Molecular bioelectricity: how endogenous voltage potentials control cell behavior and instruct pattern regulation in vivo

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ABSTRACT In addition to biochemical gradients and transcriptional networks, cell behavior is regulated by endogenous bioelectrical cues originating in the activity of ion channels and pumps, operating in a wide variety of cell types. Instructive signals mediated by changes in resting potential control proliferation, differentiation, cell shape, and apoptosis of stem, progenitor, and somatic cells. Of importance, however, cells are regulated not only by their own \( V_{\text{mem}} \) but also by the \( V_{\text{mem}} \) of their neighbors, forming networks via electrical synapses known as gap junctions. Spatiotemporal changes in \( V_{\text{mem}} \) distribution among nonneural somatic tissues regulate pattern formation and serve as signals that trigger limb regeneration, induce eye formation, set polarity of whole-body anatomical axes, and orchestrate craniofacial patterning. New tools for tracking and functionally altering \( V_{\text{mem}} \) gradients in vivo have identified novel roles for bioelectrical signaling and revealed the molecular pathways by which \( V_{\text{mem}} \) changes are transduced into cascades of downstream gene expression. Because channels and gap junctions are gated posttranslationally, bioelectrical networks have their own characteristic dynamics that do not reduce to molecular profiling of channel expression (although they couple functionally to transcriptional networks). The recent data provide an exciting opportunity to crack the bioelectric code, and learn to program cellular activity at the level of organs, not only cell types. The understanding of how patterning information is encoded in bioelectrical networks, which may require concepts from computational neuroscience, will have transformative implications for embryogenesis, regeneration, cancer, and synthetic bioengineering.

INTRODUCTION Cell behavior is regulated by numerous distinct cues that impinge on them in vivo. Alongside chemical gradients (Huang et al., 2005; Geard and Willadsen, 2009; Niehrs, 2010; Ben-Zvi et al., 2011; Gershenson, 2012) and physical forces (Beloussov and Grabovsky, 2006; Beloussov, 2008; Nelson, 2009; von Dassow and Davidson, 2011; Davidson, 2012), cell activity is orchestrated toward the creation and repair of high-order anatomical structures by a set of bioelectrical cues (Levin, 2012a,b; Levin and Stevenson, 2012). Here bioelectricity refers to endogenous electrical signaling via ion channels and pumps at the plasma membrane; specifically excluded due to length constraints is the rich literature on external electromagnetic fields (Funk et al., 2009; Cifra et al., 2011; Hronik-Tupaj and Kaplan, 2012), ultraweak photon emission (Farhadi et al., 2007; Fels, 2009; Sun et al., 2010; Beloussov, 2011), and subcellular organelle potentials (Bustamante et al., 1995; Mazanti et al., 2001; Yamashita, 2011).

All these facts, sufficiently numerous, ... will open a very wide field of reflection, and of view, not only curious, but particularly interesting to medicine. There will be a great deal to occupy the anatomist, the physiologist, and the practitioner.

Allesandro Volta (1800), communicating to the Royal Society his invention of the electric battery

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Abbreviations used: dpa, days postamputation; hMSC, human mesenchymal stem cells; hpa, hours postamputation; HPLC, high-performance liquid chromatography; 5-HT, serotonin; \( V_{\text{mem}} \), transmembrane voltage potential; VSP, voltage-sensitive phosphatase.

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The voltage potential ($V_{\text{mem}}$) at the cell membrane is produced by the movement of ions through across a cell membrane. Ions move via many different ion channels and pumps, under the control of concentration and electric gradients. Change of $V_{\text{mem}}$ is transduced into cellular effector cascades by a range of mechanisms, including voltage-sensitive phosphatases, voltage-gated calcium channels, and voltage-sensitive transporters of signaling molecules such as serotonin and butyrate. (Diagram modified, with permission, from Figure 1B of Levin, 2007.)

Bioelectrical signals feed into epigenetic and transcriptional cascades and thus trigger changes in cell properties such as proliferation, differentiation, migration, shape change, and programmed cell death. Voltage reporter dye reveals gradients of $V_{\text{mem}}$ across the anterior-posterior axis of planarian flatworms. (Taken, with permission, from Figure 2B of Beane et al., 2013.) In amputated worms, a circuit composed of proton and potassium conductances sets the voltage states at each blastema, which in turn determines the anatomical identity of each end of a regenerating fragment. (Diagram taken, with permission, from Figure 7C of Beane et al., 2011.) Manipulating this circuit in amputated planaria using pharmacological or genetic techniques that target ion flux allows the programming of stem cell–mediated morphogenesis to specific anatomical outcomes, such as the creation of two-head animals shown here.

**NEW CONTROL KNOBS: RESTING POTENTIAL DETERMINES SINGLE-CELL STATE**

In general, terminally differentiated, quiescent cells tend to be strongly polarized (bearing a more-negative resting potential),...
TABLE 1: Cell-level properties/behaviors controlled by bioelectric events.

