquely tractable control over biological form and function [3].

Origins of bioelectrics: how did tissues think before brains evolved?

Endogenous bioelectric signaling dynamics are crucial regulators of wound healing, neuronal circuit shaping, eye development, face patterning, brain and tail size, and left-right and anterior–posterior axial polarity (reviewed in [4]). Molecular-genetic and pharmacological techniques that manipulate the spatial distributions of resting membrane potentials during development, regeneration, and cancer suppression have been used *in vivo* to

Figure 1



Bioelectric signaling outside the nervous system. **(a)** The information-processing functions of the brain derive from electric dynamics implemented by ion channels, which set cell resting potentials, and gap junction synapses, which allow voltage states to selectively propagate to neighboring cells. **(b)** The same components are expressed in most somatic cells, outside the nervous system, allowing them to set up distinct bioelectric states across tissues. **(c)** On a single-cell level, voltage states are transduced by a variety of mechanisms, including KRAS clustering, cytoskeletal changes, and transporters of small signaling molecules such as calcium, serotonin, and butyrate. **(d)** Such transduction mechanisms convert bioelectric states produced by channel and pump activity to second-messenger events that regulate numerous downstream genes. Even more important than individual cell potentials **(e)** are the spatio-temporal patterns of bioelectrical state across tissue, seen in the mid-flank of a tadpole soaked in fluorescent voltage-reporter dye **(e′)**. Such distributions include both endogenous prepatterns for organ morphogenesis, such as the 'electric face' pattern that determines gene expression and anatomy of the *Xenopus* face **(f)** (red arrow points to a hyperpolarized spot that determines location of the right eye), and of pathological states corresponding to sites of tumor formation **(g)** that are detectable by aberrant depolarization signals **(h)**. Credits: a, b — Jeremy Guay of Peregrine Creative; e′ — Douglas Blackiston; f — Dany S. Adams; g — Brook Chernet.

induce entire organs (*e.g.* eyes made out of gut tissue), re-orient or duplicate primary axes, initiate regeneration of tails and limbs under non-regenerative conditions, alter the shape of regenerating heads to those of a different extant species, and reprogram oncogene-induced tumors into normal tissue (reviewed in [5•]).

Ion flows are the primary events of life [6,7]; maintaining a resting potential is arguably a basic defining feature of being alive. Indeed, the ability to exploit emergent properties driven by the physics of electricity was discovered by evolution long before multicellularity [8•]. Recent work in molecular evolution shed light on the origin of ion channels (the proteins that drive changes in the cellular bioelectric state). Most ion channel families were present in the most recent common ancestor [9], long predating nervous systems. Thus, neurons optimized far more ancient bioelectric signaling functions that were operating to move bodies' configurations through anatomical morphospace long before brains evolved to move the body through 3D space. However, the neural and pre-neural function of voltage potentials remain linked. Bioelectric gradients during development control the size and patterning of the brain [10••], while the brain in turn provides crucial embryonic patterning signals for remote muscle and peripheral innervation at the earliest stages of development [11••]. Early embryos lacking brains develop numerous malformations, even in posterior tissues, and become sensitive to chemicals that are otherwise not teratogenic. However, targeted misexpression of the HCN2 ion channel in somatic tissue largely rescues these defects, partially replacing the brain-derived bioelectric patterning signals.

New model systems for bioelectrics: new data from bacteria to man

Recent years have seen expansion of bioelectrics from aquatic workhorse models into several diverse model organisms, including bacteria and plants. Prokaryotes can propagate spatial information via remarkably brain-like K^+-based waves that integrate bacterial physiology and growth rates across their biofilm 'soma' [12••]. Even in plants, these endogenous organizing functions can be manipulated by external bioelectric stimulation to enhance complex regenerative response [13] as has been known in vertebrate limbs for many decades.

