Sparks of life: The shocking role of bioelectricity in pattern formation

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Summary

Communication between cells occurs through exchanging information in the form of signals, such as biomolecules, mechanical stress, and electricity. In the present day, most research is focussed on the communication via biomolecules. These molecules are involved in so-called biochemical signalling pathways and can thereby influence how cells behave. For example, through the activation or inactivation of certain signalling pathways by these molecules, cells can be instructed to divide or change their function. This has an effect on the subsequent structure that arises from the cooperation between the cells. The signals involved in forming multicellular structures also seem to have some interesting characteristics. It is not only the biomolecule that gives information to a cell, but also the timing and distribution of the molecule that influence how a cell behaves when it receives such a signal. However, these biochemical signals do not appear to be the only means by which cells send and receive information regarding their behaviour and their position within multicellular structures. Cells can also communicate via bioelectric signals, which are similar to the signals used by nerves and muscles. The principle is the same, the difference in charged molecules between de inside and outside of a cell generates a voltage potential across the plasma membrane, known as the membrane potential. The membrane potential of a cell is regulated by ion transporters, which are specific proteins inside the membrane that can transport and distribute the charged molecules across multicellular structures. Nerve cells can generate, distribute, and respond to electrical signals via specialized mechanisms. However, the ability of cells to use electrical signals did not evolve in nerve cells. Interestingly, researchers have observed that many different cell types use the ability to generate voltage potentials in order to send and receive signals from and to neighbouring cells. These signals are slower and weaker than those used by nerve cells. Nonetheless, they are able to influence how cells behave. It is now believed that bioelectric signals might play a role in the formation of organs, limbs, and even entire organisms during development. Furthermore, data from *in vivo* experiments suggest that bioelectricity is also involved in regeneration and tumour formation. However, further experiments need to be conducted to examine whether this is a fundamental property used by all cell types to form multicellular structures. Although many mechanisms used by cells to send and receive bioelectric signals are not fully understood, researchers are already trying to discover how these bioelectrical processes can be used to develop new insights into biology. By obtaining a deeper understanding of the role bioelectricity plays in cell communication, researchers hope to create new treatments and therapies for birth defects and diseases such as cancer. Moreover, by gaining a better control over how cells behave, new possibilities will emerge in areas such as regenerative medicine and synthetic biology, bringing about a whole new era for biomedicine.

Abstract

The role of bioelectricity in living systems has been studied for a long time. Now, with the development of novel molecular tools, advancements in fields such as machine learning, and the ever-increasing computing power, there is an increased capacity to research the significance of bioelectricity in greater detail than ever before. The prevailing belief for spatial gradient formation during morphogenesis is that only biochemical molecules are involved in this process. However, it could be argued that bioelectric gradients also provide positional information to cells during development. Therefore, by exploring the field of developmental bioelectricity, an integration between neurobiology and developmental biology, the goal is to foster a broader perspective on cellular communication and manipulation of cellular behaviour. Spatial gradient formation is a dynamic process involved in shaping multicellular structures, and the seemingly dynamic nature of the signals required for the formation of spatial gradients may extend beyond just biochemical signalling pathways. For instance, bioelectric signals consist of ion fluxes, facilitated by ion transporters, and changing membrane potentials, which are transduced and integrated into biochemical signalling pathways. This grants bioelectric signals the ability to provide instructive cues to induce cell behaviours, such as proliferation, differentiation, and apoptosis. Moreover, the ability to exchange bioelectric signals is not exclusive to the cells involved in the muscular and nervous system. Many cell types utilize bioelectric signals to form voltage gradients, and thereby can generate bioelectrical networks. These networks might be used during development to store epigenetic information, such as organism-specific anatomy, *i.e.* the target morphology. By manipulating the bioelectric properties of cells, such as the membrane potential and ion transporters, and creating quantitative models of these systems to understand collective cell behaviours, the exploration of the role of bioelectricity in cell communication and pattern formation offers exciting possibilities.

Introduction

Self-organizing patterns in biology are truly fascinating and provoke many questions on the process through which cells can form these intricate structures. Alan Turing proposed in his paper "The Chemical Basis of Morphogenesis" a reaction-diffusion model through which patterns can self-organize, and demonstrated how the diffusion of two different chemical signals can result in the formation of complex patterns, now known as Turing patterns (Turing, 1952). Currently, the prevailing thought is that the concentration gradient of these chemical signals, known as morphogens, provides positional information to cells during development and create a region in which cells differentiate according to the concentration of the morphogen signal (Tabata & Takei, 2004). This principle of spatial gradients providing positional information was first described as the French Flag Problem by Lewis Wolpert in an attempt to explain spatial scalability during embryonic development. Hence, by acknowledging the ability of biological processes to adapt and organize cells efficiently across different spatial scales via spatial gradients, and recognizing the presence of voltage gradients alongside morphogen gradients during embryonic development, it sparks curiosity for exploring the idea of multi-system involvement in providing positional information during pattern formation.

For intricate patterns to emerge during development, cells need to cooperate and organize by means of communication. Cellular communication is achieved by exchanging information via signals. These signals have the capacity to encode complex information in the form of biochemical molecules, mechanical stress, and electricity. Over the years, biochemical and mechanical signalling pathways have been studied extensively. However, the use of bioelectricity for signalling is often only recognized in the nervous and muscular systems and is primarily associated with "excitable cells". Although these cell types are indeed harnessing the power of bioelectricity, it is crucial to realize that they are not exclusive in their ability to engage in bioelectric signalling. The expression of components necessary for bioelectric signalling has been identified in more than 100 different tissue types (Churchill et al., 2019). Bioelectric signalling encompasses changes in the concentration of charged molecules, *i.e.* ions, leading to the generation of a voltage potential difference across the plasma membrane, known as the membrane potential. Regulating ion concentrations inside cells is a crucial process, as the proper functioning of proteins, gene expression, signal transduction, and hormone release all depend on a homeostatic intracellular ionic environment (Bortner & Cidlowski, 2004). However, while ionic homeostasis appears essential for numerous cellular processes, a remarkable specificity has been observed in the relationship between changes in the membrane potential and cellular behaviours. For instance, the membrane potential of highly proliferating cells, such as embryonic and cancer cells, tends to be less negative than that of differentiated, quiescent cells, indicating that bioelectric properties can serve as markers for cell characterization (Binggeli & Weinstein, 1986; Levin et al., 2017). In addition, it suggests that transmembrane voltage could be involved in a wide range of cell behaviours, including proliferation, differentiation, and homeostasis. By manipulating the ionic concentrations inside multicellular structures, researchers have demonstrated the incredible plasticity of cells to reorganize and reform without genetic modifications. For instance, researchers were able to induce embryonic eye patterning in a group of non-eye forming cells in *Xenopus Laevis* by mimicking the membrane potential of eye forming cells (Pai et al., 2012). Several different model systems have been employed to demonstrate cellular plasticity during tissue development. The reorganization and restructuring of tissue during the experiments suggest that the genome does not give rise to one specific developmental programme but rather gives rise to a highly plastic cell collective that strives to reach the organism-specific anatomy, *i.e.* target morphology. Additionally, experiments conducted on planaria demonstrated that bioelectric signalling determines both the head size and organ scaling during regeneration, suggesting a connection between cellular plasticity during tissue development and bioelectric signalling. Thus, it appears that biochemical cues provided by morphogen gradients are not exclusive in their ability to provide positional information to cells during development. Instead, bioelectric signalling, in the form of voltage gradients, also seems to offer distinctive information and enable the scaling of cells in complex pattern formations.

