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Chapter 15

Endogenous bioelectric cues as morphogenetic signals in vivo

Maria Lobikin and Michael Levin

Center for Regenerative and Developmental Biology, and Biology Department, Tufts University, Medford, MA 02155

Abstract. Complex pattern formation requires mechanisms to coordinate individual cell behavior towards the anatomical needs of the host organism. Alongside the well-studied biochemical and genetic signals functions an important and powerful system of bioelectrical communication. All cells, not just excitable nerve and muscle, utilize ion channels and pumps to drive standing gradients of ion content and transmembrane resting potential. In this chapter, we discuss the data that show that these bioelectrical properties are key determinants of cell migration, differentiation, and proliferation. We also highlight the evidence for spatio-temporal gradients of transmembrane voltage potential as an instructive cue that encodes positional information and organ identity, and thus regulates the creation and maintenance of large-scale shape. In a variety of model systems, it is now clear that bioelectric prepatterns function during embryonic development, organ regeneration, and cancer suppression. Moreover, genetic and pharmacological modulation of the prepatterns resident in physiological networks is a powerful modality for controlling growth and form. Recent data have revealed the mechanisms by which voltage gradients are transduced into downstream transcriptional cascades. Thus, mastery of the endogenous bioelectrical signaling pathways will have transformative implications for developmental biology, regenerative medicine, and synthetic bioengineering.

Correspondence/Reprint request: Dr. Michael Levin, Center for Regenerative and Developmental Biology and Biology Department, Tufts University 200 Boston Ave. Suite 4600, Medford, MA 02155, e-mail: michael.levin@tufts.edu

1. Introduction: Bioelectricity and the history of 'animal spirits'

Understanding the mechanisms by which cell-to-cell communication and large-scale pattern formation are coordinated in the developing embryo is of high priority to developmental biology, regenerative medicine, and oncology. Alongside well-characterized biochemical modes of cellular communication that regulate cellular behavior during pattern formation there exists an important and powerful signaling system that is only now beginning to be understood and integrated with canonical biochemical and genetic pathways (Adams & Levin, 2013). This system of information exchange functions through bioelectrical mechanisms.

What is endogenous bioelectricity?

Bioelectricity, in general, refers to signals carried by voltage gradients, ion flows and electric fields that all cells receive and generate. Bioelectricity is most well known in the context of neuronal excitation in which rapid changes in transmembrane potential (V_{mem}) give rise to rapid action potentials. However, long-term, steady state ion fluxes, electric fields, and pH gradients are present in *all* cells and across epithelial sheets. At the cellular level, transmembrane potentials result from the presence of ion channels and pumps within cell membranes that function to segregate ions in differing concentrations internally and externally. This segregation of charges gives rise to transmembrane voltage potentials, usually on the order of -50 mV. It is becoming increasingly clear that these bioelectric parameters serve functional roles in signaling pathways that control cell proliferation, differentiation and migration. Thusly, understanding how these mechanisms function is of high priority to developmental biology, regenerative medicine and cancer research. In complement to other work on electromagnetic radiation and other biophysical properties of cells, this chapter focuses on the endogenous patterning roles and signaling mechanisms of spatially-distributed and slowly-varying (resting) transmembrane potentials in living tissues.

A brief history

The study of bioelectricity began long ago. Original experiments date back to the 17th century to experiments done by the Dutch biologist and microscopist Jan Swammerdan who believed that muscle contraction was caused by the flow of 'animal spirits' (Cobb, 2002). Swammerdan placed frog muscle into glass vessels and observed that physically irritating nerves with scissors or another instrument caused the muscles to contract. However, it wasn't until the 18th century that evidence of 'animal electricity' was procured by the Italian physicist and physician, Luigi Galvani (McCaig et al,

2005). In his famous experiments in the late 1700's, Galvani observed that extracted frog muscles would twitch when exposed to currents produced during lightning storms. Galvani believed that the activation of these muscle movements was generated by electrical fluid carried to the muscles by nerves. This phenomenon was termed 'Galvanism', and is credited with being the underpinnings to the modern study of electrophysiology (Bresadola, 1998). Galvani fought most of his life to persuade skeptical colleagues that 'animal electricity' was a reality and it wasn't until some 75 years later that modern experimental electrophysiology was launched by Emil du Bois-Reymond's *Researches on Animal Electricity* (Abbott, 2008).

