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Bioelectric controls of cell proliferation
Ion channels, membrane voltage and the cell cycle

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All cells possess long-term, steady-state voltage gradients across the plasma membrane. These transmembrane potentials arise from the combined activity of numerous ion channels, pumps and gap junction complexes. Increasing data from molecular physiology now reveal that the role of changes in membrane voltage controls, and is in turn controlled by, progression through the cell cycle. We review recent functional data on the regulation of mitosis by bioelectric signals, and the function of membrane voltage and specific potassium, sodium and chloride ion channels in the proliferation of embryonic, somatic and neoplastic cells. Its unique properties place this powerful, well-conserved, but still poorly-understood signaling system at the center of the coordinated cellular interactions required for complex pattern formation. Moreover, disregulation of ion channel expression and function is increasingly observed to be not only a useful marker but likely a functional element in oncogenesis. New advances in genomics and the development of in vivo biophysical techniques suggest exciting opportunities for molecular medicine, bioengineering and regenerative approaches to human health.

Introduction

Regulation of the cell cycle is of significant importance to many areas of biology. During development stem cells must maintain their proliferative potential, while some terminally differentiated cells such as neurons no longer divide once specified. In wound healing and regeneration, cells must initially proliferate in order to fill damaged areas or replace lost structures, and then downregulate growth once the proper pattern has been restored. Disruption of cell cycle checkpoints can lead to uncontrolled division of cells and is highly relevant to cancer biology and human health. While tremendous progress has been made on the molecular details of biochemical checkpoint machinery (e.g., kinase cascades),[1] an important area of mitotic regulation still offers much opportunity for new discoveries: bioelectrical events controlling transmembrane voltage potential.[2]

The first correlations between membrane potential (the voltage gradient across the plasma membrane, V_{mem}) and proliferative ability came from observations that cell types with a very high resting potential such as muscle cells and neurons show little if any mitotic activity (reviewed in ref. 3). Though the early 1950's it remained unclear whether there was a causal relationship between V_{mem} and proliferation, or if both were simply characteristics related to the specialized function of these cells. This question invoked more intense research in the late 1950's and early 1960's, following a number of studies in which multiple groups reported a decrease in the membrane potential of cells following malignant transformation.[4-6] These results, in addition to observations that cultured cells under high growth conditions show a decrease in V_{mem}, were among the first to suggest a causal relationship between ion flow and the cell cycle.

These ideas were formally tested in a series of groundbreaking experiments by Clarence D. Cone, Jr. throughout the late 1960's. He first observed that V_{mem} varied through the cell cycle and postulated that the variations were directly related to progression through G_1/S and G_2/M transitions in proliferating cells.[7] In a follow up study to explicitly test causation, he altered the intracellular ionic concentration of cells and was able to induce a reversible mitotic block by mimicking V_{mem} to levels observed in neurons.[8] Even more impressively, it was shown that sustained depolarization was able to induce DNA synthesis and mitosis in mature neurons.[9,10]

Cone would later synthesize these studies, as well as the pioneering prior work, into a theory on the basic mechanism of mitotic control and oncogenesis.[11] This paper eloquently argued for a direct relationship between the cycle and electrical transmembrane potential and discussed possible mechanisms, both molecular and physiological. His commentary was notable given the limited information and methods available at the time, and Cone's innovative work and ideas provided the foundation for formal inquiry into the relationship between membrane potential and the cell cycle.

Since then, molecular, physiological and pharmacological tools have become much more sophisticated, clarifying the molecular details of biochemical pathways involving cyclins, c-myc, c-fos, numerous tumor suppressors and oncogenes. While considerable modern work underscores the link between membrane potential and the cell cycle, this fascinating bioelectric control mechanism is still not well known in the field.

Here, we briefly summarize some of the recent studies examining the relationship between V_{mem} and cell proliferation in differentiated cells, and the modulation of expression and regulation of ion channels throughout development as cells progress from

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a stem cell state to terminal differentiation (**Table 1**). We also discuss data on the role of membrane potential in neoplastic cells, and suggest that this mechanism is an attractive target for modulation in regenerative medicine and developmental biology contexts.

Membrane Potential as a Regulator of Cell Cycle in Healthy Cells

V_{mem} and cell proliferation. While rapid changes in membrane potential (V_{mem}) are best known in neurons and muscle, steady-state V_{mem} levels are associated with all cells, and exhibit cyclic fluctuations on much longer timescales than the familiar millisecond action potentials.[12,13] Transmembrane potential arises from the combined actions of numerous channels and pumps, which segregate ions across the cell surface under constraints of concentration gradient and charge. Thus, modulation of specific ion channels and transporters is both an endogenous method for controlling cellular V_{mem} and a tractable technique for functional experiments. It should be noted that a number of channels and pumps are now known to have additional regulatory roles independent of their current-passing functions.[14-17] In this review, we focus on functions specifically associated with charge transfer and voltage control.

