

Perspective

Bioelectric signaling: Reprogrammable circuits underlying embryogenesis, regeneration, and cancer

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How are individual cell behaviors coordinated toward invariant large-scale anatomical outcomes in development and regeneration despite unpredictable perturbations? Endogenous distributions of membrane potentials, produced by ion channels and gap junctions, are present across all tissues. These bioelectrical networks process morphogenetic information that controls gene expression, enabling cell collectives to make decisions about large-scale growth and form. Recent progress in the analysis and computational modeling of developmental bioelectric circuits and channelopathies reveals how cellular collectives cooperate toward organ-level structural order. These advances suggest a roadmap for exploiting bioelectric signaling for interventions addressing developmental disorders, regenerative medicine, cancer reprogramming, and synthetic bioengineering.

ANATOMICAL HOMEOSTASIS—A CENTRAL CONCEPT FOR BIOLOGY AND MEDICINE

Unicellular organisms are highly competent at managing their physiological, morphological, and behavioral needs. But one of the most important aspects of cell biology is the ability of some cells to cooperate toward invariant large-scale outcomes: building and repairing highly patterned multicellular bodies. The capacity of embryonic blastomeres to reliably self-assemble a complex metazoan organism, with the same large-scale form and function, would be surprising to a micro-scale observer who did not already know the remarkable outcome of embryogenesis, given the stochasticity and noise seen at the cellular and molecular levels. The ability of unreliable, fragile components to build robust living organisms to a precise structural and functional specification is already the envy of robotics and engineering. However, the true power of biology is seen in the further ability of cell collectives to achieve the same anatomical configuration from different starting conditions, and despite significant perturbations (Figure 1).

In some animals, regeneration (e.g., after amputation at any point along the limb) produces a perfect replacement. Furthermore, regulative development (e.g., in early mammalian embryos) makes up for even massive trauma such as complete bisection, revealing that cellular collectives can adapt to radical, unpredictable changes along their normal morphogenetic progression. But this is much more powerful than simple repair along a constant, predictable trajectory through morphogenetic stages (Pezzulo and Levin, 2016). Tadpoles must restructure their craniofacial organs to build the face of a frog; even if tadpoles are created in a “Picasso” configuration with eyes, jaws, and other organs in the wrong locations, they will produce largely

normal frog faces. Those organs will move, through un-natural paths, to implement a correct frog face, even before metamorphosis is initiated (Pinet et al., 2019; Vandenberg et al., 2012) (Figure 1A). Thus, evolution has not hard-coded a set of specific movements that reliably turn standard tadpoles into standard frogs: instead, the genome specifies a cellular collective with massive plasticity, which executes rearrangements until the correct target morphology is achieved. Salamander tails grafted to the flank slowly remodel into a limb—altering the existing tissue structure to become correct with respect to the large-scale body plan, even though there is nothing wrong in the local environment of cells at the tip of that transplanted tail (Farinella-Ferruzza, 1956) (Figure 1B). Remarkably, kidney tubules of the correct cross-sectional geometry result from the activity of either many or just one cell, showing that diverse underlying molecular mechanisms can be harnessed as needed depending on context, to achieve the same anatomical outcome (Figure 1C).

How do these systems know when to stop—recognizing that the “correct” target morphology has been achieved? Large-scale anatomical structure not only involves the emergence of system-level outcomes from local rules but also has the important property of “anatomical homeostasis”: the ability of the system to activate the necessary sequences of cell behaviors to progressively reduce the error between the current state and the species-specific target morphology. Treatment of birth defects, traumatic injury, the defections from the body plan known as cancer, degenerative diseases, aging, and synthetic bioengineering could all be revolutionized by rational control over the anatomical set point toward which cells build and repair.

While many groups are working to initiate a regenerative response in biomedical settings, a deep question of biology has received far less attention: what does the cellular collective



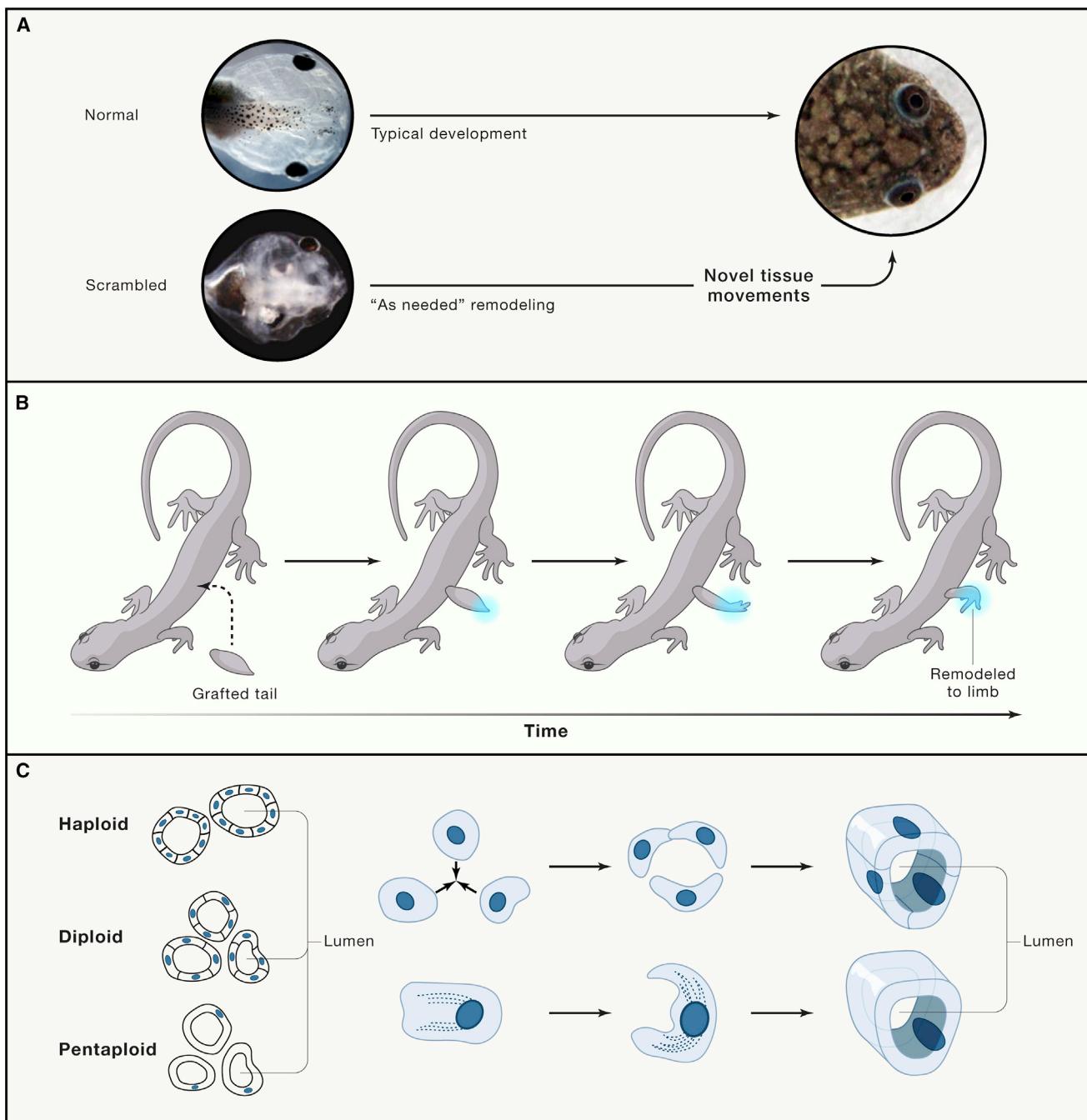


Figure 1. The challenge of anatomical homeostasis

(A) Significant remodeling processes turn tadpole heads into those of frogs. Remarkably, highly abnormal tadpole faces also make largely normal frogs as the organs rearrange and deform as needed, until a correct frog face is built.

(B) Tails transplanted onto the flank of a salamander can slowly remodel into legs—a structure more appropriate in the global context (schematized after data in [Farinella-Ferruzza \[1956\]](#) and [Holtfreter \[1955\]](#)). This includes tail-tip cells, marked in blue, which become toes, despite a normal *local* environment, illustrating the remodeling of tissue structure based on a global target morphology.

(C) Kidney tubules form with roughly correct diameter in newts despite polyploidy, which changes cell size. As the cells get bigger, the system adjusts to utilize a smaller number of cells to achieve the same geometrical endpoint as the cells enlarge due to polyploidy. Remarkably, when the cells get very large, development abandons normal multi-cellular coordination pathways and exploits cytoskeletal biomechanics to achieve the same lumen from just one cell bending around itself. This illustrates the use of diverse molecular mechanisms in novel circumstances to achieve the same large-scale anatomical target morphology. This figure was schematized after [Fankhauser \(1945\)](#).

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measure against in order to progressively reduce anatomical error, and how is this information specified in tissue? Understanding how growth and remodeling are stopped when a target morphology has been reached is critical to ensure that regenerative treatments produce organs rather than the dysregulated growth of tumors. This is also a fundamental issue for evolutionary developmental biology and the origin of specific body plans.

Considerable progress has been made on the molecular genetics of stem cell differentiation and the signaling required for normal morphogenesis. However, the ability to rationally control complex form is still largely beyond us. Profound questions remain about the relationship of genotype to anatomical phenotype. For example, some species of planarian flatworms reproduce largely by fission and regeneration and thus inherit somatic mutations over millions of years that have resulted in what seems to be a messy genome and even mixoploidy (Nishimura et al., 2015). And yet, despite all the variability in the genetics within and across planaria, their anatomy is 100% reliable when regenerating from fragments. Similarly, despite the many high-resolution studies of the transcriptional controls of neoblast fate, the field still lacks any conceptual model that will make a prediction for a simple experiment: what head shape would result from transplants of stem cells from a flat-head planarian species to one with a triangular head (Levin et al., 2019)? Would one shape be dominant to the other? Or would it result in a combination, in-between shape? Or, would the head continuously morph and remodel, as neither set of neoblasts reach the stop condition for the kind of head they should normally make? The result of such a chimera experiment is hard to predict because we largely lack a theory of how cell collectives specify anatomical-level set points for their activity and make decisions about large-scale outcomes. Thus, the current knowledge gap is in our understanding of how the genome-specified hardware of cells allows them to cooperate toward robust anatomical endpoints. It is critical to identify and control the mechanisms used by cells in coordinating across distance to interpret and act upon “stop” conditions when the correct organ-level structure has been completed.

