Reading and Writing the Morphogenetic Code

Foundational White Paper of the

Allen Discovery Center at Tufts University

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Abstract

Our ultimate goal is the top-down control of complex biological shape. The first four years of our Center's primary work will focus on exploiting developmental bioelectricity to understand how cell networks perform the computations that enable them to coordinate their activity toward robust anatomical target states. Transformative advances in birth defects, regenerative medicine, cancer, and synthetic bioengineering require mastery of these mechanisms and computational algorithms.

Learning the rules of large-scale pattern regulation will enable the ability to specify biological pattern and control its remodeling. Current technology and conceptual schemes target the level of the biological "machine code" – they are all about proteins, genes, and cells. The observables and operational parameters at this level do not refer to large-scale shape and do not facilitate its manipulation. Thus, the field faces complexity barriers with respect to rational control of morphology ("what genes must be regulated, in what ways, to change the shape of the hand, or create a new eye?").

While systems biology seeks to understand emergence of complex form from molecular mechanisms, there is a major disconnect between the plethora of high-resolution data and the ability to control patterning outcomes. A complementary top-down understanding of the information-processing and computation carried out by cells during development and regeneration is largely missing. We will address this profound gap by building new tools to exploit endogenous bioelectric pathways that implement high-level pattern homeostasis and control loops. This will greatly potentiate the impact of the existing and future results of existing bottom-up reductionist approaches, and result in highly impactful new capabilities in regenerative medicine and other fields.

We focus on the morphogenetic code: the mechanisms and information structures by which cellular networks internally represent the target morphology, and compute the cell activities needed at each time point to bring the body closer to that morphology. We will of course use the standard work-horses of modern biology: molecular genetics, biophysics, and developmental physiology. However, we will also include new kinds of computational modeling, with techniques from statistical mechanics and AI. One of the key unique aspects of this effort is that it will, for the first time, deal squarely with an informational approach to morphogenesis. Truly understanding and exploiting the morphogenetic code, especially its highly regulative aspects, requires us to understand not only the molecules and genes involved, but also the algorithms and computations that are performed by cell networks in making decisions about anatomical growth and form.

We will specifically address the current lack of conceptual apparatus for asking and answering questions of what patterning systems know, compute, and represent in their efforts to make and maintain anatomical shapes. We will develop new techniques and software for reading, writing, and rewriting the bioelectrical software that mediates between the genome and morphological outcomes. The results of these four years are expected to explore a new frontier at the boundary between biology, physics, and information science, establishing foundational technology and concepts. The next four years, and future efforts, will seek to transition these basic findings into applications in regenerative medicine, cancer biology, synthetic morphology, bioengineering, and unconventional computation.

Problem space definition

Explaining and learning to control large-scale anatomy, and its regulation toward specific shapes, is a central unsolved problem facing modern science. At its core, it is the task of understanding (and exploiting) the remarkable ability of biological systems to acquire specific 3 dimensional shape: generating spatial order on many scales, from tissues to the entire body plan. How all embryos, which begin as one stem cell (the fertilized egg), reliably build a complex defined anatomy is a major unanswered puzzle. However, stereotypical development is just the first manifestation of a more fundamental property of living things: pattern homeostasis [1, 2].

The problem of reliably generating a specific shape is hard enough, but at least the feed-forward[1] development of a zygote into a reliable anatomical outcome gives hope that complexity theory will explain the eventual reliable emergence of a specific structural endpoint. However, the bigger issue is dynamic morphological homeostasis: beyond forming a single well-defined structure, many biological systems have the ability to repair their complex shape if it's damaged in unpredictable ways [3], and remodel themselves to fit specific anatomical criteria. For example, many types of early embryos, when cut in half, will reorganize, and give rise to two perfect twins. The target morphology of a particular organism is not only a static final state, but an on-going homeostatic process that harnesses individual cell activities toward repairing and maintaining that specific shape. This occurs during aging (as individual cells die but the overall organism continues), regeneration (for example in species that can replace whole limbs, eyes, and hearts), and remodeling (a tail grafted to the flank of a salamander will, in 9 months time, be remodeled into a limb – a structure more appropriate to its new location). This process fails during carcinogenesis, as some cells ignore the normally tight morphogenetic controls and become in essence unicellular organisms, defecting from the bodyplan to grow tumors. Masters of regeneration, such as planaria [4, 5], have also solved the aging problem: body-wide immortality through continuous regeneration (while individual cells senesce and die).

Thus, the profound problem before us is that of closed loop morphogenesis: not only forming a complex shape from constant starting conditions (the fertilized egg), but repairing a shape under deformations that can not be known in advance [6]. How do systems detect when their correct shape has been altered by injury or disease, compute what steps to take to restore their correct target morphology, and decide when to stop growing (after their anatomical goal has been reached)? Regenerating animals rebuild precisely what is missing, no more no less, in the face of external injuries they could not have anticipated. When half of a planarian's head is removed, a perfect match is restored, of the right size and orientation, and then growth stops. Such systems process information about their current state and its deviation from the target morphology, perform computations about what should be done next, and make decisions about what to grow and when to stop [7, 8].

