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Review

Bioelectrical regulation of cell cycle and the planarian model system☆

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ABSTRACT

Cell cycle regulation through the manipulation of endogenous membrane potentials offers tremendous opportunities to control cellular processes during tissue repair and cancer formation. However, the molecular mechanisms by which biophysical signals modulate the cell cycle remain underappreciated and poorly understood. Cells in complex organisms generate and maintain a constant voltage gradient across the plasma membrane known as the transmembrane potential. This potential, generated through the combined efforts of various ion transporters, pumps and channels, is known to drive a wide range of cellular processes such as cellular proliferation, migration and tissue regeneration while its deregulation can lead to tumorigenesis. These cellular regulatory events, coordinated by ionic flow, correspond to a new and exciting field termed molecular bioelectricity. We aim to present a brief discussion on the biophysical machinery involving membrane potential and the mechanisms mediating cell cycle progression and cancer transformation. Furthermore, we present the planarian *Schmidtea mediterranea* as a tractable model system for understanding principles behind molecular bioelectricity at both the cellular and organismal level. This article is part of a Special Issue entitled: Membrane channels and transporters in cancers.

Contents

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1. Introduction

Efficient cellular communication is critical for the survival and perpetuation of multicellular organisms. However, the mechanism by which crosstalk among cells is translated into coordinated behavior at both the cellular and organismal levels remains poorly understood. A primitive mode of cellular communication relies on the exchange of ions between the intracellular and extracellular environment. Membrane proteins facilitate the transport of ions from one side of the plasma membrane to the other. The transport of ions is essential for the establishment of electrochemical gradients which influence the behavior of both local and distant cells in the body. For instance, the manipulation of endogenous ionic flows is able to alter patterns of cell division, migration and differentiation in a wide range of embryonic and adult stem cells of vertebrate and invertebrate models [1–4]. Although the characterization of such bioelectrical phenomena is well established, very little is known about how endogenous electric fields actually affect biological functions and the mechanism through which cells respond to their influence. Nonetheless, recent findings provide compelling evidence regarding the potential of controlling this powerful ionic flow communication system to induce regeneration of missing tissues and to stimulate, or control abnormal cell behavior observed in cancer [5–9]. This new field of research, defined as "Molecular Bioelectricity", aims to understand how voltage gradients in nonexcitable cells coordinate morphogenesis, tissue development, repair, and cancer formation [10].

Pharmacological manipulation of ionic flow provides relatively easy access to regulatory properties of cell cycle parameters [9,11,12]. We aim to offer information on the different ionic and voltage dependent variables that modulate the cell cycle during tissue regeneration, cellular turnover and cancer formation from previous research. These voltage gradients are not limited to cells, but also exist at the tissue/organ level, where they provide instructive information for specifying organ identity and large-scale anatomical order [1,10,13]. Therefore, it is essential to address the role of endogenous currents in the context of the whole organism. We propose the extremely versatile planarian model system *Schmidtea mediterranea* as a venue for exploring bioelectrical regulation at both the cellular and the organismal level to better understand the role of voltage gradients in adult tissue maintenance, repair and tumorigenesis.

2. The transmembrane potential (TMP)

All cells generate long-term, steady-state voltage gradients known as transmembrane potentials (TMPs) [3,8,14]. TMP is an ancient and evolutionarily conserved system that can be found in a variety of organisms, ranging from plants to higher vertebrates, and has been reviewed extensively [1–3,10,15,16]. It is generated by a separation of charge across the plasma membrane, leading to a negative voltage difference in respect to the extracellular environment [11,15]. However, gradient changes involved in generating TMPs are much slower and vastly different than the rapid membrane depolarizations observed in both nervous and muscle tissues [3,8]. However, similar to action potentials, TMP changes in a single cell can be transmitted over long distances via gap junction linkages [14,17–19]. TMPs are primarily maintained by the constant activity of various ion channels, pumps and transporters, collectively known as ion transport mechanisms (ITMs). These ITMs segregate charges across the plasma membrane and produce necessary current needed to generate a voltage potential [20]. An ITM of extreme importance to living systems is the sodium/potassium ATPase (Na^+/K^+ ATPase), which is essential for maintaining the transmembrane potential between 10 and −90 mV, depending on the tissue type [15]. The cell invests substantial amounts of energy to maintain TMP as changes in membrane polarity are used to drive alterations in cell behavior [14,15]. We will now explore the role bioelectric regulation of one such aspect, proliferation.

