Aging as a morphostasis defect: a developmental bioelectricity perspective

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Abstract

Maintaining order at the tissue level is crucial throughout the lifespan, as failure can lead to cancer and an accumulation of molecular and cellular disorders. We argue here that the most consistent and pervasive result of these failures is aging, which is characterized by the progressive loss of function and decline in the ability to maintain anatomical homeostasis and reproduce. This leads to organ malfunction, diseases, and ultimately death. The traditional understanding of aging is that it is caused by accumulation of molecular and cellular damage resulting from energy metabolism and mitochondrial function, and that cell growth and lifespan are limited by replicative senescence due to shortening of telomeres. In this article, we propose a complementary view of aging as a morphostasis defect, specifically driven by abrogation of the endogenous bioelectric signaling that normally harness individual cell behaviors toward the creation and upkeep of complex multicellular structures in vivo. We first present bioelectricity as the software of life, then in a second part we identify and discuss the links between bioelectricity and rejuvenation strategies and age-related diseases, and develop a bridge between aging and regeneration via bioelectric signaling that suggests a research program for addressing aging. In a third part, we discuss the broader implications of the homologies between development, aging, cancer and regeneration. In a fourth part, we present the morphoceuticals for aging and we conclude.

Running title— Aging as morphostasis defect
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1 Introduction

1.1 The challenges of next-generation aging biomedicine

Aging is a natural process that involves morphological and functional changes in cellular and extracellular components leading to a progressive decline in most biological functions [63]. The

process of aging is influenced by a variety of factors, including genetics, lifestyle, and aspects of the environment [216]. The decline of cellular function due to aging causes a gradual loss of physiological functions and increased susceptibility to a host of diseases, including cardiovascular diseases, cancer, neurodegenerative disorders, type II diabetes, and many infectious diseases, which negatively affect the quality of human life [104, 216].

Several theories have been proposed to explain the process of aging, including damage-based and programmatic theories [63, 64, 131], which of the former is more widely studied [63, 97, 104, 164]. According to this theory, inefficient repair mechanisms cause the accumulation of molecular damage, which affects crucial cellular components like the genome, telomeres, mitochondria, and proteins [97, 128, 164]. Such damage is thought to drive the aging process. In contrast, programmatic theories argue that aging is predetermined by mechanisms encoded in the genome, rather than by stochastic damage accumulation [65, 95, 266]. Recently, two groups proposed that aging is dependent instead on changes in information processing and analogized it as being dependent on the software of life [64, 316], shifting the focus from aging as cellular and molecular damage to aging as a progressive failure of biological information processing. Both of these groups located this software in the epigenome as implemented by chromatin modifications [64, 316].

In recent years, epigenetic clocks have emerged as a powerful tool to predict chronological age [93, 108] and mortality risk in humans [42, 109], as well as the age of individuals from different mammalian species [155]. These clocks rely on a relatively small number of methylation sites that become either hypermethylated or hypomethylated with age. Interestingly, epigenetic clocks tick throughout the lifespan, beginning at conception and continuing in normal body cells in vitro. However, they do not tick in embryonic or pluripotent cells [108, 123], and reprogramming with Yamanaka factors can reset them to zero [263].

Overall, while significant progress has been made in manipulating aging in model organisms through genetic, dietary, and pharmacological interventions [129, 187, 216] the underlying cause of aging in human beings remains unclear and is a subject of intense debate. Existing rejuvenation approaches still have limited effects in extending lifespan [167]. Most approaches have been bottom-up, with a focus on the cellular hardware: the genes, molecular pathways and more recently the epigenome. But micromanaging the maintenance process at the lowest level during aging may not be feasible for such complex structures as a human anatomy [141, 225, 229]. The maintenance and repair of such highly intricate and complex structures likely involves a dynamic, multi-component orchestration of events and it would be impractical to try to oversee every detail of the process. As one example, although a teratoma tumor may contain various differentiated cell types such as hair, teeth, and muscle, it does not have proper three-dimensional organization. Thus, having well-differentiated cells alone is not enough to create a functional and complex structure. To make significant advances in rejuvenation and aging bioengineering, we must understand and learn to activate resilient endogenous repair mechanisms [175]. Fortunately, the field of developmental and regenerative biology offers numerous examples of control circuits that allow for effective self-repair and the dynamic management of multicellular shapes and large-scale anatomy [146]. By understanding the communication and computational modalities that harness individual cell behaviors toward the creation and repair of complex tissues and organs (the physiological software of life), it may be possible to stop or reverse their progressive disrepair [146]. Here, we focus on one important modality for coordinating cells into multicellular collectives that navigate anatomical, transcriptional, and physiological spaces: endogenous developmental bioelectricity.

1.2 A new approach: top-down control of anatomical homeostasis via bioelectricity

Bioelectricity has long been suspected to be be an important informational layer for anatomical control in development, cancer and regeneration [54,151,152,197]. However, in recent years, molecular tools have come on line that have allowed the merging of bioelectrical mechanisms with genetics and mainstream regenerative and developmental biology (reviewed in [13, 103, 146]). Bioelectric signalling is a form of epigenetics (in the original sense of the term, as anything that is not encoded in the primary genetic sequences), which works in conjunction with other established regulatory pathways in the body relying on biophysical and biochemical interactions. Not merely an additional step in the already complex molecular processes of the body, bioelectricity provides unique and powerful signaling control dynamics, much like the electrical circuitry underlying dynamic and flexible functions of brains. Evolution discovered, as far back as bacterial biofilms [172, 232], the advantageous properties of bioelectrical networks for coordinating information across space and time, facilitating collective decision-making, and supporting reprogrammability and robustness [151]. These properties are universally acknowledged and studied in the nervous system, but they are far more ancient than brains, and enable similar dynamics (using conserved molecular mechanisms [83]) in other parts of the body as they navigate other problem spaces [85]. Importantly, just as in the nervous system (where bioelectricity enables high-level cognitive goal states to be implemented via molecular events in muscle and gland cells), somatic bioelectricity serves as a tractable interface by which high-level information (e.g. organ-level order) passes to and from molecular pathways that underlie cell behaviors.