Evolution exploits three main modalities to coordinate morphogenesis: biochemical signals, biomechanical forces, and bioelectric communication (Levin, 2014; Newman, 2019). Recent advances in bioelectrical signaling between non-neural cells are beginning to reveal how all cells, not just neurons, form electrical networks that control gene expression and cell behavior. Here, I review recent progress in the exciting emerging field of molecular developmental bioelectricity and provide a perspective on how bioelectric circuits integrate cell-, tissue-, organ-, and whole-body-level information to enable morphogenesis and pattern homeostasis. Crucially, it is becoming clear that bioelectricity is not simply one more layer of mechanism that is required alongside biochemical cues and stress forces to implement morphogenesis: it enables unique, powerful information-processing capacity that facilitates scaling of cells into complex morphogenetic collectives. These aspects shed light on the evolution of multicellular forms and provide an attractive roadmap for targeting endogenous bioelectric circuits as tractable and powerful control knobs for applications in regenerative medicine and synthetic morphology.

BEYOND NEURAL BIOELECTRICITY

Ion flows are some of the first events of life. As early as 1891, W. Roux wrote about the effect of external fields on embryonic development, and a large body of subsequent work tested the role of endogenous fields via loss-of-function (shunting) experiments and applied fields of physiological strength in a range of developing, regenerating, and neoplastic contexts (Borgens, 1982; Nuccitelli et al., 1986). Classic workers used voltmeters, vibrating probes, and electrodes to reveal the importance of endogenous ion currents and transepithelial electric fields in morphogenesis and its disorders. However, developmental bioelectricity has been reinvigorated in the 21st century by three main advances: a focus on patterns of resting potentials (voltage gradients) within tissues, mechanistic linkage with molecular genetics and biochemical pathways, and the appreciation of the evolutionary origins of bioelectricity as an information-processing modality that is ancestral to nervous system activity (Fields et al., 2020).

The familiar bioelectric activity of the brain has its evolutionary origins in ancient functions of ion channels and electrical synapses known as gap junctions (Palacios-Prado and Bukauskas, 2009). This system enabled multi-cellular computations before brains evolved and is amenable to the same conceptual framework and experimental tools used routinely by neuroscience (Pezzulo and Levin, 2015) (Figure 2). All cells, not just neurons, have ion-channel proteins and pumps that set their resting potential (V_{mem}); this is now known to be a key parameter regulating cell-level behaviors such as proliferation, differentiation, apoptosis, migration, and directional polarization in a wide range of cells from yeast to human stem cells (Blackiston et al., 2009; Chang and Minc, 2014; Li et al., 2018b). This has especially been exploited by efforts to control cell movement in wound healing and stem cell guidance contexts by electrotaxis (Feng et al., 2017). Functional roles of specific ion channels in development, cancer, and regeneration, as well as mechanisms of endogenous bioelectricity in wound healing and regeneration, have been recently reviewed (Bates, 2015; McLaughlin and Levin, 2018; Silver and Nelson, 2018).

Spatially organized bioelectric gradients (Figure 3) begin to be established as early as the two-cell stage in vertebrate embryos (Levin et al., 2002) and are already present as complex bioelectric prepatterns in oocytes in *Drosophila* (Krüger and Bohrmann, 2015). During later development, maturing cell types especially attentive to bioelectric state of their neighbors are melanocytes and the skin patterning system in general (Inaba et al., 2019; Lobo et al., 2017). These cells exploit contact-dependent bioelectrical signaling and gap-junction-mediated control of morphogens such as *Sonic hedgehog* to implement patterning of pigmentation and epithelial specializations such as feathers (Li et al., 2018a). The following is a brief overview of the key insights into non-neural bioelectricity that have emerged from a wide range of model systems.

Bioelectric hardware at the cellular level: V_{mem} meets canonical signaling

Bioelectricity works in conjunction with gene-regulatory circuits and biochemical factors, such as the linkage of transepithelial

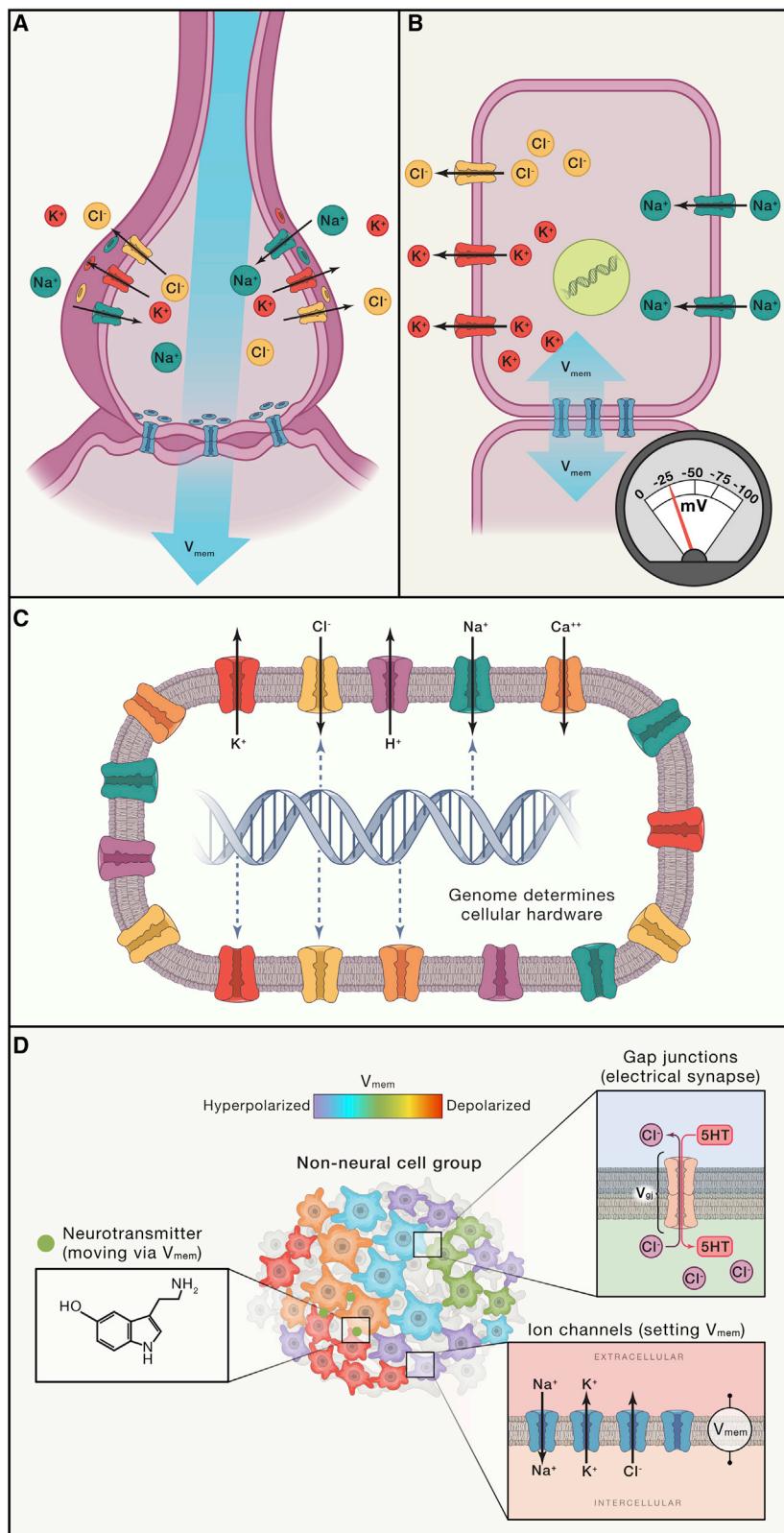


Figure 2. Developmental bioelectricity at the cellular level

(A) Neural bioelectric dynamics are created by ion channels that set the resting potential across the plasma membrane and electrical synapses that communicate electric states across networks of neurons.

(B) More ancient mechanisms present in all cells use ion channels to regulate cellular V_{mem} (resting potential across the plasma membrane) and selectively propagate those bioelectric states to their neighbors.

(C) The bioelectric state of a cell is a complex function of its past history and its neighbors' bioelectrical states, due to context-sensitive (e.g., voltage-gated) ion channels determined by the genome and transcriptional mechanisms.

(D) Tissues form bioelectrical networks similar to neural networks. Spatiotemporal V_{mem} distributions are regulated by the temporal evolution of differential voltage states of individual cells and the changing topology of connections via electric synapses (gap junctions, which are themselves voltage sensitive). Bioelectric gradients use the movement of intracellular (e.g., neurotransmitters) and extracellular morphogens (signaling molecules) to regulate gene expression and morphogenesis. Genetic, optogenetic, and pharmacological approaches target the gap junctions and ion channels, exploiting synaptic and intrinsic plasticity non-neuronal cells, just as they are routinely used in neuroscience to modulate computation in neural circuits.