3. TMP and cell cycle regulation

The cell cycle is regulated by a complex array of signals stemming from the microenvironment as well as from intracellular signals such as cyclins, cyclin-dependent kinases (CDKs), CDK inhibitors and the retinoblastoma (Rb) protein. Factors associated with ionic flow (i.e. ITMs), membrane potential, and membrane composition are known to be involved in regulating these cell cycle components [21–25]. Exciting new results in this area unveil powerful strategies to control the cell cycle, that may enhance genetic and biochemical interventions in regenerative medicine and cancer therapy [11,12]. We will discuss some of the bioelectrical mechanisms and properties known to modulate the cell cycle in vertebrates and invertebrates.

3.1. TMP and membrane polarization

Eukaryotic vacuolar-type H+-ATPases (V-ATPase) are electrogenic proton pumps that energize both the intracellular and plasma membranes by expelling H+, changing pH levels in the extracellular environment, which contribute to the maintenance of the TMP [26,27]. As intracellular pH recovers, membrane potential becomes more negative in charge, causing plasma membrane to hyperpolarize [28]. These fluctuations in TMP are particularly evident during cell cycle progression, as demonstrated in Chinese hamster lung cells [29]. During the G0/G1 transition checkpoint, there is a gradual transition of TMP from a state of intermediate depolarization to intermediate hyperpolarization. As the cell passes through the G1/S phase transition checkpoint, the TMP becomes more negative, marking the hyperpolarization of the cell membrane. During the transition through the S phase, S/G2 checkpoint and G2 phase the membrane potential is at a maximum negative voltage and remains hyperpolarized. Entering mitosis, TMP rapidly depolarizes to the lowest minimum voltage, indicating the completion of cell division (Fig. 1A) [29]. Furthermore, these fluctuations in TMP are well documented in other cell types [21–25]. These findings support the notion that TMP fluctuations through V-ATPase are an important regulatory component for ionic flow during the cell cycle and its deregulation may be associated with abnormal cell behavior.

3.2. Generation of TMP and ionic flow

Transient depolarization and hyperpolarization of the plasma membrane is mediated by the constant exchange of charged ions between the cytoplasm and extracellular environments. The V-ATPase proton pump is seen to energize the membrane through ionic gradients whereas Na+/K+ ATPases participate in maintaining the chemical gradient [30]. However, the flow of potassium ions via K+ channels eventually aids in the establishment of TMP. Inhibition of the V-ATPase reduces the proton gradient within the cell, leading to impairment of both the ionic driving force and ionic homeostasis needed for cell proliferation [31]. As intracellular concentrations of Na+ decrease, a concurrent influx of K+ ions is seen, promoting an increase in TMP. Though low in concentration, Cl− ions also play an important role throughout the cell cycle. This interplay between fluctuating states of membrane polarization and ionic flows serves as a regulator of cell cycle and cellular proliferation (Fig. 1B) [5,11,22,32]

3.2.1. Chloride dynamics

Due to nature of electrogenic V-ATPases, a parallel ion conductance must occur to aid in pH regulation and driving membrane potentials. The flux of Cl− occurs in parallel to H+ flux via V-ATPase activity and is required to maintain cellular electroneutrality [33,34]. In yeast cells, inhibition of V-ATPase strongly reduces Cl− concentration in both the vacuolar and plasma membrane [35].

Compression and swelling of a cell during the cell cycle has been attributed to a Cl−/K+ relationship, required to gather essential amino acids, metabolic substrates and materials for the synthesis of proteins