Note that morphogenesis is not just a problem of biology – the core issues of complex self-assembly, robustness, and information processing among cells and molecules, to give rise to macroscopic properties (like "organ size" and "heart is anterior

[1] A developing system progresses forward in time, and pattern emerges from physical forces and cell interactions – a so-called “open loop” process. This is in contrast with “closed loop” processes, in which a system receives feedback, allowing the effects of its actions to modify what it does in the future.

to liver") also impinge on complexity theory, information sciences, and engineering [9-12]. They involve issues of self-organized complexity (relevant for thermodynamics and information theory [13]), the inverse problem of managing outcome from low-level interactions (relevant for computability and systems optimization tasks [14]), robustness, and the control of variability (relevant for engineering and communications networks). Progress in truly solving this problem – cracking the morphogenetic code – entails being able to specify the desired large-scale shape of a biological structure and the properties by which that shape will be dynamically self-maintained. It means mastery over the guidance systems that utilize feedback and memory to *harness individual subunits (cells, pathways) toward large-scale goals like a topologically-correct structure*. Control over these processes would have transformative implications for not only biology and medicine but many other disciplines. Studying top-down control in biology puts us at the edge of a very interdisciplinary frontier, with many transformative implications for other fields that must integrate across levels of organization and control.

A novel approach to overcome current fundamental gaps

The efforts of molecular biologists have made remarkable inroads into identifying the genetic and biochemical components *necessary* for these capabilities. However, there are key gaps in our understanding about what dynamics are *sufficient* for shape homeostasis to occur. This is revealed for example by the impossibility of describing the 3D shape or remodeling properties of an organism or tissue given its gene-regulatory network or genome. We simply do not know how (in the general case) to predict large-scale patterning outcomes from the molecular-level information. The flip side of prediction is control. Consider the process of trophic memory in antler regeneration: some species of deer reproduce a particular rack pattern each year, but if a cut is made in one location, subsequent years' growth will include an ectopic tine in that location [14]. How can the stem cells at the scalp modify their genetic and biochemical pathways so that next year, one specific area of the emergent branched structure will grow an additional branch? The possibility that the growth zone at the scalp maintains an encoded map of the antlers, within which to represent damage sites, and which is used to guide subsequent growth, is a kind of model that has never been explored mechanistically: we do not have conceptual tools today to understand how representational pattern memory could be implemented by biochemical networks. This is an example of a case where large-scale pattern control is not readily reducible to cell-level pathways; however, rewriting this internal pattern memory, if we knew how, represents a much more tractable approach to being able to modify the outcome.

The mainstream paradigm in this field is largely bottom-up: it rests on the hope that systems biology and complexity theory will explain morphogenesis if we know all of the necessary mechanistic details. And some aspects of patterning have been successfully addressed this way [15-19]. However, this is not the whole story, as has been increasingly appreciated in engineering, physics, and other disciplines [20-26]. Limb regeneration for example can be seen as a feed-forward, emergent process of cell interaction guided by gene-regulatory networks and biochemical pathways. This model implies that pattern-changing interventions are to be implemented by providing specific factors in specific locations – an approach that faces important practical limitations due to complexity. Another view of the same system is as an information-processing

homeostatic system executing these steps: 1) if damaged (current pattern doesn't resemble encoded target pattern), then 2) issue commands to individual cells to bring overall pattern one step closer to the target morphology; 3) if done, stop, else go back to #2. This type of model would be defined in terms of pattern memory, anatomical measurements, and error minimization functions, performed by cellular networks. It is one of the foundational hypotheses of this Center that we begin, for the first time, to seek explanations that merge information-level descriptions and control strategies with the molecular mechanisms that implement them. A second foundational hypothesis is that rational design of interventions that manipulate systems at the level of large-scale pattern regulatory modules, not only molecular networks, will provide an essential and heretofore missing level of control over large-scale shape for applications in regenerative medicine, synthetic bioengineering, and many other areas.

Regenerative medicine will not reach its true potential until we learn to control the endpoints we actually care about: the shape and functional structure of complex organs, not only genes or protein levels. Our Center will work on developing an understanding of not only mechanism but meaning, addressing the computational referents of molecular events. Learning to control shape will require development of a "compiler" that allows one to write instructions in terms of a "high level" language of anatomy, and convert it to the "machine level" language of genes and proteins. The immense progress in information technology was made possible in large part by the development of conceptual tools integrating high-level object-oriented descriptors with the underlying physical implementation details that could be subsumed into modular, tractable controls. This enabled altering large-scale functional dynamics without having to address every underlying physical detail. Several disciplines outside of developmental biology have developed practical, quantitative formalisms for describing and analyzing causally-potent emergent controls of complex system behavior [10, 27].

