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Reprogramming cells and tissue patterning via bioelectrical pathways: molecular mechanisms and biomedical opportunities

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Abstract

Transformative impact in regenerative medicine requires more than the reprogramming of individual cells: advances in repair strategies for birth defects or injuries, tumor normalization, and the construction of bioengineered organs and tissues all require the ability to control large-scale anatomical shape. Much recent work has focused on the transcriptional and biochemical regulation of cell behaviour and morphogenesis. However, exciting new data reveal that bioelectrical properties of cells and their microenvironment exert a profound influence on cell differentiation, proliferation, and migration. Ion channels and pumps expressed in all cells, not just excitable nerve and muscle, establish resting potentials that vary across tissues and change with significant developmental events. Most importantly, the spatio-temporal gradients of these endogenous transmembrane voltage potentials (V_{mem}) serve as instructive patterning cues for large-scale anatomy, providing organ identity, positional information, and prepattern template cues for morphogenesis. New genetic and pharmacological techniques for molecular modulation of bioelectric gradients *in vivo* have revealed the ability to initiate complex organogenesis, change tissue identity, and trigger regeneration of whole vertebrate appendages. A large segment of the spatial information processing that orchestrates individual cells' programs towards the anatomical needs of the host organism is electrical; this blurs the line between memory and decision-making in neural networks and morphogenesis in non-neural tissues. Advances in cracking this bioelectric code will enable the rational reprogramming of shape in whole tissues and organs, revolutionizing regenerative medicine, developmental biology, and synthetic bioengineering.

Cellular reprogramming – a wider context

Most problems in biomedicine boil down to the control of complex biological shape[1]: its self-assembly during embryogenesis, restoration in the face of traumatic injury or degeneration, and maintenance throughout life to resist cancer and aging. Even cancer is a kind of disease of geometry, where cells stop obeying the normal patterning cues of the body in favour of tumorigenesis[2–4]. If all the problems of stem cell differentiation were solved and any single cell type could be produced at will, we would still be faced with the intractable problem of having to directly micromanage the assembly of an eye or hand from

This paper is dedicated to H. S. Burr and A. S. Presman – two early pioneers who saw the possibility of bioelectrical prepatterns of growth and form even before modern techniques made it possible to observe them directly.

No conflict of interest.

Further Reading/Resources

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individual cell types. Outside biomedicine, the field of synthetic biology must also move beyond metabolic circuits in soups of individual cells toward the bioengineering of complex autonomous structures. All of these goals require that we learn to reprogram not just single cells but the information-processing and communication pathways that orchestrate individual cell behaviours towards the patterning needs of a host organism (Fig. 1).

Examples of large-scale tissue reprogramming abound in nature. Planarian flatworms can regenerate the entire animal after almost any kind of amputation, and continuously remodel their entire body to match available cell numbers to their target morphology and correct proportions among all organs[5]. Salamanders can regenerate limbs, eyes, jaws, hearts, and portions of the brain[6] throughout life. Deer – large adult mammals – regenerate meters of bone and innervation each year[7], and even human children can regenerate their fingertips completely[8], reminding us that regeneration is not only the province of non-mammalian species. Remarkably, embryonic[9, 10] and regenerative[11, 12] environments can reprogram cancerous growth into normal anatomy[13, 14], revealing the importance of context and patterning information in regulating individual cell behaviour. While the current focus on unravelling this control circuitry has been on transcriptional networks and gradients of secreted biochemicals, recent work has highlighted the importance of another powerful control system: endogenous bioelectricity[15, 16].

All cells, not just nerve and muscle, utilize ion channels and pumps to establish specific voltage gradients across plasma and intracellular membranes (resting potential V_{mem}, Figures 2–3). While solid functional physiology over the last 40 years has implicated transepithelial electric fields as providing cues for cell migration, cell orientation, wound healing, and even limb regeneration[17–24], it is now beginning to be appreciated that V_{mem} itself is a powerful regulator of cell proliferation, differentiation, and apoptosis[25–27]. Interestingly, recent work has demonstrated that spatio-temporal patterns of V_{mem} distribution *in vivo* contain instructive information for specifying organ identity and large-scale anatomical order (Fig. 4). Distinct from effects of applied electromagnetic field exposure[28, 29] and from ultraweak electromagnetic radiations from cells[30–32], this exciting new field is “Molecular Bioelectricity” - the investigation of instructive patterning and cell:cell signalling roles of voltage gradients in non-excitable cells. The modification and interpretation of these gradients during embryogenesis, regeneration, and cancer underlies endogenous and experimentally-induced cell and tissue reprogramming. This Opinion essay discusses the current state of the field of Bioelectricity, its major open questions and opportunities for new discoveries, and implications for basic biology and biomedicine.

State-of-the-art tools of molecular bioelectricity

A number of tools have now been developed to facilitate analysis of bioelectric regulatory pathways in many model species[33]. The system in question (explanted tissue, *in vitro* organ culture, or entire developing embryo) can be interrogated with respect to the spatio-temporal profile of bioelectric properties. For example, the V_{mem} gradients that were observed in the early *Xenopus* face[34] preceded and co-localized with specific inductions of genes such as *frizzled*, suggesting the hypothesis that the observed voltage distribution played a functional role in craniofacial morphogenesis. Characterization of ion flux is best performed using ion-selective vibrating probes[35]; trans-epithelial potentials can be mapped out using standard electrophysiology[23, 36], while distributions of $V_{mem}$are detected non-invasively by soaking samples in fluorescent voltage reporter dyes coupled with quantitative microscopy[37–39]. Both vibrating probes and voltage dyes offer the opportunity of discovering multiple distinct voltage domains on the surface of individual cells[15, 33] – a potentially important combinatorial code that is obscured by traditional electrophysiology which reports only one V_{mem} value for any given cell.