Bioelectric controls, such as voltage-sensitive channels, implement feedback loops that can break spatial symmetry and negative feedback for robustness. In addition, gap junctions (electrochemical synapses) enable bioelectric signals to be sent between cells in response to voltage and other ionic parameters [214], allowing for tissue-level feedback loops that respond to changes in large-scale patterns [227]. These characteristics facilitate the precise orientation, scaling, and shaping of organs during embryogenesis, regeneration, and remodeling, through dynamic long-range coordination and anatomical decision-making [146]. Specifically, it has been argued [196,225] that these methods work because evolution exploits bioelectrical interfaces endogenously for modular control of anatomical homeostasis and repair processes [150]. Using molecular-genetic and pharmacological techniques, researchers have been able to manipulate the distribution of voltage gradients to effect alternative anatomical structure/location during development, increase regeneration after injury, and cancer suppression. They have been able to induce the growth of entire organs from different types of tissue, change the direction of primary body axes, initiate regeneration of tails and limbs in non-regenerative conditions, alter the shape of regenerating heads in planaria to resemble those of different species, and convert oncogene-induced tumors into normal tissue [144, 151, 177, 180, 283]. A variety of tools from behavioral and cognitive neuroscience have been harnessed for the control of tissue-level and organ-level information, which enables pushing much of the complexity onto the system itself via simple triggers [224, 228]. This top-down methodology for controlling anatomy opens promising avenues for therapeutic approaches to rejuvenation and the diseases of aging if we take the view that aging is a defect of morphostasis - a gradual drift from effective maintenance of anatomical homeostasis.

Here, we propose a developmental bioelectricity perspective on aging viewed as a defect of morphostasis that can be approached like other aspects of regulative development, regeneration, and cancer suppression [247, 248]. We first present bioelectricity as the software of life, then in a second part we identify and discuss the links between bioelectricity and rejuvenation strategies and

age-related diseases, and develop a bridge between aging and regeneration via bioelectric signaling that suggests a research program for addressing aging. In a third part, we discuss the broader implications of the homologies between development, aging, cancer and regeneration. In a fourth part, we present the morphoceuticals for aging and we conclude.

2 Bioelectricity as the software of life

2.1 Developmental bioelectricity: definitions

Developmental bioelectricity refers to signaling among non-excitable cells mediated by endogenous electric fields and differences in resting potential [92,152]. These electric states arise due to specific ion channels and pump proteins, which uphold voltage gradients across the cell membrane. Such gradients elicit various cellular responses, including transcriptional and epigenetic responses [143, 144, 152] (see Figure 1B). Ion channels are selectively permeable; they differentiate between the electrical charge and size of ions, allowing movement of specific ions, such as K^+, Na^+, $Ca2^+$, or Cl^-, in one direction through their pore. The flow of ions across membranes and the resulting changes in the bioelectrical pattern play a crucial role in many cellular processes, including muscle contraction, nerve signaling, secretion, tissue growth, cell proliferation and apoptosis [146]. Ion channels play a critical role in the control systems of embryonic development and have been linked to several channelopathies of of embryonic embryogenesis [14, 134, 174, 268, 273, 281], which are disorders caused by mutations in ion channel genes. These conditions have been well-studied in various animal models such as fruit flies, zebrafish, mice, frogs, and human patients, implicating several ion channels, such as the potassium channel Kir2.1, and gap junctions (connexins). This research has helped to better understand the underlying mechanisms of these disorders and the role of ion channels in normal embryonic development. In addition, dysregulation of ionic gradients can contribute to age-related declines in physiological function and dysfunction of ion channels is often linked to organ failure during aging [126]. Because of their central role, bioelectric signals are a popular target for induction of organ formation and regeneration (reviewed in [57, 180]).

Together, the voltage states induced by the action of channels, pumps, and gap junctions create a network of bioelectrical activity throughout the tissue, which ultimately triggers changes in gene expression [212] and cell behavior (migration, differentiation, shape change, proliferation, apoptosis, etc.) that lead to specific morphogenetic outcomes. This bioelectric code translates patterns of voltage into information about organ size, shape, and positioning through mechanisms such as neurotransmitter gating, calcium signaling, and voltage-sensitive phosphatases. Analogous to the way electrical neural networks of the brain control muscle movement for behavioral goals, the ancient bioelectric networks of the body control cell activities to achieve morphogenetic outcomes, by storing and implementing gradually changing anatomical patterns [144, 151, 177, 180, 283].

2.1.1 Bioelectric control of single-cell function and differentiation

The state of Vmem in a cell and its neighbours is crucial in regulating cell behavior [283], along with other modes of signaling (see Figure 1A). Usually, quiescent and differentiated cells exhibit strong polarization, while embryonic, stem, and tumor cells tend to be depolarized [20]. However, many cells, much like neurons, have several Vmem values, with a unique set of voltage domains over their surface [142]. Although the functional significance of voltage microdomains is yet to be determined, Vmem regulation is being employed in bioengineering to control cell connectivity [206],