In the following section of this paper, the concept and relevance of signalling dynamics will be discussed to highlight the potential dynamic nature of cellular signalling in pattern formation. Furthermore, the concept of bioelectricity, along with the mechanisms and dynamics involved in bioelectric signalling, will be elucidated. Subsequently, the role of bioelectricity in cellular behaviour, such as proliferation and differentiation, will be explored. The aim is to uncover the significance of bioelectricity in the formation of biological patterns and encourage a broader perspective on cellular communication and self-organization.

1. The relevance of signalling dynamics in cell communication

In order to understand how signals can propagate and induce the formation of shapes in multicellular structures, *i.e.* morphogenesis, it is important to consider the temporal organization of such signals. Dynamic properties of signals, such as frequency, duration, delay, and fold-change, can be used to encode information during signal transduction, and thereby describe the temporal evolution of a signalling system (Figure 1a) (Sonnen & Aulehla, 2014; Waddington, 2014). These parameters may manifest during signal transduction in the form of binding affinity of signalling molecules for their respective receptors, concentration gradients of the signalling molecules, and the degradation speed of the signalling molecules. Consequently, dynamic signals have the capacity to function beyond a simple on/off switch. However, propagation of a signal can be disrupted by noise originating from random fluctuations and changes in environmental conditions, such as pH levels or temperature. This, in turn, can impact the kinetics of many cellular reactions (Sonnen & Aulehla, 2014). It is therefore essential for cellular communication to have robust signals that contain the information necessary for temporal organization and thereby proper signal transduction. Moreover, cell-cell communication in pattern formation can be generally grouped into short- and long-range signal transductions (Inomata, 2017). These two types of signal transductions will be highlighted to demonstrate how temporal organization can manifest in pattern formation (Figure 1b & 1c). Thus, the potential for temporal organization, coupled with robustness to noise and high information content, characterizes dynamic signal encoding and facilitates the intriguing modes of communication between cells (Rapp, 1987).

Signalling dynamics in pattern formation

The Notch signalling pathway is highly conserved and required during both embryonic development and adult tissue homeostasis. It mediates communication between adjacent cells through cell-cell contact and thereby regulates short-range pattern formation (Bray, 2016; Inomata, 2017). A Notch ligand, Delta or Jagged, is expressed in the signal-sending cell, while a Notch receptor is expressed in the signal-receiving cell. Both are transmembrane proteins and by exerting a mechanical pulling force onto the receptor, the ligand can transduce its signal into the signal-receiving cell. The transduction of the signal into the receiving cell leads to the cleavage of the Notch intracellular domain (NICD), thereby inducing the expression of Notch target genes and initiating an autoregulatory mechanism (Inomata, 2017; Sonnen & Janda, 2021). A negative feedback loop can induce lateral inhibition, ultimately resulting in the cells to assume different identities (Sonnen & Janda, 2021). The feedback loops during signal transduction allow for self-organization and the formation of multicellular structures with equally spaced, alternating cell types, resembling a salt-and-pepper pattern (Figure 1b) (Sonnen & Janda, 2021; Sprinzak et al., 2010). The Notch signalling pathway is a great example of the interplay between mechanical and biochemical signalling, as a mechanical force is necessary for the initiation of signal transduction, leading to the induction of biochemical reactions by transducing the signal into the signal-receiving cells. Additionally, it illustrates dynamic properties of a signal, the temporal evolution that manifests in the form of feedback loops, which are important for proper signal transduction and pattern formation.