Thus, an important opportunity for fundamental advances in biology is developing a systems-control description of pattern regulation. In complement with the essential work of understanding the molecular details, we have to develop a cybernetic or control-theory view of patterning systems in terms of the information they manage and the computations they perform during pattern regulation. We currently have few conceptual tools for modeling or investigating questions of measurement, control, decision-making, or computation in patterning tissues [28, 29]. Almost all of the existing models are bottom-up, and we lack the tools to quantify and manipulate what patterning systems are measuring, storing, and computing as they remodel to specific large-scale specs. This is highly limiting – analogous to studying behavior and cognition but being limited only to molecules, and never speaking about higher-level descriptors like depth perception, memory, inference, learning, goal satisfaction, etc. Another example is doing physics by studying exclusively the motions of each molecule in a gas, and never reaching Boyle's law and thermodynamics. Even if it were possible to do science exclusively at the lowest level, advances in cognitive science, statistical mechanics, and engineering have long validated the importance of integrating and controlling multiple levels of description.

What is needed is the development of frameworks (both experimental and conceptual) to link high-level descriptors of pattern control (defined at the anatomical level) with the molecular pathways underlying them. We seek to develop a solid,

quantitative understanding of how large-scale information-processing capabilities of morphogenetic systems reduce to molecular events, and how biophysical and genetic processes integrate into systems that make decisions about things that don't exist at that level of description (organ identity, size, and topological placement). There are precedents for successful cross-level integration in the physical and information sciences[2] [33-38], but this has not been done in the biology of patterning and cell regulation. Achieving this would gain for biology what engineering has long exploited to great success: modularity and top-down guidance, which enable much more efficient prediction and control by operating at the most efficient level of intervention. Reduction and integration also make use of "implementation independence" – the low-level details can vary, as long as the functional control loops remain intact (the same computation can be done on a PC or a system of pulleys and string).

Evolutionary theory, cancer biology, and many other areas are now facing the gulf between a wealth of molecular detail and having in hand the key control parameters of a complex biological system. Synthetic and systems biology seek to address this largely via bottom-up approaches to programming metabolism and signaling among single cells in culture, but the control of shape remains largely an unsolved problem. The molecular and computational tools developed in the last decades have been ideally suited for learning about the materials of biology. This facilitates accumulating data on molecular interaction, at ever higher resolution, but eschews key issues of the information content and its encoding for optimal control. Tools for bridging the gap between molecular detail and large-scale outcomes, targeting the algorithms and information content that could enable efficient control of anatomy, have not been built.

Such approaches will inevitably reach a limit due to the inherent complexity of, for example, assembling a hand directly from stem cell progeny, or rewriting the subtle pattern of the human face to ameliorate an incipient birth defect. Bottom-up strategies face the notorious "inverse problem" – the difficulty of knowing what low-level intervention to make, in order to achieve a specific change in emergent outcomes. Ant colony simulations can explain complex behavior by large groups of ants; but which of the ants' few simple behaviors would we need to change, and how, to have them build an ant-hill with a chimney with a different shape? It is easy to plot a fractal image by iterating a formula such as $Z = Z^2+c$, but how to find the formula that will give a specific desired image? Iterative, emergent control systems are only predictable in one direction, which greatly hampers control. Such inverse problems are intractable in general, and put the goals of regenerative biologists and synthetic bioengineers into the far future, if we cannot discover how biological systems represent key large-scale states

[2] Examples include top-down control in lasers (where the emergent property of coherence is both induced by, and controls, microstates that emit light 30. Haken, H., *Synergetics of brain function.* Int J Psychophysiol, 2006. **60**(2): p. 110-24, 31. Haken, H., A. Wunderlin, and S. Yigitbasi, *An introduction to synergetics.* Open Systems & Information Dynamics, 1995. **3**(1): p. 97-130.) and virtual governors – theoretical constructs defined by the properties of several real current generators that are not real but nevertheless define an optimal control policy for regulating the entrained activity of those generators 22. Dewan, E.M., *Consciousness as an Emergent Causal Agent in the Context of Control System Theory*, in *CONSCIOUSNESS AND THE BRAIN: A Scientific and Philosophical Inquiry*, G.M. G. Globus, and I. Savodnik, Editor. 1976, Plenum Press: New York. p. 181-198, 32. Wiener, N., *Cybernetics; or, Control and communication in the animal and the machine*. 2d ed. 1961, New York,: M.I.T. Press. 212 p..

and the computations needed to harness molecular-level details to macroscopic outcomes – top-down control.

