Electrochemical Control of Cell and Tissue Polarity

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Abstract

Localized ion fluxes at the plasma membrane provide electrochemical gradients at the cell surface that contribute to cell polarization, migration, and division. Ion transporters, local pH gradients, membrane potential, and organization are emerging as important factors in cell polarization mechanisms. The power of electrochemical effects is illustrated by the ability of exogenous electric fields to redirect polarization in cells ranging from bacteria, fungi, and amoebas to keratocytes and neurons. Electric fields normally surround cells and tissues and thus have been proposed to guide cell polarity in development, cancer, and wound healing. Recent studies on electric field responses in model systems and development of new biosensors provide new avenues to dissect molecular mechanisms. Here, we review recent advances that bring molecular understanding of how electrochemistry contributes to cell polarity in various contexts.

Contents

INTRODUCTION

Cells are electrical units surrounded and regulated by electrical currents. In living cells, membrane proteins, such as pumps and channels, maintain gradients of ions across the membrane, which serves as an insulator and a capacitor. Sodium ions are usually actively pumped out of the cell, for instance, whereas potassium is pumped into the cell. Concentration differences of ions across the plasma membrane produce membrane potentials ranging typically from −10 mV to −150 mV. It is well appreciated how the dynamic regulation of ion transport generates action potentials in neurons. Electrochemical cues have also been widely studied for their role in regulating cell physiology, and defects in ion transport have been associated with numerous diseases, including cancer and kidney, liver, or heart diseases (Hollenhorst et al. 2011, Hubner & Jentsch 2002, Prevarskaya et al. 2010, Webb et al. 2011).

It is becoming increasingly apparent that electrochemistry is also an important component of cell polarity. Cell polarization for processes such as cell migration and polarized cell growth involves the localization and activation of many cellular components. Prime organizers include small GTPases such as Cdc42 and Rhos and cytoskeletal elements (Drubin & Nelson 1996). It is beginning to be appreciated that localized membrane potentials, membrane transporters, and ion concentrations at the plasma membrane also participate in regulating cell polarity and are likely to functionally interact with membrane proteins, receptors, and cytoskeletal elements. The ability of exogenous electric fields to direct cell polarization in a variety of ways illustrates the relevance of electrochemistry to cell polarization and provides a tantalizing perspective on how electric fields that surround cells could coordinate cellular behaviors in multicellular contexts.

The appreciation of electrochemistry in the cell polarity field is still in its infancy. There are several reasons why this aspect has been poorly studied by cell biologists. In general, cell biologists are accustomed to working with the localization of defined proteins, rather than electrical signals. In contrast to proteins, ions diffuse extremely rapidly and cannot be visualized directly. Deciphering specific effects of membrane potential and pH can be difficult, as these are often essential for viability and affect a large number of downstream molecules and processes in the cell. However, new fluorescent markers are being developed, for instance, to quantitate local proton concentration or membrane potential (Kralj et al. 2011, 2012; Miesenbock et al. 1998). In addition,

recent advances in studying electric field responses are invigorating this field, providing a new, promising experimental avenue to decipher molecular pathways.

Some of the basic questions in this field are as follows:

1. What is the evidence that electrochemistry is relevant to cell polarity?
2. How are electric fields, membrane potentials, pH, and ion concentrations measured in cells?
3. What are the mechanisms used to generate local effects in different parts of the cell?
4. What are the downstream effects of membrane potential and ions on the cell polarization machinery?

Investigations in a variety of systems reveal that membrane potential, electrostatics, and pH may contribute to activating small GTPases, such as Rhos and Cdc42, and cytoskeletal regulators in specific regions of the cell. In this review, we discuss basic questions of electrochemistry in cell polarity and highlight recent examples that illustrate how this organization contributes to polarized cell growth, migration, division, and tissue architecture.

ORGANIZED ELECTRICAL CURRENTS SURROUND CELLS AND TISSUES

It has been appreciated for decades that cells and tissues in our body are surrounded by organized electrical signals. In initial pioneering experiments, miniaturized, vibrating electrochemical probes that can detect currents at the subcellular scale were used to map electrical currents and fields around cells and tissues (Jaffe & Nuccitelli 1974, Reid & Zhao 2011, Reid et al. 2007). Jaffe, Nuccitelli, and colleagues demonstrated that steady electrical currents and fields are often associated with polarized behavior in a variety of cells and tissues (Jaffe & Nuccitelli 1977, McCaig et al. 2005) (**Figure 1**). At the single-cell level (**Figure 1*a***), transcellular currents with steady front-rear asymmetries have been shown to enter the back and exit the front of migrating large amoebas. These currents are of relatively small magnitude, typically 0.1 $\mu A/cm^2$, and dynamically organize with the cell's axis (Nuccitelli et al. 1977). Currents of similar organization and magnitude have been mapped in fungi, water molds, and pollen tubes undergoing steady polarized tip growth. In these instances, currents usually exit the growing tip and enter the sides (Gow 1984, Kropf et al. 1984, Schreurs & Harold 1988, Weisenseel et al. 1975). Other examples include currents associated with cell division in the early cleavage of large amphibian eggs (Kline et al. 1983), as well as those associated with polarized outgrowth in the embryo of the brown algae *Fucus* (Jaffe 1966, Nuccitelli & Jaffe 1976, Robinson & Jaffe 1975).

In the context of multicellular organisms (**Figure 1*b***), electrical currents have been associated with large-scale tissue behavior. Perhaps the most well-characterized example is in wound healing, during which steady currents are oriented toward the wound (Nuccitelli 2003, Nuccitelli et al. 2008, Reid et al. 2007). These currents may arise from a rupture in the transepithelial electrochemical organization of epithelial layers (Kucerova et al. 2011, Szatkowski et al. 2000). Electrical activity has been found in limb regeneration (Levin 2009, Nuccitelli 2003) and other morphogenetic rearrangements during vertebrate development (Hotary & Robinson 1990, Jaffe & Stern 1979). These measurements have led to speculations that endogenous electrical fields could guide the migration of cells during wound healing and development.

How are these electrical currents established? These steady electrical patterns likely arise from the superimposition of localized influx and efflux of different charges. However, generally little is known about which ions are responsible for these currents. Calcium, potassium, sodium, or even protons have been proposed in several instances (Feijó et al. 1999, Nuccitelli & Jaffe 1976, Reid et al. 2011, Robinson & Jaffe 1975), but how these steady ion fluxes may be generated by the organization of specific ion transporters at a subcellular level remains poorly documented.