Further experimentation conducted in the 19th century implicated electrical potentials in the process of wound healing. In 1831, Matteucci demonstrated the existence of action potentials in nerve and muscle cells for the first time by measuring injury potentials at cut ends using a galvanometer (McCaig et al, 2005). Injury potentials are now known to be a steady state, long-lasting direct current (DC) voltage gradient induced within the extracellular and intracellular spaces by current flowing into and around an injured nerve. Emil du-Bois Reymond built upon the initial observations of Matteucci by measuring current flowing out of a cut on his finger. This flow of current is due to the short-circuiting of the transepithelial potential (TEP) difference that occurs at a skin lesion (the TEP drives charged ions through the wound because the gap in the epithelium forms a low-resistance path for current flow). Human skin, as well as that of guinea pigs and amphibians, maintains a TEP across epithelial layers. When the skin is cut, a large, steady electric field (EF) arises immediately and persists for hours at the wound edge, as current pours out the lesion from underneath the wounded epithelium. This injury current is known to be essential for the regeneration of new limbs, where currents between 10 and 100 $\mu A/cm^2$ create a steady voltage drop of roughly 60 mV/mm within the first 125 µm of extracellular space (McCaig et al, 2005; McCaig et al, 2009).

Transcellular currents are also known to drive development and morphology. Elmer Lund carried out extensive research on electrical potentials between in the 1920s and 30s, arguing that electrical patterns are intimately related to the morphogenetic processes and vector properties of cell and tissue functions (Harold, 1982; Lund, 1947). The modern reformulation of these ideas is largely due to the work of Lionel Jaffe and his colleagues (done some 30 years later), demonstrating that electrical properties of individual cells, epithelial sheets, neural structures and limbs were necessary for growth and proper pattern and polarity establishment (Jaffe, 1981; Jaffe & Nuccitelli, 1977).

Bioelectricity in the molecular age

Several key aspects demarcate modern studies of bioelectricity from its foundations. First is an increased appreciation of spatial distribution of resting potentials. While classical works focused on electric fields and ion fluxes (mostly due to epithelia) (Borgens, 1982; Borgens, 1983; Nuccitelli, 1987; Nuccitelli et al, 1986; Robinson & Messerli, 1996; Shi & Borgens, 1995a), we now know that the spatial organization of plasma membrane voltage levels across tissues and organs carries vital patterning information that drives anatomy (Levin, 2012a). Secondly, techniques are now available for the molecular characterization of the mechanisms that both produce and respond to these gradients (Zhao et al, 2006). Together with traditional techniques such as physiological measurements and applied fields, endogenous gradients can now be manipulated with tight spatio-temporal specificity at the molecular level, via the genetic modulation of well-characterized channels and pump proteins (Adams & Levin, 2012). Thus, in addition to functional data on the electric properties themselves, the source and downstream effectors of changes in V_{mem} can now be dissected in great detail; for the first time, the patterning information encoded within dynamic bioelectrical networks are being integrated with well-known biochemical cascades and gene-regulatory networks. The results of these efforts reveal that embryonic patterning, regenerative repair and the suppression of cancerous disorganization all require continuous signal exchange between cells, tissues and organ systems (Adams, 2008; Levin, 2009).

2. The role of endogenous electric fields and voltage gradients in morphogenesis

The sources of endogenous bioelectric signals are shown in Fig. 1. Modern experimental techniques to probe animal electricity have come a long way since Galvani first made dead frog muscle twitch by applying an electric current to a nerve. Using standard techniques of molecular genetics, we can now target the expression patterns of ion channels and transporters for rational modulation. The use of knockout, RNAi, or morpholinos (antisense oligonucleotides to target specific mRNA sequences) allows gene-specific loss-of-function experiments. Pharmacological blockade, while not as specific as molecular approaches, offers the benefits of temporal control of inhibition, as well as the ability to target whole groups of ion channels or pumps at once – an important feature given that multiple ion translocators of the same family are often co-expressed and can compensate for each other, thus masking important phenotypes in gene-targeting experiments (Blackiston et al, 2009).