| Physical mechanism | References |
|--------------------|------------|
| Proliferation and cell cycle progression | Cone (1970, 1971, 1974), Cone and Tongier (1971, 1973), Cone and Cone (1976), Stillwell et al. (1973), Binggeli and Weinstein (1986), Arcangeli et al. (1993), Rouzaire-Dubois et al. (1993), Wonderlin and Strobl (1996), MacFarlane and Sontheimer (2000), Liebau et al. (2006), Morokuma et al. (2008a) |
| Apoptosis | Wang et al. (1999), Miki et al. (2001), Lauringen et al. (2003), Lang et al. (2005), Shen et al. (2013) |
| Migration and orientation | Hyman and Bellamy (1922), Anderson (1951), Stump and Robinson (1983), Schwab et al. (1995), Schwab (2001), Zhao et al. (1997), Fraser et al. (2005), McCaig et al. (2005), Pullar and Isseroff (2005), Yan et al. (2009) |
| Differentiation | Barth and Barth (1974a,b), Konig et al. (2006), Hinard et al. (2008), Sundelacruz et al. (2008), Lange et al. (2011) |
| Dedifferentiation | Cone and Tongier (1971), Harrington and Becker (1973), Stillwell et al. (1973), Cone and Cone (1976), Sundelacruz et al. (2013) |

whereas embryonic, stem, and tumor cells tend to be depolarized (closer to zero; Binggeli and Weinstein, 1986). The picture is complicated by two still poorly understood factors: the relationship of overall $V_{\text{mem}}$ to the cell cycle–dependent (sinusoidally varying) changes in voltage potential (Arcangeli et al., 1995; Higashimori and Sontheimer, 2007; Aprea and Calegari, 2012) and the fact that many cells in fact do not have a single $V_{\text{mem}}$ but bear a set of distinct voltage domains over their surface (O’Connell et al., 2006; Levin, 2012a).

Crucially, $V_{\text{mem}}$ is not simply a readout but is also a functional determinant of cell behavior, such as proliferative state and plasticity (Table 1), due to a number of mechanisms that functionally couple voltage potential changes to downstream cascades (Figure 1, B and C). These data derive from genetic experiments, as well as pharmacological screens designed to identify compounds that regulate stem cell differentiation or cancer progression (Alves et al., 2011; Sun et al., 2013). Differentiation and proliferation are controlled by changes in $V_{\text{mem}}$, as shown in human mesenchymal stem cells (Sundelacruz et al., 2008, 2013; You et al., 2012), cardiomyocytes (Lan et al., 2014), inhibitory postsynaptic currents (Jiang et al., 2009), vascular muscle (Jia et al., 2013), embryonic stem cells (Ng et al., 2010; Du et al., 2013), myoblasts (in which hyperpolarization driven by the Kir2.1 channel plays a key role; Hinard et al., 2008; Li et al., 2010), the specification of neurotransmitter types (Root et al., 2008), and the control of precursor differentiation (van Vliet et al., 2010; Yasuda and Adams, 2010; Lange et al., 2011; Liebau et al., 2011; Ring et al., 2012; Podda et al., 2013) in the developing nervous system and heart. Given the known roles of $V_{\text{mem}}$ in regulating normal migration, differentiation, and proliferation (Aprea and Calegari, 2012; Ding et al., 2012; Inaba et al., 2012; Zhang et al., 2012; Cao et al., 2013; Yamashita, 2013), it is not surprising that control of ion flux (Park et al., 2008; House et al., 2010) and membrane voltage (Morokuma et al., 2008a; Blackiston et al., 2011; Chernet and Levin, 2013a, 2013b; Yang and Brackenbury, 2013) are also increasingly implicated in the cell dysregulation of cancer (Table 2).

Bioelectric cues also provide spatially patterned signals to cells. The differential activation of voltage-responsive transduction mechanisms on opposite sides of a cell allows bioelectric signals to regulate cell polarity. This was long ago shown in the symmetry breaking and control of outgrowth point in the algae Fucus (Jaffe, 1966, 1968) and has been recently shown using high-resolution imaging and genetic techniques in yeast (Minc and Chang, 2010) and pollen tubes (Certal et al., 2008; Michard et al., 2009). The cytoskeleton is one target of such signaling (Chifflet et al., 2003; Priel et al., 2006; Sekulic et al., 2011; Campetelli et al., 2012). Positional information can likewise be dictated by voltage properties of cells (Baglioni et al., 2012) and their neighbors (Shi and Borgens, 1995). Studies of embryonic left–right patterning of the Xenopus embryo have revealed how bioelectrical processes link individual cell dynamics to axial patterning of the entire body plan (Levin and Palmer, 2007; Aw and Levin, 2009): cytoskeletal chirality within the fertilized egg drives

| Ion translocator protein | Species | References | Function |
|-------------------------|---------|------------|----------|
| NaV1.5 sodium channel | Human | Onkal and Djamgoz (2009), House et al. (2010) | Oncogene |
| KCNK9 potassium channel | Mouse | Pei et al. (2003) | Oncogene |
| Ductin (proton V-ATPase component) | Mouse | Saito et al. (1998) | Oncogene |
| SLC5A8 sodium/butyrate transporter | Human | Gupta et al. (2006) | Oncogene |
| KCNE2 potassium channel | Mouse | Roepke et al. (2010) | Oncogene |
| KCNO1 potassium channel | Human, mouse | Lee et al. (1997), Weksberg et al. (2001), Than et al. (2013) | Oncogene |
| SCNSA voltage-gated sodium channel | Human | House et al. (2010) | Oncogene |
| Metabotropic glutamate receptor | Mouse, human | Song et al. (2012), Speyer et al. (2012), Martino et al. (2013) | Oncogene |
| CFTR chloride channel | Human | Xie et al. (2013), Zhang et al. (2013) | Tumor suppressor |
| Connexin43 | Human | Sirnes et al. (2012) | Tumor suppressor |
| Acetylcholine receptor | Mouse | Felder et al. (1993) | Tumor suppressor |

TABLE 2: Ion translocators implicated in cancer.
asymmetric distribution of ion transporter proteins in the early blastomeres, and the resulting gradient drives unidirectional (preneural) serotonin flow through cell fields, eventually triggering differential gene expression on the left versus right sides of the body (Levin, 2006; Levin et al., 2006; Aw et al., 2008; Lobkin et al., 2012b; Vandenberg et al., 2012, 2013). The dissection and synthesis of such systems at the genetic and physiological levels is beginning to reveal the properties of biophysical pathways by which individual cell polarity is integrated into large-scale patterning outcomes (Marshall, 2011).

