Cracking the bioelectric code

Probing endogenous ionic controls of pattern formation

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Patterns of resting potential in non-excitable cells of living tissue are now known to be instructive signals for pattern formation during embryogenesis, regeneration and cancer suppression. The development of molecular-level techniques for tracking ion flows and functionally manipulating the activity of ion channels and pumps has begun to reveal the mechanisms by which voltage gradients regulate cell behaviors and the assembly of complex large-scale structures. A recent paper demonstrated that a specific voltage range is necessary for demarcation of eye fields in the frog embryo. Remarkably, artificially setting other somatic cells to the eye-specific voltage range resulted in formation of eyes in aberrant locations, including tissues that are not in the normal anterior ectoderm lineage: eyes could be formed in the gut, on the tail, or in the lateral plate mesoderm. These data challenge the existing models of eye fate restriction and tissue competence maps, and suggest the presence of a bioelectric code—a mapping of physiological properties to anatomical outcomes. This Addendum summarizes the current state of knowledge in developmental bioelectricity, proposes three possible interpretations of the bioelectric code that functionally maps physiological states to anatomical outcomes, and highlights the biggest open questions in this field. We also suggest a speculative hypothesis at the intersection of cognitive science and developmental biology: that bioelectrical signaling among non-excitable cells coupled by gap junctions simulates neural network-like dynamics, and underlies the information processing functions required by complex pattern formation in vivo. Understanding and learning to control the information stored in physiological networks will have transformative implications for developmental biology, regenerative medicine and synthetic bioengineering.

Introduction to Bioelectricity

It has long been known that all cells, not just excitable nerve and muscle, drive and respond to slow changes in transmembrane potential (V_{mem}).[1,2] Ion channels and pumps segregates charges to opposite sides of plasma and organelle membranes, producing slowly-changing differences in resting potential among cells in vivo (**Fig. 1**). Indeed spatial gradients of V_{mem} distributions exist also at the level of tissues and organs,[3] and have been long-known to correlate with important events in pattern formation such as gastrulation, neurogenesis and limb induction.[4-7] The classical data on developmental roles of endogenous bioelectric signals have recently been revitalized by the development of molecular-resolution genetic and pharmacological tools for the investigation and functional control of ionic signals in vivo.[8-10] Changes in resting potential regulate differentiation, proliferation, migration and orientation[11-13] of a wide variety of cell types, including stem cells, neurons and neuronal precursors and migratory populations such as neural crest. Recent data have implicated spatiotemporal patterns of V_{mem} in the regulation of embryonic development, regeneration and
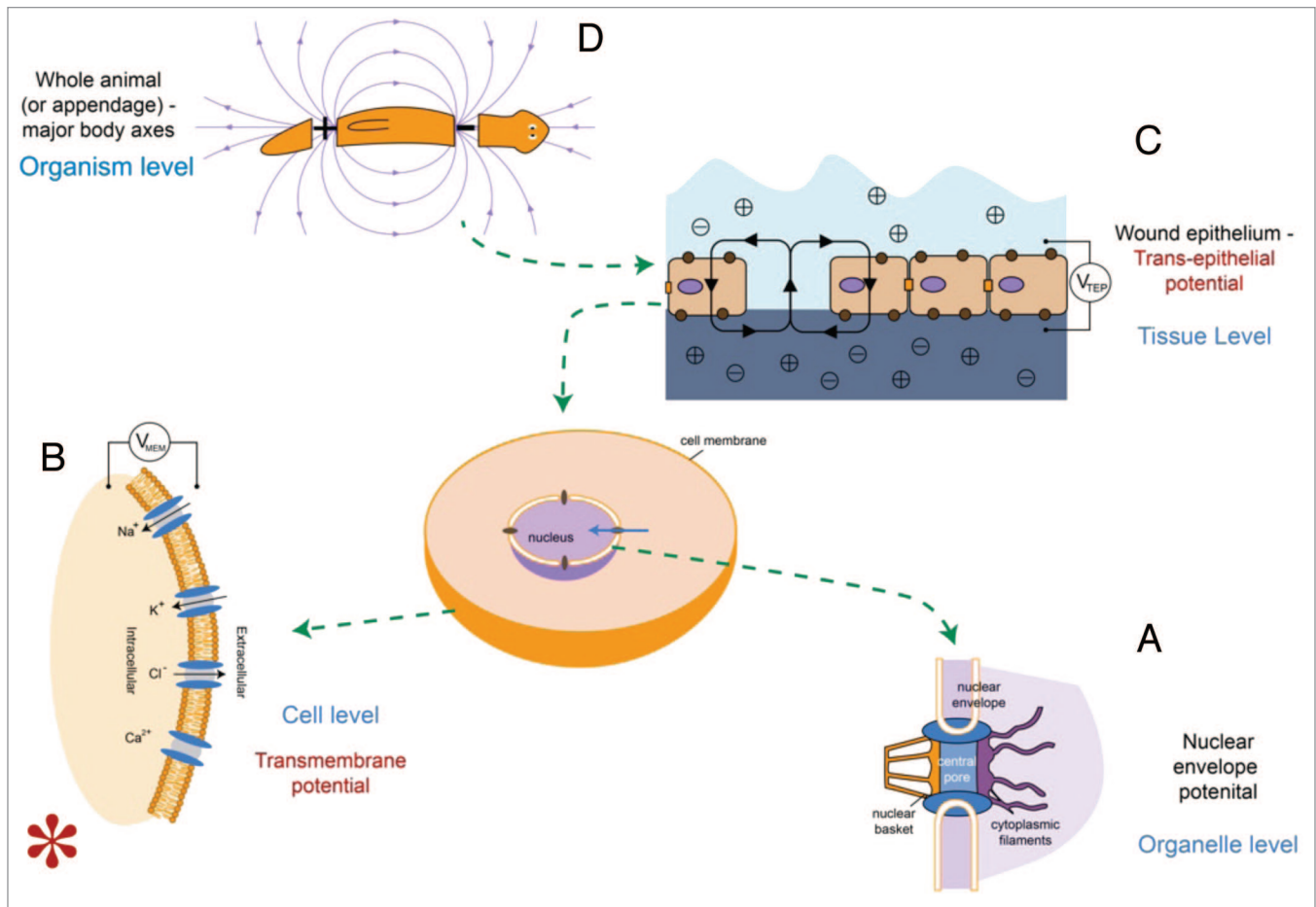
Figure 1. Multi-scale bioelectric gradients in vivo. Segregation of ions by ion channel and pump proteins, and open high-conductivity paths (through gap junctions, cytoplasm and extracellular fluids), produce gradients of voltage potential. These exist at the level of organelles (e.g., nuclear envelope potential, **A**), cells (transmembrane potential, **B**), tissues (transepithelial potential, **C**) and even whole animal axes or appendages (**D**). Schematic drawn by Maria Lobikin.