Pharmacological blockade of ion channels has been a popular method of disrupting membrane potential; while not as specific as molecular approaches (knockout, RNAi or morpholinos), it has two advantages. First, it allows a more precise control of the timing of current inhibition. Second, it often produces more informative results than gene-specific loss of function because of the extensive compensation among multiple channel types: whereas phenotypes may be masked by redundancy in single-gene knockdowns, a pharmacological approach can reveal the function of membrane potential per se, which is not necessarily dependent on any one particular gene product.

Membrane potential has been examined as a key regulator of proliferation in a number of cell types, suggesting that modulation of V_{mem} is required for both G_1/S phase and G_2/M phase transitions. Depolarization of membrane through changes in extracellular ion concentration inhibits G_1/S progression of lymphocytes, astrocytes, fibroblasts and Schwann cells suggesting hyper-polarization is a required step for S phase initation.[18-21] In B cell lymphocytes, inhibition of channels induces a reversible cell cycle arrest;[22] similar results have been noted in other cell types.[22-27] Analysis of downstream targets has revealed that inhibition of potassium channels in these cells resulted in expression changes of a number of proteins, including IL-1/2 and transferrin, both of which are implicated in cell cycle control.[18,22,23,26,28,29]

Conversely, depolarization of the plasma membrane seems to be essential for the G_2/M transition. Current models outline a rhythmic oscillation of membrane potential throughout the cell cycle, with a spike in hyperpolaration occurring before DNA synthesis followed by a prolonged period of depolarization necessary for mitosis, and appears to be conserved mechanism from early cell divisions in embryos through the normal division of differentiated tissues.[30] The exact threshold of V_{mem} necessary to drive cells though proliferative stages is not known, and is likely to vary between cell type, and in development, the stage of differentiation.

In human endothelial cells, modulation of V_{mem} through applied electric fields revealed that hyper-polarizing currents arrest cell division.[31] This arrest is characterized by downregulation of cyclin E and concurrent upregulation of the cyclin inhibitor p27, forging a direct link between transmembrane potential and known regulators of cell cycle. Conversely, depolarization of PUS-1.8 mouse macrophages results in DNA synthesis and progression through the cell cycle, and is marked by a subsequent upregulation of the transcription factors c-myc and c-fos.[32] Both of these results fit the current V_{mem} oscillatory model of cell cycle, and suggest an intimate relationship between membrane potential and the well know cyclin-dependent pathways.

While a detailed consideration of the roles of bioelectric signals with differentiation is beyond the scope of this review, differentiation is often closely associated with changes in proliferative capacity. A number of cell types have shown a strong correlation between membrane potential and differentiation, with V_{mem} becoming more hyperpolarized as cells are specified. For example, the neural crest cells of quail embryos exhibit a -35 mV resting potential early in development, but as development progresses the resting potential shifts to -55 mV.[33] These changes have been directly correlated to a turnover in potassium channels, with the early membrane voltage attributed to the expression of a K^+ channel, ERG, while at later stages ERG expression is lost and replaced with inward-rectifying K^+ channels associated with many differentiated tissues. Similarly, the normal course of human mesenchymal stem cell differentiation is accompanied by a progressive hyperpolarization; it has recently been shown that this is an instructive parameter, as artificial depolarization keeps the MSCs in the stem state, while hyperpolarization accelerates their differentiation.[34]

V_{mem} and cell cycle control in multi-cellular contexts. Cell cycle is a key parameter of cellular behavior that must be tightly regulated and coordinated during morphogenesis associated with development and regeneration. For example, in addition to normal turnover, changes in membrane potential have been linked to the proliferation of cells during wound healing. In cultured cells, modulation of V_{mem} using K^+ channel-inhibiting drugs increases wound healing in cell monolayers;[35] this is a related but distinct mechanism to the modulation of the voltage gradient across an epithelium,[36] which is also a crucial component of wound healing and normal development.[37] Regulation of gap junctional communication is known to control the proliferation of adult stem cells during regeneration in planaria,[38] although the specific ions involved have not been identified. Whereas activation of currents is necessary for proliferation, inhibition of specific currents has been shown to disrupt normal development via changes of proliferative capacity. Knockdown or inhibition of two K^+ channels (K_V1.3 and K_V1.5) resulted in cell cycle arrest at G_1 in rat oligodendrocyte precursors;[39,40] this effect was characterized by accumulation of p27 and p21. Blockade of other K^+ channels results in similar signaling cascades, suggesting convergent mechanisms downstream of the activity of many diverse channels.[40]