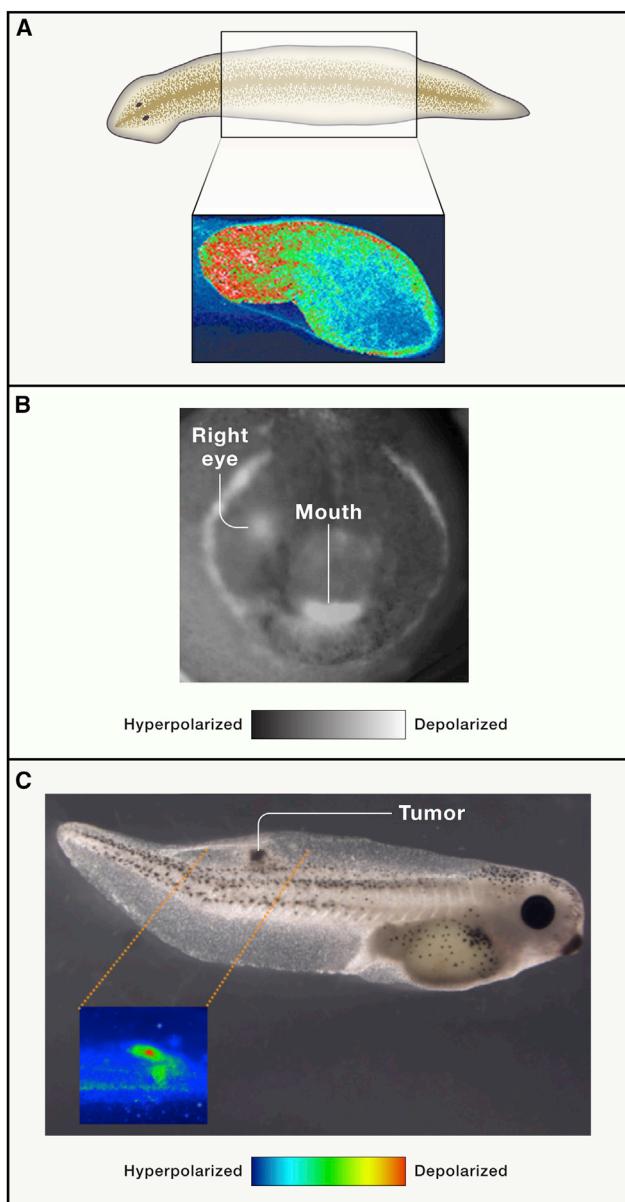


Figure 3. Tissue- and organ-level bioelectric patterns

(A) Spatial distributions of V_{mem} across tissue can be visualized by voltage-sensitive reporter dyes to reveal the depolarized head end and hyperpolarized tail end of the bioelectric circuit in a planarian flatworm fragment that determines its future regenerative anatomical polarity.

(B) Endogenous prepatterns instructively guide morphogenesis, such as this "bioelectric face" distribution on the early frog ectoderm that determines the gene-expression domains and the borders of the compartments for the eyes, mouth, arches, etc. (white signal demarcates depolarized cells, such as the embryo's right eye, appearing slightly earlier than the left). Drugs, ion-channel misexpression, or optogenetics can be used to alter this pattern resulting in predictable changes in the domains of expression of craniofacial patterning genes and subsequent head anatomy.

(C) Individual cells transforming into cancer depolarize as a very early step in the process, leading to an electrical de-coupling from tissue-level organizational cues (here shown as an early stage of a tumor in tadpoles injected with human oncogenes; voltage-sensitive fluorescent dyes reveal location and size of the cells abandoning their participation in organogenesis in favor of tumorigenesis). Image in (A) by Taisaku Nogi. Images in (B) and (C) are used with permission from Chernet and Levin (2013b) and Vandenberg et al. (2011).

gradients to physiological pathways (e.g., redox reactions Ferreira et al., 2016; Ferreira et al., 2018) and inositol phosphate and hedgehog signaling (Kucerova et al., 2012; Meng et al., 2011). Thus, the emphasis on bioelectronics as an instructive driver of morphogenesis does not diminish the critical roles of biochemical and biomechanical machinery. The key question, however, is which signaling node(s) in the complex, multi-modal network of processes that together guide morphogenesis is the most efficient target for enabling rational prediction and control of complex, large-scale anatomical outcomes. As will be discussed below, bioelectric states represent powerful, convenient pivot points for organ-level decision making among cellular collectives (an idea that complements familiar notions of biochemical master regulators). Continuous interplay of transcriptional, biomechanical, and bioelectric mechanisms implements the logic of morphogenetic control. Bioelectricity acts in concert with canonical pathways, and considerable progress has been made in linking molecular events to changes of bioelectric state in cells.

Bioelectric states are determined by ion-channel expression and function. These, in turn, are modulated by growth factors, intracellular signaling modulators, and feedback loops of pH and resting potential, in order to regulate properties such as stem cell differentiation, cancer cell motility, and transcriptional profile (Blackiston et al., 2009). As a result of channel and pump protein activity, V_{mem} changes at the cellular level are transduced to downstream effectors (2nd messenger cascades). For example, bioelectric signals can regulate the electrodifusion of neurotransmitter molecules (especially serotonin [Fukumoto et al., 2005]) in and out of cells, alter clustering of KRAS receptors in the membrane (Zhou et al., 2015), influence voltage-sensitive phosphatases (Numaga-Tomita et al., 2019), modulate integrin signaling (Becchetti et al., 2017), regulate cell volume (Yellin et al., 2018), and alter chromatin states (Matzke et al., 2019).

It has been suggested that second messenger systems integrate chemical morphogens and bioelectric cues to regulate neural system differentiation and patterning in vertebrate embryogenesis (Borodinsky and Belgacem, 2016). One hypothesis is that V_{mem} state modifies the meaning (interpretation by cells) of powerful canonical signals such as *Sonic hedgehog* (Belgacem and Borodinsky, 2015). Calcium signaling is a particularly critical downstream component, mediating bioelectric controls of patterning of the limb/wing, skin, brain, and heart during development and head-tail axis during regeneration (Atsuta et al., 2019; Beane et al., 2011; Dahal et al., 2017; Li et al., 2018a; Panáková et al., 2010). Calcium dynamics downstream of bioelectric control of voltage-gated calcium channels are often transduced by Calcineurin, as in the case of embryonic size control (Daane et al., 2018; Kujawski et al., 2014) and myoblast differentiation (Konig et al., 2006).

Changes of resting potential can regulate the expression and distribution of key developmental genes such as *Notch* in brain patterning (Pai et al., 2015b), *Shh* in left-right axis determination (Levin et al., 2002), *Sox9* and other chondrogenic differentiation genes (Atsuta et al., 2019), *BMP* and *Frizzled* in craniofacial patterning (Belus et al., 2018; Vandenberg et al., 2011), and *Wnt* in brain development, planarian head induction, and cancer (Ashmore et al., 2019; Beane et al., 2011; Lange et al., 2011; Pai et al., 2015b). Several of these pathways form control loops with

Box 1. Examples of canonical molecular components interacting with bioelectricity

The plasma-membrane variant of the V-ATPase proton pump is emerging as a key player in bioelectric controls. This versatile ion pump has been implicated functionally in left-right patterning (Adams et al., 2006), appendage regeneration (Adams et al., 2007; Monteiro et al., 2014), stem cell differentiation (Tamirisa et al., 2018), wound healing (Fraire-Zamora and Simons, 2018), spinal cord and peripheral nerve injury (Weng et al., 2020), and the biomechanical changes in cancer downstream of RAS signaling (Bartel et al., 2017). Remarkably, bioinformatics analysis comparing differentially expressed genes in regenerating plants, planaria, axolotl, and deer identified the V-ATPase as the only protein that is consistently upregulated in wound blastemas among such diverse body plans, and across independent origins of multicellularity (Srivastava et al., 2020). The Kir2.1 family of ion channels is another important regulator, playing crucial roles in the control of BMP signaling in craniofacial (mammal) and wing (*Drosophila*) development (Dahal et al., 2012; George et al., 2019), muscle cell differentiation (Konig et al., 2006), and bone development (Pini et al., 2018), providing a key example of conserved morphogenetic roles from fruit fly to human (Belus et al., 2018; Dahal et al., 2017). Bioelectric signals also integrate with aspects of biomechanics, via the organization cytoskeletal stiffness in mammalian endothelia (Evans et al., 2020) and *Drosophila* embryogenesis (Weiss and Bohrman, 2019). In turn, bioelectric states are controlled by tissue biomechanics. Physical forces, such as the Connexin43-dependent bioelectric gradients in mammary epithelium are shaped by patterns of tissue stress (required for downstream activation of Yap/Taz signaling [Silver et al., 2020]), and by biochemical signals such as IFG-1, insulin, and PDGF (Bi et al., 2013; Zaika et al., 2015).

bioelectric signals, such as the positive feedback of Notch and V_{mem} in setting the borders of the nascent brain (Pai et al., 2015b) or that of V_{mem} and Pax6 in establishing the eye field (Pai et al., 2012). PDGF upregulates I_K channel expression in vascular smooth muscle cells, implementing a negative feedback loop that controls platelet-derived growth factor (PDGF)-induced proliferation by suppressing the rise of intracellular calcium concentration and its own expression (Bi et al., 2013). These bioelectric states play crucial roles in determining cell participation in organogenesis or facilitating unicellular behaviors (such as carcinogenic conversion and metastasis; Figure 3C; reviewed in Chernet and Levin (2013a).

Numerous molecular components—proteins and biomechanical signals—are widely conserved elements that modify bioelectric state (examples are briefly discussed in Box 1), and transcriptional (and other) events have now been characterized following induced V_{mem} changes. It is therefore tempting to try to describe bioelectric controls in the familiar framework of pathways that focus on specific genes. It is important, however, to note that for understanding instructive influence in anatomical control, the focus needs to be on the *bioelectric state*, not a specific gene product. Since voltage is the result of the contributions of numerous ion types, the same voltage can be induced by the action of many different channel proteins, and the same morphogenetic outcome can often be induced by very diverse ion channels and ion types, as long as the V_{mem} pattern is correctly established (Adams et al., 2007; Pai et al., 2012).

This lack of 1:1 mapping between gene product and outcome is central to the design of loss- and gain-of-function experiments and biomedical interventions, which ought to consider V_{mem} states as the driver variable. Transcriptomic or proteomic profiling gives very limited information for bioelectronics beyond revealing to the researcher what endogenous targets exist for V_{mem} modulation—it is impossible to guess the bioelectric state of the tissue from knowing what the cells express because the channels and gap junctions (GJs) will be open or closed as a result of bioelectric dynamics, past states, and external inputs from biotic and abiotic aspects of the environment (Justet et al., 2013). The situation is even more complex for tissue-level patterns, requiring the use of recently available bioelectric computational modeling tools (Cervera et al., 2018; Pietak and Levin, 2016) to quantitatively understand the dynamics resulting from this divergence of physiology from the underlying molecular genetics. However, while this ability of the exact same cellular hardware to implement a wide range of physiological states makes experimental study more challenging, it underlies some of the most important aspects of pattern homeostasis (such as reprogrammability of target state toward which cells build), which both evolution and biomedicine can exploit.

Bioelectricity is especially relevant as a multicellular coordinating mechanism

Cells connect to their neighbors via gap junctions that allow current and small molecules to propagate among cells and stop at compartment boundaries. The study of large-scale, dynamic bioelectric properties has been advanced significantly by the use of voltage-sensitive fluorescent dyes, ion-channel misexpression, and optogenetics to specifically track and modulate these gradients *in vivo* (Levin et al., 2002). These tools allow both broader and finer profiling and control over the standing bioelectric distributions across tissues than electrodes and classic electrophysiological impalement techniques.