Alternatively, a number of screens can be used to first ask whether bioelectricity is functionally relevant in one's favourite system (and if the result is positive, then followed up with characterization of the bioelectric profile in the native state). The basic loss-of-function screen, first performed in frog and chick embryos in a left-right asymmetry patterning assay to determine whether ion flow is important[40, 41], is used to simultaneously identify the molecular-genetic origin of the signal. In this scheme[42, 43], a panel of ion channel/pump drugs with known targets is used to build a pharmacological profile of the ion conductance whose inhibition results in a specific patterning or cell behaviour change. Remarkably, in many such screens it has been possible to adjust levels of inhibition so as to dissociate housekeeping functions of V_{mem} from subtle instructive patterning roles, revealing functions in development[34, 40, 44–50], regeneration[18, 51–53], and cancerous disorganization[54–59] in the absence of generalized toxicity. The power of such inverse screens resides in the hierarchical organization of the reagents: first relatively non-specific compounds are used to test the involvement of large families of translocators (e.g., barium chloride to probe all K^+ channels). Any negative result rules out the entire family, while positive results are followed by increasingly more-specific reagents to narrow down to a single class. Such screens usually only require the use of ~10–20 compounds total; in the case of left-right patterning, the screen identified two potassium channels and two ion pumps which were subsequently validated by molecular-genetic dominant negative approaches. Because of the logarithmic reduction in the number of candidates at each step, the relatively inexpensive nature of drug experiments, and the ability of compounds to target multiple family members at once, inverse screens are a very accessible, rapid way to determine which ion translocator proteins are involved in any process of interest, and a wide variety of reagents including morpholinos, RNAi, and genetic deletion can be used for molecular validation. Moreover, unlike molecular targeting of individual genes, such drug screens overcome the high compensation (redundancy) among channel members and allow the interrogation of maternal proteins (which do not rely on zygotic transcription for their presence and are thus hard to detect in standard knockdown screens). Nevertheless, despite the drawbacks of traditional genetic screens for uncovering bioelectrical regulators of patterning, a number of channelopathies have now been uncovered as underlying specific morphological phenotypes (Table 1).

Gain-of-function strategies rely on misexpression of specific channels and pumps to perturb V_{mem} gradients *in vivo*, to test specific hypotheses generated by screens or physiomic profiling. For example, the observation that the left side of the 4-cell frog embryo pumped half as many protons per unit time as the right side led to a misexpression experiment in which a yeast proton pump mRNA was introduced into left-side cells to equalize the H^+ efflux; this resulted in specific randomization of the position of the asymmetric internal organs[40, 41], supporting a functional role for voltage gradients in embryonic left-right asymmetry. A large panel of well-characterized channels and pumps now exists that can be used to rationally modulate the bioelectric state of specific cells, and misexpression of a variety of ion channels in early frog embryos revealed the surprising reprogramming of many somatic areas into eyes[50]. These can be introduced into cells using transfection, microinjection of mRNA or DNA vectors, or viral infection. Pharmacological manipulation of natively-expressed channels or pumps (e.g., using channel opener drugs) is the method of choice in biomedical applications where gene therapy is to be avoided; for example, a recent study showed how a cocktail based on sodium flux could be used to initiate regeneration of spinal cord and muscle in a tadpole model of tail regeneration[52]. An important strategy using misexpression of wild-type and mutant channels concerns dissecting the mechanism of action. For example, a pore mutant can be used to determine whether a channel's role is scaffolding/binding or truly related to its ion translocation role. Further, by changing the V_{mem} to the same overall level using channels for different ions (K^+, Na^+, Cl^-), it is possible

to determine whether a particular ion's concentration is what matters or whether it really is the V_{mem} level that carries instructive information in the given system.

With functional and physiomic (ion flux or V_{mem} gradient) data in hand, quantitative modelling is then used to synthesize the ion conductance dynamics into a circuit with predicable V_{mem} control properties[60–62]. The last remaining piece of the puzzle is to connect V_{mem} changes to downstream transcriptional cascades. This is done through a suppression screen for transduction machinery. Given an assay in which a V_{mem} change produces a specific cell- or tissue- outcome, each of the known transduction pathways is inhibited in turn to determine which one prevents the V_{mem} change from being sensed by cells. Known transduction mechanisms that allow cells to convert bioelectrical signals into gene expression changes include voltage-sensitive phosphatases[63], gap junctions[64–66], transporters of serotonin[41, 67–69] and butyrate[54, 70, 71], and integrin signaling[72]. By identifying the transduction mechanism linking V_{mem} to transcription in a given system, the circle is closed, providing mechanistic details of every step from the expression of the channels that drive a V_{mem} change, to the mechanism that converts that change into 2nd messenger activity, to the genes (in many cases, ion channel genes!) whose activity is modified. Patterning thus occurs as a cycling, dynamical system implemented by the continuous interplay of genetics and biophysics.

Bioelectrical determinants of individual cell behavior

It has long been known that endogenous electric fields provide spatial cues for orientation, outgrowth, and migration for a broad range of cell types[36, 73–79]. Growth cone pathfinding and cell orientation guided by bioelectrical cues involves integrins, cAMP, rac, and cdc42[24, 80–86]. A particularly thorough combination of biochemistry, transgenic mouse technology, and electric field perturbation[18] dissected the mechanisms of electrotaxis showing that mammalian wound healing requires cells to sense endogenous fields (generated by trans-epithelial potential) by a PTEN - and PI(3)K-γ-dependent pathway. Cell differentiation is also controlled by changes in V_{mem}, as has been shown in human mesenchymal stem cells[26, 87], embryonic stem cells[88, 89], myoblasts (in which hyperpolarization driven by the Kir2.1 channel plays a crucial role)[90, 91], the specification of neurotransmitter types[92], and the control of precursor differentiation[93–97] in the developing nervous system and heart. Tissue engineers have also begun to take advantage of this pathway using applied electric field stimulation[98]. Given the known roles of V_{mem} in regulating normal migration, differentiation, and proliferation[99], it is not surprising that control of ion flux[58, 100] and membrane voltage[56, 57] are also increasingly implicated in cancer (Table 2). Interestingly, electric cues often dominate competing biochemical signals; for example, depolarization trumps the induction of differentiation by insulin +dexamethasone in human mesenchymal stem cells[26], while physiological-strength electric fields override opposing chemical trophic factors, contact inhibition release, and population pressure[101].

A useful heuristic (Fig. 3A) is that depolarized cells tend to be plastic, undifferentiated, and highly proliferative (stem cells, cancer cells, and embryonic cells), while strongly polarized cells tend to be the mature, somatic terminally-differentiated cells: V_{mem} is a strong regulator of cell fate plasticity and mitotic rates[102–104]. However, bioelectric cues also provide spatially-patterned signals. The differential activation of voltage-responsive transduction mechanisms on opposite sides of a cell allows bioelectric signals to regulate cell polarity. This was first discovered in the algae Fucus[105], and has been recently shown in yeast[106] and pollen tubes[107]. During left-right patterning of the *Xenopus* embryo, voltage gradients set up at the first cleavages link individual cell dynamics to axial patterning of the entire bodyplan by redistributing a long-range morphogen[108, 109110, 111]. The dissection and

synthesis of such systems, at the genetic and physiological levels, is helping to understand the properties of biophysical pathways by which individual cell polarity is integrated into large-scale patterning outcomes[112] - advances that are required for applications in tissue and organ (re)programming.