In animal models, physiological profiling and functional experiments [14,15] have identified roles for ion channel-mediated signaling via depolarization in axolotl tail regeneration. Interestingly, this signaling is mediated by transcriptional changes in downstream gene targets that are highly conserved to frog embryogenesis and differentiating human mesenchymal stem cells [16]. Even classical genetic systems such as *Drosophila* have now been utilized to understand this epigenetic control system, through the characterization of expressed electric machinery and the resulting physiological gradients [17]. Active Kir2.1 ion channels modulate BMP signaling in fly wing development [18••], while electrical synapses (innexins) modulate border cell fate [19] and eye size via DPP signaling [20]. Fascinatingly, even viruses have evolved to exploit this bioelectric control system, with genomes that misexpress viral innexins and ion channels in infected cells to manipulate growth in the host [21,22].

Bioelectrics data have now extended to mammals. Channelopathies are developmental defects caused by ion channel malfunction. Several new human syndromes, such as Keppen–Lubinsky syndrome (affecting KCNJ6 channels) result from mutations in ion channel genes, specifically affecting craniofacial patterning and other organs [23•,24•,25,26•], illustrating how unbiased investigations can lead directly into developmental bioelectrics. In addition to genetic syndromes, developmental defects can be induced by chemical agents that target ion channel-dependent signaling. This includes common compounds like alcohol (which causes fetal alcohol syndrome by targeting Kir2.1 channels) [27] and pharmaceuticals specifically designed to block or activate ion channels for epilepsy, arrhythmias, *etc.* [28].

New technology for investigating bioelectrics

Bioelectric signaling has been implicated by transcriptomic analyses, such as in a study of cellular positional memory [29] in which ion transport was the second highest gene ontology term represented. However, it is crucial to understand that bioelectric signals are not always visible at transcriptional or translational levels, because they are implemented by context-sensitive opening and closing of existing ion channel proteins. Fortunately, a number of tools for profiling bioelectric signals *in vivo* have recently been reported, including small organic dyes and other materials (nanowires, quantum dots) that fluorescently report resting potential [30,31,32•,33–35], and genetically encoded fluorescent voltage indicators (GEVIs) [36], which are ideal for systems that require constant perfusion with media. The most sensitive GEVIs that do not require high intensity laser illumination are Mermaid2 and ArcLight Q239 with a $\Delta F/F$ per 100 mV of 48.5% and 39% respectively [36]. Although Mermaid2 is more sensitive, it requires both 499 nm and 563 nm excitation wavelengths, restricting combinatorial biosensors and optogenetic actuators. ArcLight Q239 only requires a 509 nm excitation and features improved plasma membrane specificity [37]. Marina, based on ArcLight Q239 has comparable sensitivity but inverse output, showing an increase in fluorescence when depolarized [37].

The palette of optogenetic actuators has expanded greatly [38], though most are still focused on extremely fast response time (ideal for neural spiking but not for developmental bioelectrics). However, 'step function' actuators, such as Stable Step Function Opsin, BLINK2 potassium channels, and the hyperpolarizing chloride channel SwiChR++, are triggered by a short and relatively low-intensity blue light pulse and stay open for 2–30 min [39–41]. Bi-stable anion optogenetic channels such as Phobos and Aurora [42] are very promising new alternatives. A novel sodium-pumping rhodopsin chimera I_1K_6NaR is an efficient hyperpolarizer [43–45] and can even be mutated to change the ion selectivity to potassium [46,47].