**MEASURING $V_{\text{MEM}}$ IN VIVO**

The first step in analyzing a bioelectric signal is the characterization of the spatiotemporal distributions of ionic parameters and a determination of how they correlate with patterning events. $V_{\text{MEM}}$ in cells can be quantified using several approaches; unlike mRNA and protein levels revealed by sequencing or immunohistochemistry, bioelectric properties are only ascertainable in vivo and cannot be analyzed in fixed tissue. Voltage gradients can now be visualized continuously in situ using fluorescent reporters of transmembrane potential (Adams and Levin, 2012a,b; Figure 1D) and more exotic nanoscale materials (Tyner et al., 2007) suitable for use in any optically accessible tissue (Steinberg et al., 2007; Yun et al., 2007). These are a significant improvement on physiological impalement of single cells: far less invasive, and able to report multiple $V_{\text{MEM}}$ values across tissues and even within cell membrane subdomains (Lechleiter et al., 1991; Adams and Levin, 2013). Reagents include cell-permeant dyes such as CC2-DMPE and DiSBAC$_2$(3) (Adams et al., 2006; Adams and Levin, 2012b; Oviedo et al., 2008; Ozkucur et al., 2010) and genetically encoded protein reporters (Tsutsui et al., 2008; Mutoh et al., 2011; Shen et al., 2011; Akemann et al., 2012).

Additional tools for the characterization of bioelectrical events include highly sensitive ion-selective extracellular electrode probes (Reid et al., 2007; Smith et al., 2007) that reveal ion flux, microelectrode arrays (Ayasomayajula et al., 2010; Schonecker et al., 2014), and reporters of individual ion species such as protons (Tantama et al., 2011) and sodium (Tseng et al., 2010; Dubach et al., 2011a,b). Significant opportunities exist for the development of specific, bright, ratiometric dyes that localize exclusively to the desired subcellular locale (e.g., plasma membrane or nucleus). Especially exciting will be the use of multiple physiological dyes in fluorescence-activated cell sorting experiments to identify subpopulations of “pure” stem and other cell types that differ in key bioelectric properties (Mello de Queiroz et al., 2008), as has been observed for human endothelial cells (Yu et al., 2002). Of importance, such experiments on dissociated cells will clearly highlight properties that are cell autonomous versus those physiological conditions that can only be maintained within a group context.

**BIOELECTRIC SIGNALS INTERFACE WITH MOLECULAR GENETICS**

The mechanistic investigation of bioelectric cues and their interactions with canonical biochemical pathways has been enriched by several new functional techniques (Adams and Levin, 2006b, 2013; Reid et al., 2007; Song et al., 2007). The comprehensive workflow for probing developmental bioelectricity can be illustrated by two examples. In the first, a tiered pharmacological screen (Adams and Levin, 2006a) implicated a proton pump and two channels as specifically required for tail regeneration but not for wound healing or development of the primary tail (Adams et al., 2007). These loss-of-function data were confirmed using reagents with molecular specificity by misexpression of a dominant-negative form of a V-ATPase subunit protein. Marker analysis was used to show why tails failed to regenerate in V-ATPase–inhibited tails (loss of regeneration-specific gene up-regulation, lack of the obligate increase of mitosis near the wound, and abrogation of innervation into the regenerate). Fluorescent dye imaging provided physiometric profiling of the changes of $V_{\text{MEM}}$ during the stages of regeneration and confirmed that the unique voltage changes characteristic of the regenerating state were blocked by V-ATPase inhibition and were absent during stages at which tadpoles normally are not competent to regenerate their tails. On the basis of these findings, to develop a gain-of-function application, a yeast P-type proton pump was misexpressed in regeneration-incompetent animals, leading to restoration of mitosis, gene expression (MSX-1, Notch), innervation, and morphological regeneration of a complete tail. Additional rescue experiments using net-electroneutral proton exchangers allowed the independent testing of pH versus voltage signaling.

One key result was that the anatomical outcome (regeneration rescue) can be induced by a completely heterologous hyperpolarizing pump, which has no sequence or structural homology to the native Xenopus protein endogenously driving regeneration. This demonstrated that the necessary and sufficient trigger for regeneration is not a specific gene product (V-ATPase), but a bioelectric state, which can be implemented using a variety of different agents. This finding facilitated development of a purely pharmacological method of modulating ion flows in the wound to induce tail (Tseng et al., 2010) and leg (Tseng and Levin, 2013) regeneration without the need for gene therapy.