Similar to the short-range signal transduction of the Notch signalling pathway, long-range signal transduction by morphogens can regulate the formation of patterns (Figure 1c). However, morphogens are not limited to cell-cell contact and can diffuse in the extracellular space (Inomata, 2017). To illustrate the temporal organization of morphogens in pattern formation, the dorsal-ventral (D-V) axis formation in the *Xenopus Leavis* will be briefly examined. The formation of the D-V axis starts with fertilization and triggers to formation of the cortical microtubule and rotation of the egg cortex. Maternal dorsal determinants move to the future dorsal side, leading to local release of Chordin, which forms a gradient in the embryo: high concentration dorsally, low ventrally. Meanwhile, bone morphogenetic proteins (BMPs) are localized in the whole embryo and induce ventralisation. Chordin is able to inhibit the function of BMP and consequently promote dorsalisation of the embryo. Binding of Chordin to BMP results in a Chordin gradient along the D-V axis that leads to the formation of different tissue regions when a concentration threshold in reached: dorsal (high Chordin), lateral (moderate Chordin), and ventral (low Chordin) (Inomata, 2017). However, the concentration gradients are primarily regulated by morphogen synthesis, degradation, and diffusion. Emergence of the D-V axis in *Xenopus Laevis* through diffusion and interaction of Chordin and BMP demonstrates the significance of temporal organization in signal transduction. Both the Notch signalling pathway, representing the short-range signal transduction, and the D-V axis formation in *Xenopus Laevis*, representing the long-range signal transduction, indicate that the dynamic properties of signals are intrinsically involved in pattern formation and suggest that signal transduction during pattern formation might be inherently dynamic and therefore necessary.
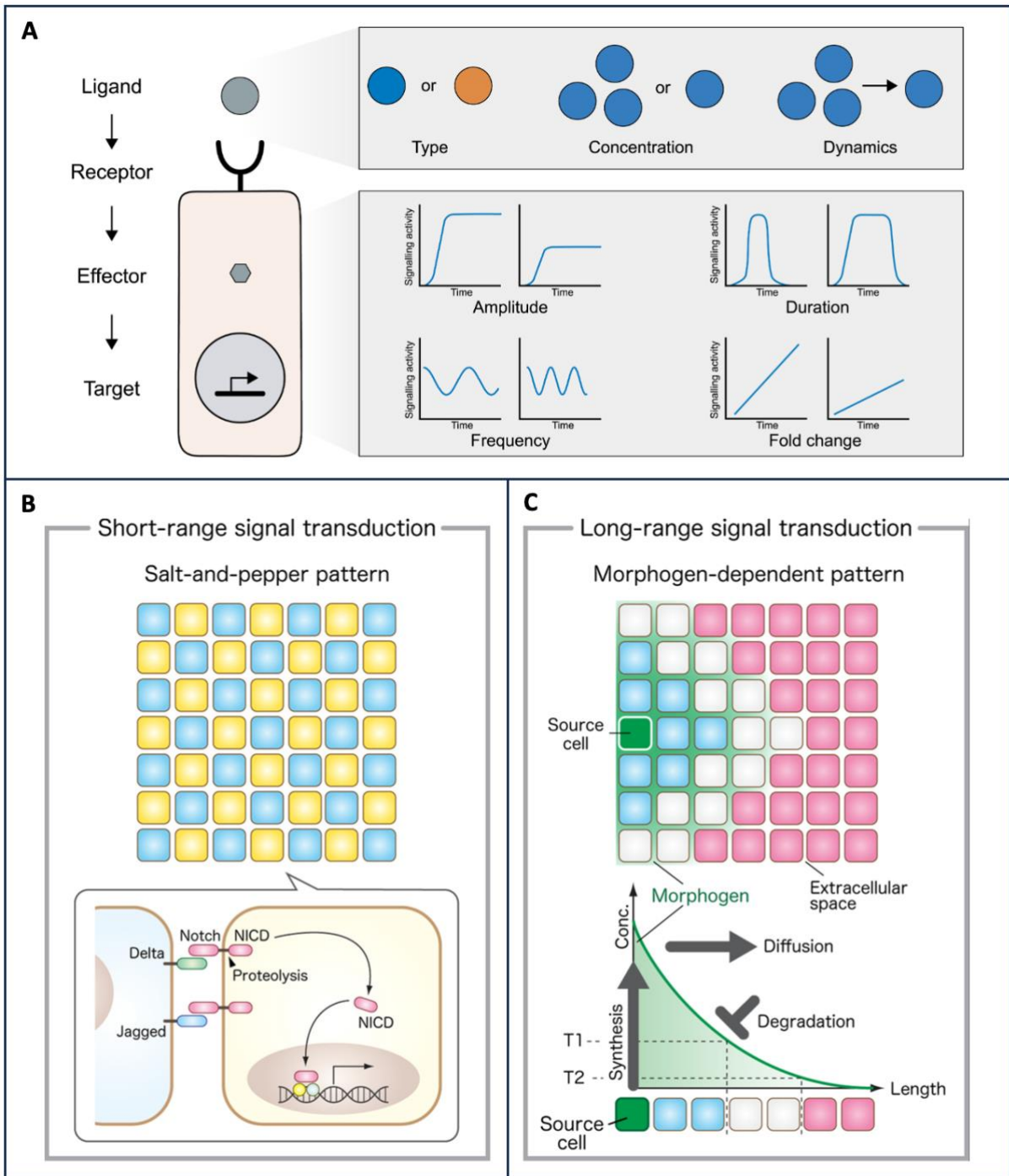
Figure 1. Cellular signalling dynamics in pattern formation.
Schematic overview of cellular signalling dynamics in short- and long-range signal transduction. (A) The initiation of signal transduction starts when a ligand interacts with a receptor. The type, concentration, and temporal properties, such as the duration of interaction between the ligand and the receptor, can be encoded in the transduced signal. The encoded dynamics of the signal can be observed by a change in amplitude, frequency, duration, or fold change of the signal effectors and targets. (B) Short-range signal transduction in the Notch signalling pathway. Delta/Jagged ligand interaction with Notch receptor results in cleavage of the Notch intracellular domain (NICD) and leads to the expression of Notch target genes, which can induce lateral inhibition and thereby create a salt-and-pepper pattern (blue and yellow pattern). (C) Long-range signal transduction of a morphogen locally secreted by the source cell. Spatial gradient is formed through diffusion of the morphogen in the extracellular space. Cells differentiate when a threshold of the morphogen concentrations is reached (T1 & T2). Blue cells indicate a high concentration of the morphogen, white cells a moderate concentration, and red cells a low concentration. The gradient is primarily regulated by morphogen synthesis, degradation, and diffusion. Adapted from Inomata, 2017; Sonnen & Janda, 2021.

2. The bioelectric properties of the cell

To demonstrate the importance of bioelectricity in pattern formation, and recognize the dynamic nature of bioelectric signals, it is first necessary to understand the bioelectric properties of cells by which they can send and receive bioelectric signals. Ion channel proteins and pumps establish a voltage potential across the membrane, known as the membrane potential, by facilitating changes in the concentration of positively charged molecules (anions) and negatively charged molecules (cations). These charged molecules are ions such as chloride (Cl^-), sodium (Na^+), and potassium (K^+), and can be distributed between adjacent cells with the help of gap junctions (Figure 2a) (Levin, 2021). Furthermore, utilization of these electrical properties of the cell is not just reserved for excitable cells such as neurons and muscle cells. Researchers have identified at least 120 different tissue types which express the components necessary for bioelectric signalling and use it to send and receive signals from and to other cells (Churchill et al., 2019).

Membrane potential and the bioelectric state

The plasma membrane consists of a lipid bilayer and separates the cells' internal world from its external environment. Embedded in the plasma membrane are many kinds of protein molecules, each performing its specific functions (Bretscher, 1985). One of these functions is to maintain ionic homeostasis. Movement of ions across the plasma membrane is facilitated by transmembrane structures known as ion channel proteins, ion pumps, and gap junctions and creates an ionic gradient between the inside and outside of a cell, resulting in a membrane potential. Cells can sense and respond to the membrane potential of their neighbouring cells through distributing ions between the cells via gap junctions. This results in the formation of bioelectric gradients, which affects the membrane potential (Levin et al., 2017). A resting membrane potential, *i.e.* the bioelectric state, is achieved if the electrochemical forces driving ion movement are equalized and ionic homeostasis is maintained (Sundelacruz et al., 2009). Ionic homeostasis is not always maintained, which is reflected in fluctuating membrane potentials. When the membrane potential of a cell is lower compared to its neighbouring cell, the cell is hyperpolarized. Conversely, if the membrane potential is higher, the cell is more depolarized (Binggeli & Weinstein, 1986). Upon measuring the resting membrane potential of various cell types, researchers observed that their bioelectric state varies (Figure 2b). Highly proliferating cells, such as embryonic cells, stem cells, and tumour cells tend to be more depolarized than differentiated, quiescent cells, which are typically more hyperpolarized, suggesting the bioelectric state of a cell is associated with its behaviour (Binggeli & Weinstein, 1986; Levin et al., 2017). Furthermore, epithelial tissue, which covers all body surfaces and serves as a barrier between the interior and exterior of the body, can also be polarized by maintaining a voltage difference between its apical and basal sides, known as the transepithelial potential (TEP). Disruption of the epithelial layer can occur during wound formation and causes the TEP to collapse at the site of the wound. The TEP of the surrounding intact epithelium generates an endogenous ion flux, also known as the injury current, directed towards the site of the wound. The injury current appears to be important in wound healing, as the disruption of the electric field created by the current leads to delayed wound healing (Tyler, 2017). Thus, the distribution of bioelectric signals between adject cells is facilitated by endogenous ion fluxes, as reflected in the distribution of the bioelectric states. This process leads to the formation of bioelectrical gradients between cells and may thereby contribute to pattern formation (Levin, 2014a).