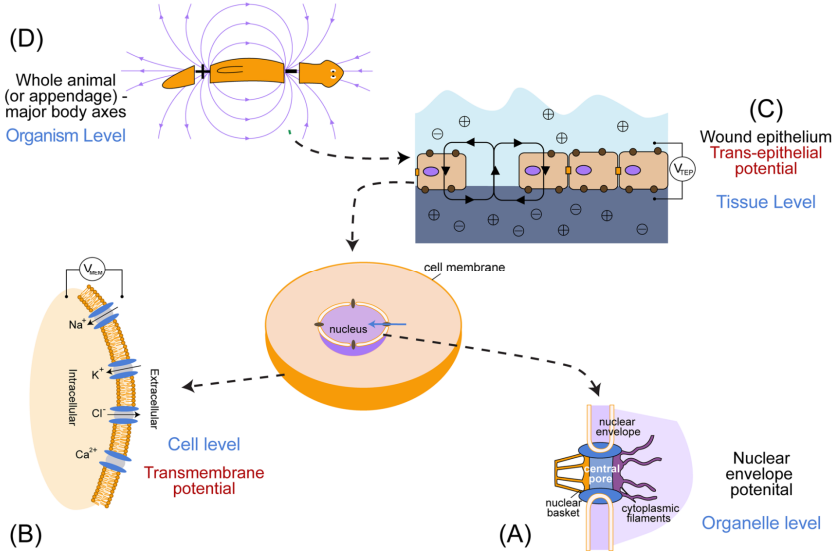

Figure 1. Sources of bioelectric signals at multiple levels of organization. Endogenous bioelectric signals comprise a set of biophysical properties that include voltage gradients, electric fields, and individual ion flows. In vivo, these originate at multiple levels of organization. (A) Organelle membranes generate voltage gradients, such as the nuclear envelope potential (largely unexplored) and the well-understood mitochondrial potentials. In recent years, the roles of resting potential across the plasma membrane of the cell (B) has become known as an important determinant of cell fate; spatial gradients of such voltage values over cell fields are now known as regulators of pattern formation in embryogenesis and regeneration. Decades ago it was recognized that the trans-epithelial electric field resulting from the parallel activities of polarized cell layers (C) was an important factor for guidance of migratory cell types during development and wound healing. Finally, at the level of entire appendages or even whole organisms (D), large-scale potential differences presage and control anatomical polarity and organ identity.

In the past decade, much work has begun to identify the endogenous ion conductances that are responsible for important patterning events, and the mechanisms by which cells can translate these signals into known gene regulatory networks (Levin, 2009). Conversely, exogenous ion channels or pumps can be introduced into cells and thus allow predictable changes in transmembrane potential to reveal gain-of-function phenotypes. These techniques have now been used in numerous model species to show that endogenous bioelectric gradients are among the most important sources of morpho-

genetic information *in vivo* (Adams & Levin, 2012a; Levin, 2012a; Levin & Stevenson, 2012; McCaig et al, 2009; Pai et al, 2012).

V_{mem} as a regulator of cell behavior

Morphogenesis broadly defined is the dynamic process by which the geometry and topology of complex biological structures is established. This occurs during embryogenesis, but is also important during remodeling and regeneration during adulthood. The establishment and maintenance of shape on many scales (cells, tissues, organs, and entire bodyplans) is regulated by a number of epigenetic factors controlling gene expression. Cells with different membrane and cytoplasm properties, but with identical DNA complements must consistently form and maintain various embryonic and adult structures. It has long been known that voltage gradients can mediate some of the necessary long-range communication through endogenous electric fields (Jaffe, 1981; Jaffe & Nuccitelli, 1977; Nuccitelli, 1988). More recent work has shown that targeted perturbation of transmembrane voltage results in specific, coherent changes of large-scale patterning. Remarkably, modulation of resting potential does not in itself impair embryonic viability, and it is often possible to dissociate subtle patterning functions of bioelectric states from basic housekeeping physiology of cells. Thus, V_{mem} levels in key groups of cells have been implicated in controlling the head-to-tail (Beane et al, 2011) and left-right (Aw et al, 2010) body axis polarity, the patterning of craniofacial structures (Vandenberg et al., 2011), the induction of eye development (Pai et al, 2012), and the initiation of *Xenopus* tail regeneration (Adams et al, 2007; Tseng et al., 2011).