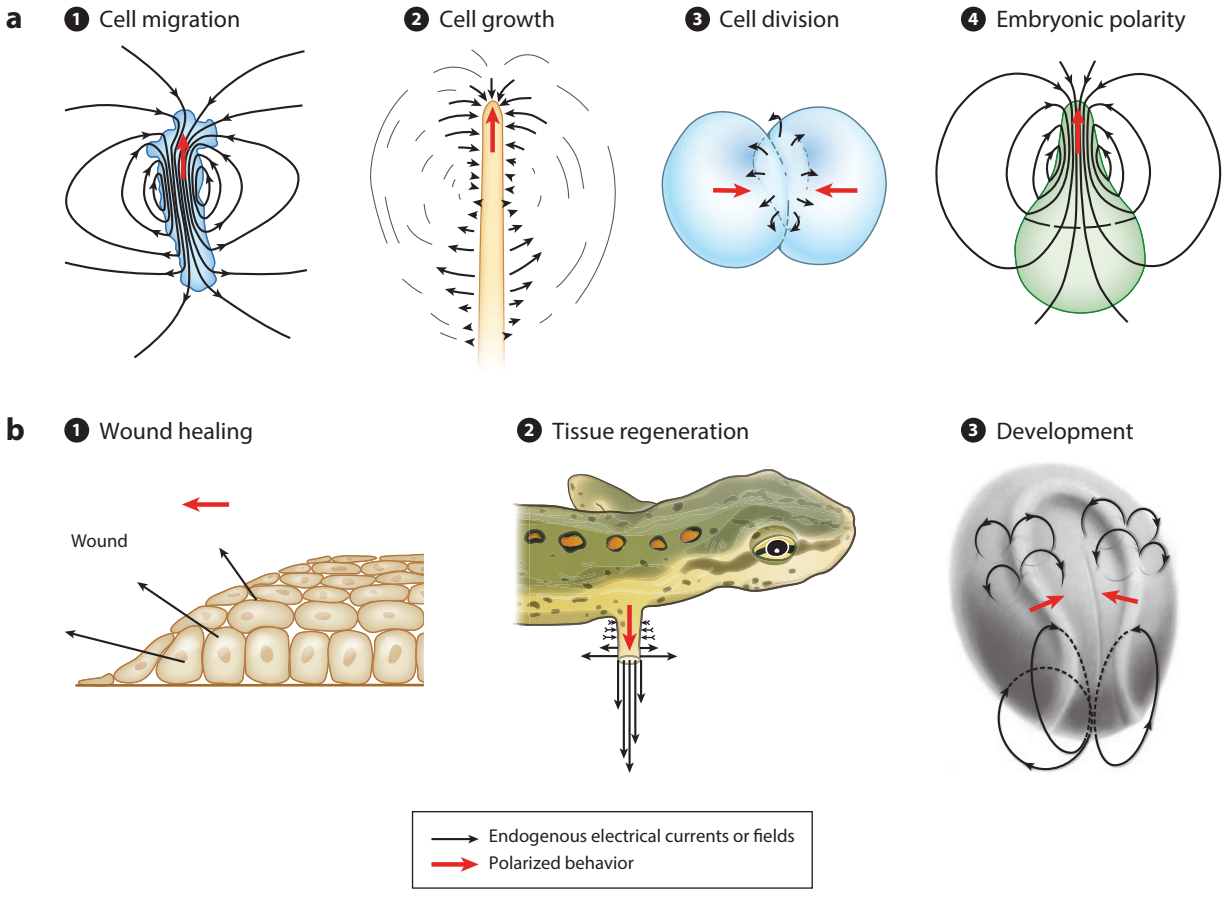


Figure 1

Electrical currents surround cells and tissues. (*a*) Electrical current patterns around single polarizing cells. The black arrows and lines are electrical currents mapped with vibrating microprobes. The red arrows indicate the direction of polarization. ① Migrating amoebas (adapted with permission from Nuccitelli et al. 1977). ② Polarized fungal growth (adapted with permission from Gow 1984). ③ Cleaving *Xenopus* embryo (adapted with permission from Kline et al. 1983). ④ Outgrowing *Fucus* embryo (adapted with permission from Nuccitelli & Jaffe 1975). (*b*) Electrical current patterns around polarizing tissues. ① Healing epithelial layer in a rat corneal wound (adapted with permission from Reid et al. 2005). ② Regenerating newt limb (adapted with permission from Borgens et al. 1977). ③ Neurulating amphibian embryo (adapted with permission from Shi & Borgens 1995).

EFFECTS OF EXOGENOUS ELECTRIC FIELDS ON POLARITY

The striking effects of applying exogenous electric fields on cells highlight the importance of electrochemistry in cell polarity. In these experiments, electric fields (EFs) of magnitudes similar to those measured in vivo have been shown to direct polarity in living cells and tissues. There is now a large body of evidence that most cells—ranging from bacteria, fungi, and amoebas to animal cells—are electrotactic and robustly orient polarity, migration, or division planes to applied EFs (**Figure 2*a***) (Brower & Giddings 1980; Hinkle et al. 1981; Korohoda et al. 2000; Lin et al. 2008; Minc & Chang 2010; Nishimura et al. 1996; Patel & Poo 1982; Pu et al. 2007; Pullar et al. 2006; Rajnicek et al. 1992, 1994; Soong et al. 1990; Zhang et al. 2000; Zhao et al. 1999, 2006). Similarly, EFs also affect cellular behaviors in multicellular tissues in the context of wound