It is our hypothesis that evolution achieved highly plastic, self-repairing systems that operate not by solving inverse problems, but by exploiting modularity and feedback loops that represent pattern goal states and computing differences among them. This strategy (control of high-level properties) minimizes the information load on underlying mechanisms and facilitates coherent pattern control. A thermodynamics analogy for using these as potent control knobs is the ability to manage "temperature" and "pressure", not the velocity of each molecule, when regulating a boiler. Synthetic biologists will continue to design effective building blocks, but important barriers stand in the way of traditional systems biology approaches to understand how to derive needed large-scale outcomes (specific patterns with self-repair capability) from those building blocks. To transition cell-level synthetic biology to true morphogenetic engineering [39-41], we must begin to understand how cells process information about current and future shape outcomes.

In a sense, our approach is the necessary top-down component to today's systems biology, learning from the modularity and *information-processing capabilities* of complex animal bodies to help achieve rational design and modification of growth and form. Our work will complement existing approaches and begin a novel way to address this type of problem, by learning from the successful examples of top-down control in the biological world around us. Many model systems illustrate control circuits operating over large-scale, not only molecular properties, and we need to learn to understand and control events at that level. The existence and transition-rules of a morphogenetic code functioning in parallel with genes and molecular pathways, forms the "dark matter" of biology today. Given that we want to control organ identity, placement, and topological arrangement, how do we practically interface (at the bench) with these high-level metrics and top-down control knobs?

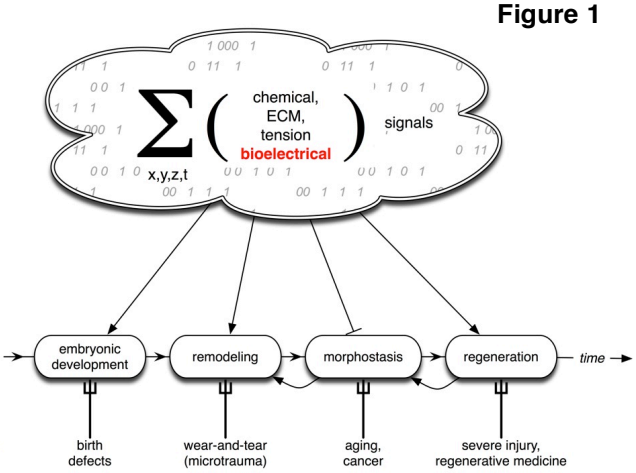
Bioelectricity: an inroad to new biology

Top-down approaches have not been attempted to date (despite classical theoretical discussions [42-45]) because current paradigms of molecular biology do not readily reveal how cells can implement information-processing functionality that makes decisions about large-scale properties and acts with respect to encoded goal states. However, we do have a robust science that has done exactly this: computational neuroscience. We definitively know from neuroscience that it is possible for cell networks to implement memory, representation, distributed processing, and goal-directed activity that integrates signals and provides flexible, robust outcomes – the brain does this routinely, harnessing molecular signaling within cells toward global cognitive states. Importantly, neither the molecular mechanisms (ion channels, electrical synapses, and neurotransmitters) nor the algorithms of decision-making and memory arose *de novo* in the brain. Instead, they evolved from far more primitive cell communication events that brains merely optimized for speed when the CNS evolved [46-48]. As we know from computational neuroscience and computer engineering, networks of flexible electrically-controlled voltage gates are an ideal medium for computation, memory, and plasticity. Crucially, all cells express these ancient components, not just neurons.

We conjectured years ago that bioelectrical signaling should have been exploited by evolution as an ideal modality for mediating the modular computation and information processing needed for top-down pattern control in development, regeneration, and remodeling. The Levin lab has been pursuing numerous predictions of this idea, and was the first to develop molecular approaches to monitor, model, and modify electrical potential distributions in patterning systems *in vivo* [49, 50]. We [51-75] and others [76-78] have now used these methods to test the implications of this unique hypothesis [79-81]. This body of work has revealed that in parallel with the genetic code (ideal for making sure the right protein components are available in the right place and time), and the epigenetic code (used for tweaking gene expression as a function of physiological history), there is also a bioelectric code – a dynamic distribution of electrical properties in somatic cell networks which mediates large-scale coordinated information processing in pattern homeostasis, orchestrating cell activity toward large-scale anatomical states.

Bioelectricity is one layer of a complex morphogenetic field (Fig. 1) that harnesses individual cell behavior towards the anatomical needs of the body. Bioelectric signals interact with chemical gradients and various physical forces, in numerous bi-directional loops. However, it is not simply yet another mechanism of single-cell control. It is a convenient and tractable entrypoint for understanding and rationally controlling information processing that maintains large-scale order *in vivo*. Much as cadherin proteins allow gene networks to harness the physics of adhesion [82] and thus exploit a whole new set of dynamics for multicellularity, ion channel and electrical synapse proteins allow cell networks to harness the unique properties of computation and implement local and long-range signaling with extensive plasticity (history, memory). Our efforts in this Center are focused on understanding and gaining control of this powerful unexplored property.