Ionic gradient formation

Transportation of ions across a membrane can be performed by ion channel proteins, ion pump and gap junctions, which all have their specific mechanisms. Passive diffusion of ions across the plasma membrane, down their concentration gradient, does not require energy. However, larger or charged ions require the assistance of ion channel proteins. This process is known as facilitated passive diffusion. Cells utilize the passive diffusion of ions as a mechanism to sense and responds to their external environment (Herrington & Arey, 2014). Many different types of ion channels have been discovered, yet all types consists of multiple subunits or domains, which surround one or multiple pores (Gabashvili et al., 2007). A pore can act as a gate for ions and can be in open (activated) or closed conformation (deactivated). The reactivation time of an ion channel ranges from milliseconds to minutes depending on the channel type and the specific conditions of its surrounding and is necessary for the channel to return to its resting closed state after initial activation. Multiple mechanisms of ion channel gating exist, each requiring a different kind of stimuli. For instance, conformational changes in voltage-gated channels can be induced by a shift in the membrane potential, while ligand-gated channels respond to the binding of specific ligands. Other interesting gating mechanisms include mechanosensitive channels, which responds to mechanical stimuli such as membrane stretching, and light-gated channels, which respond to a specific wavelength of photons. Furthermore, some ion channels selectively transport ions across the membrane, while other ion channels do not discriminate between the type of ions they transport. However, it is noteworthy that positively charged ions and negatively charged ions require different ion channel structures. The mentioned gating mechanisms do not account for the wide variety of gating mechanism utilized by ion channels. Nonetheless, they beautifully demonstrate the distinct types of stimuli involved in ionic gradient formation inside a cell.

Ions can also be actively moved against their concentration gradient by transmembrane structures known as ion pumps, which differ from ion channels in that they require energy to perform their function. This energy can be in the form of ATP (active transporters) or in the form of potential energy (secondary transporters), which is obtained from the concentration gradient created by active transporters. Furthermore, there are energy generating transporters, which produce ATP by transporting ions from a high concentration gradient to a low concentration gradient (Gadsby, 2009). Similar to ion channels, various types of ion pumps exist, each with its own functions. Nonetheless, the primary function of ion pumps is to regulate the ionic concentrations inside a cell.

Other significant contributors involved in ion distribution are intercellular channels known as gap junctions, which form through the alignment of two hemichannels between adjacent cells. Hemichannels consist of multiple integral membrane proteins, such as connexin, innexins, or pannexins (Hervé et al., 2007). Gap junctions create the opportunity for adjacent cells to directly exchange ion currents, leading to changes in membrane potential. The specificity of gap junctions is influenced by the protein composition of the two aligned hemichannels, which can vary greatly (Spray et al., 2006). Thus, ion channel proteins, ion pumps, and gap junctions play crucial roles in the regulation and distribution of ionic concentrations between cells, resulting in ionic gradient formation and changes in membrane potential. Additionally, dysfunction of ion transporters, as a result of genetic mutations or acquired through drugs or toxins, has been linked to many human diseases (Harraz & Delpire, 2024). Advancing our understanding of these membrane proteins holds promising implications for the development of novel therapeutic interventions. Furthermore, the connection between the underlying mechanisms utilized by ion transporters and their role in human diseases underscores the significance of bioelectric signalling. Thereby highlighting ion transporters as intruiging targets for manipulating ionic gradients and making them relevant in attempting to comprehend the role of bioelectricity in pattern formation.
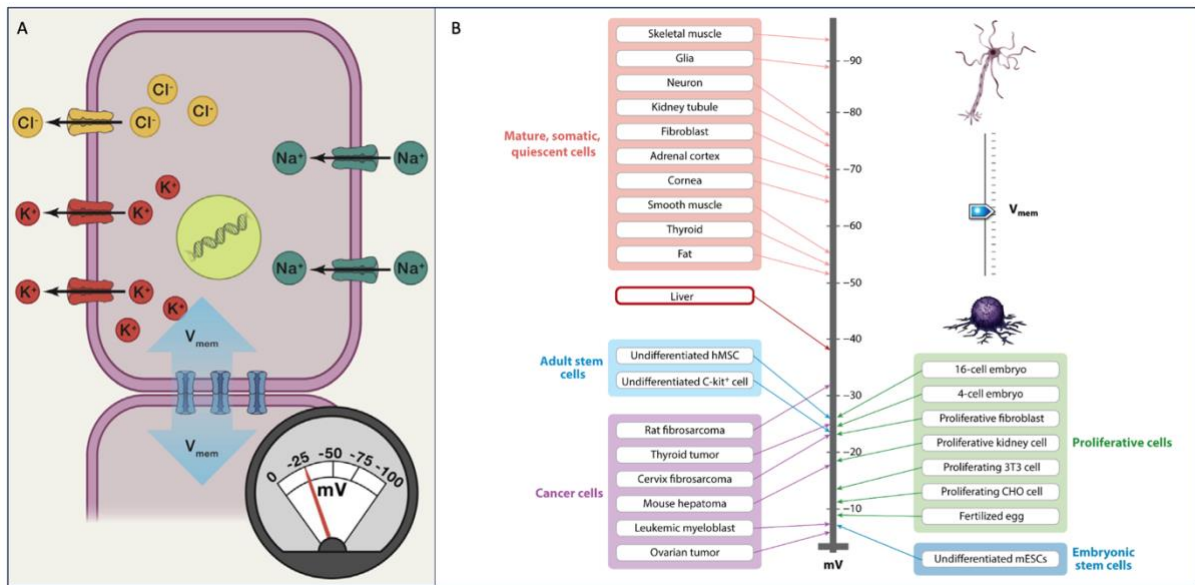
Figure 2. Resting membrane potential of different cell types.
Schematics of the resting membrane potential, *i.e.* the bioelectric state, of different cell types. (A) Simplification of mechanisms used by cells to achieve a resting membrane potential. The mechanisms involve ion transporters that distribute ions, such as chloride (Cl^-), sodium (Na^+), and potassium (K^+), to establish the bioelectric state of a cell. (B) The bioelectric state of different cell types. Terminally differentiated, quiescent cells tend to have a more hyperpolarized resting membrane potential. Conversely, proliferative, and undifferentiated cells tend to have a more depolarized resting membrane potential. Adapted from Levin, 2021 & Levin et al., 2017, respectively.