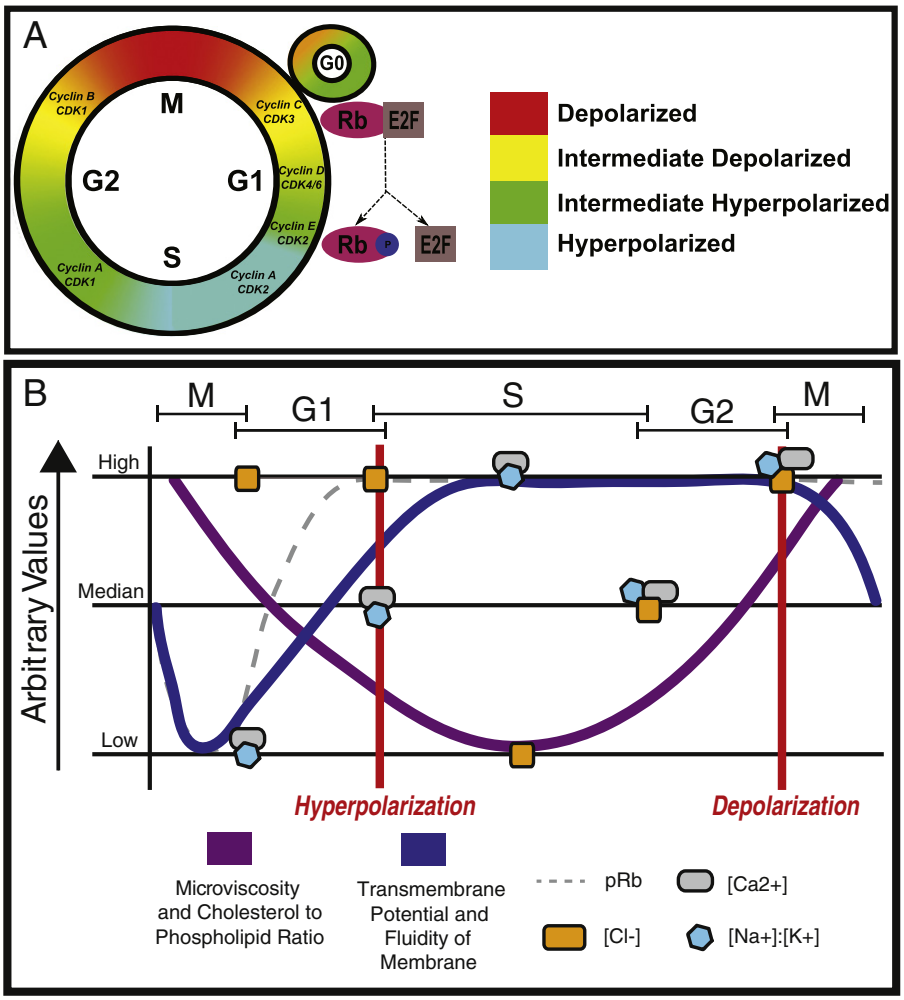

Fig. 1. a. Cell cycle modulation via transmembrane potentials, ionic gradients and gene expression. (A) Depicts the phases of cellular polarization and the regulatory checkpoints in gene expression throughout the cell cycle. M phase is a depolarized state (red), S phase is a hyperpolarized state (teal) and G0,G1 and G2 states oscillate through intermediated phases of depolarization and hyperpolarization (yellow and green). (B) Shows physical characteristics of the cell membrane and oscillation of both gene expression and ionic concentrations during cell cycle progression. The fluctuations in microviscosity/cholesterol to phospholipids ratio (purple). Transmembrane potential/membrane fluidity (blue). pRb gene expression (—) and ionic concentrations of Cl— (yellow square). Ca2+ (gray rectangle) and Na+:K+ ratio (blue hexagon) are seen to fluctuate in terms of arbitrary values throughout the cell cycle.

[36,37]. Isotonic Cl— currents in the cell cycle have been found to regulate cell volume and membrane potential [38–40]. Studies in cancer cells indicate that volume activated Cl— channels can act as regulators of cell cycle progression [41]. They show that although there is low expression of Cl— currents in the G0 phase [37], it reaches a maximum threshold in the G0/G1 and G1/S transition phases [36], reduce in the S phase and back up during the G2/M transition [42,43]. These fluctuations of Cl— currents are not isolated events, but rather correlate with the concentrations of cyclin/CDKs and CDK inhibitors throughout the cell cycle [44]. In various cell types, a decrease in intracellular Cl— concentrations during the cell cycle reduces the rate of cellular proliferation through the upregulation of p21 and CDK inhibitors; therefore, causing a downstream effect on Cdk2 and Rb expression, halting cells in the G0/G1 transition phase [37,41,45–49]. Inducing expression of a hyperpolarizing anion channel such as CLIC1 in normal cells, in the vicinity of tumors, inhibits their growth. This process occurs through the upregulation of HDAC1, an acetylating protein that in *Xenopus* embryos, is critical for cell cycle progression and rate of cellular proliferation [5,6]. This further supports that the precise maintenance of TMP and the voltage gradient via charged ions is key for cell cycle regulation.