Figure 1



Bioelectric circuits form when the function of ion channels and pumps alters resting potential, which in turn (Fig. 2A) can affect voltage-sensitive channels and electrical synapses. These circuits can exhibit complex dynamics in connected cells, and form a layer of control with their own intrinsic behavior (and self-organizing capabilities) that

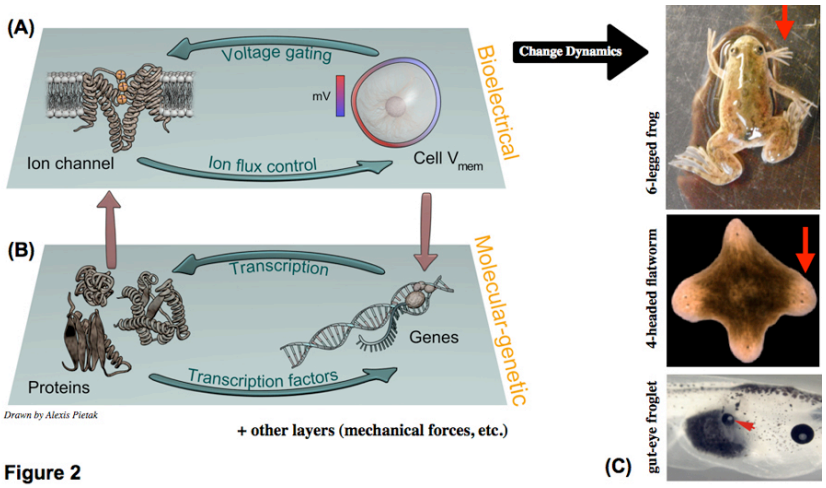


Figure 2

couples to, but is distinct from, the transcriptional networks studied by most (Fig. 2B). We now know that modulating the dynamics of this regulatory layer enables coherent large-scale patterning changes [83] (Fig. 2C).

Bioelectric signaling has several unique properties that make it an ideal pressure-point for the Center's directions. First, it operates on multiple scales of organization: we have shown that specific bioelectric states define properties at the cell [84-91], organ [52, 53, 64], and whole organism axis [66, 73, 92] levels. Second, bioelectrical states provide a convenient and tractable method for over-riding genome-default outcomes. For example, modulation of voltage states can induce eye formation in body areas where the master eye regulator Pax6 normally induce eyes [64], remodels heads to shapes belonging to different species (despite a normal genomic sequence) [57], and rescues normal brain patterning and function despite the presence of mutated neurogenesis genes such as Notch [52].

Perhaps most crucially, briefly altering the bioelectric connectivity of a cellular network enables permanent rewriting of an organism's target morphology [93]: genomically-normal worms can be changed to a 2-headed form that regenerates with 2 heads in perpetuity, illustrating the ability to stably re-wire bioelectric circuits with permanent changes to the overall anatomy (Fig. 3). These data identify exciting gaps in knowledge and opportunities with respect to novel control points for reprogramming besides genome editing: stable physiological circuits that store information.