Bioelectric dynamics in cell behaviour

The plasma membrane potential can change over time as result of fluctuating ion concentrations induced by bioelectric signalling. These bioelectric dynamics encompass signal transduction and feedback loops involved in various cellular processes, including the cell cycle. To illustrate, manipulation of the endogenous electric field of vesicular endothelial cells induced cell cycle arrest, as an increase in electric field strength resulted in decreased entry of cells into S phase from G1. The process involved downregulation of G1-specific cyclin E and upregulation of cyclin/cyclin-dependent kinase complex inhibitor p27^{kip1} (Wang et al., 2003). Furthermore, changes in membrane potential can induce transcriptional changes and create feedback loops that amplify bioelectric signals by integrating bioelectric signals with biochemical signalling pathway. For instance, expression of the K$^+$-channel TASK3 can be regulated by the membrane potential via the calcineurin pathway. Depolarization of the membrane induces Ca^{2+} influx, thereby activating downstream targets of the calcineurin pathway that regulate TASK3 expression (Zanzouri et al., 2006). Distribution of bioelectric signals also depends on the gap junctions in the plasma membrane between adject cells, as these intracellular structures allow for directly transferring ions between cells. Fluctuations in ionic concentrations leads to voltage gradients and induces the formation of bioelectrical networks between cells. The voltage gradients can provide positional information to surrounding cells (Shi & Borgens, 1995). For instance, researchers observed that proton dynamics induced by H$^+$-ATPases activity may underlie basic mechanisms of polarity and spatial regulation (Certal et al., 2008). Thus, bioelectric signals can be distributed on a multicellular scale, can lead to changing membrane potentials which transduce the signal, and can amplify themselves through feedback loops involved in the transduction of the signals. Hence, these characteristics highlight the dynamic nature of bioelectric signals.

Bioelectric signalling is a function of ion transporters and can be initiated in several ways. Signalling can arise through a change in membrane potential of an individual cell, can arrive in a cell through a gap junction, or can be initiated through a break in an epithelial layer that carries a TEP (Sundelacruz et al., 2009). After initiation of the bioelectric signal, transduction of the signal into the cell occurs through integration with biochemical signalling pathways. While not all aspects of bioelectric signal transduction have been uncovered, progress has been made in elucidating the molecular details of some signal transductions pathways (Figure 3). Integration of bioelectrical signals with biochemical signalling pathways can provide instructive cues for cellular behaviours involved in pattern formation, such as proliferation, differentiation, and apoptosis (Levin et al., 2017; Lobikin et al., 2012). Transduction of bioelectric signals starts by alterations in physiological processes such as changes ion fluxes, pH gradients, the membrane potential, and electric fields (Sundelacruz et al., 2009). Numerous mechanisms serve as biophysical receptors to detect these physiological changes, and transduce it into second messenger cascades (Sundelacruz et al., 2009). A well-known example of such a second messenger cascade is calcium signalling. Voltage-gated calcium channels respond to changes in the membrane potential, leading to an influx of calcium ions into the cell (Greer & Greenberg, 2008). This influx of calcium ions results in the activation of various calcium sensors, thereby transducing the signal. Calcium signalling is essential for many types of cell behaviour and is also involved in pattern formation (Sundelacruz et al., 2009). For instance, the breaking of the initial bilateral symmetry in vertebrate embryos is initiated by left-right differences in H$^+$/K$^+$-ATPase activity, which affect extracellular calcium concentrations and leads to asymmetric activation of Notch. Consequently, this results in asymmetric gene expression (Raya et al., 2004). Other bioelectric signal transductions mechanisms include integrin-linked signalling, involving human *ether-à-go-go* (EAG)-related type 1 channel (hERG1), voltage-sensitive phosphatases operating through the phosphoinositide kinase pathways, and voltage-dependent changes in the function of intracellular transporters of signalling molecules such as serotonin (Arcangeli & Becchetti, 2006; Iwasaki et al., 2008; Sundelacruz et al., 2009). Moreover, ionic concentrations can be converted into butyrate movement by the sodium-butyrate exchanger SLC5A8. Butyrate is involved in epigenetic regulation as it acts as a histone deacetylase inhibitor, thus suggesting a connection between ionic concentrations and the chromatin state (Ganapathy et al., 2008). Furthermore, it was observed that depolarization of the membrane can induce nanoscale reorganization that leads to clustering of protein receptors involved in RAS signalling, thereby amplifying K-Ras-dependent mitogen-activated protein kinase (MAPK) signalling. Conversely, clustering of the receptors is disrupted after membrane repolarization and inhibits MAPK signalling (Zhou et al., 2015). In essence, bioelectric signal transduction occurs through integration with biochemical signalling pathways. Furthermore, the discussed biochemical signalling pathways are involved in many types of cell behaviour, including proliferation and differentiation. Thus, indicating bioelectric signals play a role in proper cell behaviour, facilitating pattern formation and pattern homeostasis (Adams & Levin, 2013).
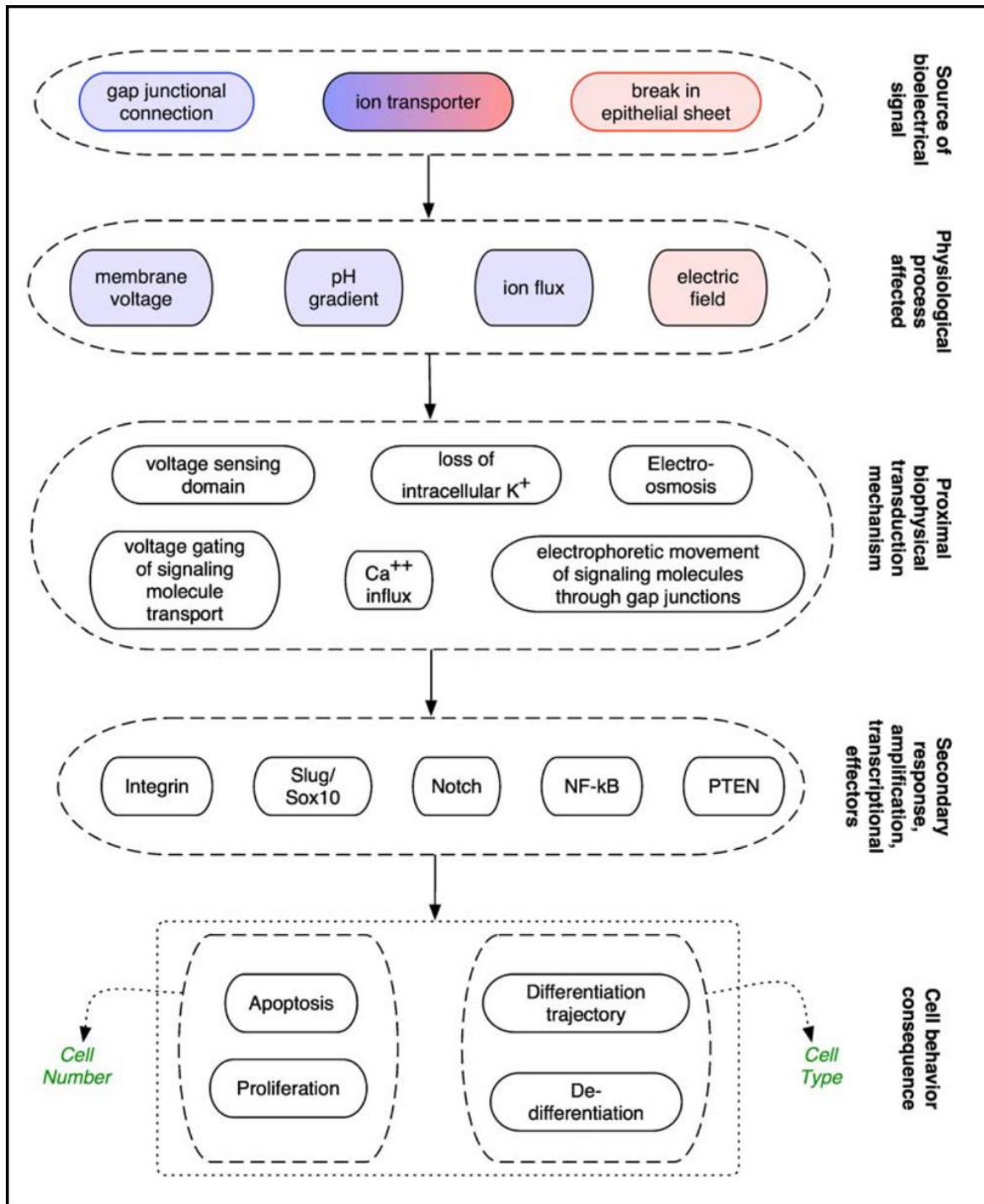
Figure 3. integration of bioelectric signals with biochemical signalling pathways alters cell behaviour.
A schematic overview of the integration of bioelectric signals with biochemical signalling pathways. Gap junctions, ion transporters, and breaks in epithelial layers can all be a source of bioelectric signals. The signals induce changes of physiological processes, such as membrane potential, pH gradients, ion fluxes, and electric field effects. The change in the physiological processes is then detected by specific biophysical transduction mechanisms, resulting in calcium influx, voltage gating signalling molecule transport, etc. The biophysical transduction mechanisms trigger a secondary response, thereby inducing transcriptional effectors. The resulting transcriptional cascade can control all aspects of cell behaviour, including proliferation, differentiation, and apoptosis. Transduction of the bioelectric signals into the secondary response pathways allows these signals to control aspects such as cell number and cell type. Adapted from Sundelacruz et al., 2009.