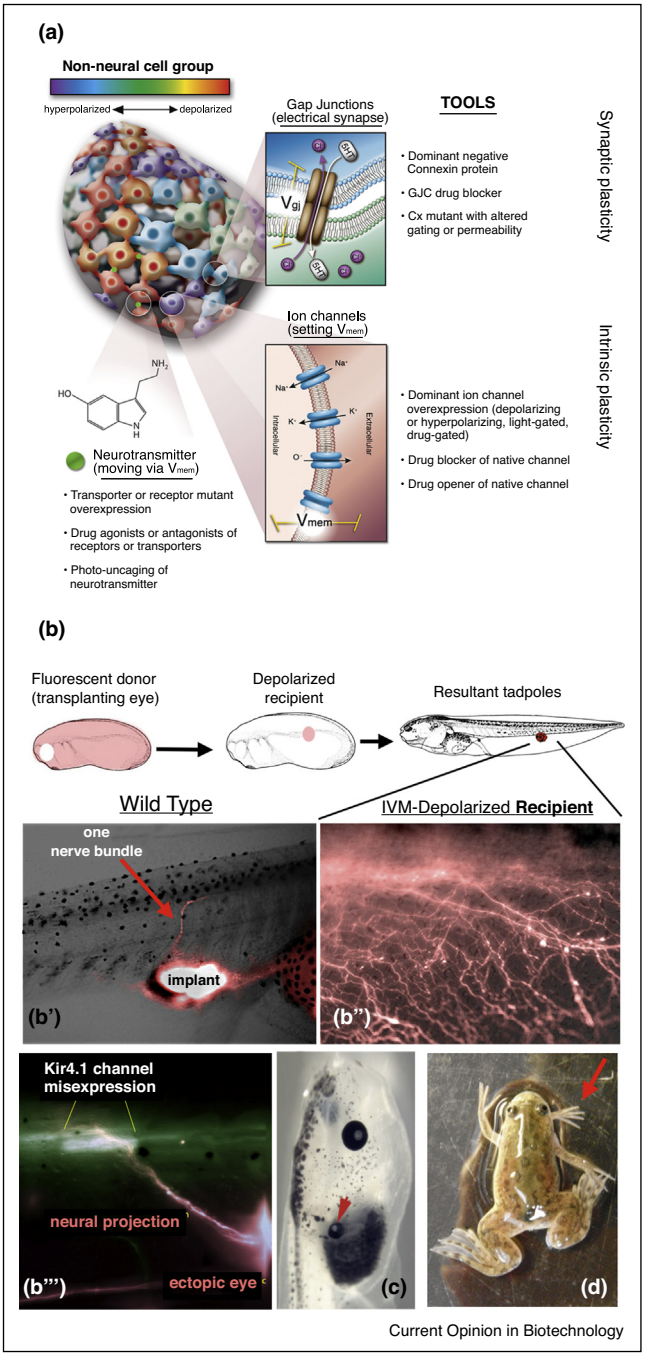
Novel methods for spatially and temporally reading and actuating membrane potential without GEVIs or optogenetics have also been advanced, which is an advantage for eventual biomedical applications without gene therapy. High-density nanowire arrays that record membrane potential with high sensitivity, spatial resolution, and signal to noise ratio can be addressed individually via a unique nanowire-on-lead approach [48]. The use of poly (3,4-ethylenedioxythiophene):polystyrene sulfonate conducting polymer microwires can depolarize cells with high spatial resolution and no toxicity [49].

It is also vital to control tissue-level bioelectric states (Figure 2). Regenerative applications include using wearable bioreactors [50] to facilitate the delivery of bioelectric modulating reagents; these have not yet been integrated with optogenetic stimulation, but utilize blends of compounds that alter membrane potential. Prior work inducing regeneration and altered patterning via spatially homogenous treatments are being complemented [51,52••] with approaches that take advantage of the exquisite spatio-temporal specificity enabled by optogenetic actuators driven by patterned light delivery [53••] and advanced nanomaterials that predictably affect cell V_{mem} [49,54] to lay down arbitrary masks of activation and thus induce desired bioelectric prepatterns.

Bioelectrics at the cellular level

Progress has been made on the bioelectrical control of single-cell functions, and even subcellular components, such as microdomains in plasma membranes, which bear distinct bioelectric states within a single cell [55,56], and the role of the nuclear envelope potential [57]. Studies in a wide range of mammalian stem and somatic cell types, have revealed roles in axon guidance [58••], cell migration [59], stem cell differentiation [60], and proliferation [61].