The available tools enable a multistep strategy that combines pharmacological screening, physiological imaging, and molecular-genetic tools to generate loss- and gain-of-function data showing how a bioelectric pathway normally works and how it can be exploited to trigger pattern formation. A similar approach was taken with an initial gain-of-function screen, misexpressing ion channels in frog embryogenesis. One of the outcomes was the finding that a specific $V_{\text{MEM}}$ range was necessary and sufficient to trigger ectopic eye development (Pai et al., 2012). Dye imaging data showed that the location of the endogenous eyes is demarcated by a prepatter of $V_{\text{MEM}}$ states in the anterior neurectoderm and that experimental alteration of this prepatter results in abnormal craniofacial gene expression and eye and facial malformations (Vandenberg, 2011; Pai et al., 2012). To complement the data showing that bioelectric states are an endogenous component of eye development, it was then shown that driving eye-specific $V_{\text{MEM}}$ states in other body regions (by misexpression of ion channels) was sufficient to induce anatomically complete (well-formed) ectopic eyes (Figure 2A). Marker analysis revealed that this occurs via establishment of a positive feedback loop between hyperpolarization and Rx1/Pax6 expression, whereas a suppression screen of transduction mechanisms implicated voltage–gated calcium signaling as the transduction mechanism. However, note that, by themselves, “master” eye genes such as Pax6 do not produce eyes outside the head in vertebrates (Chow et al., 1999). Moreover, as with the tail, individual cell types appropriate to the eye did not have to be specified. Together these data revealed the unique properties of bioelectric triggers to reprogram body regions at the level of organ identity and overcome lineage specification limits observed with biochemical inducers.

Of interest, many forward genetic approaches have identified ion channel genes responsible for patterning phenotypes, as have unbiased transcriptional network analyses in development (Langlois and Martyniuk, 2013) and cancer (House et al., 2010). These include patterning of the face, limb, brain, and viscera in a range of model systems and a number of channelopathies that form an important
FIGURE 2: Bioelectric properties specify instructive, non–cell-autonomous patterning cues. (A) Targeted V_{mem} change, via misexpression of ion channels in the frog embryo, induces the formation of ectopic structures such as complete eyes, even in regions normally not competent to form eyes (such as on the gut). (Used, with permission, from Figure 3G of Pai et al., 2012.) (B) Tracking the ion channel expression using a lineage marker reveals that the effect is not cell-autonomous: in a lens created in the tail of a tadpole by ion channel expression, only about half of the ectopic cells express the heterologous ion channel (revealed by blue lacZ staining); the other half of the induced structure consists of host cells recruited to participate in making the appropriate shape but not themselves targeted by the V_{mem}-altering reagent. (C) Melanocytes seen in a cross section of a Xenopus tadpole are normally few in number, round, and confined to their normal locations. (D) Depolarization induced by ion channel modulation induces these cells to overproliferate, acquire an elongated shape, and invade many organs (red arrow). Of importance, this effect is also not cell autonomous, as seen in the melanocyte phenotype, which results when cells (marked by ion channel expression construct lineage label in blue) depolarized at a considerable distance from the melanocytes. (Taken, with permission, from Figure 6A of Chernet and Levin, 2013b.) (E) A normal planarian has a head and tail and regenerates each at the appropriate end of an amputated fragment. When it is cut into thirds and the middle fragment is briefly exposed to octanol, which temporarily blocks long-range bioelectrical signaling between the wound and mature tissues, a two-headed worm is generated. This change, via misexpression of ion channels in the tail of a planarian, induces the formation of ectopic structures such as complete eyes, even in regions normally not competent to form eyes (such as on the gut). The process described here is not cell autonomous: in a lens created in the tail of a planarian by ion channel expression, only about half of the ectopic cells express the heterologous ion channel (revealed by blue lacZ staining); the other half of the induced structure consists of host cells recruited to participate in making the appropriate shape but not themselves targeted by the V_{mem}-altering reagent.