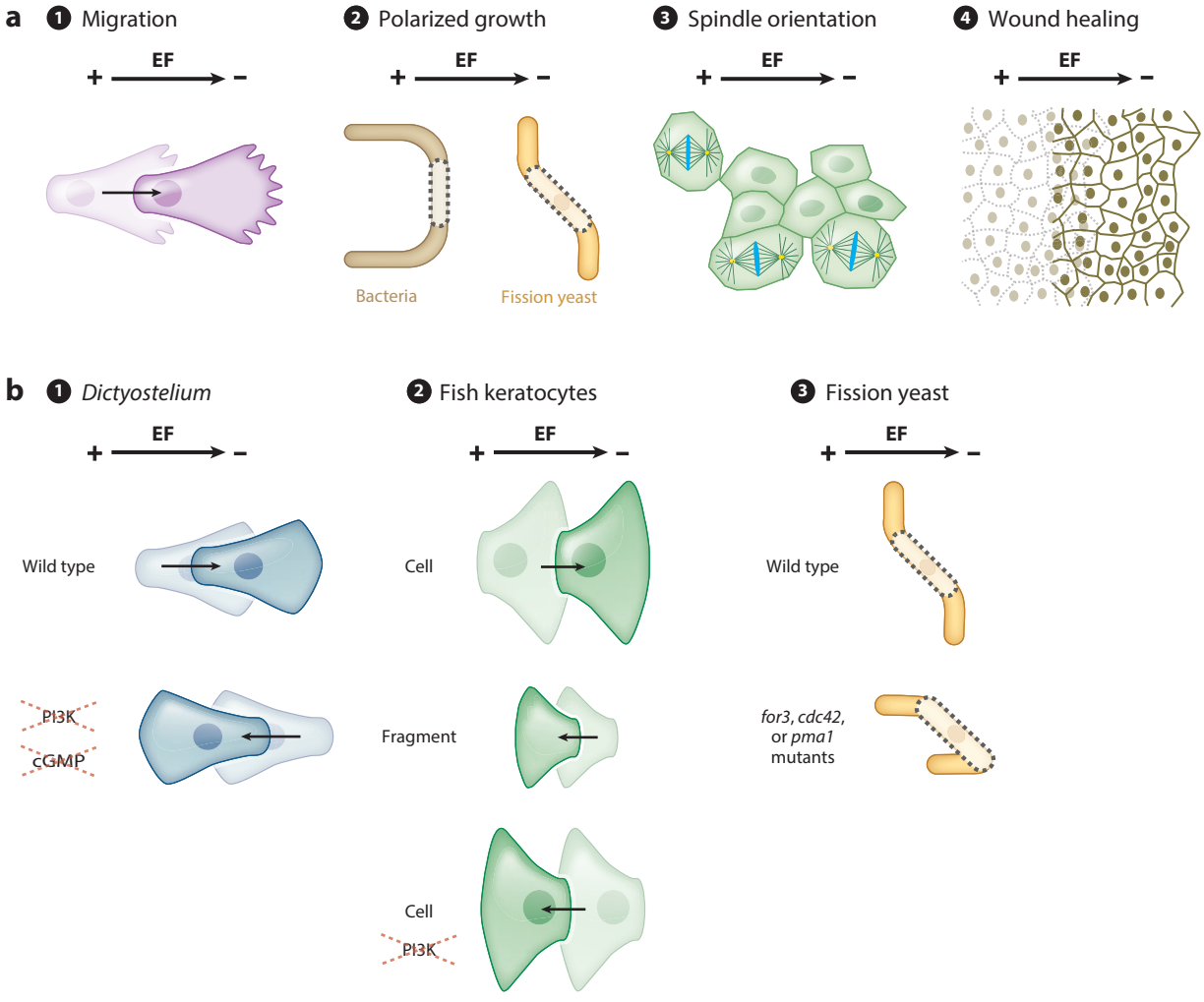


Figure 2

Application of exogenous electric fields can direct cell polarity. (*a*) Exogenous electric fields (EFs) can orient ① polarized migration, ② polarized growth, ③ cell division, and ④ wound healing. (*b*) ① *Dictyostelium* amoeba migrates toward the cathode of an applied EF and to the anode when both PI3K and cGMP are inhibited (adapted from Sato et al. 2009). ② Fish keratocyte cells orient to the cathode of the EFs, whereas cell fragments migrate to the anode, and cells inhibited for PI3K migrate to the anode (adapted from Allen et al. 2013, Sun et al. 2013). ③ In fission yeast cells, which normally grow as rods, EF causes cells to reorient growth perpendicular to the EF direction. This reorientation depends on the proton ATPase pump Pma1, the small GTPase Cdc42, and the formin For3. *pma1*, *for3*, or *Cdc42*, mutants orient instead to the anode (adapted from Minc & Chang 2010).

healing, regeneration, and development (Hotary & Robinson 1992, Levin 2009, Zhao 2009, Zhao et al. 2006). These findings suggest that electrotactic signals may be as potent or important as chemotactic or mechanotactic signals for guiding cell polarization.

A puzzling, but potentially revealing finding is that different cell types respond to EFs by orienting to different directions. Many migrating cells, including epithelial cells, fibroblasts, and neutrophils, respond by moving toward the cathode of the EF (negative electrode) (Zhao 2009). In contrast, breast cancer cells and some endothelial cells migrate in the opposite direction, toward

the anode (Chang et al. 1996, McKasson et al. 2008, Pu et al. 2007, Zhao et al. 2004). Most bacteria grow and bend toward the anode, whereas some fungi, such as *Candida albicans*, elongate toward the cathode. Other mycelia fungi and the fission yeast *Schizosaccharomyces pombe* reorient polarity to grow perpendicular to the EF (Crombie et al. 1990, McGillivray & Gow 1986, Minc & Chang 2010, Rajnicek et al. 1994). These different orientations highlight the complexity of these responses and may arise because of the existence of competing signaling platforms used to steer cells.

How cells sense EF signals and reorganize the cytoskeleton and polarity machinery is not well understood. EFs likely affect only processes outside or close to the plasma membrane (Jaffe 1977). One proposed effect of EFs is to move proteins and other cellular components by simple electrophoresis (Allen et al. 2013, Minc & Chang 2010, Poo 1981, Poo & Robinson 1977, Poo et al. 1978). However, this is certainly not the only mechanism. EFs may influence ion transport and/or membrane potential locally around the cell (Gross et al. 1986; Jaffe & Nuccitelli 1977; Kotnik & Miklavcic 2000, 2006; Kralj et al. 2011). Recent genetic characterization of EF effects has begun to shed light on molecular mechanisms regulating polarity by external EFs and their general relevance to polarity regulation. Important studies that integrate quantitative and molecular approaches in fission yeast, slime mold, and keratocytes are discussed in greater detail below.