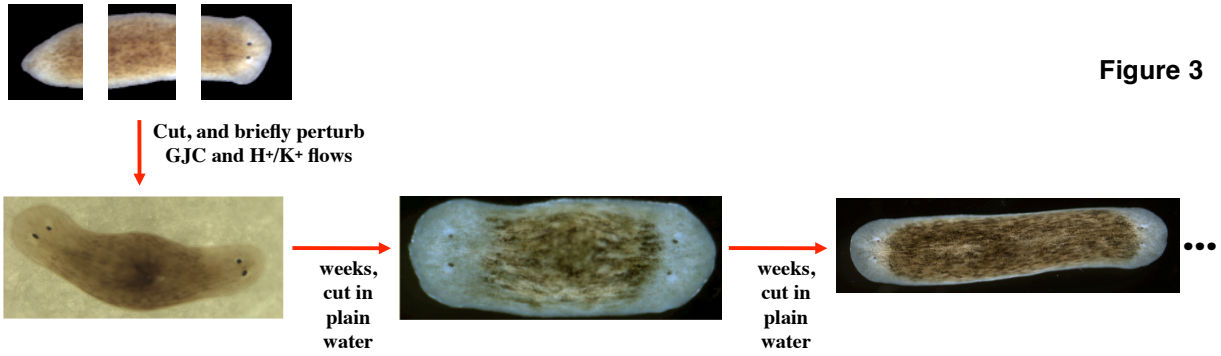


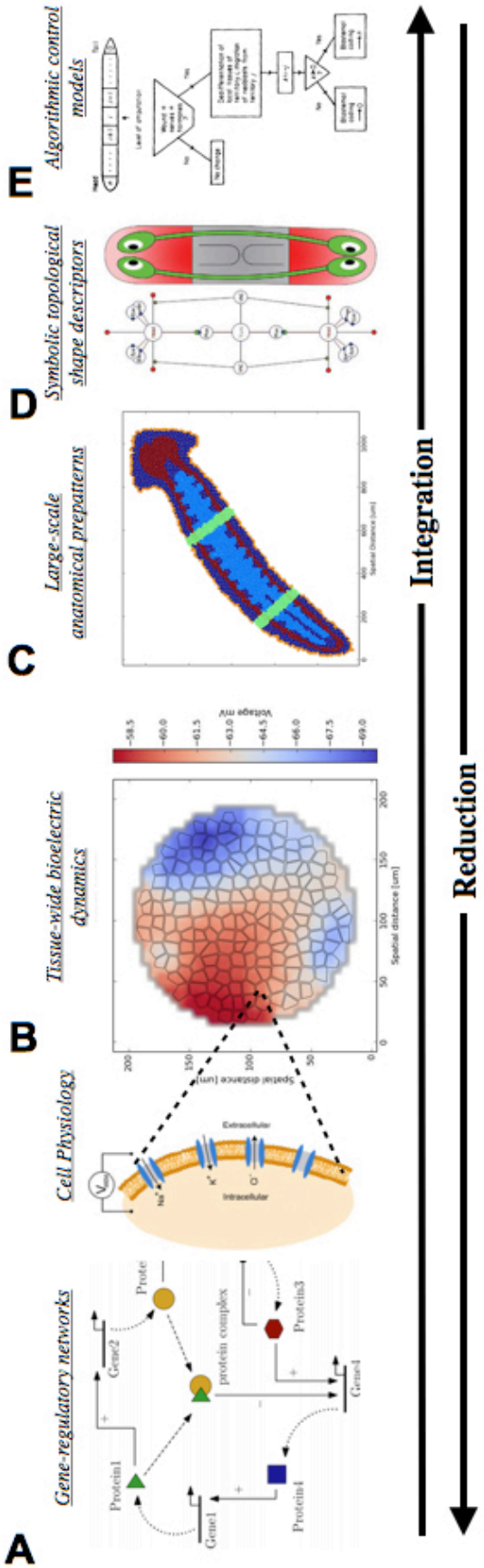
Figure 3

The "bioelectric code"[3] is defined as the mapping of real-time electric circuit dynamics among tissues to the pattern-regulatory functions that cells carry out. What we have learned, after 16 years of focused effort in this field, is that bioelectrical signaling 1) exerts profound control over large-scale morphogenetic properties in a range of model systems [64, 66], 2) facilitates exploiting native modularity (such as triggers complex downstream patterning outcomes as a kind of master regulator) [75, 94], 3) is transduced by a set of known mechanisms into downstream chemical signals (neurotransmitters and other morphogens) [65, 95] and gene transcription changes [51], and 4) forms feedback loops with genetic pathways, often over-riding competing signals from other modalities [64, 89, 96].

Fundamental gaps in knowledge include [9, 83, 97-102] answers to several questions. How is the mapping defined by cells (the origin of the code)? What level(s) of organization is the interpreter of the code (molecular networks, cells, tissues, etc.)? How can the code be interpreted (by cells or by morphogenetic engineers)? Whence do the

[3] The definition of this process as a "code" (implying messages, senders, and receivers) is consistent with our plan to include approaches of control and signal theory to address this fascinating aspect of biology.

Figure 4



initial bioelectric prepatterns arise? What are the transition rules between specific bioelectric states? How does the code change during development, regeneration, and aging? What is the best framework for computing interventions that can be applied to re-write encoded pattern memories and thus control morphogenesis top-down? What are the limits of the morphogenetic editing allowed by this code (what shapes are possible to achieve). Answering these questions, and harnessing new modalities for reading and writing the bioelectric code toward pattern control would be the goal of this inaugural Allen Discovery Center. Our work will thus have several parallel components: understanding the information processing (theory), and developing practical mechanisms for manipulating the pattern control loops *in vivo*.

Implementation plan: 1st four years

Driving vision hypotheses

- Instructive information is encoded and communicated in tissues via bioelectric code
- This code facilitates top-down, modular control of pattern formation
- It is possible to learn to interpret and write the code, for modifying the information control layer of patterning (1st 4 years)
- This will have huge implications for evolution, regenerative medicine, birth defects, cancer, bioengineering, and computer architectuires, all of which we can exploit via specific applications in the subsequent 4 years.

Our sub-goals will be to (1) learn to read the code, (2) learn to interpret the code, and (3) learn to write the code *in vivo*, in complex animal models. The intellectual mindmap of our project components is illustrated by the schematic on the left of this page.