Anisotropic activity of ion channels and gap junctions within cell sheets result in slow, gradual changes in large-scale bioelectric maps across tissue (Figures 3A and 3B). Functional experiments altering these patterns by channel misexpression, knockdown, and in-place optical or pharmacological stimulation have shown that they serve as instructive regulators of gradients of morphogens to align anterior-posterior (Durant et al., 2019) and left-right (Adams et al., 2006; Levin et al., 2002) axes and set the borders of gene-expression domains that establish organs such as limb (Atsuta et al., 2019; Borgens, 1984; Monteiro et al., 2014), face (Adams et al., 2016; Vandenberg et al., 2011), brain (Pai et al., 2015a, 2015b, 2018, 2020), eye (Nuckels et al., 2009; Pai et al., 2012), and heart (Pitcairn et al., 2017).

Numerous examples (Figure 4) in diverse model systems (including *Drosophila*, zebrafish, frog, planaria, chick, and mouse) show that slowly changing spatial distributions of bioelectric parameters across tissue guide organ-level geometry, regulating size (Beane et al., 2013; Iovine et al., 2005; Perathoner et al., 2014; Sims et al., 2009), shape of organs such as the limb (Atsuta et al., 2019) and heart (Iovine et al., 2005; Pai et al., 2017; Pitcairn et al., 2017), compartment boundaries for organ primordia (Emmons-Bell et al., 2020; Pai et al., 2015b),

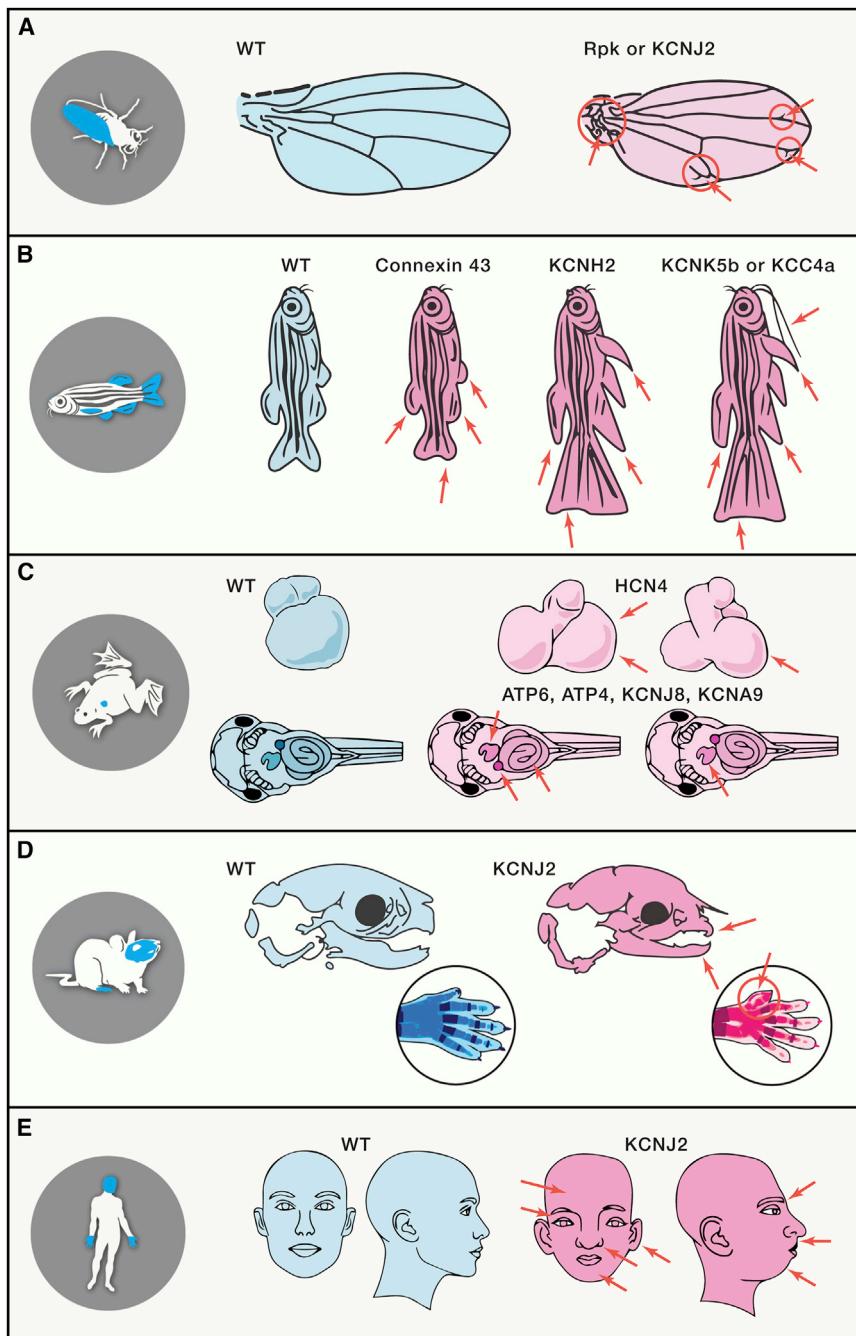


Figure 4. Ion-channel loss- and gain-of-function developmental phenotypes reveal non-neuronal functions of bioelectricity across phyla

Red arrows indicate malformed features. "WT" denotes "wild type" normal morphology for each species' feature.

(A) *Drosophila* wings that develop without functional ion channels such as *irk2*, which encodes a potassium channel or the *Rpk* (Ripped Pocket or ENaC) sodium channel have characteristic defects: they exhibit bristle malformations, abnormal vein patterns, hinge defects, and can be reduced in size or almost completely missing. Schematics are based on phenotypic data from Dahal et al. (2012), (2017) and George et al. (2019).

(B) Zebrafish mutants reveal ion channels essential for developmental control of proportion. Compared to wild-type fish (WT), mutations in *Connexin43* (*shortfin*) exhibit reduced fin size, while gain-of-function mutations in *kcnh2a* (*longfin*), *kcnk5b* (*another longfin*), or *kcc4a* (*scheleir*) induce increased fin size. Barbels, sensory whiskers of the fish, also show variable proportion in *shortfin* and other *longfin/scheleir* mutants, but not in *longfin*. This difference, as well as different characteristics of growth in the mutant classes, suggests that bioelectrics is regulating separate developmental processes during appendage formation. Schematics are based on phenotypic data from Daane et al. (2018), Iovine et al. (2005), Lanni et al. (2019), and Perathoner et al. (2014).

(C) *Xenopus* embryos in which the function of the HCN4 (hyperpolarization-activated cyclic nucleotide-gated potassium channel) is abrogated exhibit cardiac malformations such as twisting, rotation, failure to loop, and double ventricles (Pai et al., 2017; Pitcaim et al., 2017). Targeting ATP6 (V-ATPase), ATP4 (H,K-ATPase), KCNJ8 (Kir6.1), or KCNA9 (KCNO1) results in randomization of left-right asymmetry, including independent reversals of the heart, gut, and gall bladder.

(D) *Kcnj2* (Kir2.1) potassium channel mutations in mice result in deformations in craniofacial and limb structures. Phenotypes include cleft palate, hypoplastic tympanic ring, nasal bones, maxilla, premaxilla, mandible, and enlarged fontanelle. Limb defects include extra digits and digits that are reduced in size. Schematics are based on phenotypic data from Belus et al. (2018) and Dahal et al. (2012).

(E) Human patients with mutations in *kcnj2* (Kir2.1) potassium channels exhibit Andersen-Tawil syndrome, with craniofacial malformations that include a cleft or high arched palate, broad forehead and nose, wide-set eyes (hypertelorism), low-set ears, and a small lower jaw (micrognathia). Individuals with mutations in *kcnj2* often also have fused digits. Schematics based on Adams et al.

digit defects such as clinodactyly (abnormal curvature), brachydactyly (shortened digits), and syndactyly (2016), Bates (2013), and Yoon et al. (2006).

All schematics are courtesy of Jeremy Guay of Peregrine Creative.

and the size and morphology of entire heads (Beane et al., 2013; Emmons-Bell et al., 2015).

The importance of endogenous bioelectronics *in vivo* is reinforced by emerging mouse and human data on genetic channelopathies that present with anatomical defects (reviewed in Srivastava et al., 2020) and unique phenotypes in traditional models such as zebrafish, *Drosophila*, and mouse (Belus

et al., 2018; Daane et al., 2018; Dahal et al., 2012, 2017; George et al., 2019; Lanni et al., 2019; Perathoner et al., 2014; Villanueva et al., 2015). While the developmental bases of these disorders are currently the focus of much research, it is already clear that endogenous non-neuronal bioelectric signaling via potassium channels and gap junctions is essential for normal development (Belus et al., 2018; Lanni et al.,

2019; Pai et al., 2012, 2017; Perathoner et al., 2014; Pitcairn et al., 2017) and regeneration (Adams et al., 2007; Chan et al., 2017; Franklin et al., 2017). In addition to demonstration of necessity, misexpression of ion channels is sufficient to induce predictable bioelectric changes that push developing systems to novel phenotypes, for example, expanding the map of tissues with competency to form organs such as eyes (Pai et al., 2012). During regeneration, induction of novel bioelectric states can permanently modify anterior-posterior axial determination (Durant et al., 2017) or induce regeneration of complex anatomical features appropriate to other species (Emmons-Bell et al., 2015). Thus, human disease variants and mutation of channels and their regulators in model systems have shown that specific bioelectric signaling is both sufficient and necessary for developmental patterning (Bates, 2015).