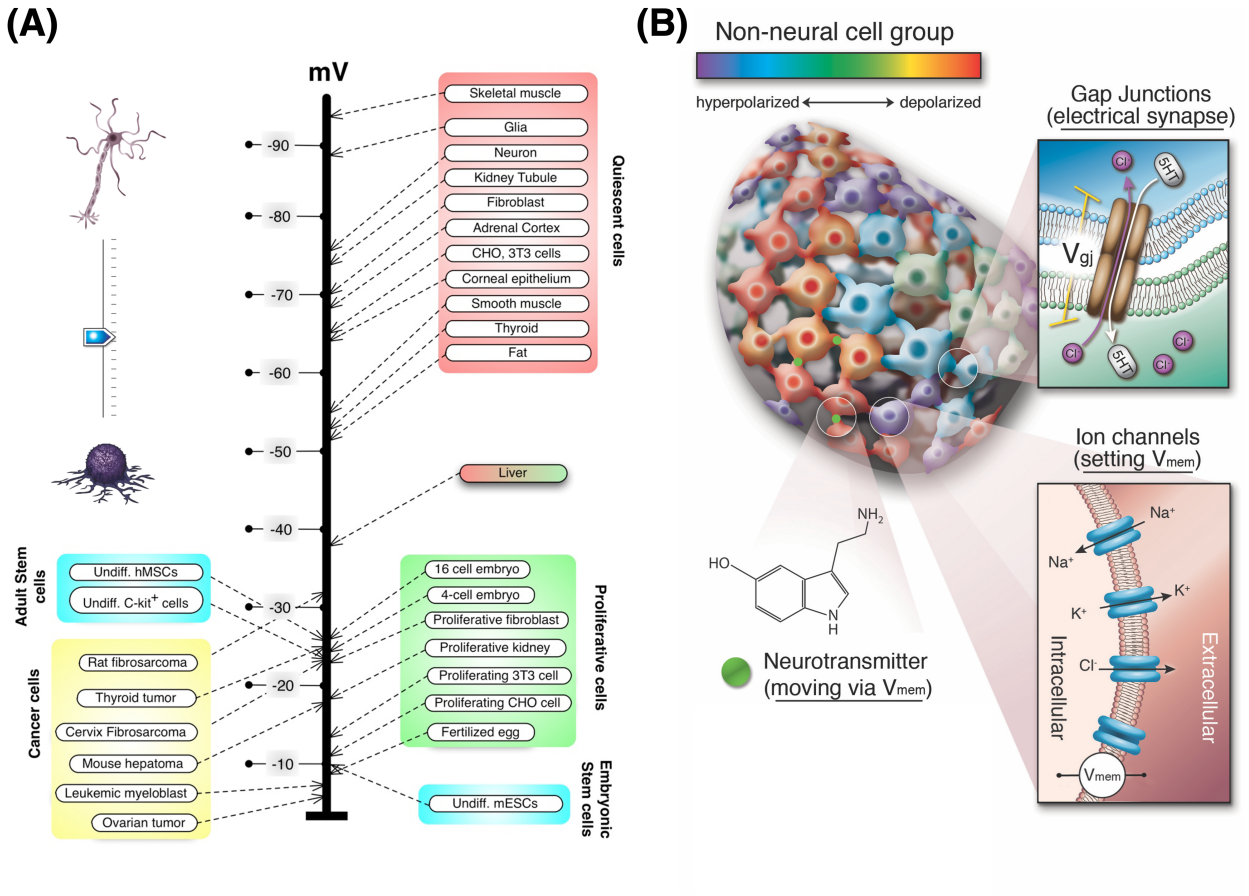

Figure 1: Bioelectricity in cells and non-neural cell groups. **(A)** Highly adaptable cells such as embryonic, stem, and cancer cells tend to have a relatively depolarized state, whereas mature and fully differentiated cells are hyperpolarized (data from [20]). **(B)** The process of developmental bioelectricity involves ion channel proteins on cell surfaces, which contol the movement of ions and establish a resting potential (Vmem) across the membrane. This cell Vmem can also be communicated to other cells through electric synapses, or gap junctions. The resulting interactions between cell voltages lead to stable distributions of Vmem across tissues, as seen in the frog embryo face, where they determine the placement and number of craniofacial organs like eyes and mouth. These gradients can be manipulated using interventions that target ion channels, gap junctions, and small molecular signals like neurotransmitters. Panel A and B reproduced with permission respectively from [154] and [229].

differentiation [283, 286], and wound healing [284].

Changes in Vmem are involved in controlling differentiation and proliferation in various cell types, including human mesenchymal stem cells [284, 323], cardiomyocytes [136], iPSCs [117], vascular muscle [116], embryonic stem cells [194], myoblasts [106], as well as in neurotransmitter specification [246] in the developing nervous system and heart. This implication of Vmem in migration, differentiation and proliferation is well-established. Crucially, the key role of bioelectricity extends far beyond the single cell state and underlies its control of large-scale order in regeneration, developmental defects, and disorders such as cancer [147, 151].

2.1.2 Bioelectric control of large-scale anatomy

The dynamics of bioelectric signaling within the body play a crucial role in regulating diverse processes including wound healing, the formation of neural circuits, eye development, facial patterning, brain and tail size, and the determination of left-right and anterior-posterior body axis (reviewed in [152]).

Manipulation of bioelectrical patterns has emerged as a promising approach for controlling morphogenesis and advancing regenerative medicine [146, 152, 229], especially because it offers the potential of using a high-level interface where simple signals can serve as triggers of complex, downstream self-limiting cascades that do not have to be micromanaged by the bioengineer. These interventions can be implemented at various levels, such as using electrotaxis to control cell movement in wound healing [80, 327] or by manipulating ion channels using different types of drugs known as morphoceuticals [229] to establish specific bioelectric prepatterns for organogenesis or appandage induction. Studies have demonstrated that malformations caused by mutations and teratogens can be reversed by enforcing the correct voltage maps using drugs, optogenetics, or channel misexpression [47, 209–211]. Additionally, profound defects in brain structure and function caused by teratogens can be reversed by restoring normal endogenous voltage pre-patterns in Xenopus [213]. Furthermore, research has shown that developmental defects in the brain caused by mutations in key neurogenesis genes, such as Notch, can be rescued by the overexpression or activation of native HCN2 (hyperpolarization-activated cyclic nucleotide-gated) channels [210]. As voltage-regulated ion channels, HCN2 channels are sensitive to their context, increasing hyperpolarization in slightly polarized cells but having no effect on depolarized cells, thus functioning as "contrast enhancers" that sharpen weakened differences in Vmem across compartment boundaries, similar to how a sharpen filter emphasizes order in a fuzzy image. Importantly, application of HCN2 opener drugs was systemic, without the need to precisely control the spatial properties of delivery.