Changes in V_{mem} and channel function during the cell cycle. Alongside the functional control of proliferation by ion channel function, observations from a wide array of cells have also shown the converse—control of physiology as a function of the cell cycle, suggesting bi-directional regulatory circuits. An increase in the expression or activity of potassium channels was observed after exposure to mitogens,[19,41-48] demonstrating channel expression downstream of proliferation checkpoints. It remains unclear which mitogen activated pathways are responsible for the regulation of potassium channels, although the involvement of p21 and its downstream targets, the protein kinase Raf and GTPase Ras, has been shown.[49] It is probable the multiple downstream signaling cascades exist, as the regulation of ion channels by mitogens is likely to be dependent on both cell type and the specific mitogenic signals used.

A number of ion channels show variation of expression or activity across stages of the cell cycle. During the G_1/S transition, multiple families of K^+ currents become active, including ATP-sensitive K^+ channels, outward rectifying currents (K_{IR}), and Ca^{2+} activated K^+ channels.[50-53] During the G_2/M transition, increases in potassium channel EAG currents have been noted.[54] In addition, progression to M phase is also characterized by an increase in chloride flux. Voltage activated chloride currents show a strong increase during G_2; the NCC27 channel is activated, and ClC-2 shows M phase-specific expression and phosporylation.[55-57]

Alongside characterization of individual cells in vitro, ion channel and gap junction function changes across the cell cycle have also been observed in embryonic development.[58-60] The complex bidirectional relationship between ion transporter function and cell cycle suggests this set of mechanisms as a powerful and versatile physiological network which can be used during pattern formation in a flexible and highly dynamic control mechanism to synchronize cell division.

Membrane Potential and Cancer

Neoplasia has long been associated with aberrant changes in cell cycle.[61,62] Alterations in membrane potential and ion channel expression/function have been observed in a wide array of cancers.[63] Likewise, alongside ion-independent roles of GJs in neoplasm,[64] it is clear that gap junctions are an important component of V_{mem}, regulating its fluctuations,[65] establishing iso-potential cell fields, and modulating cellular responses to external electric fields.[66] For brevity, the well-known role of gap junctions in cancer is not discussed here (reviewed in refs. 67 and 68).

Potassium channels. The proliferation of some tumor cells is dependent on voltage-gated potassium channels.[69,70] hERG channels are particularly implicated,[71-76] as are 2-pore channels such as KCNK9.[77] In the case of KCNK9, it is known that its oncogenic potential depends on K^+ transport function, not some other role of the protein,[78] and in human colorectal tissue KCNK9 K^+ channel expression was shown to be significantly elevated.[79] A screen of several cervical cancers found the K^+ channel EAG expressed in 100% of the biopsies analyzed, and overexpression of EAG in human cells resulted in more quickly dividing progeny in culture.[80,81] This result was replicated in vivo using mice implanted with human EAG (hEAG) expressing CHO cells. All of the mice receiving CHO hEAG injections formed tumors while none of CHO controls formed a growth greater than 1 mm in size.[81] hEAG-1 is a true oncogene since its overexpression drives mammalian cells into uncontrolled proliferation and favors tumor progression in cells injected into immune-suppressed mice.[81] Likewise the an EAG relative, hERG, is not normally present in most differentiated cells besides the heart but has been observed in a number of human cancers and neoplastic transformation in prostate epithelium.[73,82] In these cells, hERG appears to recruit tumor necrosis factor receptor (TNFR) to the plasma membrane and cause a subsequent increase in NFκB, a known proliferation control gene. In addition to modulations of single channels, some cancers are characterized by the activation of multiple potassium currents, such as in the case human melanoma lines which express of both hEAG1 and Ca^{2+}-activated K^+ channels.[83] Indeed, complex interactions by multiple channels likely exist, and currents driven by diverse families of potassium channels (including calcium activated, inward rectifying K_{ir}, EAG and ERG) have all been correlated with cancerous tissue.