Recent work in *Xenopus* has shown that the strong defects of brain structure and function induced by teratogens can be specifically reversed by reinforcing specific bioelectrical states (Pai et al., 2018). Remarkably, even the devastating effects of mutation of key neurogenesis genes such as *Notch* can be rescued (Pai et al., 2015b), resulting in normal brain morphology and tadpole learning capacity by misexpression or by activation of native, HCN2 (hyperpolarization-activated cyclic nucleotide-gated) channels. These voltage-regulated channels act as context-sensitive “contrast enhancers” that sharpen differences in V_{mem} across compartment boundaries by hyperpolarizing slightly polarized cells and not affecting depolarized ones. The ability to use these channels to reinforce the native V_{mem} difference that demarcates the edges of the nascent brain (thus setting brain size and shape) was made possible by building a computational model of the endogenous circuit that sets the brain prepattern. This allowed us to understand its spatial properties and how they change during teratogenesis, and to simulate potential bioelectric interventions *in silico* (Pai et al., 2018). The computational modeling not only makes it possible to identify already human-approved bioelectric drugs that reverse severe brain defects in this vertebrate model but also explains why the effect works at long range (via propagation of bioelectric state across tissues), such as from implanted HCN2-overexpressing cells outside the nervous system (Pai et al., 2015a, 2020).

Thus, the field of developmental bioelectricity is entering a maturation phase in which rational control of growth and form can be induced by bioelectric changes specifically derived from quantitative, predictive models. The development of models is essential to understand the origin of bioelectric patterns, and how their dynamics differ from familiar diffusion-based signaling. Elegant recent advances include the modeling of spatiotemporal coordination of bioelectric states in the proto-bodies formed by bacterial biofilms (Larkin et al., 2018; Liu et al., 2017). All of these studies reveal how collective multicellular states show electrical coupling mechanisms that are not readily deduced from biochemical descriptions at the individual cell level (Cervera et al., 2016).

Some of the highest-resolution work merging theory and experiment in this area has been done in synthetic bioelectric tissues, such as cultured cells engineered with a defined set of channels, using optogenetics to control and monitor the estab-

lishment and evolution of bioelectric patterns over time (McNamara et al., 2016). Computational modeling and validation in real cells have enabled the building of bioelectric circuits from the ground up, demonstrating mechanisms that can stably store information in the dynamical state of the bioelectric pattern (Lou et al., 2016; McNamara et al., 2016, 2018, 2020). Moreover, this synthetic platform has revealed the mechanisms by which different geometries of homogeneous tissues support the emergence of specific bioelectric patterns, including shifting domain walls as observed in developmental compartments *in vivo* (McNamara et al., 2018; Silver et al., 2020). This minimal system, highly amenable to quantitative modeling, demonstrates symmetry breaking and epigenetic information storage in the bioelectric layer of cellular physiology (McNamara et al., 2019, 2020), with numerous implications for understanding endogenous morphogenesis as well as the design of synthetic patterning systems that exploit bioelectric mechanisms.

THE SOFTWARE OF LIFE: BIOELECTRIC CONTROLS AS A UNIQUE, REPROGRAMMABLE MEDIUM

Bioelectric controls are a type of epigenetics, in Waddington’s original, broad sense of the word; they constitute control information that resides outside of the genomic sequence. They are tightly integrated into upstream and downstream cascades of canonical regulatory pathways, doing their work within a network of important reciprocal biophysical and biochemical interactions. However, it is crucial to note that bioelectronics is not just another mechanism to simply include in the bewildering cascade of molecular steps that are required for cells to function. Bioelectronics allows evolution to exploit unique and powerful signaling dynamics, as amply illustrated by the importance of electrical circuitry in brains and in engineered computing devices. For example, many channels are themselves voltage-sensitive, implementing feedback loops that enable spatial symmetry breaking (positive feedback) and robustness (negative feedback). Likewise, gap junctions propagate bioelectric signals across cell fields in a manner gated by voltage and other ionic parameters (Palacios-Prado and Bukauskas, 2009), enabling feedback loops to occur on a tissue level, thus setting and responding to changes in large-scale patterns (Pietak and Levin, 2017).

The ability of ion channels and gap junctions to conditionally propagate signals based on the physiological state established by their prior activity implements a historicity—a memory of physiological state. This experience-dependent plasticity is consistent with their role as the precursors of modern neural synapses and enables a very rich time-dependent evolution of bioelectric patterns over time in a wide range of tissues. Thus, channels and gap junctions, being voltage-gated current conductances, are in effect biological transistors, and enable the *de novo* formation and maintenance of large-scale complex V_{mem} patterns changing slowly over developmental time.

These dynamics enable powerful advantages over chemical diffusion alone. Recent computational modeling analyses of single-cell and multicellular bioelectric circuits have shown how combinations of ion channels and gap junctions readily implement single-cell voltage memory, synchronized temporal coordination of voltage patterns across distance, and spontaneous

spatial symmetry breaking. They readily support the emergence of voltage patterns in a transcriptionally homogeneous cell field that can induce Turing-like patterns of spots and stripes in downstream biochemical layers, regulate the steady-state concentration of electrodiffusing molecules in cells, homeostatic control and re-scaling of bioelectric patterns across tissue fragments, and bi-stability (Brodsky, 2018; Cervera et al., 2020; Law and Levin, 2015; Pietak and Levin, 2016, 2017). These features in turn enable the dynamic, long-range coordination and anatomical decision making that is required to orient, scale, and sculpt organs precisely in accordance with the rest of the body during embryogenesis, regeneration, and remodeling. While living tissue is not similar to today's familiar computer architectures, biological regulation exploits three key aspects of the deep concept of "software" as most clearly exploited in computer science. The first of these is reprogrammability—the ability of the exact same hardware to implement different dynamics based on inputs, or experiences/stimuli, not necessarily rewiring of the components or their connections. The second is the separation of instructive data from the machine that acts based on that information. And the third is modularity—the ability to kickstart defined, complex cascades of activity with simple triggers in different spatiotemporal contexts. One of the key aspects of software modularity (and a powerful element of evolvability) is that the trigger that initiates a module does not have to be any one specific gene, mechanistically tied to the mechanisms that follow. In the context of bioelectricity, this is implemented via patterns of V_{mem} : for example, evidence suggests a specific V_{mem} pattern triggers eye development, anywhere in the body, regardless of which ion-channel protein (or even which ion) is used to implement that state (Pai et al., 2012).

Reprogramming anatomy

The ability to stably derive a different anatomical structure from a wild-type genome was originally described in the inheritance of cortical surgical interventions in propagating lines of ciliates (Beisson and Sonneborn, 1965). However, recent work has shown that permanent reprogramming of the entire body plan is possible in complex multicellular organisms as well (Figure 5). A bioelectric V_{mem} prepatterning determines head number and position in planaria, acting within 3 h of amputation (the earliest known steps in anterior-posterior axial patterning in planaria), which drives the correct pattern of downstream head- and tail-specific genes (Durant et al., 2019). Transient perturbation of the circuit via gap-junction blockers that inhibit network connectivity, or via ion-channel drugs that directly reset V_{mem} states, leads to a change of the circuit dynamics that results in a V_{mem} pattern that is depolarized on both ends. This produces viable mirrored two-head worms, and a converse change (targeting the H⁺/K⁺-ATPase) can induce no-head worms, illustrating instructive activity of the V_{mem} prepatterning (Beane et al., 2011).

One remarkable aspect of this circuit is that it exhibits memory (Figure 5C), a property that is shared by even bacterial biofilms (Yang et al., 2020). The animals that are produced by modulating endogenous bioelectric distributions continue to regenerate as two headed when cut in plain water, in perpetuity, weeks or months after the initial brief drug treatment (Oviedo et al.,

2010). In each cut, despite the fact that the ectopic heads are discarded and the genomic sequence has not been altered, normal trunk fragments re-grow 2 heads consistent with the bioelectric pattern memory that has been altered. It should be noted these lines of two-headed worms are the only permanent "lines" (different from wild-type anatomy) that exist in the planarian model system; no naturally occurring genetic lines of divergent morphology have been recovered in planaria, other than this bioelectric induction. This example reveals how the genome builds tissue that can support large-scale physiological (V_{mem}) distribution patterns that are different from the species' default pattern; these patterns are used to guide overall growth and form after injury. This stable change of the pattern to which animals regenerate upon damage does not require transgenes. Indeed, in all three cases—one-, two-, or zero-head worms—the information that determines the body's axial patterning in future rounds of regeneration is invisible at the level of the genomic sequence—it can only be detected (for prediction of morphogenetic outcome) by physiological profiling.

The permanent change of the planarians' target morphology is triggered by a brief modulation of bioelectric circuit states. The ability of short-term stimuli sensed by ion-channel and electrical synaptic mechanisms to affect long-term behavior is a familiar situation in the nervous system (ranging from LTP to complex memory), utilizing both synaptic (in the case of electrical, or GJ, synapses) and intrinsic plasticity. It is now clear that a precedent for this in behavioral contexts also exists in the morphogenetic arena. Multi-stable bioelectric states in non-neural tissues are thought to be similar to neural memory (Pezzulo and Levin, 2015) and use the same, conserved ion-channel and electrical synapse machinery; but they have also been observed in skeletal muscle (Rosenberg et al., 2004), heart (Zoghi, 2004), and cancer cells (Lansu and Gentile, 2013).

Bioelectric control in planaria extends not only to the number of heads but also to their morphology, potentially shedding light on evolutionary transitions. Brief inhibition of gap-junction communication leads to a fragmentation of native bioelectric compartments (Emmons-Bell et al., 2015), resulting in regenerates with radical changes in the external shape of the head (among triangular, rounded, and flat morphologies). Morphometric analysis of the head shape, brain shape, and distribution of neoblasts showed that these regenerates were very similar to other known species of planaria (Figure 6A): without genomic editing, a planarian fragment can be induced to produce heads appropriate to other species across ~100 million years of evolutionary distance, including the species-specific distributions of stem cells (Figure 6B) and brain shapes (Figure 6C). The specific outcome was stochastic, with frequencies of these different species' heads being produced proportionally to the evolutionary distance between them (Emmons-Bell et al., 2015).