Bioelectric signaling has also been found to be a viable method for inducing the regrowth of entire body parts [17, 139, 157, 200, 265]. Studies on rats have demonstrated that limb regeneration can be enhanced through the use of bioelectric signals. Additionally, in a frog model of tail regeneration, complete regrowth of muscle, spinal cord, blood vessels, and skin has been observed subsequent to one-hour treatment with an ionophore, which induces a pro-regenerative state in the wound and does not need to be maintained during the subsequent regeneration process [296].

These bioelectrical networks in the tissues store the target morphology and coordinate cellular activity across distances [83, 224]. Because a cell's bioelectric state is influenced by prior experiences, extracellular signaling, and signals from neighboring cells, these post-translational events are invisible to traditional omics approaches. While complicating modeling and experimentation, this historicity property offers a unique target for biomedicine: reprogrammability.

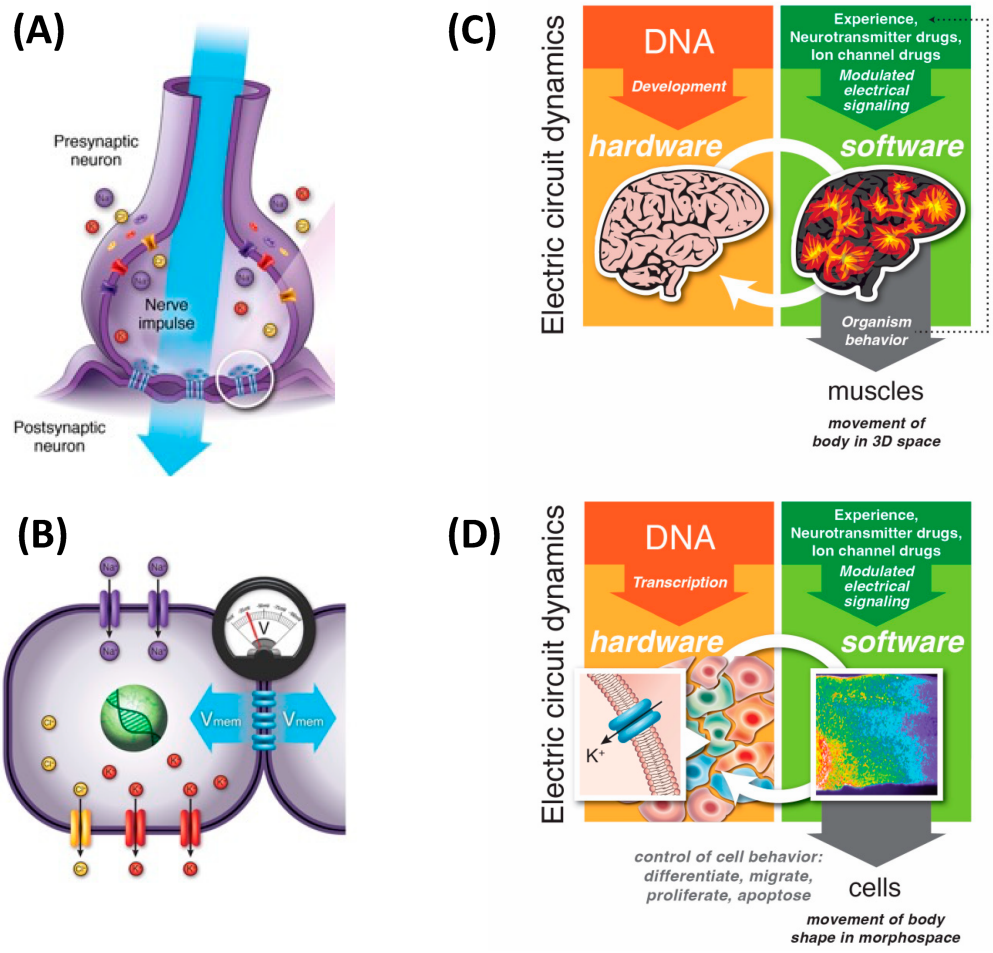

Figure 2: Bioelectricity as the software of life, comparison with brain information processing. **(A)** Neural cells compute by forming networks in which each cell can use ion channels to establish a specific resting potential and selectively communicate it to connected neighbors through electrical synapses known as gap junctions. **(B)** Neural dynamics represent an optimized version of an older system that is present in all cells, where ion channels are utilized, and most cells form electrical connections with neighboring cells. **(C)** DNA-specified ion channel hardware present in neurons in the brain facilitates bioelectric computation, which represents the software that can be influenced by stimuli or experiences. The synapses illustrated in (A) allows for quick communication over long distances, which helps the brain regulate muscle cell physiological dynamics and achieve three-dimensional movement of the body. **(D)** Before the emergence of specialized, high-speed neurons, preneural bioelectric networks utilized the same physiological software architecture and ion channel hardware. Bioelectrical networks' memory and information-processing features were used to regulate cell behaviors and control the body configuration's movement through morphospace [83,146]. The figure is reproduced with permission from [85]. Images by Jeremy Guay of Peregrine Creative.