A fundamentally physical event such as ion flow or V_{mem} change needs to be transduced into genetic responses (Fig. 3B). Several distinct mechanisms convert slow changes in resting V_{mem} levels into second-messenger cascades in non-excitable cells that ultimately drive transcriptional responses (reviewed in detail in[113]). These include: electrophoretic redistribution of small signalling molecules through gap junctional paths, voltage-based regulation of membrane transporters of signalling molecules like calcium and various neurotransmitters, and electrophoretic separation or clustering of protein complex subunits within the plane of the cell membrane.

The most recent transduction mechanism to be described involves the SLC5A8. This protein, already implicated by genetic data in colon cancer[114–117], converts ion levels into the movements of butyrate, which in turn is an important regulator of histone deacetylases and thus of epigenetic chromatin state[118]. Recent attempts to reprogram cancer in a frog model showed that V_{mem} is not only a promising diagnostic modality for non-invasively revealing tumour sites and margins, but is also a functional parameter regulating oncogene-mediated tumorigenesis. Cells artificially hyperpolarized by genetic or pharmacological means resisted forming tumours despite high levels of oncogene expression[54, 55]. This suppression effect was mediated by SLC5A8, which linked voltage change to HDAC activity modulation by butyrate. A similar pathway has been found to occur in limb and tail regeneration[119, 120]. Thus, as has been seen with voltage regulation of serotonin movement and signalling in non-neural cells during left-right patterning[41, 67–69], V_{mem} control of small molecule flow and signalling may be a conserved module for the regulation of programs that mediate cell activities into anatomical structures and away from tumorigenesis.

Bioelectric gradients determine large-scale pattern

Large-scale morphogenesis emerges from the orchestrated interactions of individual cells. Recent data has shown that contact-dependent depolarization regulates the interactions of distinct cell types[121], as well as regulates the paths taken through the body by migratory cell types[57]. Screens have also revealed new roles for bioelectric signals in endogenous regulation of complex organ patterning programs in vivo (Fig. 4). In regenerating planaria, a circuit driven by the H,K-ATPase has been described that regulates whether a head or tail appears at an amputation site[61, 122]. By experimentally regulating the V_{mem} at the wound and the physiological communication among cells via gap junctions, 0-, 2-, or even 4-headed (rhombus-shaped) worms can be created – a remarkable control over large-scale shape[123]. This is an example of bioelectric cues directly regulating what structure is built by the adult stem cells (neoblasts), and serves as an important model for the use of bioelectric signalling to coax stem cell-mediated growth in biomedical contexts.

Recent data in *Drosophila* embryos linked the Kir2.1 channel to the important TGF-β patterning pathway in wing patterning[34]. In Xenopus embryogenesis, regionalization of the anterior field by patterns of hyperpolarized and depolarized cells specifies a prepattern for gene expression and subsequent anatomy of the face. Specific endogenous patterns of differential V_{mem} in the naïve tissue preceded and controlled the position of eyes[50, 124] and many components of the face[34], while experimental alterations of this native pattern produced predictable craniofacial defects. Bioelectric regulation of the embryonic face is likely to be highly relevant to human medicine, as several channels have now been implicated in craniofacial dysmorphias[125–134], including Kir2.1 (Andersen-Tawil

syndrome), GABA-A (Angelman syndrome), and KCNQ1 (Beckwith-Wiedemann syndrome). Reprogramming tissue for applications addressing these birth defects will require increased understanding of the origin and role of the patterns of V_{mem} during craniofacial patterning.

One important area for future work in synthetic biology and bioengineering is to understand the links between V_{mem} and mechanical properties of tissues[135]. It is now clear that electrical stimulation can alter biomechanical outcomes[136–138], and future work must distinguish the individual roles of electric fields, resting potential, and the roles of proteins that transduce bioelectricity to and from mechanical deformation, such as prestins[139] and stretch-activated ion channels[140].

Manipulation of V_{mem} gradients: applications

In addition to the endogenous roles of bioelectric gradients that have been discovered by loss-of-function approaches, the most exciting data for reprogramming applications come from gain-of-function studies that ask what shape changes are *possible* to make by appropriate modulation of V_{mem}[16, 113, 141, 142]. Such data were first derived from screens in which ion channels and pumps were randomly misexpressed in frog embryos to gauge the range of phenotypes that might be obtained by perturbation of endogenous bioelectric gradients, followed by focused experiments designed to induce specific physiological states (e.g., ones associated with regenerative response[53, 143]).

The *Xenopus* larva regenerates its tail – a highly patterned appendage containing a spinal cord, muscle, peripheral innervation, vasculature, and connective tissues[144, 145]. Interestingly, tadpoles undergo age-dependent decline of regenerative ability, as do human beings. The non-regenerative (refractory) state in the *Xenopus* tail can be overcome by transgenes driving strong proton efflux[53] or by a cocktail that modulates sodium content[52]. In either case, the downstream sequellae of the regeneration-specific physiological state are induction of regenerative genes (such as Notch and BMP4), a strong increase in cell proliferation at the wound, and extensive innervation towards the outgrowth. There are several important aspects to this pathway. First, the whole complex cascade of organ regeneration can be triggered by an extremely simple event (proton pumping "on" – likely only 1 bit of information since no attempt was made to tune the strength, pattern, or timing of the flux). Indeed in the case of the sodium-based cocktail, an exposure of just 1 hour was sufficient to kick-start the whole regenerative process. Moreover, what formed was a normal tail of the right size, shape and orientation – not a small tail or tumour, suggesting that in this case (unlike in the case of the craniofacial prepattern) what is encoded by V_{mem} here is a "master regulator" or top network node signal – a sort of subroutine call that activates a complex, self-limiting downstream developmental module that builds the tail. More recently, a preliminary study showed that the same cocktail initiates leg regeneration after amputation[146], suggesting that this system interacts with positional information cues to specify a "build whatever structure normally goes here" signal, not a set of cues that directly micromanage the morphogenetic process.