One example of how V_{mem} values regulate the behavior of key cell populations *in vivo* is demonstrated by the discovery of a set of cells in the frog embryo that can confer a neoplastic-like phenotype upon stem cell derivatives, resulting in an embryo-wide 'hyperpigmentation' phenotype (Blackiston et al., 2011). The expression of the glycine-gated chloride channel (GlyCl) demarcates a widely, yet sparsely distributed cell population that can be specifically targeted by exposing embryos to the potent GlyCl channel agonist, ivermectin (Ottesen & Campbell, 1994). Then, controlling the extracellular concentrations of chloride in accordance to the Goldman equation, the membrane potential of GlyCl-expressing cells can be specifically modulated to known levels (and monitored with voltage-reporting fluorescent dyes). When depolarized, these GlyCl-expressing cells instruct, over a significant distance (mediated by regulation of serotonin signaling), the neural crest cell-derived melanocytes to undergo a neoplastic-like conversion acquiring three major properties commonly associated with metastasis: they hyperproliferate, become highly invasive, and undergo a change in shape, as well as up-regulating genes associated with neoplasia – SLUG and Sox10 (Morokuma et al, 2008). Crucially, this metas-

tasis-like phenotype can be reproduced by misexpressing mRNAs encoding sodium, potassium, or proton transporters, and can be rescued by the simple manipulation of extracellular ion content or through misexpression of opposing (hyperpolarizing) channels that drive the bioelectric state of the instructing cells back to normal. Together these data demonstrate that the control of instructor cell-derived signaling is driven by voltage *per se*, not necessary any one specific channel protein or type of ion.

How is V_{mem} change transduced into specific cellular responses?

Several known mechanisms (Fig. 2) convert long-term changes in V_{mem} levels into second-messenger cascades that ultimately drive transcriptional responses (Levin, 2007). Voltage-driven conformational changes of molecules such as integrins (Arcangeli & Becchetti, 2010; Arcangeli et al, 1993) and phosphatases (Lacroix et al, 2011; Okamura & Dixon, 2011), as well as voltage-regulated movement of signaling molecules through calcium channels (Varga et al, 2011), gap junctions (Brooks & Woodruff, 2004; Fukumoto et al, 2005), and neurotransmitter transporters (Levin et al, 2006), can all play a role in linking biophysical events to changes in gene transcription. These processes then feed into several known genetic mechanisms, often involving changes in expression or function of genes such as PTEN, Integrin, SLUG/Sox10, Notch, SIK, and NF-kB. This, in turn, leads to changes in cell cycle, position, orientation and differentiation. It is now known that the nuclear membrane also possesses its own complement of ion transporters (Bustamante, 1994; Bustamante et al, 1995; Bustamante et al, 1994; Mazzanti et al, 2001); although the function of the nuclear envelope potential has not been explored in developmental patterning, it is possible that the current picture of bioelectric signaling needs to be expanded beyond cell surface events. Thus, V_{mem} changes and ion flows can function as one link in the continuous interplay between genetic networks (which establish patterns of ion channel and pump expression) and the biophysical events that redistribute signaling molecules and control cell behavior within the long-range signaling pathways that occur during development and regeneration.