metastatic transformation.[14] Thus, bioelectric cues function alongside chemical gradients, transcriptional networks, and haptic/tensile cues as part of the morphogenetic field that orchestrates individual cell behavior into large-scale anatomical pattern formation.[15,16]

Recent work in tractable model systems has shown that bioelectric cues serve as mediators of positional information[17] and determinants of anatomical identity during growth and morphostasis.[18,19] Endogenous bioelectric fields mediate wound healing by coordinating epithelial closure,[20] initiate complex appendage regeneration,[21,22] regulate neoplastic transformation,[23,24] determining the left-right asymmetry of internal organs,[25,26] control differentiation of mammalian adult[27] and embryonic[28] stem cells, and even dictate the

large-scale anatomy (producing head vs. tail) of tissues derived from the blastema in regenerating flatworms.[18] Importantly, these coherent changes of anatomy have been analyzed to reveal the mechanistic steps leading from voltage change to cell behavior,[10,29] revealing regulation of small signaling molecules' movement through transporters as a common scheme for transducing bioelectrical changes into transcriptional[23,30,31] and epigenetic[29,32,33] readouts.

Importantly, in a number of cases, it has been shown that the patterning change produced by a specific bioelectric signal (e.g., eye induction, neoplastic transformation, left-right asymmetry generation) is dependent only on the voltage itself, not on the genetic identity of the channel or the chemical species of the ion involved; in most cases, the same

downstream effects on cells' behavior are induced by a given V_{mem} state regardless of whether that state was achieved by movement of potassium, sodium, or chloride ions,[19,23,26,30,34] or of which ion translocators' activity resulted in the given potential change. Because the V_{mem} of cells can be set by post-translational gating of ion channels, pumps and gap junctions (not just at the level of gene expression), this is a true epigenetic layer of control (in Waddington's original sense of the word.) The instructive signal is often borne by the physiological state itself, not the genetic identity of any particular ion channel or pump gene.