Proton, chloride and sodium flux. Manipulation of membrane H^+ flux can confer a neoplastic phenotype upon cells,[84] and voltage-gated sodium channels potentiate breast cancer metastasis.[85] Studies in glioma lines have revealed a role of the ClC-3 chloride efflux channel in cellular division.[86] Following a chloride buildup, Cl^- efflux is a known driving force in cytoplasmic condensation and is required for mitosis to progress. Expression of ClC-3 is localized to both the plasma membrane and mitotic spindle of D54-MG and U251-MG glioma cells, and inhibition of the channel though hairpin RNA constructs resulted in the loss of premitotic condensation and arrest of the cell cyle. These results have been supported in studies of human prostate cancer lines, and support the role of chloride channels as key regulators of proliferation through cell size regulation.[87,88]

Sodium channels have been implicated in mouse cancers in vivo. The knockout $APC^{min/+}$ line share a mutation found in many human colorectal cancers, and subsequently develop multiple intestinal neoplasias.[89] In vivo transepithelial voltage recordings in this line revealed an increase in Na^+ compared to wild type mice that was the result of an increase in expression of the ENaC Na^+ channel. The downstream targets of Na^+ signaling are not well known, but it was noted that neither Cl^- nor Ca^{2+} absorption were not altered in the $APC^{min/+}$ line.

Metastatic potential correlates with voltage-gated inward sodium current and it has been suggested that some sodium channels may be oncofetal genes.[85,90-93]

Molecular medicine. Unique bioelectrical properties of tumor tissue have been recognized for a long time.[94-109] How relevant the changes of ion flux are to neoplasm in general will require much future work. The majority of studies has examined the presence of ion channels in cancerous tissues but have not explicitly examined the physiological role of V_{mem} changes in the cell. Nor do we know in many cases at which stage of neoplasm development the bioelectric signals are relevant. Cancer cells have been observed to be depolarized with respect to normal healthy epithelial tissue,[110-113] and hyperpolarization therapy

(molecular-genetic or pharmacological) remains to be tested as a therapeutic approach.

Characterization of ion channels involved in cancer will undoubtedly be of high interest in human health. First, expression of ion channels may prove to be highly relevant markers when screening tissue if unique among the transformed tissue, or if expression levels are significantly elevated.[80,114-116] Voltage sensitive dyes have been used successfully in vivo to image the action potentials of the visual cortex, and modification of these techniques has been suggested for cancer screens. Double dye systems have more recently been developed to examine the membrane potential of non-nervous tissue in vivo.[117] Application and analysis of these dyes could potentially reduce cost, invasiveness and time involved with diagnosis compared to more traditional biopsies, and in the case of epidermal malignancies such as melanoma may involve no invasiveness whatsoever if they could be applied and examined in intact skin.

Second, inhibition of ion channels through pharmacological treatment has been proposed as a potential cancer treatment.[118] Drugs that target membrane voltage-generating transporters have shown clinical promise in cancer.[119] Indeed, evidence in cancer lines supports this theory. In human prostate cells inhibition of Ca^{2+} dependent K^+ channels lead to a decrease in cell proliferation,[120] and knockdown of EAG with antisense oligonucloetides reduced division rates in somatic cancer lines.[81] Growth suppression of pancreatic tumor cells occurs after selective blockade of IK-type channels.[121] Control of tumor growth though pharmacology is especially exciting in cancers which display ion channels that are non-existent throughout the rest of the body as there would be little chance of the drug interacting with healthy tissue in the donor. For ion channels which are present in both tumors and surrounding tissues, targeted delivery remains an active area for investigation.

Challenges and Opportunities for Regenerative Medicine

Modulation of the membrane voltage as a novel parameter controlling cell proliferation offers unique opportunities for guiding morphogenesis in vitro (tissue engineering) and in vivo (regenerative medicine).[2,122,123] For example, V-ATPase proton pump activity in the zebrafish eye is needed for retinoblast proliferation and survival,[124] while induction of proton efflux from wound tissue has been shown to induce complete regeneration of the tail in Xenopus tadpoles.[125] The molecular details of epigenetic bioelectrical pathways must be considered in developing strategies for rational modulation of cell behavior based on bioelectric controls.

Mechanisms. How are changes in V_{mem} transduced into alterations of mitotic behaviors (**Fig. 1**)? One likely mechanism is regulation of Ca^{2+} entry into the cell, and the positive feedback loop that would occur between Ca^{2+} entry and Ca^{2+} dependent potassium channels.[126] For example, increasing intracellular calcium concentration with the ionomycin restored normal division in cells which were cultured in high potassium media, a condition normally inhibitory to division.[28] In addition, Na^+ influx is required for the uptake of metabolic substrates and subsequent progression through G_1; hyper-polarization of the plasma membrane through potassium channels has been suggested to increase the rate influx and intracellular concentration of Na^+.[47] However, these links remain poorly understood and further research will be necessary to determine the direct and indirect downstream signal cascades resulting from V_{mem} flux.