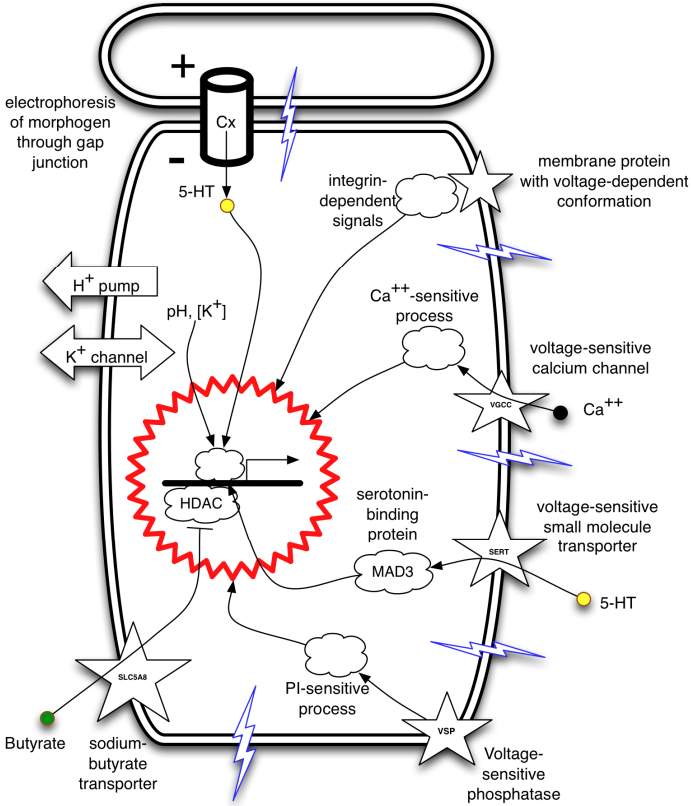

Figure 2. Mechanisms for converting membrane voltage change into transcriptional events. Multiple mechanisms exist within cells to transduce changes in V_{mem} (a biophysical event) into genetic responses. Transcriptional cascades are initiated by second messenger systems that are voltage-regulated, including the movement of small molecules such as serotonin (5HT) through gap junctions via electrophoresis (voltage gradient between two connected cells) or through voltage-powered transporters such as the serotonin transporter SERT. Other molecules, such as integrin receptors and voltage-sensitive phosphatases can convert changes in V_{mem} into powerful integrin- and PTEN-dependent downstream signaling. Additional small molecules include Calcium, mediated by voltage-gated calcium channels, and butyrate/sodium transporters (such as SLC5A8) that allow voltage to control the import of key epigenetic regulators such as butyrate. Legend: star indicates membrane protein. Cloud indicates a process (chain of signaling steps). Lightning bolt indicates local change in transmembrane potential. Cylinder indicates a gap junction pore to neighboring cell. Colored circles represent small signaling molecules.

Techniques for identifying voltage transduction mechanism

How can the particular transduction mechanism mediating any bioelectric effect be identified in a specific assay? One example is provided by the identification of the 'instructor cells' that, when depolarized, cause a hyperpigmentation phenotype in *Xenopus laevis*. In this case, as well as other similar examples *in vivo*, the mechanism by which long-term depolarization is transduced into transcriptional and cell behavior changes was identified through a suppression drug screen. In such a loss-of-function approach, each possible signal transduction candidate is probed by inhibiting it to determine whether this suppresses a given effect of V_{mem} change (Adams & Levin, 2006; Adams & Levin, 2012). In the case of melanocytes, inhibitors of Ca^{2+} influx, of serotonin transporter (SERT) function, or of gap junctional connectivity were used together with the depolarizing ivermectin treatments. Only exposure to the specific inhibitor of the serotonin transporter (fluoxetine) blocked ivermectin-induced hyperpigmentation in all of the treated embryos, suggesting that SERT is required for the transduction of this bioelectrical signal (Blackiston et al, 2011). Consistently with this model, embryos treated directly with extracellular serotonin also resulted in consistent hyperpigmentation. Similar screens have resulted in the identification of the various transduction mechanisms in various morphological events (summarized in Table 1).

Developmental role	**Key biophysical event**	**Transduction mechanism**	**Reference**
Tail regeneration in *Xenopus*: 1° step	Voltage change (repolarization)	Guidance of neural growth	(Adams et al, 2007)
Tail regeneration in *Xenopus*: 2° step	Intracellular sodium content	SIK2 (salt-inducible kinase)	(Tseng et al, 2010)
Proliferation of progenitor cells	Voltage change	Ca^{++} flux through voltage-gated calcium channels	(Ring et al, 2012)
Neoplastic conversion of melanocytes in *Xenopus* tadpoles	Voltage change (depolarization)	Serotonin movement through SERT	(Blackiston et al, 2011; Morokuma et al, 2008)
Polarity determination in planarian regeneration	Voltage change	Ca^{++} flux through voltage-gated calcium channels	(Beane et al, 2011)
Left-right patterning in *Xenopus* embryos	Voltage change	Serotonin movement through gap junctions	(Adams et al, 2006; Fukumoto et al, 2005a,b; Levin et al, 2002)
Trachea size control in *Drosophila*	Ion-independent function	Planar polarity, septate junction structure	(Paul et al, 2007)

Table 1. Known transduction mechanisms by which ion flows impact morphogenesis.