One conceptual framework for understanding these phenomena identifies morphogenetic outcomes with attractors in a high-dimensional landscape. This is a familiar idea in developmental biology with respect to cell properties and transcriptional spaces (Huang et al., 2009; Jaeger and Monk, 2014) and can now be extended to the coordination of large-scale order by bioelectric properties. Each attractor in the state space of the bioelectric circuits guiding cell behavior during head regeneration (Figures 5D,

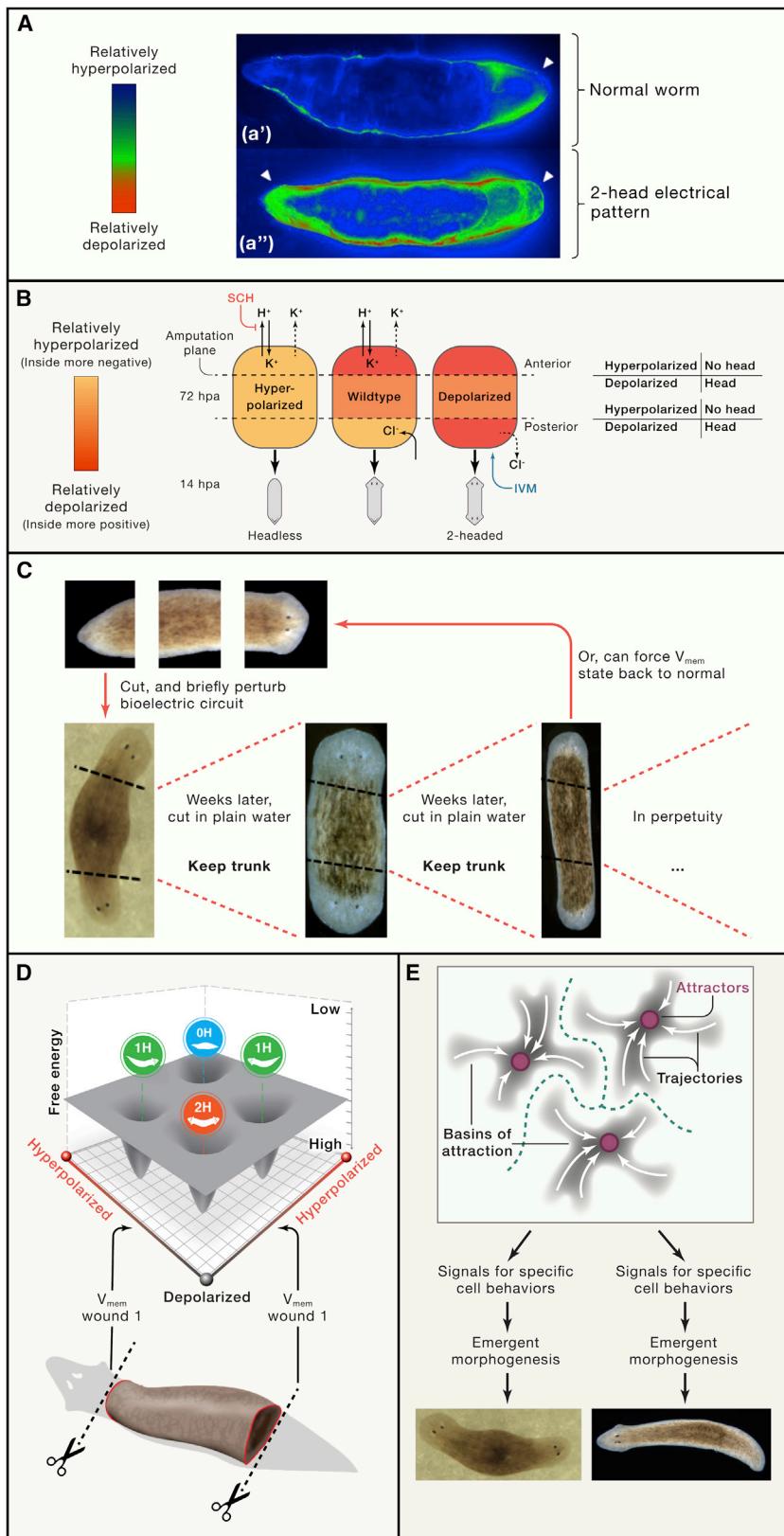


Figure 5. Reprogrammable biological hardware

(A) A standing bioelectrical pattern of resting potentials exhibits one depolarized region in wild-type worms (a') and a mirror-image bipolar pattern in worms that are, or are going to be, two headed (a'').
 (B) An electrical circuit composed of a proton/potassium exchanger pump and several potassium and chloride channels is instructive for anatomy: using RNAi or drugs to target the channels to duplicate or remove the depolarization alters the downstream expression of canonical axial polarity genes and produces two-headed or no-headed worms, respectively.

(C) Two-headed worms made this way reveal a permanent revision of the target morphology: subsequent rounds of regeneration in plain water, long after the reagent is gone from the tissue, continue to make two-headed worms. The pattern to which these cells will build upon damage is stably re-written by a brief (~2-day) modulation of their bioelectric state despite their wild-type genome.

(D) A dynamical state portrait of the combined electrochemical circuit illustrates various attractors that represent stable morphologies for this system, as stable primitive pattern memories in a non-neural connectionist network.

(E) One way to model how bioelectric circuits encode pattern memories is provided by the field of artificial neural networks, where specific memories are represented by attractors in the state space of the network. The tissue-level bioelectric patterns corresponding to each stable attractor in this network trigger different downstream changes in signaling pathways, distributions of morphogens, and transcription. These changes in turn result in emergent large-scale morphogenetic features. The connectionist paradigm from neuroscience thus facilitates models in which information processing events on multiple scales (pattern memories and cellular signaling events) are functionally integrated in the same model.

(A) and (B) are used with permission from [Pezzullo et al., 2021](#) and [Beane et al. \(2011\)](#), respectively. (D) and (E) were drawn by Justin Guay of Peregrine Creative.

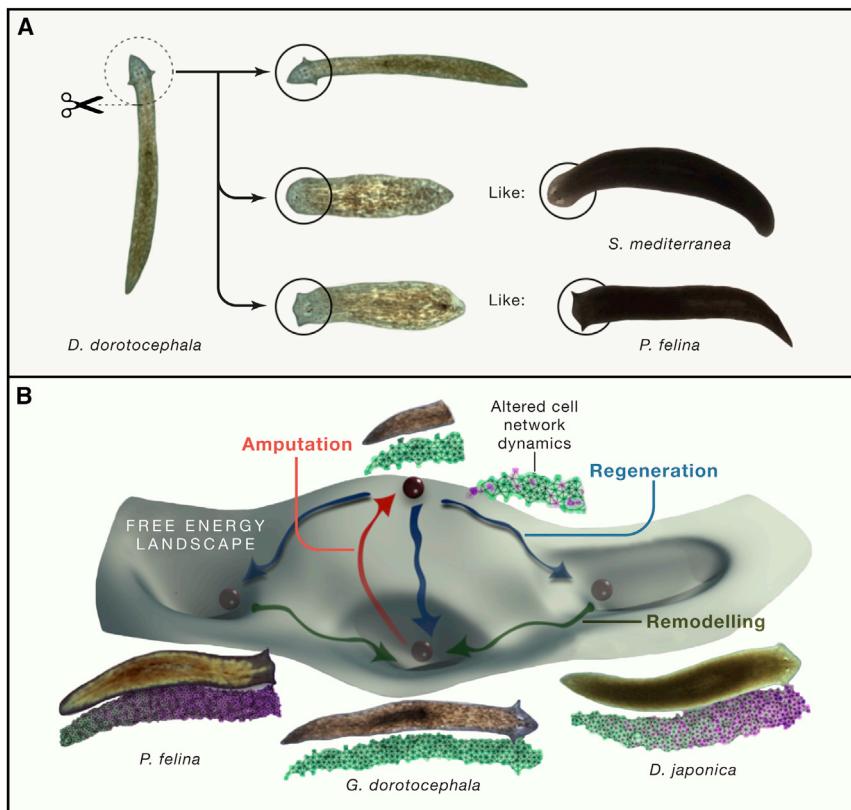


Figure 6. Exploring regions of morphospace belonging to different species, without genomic change

Temporary reduction in bioelectrical coupling within *G. dorotocephala* leads to a regeneration of heads that resemble other species of planaria, including the round heads of *S. mediterranea* and *P. felina* (A). The process is stochastic, and the frequency of each type of head is proportional to the evolutionary distance between them and the original species (Emmons-Bell et al., 2015). One conceptual way to model this is as a morphospace shaped by the bioelectric and biochemical circuits that dictate head shape. This space has several attractors, and the act of decapitation raises the system out of this stable attractor, to a high-energy configuration that it will reduce through regeneration (B). If bioelectric signaling in the network is impeded during the process, the system can stochastically drop into one of the other attractors instead of the system default shape, which it uses reliably during normal regeneration. Cracking the morphogenetic code will involve mapping the circuits to understand all of the endogenous attractors, their role in evolution, and the manipulations that can push the system toward desired ones in biomedical settings. Images of planaria courtesy of Junji Morokuma and Richard Grawne. (B) was used with permission from Sullivan et al. (2016).

5E, and 6B) represents a possible stable bioelectric state, which helps drive specific distributions of morphogens and regulates cell migration and proliferation toward unique anatomical outcomes. The strongest attractor corresponds to the species-default shape, but when the network is transiently disconnected and then allowed to settle back down (minimizing free energy in the connectivity space of the network as gap-junctional links re-establish), the system does not always land in the original region of the bioelectric state and may end up in an attractor corresponding to other head shapes. Interestingly, the transitions of body shape through anatomical morphospace by deforming coordinate grids were modeled by classic work of D'Arcy Thompson (Briscoe and Kicheva, 2017; Stone, 1997), but at the time there were no mechanisms known that could underlie this. With the discovery of chemical (Niehrs, 2010) and bioelectrical (Shi and Borgens, 1995) coordinate grids, it is now possible to formulate and test quantitative models of the potentially multiple stable modes available to any tissue circuit (genetically specified set of expressed channels). Next efforts in this field will continue to flesh out these modes' relationship to anatomical deformations and the physical forces that drive them (Bessonov et al., 2015; Pietak and Levin, 2017; Tosenberger et al., 2015).

Separating instructive information from the execution machinery

Recent computational models have compared the self-organization and robustness properties of bioelectric versus biochemical gradient prepatters (Cervera et al., 2018; Pietak and Levin,

2017, 2018). While biochemical, bioelectrical, and biomechanical processes are all crucial and work together for the control of morphogenesis, there are several unique aspects of bioelectrical signaling. In this context, "reprogram" is used in an expanded sense from its familiar meaning in stem cell biology. It denotes changing the logic of complex, multicellular patterning decisions without having to alter the physical structure of the cellular hardware that implements them.