Electric Field Effects in *Dictyostelium*

Dictyostelium discoideum amoebas have been instrumental in dissecting molecular mechanisms of directional cell migration (Chen et al. 1996, 1997; Devreotes & Zigmond 1988; Kim et al. 1997; Parent & Devreotes 1996). When exposed to homogeneous concentrations of cyclic adenosine monophosphate, these cells migrate in random directions. In the presence of small EFs, they orient migration to the cathode within minutes (**Figure 2*b***) (Song et al. 2002). Downstream signaling modules regulating directional cell migration, for instance, during chemotaxis, include PIP and intracellular cyclic guanosine monophosphate (cGMP) signaling. These effectors promote actin polymerization at the leading edge for migration (Veltman & Van Haastert 2006, Veltman et al. 2008). Sato et al. (2009) tested the role of these modules in electrotaxis. Mutants displaying reduced levels of cGMP exhibited attenuated cathodal migration, and similar phenotypes were obtained when PIP signaling was repressed through PI3-kinase (PI3K) inhibition. Strikingly, when both PIP2 synthesis and cGMP pathways were knocked down, cells migrated in the opposite direction, to the anode of the EF, which suggests the existence of parallel pathways participating in regulating electrotaxis and puts forward the existence of a third pathway promoting anodal migration (Sato et al. 2009). These studies support the role of PIP signaling for electrotaxis and provide additional details for the mechanisms involved. PIPs have been proposed to mediate EF-induced oriented migration in other systems, for instance, in wound healing and in fish keratocytes (McCaig et al. 2002, Sun et al. 2013, Zhao et al. 2006). Cross talk between EFs and polarity in *Dictyostelium* may be mediated by calcium transport and membrane potential at the plasma membrane (Gao et al. 2011, Onuma & Hui 1988, Pullar & Isseroff 2005, Shanley et al. 2006), but the details of this transduction remain to be established.

Electric Field Effects in Fish Keratocytes

Another important model for cell migration is the fish keratocyte. These cells, which are normally assembled in monolayers in the fish scales, can easily be extracted and cultured from commercially available fish. These highly regular cells migrate with directional persistence and rapid speed, even in the absence of a guiding cue. In addition, it is still debated whether fish keratocytes may

use any form of chemotactic signals to orient migration in vivo, and thus electrotaxis might be a prevalent mode for directing these cells in tissues. Keratocytes are highly electrotactic and normally migrate to the cathode (Cooper & Schliwa 1985). In two recent studies, Thériot, Mogilner, Zhao, and colleagues revisited the mechanisms by which these cells may sense EFs, using quantitative analysis of migration speeds and trajectories and a suite of pharmacological inhibitions and physical perturbations (Allen et al. 2013, Sun et al. 2013). Whereas intact cells migrate toward the cathode, cell fragments migrate toward the anode of the field (**Figure 2*b***). As in *Dictyostelium*, cathodal migration of cells depends on PI3K and PIP signaling, and anodal migration of fragments depends on calcium and myosin activation. The authors propose a model in which the EF may bias protrusive and contractile actomyosin networks to different directions and differentially drive cells or fragments, which use a different balance of these two motility modes (Sun et al. 2013). Through quantitative analysis, and by modulating ion concentration in the medium, they further suggest that the driving mechanism for EF response is the electrophoresis of certain membrane proteins, although they do not yet provide any direct evidence for the involvement of a transmembrane-charged protein in these responses (Allen et al. 2013).

Electric Field Effects in Fungi

Fungal cells are generally nonmotile but grow in a polarized manner. Both budding and fission yeast are genetically tractable models to determine conserved molecular mechanisms of cell polarity regulation (Chang & Martin 2009, Chang & Peter 2003). Ion transporters and membrane potential regulators are also widely shared between fungi and higher organisms, but their contributions to cell polarization are not well understood. Many fungal cells have been shown to display strong electrotactic responses (Crombie et al. 1990, McGillivray & Gow 1986, Harold et al. 1985). EFs may be present in natural fungal habitats, and some fungi and molds have been suggested to target wounds by following wound-induced EFs (van West et al. 2002).

The fission yeast *S. pombe* has been established recently as an excellent model to study EF effects (Minc & Chang 2010). These are normally straight, rod-shaped cells that grow by tip extension. Application of an EF in small microfluidic chambers causes the cells to reorient their growth axis by bending perpendicular to the EF, creating cells with a bent morphology (**Figure 2*b***). Candidate genetic screens of mutants revealed that this EF response depends on the formin For3 and the small GTPase Cdc42, which regulate actin cable polymerization for cell polarity (Feierbach & Chang 2001, Martin et al. 2007). Interestingly, this screen further identified a conserved plasma membrane ion pump, the proton ATPase Pma1, which regulates pH and membrane potential in yeast and fungi, as a mediator of EF effects; other transporters lacked apparent effects. One interesting result is that mutants in these different genes still oriented to the EF but in the wrong direction, toward the anode of the EF. Modeling of biophysical EF effects coupled with experimental data suggests that the EF reoriented cell polarity perpendicular to the EF by altering the spatial regulation membrane potential and local pH effects. These effects may then redirect the polarity machinery, including Cdc42 and its target formin, which reorganize the axis of the actin cytoskeleton and membrane trafficking. In contrast, EF reorients polarity in a different direction (toward the anode) in *pma1*, *for3*, and *cdc42* mutants; this effect may rely on the anodal electrophoresis of transmembrane cell wall enzymes that possess negatively charged extracellular domains (Minc & Chang 2010).

EF effects have also been studied in the hyphal fungus *C. albicans*. Hyphae grow toward the cathode of an EF (Crombie et al. 1990). This orientation depends on Ca^{2+} transport mediated by the voltage-gated Ca^{2+} channel CaCch1 (Brand et al. 2007) and may be mediated by the activation of Cdc42 and the actin-nucleator formin Bnr1 (Brand et al. 2008, 2014).

HOW DO ELECTRICAL ACTIVITIES REGULATE CELL POLARITY?

These EF studies in different organisms reveal the importance of electrical aspects of cell polarization in numerous contexts. Identification of mutants that show altered responses is proving to be an important entry into defining molecular mechanisms, revealing, for instance, roles of pumps, pH, Ca^{2+}, small GTPases, lipid signaling, and actin regulation factors. We envision that these elements regulate each other, although in general, the connections between these elements remain to be defined. These elements are likely to be critical in the normal electrochemical regulation of polarity and cytoskeletal elements. In this section, we examine these elements and what is known about how they affect cell polarity.