Bioelectric signals for coordination of non-local morphogenesis

Large-scale pattern formation requires the orchestration of numerous cell-level processes. Bioelectric gradients are an ideal mechanism for implementing such coordination because they function across a range of size scales (Adams & Levin, 2013; Blackiston et al, 2009; Sundelacruz et al, 2009) and control basic cell behaviors such as cell cycle progression (Binggeli & Weinstein, 1986; Sundelacruz et al, 2009) and differentiation (Konig et al, 2006; Konig et al, 2004), in a wide range of cell types, including human mesenchymal stem cells (Sundelacruz et al, 2008), embryonic stem cells (Ng et al, 2010), and mature somatic cells (Cone & Cone, 1976; Cone, 1970). Many studies have also examined the effects of V_{mem} on cell migration and orientation, and significant progress has been made on dissecting the molecular mechanisms driving these processes in the context of wound healing (McCaig et al, 2005; McCaig et al, 2009; Schwab, 2001; Zhao et al, 2006) and whole-body embryogenesis (Pan & Borgens, 2010; Shi & Borgens, 1995).

Given the abilities of voltage gradients to exert influence both cell-autonomously and over long distances, what kind of patterning information can bioelectric signals mediate? Transmembrane potential can specify issue identity at the level of cell groups (Levin, 2012) as evidenced by recent findings showing that the artificial manipulation of V_{mem} (hyperpolarization to a specific level) in developing *Xenopus* embryos can turn cell groups far from the anterior neuroectoderm to an eye fate (Pai et al, 2012). V_{mem} changes can also control large-scale axial polarity, such as the head-tail polarity of regenerating planarian fragments (Beane et al, 2011; Marsh & Beams, 1947; Marsh & Beams, 1952), and the left-right patterning of the early frog embryo (Levin, 2006; Levin et al, 2006). In the latter series of studies, a pharmacological screen first implicated several ion transporters in establishment of correct laterality (Levin et al, 2002); serotonergic mechanisms mediating the effect were later found using a suppression screen (Fukumoto et al, 2005; Fukumoto et al, 2005). Transmembrane voltage patterns across tissues can also provide positional information to guide migratory cells in vertebrate neurulation (Shi & Borgens, 1995) or specify the spatial patterns of gene expression during craniofacial morphogenesis (Vandenberg et al, 2011) – a kind of subtle prepattern that underlies the biochemical and genetic prepatterns that drive anatomy. In addition to providing large-scale anatomical identity and controlling the geometry of gene expression, bioelectric gradients can act as master regulators, triggering highly-orchestrated, self-limiting downstream patterning cascades such as regeneration of an entire appendage. For example, regeneration of the tadpole tail can be induced by very simple signals consisting of modulations of proton or sodium ion movement in the blastema during non-regenerative stages (Adams et al, 2007; Tseng et al, 2010).

Given this epigenetic control of cellular processes, it should come as no surprise that bioelectric properties are essential to many developmental processes that require the proliferation, differentiation, migration and orientation of a vast number of cells. These same signals that are necessary in the regeneration and remodeling of complex tissues also participate in the continuous battle of multicellular organisms to avoid the runaway growth of cancer.

3. Endogenous electric fields & ionic flow in the detection & treatment of cancer

The same signaling mechanisms required for stem cell specification and lineage restriction during embryonic pattern formation also play fundamental roles in adult tissue regeneration and cancer. Indeed, cancer can be described as a lack of morphostasis, or a disruption in the ability to maintain target morphology (Oviedo & Beane, 2009; Rubin, 1985; Tsonis, 1987).