By taking advantage of the ability of bioelectric circuits to spatially integrate, stably store, process, and propagate information, biological systems acquire an important degree of plasticity. The rules governing the dynamic bioelectric patterns in tissues are in effect a versatile physiological "software" layer functioning between the cellular hardware (specified by the genome) and the variety of anatomical outcomes at multiple scales that can emerge from it. Bioelectric circuits enable considerable divergence between the default states associated with wild-type genomes and actual anatomical outcomes in vertebrates as well as planaria. Tissues bearing strong oncogenic KRAS mutations (otherwise sufficient to drive cells toward tumorigenesis) can exhibit completely normal histomorphology if forced to the normal bioelectric state of their neighbors (Chernet and Levin, 2013b), while normal (wild-type) genomes can support body-wide metastatic phenotypes in the context of forced alterations of bioelectric communication between cell types without exposure to carcinogens or DNA damage (Morokuma et al., 2008). Morphogenesis can also be altered in the absence of genetic change by ion-channel-targeting

compounds, such as topiramate, which act as teratogens (Hernández-Díaz et al., 2012) by modulating bioelectric dynamics despite normal (wild-type) protein machinery. In addition to endogenous (default) bioelectric dynamics during morphogenesis, and their manipulation by experimenters, bioelectric pathways can also be hijacked by genomes other than the host's—for example, by commensal bacteria that produce compounds such as indole that target ion transport (Chimerel et al., 2013); indeed, indole-producing bacteria species can alter head number and visual system patterning in regenerating planaria (Williams et al., 2020).

One key concept is the separation of instructive data from the machine that executes it, a design principle central to many of today's information technology architectures. Biologically, it means that the same cellular machinery can build one of several anatomies, based on specific information-bearing physiological states (Pezzulo et al., 2021). One powerful example is the planarian flatworm, in which several different permanent body plan patterns can be encoded via the bioelectric circuit state. Based on the body-wide pattern of resting potentials, genetically wild-type cells readily build either a default one-headed animal or a two-headed form. The description of head number and layout is contained in the bioelectric circuit and can be permanently edited independently of the genetic specification of the cells themselves (Durant et al., 2017, 2019; Oviedo et al., 2010). In this model, the outcome is defined by the pattern held by the circuit, whether it is the default pattern or a different one. While this has been most clearly seen in planaria, similar phenotypes have been reported for vertebrate face and tail development (Sullivan et al., 2016). This exciting aspect of biological control is becoming especially tractable with advances in synthetic morphology (Kamm et al., 2018) that will push the limits of what wild-type cells are willing and able to make, given appropriate informational inputs. It is an open question as to whether cells are truly universal constructors and could be coaxed to build any desired morphology, once the mapping between bioelectric states and anatomical outcomes is fully understood.

The same advantages that such an architecture offers computer science also greatly improve evolvability of the biological system. It is much easier for evolving organisms to extend phenotypic outcomes if structure and function can arise through plasticity and emergent changes to the set points of anatomical homeostasis; successful variants then can be canalized back into the genome over time. The same property (separation of instructions from execution machinery) has been pointed out for DNA, which can be altered by the experimenter or by evolution and the same cell can execute it. It is tempting to speculate that the massive advantages of bioelectrically based plasticity that brains have capitalized upon had their origins in the somatic discovery of those same advantages for the adaptive navigation of morphospace (Fields et al., 2020). The concept of software reprogrammability goes hand-in-hand with anatomical homeostasis: while the machinery that enables cellular collectives to work toward an anatomical set point (and stop remodeling when it has been achieved) can be kept constant, the biophysical specification of that set point can be changed by processes that, while inextricably linked, can evolve separately.

This important aspect facilitates both evolution and biomedicine; the separation of instructive data from execution machinery in bioelectric control implements a more direct encoding of large-scale phenotypes from tractable physical properties (Lobo et al., 2014). Because DNA does not directly specify anatomy, the mapping from genotype to phenotype is highly indirect—it involves many iterative processes that are nonlinear and hard to predict. The genomic sequence does not directly map onto size, shape, symmetry types, and geometry of the body in general. This gives rise to a crucial inverse problem facing genomic editing efforts: which genes do we target, and how do we tweak them, in order to achieve desired changes in a complex anatomy? In contrast, the mapping for information stored in the bioelectric circuits (and their resulting distributions of V_{mem} across tissues) can be more direct: for example, the bioelectric pattern that guides the development of the vertebrate face reveals the components of the facial structure and their number and locations, while the bioelectric prepatterning in planaria clearly indicates where heads should be formed. This direct mapping feature is what enabled a straightforward computational model that could be analyzed to extract a highly effective repair intervention for the very complex process of brain patterning (Pai et al., 2018). Of course, prepatters of transcription factors and morphogens also exploit this direct encoding property.

Other morphogenetic features (e.g., precise shapes of appendages) have not yet been predictably extracted from voltage data, and much opportunity exists in this field to develop computational pipelines that can infer future anatomical outcomes from bioelectric profiling data. It is likely that progress in understanding bioelectric states and how they map to downstream cellular behaviors will make it much easier to achieve desired complex outcomes by editing this physiological encoding—in effect, by programming cellular collectives at the “software level.” This can be done by discovering and triggering modular patterning subroutines (such as “build an eye” or “regenerate a tail”) by using input stimuli: transient bioelectric patterns induced by opening or closing existing channels or gap junctions. Genetic rewiring of the available protein hardware is not necessary to shape the patterns that endogenously define the geometric targets of cellular activity. This strategy has many implications for biomedical strategies. As molecular characterization of bioelectronics has identified capabilities and phenotypes that have proved very difficult to reach using traditional molecular-genetics strategies (eyes forming outside of the anterior neural field, which the molecular “master eye inducer” Pax6 cannot do in vertebrates [Chow et al., 1999], induction of complete appendage regeneration [Tseng et al., 2010], normalization of tumors [Chernet et al., 2016], and permanent lines of animals with altered morphologies [Oviedo et al., 2010], etc.) in model systems that otherwise offer no genetic mutant lines with permanently altered anatomy. Of course, the native biological software was not written by a programmer but rather is the result of evolutionary selection for optimal plasticity and control features resulting from specific ion-channels' exploitation of the laws of physics and computation. Importantly, recent work in evolutionary computation has revealed how flexible, robust, parallel algorithms can emerge without *a priori* design (Koza, 1992). The next generation of researchers in synthetic morphology will be able to use a

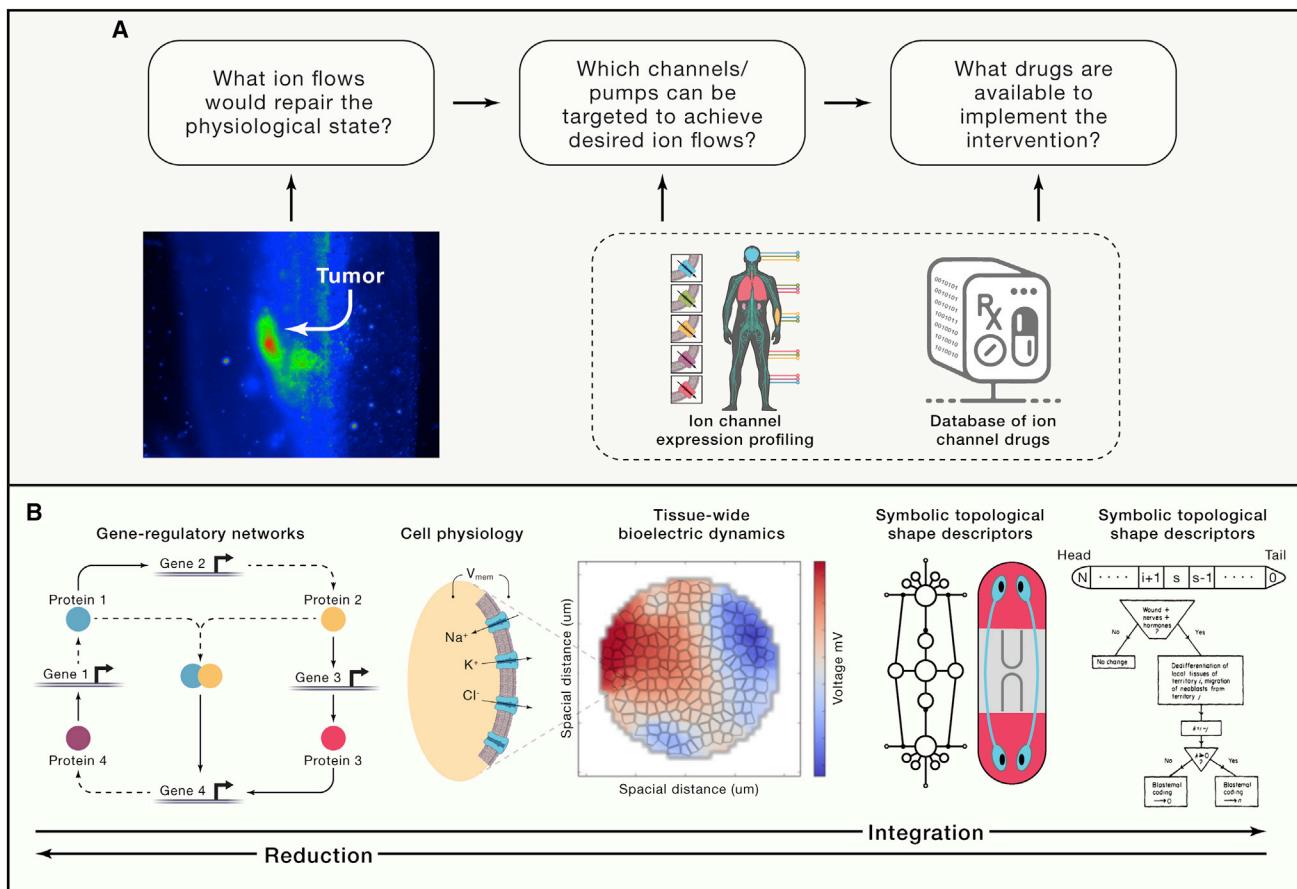


Figure 7. A roadmap for electroceutical biomedicine

(A) The large set of known ion-channel drugs, many of which are human approved, can be exploited in regenerative medicine. Existing transcriptomic databases reveal which ion channels are potential targets for intervention in any tissue and serve as inputs to a computational environment that simulates bioelectric circuits and thus can predict the large-scale patterns that would result from opening or closing specific channels. These *in silico* models can be used to derive candidate interventions—blends of specific small-molecule compounds that target tissue-specific ion channels to induce the desired bioelectric state to trigger repair, remodeling, or normalization, as needed. The image was taken with permission from Churchill et al. (2019).