Additional mechanisms for sensing changes in plasma membrane polarization levels include: proteins that change conformation upon V_{mem} changes and activate integrin-dependent[127] or PTEN phosphatase-dependent cascades,[128,129] depolarization-dependent nuclear translocation of the NRF-2 transcription factor,[130] induction of specific kinases such as KID-1,[131,132] and the influx of mitogens such as serotonin,[133-137] which is controlled by V_{mem} through the voltage-powered serotonin membrane transporter SERT and through gap-junctional paths via electrophoresis.[138]

Special features of V_{mem} signalling. The control of cell functions by membrane voltage is highly non-linear because the existence of multiple feedback loops (**Fig. 2**). For example, many of the channels, pumps and gap junctions that determine transmembrane potential are themselves pH- and voltage-sensitive, leading to complex recursion of effects. This is a very powerful mechanism for buffering evolutionary control mechanisms, sometimes resulting in positive feedback loops (such as NFκB, which is turned on by K^+ loss, downregulating transcription of the potassium importer HK-ATPase[139]) as well as negative feedback loops (e.g., depolarization can activate the V-ATPase hyperpolarizing pump). Thus, quantitative mathematical modelling will be necessary to integrate the temporal dynamics of multiple ion fluxes and the resulting V_{mem} changes and thus develop strategies for placing cells into specific V_{mem} states in biomedical applications.

The non-linear and non-local aspects of bioelectric signals result in some very interesting features of V_{mem} control over proliferation during morphogenesis.[140-143] For example, mitotic upregulation induced by the V-ATPase is limited to the regenerating region (not the rest of the tadpole) when Xenopus tadpoles regenerate their tails by a voltage-dependent mechanism.[125] Alongside this spatial control, temporal control can also be used with high resolution: in depolarization-induced overproliferation of melanocytes, these cells undergo no more than 1 extra cell cycle, despite the continued presence of the depolarizing potassium channel mutant protein.[117]

In our lab, gain-of-function studies altering bioelectric signals during embryogenesis by misexpression of specific ion transporters and their mutants have revealed a wide variety of subtle phenotypes related to proliferation and differentiation (**Fig. 3**). The ability to integrate growth control with morphogenesis via bioelectric parameters is only beginning to be understood, and further molecular investigation will surely reveal details of significant importance to biomedicine. Interestingly, we have observed that not only long-term, but also transient, modulation of V_{mem} has the ability to activate proliferation. Electroporation, a technique in which exogenous DNA is introduced into cells by square electric pulses on a millisecond timeframe, is widely used in cell biology, developmental biology and regenerative medicine.[144-148] Surprisingly, the process of electroporation itself, using no DNA,

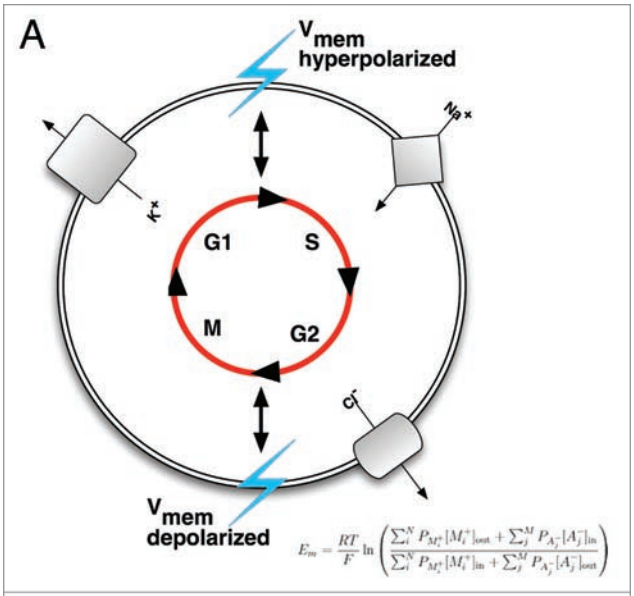
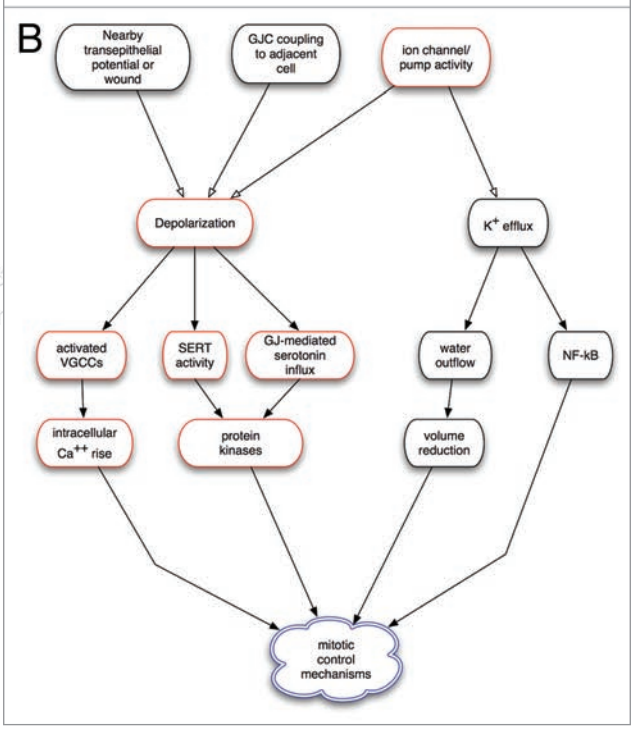