The molecular physiology of cancer

Many of the same signaling pathways (i.e. TGFß, Wnt, Notch, etc.) regulate self-renewal in both stem cell and cancerous cell types (Al-Hajj & Clarke, 2004; Bjerkvig et al, 2005; Reya et al, 2001; Wicha, 2006). While the unique bioelectrical properties of tumor tissue have long been recognized (Burr, 1941; Cameron & Smith, 1989; Koch & Leffert, 1979; Rozengurt & Mendoza, 1980), it is only in recent years that ion channels and bioelectric communication have emerged as important players in cancer-related processes. Many ion channels have been found to be involved in cancer-related cellular behaviors such as proliferation, apoptosis, migration and angiogenesis (Blackiston et al, 2011; Fiske et al, 2006; Kunzelmann, 2005; Morokuma et al, 2008; Pardo et al, 2005; Prevarskaya et al, 2007; Roger et al, 2006). In fact, ion channels are involved in each of the six traditional hallmarks of cancer: 1) self-sufficiency in growth signals, 2) insensitivity to antigrowth signals, 3) evasion of programmed cell death (apoptosis), 4) limitless replicative potential, 5) sustained angiogenesis, and 6) tissue invasion & metastasis (Hanahan & Weinberg, 2000; Prevarskaya et al, 2010).

The bioelectric profiles of different cell types demonstrate the link between membrane voltage and proliferative potential. The resting V_{mem}'s of various cell types vary widely (generally -10 mV to -90 mV) with plastic, embryonic, stem and tumor cells being relatively depolarized, whereas quiescent, terminally differentiated cells are relatively hyperpolarized (Binggeli & Weinstein, 1986; Sundelacruz et al, 2009). Membrane potentials are involved in the control of mitosis rate, as the modulation of V_{mem} has been shown to be required for both the G_1/S and G_2/M phase transitions

(Blackiston et al, 2009; Freedman et al, 1992). Mitotic arrest can be achieved by hyperpolarizing Chinese hamster ovary cells to -75 mV, and reversed by depolarizing to -10 mV (Cone & Tongier, 1973). Depolarization is also responsible for the hyper-proliferation of melanocytes in *Xenopus* embryos (Blackiston et al, 2011; Morokuma et al, 2008). V_{mem} thus provides a convenient target for the modulation of proliferative potential.

A number of ion channels have also been implicated in enhanced cell migration, motility and invasion; all crucial components of tumor metastases. For example, voltage-gated sodium channels have been detected in biopsies of metastatic breast, prostate and cervical cancers as well as in metastatic cancer-derived cell lines (Diaz et al, 2007; Diss et al, 2005; Fraser et al, 2005; Roger et al, 2007). Potassium and chloride channels have also been implicated in the dynamic changes in cell shape and volume required for the capacity to move and invade extracellular spaces in glioma cells (McFerrin & Sontheimer, 2006; Prevarskaya et al, 2010). A number of these studies indicate that highly metastatic cancers express embryonic isoforms of voltage gated sodium channels, further supporting the notion that cancer is a recapitulation of a developmental state. More recently, two studies revealed that depolarized membrane voltage is both a physiological signature by which nascent tumors can be non-invasively detected using fluorescent reporter dyes, and a functional parameter that can be used to control tumorigenesis: artificial hyperpolarization of oncogene-expressing cells by a range of ion channel types significantly reduces the formation of tumors in an amphibian model (Chernet & Levin, 2013; Lobikin et al, 2012).

Cancer: rogue genetics or loss of tissue organization?

In fact, developmental systems are a convenient model for the studies of cancer biology, providing access to a number of stem cell populations that are present throughout embryogenesis, many of which have been implicated with neoplasms. Perturbations in embryonic systems that can induce neoplastic-like phenotypes thus allow significant insights into the signaling mechanisms that may give rise to the creation of cancerous stem cells. Stem cells can be regarded as the center of the regeneration-development-cancer triad (White & Zon, 2008) and the backbone of the cancer stem cell hypothesis (Dean et al, 2005). Melanomas, for example, are tumors of pigmented cells known as melanocytes. Recent studies have highlighted that melanoma cells seem to revert to a more stem cell-like phenotype as they become more aggressive, showing decreased expression of the micropthalmia-associated transcription factor (MITF) and tyrosinase-related protein 1 (TRP1) (Bittner et al, 2000; Hendrix et al, 2003). This de-differentiation might make highly aggressive tumors more difficult to identify in routine histopathology amplifying the need for different classification standards.