Membrane Potential and Polarity

The membrane potential of a cell results from gradients of charges segregated across the insulating plasma membrane. Membrane potential is dynamically regulated by ion channels and pumps, which function in exporting and importing anions and cations through the membrane. Values of resting membrane potentials may vary largely between different cell types, possibly ranging from −10 mV to −150 mV (Levin 2012). Some cells globally modify their membrane potential to perform specific functions or during different periods of their life cycle, for instance, during egg fertilization or cell differentiation (Blackiston et al. 2009, Wessel & Wong 2009). Metastatic cancer cells are often associated with depolarized (reduced) membrane potential (Binggeli & Weinstein 1985, Binggeli et al. 1994). Membrane potential may feed back on ion transport, intracellular pH, or membrane surface charges at the membrane inner and outer leaflet. It has long been suggested as a potential cue regulating patterning of embryonic tissues (Jaffe & Nuccitelli 1977). Tissue-scale membrane-potential gradients could yield electrophoresis of morphogens through gap junctions or other cell-cell connections (Bohrmann & Gutzeit 1987, Esser et al. 2006, Levin et al. 2002, Woodruff & Telfer 1980). Alternatively, membrane potential could indirectly influence downstream cytoplasmic factors or even gene transcription (Levin 2012).

Levin and colleagues have promoted the idea that membrane-potential regulation at a tissue-scale level could influence cell fate, cell behavior, and consequent morphogenetic processes, including organ regeneration, embryonic patterning, and tissue architecture. In this view, cells in a specific part of a tissue may express different membrane potential–regulating ion channels (like K^+ channels or H^+-ATPases) and display largely different membrane potential than cells in a neighboring tissue, which could influence differentiation, cell cycle, or growth by yet poorly understood mechanisms (Blackiston et al. 2009, Levin 2009). Such studies have been performed in the context of various stages of *Xenopus* development (Levin et al. 2002, Morokuma et al. 2008, Pai et al. 2012) and tadpole regeneration (Adams et al. 2007) and during planarian regeneration (Beane et al. 2011, 2013). Supporting evidence includes forward genetics, the ectopic expression of membrane-potential regulators, local applications of ion-transport drugs, or the use of optogenetic tools to manipulate membrane potential (Adams et al. 2007, 2013; Beane et al. 2011; Levin et al. 2002; Morokuma et al. 2008; Pai et al. 2012).

A recent study on zebrafish skin cells presents an interesting example of the effect of membrane potential changes on cell migration and tissue patterning (Inaba et al. 2012). Zebrafish have pigmented skin stripes of alternating blue and gold (for males) and blue and silver (for females) that run along their body (**Figure 3**). Each stripe is typically composed of a pigment cell type. The melanophores comprise the gold stripe, and the xanthophores are in the blue stripes. Mechanisms for how these two cell types stay apart to regulate stripe patterning are lacking, but several fish mutants that display defects in stripe patterns have been identified (Iwashita et al. 2006, Maderspacher & Nüsslein-Volhard 2003). One such mutant, called Jaguar, has a mutation in a gene encoding an

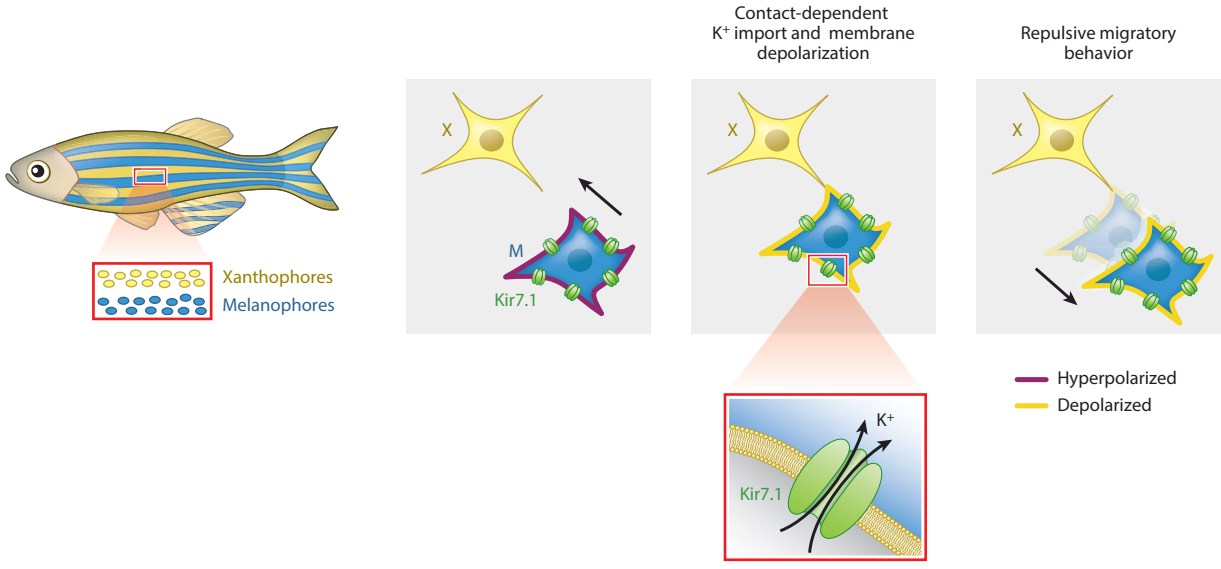


Figure 3

Membrane potential affects cell migration for tissue-scale patterning. Zebrafish have a regular pattern of blue and yellow stripes, which are formed by the pigment cells melanophores (M) and xanthophores (X), respectively. Stripes may be generated by the repulsive behavior of M contacting X, which is regulated by a contact-dependent membrane depolarization of M driven by the membrane potential–rectifying K^+ channel, Kir7.1 (*green*).