A considerably different picture of the role of voltage gradients is painted by another recent finding. By modulating V_{mem} states *in vivo* in frog embryos, any location in the tadpole could be turned into eye tissue –in some cases, a complete eye with all of the normal tissues arranged in proper morphology[50]. The effect takes place via a feedback loop in which a hyperpolarized voltage state activates the Pax6 eye gene and vice versa. However, the mechanism must involve much more than upregulation of Pax6 because Pax6 itself only makes eyes when it is misexpressed in the anterior neuroectoderm but not outside the head. Appropriate misexpression of ion channels was able to induce eyes anywhere, including in

the gut, tail, and lateral plate mesoderm. Since it was previously thought that only neurectoderm was competent to make eye, these data suggest that bioelectric pathways may necessitate a revision to current lineage restriction maps, and may be a powerful way to control differentiation of iPS cells, embryonic stem cells, and somatic cells that need to be reprogrammed. As in tail regeneration, the whole eye was induced without having to specify the details of its construction (a desirable property for applications in regenerative medicine); however, because eyes could be formed anywhere (in ectopic, inappropriate locations) simply by achieving a specific range of V_{mem} (about 15 mV wide), the authors hypothesized that individual organs could be encoded by specific ranges of V_{mem} values. In addition to "form whatever normally goes here" (the kind of effect observed in the induction of tail/limb regeneration), it might be possible to coax cells to build appropriate organs as needed on demand. The mapping between V_{mem} ranges and other structures besides eye remains to be elucidated. Thus, bioelectricity offers opportunities for control at multiple levels: reprogramming of cell behaviors such as de-differentiation and mitotic control, and regulating large-scale morphogenesis at the level of anatomical polarity, growth control, and organ specification. Future work must delineate the precise methodology and bioelectrical parameters that induce the former or latter mode of signalling from V_{mem}change.

Unique features of bioelectrical signalling

A major area of inquiry remains the cracking of the bioelectric code – detailing the mapping between V_{mem} state distributions and the resulting anatomy. It is necessary to more clearly understand the role of V_{mem} gradients in kick-starting complex developmental modules that build whole organs vs. serving as direct templates for tissue patterning. To fully capitalize on the power of bioelectrical cues for cell and tissue reprogramming, it is important to exploit several major distinctions between bioelectrical controls and the biochemical/genetic signals for which modern molecular and cell biology tools are optimized. First, there is not always a simple one-to-one correspondence between genetics and bioelectricity. The post-translational gating of channels means that it is impossible to determine the V_{mem} state of a cell from expression data describing which channel proteins are present. Thus, the real-time physiological prepatterns of V_{mem} in tissue will be invisible to high-resolution mRNA or protein profiling technologies. V_{mem} should be thought of as an aggregate, higher-level property akin to "pressure" or "temperature", resulting from the ensemble properties of many individual gene products. The fact that V_{mem} is determined by a contribution of all of the ion conductances in any cell means that single channel knockouts will fall prey to false negatives (due to compensation and redundancy). Understanding and controlling the bioelectrical gradients that encode the programs of pattern formation will require deep physiomic profiling data, and continued development of tools for regulating the V_{mem} of tissue regions at will. One such technology is optogenetics[147]; although so far these reagents have been used largely in neural and excitable cells, the application of light-based control of ion flows[148, 149] to non-neuronal, somatic cells will enable the construction of true computational tissues into which bioelectric patterning information can be dynamically written and read from, for guided self-assembly of physiological and thus genetic and anatomical patterns. The first steps have been taken, as a recent report showed the induction of tail regeneration by optical modulation of bioelectric state after amputation[150].

One exciting yet speculative hypothesis is suggested by the recent advances in bioelectricity (Fig. 5). The complex feedback dynamics between physiological parameters and proteins that are both regulated by, and regulate, those bioelectric states (e.g., voltage-sensitive potassium channels) can result in multiple stable attractors for cell physiology. This in turn means that cells could store information in their bioelectrical states[151]. Moreover, since non-neural cells are able to participate in electrical communication with their neighbours using electrical synapses (gap junctions[152, 153]), there may be no *fundamental* difference between

electrically-communicating somatic tissues and neural networks. It has been suggested that synapses are an evolutionary innovation based on much more primitive cell:cell signalling modes using gap junctions, voltage regulation, and neurotransmitter molecule movement[154]; thus it is tempting to speculate that the information processing abilities of neural networks are also not restricted to brains. Indeed, non-neural cells may be able to store immense amounts of information encoded in the stable many-valued V_{mem} levels (at each of many domains across the cell surface). The development of new technologies for tracking and modulating the bioelectrical communication among actively patterning tissues will allow the testing of this hypothesis, and may reveal memory, decision-making, and other functions formerly reserved for neural systems implemented in somatic tissues[155, 156] – a finding that would have important implications not only for strategies to reprogram morphogenesis but also the design of novel architectures for computer technology[157, 158].

Conclusion

The true potential of biological reprogramming will only be realized when we gain control of shape at the level of large-scale multicellular structures[166]. The next phase of synthetic bioengineering will usher in the development of computational tissues: multicellular biological constructs that can be programmed as an excitable medium[158, 167]. The output of such programs will be complex 3-dimensional structures such as eyes, limbs, and synthetic constructs with shapes never before seen in the evolutionary history of Earth. The “master regulator” properties of bioelectric and other signalling modalities will allow bioengineers to offload some of the incredible computational complexity onto the organism itself, which is already ideally suited to carry out the assembly and repair process. When integrated with new bioreactors[168, 169] and delivery methodology that can tweak the time evolution of complex systems by providing instructive cues at key decision times, such guided self-assembly processes will greatly expand our ability to produce required structures for regeneration *in situ* or for transplantation. This will revolutionize not only regenerative approaches but also the creation of hybrid biological robotic devices[170].

Such advances require the development of new theory and methodology. On the theoretic front, both analysis and synthesis of complex shapes will benefit from the development of new models for understanding how information can be stored and manipulated in physiological networks, and of next-generation artificial intelligence tools supporting a bioinformatics of shape and pattern control. In technique development, advances in fields such as optogenetics will greatly facilitate hypothesis testing by allowing the bioelectric signals within complex tissues to be altered as needed. Next frontiers in molecular bioelectricity include the functional relevance of nuclear envelope voltage potentials, and the significance of multiple V_{mem} domains across the surface of cells. The development of mature bioelectric technology will revolutionize the field of molecular physiology and reprogramming as the development of restriction enzymes did for molecular cell biology. Just as the understanding of cell function was transformed by the ability to construct desired DNA sequences, the cracking of the bioelectric code will propel the field to exciting new vistas in the control of growth and form, with biomedical applications in birth defects, regenerative repair, cancer, and synthetic biology.