Figure 2



Manipulation of bioelectric networks result in patterning changes *in vivo*. **(a)** Networks of electrically connected cells can be manipulated in several fundamental ways: altering the electrical connectivity (network topology) via dominant-negative, mutant, or wild-type connexin proteins (synaptic plasticity), altering the resting potential of individual cell groups by introducing new channels or modifying existing channels pharmacologically (intrinsic plasticity), or directly altering the normally voltage-guided movement and signaling of small molecules (such as neurotransmitters) through the network. Altering V_{mem} *in vivo* (regardless which ion movement is used to achieve it) predictably induces changes such as increasing the innervation from eye transplants in frog embryos **(b)**, which normally grows out one new nerve bundle **(b′)**, but exhibits extensive new innervation when the surrounding host tissue is depolarized **(b″)**; the same method can be used to target innervation growth to regions of specific V_{mem} **(b‴)** shows neuronal targeting of a region expressing the hyperpolarizing Kir4.1 channel). On an organ level, inducing eye-specific bioelectric patterns by ion channel mRNA misexpression can induce eye formation anywhere in the body, even outside the anterior neural field, such as on the gut (**(c)** red arrowhead indicates ectopic eye made in endoderm), or ectopic limbs (red arrow in **(d)**, induced in a frog overexpressing an optogenetic activator). Credits: a — Jeremy Guay of Peregrine Creative; b-b‴ — Douglas Blackiston; d — EnPAC transgenic frog made by Gufa Lin. Photos by Dany S. Adams and Erin Switzer.

These advances enabled the application of bioelectric controls to target the function of the adaptive [62] and innate [63] immune systems, and demonstrate electrical communication between macrophages and cardiac cells [64].

Light-based control of ion channels has been increasingly used in targeting bioelectric mechanisms *in vitro*, including the optogenetic control of differentiation in glial progenitor [65••] and tumor cells [51]. An elegant synthetic biology study created an electrically excitable tissue by misexpressing several ion channels in a non-neural cell line, demonstrating memory (entrained ring oscillator) and the optical establishment of boundaries between compartments with distinct V_{mem} [66••].

Important molecular advances are being made in understanding how bioelectric states couple to downstream genetic response cascades, via transcriptional profiling that identified highly conserved classes of response genes between human, axolotl spinal cord, and frog embryo depolarization events [16], and targeted experiments that identified specific downstream genes such as Notch [10••], Wnt [67•], CREB, MEF2, and SP4 [68]. How do bioelectrical signals control gene expression? In addition to well-known mechanisms, such as Ca^{2+} and the flux of neurotransmitters through gap junctions and transporters such as SERT (Figure 1c), newly discovered transduction mechanisms include changes in actin and stiffness of the membrane [69•], integrin-dependent phosphorylation of focal adhesion kinase [70], and phospholipid dynamics of signaling molecules such as KRAS [71].

Elegant recent work revealed the bi-directional interplay between bioelectric states and canonical pathways such as BMP and Hedgehog; second messengers including calcium and neurotransmitters integrate bioelectric signals in very early neural patterning, revealing how bioelectrics cooperates with trophic factors and morphogens to specify neural cell fate [72•]. Importantly, V_{mem} modifies the meaning (interpretation) of canonical signals by cells [73••], a theme discussed below in the context of bioelectrics as part of the decision-making apparatus of cell collectives.

Recent successes in the control of growth and form

One of the most exciting developments in this field is the demonstration that endogenous bioelectric circuits not only help explain endogenous pattern regulation but also serve as tractable control points for making coordinated, high-level changes to anatomy. Targeted ion channel misexpression and pharmacological targeting of endogenous channels have been used to demonstrate control over the extent and targeting of innervation in organ transplants [74] (Figure 2b) and the behavior of cell sheets to augment wound healing [75,76]. Experiments in the developing frog brain showed that artificially enforcing the normal bioelectric brain prepattern can counteract the normally devastating effects of a dominant mutant *Notch* protein [10••], largely rescuing morphogenesis, gene expression, and learning/behavioral capacity in tadpoles despite a defect in this key neurogenesis gene. The ability to override genome-default states (also seen during bioelectric suppression of KRAS mutant-induced tumorigenesis [51] and inducing head shapes appropriate to other species from a wild-type planarian body [5•]) is a recurring theme in data on the roles of bioelectric circuits in the relationship between the genotype and phenotype.