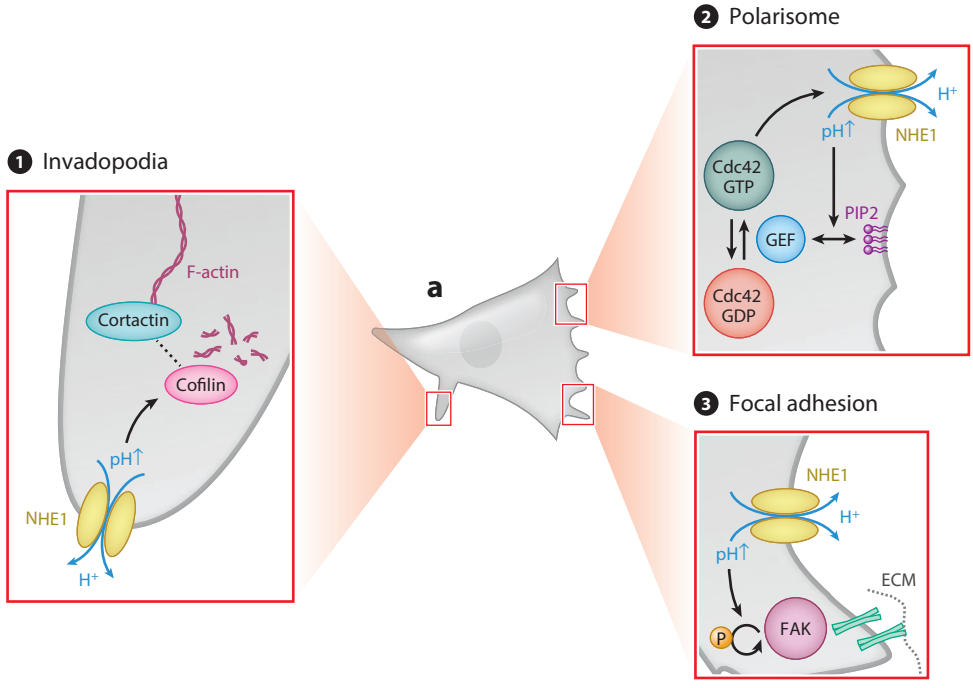
inward-rectifying potassium channel, Kir7.1. This channel regulates the resting membrane potential by promoting the entry of potassium in the cytoplasm and is expressed in the melanophore cells of the fish skin, but not in the xanthophores. Dynamic tracking of the melanophore membrane potential visualized with sensitive fluorescent dyes reveals a Kir7.1-dependent membrane depolarization when these cells contact a xanthophore. Contact is mediated by long, dendrite-like protrusions that extend far from the cell body and cause a concomitant fast membrane depolarization and polarity switch, causing the melanophore to migrate away from the xanthophore. In Jaguar mutants, membrane potential adaptation is impaired, and no polarity switch is observed, causing the intermixing of pigment bands at the animal level. Thus, cellular membrane potential values are sensitive to cell-cell contacts and may regulate cell polarity and large-scale tissue patterning. Although it remains to be established how signal transduction occurs down to the cytoskeleton and migration machineries, we note that Kir channels have been shown to tightly associate with polarity-mediating lipids, such as phosphatidylinositol (Hansen et al. 2011).

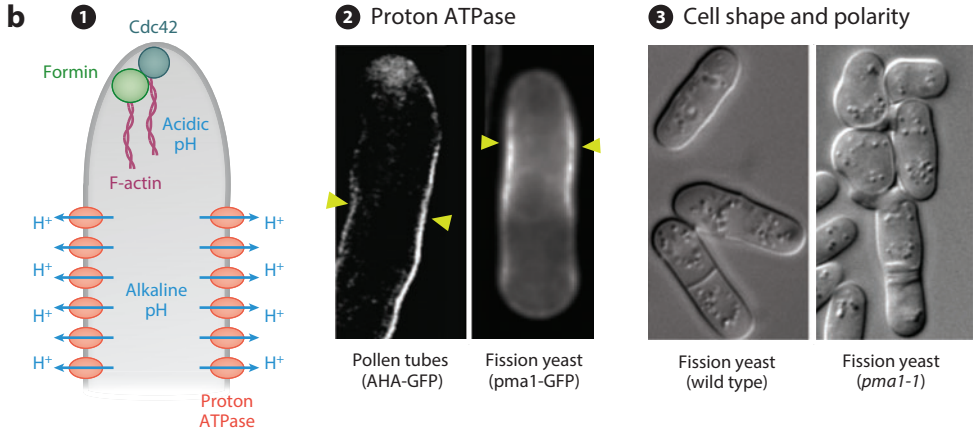
Intracellular pH and Polarity

A key regulator of cell polarity may be intracellular pH. pH is not held strictly constant in the cell but may exhibit dynamic spatiotemporal gradients. Several lines of evidence suggest that transporters that regulate pH at the plasma membrane are critical for cell migration and polarized cell growth (**Figure 4**). In fibroblasts, mutations in the Na-H pump NHE1 cause cell-migration defects; cells still move but lack directionality and exhibit defects in focal adhesion remodeling (Choi et al. 2013, Denker & Barber 2002). NHE1 is necessary for efficient binding of a Cdc42 GEF to PIP2 at the leading edge and consequent Cdc42 activation (Frantz et al. 2007). In turn,

Cdc42 activity helps to localize NHE1, producing the makings of a positive feedback (**Figure 4*a***). pH regulation by NHE1 has also been implicated in regulation of cell invasion in cancer cells (Magalhaes et al. 2011). Manipulations and intracellular pH measurements show how pH fluctuations are associated with oscillatory behavior of invadopodia elongation. pH was shown to release cortactin from cofilin, allowing cofilin to stimulate local actin polymerization (**Figure 4*a***).

Transcellular pH gradients have been measured in plant cells, such as pollen tubes and fucoid eggs (Feijó et al. 1999, Gibbon & Kropf 1994), and in fission yeast (N. Minc & F. Chang, unpublished results). In general, the pH at growing tips is more acidic than in the rest of the cytoplasm (**Figure 4*b***). Pma1p is a H^+-ATPase pump at the plasma membrane that regulates pH in fungi.





Remarkably, a single Pma1 molecule can pump out ~100 protons per second, and given that there are typically ~100,000 of them at the plasma membrane, they could in principle extrude the whole proton cytoplasmic pool of a yeast cell within milliseconds (Volkov 2012). Pma1 is localized to the sides of cells in fungi and yeasts and is largely excluded from growing zones (in a pattern opposite to most polarity factors) (Fajardo-Somera et al. 2013, Malinska et al. 2003, Minc & Chang 2010). The rapid proton flux and this localization pattern are predicted to set up a polarized current of ions throughout the cell. In fission yeast, although *pma1*-null cells are not viable, a hypomorphic allele, *pma1-1*, is alive but has polarity and morphogenesis defects: These mutants display disorganized and faint actin cables but still exhibit active, localized Cdc42 activity (Minc & Chang 2010), suggesting that Pma1 and possibly pH are required for formin-dependent actin cable formation at some step downstream of Cdc42. These mutants also have defects in directing patterns of monopolar and bipolar growth after cell division. Pma1 was found in a screen for mutants defective in EF responses, suggesting a role of pH in mediating electrical effects on cell polarity in these cells (Minc & Chang 2010). A proton ATPase has also been found to mediate pH-dependent polarized growth of pollen tubes in plants. This pump, Nt ANA, like pma1p, is also located on the sides of the tube and excluded from the growing tip. Inducing proton influx using an antibiotic that forms a cation pore is shown to reorient polarity (similar to effect of EFs) (Certal et al. 2008, Feijó et al. 1999).