3.2.2. Sodium and potassium dynamics

The efflux of H+ by the Na+/H+ transporter into the extracellular environment of the cell generates a driving force for the influx of Na+ into the cytoplasm [30,50–52]. This influx of Na+ into the cell by the Na+/H+ transporter is then counterbalanced by the Na+/K+ ATPase pump to maintain the electrochemical gradient. The ratio [K+]i/[Na+]i created by the interplay between Na+, the most abundant inorganic cation and K+, the second most abundant cation in the intracellular fluid attributes to a heavily regulated electrochemical gradient, as they run parallel in concentration [53]. However, K+ ions and TMP play important roles as they are responsible for creating the driving force needed for the release of intracellular Ca2+ [54,55]. Through the inhibition of membrane bound K+ channels (e.g. Kv), cell cycle progression has been seen to halt during the early and late Gl phase, G1/S transition and G2/M [56,57]. It has been shown in mouse neuroblastoma cells, Na+/K+ ATPase pumping oscillates during cell cycle progression [58]. Membrane permeabilities to K+ and Na+ are reported to be high in M phase (membrane is in depolarized state), decreases by threefold in G1 phase (membrane is in hyperpolarized state) and rises through the G1/S transition into S phase [58]. As the cell progresses into G2 phase, K+ and Na+ permeabilities decrease and rapidly increase through the G2/M transition phase [58]. Studies in several cancer cell lines provide support for the ability of potassium channels to modulate cellular proliferation [59–62]. For example, the overexpression of K+ channels in Glioma cells maintains a depolarized membrane potential, resulting in high rates of cellular proliferation [63,64]. These findings highlight the direct relationship

between TMP, ionic gradients and cellular proliferation through cell cycle.

3.2.3. Calcium dynamics

Cell volume oscillations and K+ influxes during the cell cycle regulates cellular proliferation by modulating the release of intracellular Ca2+ ions [65,66]. The rate of K+ ion permeability through the membrane provides a driving force for Ca2+ influx activating calmodulin. This protein is known to activate crucial proteins such as Rb and cyclin/CDK complexes, necessary for the cell cycle progression. Rb activation is closely regulated by Cyclin D and Cdk4/6 release in early and mid G1 phase [57]. Rb is increasingly phosphorylated through the G1 to S phases, at which point it remains hyperphosphorylated until the completion of M phase [67,68]. It is known that Ca2+ influx is required for the initiation of S phase and the completion of M phase, possibly through its contribution to a more negative TMP [67–69]. Ca2+/calmodulin mediates the activation of Cdk1 and Cdk2, which are known to be upregulated through the S to M phases [70,71]. The activated Cdk2 hyperphosphorylates Rb, releasing E2F allowing the cell to progress through the cell cycle. Inhibition of Ca2+ binding protein calmodulin prevents Rb hyperphosphorylation and ultimately leads to cell cycle arrest in G1 phase with subsequent down regulation of Cyclin-D expression and loss of Cdk4/6 and Cdk2 [72]. These results strongly support a direct role of Ca2+ currents and TMPs in the regulation of protein activity throughout the cell cycle.

3.3. Plasma membrane dynamics

TMP regulation of lipid–protein interactions in the cellular membrane becomes another point of interest in cellular proliferation. Microviscosity, the physical state describing the degree of membrane fluidity, is a key regulator of membrane protein mobility. It is thought to do so by modulating microtubules, microfilaments, and membrane bound enzymes [58,73,74]. TMP has been shown to regulate microviscosity by influencing the conformation of transmembrane ionic channels and activity of enzymes within the phospholipid matrix [23,24,75]. Microviscosity of the membrane lipids varies throughout the cell cycle and is highest during mitosis, rapidly decreases through G1 phase, reaching a minimum during S phase and finally increases during G2 phase as the cell enters mitosis (Fig. 1B) [23].

Membrane microviscosity and fluidity also regulate cellular proliferation through the modulation of membrane binding phospholipids, ionic headgroups, hydrophobic acyl chains and/or the binding of cholesterol [73,76,77]. Expression and accessibility of surface antigens/receptors of a membrane are mediated through fluctuations between membrane microviscosity and membrane fluidity, which are reciprocals of one another [78]. A key relationship between microviscosity and membrane lipids is the ratio of cholesterol to phospholipids that affects internal membrane viscosity, lipid motion and regulates cellular behavior (e.g. cell migration) [24]. As a cell reaches maximum microviscosity, the fluidity of surface membrane lipids are at a minimum while the ratio of cholesterol to phospholipids is at its highest peak [24]. Furthermore, during this microviscosity state, surface antigens and receptors increase in expression, exposure and accessibility, leading to membrane saturation [23]. The reduction of membrane surface area happens concurrently with a decrease in TMP (Fig. 1B) [75]. As a consequence of oscillatory microviscosity levels, the cell surface proteins that are indirectly recruited to the membrane play a key role in the regulation of the cell cycle.