On an even larger scale, recent work in regenerating planaria (Figure 3) has revealed the bioelectric encoding of pattern memory that dictates how many heads the animal will have if it regenerates after damage [77••]. A brief (transient) pharmacological shift of the bioelectric circuit into another attractor state generates a permanent line of animals that always regenerate double-headed if cut in the future (with no more external manipulation), revealing that pattern memories encoded in somatic bioelectric circuits can be re-written away from default target morphologies without editing the genomic sequence. Moreover, these data showed that the bioelectrically encoded regenerative set-point could be edited in an animal that is normal (one-headed) in terms of its anatomy and molecular histology, revealing that (as with any hardware-software system), the outcome can be permanently changed without altering the tissue structure or genetics. The same body can apparently store (at least) two different bioelectric 'memories' which are latent but will guide regenerative patterning if the animal is cut at some future time.

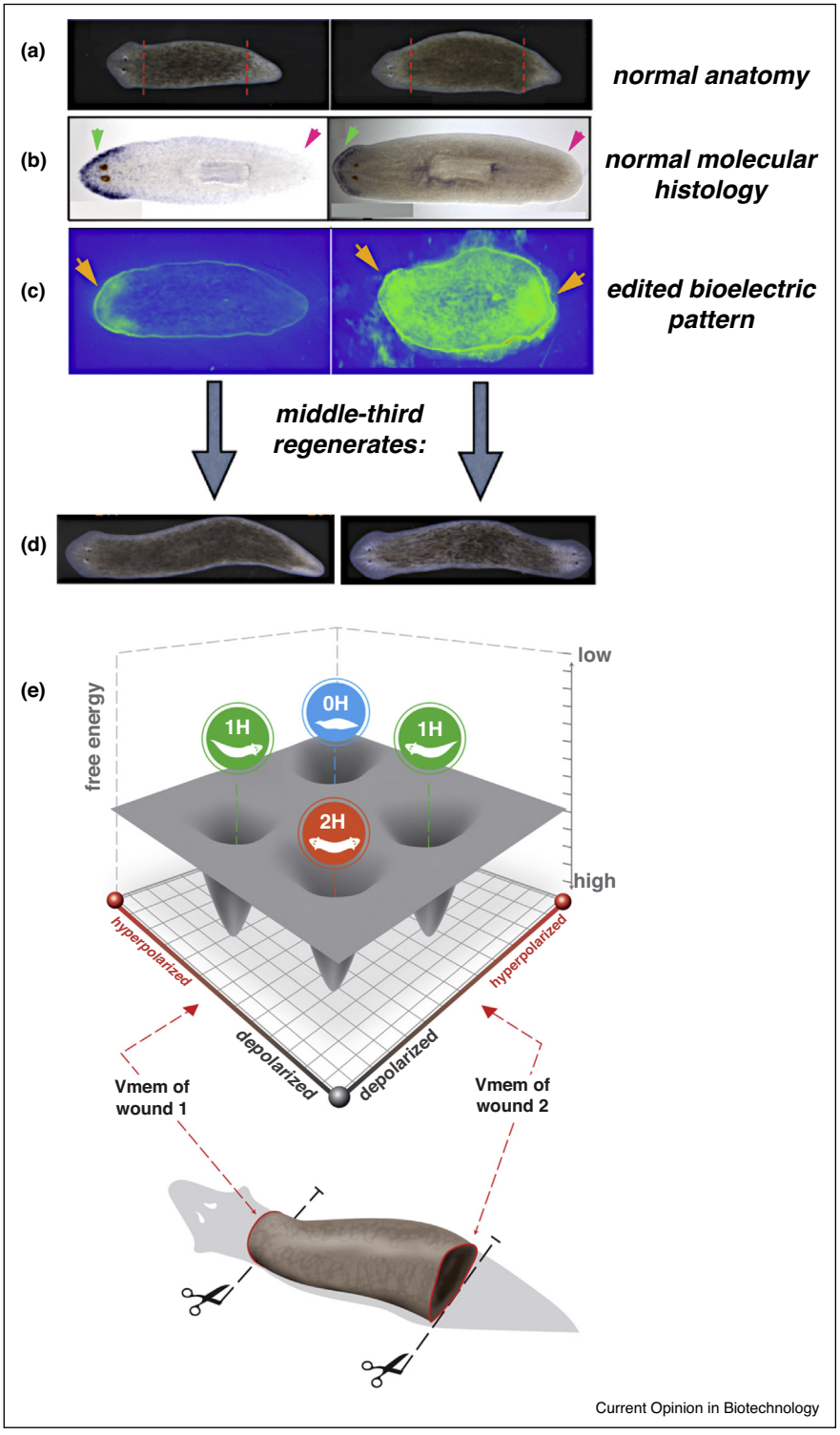
Together, the data from stem cell controls, wound healing applications, and large-scale pattern editing suggest a new strategy for regenerative medicine. Ion channel targets are the third best-selling group of prescribed drugs, with worldwide sales of $12 billion annually [78]. Thus, the plethora of ion channel drugs, many of which are already approved for human use (anti-epileptics, anti-arrhythmics, *etc.*) form a remarkable pool of 'morphoceuticals' — compounds that can be immediately re-purposed for control of coordinated cell behaviors in regeneration, birth defect repair, and tumor reprogramming — areas in which micromanagement from the molecular level up faces daunting complexity limits. The ability of single exogenous ion channel function to mimic the brain's native protection of an organism from teratogens [11••] suggests numerous opportunities for biomedical intervention. The next enabling step in this roadmap (Figure 4) is the creation of machine learning-based computational platforms [79] that combine predictive bioelectric simulators [80•] with databases of ion channel expression profiles in various human tissues and chemical databases of known drugs targeting each channel. An appropriate expert system will invert bioelectric circuit models to suggest drug cocktails that target specific ion channel combinations (with restricted spatial expression in the body) and force desired patterns of V_{mem} in selected body sites for pattern repair.