How does pH regulate protein function? pH has direct, specific effects on the conformation and activities of many proteins. A classic example is the regulation of hemoglobin (Giardina et al. 2004). The addition of a proton to specific amino acid residues, termed protonation, is a posttranslational modification that is still largely underappreciated (see Casey et al. 2010 and Schonichen et al. 2013 for recent reviews on protonation). Somewhat like phosphorylation, protonation can influence electrostatics of titratable residues, like histidine or aspartate, and impact protein conformations, protein-protein interactions, or protein-lipid interactions. In the context of cell polarization and migration, pH has been shown to regulate the activities of actin-associated factors, such as ADF/cofilin, villin, talin, focal adhesion kinase, and certain guanine nucleotide exchange factors (Choi et al. 2013; Frantz et al. 2007, 2008; Srivastava et al. 2007, 2008). One well-studied example is the actin-severing protein ADF/cofilin, which is activated by deprotonation at alkaline pH at a C-terminal histidine residue and directly affects cofilin binding to phosphoinositide (Frantz et al. 2008).

The role of pH in the regulation of actin is also demonstrated in an in vitro crude extract system (Kohler et al. 2012). In this work, droplets of *Xenopus* extracts are assayed for the ability to form

Figure 4

Influence of pH on polarization processes. (*a*) ① Alkalinization of intracellular pH, regulated by the sodium-exchanger NHE1, mediates the interaction between actin-associated factors cofilin and cortactin to regulate actin polymerization and invadopodia formation (adapted from Magalhaes et al. 2011). ② NHE1 influences Cdc42 activation by tuning local internal pH, which impacts the binding of a Cdc42 GEF to lipids (adapted from Frantz et al. 2007). Cdc42, in turn, promotes local activation of NHE1, thereby generating a positive feedback. ③ NHE1 and pH influence focal adhesion assembly by mediating a pH-dependent autophosphorylation of the focal adhesion kinase (FAK) (adapted from Choi et al. 2013). (*b*) ① Proton ATPases that regulate pH in pollen and fungi are excluded from the tip, yielding a more acidic tip that may serve in polarized cell growth. ② Localization of proton ATPases fused to GFP (AHA in pollen tubes and Pma1 in fission yeast) to the sides of cells (*arrow*). ③ Fission yeast *pma1-1* mutant cells have defects in the direction of cell polarization after cell division. Morphological defects of fission yeast in a *pma1-1* mutant with reduced ATPase activity (adapted with permission from Certal et al. 2008 and Minc & Chang 2010). Abbreviation: ECM, extracellular matrix; P, phosphorylation.

contracted, crosslinked actin networks. Contractility is highly sensitive to pH, with effects seen in pH changes of as little as 0.1 units. A localized injection of pH buffer is capable of breaking symmetry and causes a rapid formation of actin bundles at the injection site, demonstrating how pH is capable of inducing quite local changes in contractility. A recent paper also demonstrates that the polymerization activities of actin by itself are also sensitive to pH (Crevenna et al. 2013). These findings, and others, suggest how localized pH gradients set up gradients in contractility (Kohler et al. 2012), actin network properties (Schmoller et al. 2012), and actin nucleation (Crevenna et al. 2013) that contribute to cell polarization, migration, and cytoplasmic flows.

Membrane Electrostatics and Cell Polarity

In addition to regulating proteins, intracellular pH likely modulates effective lipid charges at the inner leaflet. One important potential effect of membrane electrostatics is on the binding of membrane-associated polarity factors.

Studies on planar cell polarity pathway regulation in *Drosophila* epithelial tissues provide a good example for these concepts (Simons et al. 2009). This conserved signaling pathway mediates epithelial tissue polarity and architecture in different organisms (Goodrich & Strutt 2011) and relies on the recruitment of the Wnt receptor transmembrane protein Frizzled (Fz) at cell-cell contact along the tissue axis. Fz binding to Dishevelled (Dsh) at the inner leaflet activates the recruitment of cytoskeleton elements for cell polarity, growth, and division (Goodrich & Strutt 2011, Segalen & Bellaiche 2009). Fz binding to Dsh requires the targeting of Dsh to the plasma membrane. In a genome-wide RNAi screen, Simons et al. (2009) identified the sodium/proton exchanger Nhe2 as a key regulator of Dsh targeting to the membrane. This protein shares homology with the human NHE family (in particular with hNHE3) and regulates intracellular pH, as discussed above for other NHE pumps. Simons et al. (2009) proposed that pH values regulated by Nhe2 influence head negatively charged phospholipid (whose pKa are close to neutral) protonation levels, impacting the binding of the polybasic stretch of Dsh DEP (Dsh, Egl-10, pleckstrin) to the plasma membrane inner leaflet (**Figure 5*a***). Thus, Nhe2, pH, and effective membrane charges on lipids regulate Dsh targeting to the membrane, which allows this protein to interact with Fz. Fz localization also relies on pH regulation, through the action of a V-ATPase proton pump that extrudes protons from organelles and cells by consuming ATP energy (Hermle et al. 2010). These studies thus illustrate the importance of proton transporters, pH regulation, and membrane electrostatics for the proper stabilization of a polarity axis in a tissue context (Hermle et al. 2011).

Membrane electrostatics may contribute to the targeting of many other membrane-associated factors (Yeung et al. 2008). Importantly, many well-known GTPases, such as Cdc42 or Rho, display net positive charges; thus, in addition to their hydrophobic, prenylated tails, which help them bind to the membrane, charge interactions with negative lipid heads may contribute to their stability and consequently to cell polarity regulation (McLaughlin & Aderem 1995).