4. TMP and cancer

Since TMP is known to control key cellular processes such as proliferation, migration, and growth, its proper maintenance is crucial to body homeostasis [12,79]. Deregulation of membrane potential can induce drastic changes in cells, and in some cases, cause them to become tumorigenic. In the same virtue, TMP may also be manipulated to prevent tumor progression [5–7,20,62].

4.1. Cancer progression

Numerous studies have shown that deregulation of TMP can lead to tumorigenesis [20,40,80–82]. There is a general correlation between the proliferative capacity of a cell and it's TMP, which cancer cells often use to their advantage [1,22]. A common trait among tumor cells is that they are more depolarized at any given time, comparable to the TMP excitable cells discussed previously [11,79]. A panel of breast cancer cells shows an increase in depolarization as compared to controls [83]. For example, MCF-7 cells exhibit lower TMP values in both the G1 and S phases as compared to normal breast cells [25]. In addition, a K+ channel, hERG1, normally expressed in differentiated cardiac myocytes is upregulated in various types of cancer cells [62,65,84]. Lastly, elevated expression of Kv10.1. and Kv11.1 channels are correlated with an increased probability of relapse and a lower survival rate in human patients [9,12]. Intracellular Na+ levels are usually elevated in cancerous tissue, supporting the notion that a state of depolarization is critical for cancer transformation [9,20,40].

Deregulation of V-ATPase pumps can often lead to excessive cellular proliferation. For example, in many breast cancer cell lines, there is an upregulation in the expression of V-ATPases in the cell membrane whereas overexpression in normal cells confers a neoplastic phenotype [85,86]. Another voltage-gated proton pump Hv1, critical for proton transfer, is overexpressed in high-grade metastatic human breast cancer cell lines such as MCF-7, but shows minimal expression in low-grade metastatic breast tumors [87]. Overall, these studies suggest that proper ionic flow in cells is critical for the proper cellular maintenance.

4.2. Escaping cell death

TMPs not only play an important role in the proliferation required for tissue homeostasis but also maintains constant cellular turnover. It does so through the activation of pathways that results in cellular degeneration via the inhibition of the cell cycle machinery or through both the intrinsic and extrinsic components of the apoptotic pathway [62]. Cells unresponsive to environmental cues may be forced to enter a stage known as senescence, mediated by TMP [88], which results in permanent cell cycle arrest, through secretion of antigrowth signals that prevent oncogene related growth [89]. This change in the TMP may lead to an upregulation of p16-pRb and p53, the primary mediators of senescence [88].

Proper maintenance of TMP by ITMs is critical for determining cellular fate. For instance, downregulation the Na,K-ATPase has been shown to markedly increase apoptosis in normal and tumor cells [90,91] K+ channels are critical modulators of cell fate decisions, such as controlling the onset of apoptosis [62,65,84]. For example, expression of apoptosis-related KCNA1 channel is significantly reduced in human cancers [92]. Blocking hERG1 channels can stop the flow of K+ into the cell, leading to apoptosis [88]. Similarly, efflux of K+ ions is seen to be a major perquisite behind caspase-3 activated apoptosis in HeLa cells [93]. On the other hand, upregulation of hERG1 results in G0/G1 arrest without undergoing apoptosis, consistent with the state of replicative senescence [20,94]. These findings suggest that TMP plays an important role in the maintenance of cellular homeostasis and the deregulation can lead to excessive activation of apoptotic pathways leading to massive cell death.

4.3. Cancer cell migration

Stages of invasive cancer progression begins with the loss of cell adhesion from the primary tumor site, followed by the invasion of cells into the circulatory system and lastly seeding of distant tissues to form secondary tumors [95]. Although TMP is important in abnormal cellular proliferation, it also plays an indirect role in cell migration by modulating intracellular Ca2+ ion concentrations [9,40]. For example, overexpression of Kca2.3, key for maintaining a hyperpolarized

membrane potential, increases the cell's ability to migrate through the release of intracellular Ca2 + [96]. Deregulation of K + channel GIRK1 is correlated with higher incidence of lymph node metastasis and poor prognosis in a small sample of patients and is overexpressed in breast tumors [97]. RT-PCR and immunocytochemistry experiments demonstrated that the expression levels of voltage-gated proton pump Hv1 is increased among different breast cancer cell lines [87], indicating that proper maintenance of TMP may be necessary for cellular adhesion and function.