reprogrammed to a head fate, are discarded at each cut: the information encoding a bipolar two-head animal is present even in the normal gut fragment—it is distributed throughout the body. We propose that this information is a kind of memory, encoded in electrical networks of somatic cells coupled by gap junctions, and is stored at the level of bioelectrical dynamics. (E–I taken, with permission, from Figure 2 of Levin, 2014; photographs of planaria taken by Taisaku Nogi, Children’s Health Research Institute, Canada, and Fallon Durant.)
| Protein                                      | Morphogenetic role or loss-of-function phenotype                                                                 | Species          | References                                                                 |
|----------------------------------------------|------------------------------------------------------------------------------------------------------------------|------------------|---------------------------------------------------------------------------|
| TMEM16A chloride channel                     | Tracheal morphogenesis                                                                                            | Mouse            | Rock et al. (2008)                                                         |
| Kir7.1 potassium channel                     | Melanosome development                                                                                            | Zebrafish        | Iwashita et al. (2006)                                                    |
| Cx41.8 gap junction                          | Pigmentation pattern                                                                                            | Zebrafish        | Watanabe et al. (2006)                                                    |
| Cx45 gap junction                            | Cardiac defects (cushion patterning)                                                                             | Mouse            | Kumai et al. (2000), Nishii et al. (2001)                                 |
| Cx43 gap junction                            | Oculodentodigital dysplasia, heart defects (outflow tract and conotruncal), left–right asymmetry defects, eye defect, osteoblast differentiation in bone patterning, syndactyly, microphthalmia | Human, mouse     | Britz-Cunningham et al. (1995), Reaume et al. (1995), Ewart et al. (1997), Pizzuti et al. (2004), Debeer et al. (2005), Civitelli (2008), Zoidl and Dermietzel (2010), Gabriel et al. (2011) |
| Kir2.1 potassium channel                     | Wing patterning                                                                                                | Drosophila       | Dahal et al. (2012)                                                        |
| Cx43 gap junction                            | Fin size and pattern regulation; craniofrontonasal syndrome                                                      | Zebrafish, mouse | Iovine et al. (2005), Davy et al. (2006), Hoptak-Solga et al. (2008), Sims et al. (2009) |
| Kir2.1 potassium channel                     | Andersen–Tawil syndrome, craniofacial and limb defects                                                           | Mouse, human     | Bendahhou et al. (2003), Dahal et al. (2012)                               |
| CFTR chloride channel                        | Bilateral absence of vas deferens                                                                               | Human            | Uzun et al. (2005), Wilschanski et al. (2006)                              |
| KCNK9, TASK3 potassium channels              | Birk–Barel dysmorphism syndrome, craniofacial defects                                                           | Human            | Barel et al. (2008), Veale et al. (2014)                                   |
| Girk2 potassium channel                      | Cerebellar development, retina patterning                                                                       | Mouse            | Rakic and Sidman (1973a,b), Hatten et al. (1986), Patil et al. (1995), Tong et al. (1996), Savy et al. (1999), Liesi et al. (2000) |
| GABA-A receptor (chloride channel)           | Angelman syndrome, craniofacial patterning (e.g., cleft palate) and hand defects                                | Mouse, human     | Wee and Zimmerman (1985), Culiat et al. (1995), Homanics et al. (1997)    |
| KCNH2 K⁺ channel                             | Cardiac patterning                                                                                            | Mouse            | Teng et al. (2008)                                                         |
| NHE2 Na⁺/H⁺ exchanger                        | Epithelial patterning                                                                                            | Drosophila       | Simons et al. (2009)                                                       |
| V-ATPase proton pump                         | Wing-hair patterning, pigmentation and brain patterning, left–right asymmetry, eye development, tail regeneration, craniofacial patterning | Drosophila, medaka, human, chick, Xenopus, zebrafish | Hermle et al. (2010), Muller et al. (2013), Borthwick et al. (2003), Adams et al. (2006), Nuckels et al. (2009), Vandenberg et al. (2011), Monteiro et al. (2014) |
| Kv channel                                   | Fin-size regulation                                                                                            | Zebrafish        | Perathoner et al. (2014)                                                   |
| KCNQ1 potassium channel                      | Abnormalities of rectum, pancreas, and stomach, left–right patterning, Jervell and Lange-Nielsen syndrome, inner ear and limb defects | Mouse, Xenopus   | Chouabe et al. (1997), Casimiro et al. (2004), Rivas and Francis (2005), Morokuma et al. (2008b), Than et al. (2013) |
| Kir6.2 potassium channel                     | Craniofacial defects, left–right patterning                                                                    | Human, Xenopus   | Gloyn et al. (2004), Aw et al. (2010)                                     |
| NaV 1.5, Na⁺/K⁺-ATPase                       | Cardiac morphogenesis                                                                                            | Zebrafish        | Shu et al. (2003), Chopra et al. (2010)                                   |
| H⁺,K⁺-ATPase                                 | Left–right patterning, polarity during regeneration                                                             | Xenopus, chick, sea urchin, zebrafish, planaria | Levin et al. (2002), Kawakami et al. (2005), Aw et al. (2008), Beane et al. (2011) |
| Innexin gap junctions                        | Foregut, cuticle (epithelial) patterning defects                                                                 | Drosophila       | Bauer et al. (2002), Bauer et al. (2004)                                  |
| TRH1 K⁺ transporter                          | Root-hair patterning                                                                                            | Arabidopsis      | Rigas et al. (2001)                                                       |

**TABLE 3:** Ion translocators implicated in patterning by genetic approaches.
has been implicated in control of growth-cone turning (Nishiyama et al., 2008), eye patterning (Pai et al., 2012), and flatworm regeneration (Nogi et al., 2009; Beane et al., 2011; Zhang et al., 2011). Another uses the voltage gradients among cells to move small signaling molecules such as serotonin through gap junction-coupled cell fields, as occurs in left–right patterning (Fukumoto et al., 2005b; Adams et al., 2006) and control of neuronal pathfinding (Blackiston et al., 2015). Finally, voltage-sensitive phosphatases couple \( V_{\text{mem}} \) change to the plethora of events regulated by PTEN phosphatases (Murata et al., 2005; Okamura and Dixon, 2011).

Of interest, when they conflict, bioelectrical cues tend to trump chemical signals. One example is the guidance of cell motility: if a chemical gradient and an electric field are set up in opposite directions, the bioelectric vector trumps the chemical cue in directing cell movement (Zhao, 2009; Cao et al., 2011). Another example is the differentiation of human mesenchymal stem cells (hMSCs), which normally hyperpolarize as they differentiate; despite the presence of potent chemical inducers, hMSCs will not differentiate if kept artificially depolarized (Sundelacruz et al., 2008). Indeed, the voltage state can even partially reverse the differentiation state, inducing plasticity in differentiated hMSCs (Sundelacruz et al., 2013).

By identifying the specific ion channel genes that set \( V_{\text{mem}} \) states, the transduction mechanisms that sense \( V_{\text{mem}} \) change, and the downstream transcriptional or epigenetic targets (which include ion channels themselves), recent work has established the causal chain integrating bioelectrical cues with chemical pathways (Table 5). Neither signaling mode is entirely “upstream” of the other—cellular processes are regulated by the continuous cyclical interplay between transcriptional control of ion channel profiles within cells and the regulation of transcription by voltage dynamics. Future work will identify new ion channel genes important for specific functions, additional transduction mechanisms by which cells sense their depolarization and hyperpolarization, and genome-wide (next-generation sequencing (NGS) or microarray) profiles of transcriptional programs triggered by specific \( V_{\text{mem}} \) change.