Each of our efforts falls somewhere along the scale (represented here in planarian regeneration) from (Fig. 4A) molecular networks (GRNs), to (Fig. 4B) bioelectric

circuits in single cells and tissues [56, 103] (image obtained from work by Alexis Pietak), to (Fig. 4C) large-scale gradients [66, 104], to (Fig. 4D) symbolic representations of the kind we use for AI-based model inference [105-109], to (Fig. 4E) algorithmic descriptions of the growth process (taken from [15, 110]). The goal is to learn to control the high-level properties such as organ placement, size, and shape (Fig. 4D,E), by interventions that manipulate properties of networks that encode these large-scale features (Fig. 4B). We will learn how to make changes in the algorithmic process that regulates large-scale features (Fig. 4E) by taking advantage of the representation and mapping of these features within cell signaling dynamics (Fig. 4B-C).

Each of the participants' subprojects contributes to a key aspect of the overall vision and will be merged together into a multi-scale integration of low-level components with maximal molecular realism, with high-level features (with optimal enablement of predictive control). The current subprojects include: understanding the mechanistic interactions between voltage and small RNA/chromatin pathways (Fig. 4 A←→B), developing and testing models of self-organization, time-dependent evolution, and optimal control of bioelectric patterns (Fig. 4 B←→C), identifying technologies for, and endogenous modifiers of, bioelectric gradients (Fig. 4 C←→D), and characterizing and exploiting mechanisms of bioelectrically-mediated decision-making on a global scale (Fig. 4 D←→E). Each project implements the *intersection of 2 components* (2 panels of the mindmap, not one) in line with the goal of ensuring that everything melds together into a coherent body of knowledge.

Overall Approach

The morphogenetic code (Fig. 2) is a large problem; it requires new technical capabilities and new conceptual insights, not simply more molecular "omics" data. Our plan for the first four years of the Allen Discovery Center at Tufts is a focused attack on this next-generation problem. The overarching approach is to develop tools and models that facilitate the control of large-scale pattern *in vivo* (re-writing pattern), and gain a mechanistic understanding of patterning systems at a computational level - the missing flip side of molecular reduction strategies. The project will be performed not only at the Levin lab at Tufts, but also by a set of key participants, both external faculty in specific areas, and internal personnel. Inclusion of these other labs not only provides essential expertise for important project areas but also enables the degree of parallelization needed to make significant impact in a 4-year timeframe. The participants list is meant to be kept fluid during the 8-year timeframe – early efforts will identify the most promising inroads, and additional expertise can be brought in later as needed.

Our plan is to immediately begin to construct necessary instrumentation and produce transgenic animal models. At the same time (year 1), work will immediately begin on identifying mechanisms by which the bioelectric code controls, and is controlled by, 2 key biochemical pathways that have never been linked to voltage regulation: small RNAs and chromatin remodeling. This is an essential component to fully dovetail bioelectrics with the existing body of mechanistic work on morphogenesis and expand the understanding of how physiological state transitions can be expected to regulate significant other signaling modalities. Since the DNA content of different cells in multicellular organisms is essentially the same, cellular memory, which is key for any developmental process, is stored, maintained and read in part by epigenetic processes.

There are multiple epigenetic layers, and in the project we will focus on the connection of two main mechanisms, chromatin modifications and regulatory small RNAs, to bioelectric pathways. Recent work shows that transgenerational memory can be transmitted in part through heritable small RNAs and chromatin marks [111]. In some model organisms, it is now clear that the expression of every gene in the genome is further regulated by heritable epigenetic information; thus, information encoded epigenetically allows both stable and dynamic control over gene expression [112]. Bioelectric gradients are an ideal mechanism for allowing cell-level memory (mediated by small RNAs and chromatin states) to amplify into body-wide stable pattern storage.

By year 2 we will be constructing quantitative models of the bioelectric code at several levels (Fig. 4), and begin developing specific proof-of-principle applications that test, validate, refine, and utilize those models to predict the nature of novel patterning control. Our model systems include a mix of regenerative non-mammalian models (in which to learn about the rich endogenous mechanisms) and mammalian tissue culture applications which are important to maintain continuous relevance to eventual biomedical endpoints as well as to serve as test-beds for validating novel concepts from the ground up. Because this project is necessarily “compass-driven” (it’s a frontier area and the major discoveries cannot yet be predicted), our goal is to perform work in the first year to help identify the most promising areas and help shape the emphasis of subsequent years. A nimble directionality will be used to set priorities in all years, but it is important to note that especially in the beginning we will explore several promising directions and then will narrow down to the ones whose data suggests them to be the most likely to be impactful and rewarding (not try to pursue everything at once).

Our plan has 2 global components, addressed via a highly interdisciplinary combination of new theory, device development, and focused experiments. We will 1) answer several fundamental questions for the first time, and 2) enable new capabilities for pattern control. Our process is meant to be iterative feedback between the development of new theory, instrumentation, and specific results. The first year will be largely mechanistic investigations and platform development. But by year 2, we will begin testing models and immediately performing key experiments in pattern control that will allow us to revise the models, alter apparatus/protocols if needed, and identify new but important unknowns to be addressed. Our deliverables will not only facilitate this Center's effort, but be widely enabling technologies for others, multiplying the impact on many subfields. Our second goal is to use these new tools and conceptual apparatus to answer key questions and generate new basic knowledge that alters prevailing paradigms in this area, and rewrites fundamental assumptions present in textbooks in the field. In the second phase, we plan to continue developing new knowledge in areas identified in phase 1, and to begin to transition the new knowledge into proof-of-principle applications targeted towards regenerative medicine and engineering.