4.4. Abrogation of cancer growth

Manipulation of ITMs to induce changes in TMP is another potential therapy target to prevent the transformation of normal cells into cancer cells [11]. Membrane hyperpolarization leads to a decrease in proliferation by sustaining high levels of K + and Ca2 +, which can inhibit cell cycle progression (Fig. 1B) [95]. Interestingly, tumor-like structures generated in *Xenopus* embryos were later inhibited by the upregulation of the anion channel CLIC1 in the stroma leading to the hyperpolarizion of the tumor-like cells [5,6]. Inhibition of certain voltage gated Na + channels reduces the metastatic ability of prostate cancer cells in a rat model by abrogating their motility [96,98]. Numerous studies suggest that the pharmacological or genetic inhibition of K + channels can reduce the proliferation of cancer cells [99–101]. For instance, blocking the Kv10.1 channel causes a cessation of cellular proliferation and migration in several myeloid leukemia cell lines [12].

A negative membrane potential has been associated with ATP induced cell death [102,103]. The microenvironment of cancer cells tend to be hypoxic, resulting in increased K + efflux in the extracellular space [102,103]. The increase in K + in the microenvironment can inhibit Pannexin-1, leading to apoptosis via caspase-3 activation [104]. This is supported by the observation that normal concentrations of K + inside a cell inhibit the activation of the apoptosome by preventing the actions of Apaf-2 [105]. TMP across the mitochondria could also decide whether a cell undergoes apoptosis. Loss of normal mitochondrial TMP triggers the release of cytochrome c, into the cytoplasm, signaling the formation of the apoptosome and the death of the cell [84]. These studies suggest that TMP modulation maybe a potential target for directed-apoptosis and proper understanding of TMP and its effect on cell dynamics will be essential for developing novel cancer therapies.

5. *S. mediterranea* and TMP

Ion transporters and channels serve as excellent targets for cancer therapy because they are present on the surface of cell membranes and many molecular tools are readily available for manipulating them [61,94,95]. Most drugs used to block or enhance ion transporter's activity have been characterized extensively and a large number have FDA approval [79], which offers the possibility of quick translation into therapies. Pharmacological deregulation of ion channels is an effective method for disrupting TMP. It allows for precise control and timed inhibition while delivering a more informative result than a gene knockout method where there could be many complications due to functional compensations [10,14,15]. Dissecting the molecular mechanisms by which biophysical properties regulate tumorigenesis and metastatic processes requires a model system that is tractable to both biophysical/physiological techniques and state-of-the-art molecular genetics [11].

The planarian model organism *S. mediterranea* provides an excellent base of study for understanding bioelectric regulation of tissue regeneration as they possess a simple anatomy along with a remarkable ability to rapidly regenerate [19,106–112]. This regenerative ability extends to healing any part of its body after receiving an injury, including the neural tissue, digestive system, brain, photoreceptors and connective tissue (Fig. 2A) [19,107,109,110]. Its robust regeneration is fueled by an abundant population of adult pluripotent stem cells known as neoblasts that is responsible for cellular turnover as well as tissue regeneration [19, 107–110,113]. The genome of *S. mediterranea* has been sequenced and many of molecular tools have been developed to study genetics, physiology, and biochemistry in this model organism [19,107–110, 113].

Immunohistochemistry and in situ hybridization are often used to visualize cell migration and differentiation patterns in planarians [114]. These techniques can determine anatomical structural differences, neoblast state and abnormalities in tissue patterning (Fig. 3A). Neoblasts in different phases of the cell cycle can be visualized through the use of anti-phospho histone H3 antibody (H3P) and bromodeoxyuridine (BrdU) [115]. Fluorescent activated cell sorting (FACS) is a unique method of isolating planarian neoblast populations. Dyes such as Hoechst 33342 and calcein AM are used to stain live cell DNA content and cytoplasmic activity [116]. FACS allows for the isolation of cell populations enriched in radiation-insensitive differentiated cells (Xins) or radiation-sensitive adult stem cells fractions (X1 and *X*2) (Fig. 3B) [106,116–119]. Cell cycle analysis is also possible through flow cytometry [120,121].