Of importance, however, \( V_{\text{mem}} \) regulation extends beyond the state of single cells. Cells can sense the voltage states of their neighbors through gap junctions (GJs)—versatile (and themselves voltage-sensitive) channels allowing the direct sharing of current and other small molecules between cells (Palacios-Prado and Bukauskas, 2009; Pereda et al., 2013). The importance of GJ-mediated cues for cellular decision making has been shown, for example, in the development of the neocortex (Sutor and Hagerty, 2005) and more broadly in setting up the patterns of chemical syndasps (Anava et al., 2013). Cells can also read the bioelectrical state of distant regions via the chemical molecules redistributed (and transported or diffused) across long distances by bioelectric state change. This was long ago suggested by Burr, who used voltage readings at remote locations of the body to detect transplanted or induced tumors (Burr et al., 1940; Burr, 1941). Recent data in the frog model implicate long-range signaling via bioelectrical control of butyrate (Chernet and Levin, 2014) and serotonin (Blackiston et al., 2011; Lobikin et al., 2012a) in tumorigenesis and metastatic induction. Additional modes for nonlocal bioelectrical signaling include tunneling nanotubes (Chinnery et al., 2008; Wittig et al., 2012) and exosomes, which contain numerous ion channels (Lotvall and Valadi, 2007; Valadi et al., 2007; Wahlgren et al., 2012) and could regulate bioelectric states of cells that incorporate them. Because bioelectrical gradients mediate signaling beyond the single-cell level, they form a versatile medium for carrying information.

### TABLE 4: Known transduction mechanisms by which ion flows affects cell behavior.

| Developmental role | Key biophysical event | Transduction mechanism | References |
|--------------------|-----------------------|------------------------|------------|
| Tail regeneration in Xenopus: first step | Voltage change (repolarization) | Guidance of neural growth | Adams et al. (2007) |
| Tail regeneration in Xenopus: second step | Intracellular sodium content | SIK2 (salt-inducible kinase) | Tseng et al. (2010) |
| Neoplastic conversion of melanocytes in Xenopus tadpoles | Voltage change (depolarization) | Serotonin movement | Morokuma et al. (2008a), Blackiston et al. (2011) |
| Polarity determination in planarian regeneration, length control of zebrafish fin | Voltage change | Ca\(^{2+}\) flux through voltage-gated calcium channel | Beane et al. (2011), Zhang et al. (2011), Chan et al. (2014), Kujawski et al. (2014) |
| Left–right patterning in Xenopus embryos, melanocyte transformation toward meta-static behavior | Voltage change | Serotonin movement | Levin et al. (2002), Fukumoto et al. (2005a,b), Adams et al. (2006), Blackiston et al. (2011), Lobikin et al. (2012a) |
| Trachea size control in Drosophila | Ion-independent function | Planar polarity, septate junction structure | Paul et al. (2007) |

BIOELECTRIC STATES CAN ACT AS NECESSARY, SUFFICIENT, AND INSTRUCTIVE PATTERNING SIGNALS

Spatiotemporal gradients of \( V_{\text{mem}} \) among cells in vivo are now known to regulate organ identity, positional information, size control, and polarity of anatomical axes. One mode of \( V_{\text{mem}} \) signaling is as a prepattern. Much like Hox genes, whose combinatorial patterns of gene expression encode specific body regions during development, it has recently been shown that bioelectric prepatterns in the developing face of the frog and planarian models regulate the gene expression, size, and shape of craniofacial components (Vandenberg et al., 2011; Beane et al., 2013). In the frog, for example, patterns of hyperpolarization in the nascent face reveal the prospective locations of the eyes and other structures; experimental perturbation of these distributions alters the boundaries of expression of face patterning genes such as Frizzled, with the expected effects on craniofacial anatomy. Bioelectric gradients also specify orientation of the left–right axis in frog and chick embryos (Levin et al., 2002; Adams et al., 2006) and set the size of regenerating structures in segmented worms and regenerating...
Borgens (1986), References

Table 5: Data on endogenous bioelectric signal roles in morphogenesis.

| Role                                      | Species/system | References                        |
|-------------------------------------------|----------------|-----------------------------------|
| Cellular polarization (anatomical asymmetry of cell or epithelium) | Alga Fucus, yeast | Jaffe (1982), Minic and Chang (2010) |
| Migration of neurons and positional information | Chick, amphibia | Shi and Borgens (1995), Pan and Borgens (2010) |
| Patterning in gastrulation, neurulation, and organogenesis | Chick, axolotl, frog | Stern (1982), Hotary and Robinson (1992), Borgens and Shi (1995), Shi and Borgens (1995), Levin et al. (2002), Adams et al. (2006) |
| Directional transport of maternal components into the oocyte | Moth, Drosophila | Woodruff (2005) |
| Growth control and size determination | Segmented worms | Kurtz and Schrank (1955) |
| Neural differentiation | Xenopus embryo | Uzman et al. (1998), Lange et al. (2011) |
| Polarity during regeneration | Planaria, plants, and annelids | Marsh and Beams (1947, 1949, 1950, 1952), Marsh and Beams (1957), Bentrop et al. (1967), Novák and Bentrup (1972), Novak and Sirnoval (1975), Beane et al. (2011) |
| Induction of limb and spinal cord regeneration | Amphibia | Borgens (1986), Borgens et al. (1986, 1990) |
| Control of gene expression and anatomy in craniofacial patterning | Xenopus embryo | Vandenbergen et al. (2011) |
| Induction of eye development | Xenopus embryo | Pai et al. (2012) |

The voltage is what matters for the outcome, not which ion or channel was used to set it.