Thus, patterning information at the level of physiology regulates morphogenesis in a number of systems. In parallel with the genetic and epigenetic codes, this implies the existence of a bioelectric

code—a quantitative mapping between ionic properties of cellular structures and anatomical outcome. Further progress necessitates a synthesis—a conceptual understanding of how biophysical properties of cells may be interpreted by growing tissues into specific changes of growth and form. Interestingly, recent data suggest three different ways by which biological structures decode bioelectric patterns.

Eye Induction by Control of V_{mem}

The development of voltage-sensitive fluorescent dyes has facilitated the non-invasive tracking of ionic gradients within single cells (**Fig. 2A**) and during complex pattern formation (**Fig. 2B**).[35,36] Moreover, strategies for targeted misexpression of well-characterized ion translocator proteins allow the investigator to specifically depolarize or hyperpolarize select cell groups in vivo during loss- or gain-of-function experiments.[37] These techniques recently converged to shed new light on a paradigm case of embryonic pattern formation: eye development in the frog *Xenopus* laevis.[19]

A survey of spatiotemporal distributions of V_{mem} during craniofacial development[35] revealed two spots of hyperpolarized cells that become eyes; artificial depolarization of these cells by misexpression of a cation channel altered expression of endogenous eye markers and induced eye defects. Conversely, misexpression of a number of channels could drive non-eye-field cells into the resting potential range associated with eye induction (**Fig. 2C**), initiating a feedback loop between hyperpolarization and Pax6 expression, and causing the formation of complete eyes (**Fig. 2D**). Most excitingly, this could occur far outside the anterior neural field (where exogenous Pax6 alone cannot initiate eye formation): eyes could be formed in the gut, tail, or lateral plate mesoderm! These data significantly extend our understanding of lineage restrictions and competence during development; a specific range of resting potential was able to drive cells well outside of the anterior neurectoderm into an eye fate and form complete organs. What are the implications of these data in the wider context of the field of bioelectricity and molecular developmental biology?

## Major Open Questions About the Bioelectric Code

For any code, it is crucial to ask what outcomes are mapped onto which observable properties of the coding medium. For example, the well-established genetic code maps the structure of DNA into the sequence of protein building blocks, but it does not directly specify morphology. Precisely what aspects of pattern are encoded by biophysical properties of cells? Three major (non-mutually-exclusive) possibilities have been suggested by the latest data.

The first is that of spatiotemporal gradients of V_{mem} within cells and tissues as direct prepattern for growth and form, an idea that was first proposed decades ago.[38,39] Recent studies of bioelectric changes in the developing amphibian face[35] reveal that iso-electric domains of voltage regionalize the growing tissue-bioelectric properties distributed across ell fields directly form a template controlling gene expression much as patterns of gene expression (e.g., HOX code) underlie the anatomy of vertebra, limbs and whole embryos. According to this hypothesis, the way to understand bioelectric influences is at the level of control of gene expression domains in tissue, and that this code can be capitalized upon by imposing desired patterns upon tissue by manipulation of the V_{mem} (e.g., repairing birth defects by inducing the right pattern of voltage gradients within tissue primordial that thus correct the expression patterns of key downstream patterning genes). We are currently developing optogenetic[40] strategies that would allow the real-time rewriting of bioelectric patterns in vivo.

A second possible function of the bioelectric code is a mapping of voltage ranges to specific anatomical structures. For example, a narrow range of transmembrane potential is necessary and sufficient for demarcating the eye field (**Fig. 2C and D**) while depolarized or hyperpolarized V_{mem} determines whether a head or tail forms at a wound blastema in planaria (**Fig. 2E and F**). On this model, there may be specific voltage ranges corresponding to individual organs, such as eyes, heads, or hearts (**Fig. 3A and B**); indeed, given the fact that each cell has not one but many domains of different V_{mem} along its surface (**Fig. 2A**), the spatial distribution of voltage values could form an even richer combinatorial code and store a significant amount of information for a cell as well as its neighbors. Thus, it is imperative to discover whether additional voltage ranges are associated with the formation of various organ structures, and if so, whether the quantitative nature of this mapping is the same across different model systems and across individuals of the same species. Note that this view focuses on one organizational level higher than the prepattern model, since here the functional association of specific V_{mem} values is with an entire complex organ, not with direct control of transcriptional patterns within cell fields.