The adult body of *S. mediterranea* provides unique opportunities to analyze regulation of cell proliferation by TMP [1,13,19,122]. Localized or systemic neoblast proliferation could be altered by metabolic changes (feeding or starvation) and tissue injury [17,108,121]. Furthermore, neoblast overproliferation and tumor formation can be induced by manipulation of well-characterized tumor suppressor genes (e.g. p53, PTEN) (Fig. 2B) [123,124]. The pattern of regeneration of entire body parts is susceptible to molecular manipulation of ion flows, gap junctional communications, and conserved signaling pathways such as Wnt and B-catenin [17,18,107,125,126]. Regeneration in planarians proceeds through activation of cell proliferation and application of bioelectric fields are known to modulate repair and tissue polarity in flatworms [127,128]. Membrane potential across the whole planarian body could be monitored using DiBAC (4) (3) staining, and the newly developed approach termed Planarian Immobilization Chips (PICs) to visualize bioelectrical changes in real time while minimizing tissue damage [13,122,129,130].

The H,K-ATPase is a major player in the regulation of tissue maintenance and regeneration in the planarian model. This ion transporter is essential for both the proper development of organ size during planarian regeneration and the anterior polarity in regenerating worms [13, 122]. Functional disruption of H, K-ATPase by RNA interference (RNAi) leads to failure in tissue remodeling and proportion adjustment of regenerated structures [13]. These results in planarians are consistent with zebrafish studies that found bioelectric signaling regulates fin allometric scaling and coordination of growth [131]. Similarly, ion transporters are required for proper regeneration of lost tissue as well stem cell maintenance in *Xenopus* and mice [132,133]. Together, findings in both vertebrate and invertebrate models demonstrate that TMP and ITMs modulate central issues of regeneration and development, such as cell fate decisions, the establishment and maintenance of tissue proportion, and the growth of complex structures, through well conserved mechanisms.

Furthermore, planarians are also amenable to chemical treatments aimed at targeting ion transporters and recordings of TMP in real time [18,122,125,129]. Drug-induced changes in TMP could be used as a venue to perform gain of function studies in planaria, as there are currently no other means for doing such research in this system. Increased knowledge of bioelectricity in living systems will contribute to our understanding of how ion flows can be used in clinical settings to influence cellular proliferation, migration and differentiation to control tissue function. For instance, modulation of ion transport with chemicals could be a powerful tool to halt cell cycle progression in abnormally proliferating cells [134] or an instrument to prompt cellular division to re-establish form and function to lost or damaged tissues [133]. The molecular basis of these processes could be readily investigated in the planarian model and later validated in more complex systems.

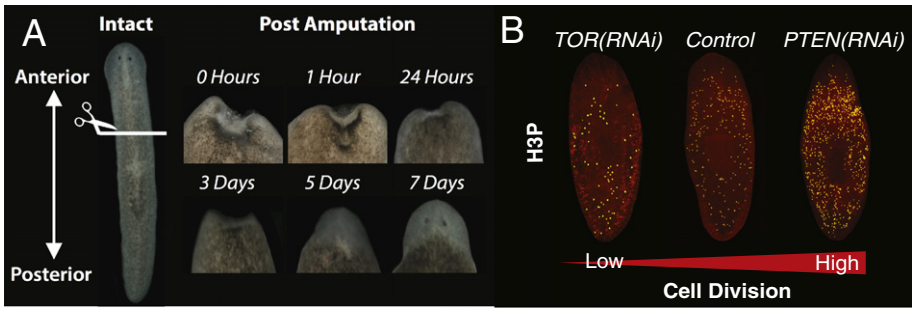

Fig. 2. Planarian tissue regeneration and maintenance. (A) Images of a live worm (left) amputated pre-pharyngeal; images on the right show the regeneration of the anterior portion including the head. Time-lapse images depict proper wound healing response and blastema formation in a regenerating planarian. By Day 7, the fragment has regenerated into a completely functional animal. (B) Image depicts cell division (yellow dots, H3P immunostaining) after RNAi of TOR and PTEN genes in comparison to control (mock-RNAi). TOR(RNAi) reduces mitotic activity while PTEN(RNAi) induce the opposite effect.