3. The underappreciated potential of bioelectricity

An interesting analogy can be drawn between a collective of cells and a computer. The genes and proteins of the cells can be viewed as the hardware, and the bioelectricity running through the cells can be seen as the software. Instead of tinkering with the hardware, *e.g.* manipulating the genetics, it would be easier to modify the software, which could be viewed as a bioelectric code that maps the stable bioelectric states of cells to their subsequent anatomical outcome (Durant et al., 2017). Deciphering this bioelectric code could provide valuable insights into how cells are able to self-organize and form multicellular structures (Zanzouri et al., 2006). Numerous experiments have been conducted *in vivo* regarding the collective behaviour of cells, demonstrating that the genome does not merely give rise to a specific developmental programme. Instead, it generates a collective of cells capable of dynamically responding to its environment (Levin, 2021). The mechanisms through which cells dynamically respond to their environment remain incompletely understood. A potential mechanism might involve bioelectric signalling. As mentioned earlier, a break in an epithelial layer causes a disturbance of the TEP and creates an endogenous ion flux that guides cells to the site of the break (Tyler, 2017). The endogenous ion flux generated by ion channels and maintained by gap junctions, creates weak electric fields that are utilized by cells in directional migration. This ability of cells to respond to weak electric fields is called electrotaxis, also known as galvanotaxis (Cortese et al., 2014). However, the exact mechanisms underlying electrotaxis have not been fully elucidated. Hence, researchers have been conducting *in vivo* experiments to understand the role and mechanisms of bioelectricity in the collective behaviour of cells. The findings of these experiments will be highlighted to demonstrate the effect of manipulating the bioelectric dynamics of a cell collective and to suggest a connection between cellular plasticity and bioelectric signalling.

Cellular plasticity

Salamanders exhibit remarkable regenerative ability, enabling them to regrow tissues, organs, and even whole-body parts. Consequently, salamanders have been utilized to study the underlying mechanisms by which cells are able to sense missing or damaged parts (Joven et al., 2019). By grafting the tail of a salamander to its flank, the tail remodelled itself into a functioning leg (Farinella-Ferruzza, 1956). The structure of the tail changed to become more in line with the large-scale body plan and took place even though the local environment of cells at the tip of the transplanted tail was normal. This illustrates that the remodelling of tissue structure is based on global target morphology and not on the local environment (Farinella-Ferruzza, 1956; Levin, 2021). However, the exact mechanisms behind the remodelling of tissue remains unclear. Recently, researchers have been conducting more experiments which could help explain some of the mechanisms behind the collective behaviour of cells to conform to their target morphology.