Two recent studies in budding yeast illustrate how lipid charges in the membrane influence polarity (**Figure 5*b***). The small GTPase Cdc42 is a central polarity factor in budding yeast (Drubin 1991). Many membrane domains segregate to the polarized growth site (Bagnat & Simons 2002a,b), but their function in cell polarization has not been clear. Fairn et al. (2011) showed that phosphatidilserine (PS) lipids, which account for most of the negative charges at the plasma membrane inner leaflet, accumulate at sites of polarized growth and contribute to the recruitment of Cdc42. The amount of PS at these sites is dynamically regulated by a lipid flippase complex, which flips PS and neutral phosphatidylethanolamine across the membrane (Das et al. 2012). In addition to flippase complexes, ion gradients and membrane potentials may also regulate charged

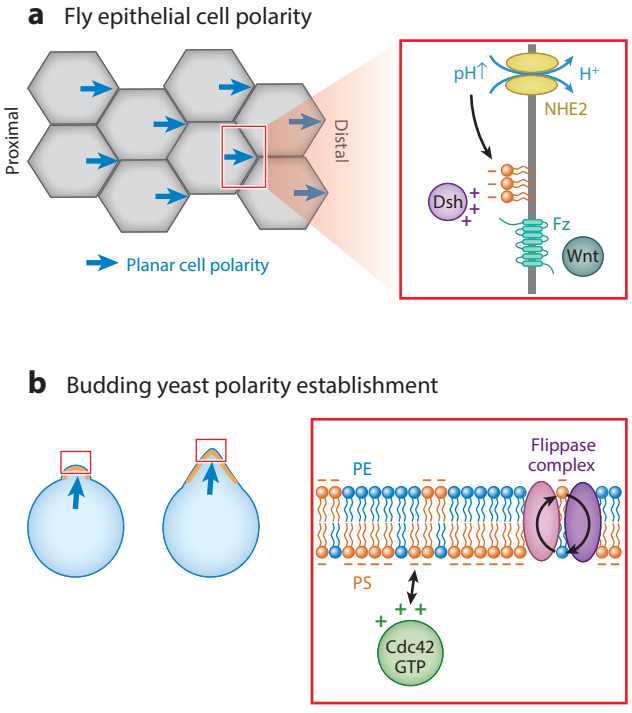


Figure 5

Effect of membrane charge on cell-polarization processes. (*a*) Planar cell polarity (PCP) controls polar organization in fly epithelium. PCP activation requires the binding of Frizzled (Fz) and Dishevelled (Dsh). NHE2 activation causes local alkalinization of internal pH, which increases available negative charges on lipid heads, and promotes positively charged Dsh targeting to the membrane and subsequent binding to Fz. (*b*) Polarized distribution of negatively charged phosphatidylserine (PS) during yeast polarization is regulated by polarized trafficking and a flippase complex, which leads to accumulation of PS at sites of growth (bud or mating tip emergence). Polarized PS provides a favorable electrostatics environment for the binding of Cdc42 at the membrane. Abbreviation: PE, phosphatidylethanolamine.

lipid flipping, as proposed in different theoretical and in vitro studies (Hall 1981, McLaughlin & Harary 1974, McNamee & McConnell 1973). Thus, lipid charge could act as a platform to transduce signals across the membrane in the regulation of polarity components.

CONCLUSIONS AND OPEN QUESTIONS

In this review, we discuss the effects of electric fields, ion transport, membrane potential, and electrostatics in regulating cell polarization. These elements have been stitched together into a working model for how electrochemistry may control cell polarization. We postulate that polarization cues lead to asymmetric localization or activation of transporters that regulate pH and other ions at the plasma membrane. pH has many effects, including the activity of actin regulatory proteins, small GTPases, and membranes. Positive feedback loops between these elements help to establish a robust polarity axis. Electric fields surround cells, even individual ones, and are able to direct polarity processes by reorganizing membrane potentials that then trigger pH and other elements in the polarity pathway. One exciting possibility is the function of EFs, which act as

long-range and fast-propagating signals, in controlling cell polarity not only in the context of single cells but also in coordinating polarity in tissues or even over whole organisms.

This perspective represents a new dimension in cell polarization. This view may inform on large-scale screens that identify novel, unusual candidates that affect cell polarity. For instance, in fission yeast, a recent genome-wide screen for morphogenesis defects identified 62 membrane-transporter mutants with abnormal cell shapes (Hayles et al. 2013). Genetic studies have revealed elements in electrochemical cues as important for processes broadly related to polarity and morphogenesis, like aging in budding yeast (Hughes & Gottschling 2012) and mitotic rounding in animal cells (Stewart et al. 2011). Genetic screens for mutants with abnormal EF responses promise to be fruitful in this arena (Zhao et al. 2013).

An important open question is whether steady-state electrochemical gradients may exist inside cells and, if so, how they may be established and maintained. Although we are only beginning to understand how gradients of cytoplasmic and membrane-bound proteins are established in cells (Goehring et al. 2011, Hachet et al. 2011, Saunders et al. 2012), one challenge for ions is that they diffuse much faster than large proteins and have the potential to bind and interact with many factors and complexes in cells. Evidence for gradients of pH has been revealed by the use of sensitive probes at focal adhesion, invadopodia, and sites of macropinocytosis and fungal tip growth (Choi et al. 2013, Feijó et al. 1999, Frantz et al. 2008, Koivusalo et al. 2010). Because some pumps and transporters can be sharply localized and transport with impressive activity, they may create subcellular domains with specific electrochemical activities with defined pH and membrane potential. Alternatively, there could be depletion mechanisms generated by a subcellular accumulation of proteins or protein complexes, which bind and consume specific ions, thereby acting as local sinks for gradient generation (Schonichen et al. 2013).

Another open question is how these cues may signal down to the regulation of the cytoskeletal network or to the targeting and activation of polarity modules. Although studies in various in vivo and in vitro systems have so far given no general consensus, some broad principles are emerging. It thus remains to be clarified whether the specificity in these signaling events could be cell-type or cell-state dependent. The large directional variations in EF responses begin to address these questions and suggest there may be multiple layers that can be activated through positive feedback and steer polarity in different directions.

The development of new quantitative sensors and actuators of electrochemical cues (Crevenna et al. 2013; Fenno et al. 2011; Kralj et al. 2011, 2012) promises to facilitate future work in this field. A wealth of information on the molecular and structural basis of ion-transport systems is already available in the literature. Exciting future discoveries promise to define the electrical bases for how these systems contribute to the spatial organization of cells and tissues.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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