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Sidebar: A new bioinformatics of shape

"There is an obvious discrepancy between the single-cell genetic input and the multicellular geometrical output. To bridge the gap, a mathematical proof, usually in the form of computer simulations, becomes necessary"[159]. While the tools of bioinformatics have revolutionized molecular, cell, and evolutionary biology, they are largely limited to operating with genomic, transcriptional, or proteomic data – at the level of molecules. Likewise, recent work in the robot scientist field[160, 161] is most suited to biochemistry, drug discovery, and metabolism. Computational efforts in synthetic biology[162] have begun to develop frameworks for guiding top-down design of complex pathways and control circuitry. However, in the fields of developmental and regenerative biology, scientists are still required to manually attempt to derive testable models from functional data. Organisms such as salamanders, planaria, and deer provide striking proof-of-principle that complex organ repair in adulthood is an achievable goal. As we probe these model systems to uncover the rules guiding dynamic remodelling (reprogramming) of cell behaviour and organ shape, an ever-increasing deluge of functional data floods the literature. What are needed are constructive (algorithmic) models that specify the actions and decisions made at each step[163, 164]. A true explanation of pattern regulation must specify the information and energy flow at step that is *sufficient* to produce the self-repairing systems we seek to emulate – not only outline gene-regulatory networks based on loss-of-function data that reveal what components are *necessary* for the system to work correctly. However, the mountain of information on experimental perturbations that result in specific changes of anatomical shape is already so large and complex that scientists can rarely produce algorithmic models that fit those data. Additional data inhibit, not help, the efforts of human scientists to keep up with the facts and try to mentally construct a model whose behaviour fits all of the data. A fundamental piece of this problem is that feedback, emergence, and complexity (in the dynamical systems sense) render it extremely difficult to know in advance what dynamic behaviour will result from a model specified in terms of components and rules. Thus, one key pillar of continuing advances past the current plateau is the development of a new Bioinformatics of Shape. This marriage of bench biology and true computer science is beginning to give rise to artificial intelligence tools[165] to help mine the database of functional experiments on pattern formation and suggest testable, mechanistic, algorithmic models of biological systems that acquire, modify, and repair their shape.

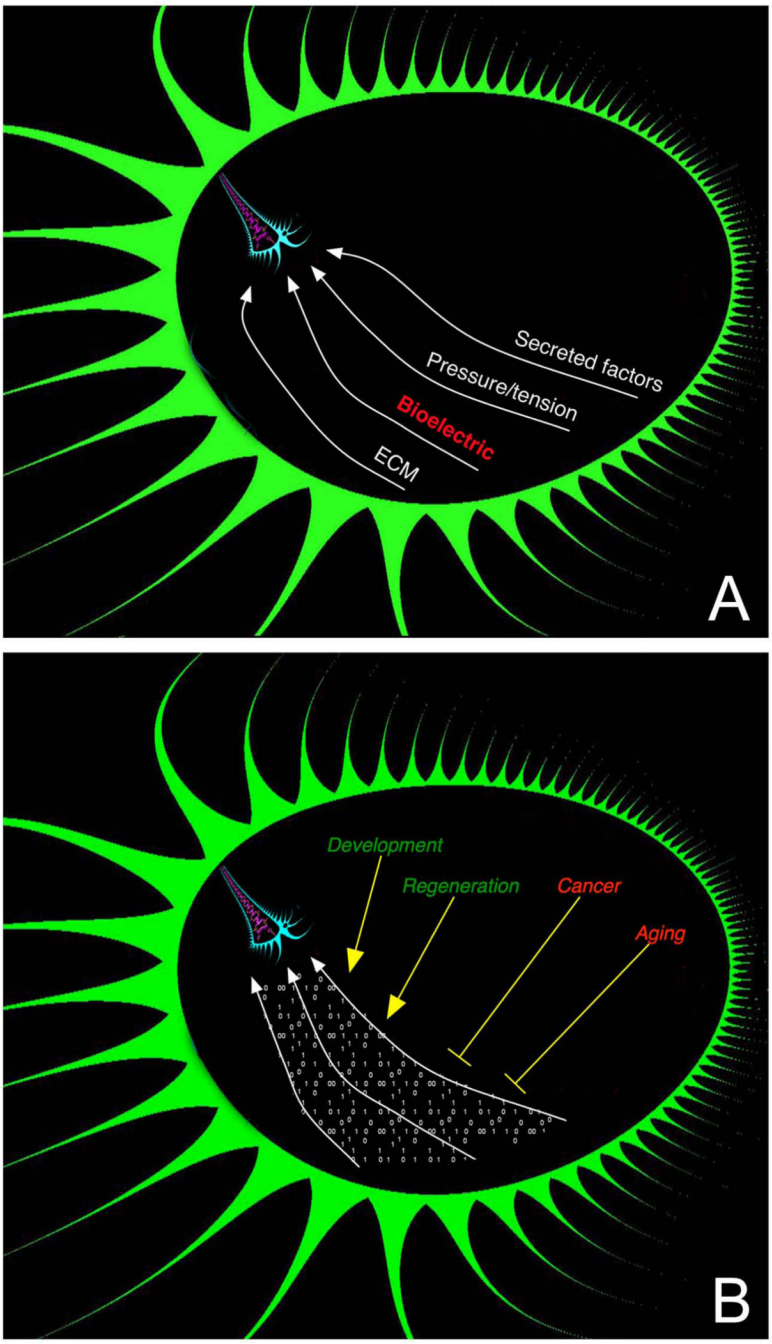

Figure 1. the morphogenetic field

(A) Cell activity is guided by a complex, spatially-distributed set of signals from the host organism mediated by diffusing chemical, extracellular matrix, tension/pressure, and bioelectrical properties. (B) This morphogenetic field orchestrates cell behaviour towards large-scale anatomical programs during development and regeneration; its influence is subverted during oncogenic transformation and aging. Mastery of the information stored in this field, and of the mechanisms by which cells interact with it, will result in the ability to reprogram large-scale tissue and organ shape, with transformative implications for the fields of birth defects, regenerative medicine, cancer, and synthetic bioengineering.