Figure 1. Linkage between bioelectric signals and cell cycle control via V_{mem} changes. (A) Schematic illustrating the linkage of membrane potential modulation to ion channel dynamics during the cell cycle. During G_1/S transition, the membrane potential becomes hyperpolarized relative to the normal resting potential. Potassium channels from the ATP-sensitive, voltage gated and Ca^{2+}-activated families become active allowing for potassium efflux from the cell; sodium channels also become activated. During the G_2/S transition the membrane becomes depolarized, and there is a decrease in potassium channel activity. In addition, G_2/M is characterized by the activation of chloride channels and a subsequent efflux of chloride. While the role of potassium channels are the most well-studied in relation to the cell cycle, a number other ion gradients are involved, each contributing to the net membrane potential as described by Goldman-Hodgkin-Katz equation. (B) Membrane voltage in a cell can be altered by a variety of factors, including channel/pump activity in it's own membrane (cell-autonomous effects), gap junctional communication to neighboring cells of different potential, or nearby electric fields and ion flows from wounded and intact epithelia (the latter two being non-cell-autonomous control mechanisms). In turn, changes in ion flow can be transduced into alterations of the mitotic program by voltage-gated calcium channels and calcium-dependent second-messenger pathways, changes in cell volume, and alterations of transport of mitogens such as serotonin. These can arrive in cells by two voltage-dependent mechanisms: electrophoresis through gap junctions, or changes in the activity of transporters like SERT that are powered by transmembrane potential.



is sufficient to activate not only de-differentiation[149] but also hyperproliferation (**Fig. 3E–E'''**). This is surprising, given the rapid nature of electroporation compared to the time-scale of cell division, and indicates that significant care must be used using any methods that disrupt transmembrane potential.

Future. Membrane voltage is a well-conserved and probably ancient control system, functioning in phyla ranging from plants[150] to higher vertebrates. Several areas of this field suggest exciting future advances.

First, physiological parameters such as membrane voltage and specific ion content may be used as in vivo markers to identify special subpopulations of cell types. For example, human mammary tumor cells fall 4 Gaussian distributions of voltage with means of -9, -17, -24 and -2 mV.[151] The functional significance and the value of this as a marker remain to be tested, but given the important regulatory roles of V_{mem}, it is likely that these data are informing us of important distinctions among subtypes of the population.

Second, it is abundantly clear that the original hypothesis of depolarization inducing growth[3] is too simple. It is much more likely that types of cells (e.g., embryonic, committed, neoplastic, etc.,) can be sorted into distinct regions in a multi-dimensional

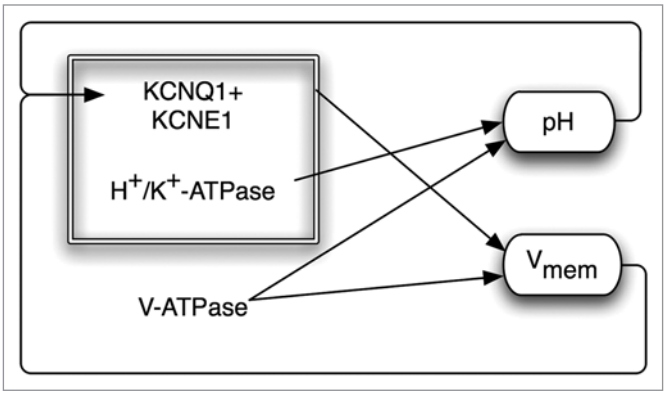


Figure 2. Sample of recursive feedback among physiological parameters and ion channel/pump activity. Bioelectric controls of cell functions are inherently non-linear because channels and pumps produce effects on voltage and pH that in turn regulate those same channels and pumps. Here is shown one example taken from a circuit used in vertebrate left-right patterning.[166] The V-ATPase creates both a pH gradient and contributes to membrane hyperpolarization. At the same time, the H,K-ATPase functions together with a K^+ channel to regulate V_{mem}; however, both of these components are themselves voltage- and pH-sensitive. Quantitative models of such networks, which take into account both the molecular biology of components expressed in relevant cells and the time-dependent physiology of the resulting circuit.