2.1.3 Reprogramming anatomy: the software analogy

Although the mechanism of biological information processing in living systems differs from that in computer architectures, it leverages three essential aspects of the "software" concept utilized in computer science. These are reprogrammability, then the storage of malleable data that determines the subsequent behavior of the hardware, and modularity, which allows for the initiation of a complex activity by simple triggers in various spatiotemporal contexts because much of the functionality is in the interpretation of the pattern by the target machinery. Software modularity is particularly significant because the trigger that starts a module does not have to be a specific gene that is mechanistically linked to the mechanisms it triggers. In bioelectricity, this is achieved through patterns of Vmem. For example, a particular Vmem pattern triggers eye development anywhere in the body, independent of which ion-channel protein or ion is employed to implement that state [208]. In planaria, the bioelectric prepattern that determines head number and shape (reviewed in [72]) can be readily re-written by brief drug exposures [71], after which the cells build new heads of the right shape, size, and composition. This offers the possibility that through this interface, we would be able to re-specify the setpoints for anatomical homeostasis (see Figure 3), and/or improve cells' ability to implement those setpoints: use low information-content triggers to help the body maintain its correct, healthy target morphology at the tissue and organ levels throughout the lifespan. Two simple hypotheses suggest themselves. Could aging be a decline in the precision of the bioelectric prepattern over time ? And/or, could it be due to a reduction in the cells' ability to sense and implement it? Both possibilities seem tractable given the state of the field today

3 Aging as a morphostasis defect: a developmental bioelectricity perspective

3.1 Rejuvenation strategies and bioelectricity

Strategies to delay and potentially reverse the aging process have recently been explored including blood factors, metabolic manipulations, senolytics and cellular reprogramming [167]. Here, we review their links with bioelectricity.

3.1.1 Blood factors

Blood factors seem to play a major role in rejuvenating effects [37, 250, 304]. This has been shown using heterochronic parabiosis, in which the circulatory systems of a young mouse and an aged mouse are fused. This technique can revitalize muscle stem cells and reverse the decline in stem cell activation and number [27, 53]. It reduces genomic instability [264] in the aged mice and reverses age-associated gene expression signatures [304]. It also has positive effects on several other organs, including the liver, heart, and brain [127, 163, 267, 303].

It seems that the reverse is also true: there are pro-ageing effect of aged blood is higher than the pro-rejuvenation effect of young blood on the liver, muscle and brain [241]. Systemic pro-ageing factors have been identified through heterochronic parabiosis, and includes eotaxin (also known as CCL11) and β2-microglobulin. The levels of these two factors increase with age, and it has been shown that they inhibit neurogenesis and cognition in young mice [267, 303]. The excessive Wnt signalling that underlies the differentiation bias of aged muscle stem cell is also reversed by heterochronic parabiosis [26, 27]. Interestingly, Wnt signalling has been linked to bioelectrical