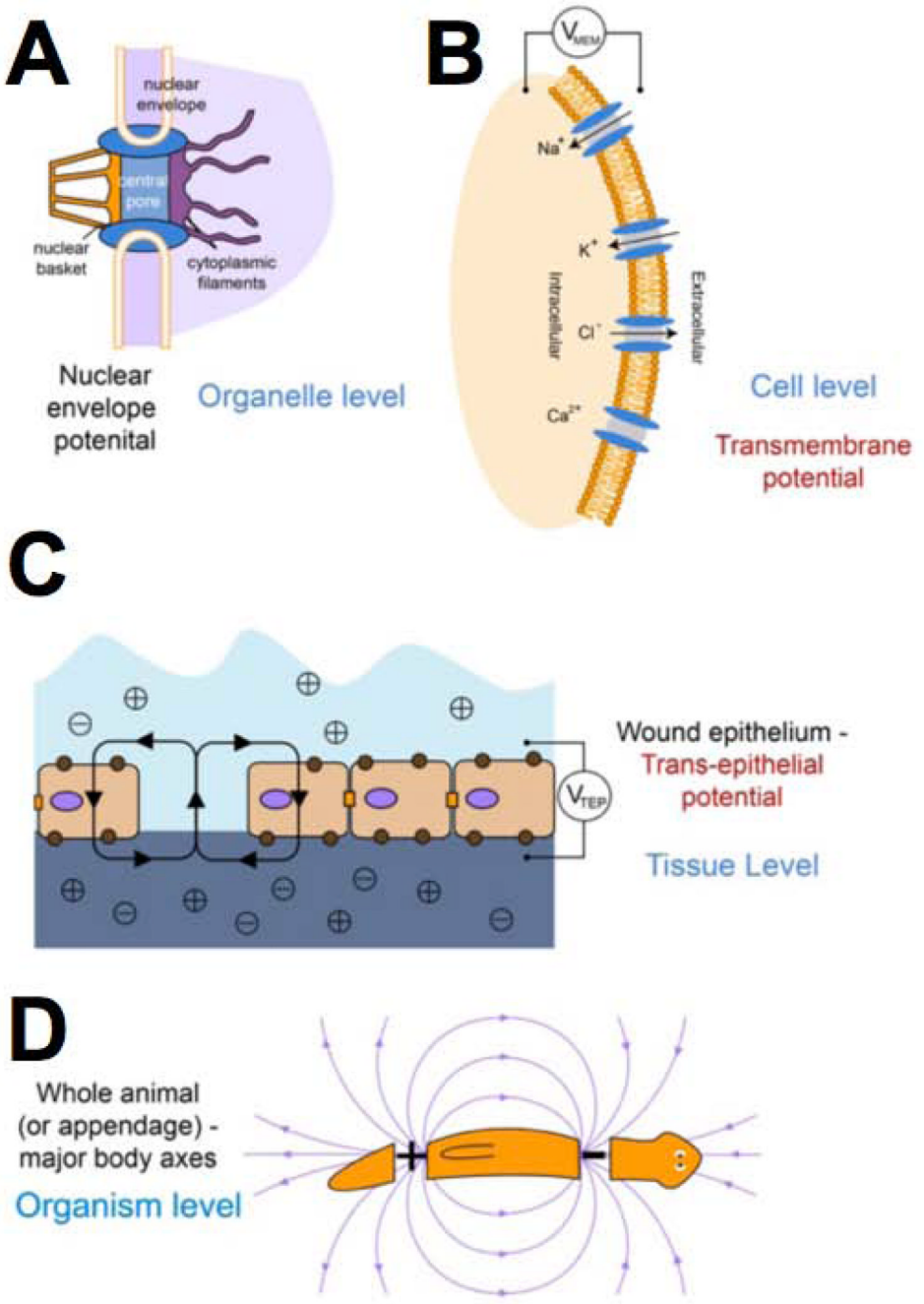

Figure 2. bioelectric gradients exist at multiple scales of size and levels of biological organization

Organelles (A) and whole cells (B) are bound by membranes containing ion channel, pump, and transporter proteins. The activity of these ion translocators give rise to differences in resting potential (V_{mem}) across the membrane. Stacked in parallel, cells also give rise to a trans-epithelial potential (C), and electric fields have been characterized that correspond to appendages (limbs) or entire body axes (D). This overlapping set of cues provides positional information, organ identity, and other cues for cell behaviour and morphogenesis. This figure was drawn by Maria Lobikin, and is used with permission.

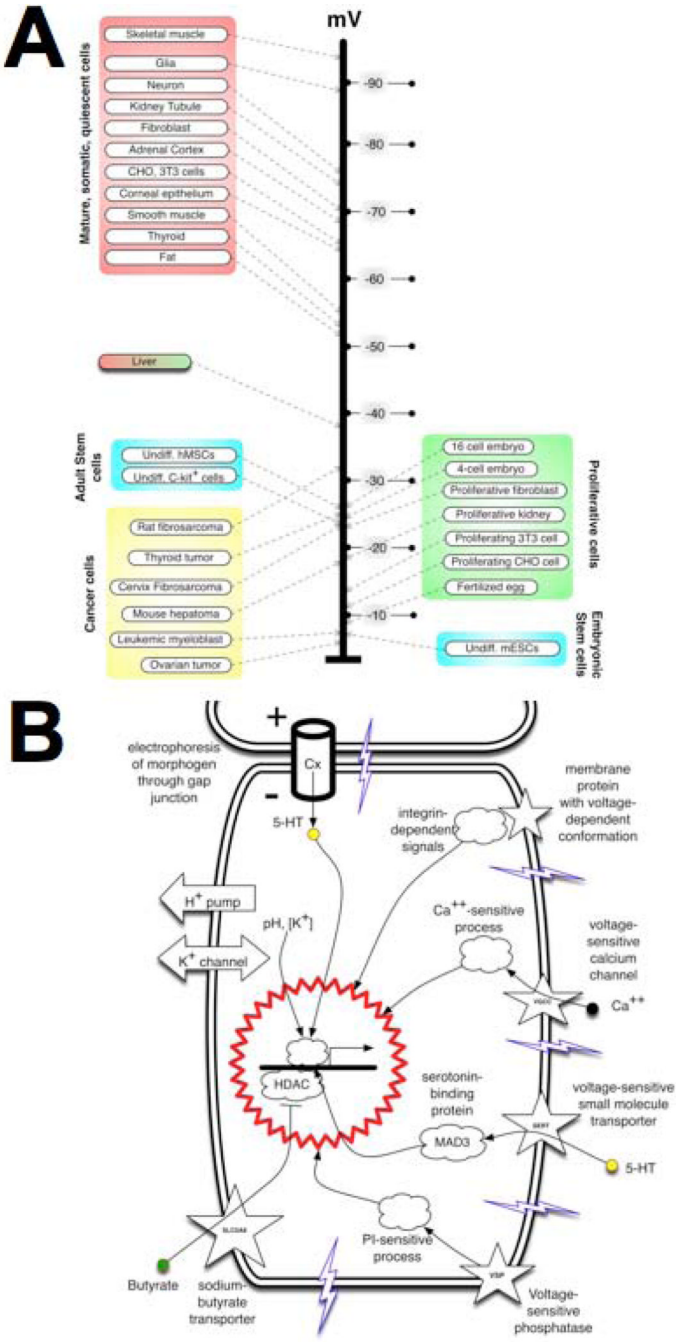

Figure 3. V_{mem} at the level of single cells: its transduction impacts cell states