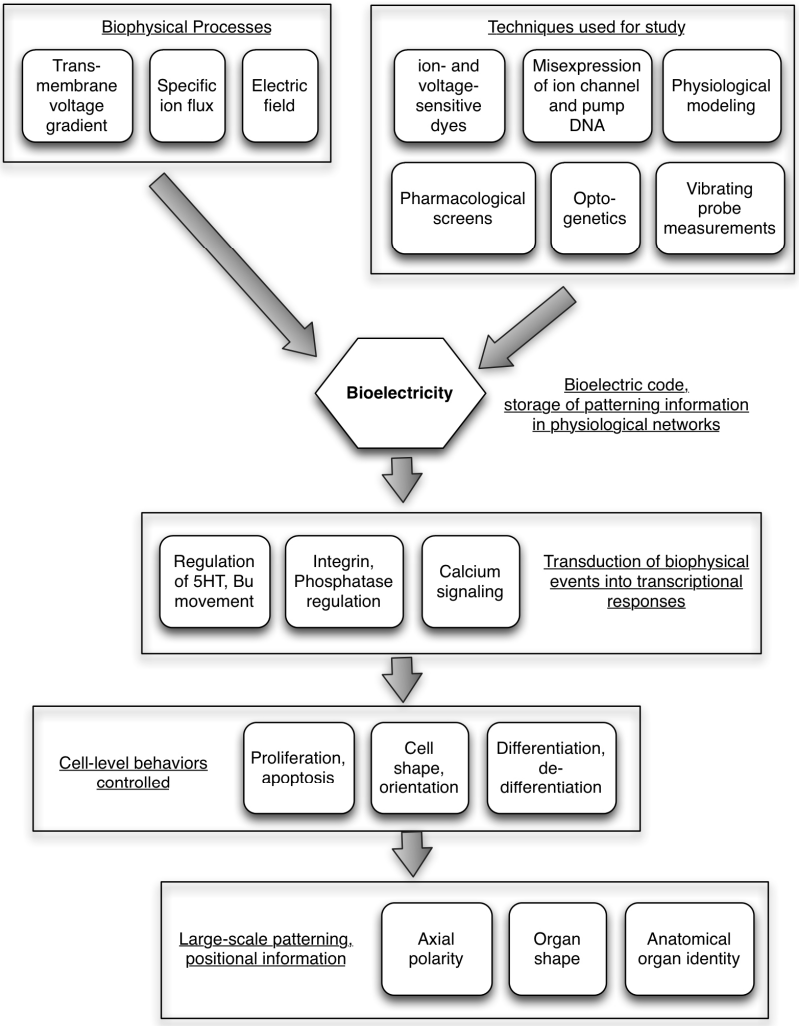

Figure 3. A mind-map of the field of bioelectricity.

There is currently significant debate as to whether stem cell disregulation and genetic mutation (Vaux, 2011a; Vaux, 2011b), or epigenetic signals from the microenvironment (Hendrix et al, 2007; Sonnenschein & Soto, 2011; Soto & Sonnenschein, 2004; Soto & Sonnenschein, 2011) are the better perspective from which to understand cancer. Importantly however, bioelectric mechanisms have now been shown as central players in both types of events (Levin, 2012b). Regardless of which view turns out to be the more accurate, continued advances in the understanding of regulation of stem and somatic cells by voltage gradients, and the interplay between biophysical and genetic regulators, are likely to have significant implications for the cancer problem.

4. Conclusion

Endogenous membrane voltage are one key component of the rich set of electromagnetic events taking place in living tissues; their spatio-temporal distribution represents important, yet still under appreciated, sources of instructive information in the control of morphogenesis. Recent work, making use of modern experimental techniques, has allowed scientists to probe the connections between these biophysical signals and the molecular-genetic downstream pathways that control cell behavior and thus large-scale patterning. However, we are only beginning to scratch the surface, and much development of technology and conceptual apparatus must take place before a full understanding of self-generated order and information storage in physiological networks can be gained. This includes development of theoretical formalisms for modeling information storage in real-time physiological (not genetic) networks, comprehensive (quantitative) physiomic profiling of morphogenetic model systems *in vivo*, and the application of tools such as optogenetics to allow the experimental re-writing of bioelectric patterns in living tissues. Bioelectricity (Fig. 3) still represents a novel area of research in the life sciences, and improvement in the ability to control bioelectrical information is sure to be transformative for regenerative medicine, bioengineering, and synthetic biology.

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