Some fundamental scientific details

One unique aspect in our work is that we will address bioelectric signaling: the ways in which networks of all cells (not just excitable nerve and muscle) use gradients of resting potential to regulate morphogenesis. Our lab and other groups using our technology have begun to show bioelectrical control of stem cell function, organ size,

tumor normalization, craniofacial and brain patterning, induction of limb regeneration in non-regenerative settings, and production of complete eyes, limbs, and other organs from other types of somatic tissue *in vivo* [79, 81, 83, 97-99]. It is now abundantly clear that modulating bioelectric gradients can induce rational, coordinated changes in large-scale anatomy; it is extremely efficient in exerting top-down control over shape, and coordinates numerous downstream steps that would be too difficult to micromanage directly. It is an ideal modality for exploiting developmental modularity to activate morphogenetic "subroutines". What is not known is how cellular networks can represent target morphologies and enable the step-wise, iterative computations by which bodies determine what's missing and coordinate cell-level instructions toward large-scale goals.

Elucidating the large-scale representation of somatic pattern by bioelectric states is a key next challenge in this field and our plan includes a concerted attack on this fundamental problem. Our work will not focus exclusively on bioelectricity – clearly, the complete answer will require the integration of bioelectrical signaling with biochemical gradients, gene-regulatory networks, and physical forces, because these other properties are used by cells during morphogenetic processing. However, bioelectric signaling is an essential, novel element in cracking this puzzle, because a significant part of the morphogenetic code is encoded in bioelectric properties and because it seems to offer control over coherent modules, not only cell-level details.

Cells communicate using a variety of modalities, including secreted chemicals and pressure/tensile forces. However, bioelectricity is special. It is not an accident that evolution has chosen bioelectricity as the modality that underlies information processing in the brain. It is likewise not an accident that our information technology relies on electricity for computation. Electrical dynamics are ideally suited for computation - precisely what is needed for cells to continuously regulate after unpredictable injury and perturbation. Also, bioelectrical signaling is uniquely suited for integrating long-range signaling, which is required in order to coordinate individual cell behaviors across the whole organism (like the remodeling that ensures that embryonic organs and regenerating structures grow in perfect proportion with other regions of the body). The fundamental unit of computation, the logic gate, is easily constructed of transistor-like elements, which - both in the brain and in the body - is made of gap junctions: channels between cells that regulate current flow based on local voltage. Modulation of gap junction-based somatic networks is a key aspect of developmental bioelectricity, because this is an untapped entry-point into how cell networks represent patterning information.

The striking data showing that rewriting the bioelectric circuit dynamics leads directly to the reprogramming of shape *in vivo* suggest a new metaphor for understanding morphogenesis. The current textbooks say that the DNA is the software while the cell is the hardware that interprets it. We believe this metaphor needs to be revised to encompass the true circularity of the process: the DNA determines the hardware (by encoding the specific gap junction and ion channel proteins that can support electrical dynamics in cell networks), while the resulting bioelectric circuits have their own dynamics that regulate gene expression, which in turn may affect the number and type of channels, in a continuous interplay between genetics and physiology. The hardware is important, in that it limits what can happen. But it does not fully determine the outcome - the morphogenetic outcome is in large part the result of bioelectric

software - circuit dynamics that run on the cellular ion channel hardware (very much like what happens in the brain, where cognitive content and specific memories derive from the bioelectric software, not the genome directly). Learning to manipulate the bioelectric software running on somatic cell networks is the key to top-down programming of morphogenesis.

Conclusion

The Allen Discovery Center at Tufts will make fundamental progress on the understanding and control of the morphogenetic information encoded in bioelectric properties, the mechanisms by which these relate to genetic signaling, and the strategies for their optimal control. Only the edge of this field is known, while the depths remain largely uncharted. Organisms homeostatically regulate their structure toward a specific patterning. Characterizing these dynamics will help move the field beyond the machine language of genes and proteins toward high-level modular control of overall morphology. Our focus is on the information structures that represent an as yet-uncharacterized layer of control. By manipulating the pattern memories encoded bioelectrically in somatic tissues, and developing techniques to harness the computations that drive cells toward high-level patterning goals, a very exciting set of novel capabilities will result, with many broad implications. Bioelectricity couples the genome to the unique dynamics of computations, as adhesion proteins allow the genome to exploit various physical forces. Understanding the circumstances under which bioelectric networks can process information that can override genomic sequence and chromatin epigenetics will enrich our understanding of the origins of biological order and pave the way to transformative biomedical applications.

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