Interestingly, recent data demonstrated the existence of an even higher-level effect. In the tadpole tail, during the refractory period at which the tail normally does not regenerate (**Fig. 3C and E**), induction of proton pumping or sodium influx can kick-start the regeneration of an entire appendage including spinal cord, muscle, vasculature and peripheral innervation. In both cases, very little information was provided by the initiating bioelectrical signal, as we did not modulate the ion flux in any way. These findings were most intriguing since following amputation, we could induce the regrowth of an appendage without knowing, or having to specify, the detailed structure of the organ (a finding with obvious implications for regenerative biomedicine). Instead of micromanaging its assembly (a task beyond today's bioengineering capabilities), the appropriate bioelectric signal is apparently able to activate the host's coherent, self-limiting morphogenetic subroutine corresponding to tail formation. Note that unlike the ectopic eyes and posterior heads (**Fig. 2**), in this case the appropriate structure (heart or tail) is formed at the appropriate location (**Fig. 3**) without our matching the signal to any specific property of these structures. Thus, we wondered whether in some cases, the bioelectric signal also activates a positional information pathway
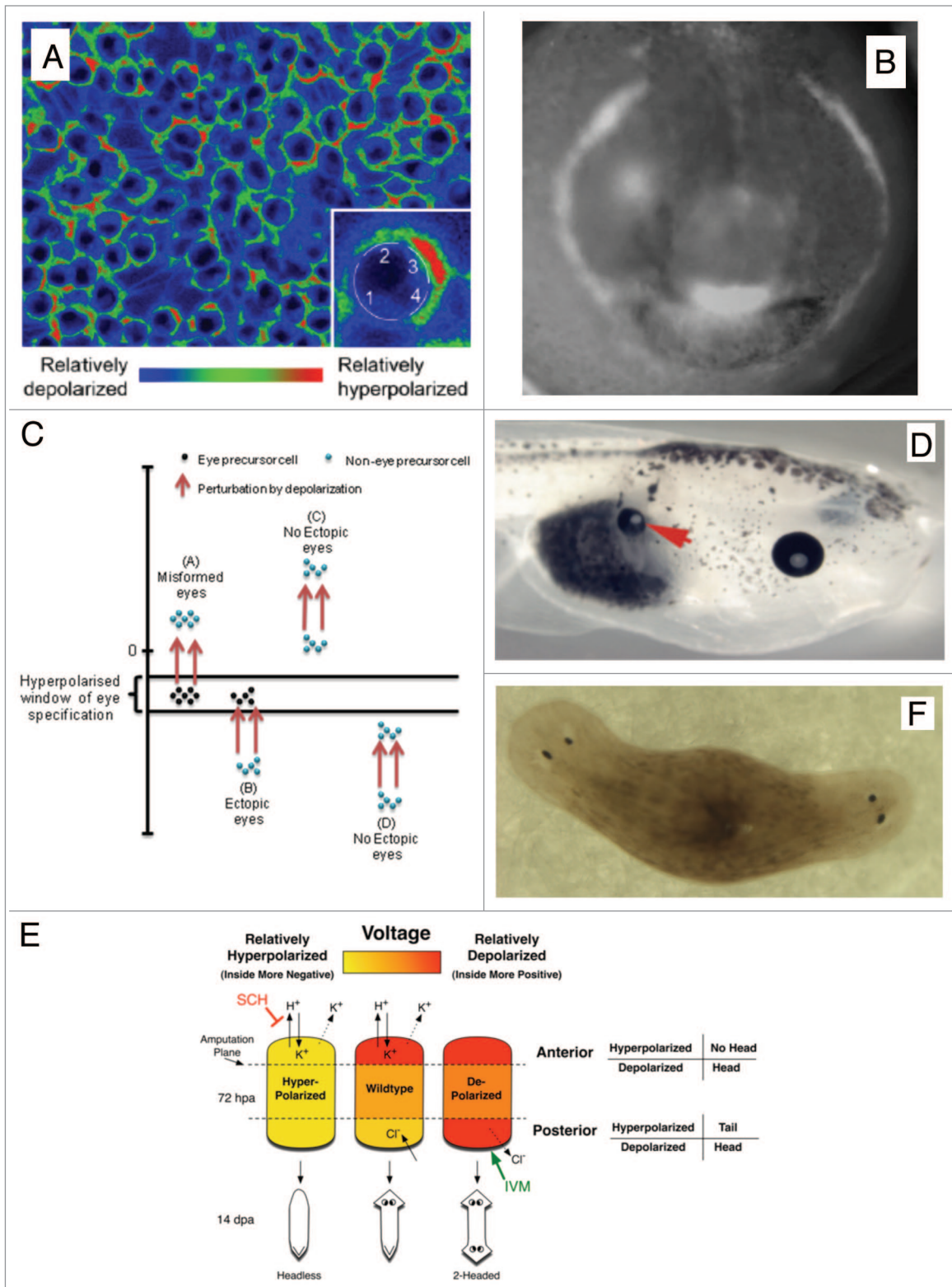
Figure 2. Bioelectric properties regulate large-scale morphology. (**A**) Voltage-sensitive dyes reveal domains within membranes of individual cells in culture—each cell contains not one but a manifold of transmembrane voltage values.[37] (**B**) At the level of organogenesis, patterns of hyperpolarized cells (lighter stain) drive craniofacial patterning in *Xenopus* laevis. These patterns determine gene expression domains (e.g., *Frizzled*) and are instructive for morphogenesis of the face.[35] Artificially setting the resting potential in embryonic frog cells in vivo to a narrow range that corresponds to eye fate (**C**) forces cell groups far outside the anterior neural field (e.g., gut or mesoderm cells) to form complete eyes (**D**).[19] Beyond single organs, manipulation of V_{mem} in accordance with a predictive model (**E**) allows rational control of large-scale pattern produced by the activity of adult stem cells in planaria; panel **F** shows a 2-headed worm in which a pharmacological technique was used to reset posterior blastema identity to that of a head by inducing the bioelectrical polarization value that determines anterior tissue fate.