The future: not just mechanism but meaning, of bioelectric states

One of the most important steps in this field is the development of deep new theory for cracking the bioelectric code — truly understanding its global information semantics to complement the focus on high-resolution subcellular mechanisms [81]. The higher-order dynamics of gene-regulatory networks and biochemical systems have been studied for many decades, but the information-processing and spatial self-organizing properties of somatic bioelectrics are only now beginning to be appreciated. The work in neural decoding (extraction of semantic content from electrical activity in the brain) provides welcome guidance [82,83•]. It is crucial to move beyond single-cell pathway analysis to understand how large-scale voltage properties are exploited by evolution to implement the computations needed for pattern maintenance and self-assembly *in vivo*. In the context of biomedicine, this means moving to circuit disorders and 'computational psychiatry' [84,85•] of the body beyond channelopathies at the cellular level. This effort is being augmented by bio-realistic quantitative modeling approaches, via equivalent circuit models [86] and as fully spatialized simulation environments [80•,87] that allow detailed simulation of the interplay between bioelectric and biochemical pathways, and their self-organizing and long-range pattern-regulation properties.

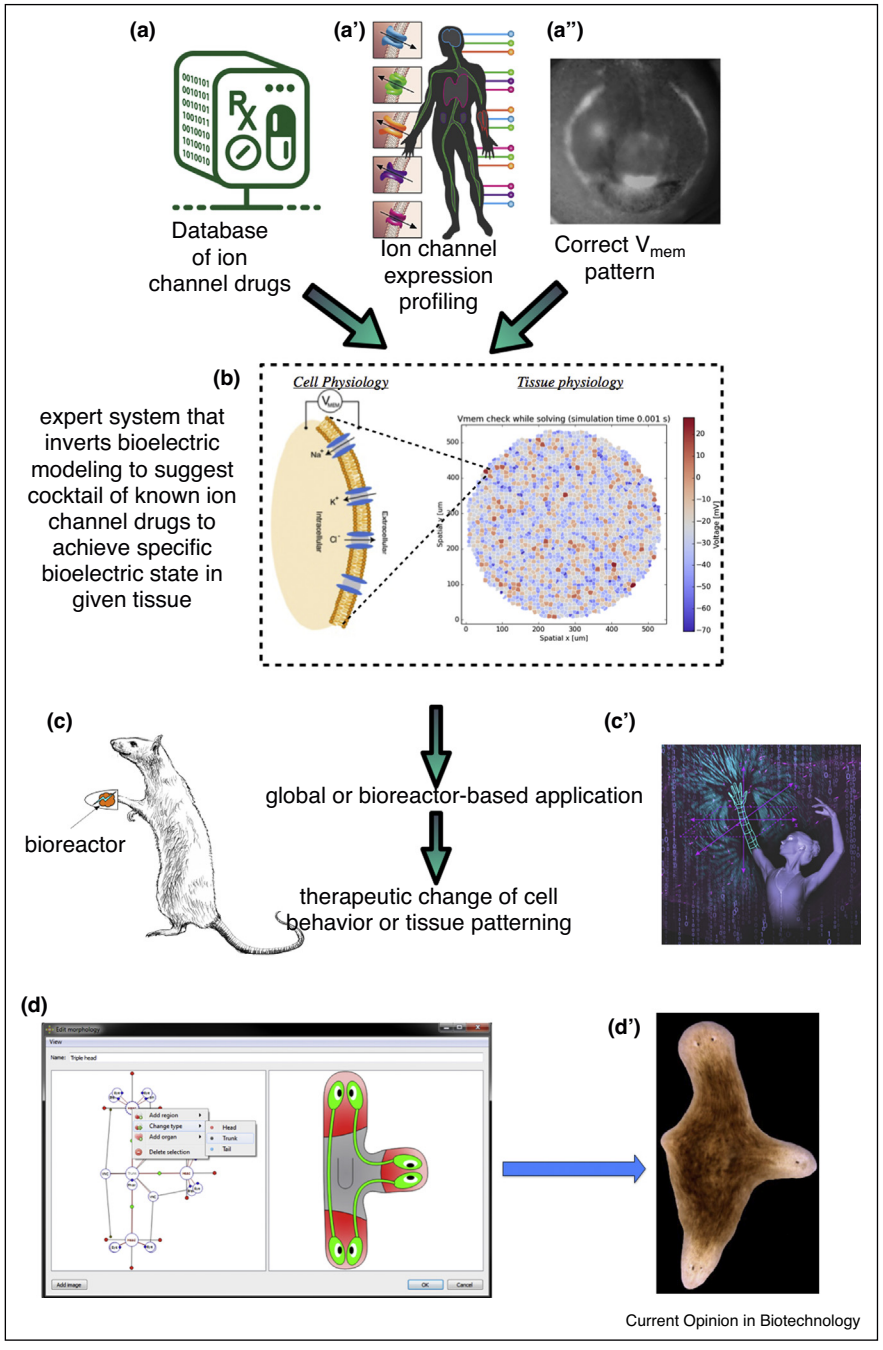
'Nervous systems are foremost spatial organizers' [88], and this is a function they inherited from their pre-neural past in which bioelectric circuits were used mainly for pattern control. Ion translocators are computational elements [89] themselves, supporting physiological state memory. For example, when cancer cells are moved out of a solution containing a hERG1 activator compound, they stay reprogrammed — an example of the consolidation of short-term memory (transient V_{mem} state) into long-term biochemical (phosphorylation, gene expression) states. The same thing occurs *in vivo*, for example in planaria, where a brief

Figure 3



Bioelectric prepatterns store target morphology memories. Planaria that have normal anatomy (one head, one tail **(a)**) and normal molecular histology (head marker expressed only in the head end **(b)**), but are transiently modified (using pharmacological targeting of bioelectric circuit) to have depolarized (green) regions in both ends versus the normal unilateral pattern **(c)** will regenerate from middle third fragments as one or two-headed (bipolar heteromorphosis) forms respectively **(d)**. Recent computational modeling of the bioelectric circuit (the state space of which is schematized in **(e)**) reveals how its attractors encode stable (permanent) pattern memories that guide future rounds of regeneration toward distinct anatomical outcomes. Credits: a–d from [77••]; e — Jeremy Guay of Peregrine Creative.