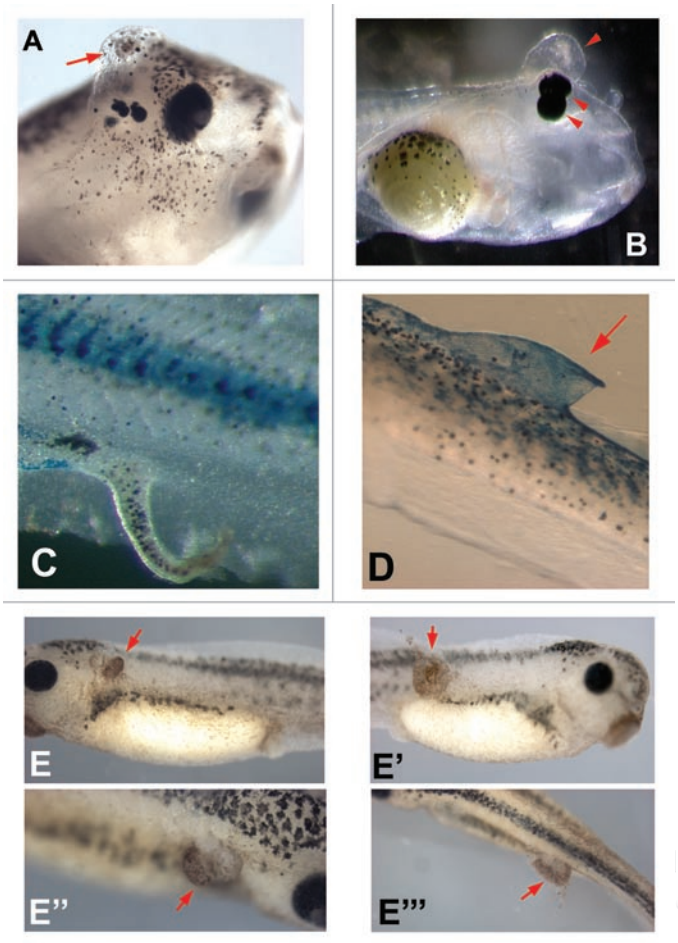


Figure 3. Perturbation of growth in Xenopus embryos caused by manipulation of ion channels and electroporation. A variety of mRNAs encoding wild-type and mutant channels were microinjected into frog embryo blastomeres to screen for bioelectrical signals with roles in growth and pattern control. The VSOP[167] proton channel (kindly provided by Yasushi Okamura) (A), the Cx32 gap junction subunit (plasmid kindly provided by Dan Goodenough) (B and C), and the HERG K^+ channel (plasmid kindly provided by Annarosa Arcangeli) (D) result in ectopic growth and abnormal duplication of body structures such as eyes, sometimes forming extensive fin-like protrusions that are clearly associated with increases in cell growth. (E–E''') Electroporation of embryos at stage 33, with no DNA, (95 msec interval, 5 msec pulse, 10X repeated) results in significant areas of ectopic growth 24 hours later. Red arrows indicate hyperproliferation.

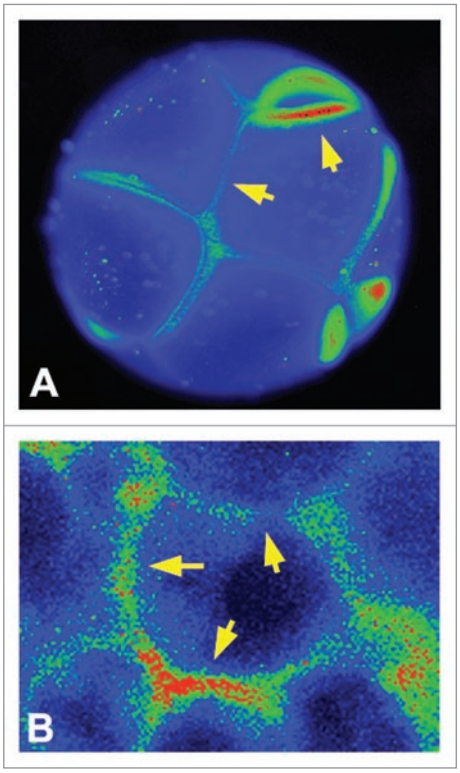


Figure 4. Membrane voltage levels are not homogenous around the cell surface. Using the voltage-sensitive fluorescent dye DiBAC,[168] early frog embryo blastomeres (A) as well as COS cells in monolayer culture (B) exhibit significant variations of membrane voltage level around the cell surface, indicating that a single V_{mem} number for a given cell drastically under-estimates the amount of information that can be encoded in the plasma membrane's physiological state and potentially communicated to neighboring cells. Images courtesy of Dany S. Adams.

state space with axes corresponding to physiological parameters (of which membrane potential is just one). Moreover, because of the V_{mem} oscillations occurring during the cell cycle, it is clear that temporal variation must be added to models of this signalling system.