that guides the induced growth toward the locally-appropriate anatomical identity.

To test this hypothesis, we used the same cocktail that induced tail regeneration (**Fig. 2E and F**) on a tadpole hindlimb amputation. We found (**Fig. 4**) no instances of a tail or any other non-limb structure forming at the wound site; instead, a number of (normally non-regenerative) animals grew back hindlimbs, including the most distal components (toes and even toe-nails); these limbs were sensitive to touch and motile (see Video S1). This remarkable effect is not only promising with respect to biomedical use of this technology to induce repair and regeneration of complex structures, but also reveals that the same biophysical signal can induce different (appropriate) structures at distinct anatomical locations.

Conclusion and Hypothesis: What's Next for Molecular Bioelectricity?

The field of bioelectricity is at an extremely exciting juncture. Molecular-level tools now exist, and a number of recent papers have demonstrated the importance of these physiological signals and shown how they interface to canonical biochemical/genetic pathways.[9,10,13] These data have taught us a number of important lessons. First, that resting potential is not simply a basic parameter needed for cell health. Much as information is routinely encoded in a subtle modulatory signal coexisting with a strong carrier wave in radio-communications, it is possible to experimentally dissociate the bioelectricity needed for housekeeping processes from the developmental roles of voltage patterns. Second, experimental modulation of voltage in vivo is a powerful method for making large-scale, coherent changes in anatomy. Thus, patterns of ionic properties among non-excitable cells are endogenous instructive parameters that not only control basic behaviors of individual cells, but also serve as regulators of shape at the organ and whole organism level. As such, these pathways are a tractable and attractive control point for biomedical strategies in the areas of birth defects, regeneration and cancer suppression.
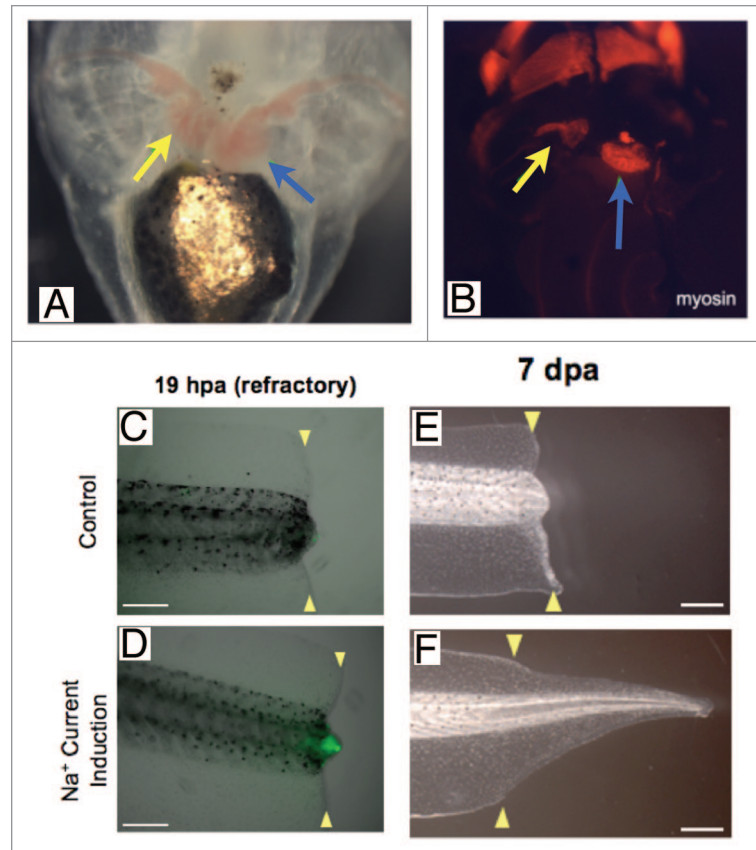