Figure 4



Predictive computational platforms for biomedical applications: a possible roadmap. Existing bioinformatics resources, including databases of known ion channel drugs **(a)**, expression profiles of channels and pumps in various healthy and diseased human tissues **(a′)**, and known correct bioelectric prepatterns for specific cellular tissues and structures **(a′′)**, are inputs into expert systems being developed in our center **(b)**. These machine-learning platforms invert bioelectric modeling tools [79] to infer what interventions can be performed (which ion channels, pumps, or gap junctions need to be activated or deactivated) to induce desired bioelectric states. Using systemic application or bioreactor-based delivery in model systems **(c)** or human patients **(c′)**, these will someday be able to address bioelectric circuit disorders that comprise many degenerative, birth defect, or carcinogenic conditions, as well as induce regenerative repair. The ultimate goal of this research program is a 'biological compiler' that can convert anatomical specifications **(d)** into biophysical signals that modify target morphology encodings causing cells to build to the spec (illustrated via planarian bodyplan **(d′)**). Credits: a, a′ — Jeremy Guay of Peregrine Creative; a′′ — Dany S. Adams; b — Alexis Pietak; c — Jay Dubb; c′ — Jeremy Guay of Peregrine Creative; d — Daniel Lobo; d′ — Junji Morokuma.

bioelectric circuit change can permanently shift the pattern to which regeneration repairs upon future injuries [77••,90••]. The principles of computation in genetic circuits are becoming understood via the work of synthetic biology, but are largely limited to transcriptional machinery [91]. The next decade will doubtlessly extend these efforts for bioelectrics; physical limits on the powers of purely chemical gradients [92,93] can be greatly assisted by bioelectric mechanisms which are ubiquitously exploited by evolution and modern information technology as a uniquely tractable set of physics principles for implementing memory, computation, and spatially integrated decision-making.

Exciting recent efforts at the intersection of cell biology and primitive cognition have begun to explore the perception space of cells [94•,95,96••], to understand how living tissues represent internal models of themselves and their environment. Parallels between developmental and neural computation were noticed long ago [97••], and emerging concepts in neuroscience such as active inference [98] might be well-applied to cellular interactions via bioelectric states. We believe it is likely that as in neuroscience, the information content of bioelectric states during pattern control may be most efficiently understood and manipulated via connectionist approaches such as artificial neural network models in which stable attractors of bioelectric state correspond to individual pattern memories for distinct anatomical outcomes [83•,99,100].

This idea is not only a roadmap for computational analysis but has specific implications for experiments. How does regeneration and regulative development proceed toward invariant target morphologies from diverse starting states? Understanding this process as an error minimization scheme suggests novel approaches for manipulating bioelectric memories as set-points for least-action (free energy) control systems, which would provide a very efficient top-down strategy [99,100] for regulating growth and form. More specifically, a view of somatic bioelectric circuits as pre-neural computational networks predicts that it should be possible to train living tissues for specific patterning outcomes via behavior-shaping (using appropriate rewards and punishments) — these experiments are currently on-going in our lab. Thus, we suggest that a view of bioelectric networks as fundamentally information-processing agents operating to enable cell cooperation toward large-scale patterning goals is an enabling formalism for next-generation applications in regenerative medicine and synthetic morphogenic engineering. The recent concordance of reagents, tools, functional data, quantitative biophysical modeling, and conceptual advances define a vibrant new field at the intersection of molecular genetics, developmental biophysics, and cognitive neuroscience with incredibly exciting and important implications for basic science and biomedicine.

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