(B) The long-term strategy is to achieve a multi-scale understanding of anatomical homeostasis that integrates from transcriptional networks that produce ion-channel and gap-junction proteins, to single-cell voltage states, which scale up to tissue-level bioelectric dynamics and implement body-wide circuits that make modular decisions about large-scale anatomical features. Applying interventions at this level will make it much easier to induce and shape complex anatomical outcomes for regenerative medicine top-down, overcoming the complexity barriers that limit bottom-up rewiring approaches. Panels are by Jeremy Guay, Alexis Pietak, Daniel Lobo, and Jonathan Slack.

combination of evolutionary and rational design to create and modulate the regulatory logic of existing organisms or synthetic biobots (Kriegman et al., 2020).

BIOMEDICAL IMPLICATIONS: TOWARD MORPHOCEUTICALS

Bioelectric states are an extremely convenient control parameter for rational control of growth and form, thus becoming an attractive target for design of therapeutics in a biomedical context, for four main reasons. First is their ability to trigger morphogenetic cascades that are too complex to micromanage directly. For example, relatively simple voltage states can induce the formation of entire planarian heads (Beane et al., 2011), or frog tails, including the correct pattern of spinal cord, vasculature, muscles, etc. (Tseng et al., 2010). Second, the bioelectric circuits

tend to function as the early decision-making switch that selects among downstream morphogenetic programs that function long after the initial stimulus is gone. For example, prolonged osteogenic effects occur long after discontinuing electrical stimulation (Eischen-Loges et al., 2018), and the 8–10 days of regeneration of planarian heads and tadpole tails is kickstarted by an electrical state occurring during 3 or 1 h, respectively (Durant et al., 2019; Tseng et al., 2010).

Third, ion-channel-targeting drugs are the 3rd best-selling group of prescribed drugs, and only a few of the estimated 400 annotated ion-channel genes predicted in the human genome have yet been targeted (Wilkinson et al., 2015). The existence of human-approved reagents that induce organ regeneration, repair of birth defects, and cancer normalization (Chernet and Levin, 2013b; Pai et al., 2018; Tseng et al., 2010) reveals ion-channel blockers and activators as a powerful

Box 2. Future steps: Tools, approaches, and opportunities

The future of bioelectronics research includes the following:

- (1) Better integration with genetics: although it is clear that many bioelectric phenotypes involve channel opening/closing, not loss or gain of function at the genetic level, there is still much room for better understanding of the role of mutation in bioelectric regulation, and for understanding the contribution of bioelectronics to genetic change. Transcriptomic analyses often identify channels as differentially expressed genes; for example, in a recent study of positional memory in the zebrafish fin, electrogenic genes were the second highest category observed (Rabinowitz et al., 2017). In a recent screen for size control mutants, the only genes identified were all ion channels and GJs—not transcription factors (Perathoner et al., 2014). Recent work has firmly integrated genetic models such as *Drosophila* (Emmons-Bell et al., 2020; George et al., 2019; Lautemann and Bohrmann, 2016; Weiss and Bohrmann, 2019) and zebrafish (Inaba et al., 2012; Perathoner et al., 2014) with bioelectronics research. The use of optogenetic reporter and actuator lines in these systems, together with the very powerful imaging capabilities, tuned to slowly changing embryo-wide bioelectric states (as opposed to the current focus on neural optogenetics) will lead to significant advances. It's important to note, however, that due to compensation by different channels, single-channel knockouts exhibit a huge false-negative rate with respect to discovery of bioelectric controls. Drug experiments and introduction of dominant negatives targeting a whole family of channels can be a very effective tool to overcome this. Closely related to the integration with genetics is the need for integrating bioelectric signaling with biomechanics, for example, as part of the pathway that involves stretch-activated ion channels in the control of human neural stem cell lineage choices (Pathak et al., 2014). As important as the study and discovery of new channelopathies is, it is essential to scale up the models in this field to the understanding of somatic *circuit disorders* in development and remodeling, as is occurring in neuroscience (O'Leary et al., 2014).
- (2) In addition to drugs and optogenetics, nanomaterials are an exciting new class of tools. Induced bioelectric changes are an important class of effects to consider for bioengineers using nanoparticles (Warren and Payne, 2015). These new biomaterials are being used to control stem cell function via ionic signals (Guo et al., 2016). Macroscopic electrode applications are being revisited, moving beyond neural targets to regenerative contexts such as limb repair (Eischen-Loges et al., 2018; Leppik et al., 2018).
- (3) One of the big barriers in the field is the lack of physiomic profiling datasets, measuring bioelectric state in large numbers of cells from diverse tissues. Indeed, quantification of bioelectric state is an essential component to understanding cell identity and function in the single-cell era (Morris, 2019). New tools are coming on-line and need to be deployed in morphogenetic contexts, such as nanoelectrode arrays (Staufer et al., 2019), photoacoustic imaging (Rao et al., 2017), new fluorescent voltage indicators (Treger et al., 2015), and even label-free V_{mem} imaging (Oh et al., 2012).
- (4) Conceptually, a key future area is the origin of endogenous bioelectric patterns, such as the electric face or the planarian head-tail voltage map. It's clear these can be modified by external influence, like neural patterns shaped by experiential input or drugs, but the default bioelectric prepatterns produced by the genetically specified cellular hardware during embryogenesis are very stable (akin to the instinctual behavioral patterns with which many animals are born as a result of the genetic boundary conditions on brain bioelectric circuits). Computational modeling of symmetry breaking and self-organization in bioelectric states (Brodsky, 2018; Pietak and Levin, 2017) needs to catch up to the huge body of work on Turing-like patterns in the biochemical layer, to understand the conditions under which specific patterns can arise in a field of cells with given ion-channel expression. This will likely play a large role in a better appreciation of physiological sources of stochasticity, such as non-genetic variability. Computational analysis is essential to understand complex features such as memory, bi-stability, oscillation, traveling waves, establishment of stable and moving compartment domain walls, and homeostat controls in bioelectric circuits and their control of gene-expression and morphogenetic outcomes (Cervera et al., 2019; McNamara et al., 2016). The computational modeling of bioelectric states in the body now also has the opportunity to benefit from information theory, as occurred for the bioelectronics of the brain, using new advances in causal information methods to analyze and better control cellular signaling and collective behavior—not merely the mechanics—of bioelectric events at different scales (Moore et al., 2018).
- (5) Synthetic morphology: expanding on recent advances in cell-level synthetic biology, the future of this field involves taking advantage of cells' plasticity to bioengineer novel body architectures (Kamm et al., 2018). By including bioelectric pattern memory reprogramming in the design of these synthetic living machines, it may be possible to achieve top-down programming of their morphogenesis to complex functional specifications with desired behaviors, using a wide variety of cells to explore regions of morphospace far beyond their normal genomic default anatomy.

toolkit of potential electroceuticals—more versatile than electrode-based devices for triggering nerves. Modulation of native ion-channel circuits in all tissues may be a path forward to rapid repurposing of ion-channel and neurotransmitter drugs for regenerative medicine (Birmingham et al., 2014; Gupta et al., 2013). A key component of a operational roadmap (Figure 7) is the development of computational platforms such as EDEn (Churchill et al., 2019), which uses machine learning and computational modeling of bioelectric circuits to infer which channels should be opened or closed to induce the desired large-scale bioelectric state in a tissue of interest (Churchill et al., 2019). This is an essential step toward identifying blends of drugs for reversing disease states.

Finally, bioelectric circuits are not local in their effects, because voltage patterns propagate (McNamara et al., 2020).

For example, limb injury is rapidly mirrored in the bioelectric state of the untouched, contralateral limb (Busse et al., 2018), while both brain repair (Pai et al., 2015a) and tumor normalization (Chernet and Levin, 2014) can be induced by voltage states induced at some distance from the target tissues. These properties suggest the possibility of surrogate site diagnostics and interventions for hard-to-reach locations *in vivo*. However, it is important to note that biomedical applications will require much more work on basic mechanisms in clinically relevant (mammalian) models. It is important to merge the genetic models with physiological datasets, to truly understand how bioelectric interventions will impact canonical regulatory pathways and ensure that such treatments do no harm while respecifying native bioelectric states, biochemical signaling, and cellular behaviors.

An important corollary to the above features is that the ideal bioelectric interventions will permanently re-set patterns toward which cells work. The role of bioelectric circuits in implementing large-scale decision making in cell collectives offers the opportunity to induce repair of disease states (and not simply to address symptoms) by regulating morphogenetic set points for cellular collectives. This class of top-down interventions—“morphochemicals”—contrasts and complements most current approaches that target pathways bottom up. Strategies that force outcomes by manipulation at the level of cell control pathways, as opposed to resetting long-term homeostatic set points, tend to ameliorate symptoms only as long as the reagent is present; they also tend to focus on narrow outcomes that are not integrated with other aspects of the organism and may cause incompatibilities and unwanted side effects. In contrast, triggers of high-level anatomical set points can induce highly coordinated downstream effects, such as the simultaneous, integrated control of organ identity and size, which otherwise need to be individually addressed by direct manipulation of the implementation pathways (Durant et al., 2019).

Computer science achieved a revolution in information technology by moving from programming by physical rewiring of circuit boards in the 1940s to the implementation of hardware sophisticated enough to be reprogrammable, allowing the device to be controlled by “stimuli”—brief inputs (experiences) that take advantage of its intrinsic modularity, decision-making, and information-processing capacity. Analogous to the maturation of computer science, control of the bioelectric dynamics that regulates “organ”-level anatomical subroutines can help move biomedicine beyond the current focus on rewiring the “hardware” of cells to an exploitation of the flexible “software” that guides multi-scale morphogenesis (Box 2). Offloading much of the complexity of building functional organs onto the system itself, by taking advantage of high-level controls of innate plasticity and modularity, may bring about regenerative outcomes on a much faster timescale than attempting to recreate them by micromanaging individual stem cell derivatives and morphogen gradients by hand.

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DECLARATION OF INTERESTS

M.L. is a co-founder of Morphochemicals Inc.

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