Figure 3. Bioelectric signals produce appropriate organs at appropriate locations. (**A and B**) Misexpression of specific ion channels (unpublished data, obtained by Sherry S. Aw) induces beating, ectopic hearts (indicated by yellow arrows) to be formed next to the original (primary heart, indicated by blue arrows). Likewise, at a stage when tails no longer regenerate in tadpoles (**C and E**) simple induction of a 1 h sodium flux into the tail (**D**) causes regeneration (**E**) of the entire tail.[21] In both cases, the information content of the bioelectric event is very low—a simple signal can kickstart a complex, downstream morphogenetic cascade appropriate to the location within the host.

The next steps in this field[41] must involve expanded efforts to obtain comprehensive physiomic profiling data that can be mined for quantitative analysis of the bioelectric code. Since many different ion channels can contribute to the same V_{mem}, and many different V_{mem} levels can arise from the post-translational gating of exactly the same set of channels and pumps, proteomic or transcriptome analysis inevitably miss information inherent in bioelectric properties. The profiling data, using combinations of electrodes and reporter dyes, must be merged with functional analyses using state-of-the-art methods for manipulating V_{mem} in vivo. One of the most exciting future lines of research concerns the development of chemical strategies for conferring light sensitivity to native ion channels,[42,43] allowing optical control of ion flux with heretofore unprecedented spatiotemporal resolution. As these pathways become better understood, bioelectric elements will be encapsulated as modules that can be plugged into existing bioengineering frameworks, adding greatly to the power of the current set of building blocks in synthetic biology.[44,45]