In addition to specifying directly the pattern of subsequent anatomy, some bioelectric signals seem to trigger whole developmental modules. In the case of tail regeneration in Xenopus, genetic, optogenetic, and pharmacological experiments have been used to recapitulate a regeneration-specific bioelectric state in nonregenerative animals and induce complete regrowth of this complex neuromuscular appendage (Adams et al., 2007; Tseng et al., 2010). Not only could appropriate \( V_{\text{mem}} \) state overcome physiological, chemical, and age-dependent blockade of regenerative capacity, but it was seen that a very simple (low information content) stimulus, such as “pump protons,” could be sufficient to trigger a complete and self-limiting cascade of events that rebuilt the appendage (Tseng and Levin, 2013), in essence providing a “build whatever normally goes here” signal. These examples reveal that bioelectric state can function as a sufficient signal or master regulator; this bodies well for the use of this approach in regenerative medicine, as we may not need to micromanage the morphogenesis of complex structures but instead rely on patterning subroutines already present in the host.

Bioelectric signals can also set the identity of whole embryonic regions to different organs. The morphogenesis of new regeneration blastemas in planaria (Figure 1, D–F) can be directed to make heads or tails by appropriate modulation of resting potential (Beane et al., 2011, 2013). In vertebrates, whole-eye formation can be induced ectopically, far outside the head, even in mesoderm or endoderm (Figure 2A) by misexpression of specific ion channels in vivo (Pai et al., 2012); this process is mediated by a feedback loop between hyperpolarization and expression of eye-specific genes such as Rx1 and Pax6, which in its absence cannot initiate eye formation outside of the head. It is also interesting that this signaling is not cell autonomous: cells with unique voltage characteristics serve as organizers, recruiting wild-type host tissues to participate in the ectopic morphogenesis (Figure 2B).

These examples illustrate the fact that bioelectric state provides instructive information to patterning processes and reveal that cell groups can be programmed at the level of complex organs, not only at the level of specifying individual cell types. Understanding in detail the mapping between bioelectric states and the anatomical outcomes—quantitatively cracking the bioelectric code—is a major open direction in this field. Possibilities for the parameters that functionally determine distinct organ types include spatial distribution of absolute \( V_{\text{mem}} \) values within a cell group, relative differences in \( V_{\text{mem}} \) across cell borders, and/or time-dependent changes of \( V_{\text{mem}} \) within cells. One technology that is likely to be instrumental in testing hypotheses about the bioelectric code is optogenetics (Knopfel et al., 2010; Liu and Tonegawa, 2010), which will facilitate the reading and writing of bioelectric patterning information in vivo. The first steps have been taken, showing regulation of stem cells via optogenetic signaling (Stroh et al., 2010; Wang et al., 2014), and a recent report showed the induction of tail regeneration by optical modulation of bioelectric state after amputation (Adams et al., 2013).

BIOELECTRICITY DOES NOT REDUCE TO MOLECULAR GENETICS

The information-bearing signal (the necessary and sufficient trigger) for events such as eye induction, head determination, and tail regeneration via \( V_{\text{mem}} \) change is a physiological state, not a gene product (Levin, 2013; Tseng and Levin, 2013). Studies reveal that the exact identity of the channel or pump used to trigger such morphological changes is often irrelevant—many sodium, potassium, chloride, or proton conductances can be used, as long as the appropriate...
The resting potentials across a tissue can arise from preexisting differences in ion channel transcription, but that is not the only way (Justet et al., 2013). Such regionalized patterns of $V_{\text{mem}}$ can also form de novo in transcriptionally and proteomically identical cells because cells coupled by gap junctions (electrical synapses) form a (slow) electrically excitable medium; this is a particularly interesting aspect because such media are known to have powerful computational capabilities (Fenton et al., 1999; Gorgcki and Gorgcka, 2007; Adamatzky et al., 2011). Positive feedback loops implemented by elements such as voltage-gated ion channels, which both set and respond to $V_{\text{mem}}$ changes, can drive spontaneous symmetry breaking and amplification of physiological noise. Considerable self-organization dynamics can take place without a need for preexisting chemical prepattern (Toko et al., 1987; Schiffmann, 1991, 1997; Palacios-Prado and Bukauskas, 2009) or transcriptional activity; for example, human red blood cells have a physiological, not genetic, circadian clock rhythm driven by a slow ionic oscillation (Chakravarty and Rizvi, 2011; O’Neill and Reddy, 2011). Such dynamics has been studied in nerve and muscle (Żykov, 1990; Chen et al., 1997; Boettiger et al., 2009; Boettiger and Oster, 2009), and Turing-type self-organization has long been appreciated in chemical signaling (Takagi and Kaneko, 2005; Muller et al., 2012; Sheth et al., 2012). However, capabilities and properties of self-organization of voltage patterns in groups of nonneural cells remain to be formally analyzed. Quantitative analysis of in silico models of bioelectric dynamics will need to be integrated with deep new data sets from appropriate physiometric technologies to fully understand and control developmental patterning in vivo.

One unexpected recent finding illustrates the storage of patterning information in physiological networks and has significant implications for evolution. Planarian flatworms have the remarkable ability to regenerate completely from partial body fragments (Reddien and Sanchez Alvarado, 2004; Salo et al., 2009; Lobo et al., 2012). After a surgical bisection, the cells at one edge make a tail, whereas those at the other edge make a head, revealing that the adult stem cells that implement regeneration are not locally controlled (since the cells were direct neighbors until the scalpel separated them) but must communicate with the remaining tissue to decide what anatomical structures must be formed. It was shown that this long-range communication occurs via gap junction–mediated electrical synapses (Scemes et al., 2007; Marder, 2009; Pereda et al., 2013), and works together with a bioelectric circuit that determines head versus tail identity in each end’s blastema (Beane et al., 2011, 2013). Brief inhibition of this gap junction–mediated communication results in worms developing heads at both ends (Nogi and Levin, 2005; Oviedo et al., 2010).