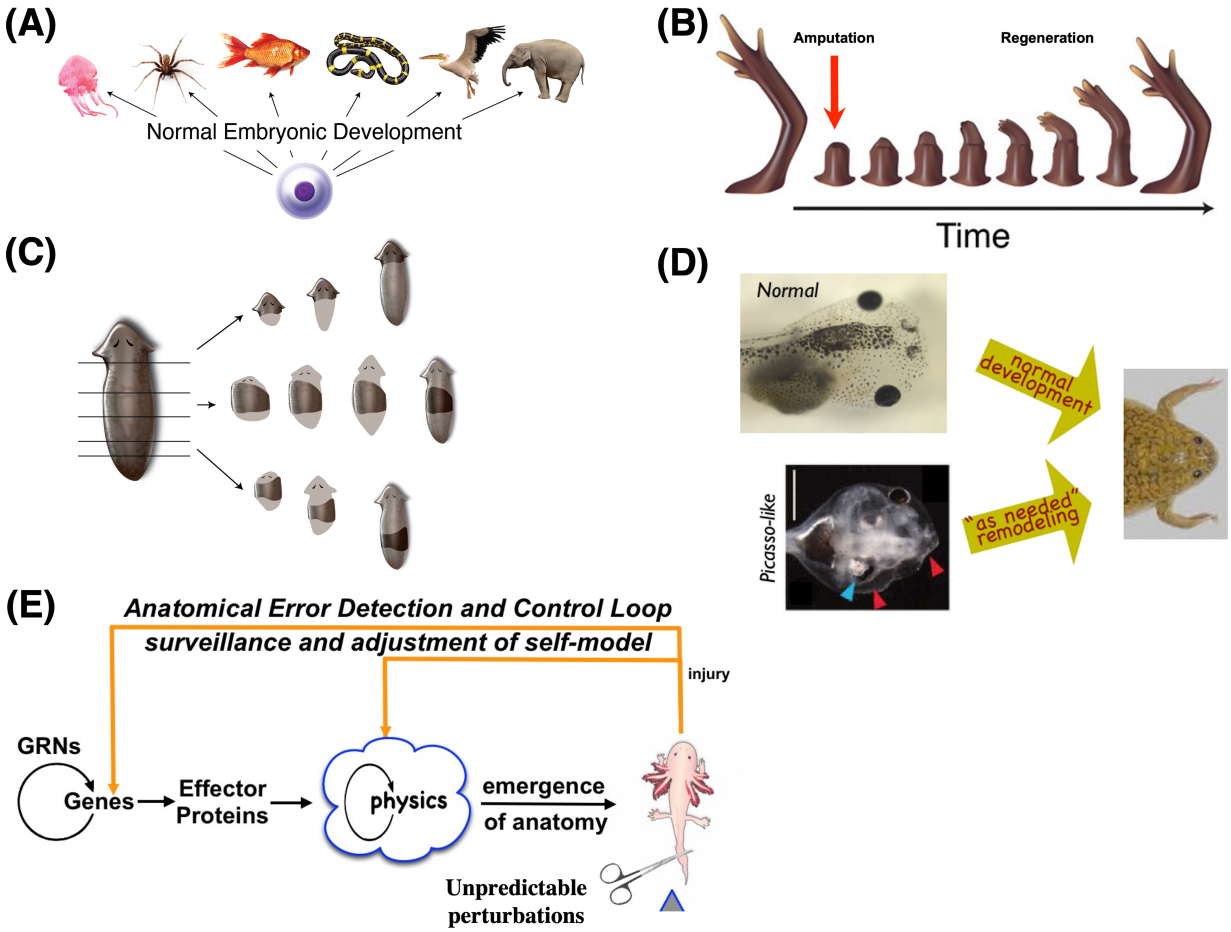

Figure 3: Anatomical homeostasis or the ability to reach the target morphology from different starting points. (A) The normal development of an embryo results in complex structures originating from a single egg cell, essentially reconstructing an entire body from one cell. This process consistently follows a complex path in morphospace to achieve the correct final form. (B) Regeneration of a limb after amputation. (C) Fragments of planaria have the ability to regenerate and reconstruct the complete organism, adjusting their size as necessary to maintain proportional dimensions. (D) During the transition from tadpole to frog, the head undergoes significant remodeling. Even when tadpoles have highly abnormal facial features, the various anatomical components migrate and rearrange until they reach the desired normal head morphology in frogs. (E) Anatomical homeostasis is reached through an error-minimization scheme. Panel A-B is reproduced with permission from [229], panel C-D-E respectively from [148], [300], and [150]. The illustrations in panels A-C were created by Jeremy Guay of Peregrine Creative.

changes and is aberrantly activated in several human diseases including cancer [188, 238]. Wnt signaling is an important pathway mainly active during embryonic development and is implicated in the control of cell proliferation. Ion channels regulate Wnt signalling in several ways. Fei et al. found a downregulation of $Ca_V1.2$ in aging models characterized by an altered osteogenic differentiation caused by the inhibition of the Wnt signaling pathway [79]. More generally, the variation in K+ and Ca2+ fluxes across cell membrane induced by changes in transmembrane potential can modulate canonical Wnt signalling [30, 239, 255]. Other channels (i.e., Kv 7.1, CFTR for example) may also be implicated in the formation of multiprotein membrane complexes that modulate the correct cytoplasmic-nuclear localization of β-catenin by physical interaction [30, 239]. The The Wnt/β-catenin pathway is also the pathway most associated with channels regulation development and progression of cancer progression [238]. Cancer has also been presented as a disease due to aberrant cell-cell information processing [143, 147, 228].

Extracellular vesicles from young animals have also been shown to rejuvenate aged cell bioenergetics and induce skeletal muscle regeneration [251]. In that study, they enriched gene ontology terms associated with the 313 genes that were altered in the presence of extracellular vesicles in order to investigate their regulatory role in young serum on the transcriptomic profile of injured aged skeletal muscle. Interestingly, they found that the top altered processes, are related to the regulation of ion channels, specifically calcium (Ca2+) and potassium (K+) channels. Indeed, the regulation of ion gradients is critical for mitochondrial health and function [61, 94, 190].

Another blood factor potentially involved in the rejuvenating effect necessary for muscle maintenance and regeneration is oxytocin [76]. Oxytocin levels in the blood decrease with age and it has several roles in ion channel regulation. For example, its action as a neuromodulator [274] is mediated via ion channels regulation of depolarization, integration and burst firing in CA2 pyramidal neurons [159]. Oxytocin also regulates acid-sensing ion channels [233].

3.1.2 Metabolic approaches

Long-term dietary restriction including fasting regimens are known to extend healthspan and lifespan across several species [60, 125]. mTOR and insulin–IGF signalling could play a key role in nutrient-sensing pathways with a rejuvenating effect [112, 121, 129]. Rapamycin, an mTOR inhibitor, improves haematopoietic stem cell function in aged mice and extends lifespan with a short-term treatment (6 weeks) [43]. Autophagy, the process of cellular self-degradation, is also known to be regulated by the insulin and mTOR signaling pathways [112, 121, 129]. mTOR could be central in mediating the beneficial effects of dietrary restrictions, raising the possibility that mTOR inhibitors could be used to rejuvenate ageing tissues.

Rapamycin and the mTOR signaling pathway have several impacts on bioelectricity. Rapamycin inhibits voltage-gated K+ channels in dendritic cells [299] and the reciprocal relationship between ion channels and pumps in autophagy has been shown to be mediated via mTOR pathways [2]. Importantly, autophagic progression is regulated by calcium signaling produced by TRPML1 (transient receptor potential cation channel), which induces biogenesis of autophagic-lysosomal organelles, activation of mTORC1 (mechanistic target of rapamycin complex 1) and degradation of autophagic cargo. Two critical components of the autophagic network regulate TRPML1 function: LC3 (microtubule-associated protein light chain 3) and mTORC1. Autophagy is intrinsically linked to the degradative function of the lysosome. Lysosome-associated ion channels implicated in autophagy such as TRPML1, TPCN and TMEM175 have been linked to neurodegenerative and age-related diseases, including Parkinson's disease and Alzheimer's disease [119,259,312]. Rapamycin also has anti-cancer effects and has been shown to inhibit both oncogenic intestinal ion channels

and neoplasia in APCMin/+ mice [133].