It is known that embryonic development has the capacity to adapt its morphogenetic processes in responds to external perturbations. To better understand the mechanisms behind this adaptability of cells, researchers induced craniofacial defects in *Xenopus Laevis* embryos and then tracked the so-called "Picasso" tadpoles with craniofacial deformities (Levin, 2021). The researchers observed that the craniofacial deformities were naturally corrected during the first few months of tadpole development. However, the movement of the misplaced craniofacial structures to their correct position was significantly different from their normal movement, suggesting the craniofacial structures use a mechanism to asses and adjust their location relative to other local organs (Vandenberg et al., 2012). This mechanism could be the same mechanism that is used by the cells in the salamander tail to become more in line with the target morphology. Both experiments demonstrate that evolution has not predetermined specific means by which cells must undergo development. Instead, the data suggests that the genome governs a highly plastic cellular collective that undergoes rearrangement until the correct target morphology is achieved (Levin, 2021). However, the question how cells know what the target morphology is, remains.

Bioelectric dynamics in pattern formation

An interesting case could be made for the role of bioelectricity in coordinating cellular behaviour to reach the target morphology, as it can provide instructive cues in the form spatiotemporal patterns of voltage potential across the plasma membranes (Levin, 2014b). It was observed that during embryonic morphogenesis, complex structures can be induced by manipulating the membrane potential. For instance, researchers observed that during normal embryogenesis of the *Xenopus Laevis*, a specific group of hyperpolarized cells that will form the eyes can be demarcated in the anterior neural field (Pai et al., 2012). Initially, they noted that the eyes originating from these cells were malformed when the dorsal lineages in which these cells reside were depolarized instead of being hyperpolarized. Subsequently, they discovered that by manipulating the membrane potential of non-neural progenitor cells during the embryogenesis of *Xenopus Laevis*, well-formed ectopic eyes could be induced. Remarkably enough, these structures were morphologically and histologically similar to endogenous eyes. Even more astonishing was the fact that these structures were able to form far outside of the anterior neural field. Interestingly, the researchers observed that the transcription factors involved in the formation of the specific group of hyperpolarized cells that will form the eyes, is regulated by a calcium-channel dependent pathway that transduces the membrane potential. This revealed an instructive role of the membrane potential during embryogenesis in *Xenopus Laevis*, as it was found to be an upstream signal for eye development.

Identification of mechanisms responsible for morphogenetic events, such as organogenesis and regeneration, are extremely valuable for their applications in fields such as regenerative medicine and synthetic biology. *Dugesia Japonica*is, also known as planaria, is a powerful model system to study functional manipulation of morphogenesis, as these flatworms have a highly regenerative ability where they can regenerate any body structure after significant damage. To illustrate, it has been observed that 1/279th piece of a planarian was still able to regenerate into a fully formed planarian (Handberg-Thorsager et al., 2008). By employing this fascinating model system, researchers have demonstrated that voltage-dependent bioelectric signalling via the cell membrane determines both the head size and organ scaling during regeneration in planaria (Beane et al., 2013). Manipulating the membrane potential of regenerating planaria, such that it became hyperpolarized, resulted in disproportional sized heads and pharynges in the regenerated planaria. Subsequently, the researchers noted that the disproportional sized heads and pharynges resulted from a lack of apoptosis, which is normally required to adjust organ size and placement, indicating apoptotic remodelling as a connection between bioelectric signalling and pattern formation.

In other fascinating experiments conducted on planaria, researchers demonstrated that the normal anterior-posterior pattern in regenerating planaria can be permanently rewritten through a brief disturbance of their endogenous bioelectrical networks (Durant et al., 2017). This was achieved by blocking specific gap junctions, thereby interfering with gap junctional communication and reducing the ability of cells to communicate ionically. The disturbances in the bioelectric dynamics during regeneration stochastically gave rise to regenerated planaria with two heads and regenerated planaria with normal morphology. The two-headed planaria do not differ from the wild-type planaria, as their histology, expression of key polarity genes, and neoblast distribution is the same. Instead, it seems that the altered regenerative body plan is stored in the global pattern of the bioelectric states. These global bioelectric state patterns can be viewed as a multi-stable epigenetic switch. Experimental reversals of the two-headed bioelectric state pattern reset the subsequent regenerative morphology of the regenerated planaria back to wild-type. These experiments beautifully highlight the involvement of bioelectricity in pattern formation and demonstrate the direct effects of manipulating the bioelectric dynamics during these processes. Additionally, the research on planaria elegantly illustrated the ability of bioelectrical networks, in the form of global bioelectric state patterns, to stably override genome-default target morphology, suggesting one genome can give rise to more than one target morphology (Durant et al., 2017).

Bioelectricity in carcinogenesis

The ability of cells to adapt to their surroundings seems to be fundamental in how they operate, especially during morphogenetic event, such as organogenesis and regeneration. In these processes, cells intricately respond to both chemical and bioelectrical cues, orchestrating the dynamic interplay between cell proliferation, differentiation, and spatial organization. Interestingly, cells' ability to adapt is not limited to normal functions. It also plays a role in diseases, especially in the process of carcinogenesis. Carcinogenesis can, according to the Somatic Mutation Theory (SMT), be characterized by changes at genetic, epigenetic, and cellular level, resulting in abnormal cell proliferation. However, this theory is increasingly being challenged by experimental observations it cannot explain. For instance, a neoplastic phenotype can be reversed when cells from a neoplasm are placed in a normal environment (Soto & Sonnenschein, 2004). Similarly, during previously mentioned remodelling experiments in salamanders, cells from an already differentiated tissue demonstrated the ability to adapt to their surroundings. Both observations suggest an interesting connection between abnormal cell behaviour and adaptability to the cellular surrounding.

An interesting alternative theory for carcinogenesis is the Tissue Organization Field Theory (TOFT), which poses that carcinogenesis is mainly a tissue regulation disease rather than having a cellular origin (Carvalho, 2021). It is a thought-provoking alternative, which does not exclude oncogene mutations as an important driver for cancer formation, rather it highlights the importance of proper cell communication in tissue organization. It suggests carcinogenesis can be addressed by targeting tissue-wide regulation mechanisms instead of focussing on specific cellular processes. Underscoring this argument, researchers have identified that biochemical signalling pathways are frequently altered in cancer cells, as oncogenic mutations can disrupt proper signal transduction (Yip & Papa, 2021). For instance, the dynamic signal encoded in the Ras-ERK pathway is disrupted in cancer cells containing particular B-raf mutations. These mutations lengthened the ERK signal by increasing its half-life, thereby leading to a misinterpretation of the signal, which affected proliferation (Bugaj et al., 2018). Thus, an alteration in a dynamic signalling pathway can lead to disruption of normal tissue organization, the latter of which can also be seen in carcinogenesis (Seferbekova et al., 2023). By viewing carcinogenesis as dysregulated behaviour of a cell collective during tissue organization, and understanding the mechanisms that underly this behaviour, new perspectives to address the problem of carcinogenesis could be obtained. This citation provides an additional intriguing perspective on carcinogenesis by combining elements of SMT and TOFT: "Cancer may be a disease of geometry: a misregulation of the field of information that orchestrates individual cells' activities towards normal anatomy" (B. Chernet & Levin, 2013). Nonetheless, it raises the question of what might constitute this field of information that orchestrates cellular activity. It describes cancer as a disease of dysregulated morphogenesis, as the information that orchestrates individual cells' activities towards normal anatomy could be provided by the same underlying mechanisms that are currently thought to be employed in morphogenesis, *i.e.* spatial gradients providing positional information.