Looking further ahead beyond the immediate biomedical and engineering applications of bioelectricity, fully unlocking the promise of bioelectricity will require a novel conceptual apparatus with which to understand and learnt to exploit the dynamics of information encoded in the real-time dynamics of physiological (electrical) networks among tissues (**Fig. 5A**). Bioinformatics and modeling tools must be developed
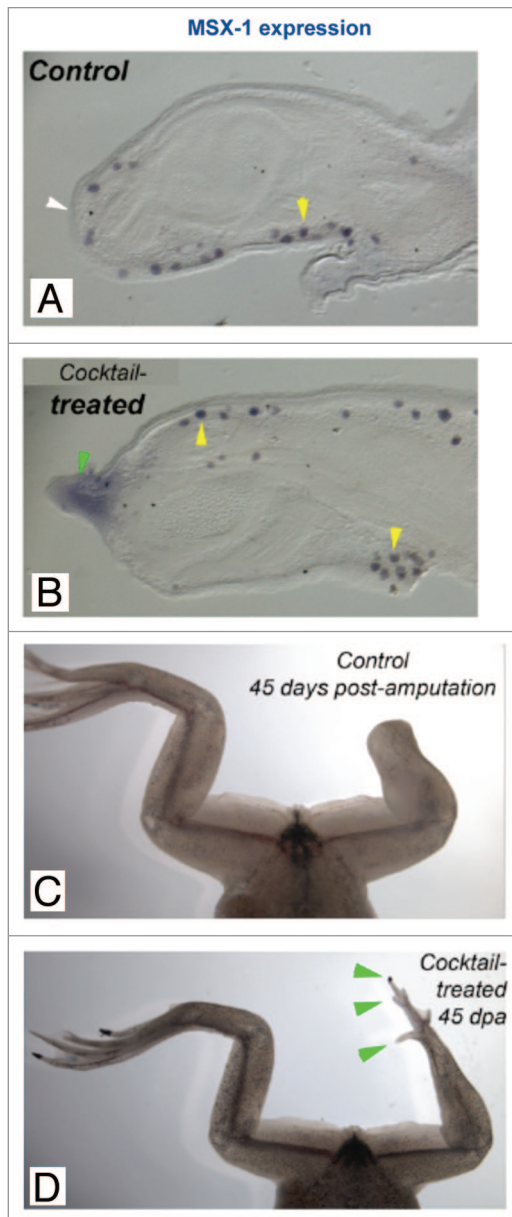
Figure 4. Na⁺ induction promotes limb regeneration. To determine whether the sodium influx that triggered tail regeneration was specific for tail identity or triggered events that interacted with positional information cues (initiated a spatially-appropriate response), we tested the same monensin cocktail that initiated tail regeneration on a tadpole leg amputation model. Hind limbs of st. 57 tadpoles were amputated at the tibia-fibula location. At 1 dpa, tapoles were treated either with 0.1% Ethanol (Control) or with a cocktail of 20uM Monensin and 90mM Na-Gluconate in 0.1XMMR for 1 h. Tadpoles were allowed to recover and grown at 23C until froglet stage. Sectioning during early stages revealed that compared with control limbs (**A**), cocktail-treated limbs upregulated MSX-1, a regenerative marker at the wound edge [green arrowhead, compare with lack of MSX-1-positive region in (**A**) indicated by white arrowhead (**B**)]. Yellow arrowheads indicate additional cells expressing MSX-1 away from the wound (present in all limbs, treated or controls). Within 45 d, compared with very little or no (**C**) growth in controls, relatively improved regrowth at the amputation site was seen in the treated animals (**D**). These regenerated hindlimbs often included toes and toenails (green arrowheads), indicative of proper distal morphogenesis. Notably, in no cases (n = 28) were non-limb structures observed.

models of cardiac and brain physiology are not directly suitable because they focus on action potentials and spiking dynamics, rather than the very different propagation and slow changes of resting potential that occur in non-excitable tissues.

However, we speculate that the tools of cognitive neuroscience can be extended to understand the electrical communication among non-neuronal cells. During regeneration and embryogenesis, organs frequently determine their size, ascertain their shape relative to a target morphology, recognize specific stimuli, exhibit habituation and sensitization to signals, and make decisions based on current information and data stored from prior events. We now know that many of these processes are under control of bioelectrical mechanisms. Is it possible that the ability of organs and tissues to remodel and dynamically maintain/restore specific 3-dimensional shape is a result of computations they perform via electric signaling[46]? The cognitive properties of neural networks, and the success of information processing technologies (e.g., computer science) strongly suggest that bioelectrical communication is a universal and convenient medium by which to control complex events like pattern formation. Although this remains to be tested, such a hypothesis is highly compatible with evolutionary conservation of fundamental mechanisms and the increasingly-observed parallels between developmental mechanisms and neural information processing.[47]

As is beginning to be appreciated for astrocyte and glial networks,[48,49] and has been suggested for bone[50] and even plant cells,[51] many different interconnected cell types could be functionally isomorphic with neural networks (**Fig. 5B**). Resting V_{mem} levels are analogous to the "node activation" of neural net models, while voltage-sensitive, often asymmetric gap junctions mediating cell:cell links can readily play the role of synapses. Thus, our hypothesis is that the existing highly successful theoretical apparatus for information processing in neurobiology could be extended to understand the properties of highly dynamic, self-repairing tissues and organs.[15,52] We are embarking on a

that truly capture the unique signaling properties of physiological networks and merge them with the current crop of techniques that focus on gene-regulatory networks in multi-scale models of complex biological systems. The existing

research program to determine whether quantitative, predictive models of information storage and exchange can be used to explain the cognitive-like functions of morphogenetic systems. This is an inter-disciplinary effort that blurs the line between the mechanisms of spatial information (shape, target morphology) and those of temporal information (pattern in time-dependent signals, learning and memory). We hypothesize that the rich and deep techniques of computational neuroscience can be applied to understand and manipulate the functions of dynamically remodeling tissues. If true, such unification would result in truly transformative advances in synthetic morphology, bioengineering, hybrid cybernetic bio-robotics and regenerative medicine.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Supplemental Material