Other nutrient-sensing pathways could also be involved in the rejuvenation effects of dietary regimens including metformin and resveratrol [167]. Metformin is known to increase AMPK activity [25, 112] which preserves mitochondrial function and decreases inflammation when administered starting at middle age [171]. Metformin has several effects on bioelectricity. It restores electrophysiology of small conductance calcium-activated potassium channels in the atrium of GK diabetic rats [90]. Chloride intracellular channel 1 regulates the antineoplastic effects of metformin in gallbladder cancer cells [160]. This drug also may increase cardioprotection in diabetic patients. Indeed, in adult rat myocytes, metformin has been shown to normalize aberrant intracellular Ca2+ clearing induced by high glucose [243]. Resveratrol, a red wine antioxydant, can activate sirtuins and other nutrient-responsive pathways. It reduces inflammation and improves cognitive and renal functions in rodents when initiated at mid-to-late life [98, 130, 219]. Resveratrol derivatives modulate voltage-gated potassium channels [201]. It also regulates intracellular calcium signalling via ion channels [179].

3.1.3 Senolytics

Cellular senescence is a process characterized by the cessation of cell division. It is induced by stress and prevents the development of damaged cells [34, 105]. Senescence is known to be involved in the prevention of tumour development [34], tissue remodeling during embryogenesis [189, 275], wound healing [67] and aging [9, 199, 329]. Cellular senescence has long been thought to be a marker of organismal aging [34], although whether it is a cause or consequence is only now beginning to be resolved. Indeed, elimination of senescent cell using compounds or via specific mouse models have revealed that it can reverse or delay aspects of the ageing process [9, 10, 41, 115, 199, 314, 329].

The cell-cycle inhibitors p16INK4a and p21CIP1 are markers of cellular senescence, as are many secreted inflammatory factors (collectively referred to as the senescence-associated secretory phenotype (SASP)) [34, 105]. The mRNA levels of p16INK4A were elevated by 1.4-fold in TRPM7-deficient BxPC-3 cells suggesting a direct link between the bioelectrical pattern and senescence [322]. TRPM8 ion channel is also aberrantly expressed and required for preventing replicative senescence in pancreatic adenocarcinoma [321].

Several senolytic drugs have been developed: we have inhibitors of the Bcl protein family (for example, navitoclax, also known as ABT263) [41], of kinase (like dasatinib and quercetin) [329], and of heat shock protein 90 [91]. Interestingly, bcl-2 protein family has been seen as ion channels [254]. And navitoclax is an inhibitor of these bcl-2 proteins [328] and therefore affects directly the bioelectric pattern. Quercetin is also known to regulate ion channels function [242]. Quercetin potentiated KCNQ1/KCNE1, KCNQ2/3 and KCNQ4 currents [242] and regulate ligand-gated ion channels [138]. Several studies have also demonstrated the potential role of quercetin action on voltage-gated ion channels [118, 165, 319]. HSP-90 has also a role in intracellular calcium homeostasis [156]. HSP-90 also promotes HERG potassic channel maturation [82].

3.1.4 Cellular reprogramming

Cellular reprogramming allows the conversion of terminally differentiated somatic cells into pluripotent cells (iPSCs) via somatic cell nuclear transfer (SCNT) (the transfer of the nucleus of an adult somatic cell into an enucleated oocytes), or forced expression of Yamanaka factors (Oct4, Sox2, Klf4, and c-Myc) [290]. Ion channels have also been shown to be regulators of cell differentiation.

Bioelectric signaling has been shown to regulate a range of cellular characteristics, such as

differentiation in mesenchymal stem cells [282, 284, 285, 287]. Ion channels and transporters can also influence skeletal muscle myoblast differentiation, cardiac differentiation from pluripotent stem cells, as well as vascular smooth muscle cell differentiation [44]. And TRPV4 ion channel has been shown to be a novel regulator of dermal myofibroblast differentiation [260]. In addition, mechanosensitive ion channels and stem cell differentiation are intrinsically linked [70].

3.2 Aging-related diseases as channelopathies ?

Recent research is starting to uncover the connections between aging, aging-related diseases, and bioelectricity primarily by recognizing the central role of ion channels [236,253,276,301]. Ion channels play a key role in maintaining physiological homeostasis by regulating membrane potential, signal transduction pathways and tissue information processing. Further, it is believed that abnormal changes in ionic gradients may be responsible for the decline of physiological functions as we age. [236, 253]. Studies have shown that ion channels can regulate rates of cellular or organismal aging, and that ion channels are involved in aging-related diseases such as heart disease, brain disease, cancer, and eye disease [236], as well as in aging-related inflammation via mitochondrial functions [276]. Could it be possible that age-related diseases, in large part, chanellopathies, the result of ion channel dysfunction ?

3.2.1 Cardiovascular diseases

Vascular dysfunction, which is characterized by changes in the structure and function of blood vessels, is a leading factor in the development of aging-related diseases such as cardiovascular and cerebrovascular conditions. Ion channels play a crucial role in this process by modulating blood vessel dilation through activation of potassium channels or inactivation of calcium channels. Studies in aging coronary smooth muscle have shown a decrease in voltage-gated calcium-activated potassium channels [170]. Altered regulation of ion channels due to aging-related decreases in testosterone and estrogen may also play a role in cardiovascular disease of aging. Testosterone has an inhibitory effect on vasodilation [257] and hormones such as testosterone and estrogen participate in vasorelaxation by modulating ion channels and activating signaling pathways, such as the phosphoinositide 3-kinase (PI3K)/Akt-dependent pathways, in vascular endothelial cells [107]. Both hormone receptors and calcium voltage-gated channels are found on the plasma membrane, suggesting that similar signaling pathways may be involved [198]. Toro et al. also reported an aging-related decrease in the density of the the voltage- and calcium-gated potassium channel BKCa alpha subunit in coronary smooth muscle, associated with a reduction in the release of nitric oxide (NO) [294]. Similarly, dysfunction of the sinoatrial node (SAN) has been linked to alterations in the activity of ion channels that regulate the directionality of ionic fluxes in the SAN and cardiac muscle [236]. In addition, functional interaction among KCa and TRP channels, important for normal cardiovascular physiology, is altered during aging [18]. As a result, ion channels are becoming a focus of cardiovascular drug development, with ion channel modulators being considered as promising therapeutics for reducing age-related physiological changes [19, 207, 292].