Third, it should be noted that assigning a single V_{mem} value to a cell is also a significant oversimplification. In fact, a number of embryonic blastomeres (**Fig. 4A**) and mammalian cells in culture (**Fig. 4B**) exhibit distinct domains of membrane voltage around the cell periphery. While the physiological literature often reports one particular mV reading for a cell this neglects the considerable complexity of microdomains of V_{mem} on cells, presumably established by distinct population of channel/pump proteins on lipid rafts[152,153] and fence functions performed by plasma membrane and cortical/cytoskeletal components. Thus, since different V_{mem} values can be present in domains as small as 2 μ (reviewed in ref. 154), each cell potentially contains a 2-dimensional surface which encodes a tremendous amount of information that can be transmitted to distinct intracellular second-messenger pathways as well as neighboring cells.[155]

Finally, it must be observed that transmembrane potential is only the best-known and most tractable of the cellular bioelectric parameters. Subcellular organelles such as mitochondria, endosomes, phagosomes, ER and nucleus all possess a transmembrane potential due to specifically-localized ion channels.[156-164] Future efforts must develop subcellularly-targeted voltage reporter proteins[165] and mutant channels that can be used to study and functionally alter the bioelectric signalling in distinct intracellular locales.

The detailed understanding of the contribution of transmembrane potential to cell cycle control, and especially the integration of this mechanism into biochemical and genetic mechanisms occurring during complex morphogenesis, will reveal fascinating aspects of interdisciplinary biophysics and will offer significant opportunities for the biomedicine of birth defects, cancer and regenerative medicine.

Table 1. A sample of some of the most well characterized modulations of membrane potential or ion channel activity, and the effect on cell proliferation

V_{mem} or channel examined	Effect	Cell type	Ref.
V_{mem} hyperpolarized through physiology	arrest	vascular endothelial	31
V_{mem} depolarized through high K^+ media	proliferation	mouse macrophage cell line (PU5-1.8)	32
ClC-3 Cl^- channel activity	proliferation	glioma cell lines D54-MG and U251-MG	86
ENaC Na^+ channel expression	proliferation	mouse colonic epithelium	89
EAG K^+ channel activity	proliferation	multiple human melanoma lines	83
EAG K^+ channel activity	proliferation	multiple human carcinoma lines	81
EAG K^+ channel expression	proliferation	xenopus oocyte	160
ERG K^+ channel activity	proliferation	multiple human cancer lines	73
ERG K^+ channel activity	proliferation	quail neural crest	33
KCNK9 K^+ channel activity	proliferation	human colorectal cancer tissue	79
$K_V1.3$, $K_V1.5$ K^+ channel activity	proliferation	rat oligodendrocyte progenitors	39
C^{2+} dependent K^+ channel activity	proliferation	prostate cancer cell line LNCaP	120
Ca^{2+} sensitive K^+ channels inhibition	proliferation	colon carcinoma cell line T84	35
K^+ channel inhibition	arrest	human and mouse lymphocytes	18, 22
K^+ channel inhibition	arrest	chick astrocytes	20
K^+ channel inhibition	arrest	hamster fibroblasts	21
K^+ channel inhibition	arrest	rat Schwann cells	19

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Glossary

KID-1: a member of the pim family of proto-oncogenes.[169]

Oncofetal: a protein form present in tumors and embryos, associated with the rapid growth and undifferentiated phenotype characteristic of neoplastic and embryonic states.

EAG/ERG: Members of the ether-a-go-go gene family and major component of a delayed rectifiers current that allow potassium to exit the cell when active.[170]

K_{IR}: Inward rectifying potassium channel family involved in both depolarization and repolarization of the plasma membrane. The family is characterized by passing K^+ ions more inward than outward.[171]

ClC's: Chloride channels involved in the maintenance of membrane potential that form a diverse family activated by various methods including V_{mem}, Ca^{2+} concentration, or ligand gating. Includes ClC-1 (NCC27), ClC-2, ClC-3.[172]

KCNK9: a member of the two pore domain potassium channel family.[173]

ENaC: a ion channel in epithelium permeable to Na^+ and Li^+ ions.[174]

IK: Intermediate conductance, Ca^{2+}-activated potassium channels.[175]

V-ATPase: a protein pump that moves protons out of cells via ATP hydrolysis.[176]

$K_V1.3/K_V1.5$: members of the voltage gated potassium channel family. Both are considered delayed rectifiers.[177]

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