Supplemental material may be found here: www.landesbioscience.com/journals/cib/article/22595

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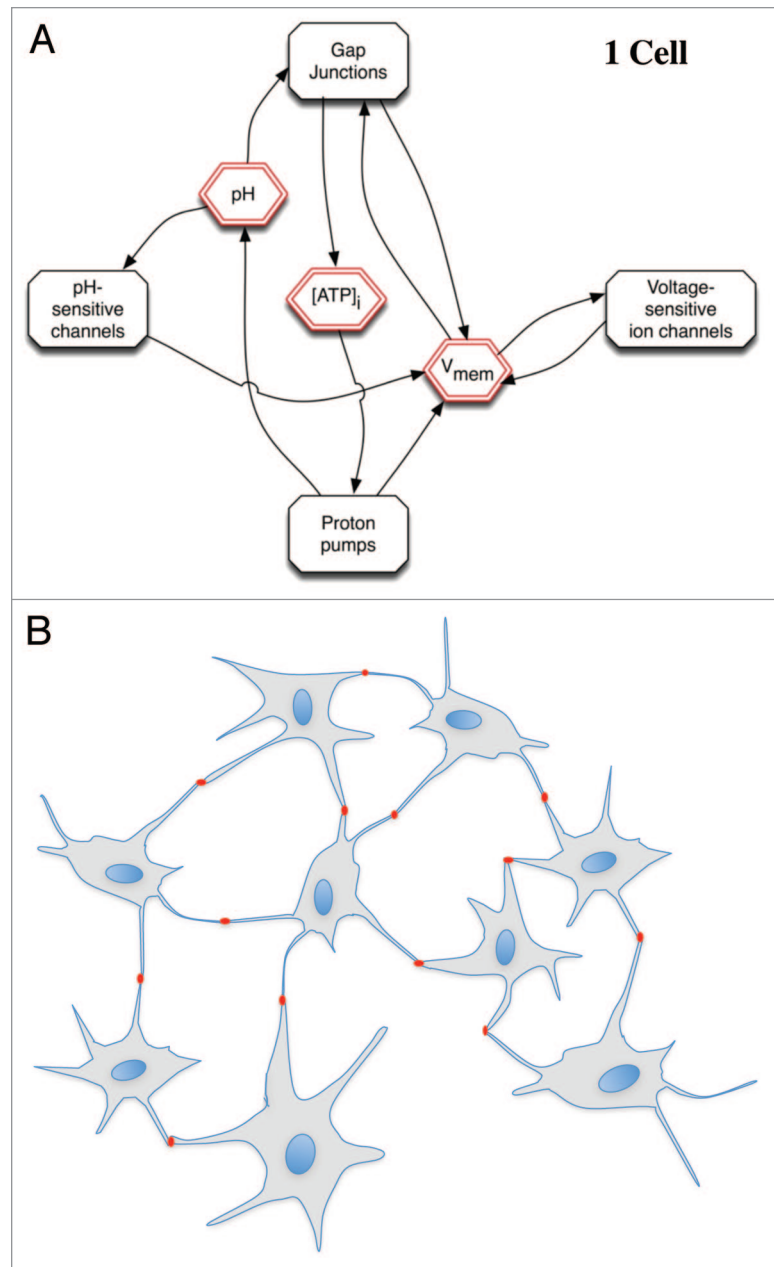
Figure 5. Physiological networks can store information. (**A**) At the level of a single cell, ion translocator proteins are both regulated by, and regulate, voltage. The network of physiological parameters and post-translational states of ion channels and pumps implements rich feedback loops, which can support multistable attractor states and thus store information in the resting potential state of cells.[53] (**B**) Collections of cells coupled by gap junctions implement neural-like networks, since the voltage-sensitive gap junctions play the role of synapses, and each cell has the ability to occupy multiple V_{mem} states based on electrical signals from its neighbors. It is thus very likely that computational tissues could be made from non-excitable cells.

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