6. Concluding remarks

TMP and the many proteins that are involved in its maintenance, are of great importance to the normal cellular function and to a greater extent, the organism. The complex interplay between K+, Na+, Cl− and Ca2+ ions in and out of the cell determines the polarization of TMP at any given moment. Ionic oscillations during the cell cycle are able to control passage of cells through critical checkpoints by regulating key proteins such as Cyclin/CDKs and Rb. The deregulation of a small subset of proteins in this intricate system can lead to abnormal cell behavior, ultimately leading to the onset of tumorigenesis. Most studies that have been performed to explore this phenomenon have been through in vitro methods, however, this only provides a partial view of the bioelectrical phenomena. In order to gain a comprehensive understanding of TMP's role in cellular processes, it is critical to perform in vivo analyses of cell cycle progression and cancer transformation in the adult body and the planarian *S. mediterranea* is a well-suited model organism for this task.

Planarians provide an excellent model for studying mechanisms of bioelectric regulation of cell cycle as they are host to a population of mitotically active, pluripotent stem cells that maintain their high rate of cellular turnover and impressive regenerative capability. They are

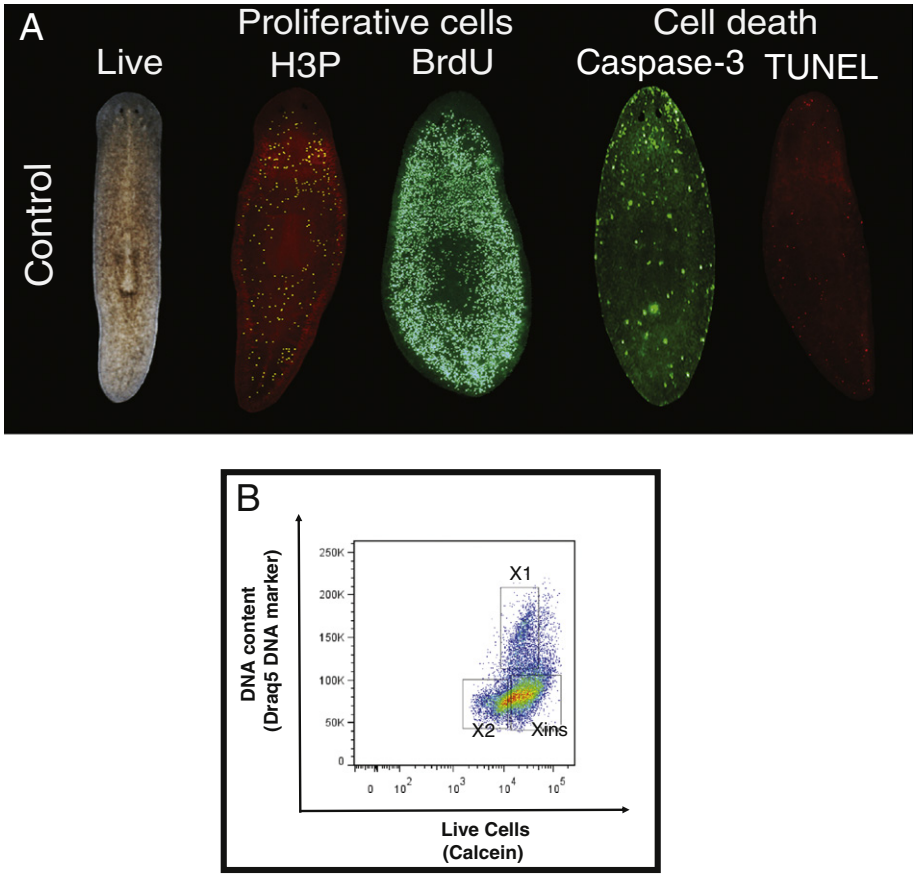

Fig. 3. Planarians allow for analysis of stem cell proliferation, differentiation and cell death in the whole organism. (A) Left to right. Live worm, whole-mount immunohistochemistry (WIHC) to stain cells in different parts of the cell cycle, including mitotic cells alone (H3P), cells entering S phase of the cell cycle (BrdU positive cells, green dots indicate proliferative cells), cell death showing Caspase-3 and TUNEL (fragmented DNA of apoptotic cells) (B) FACS plot shows gates used to isolate irradiation sensitive (X1 and *X2*) and insensitive (Xins) populations based on DNA content and viability.

amenable to analysis of cellular transformation by chemical treatment or genetic manipulation of tumor suppressor genes. The many tools that have been developed to study these animals will prove useful for manipulating various facets of their internal characteristics and eventually enable us to address how endogenous electric fields contribute to tissue homeostasis and regeneration of the whole organism. Increased efforts in this area will propel the field into creating applications, which can eventually lead to a better understanding of human tissue homeostasis and regeneration.

Conflict of interest

The authors Barghouth et al. declare no conflict of interest.

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