What is remarkable (Figure 2, E–I) is that weeks later, when these two-headed animals have their heads and tails amputated again (in just water, with no further perturbation), the same two-headed phenotype results, and this is repeated upon subsequent amputations. Thus a transient perturbation of physiological cell–cell communication stably changes the pattern to which the animal regenerates upon damage, despite normal genomic sequence. This again illustrates the potential divergence of genetic versus physiological information, especially since the phenotype is stable across fission (this animal’s most frequent reproductive mode), and thus could have significant implications for evolution. Although epigenetic processes may be involved, chromatin modification mechanisms alone are not a sufficient explanation, since the ectopic heads (tissue that might be suggested to have been epigenetically reprogrammed into a head state from its original tail identity) are thrown away at each generation of cutting. What remains is a gut fragment, which somehow knows that it is to form two heads, not one, upon further

**Bioelectric Gradients Have Distinct, Autonomous Dynamics**

Bioelectric patterns are clearly important drivers of cell behavior and pattern formation, but how do these patterns originate? Diverse resting potentials across a tissue can arise from preexisting differences in ion channel transcription, but that is not the only way (Justet et al., 2013). Such regionalized patterns of $V_{\text{mem}}$ can also form de novo in transcriptionally and proteomically identical cells because cells coupled by gap junctions (electrical synapses) form a (slow) electrically excitable medium; this is a particularly interesting aspect because such media are known to have powerful computational capabilities (Fenton et al., 1999; Gorgcki and Gorgcka, 2007; Adamatzky et al., 2011). Positive feedback loops implemented by elements such as voltage-gated ion channels, which both set and respond to $V_{\text{mem}}$ changes, can drive spontaneous symmetry breaking and amplification of physiological noise. Considerable self-organization dynamics can take place without a need for preexisting chemical prepattern (Toko et al., 1987; Schiffmann, 1991, 1997; Palacios-Prado and Bukauskas, 2009) or transcriptional activity; for example, human red blood cells have a physiological, not genetic, circadian clock rhythm driven by a slow ionic oscillation (Chakravarty and Rizvi, 2011; O’Neill and Reddy, 2011). Such dynamics has been studied in nerve and muscle (Żykov, 1990; Chen et al., 1997; Boettiger et al., 2009; Boettiger and Oster, 2009), and Turing-type self-organization has long been appreciated in chemical signaling (Takagi and Kaneko, 2005; Muller et al., 2012; Sheth et al., 2012). However, capabilities and properties of self-organization of voltage patterns in groups of nonneural cells remain to be formally analyzed. Quantitative analysis of in silico models of bioelectric dynamics will need to be integrated with deep new data sets from appropriate physiometric technologies to fully understand and control developmental patterning in vivo.
cutting; the information about basic anatomical polarity and body organization must be stored in a distributed form throughout the animal. Quantitative, field-like models of this circuit remain to be developed to understand precisely how information guiding specific shape outcomes is encoded in (represented by) bioelectric states among cells.

CONCLUSION: NEXT STEPS AND BEYOND

Major open questions for future progress include the mechanisms by which cells compare bioelectric state across distances, additional molecular details of the interactions of bioelectrical signals with chemical gradients and physical forces, and the development of quantitative models of bioelectric circuits that store stable patterning information during morphogenesis. Expansions of the toolkit of synthetic biology will soon allow the rational top-down programming of bioelectric circuits, which will have important implications for regenerative medicine, cancer biology, and bioengineering (Reid et al., 2011a; Levin, 2013). Optogenetics, once expanded to facilitate the control of stable $V_{\text{mem}}$ in large, nonexcitable cell groups, will play a large part, and there is significant room for advances in better voltage reporters and techniques for in vivo modulation of bioelectric state. One hypothesis for the development of deep, quantitative theory in this field is that patterning information may be stored within nonneural bioelectric cell networks using the same molecular mechanisms and information-processing algorithms that underlie behavioral memory in the nervous system. This is being tested in our lab. It is thus possible that the techniques such as those now used to extract mental imagery from electrical measurements of living human brains (Nishimoto et al., 2011) may shed crucial light on the encoding of anatomical pattern in the electrical circuits of somatic cells; conversely, the cracking of the bioelectric code in development and regeneration may have important benefits for the understanding of the semantics of electric states in the brain.

In practical terms, the molecular biologist needs to consider not only transcriptional and protein profiles when working to understand regulation of single-cell behavior and pattern formation. Significant instructive information is generated at the level of bioelectricity; ion channels and gap junctions are the molecular elements of such circuits, but bioelectrical signaling has its own unique dynamics that will become increasingly tractable with development of new technology specifically targeting stable $V_{\text{mem}}$ states. The existence of bioelectric signaling among most cell types, not only neurons, suggests that the field of applicability of electrochemicals (Famm et al., 2013; Sinha, 2013; Birmingham et al., 2014) is much wider than anticipated by current plans to target neural function. More broadly, to the extent that the data of developmental bioelectricity are erasing artificial distinctions between neural and nonneural cell types, the insights of computational neuroscience and cognitive science will become relevant to cell and developmental biology. It is possible that the most effective ways to understand high-order (anatomical-level) outcomes will involve not only bottom-up models of molecular pathways but also top-down models in which information and control theory concepts play central roles. In this way, molecular bioelectricity may be revealing a mechanistic path toward understanding the intelligence exhibited by cell behavior and harnessing it toward transformative advances in biomedicine and the information sciences (Albrecht-Buehler, 1985; Rubenstein et al., 2009; Marshall, 2011; Aur, 2012).

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