(A) A sample survey of many cell types (modified and updated after[102]), and recent functional data[25, 103, 104, 171–174], reveals that at the level of single cells, V_{mem} determines cell plasticity and proliferation potential. Depolarized cells tend to be rapidly proliferating and undifferentiated (e.g., embryonic, stem, or tumor cells) while terminally-differentiated somatic cells tend to be highly polarized. Importantly, cell state can be functionally altered (switched between these two classes, in either direction) by artificial change of V_{mem}. This panel is modified after Fig. 1 of[15]. (B) A range of mechanisms have now been characterized that transduce alterations of V_{mem} into downstream effector cascades (transcriptional changes). These include signalling proteins with a voltage-sensitive conformation (e.g.,

integrins and voltage-sensitive phosphatases) and transporters of small signalling molecules whose activity is regulated by V_{mem} (such as gap junctions, voltage-gated calcium channels, and solute carriers, which allow V_{mem} changes to signal via serotonin, Ca^{++}, butyrate, and likely many other yet-to-be-discovered compounds). This figure is modified after Fig. 1B of[113].

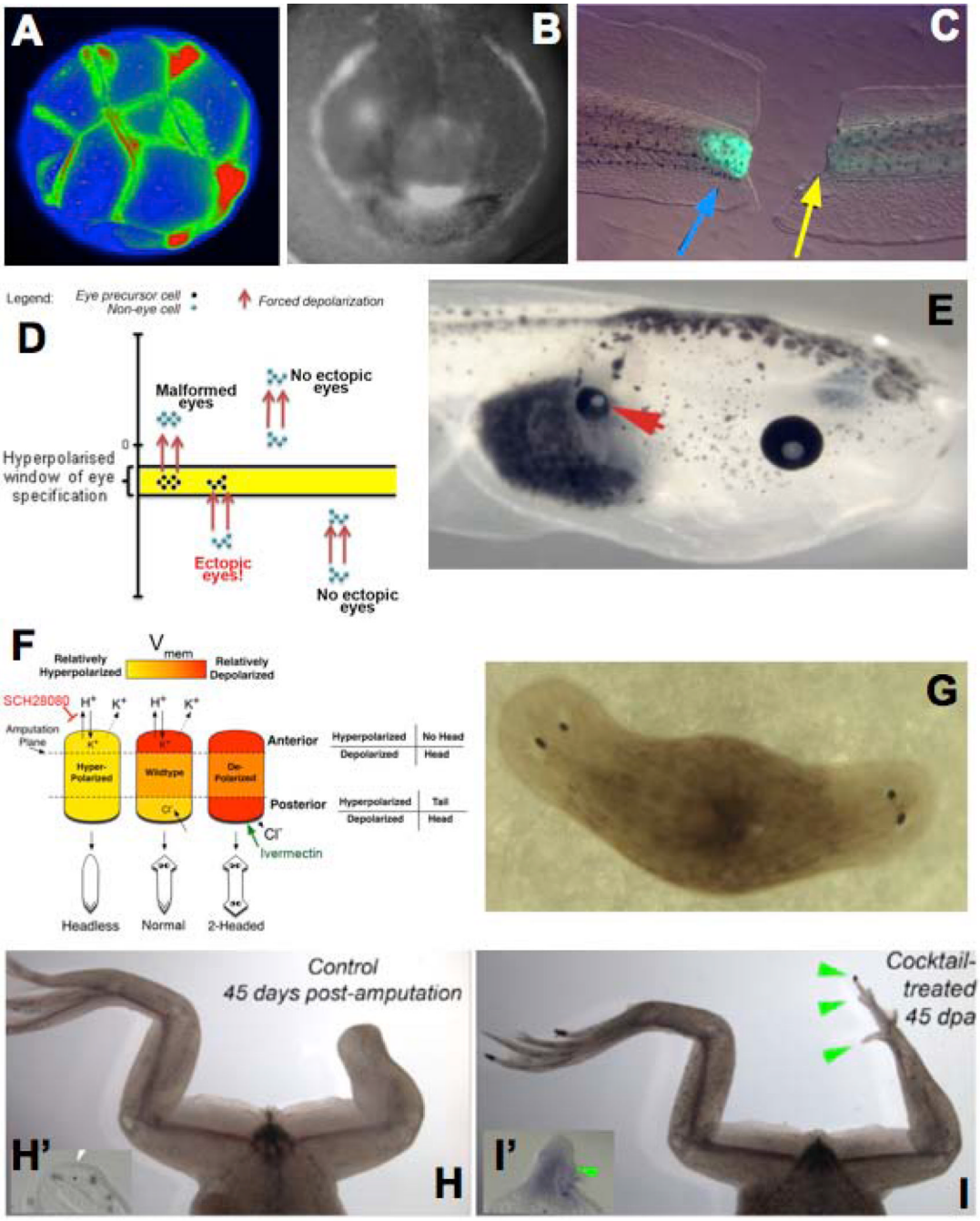

Figure 4. Large-scale tissue and organ reprogramming by V_{mem} gradients

Voltage-sensitive fluorescent dyes reveal spatio-temporal patterns of bioelectrical gradients *in vivo*. Examples of gradients in the Xenopus laevis (frog) model include cleavage stages (A), craniofacial patterning (B, showing the hyperpolarizations in tissues that will become eye, branchial arches, and cement gland), and tail regeneration (C, regenerating tail on the left, and one that is prevented from regeneration by inhibition of V-ATPase activity on the right; green fluorescence signal indicates the normal repolarization of the wound (blue arrow), and when the repolarization is experimentally prevented (yellow arrow), regeneration is blocked). Functional data reveal a model in which a narrow range of V_{mem}

(D) forces somatic cells, such as gut cells, to form a whole complete eye (E, arrowhead). A similar bioelectric circuit model describes the pathway regulating head vs. tail identity of regenerating tissue in planaria (F); experimental control of V_{mem} in this model results in 2-head animals after amputation (G), revealing the ability to control the shape of organs constructed by adult stem cells by bioelectric signalling. Brief treatment with sodium ionophore cocktail induces froglet hindlegs, which normally do not regenerate (H, showing wound region and lack of expression of the blastema gene MSX1 in inset panel H') to regenerate legs with toes and toenails (I, showing blastema and induction of MSX1 expression in inset panel I'). This figure is modified after figures from references[16, 50, 61, 146].