As previously discussed, the bioelectric properties of cells are intrinsically linked to control mechanisms that regulate cellular behaviour. Additionally, researchers have tested the role of membrane potential in carcinogenesis mediated by canonical oncogenes in *Xenopus Laevis* (B. T. Chernet & Levin, 2013). They induced the formation of tumour-like structures (ITLs) by overexpressing known oncogenes. It was observed that a depolarized membrane potential was a common characteristic feature of these ITLs, which could even be used as a marker to reveal ILTs before they became histologically and morphologically apparent. In addition, they recognized that the membrane potential also had a functional importance, as hyperpolarization of the membrane potential, through induced overexpression of ion transporters in these ILTs, resulted in a return to the normal membrane potential of the tissue and significantly reduced ITLs formation *in vivo*. The researchers further observed that changes in the membrane potential were transduced via SLC5A8-dependent mechanisms, which leads to a change in the chromatin state (B. T. Chernet & Levin, 2013). Thus, the data suggests that upstream bioelectric signals might be responsible for epigenetic modifications that facilitate the suppression of ITLs. The functional involvement of the membrane potential in the deviation of ITLs cells from their usual tumour development programs highlight the importance of bioelectric signalling in carcinogenesis. Hence, through the dynamic nature of bioelectric signalling, it is able to form bioelectrical networks between cells via bioelectric gradients, thereby providing spatial information and instructive cues. These factors contribute to the formation of multicellular structures, such as tumours, organs, and whole organisms. Additionally, the involvement of bioelectric signalling in the incredible plasticity of cells to reorganize and reform without genetic manipulation highlights the profound involvement of bioelectricity in pattern formation and morphogenesis.

Discussion

It has become evident that bioelectricity is not merely a property used by excitable cells, such as neurons and muscle cells, to send and receive information. Many different cell types utilize the fascinating capacity of bioelectricity to exchange information and induce various cellular behaviours, such as proliferation and differentiation. To unravel the mysteries of cell communication in pattern formation, it is important to understand the underlying principles of signal transduction. There is a growing body of research demonstrating that biochemical signalling pathways involved in pattern formation are inherently dynamic. Timing and distribution are some of the key mechanisms underlying these dynamics. However, temporal organization is not exclusive to biochemical signalling. It appears that bioelectric signalling is also inherently dynamic, as the distribution of ions across multicellular structures forms bioelectric gradients, which induce feedback loops that amplify signals and change cell behaviour. These factors enable large-scale patterning and pattern homeostasis and is achieved by transduction of the bioelectric signal into biochemical pathways. The seemingly dynamic nature of bioelectric signalling provokes an interesting perspective on how cells operate collectively to form intricate patterns. Instead of viewing cells as individual units, they should be considered as elements working together in a dynamic system, forming a network that is connected through the continual exchange of signals, both biochemical and bioelectrical.

Cells in a collective exhibit emergent behaviour that can be accessed and modified by manipulating the bioelectric properties of cells. Bioelectricity has been studied for a long time, and pursuing this has not always been an easy task, as the bioelectric properties of cells immediately disappear upon cell fixation or cell death and can therefore only be observed *in vivo* (Levin et al., 2017). However, In the recent years, novel molecular tools have been developed that can manipulate and quantify bioelectric patterns as well as identify downstream targets and molecular sources of bioelectric signals. For example, the electrical connectivity between cells or the bioelectric state of specific cells can be altered through specific ion channel drugs or other methods such as optogenetics (Adams & Levin, 2013). Additionally, visualization of spatiotemporal distribution of voltage gradients and quantification transmembrane potential patterns can be accomplished with fluorescent reporters of transmembrane potential such as genetically encoded voltage indicators (GEVIs) (Treger et al., 2015). Moreover, bioelectric properties of cells can be studied through quantitative modelling to create a better understanding of how voltage patterns arise and how resting membrane potentials evolve over time (Levin et al., 2017). An example of such a modelling environment is the BioElectric Tissue Simulation Engine (BETSE), which enables quantitative experiments and modelling of biochemical signalling and physiology within an integrated virtual tissue (Levin et al., 2017; Pietak & Levin, 2016). Novel molecular tools and quantitative models like BETSE will contribute to improving our understanding of the role of bioelectric signalling in specific patterning events and morphogenesis.

Applying the already mentioned analogy between a collective of cells and a computer, suggests that reprogramming the software of a cell collective could be explored with models like BETSE, to find out which specific ion transporters have to be manipulated to form specific structures. However, before bioelectric programming becomes truly viable, it will be necessary to decipher the bioelectric code that maps the resting membrane potential patterns of cells to their subsequent anatomical outcome. Subsequently, every species could have its own software, as every genome gives rise to a different composition of hardware and could thereby enable different modes of bioelectric signal transduction. This is, of course, assuming there is such a thing as a bioelectric code. Nonetheless, an increasing body of evidence is being brought to the surface that suggests there might be something of this nature and it could prove helpful in unravelling the mysteries of cellular signalling in pattern formation and morphogenesis (Levin & Martyniuk, 2018). Advancing our understanding of cellular communication will enable the development of novel therapeutic treatments for birth defects and diseases such as cancer. Moreover, research fields like regenerative medicine and synthetic biology will benefit greatly from this knowledge, as regeneration of specific tissues and generation of synthetic life forms become reality. Additionally, areas such as soft robotics are advancing as cells and their electrical properties are being used to create so called "electrophysiological robots", whose actions are guided by the electrical signals flowing through the tissue of these soft robots (Cheney et al., 2014). By illustrating the profound implications of bioelectricity in pattern formation and morphogenesis, the intention is to foster a broader perspective on cellular communication and manipulation of cellular behaviour, thereby aiming to further increase our understanding of the underlying principles of biology. Despite the continual expansion of the field of developmental bioelectricity, it is still in its infancy. Substantially more research needs to be conducted to generate more practical applications. Fortunately, the groundwork has been laid, and with the development of novel molecular tools, ever-increasing computational power, and rapid progress in areas such as machine learning, the exploration of bioelectricity holds great potential.

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