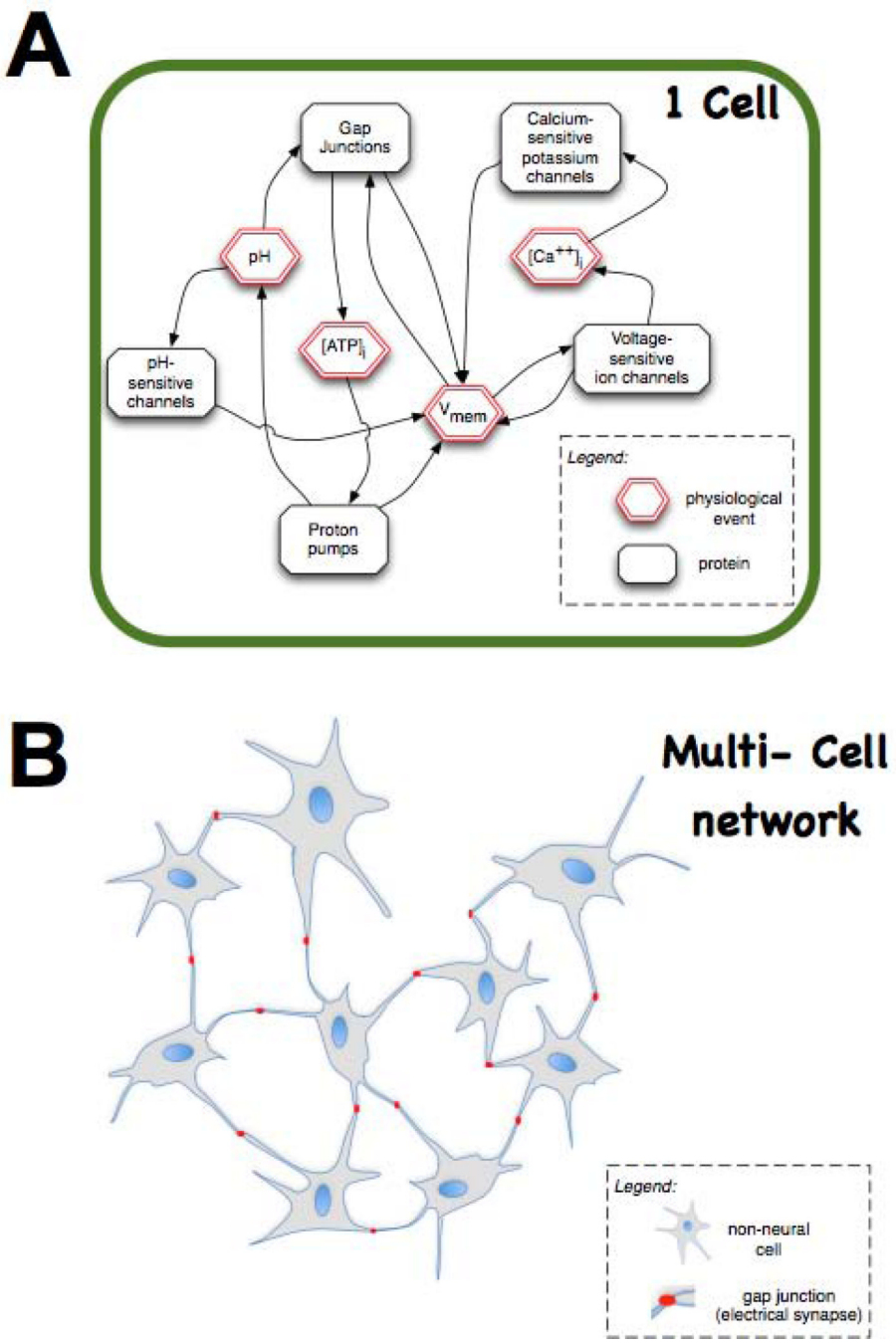

Figure 5. Bioelectric signals enable non-neural cell fields to function as a computational medium

(A) At the level of single cells, elements such as voltage-sensitive ion channels result in feedback loops between the activity of ion translocator proteins and physiological parameters such as V_{mem}. These feedback loops ensure that physiological networks have non-obvious (emergent) behaviour dynamics, which can display hysteresis and multiple attractor states – thus able to store information encoded in stable V_{mem} states (e.g., depolarized = 1, hyperpolarized = 0) that would be invisible to genetic or proteomic profiling. (B) Even more interestingly, multiple (non-neural) cells communicating electrically via gap junctions (electrical synapses) could potentially store information and make decisions in the same way as do neural networks. The testing of this speculative

hypothesis (using paradigms well-developed in computational neuroscience) may reveal entirely novel ways to understand and manipulate tissue-wide information that directs morphogenesis, and new approaches for the development of new (biologically-embedded) computational platforms. This figure is modified after figure 5 of reference[146].

Table 1

Channelopathies with patterning phenotypes

Protein	Morphogenetic role	Species	Reference
TMEM16A chloride channel	Tracheal morphogenesis	Mouse	175
Kir7.1 potassium channel	Melanosome development	Zebrafish	176
Cx41.8 gap junction	Pigmentation pattern	Zebrafish	177
Cx43 gap junction	Fin regeneration	Zebrafish	178
Kir2.1 potassium channel	Wing patterning	Drosophila	44
Cx43 gap junction	Fin size regulation, Craniofrontonasal syndrome	Zebrafish Mouse	179, 180
Cx43 gap junction	Osteoblast differentiation	Mouse	181
Kir2.1 potassium channel	Craniofacial (Andersen-Tawil syndrome) and limb patterning	Mouse	44, 127
CFTR chloride channel	Bilateral absence of vas deferens	Human	182, 183
Girk2 potassium channel	Cerebellar development	Mouse	184–187
GABA-A receptor (chloride channel)	Craniofacial patterning, (Angelman Syndrome)	Mouse	188
KCNH2 K^+ channel	Cardiac patterning	Mouse	189
NHE2 sodium/proton exchanger	Epithelial patterning	Drosophila	190
V-ATPase proton pump	Wing hair patterning	Drosophila	191
KCNQ1 potassium channel	Hypertrophy of tongue, liver, spleen, pancreas, kidneys, adrenals, genitalia – Beckwith-Wiedemann syndrome; craniofacial defects	Human, Mouse	132, 192, 193
Kir6.2 potassium channel	Craniofacial defects	Human	134

Table 2

Ion translocators as oncogenes

Protein	Species	Reference
NaV1.5 sodium channel	Human	58, 194
EAG-1 potassium channel	Human	195
KCNK9 potassium channel	Mouse	196
Ductin (proton V-ATPase component)	Mouse	197
SLC5A8 sodium/butyrate transporter	Human	71
KCNE2 potassium channel	Mouse	198
KCNQ1 potassium channel	Human	132, 199