3.2.2 Brain diseases

Dysfunction of certain types of sodium, potassium, and calcium channels has been linked to conditions such as dyskinesia, seizures, epilepsy, and ataxia [262]. A variety of neurological disorders are associated with dysfunction or genetic mutations in ion channels, including Alzheimer's, Parkinson's,

and Huntington's diseases, and multiple sclerosis [135]. In particular, changes in calcium ion channels cause dysregulation of intracellular calcium homeostasis, which plays an important role in age-related neurological disorders [192]. Studies have also demonstrated that e CaV3.1 T-type calcium channel is downregulated in cell and mouse models of Alzheimer's disease [244].

Another contributor to aging-related dysfunction in the brain is the dysregulation of calcium and potassium homeostasis caused primarily by oxidation of thiol groups by the reactive oxygen species produced in cells during aging. Changes in calcium levels have a significant impact on cellular properties such as phosphorylation and excitability, both directly and indirectly through alterations in the threshold for activation of calcium-activated potassium (K(Ca)) channels. Additionally, direct oxidation of voltage-gated potassium channels disrupts potassium homeostasis, resulting in hyperexcitability, inflammation, and neuronal loss. All of these effects contribute to the cognitive decline seen in both normal aging in neurogenerative disease [217].

Changes in calcium homeostasis have been linked to the decline in neuronal activity that occurs with aging [81, 293]. However, the specific mechanisms underlying this relationship are not yet fully understood. Some studies have suggested that a decrease in the number or function of calcium channels may contribute to cognitive decline, while others have proposed that an increase in intracellular calcium may be harmful to neurons [52, 315]. Furthermore, research in the field has shown that changes in the expression and function of specific calcium regulatory proteins, such as the type 2 RyR protein variants, may play a role in antiapoptotic function associated with mild cognitive impairment and Alzheimer's disease [31].

The transient receptor potential (TRP) ion channel family has been found to play a significant role in mediating neurogenic inflammation and pain signaling. Members of this family are co-expressed in a large proportion of nociceptors, with at least 25% being found to have such co-expression [15]. Specific members of the TRP family, such as TRPA1, have been shown to mediate the inflammatory actions of environmental irritants and analgesic agents and to enhance pain and inflammation [178]. Cognitive decline and neuroinflammation often occur together in the aging brain, with inflammatory molecules negatively impacting spatial memory [24, 183].

The brain contains a significant number of non-neuronal cells such as glia, microglia, astrocytes, and oligodendrocytes which play important roles in maintaining brain homeostasis [182] and neuronal ion homeostasis [6], and providing protection as the first line of defense against inflammation. Microglia, in particular, act as the resident immune cells in the brain, responding to and propagating inflammatory signals by producing pro-inflammatory cytokines that can have cognitive consequences. Studies have shown that increased oxidative stress and inflammation are associated with brain aging [89]. Furthermore, research has demonstrated that microglial activation is dependent on the expression of the Kv1.5 potassium channel, and that the β-amyloid peptide can induce the expression of these channels in microglia [50]. This suggests that increased oxidative stress in aging could lead to inflammation by upregulating potassium channels in microglia and that changes in ion channels could be a potential cause of neuronal dysfunction associated with aging.

3.2.3 Cancer

A wide variety of external factors (e.g. smoking, sun exposure) and internal factors (e.g. inherited genetic mutations, infections) have been implicated in cancer. Other work emphasizes cancer as a disorder of multicellular collective intelligence (reviewed in [46, 147, 269, 270]). While the incidence of cancer can vary widely among different populations and exposure profiles, the greatest risk factor for developing cancer is aging [88].

It is well-established that tumors have distinct bioelectric signatures and abnormal electrical

connections compared to normal tissue [1, 46, 162, 295], as well as an accumulation of excessive sodium [140]. Additionally, the onset of tumorigenesis is associated with the bioelectric disconnection of cells from the larger somatic morphogenetic network. Metastatic melanoma can be induced experimentally by dysregulating bioelectric signaling between cells, in the absence of carcinogens or oncogenic mutations. In addition, some experts have described cancer as a specific type of channelopathy, the onco-channelopathies [28, 69, 111, 202, 218, 231, 317, 318]. However, it is important to note that while abnormal bioelectric states can certainly be caused by mutant ion channels, the dynamic nature of channel signaling allows for a wide range of physiological events to induce these states without genetic mutations [181].

Hyperpolarization, has been shown to produce cancer normalization even after tumors have formed, using neuroscience techniques such as optogenetics [47], through a mechanism dependent on gap junctions, suggesting potent therapeutic potential for hyperpolarizing agents [48, 49]. Thus, hyperpolarization via pharmacological, genetic or light-based stimulation methods may be able to prevent or reverse tumors, and researchers are currently exploring the role of ion channels in tumor resistance [7]. Gap junctions and connexins, which play a crucial role in spreading morphogenetic signals throughout the body, are being considered as potential therapeutic targets for cancer [66,124]. Recent research by Mathews et al. has demonstrated ion channel drug-induced suppression of the cancer phenotype in glioblastoma cell lines [176]. Several different authors proposed ion channels as a target to suppress cancer [35, 68, 237].

3.2.4 Inflammaging

As people age,the balance of pro- and anti-inflammatory cytokines in the blood is disrupted, leading to three- or four-fold increase in pro-inflammatory cytokines in older adults. This is referred to as "inflamm-aging". Research has suggested that various age-related conditions such as muscle and bone loss, anemia, immune dysfunction, and cognitive decline are linked to this disruption in cytokine balance [186, 240].

Inflammation can affect the expression and function of ion channels through various mechanisms. Cytokines, prostaglandins, leukotrienes, and reactive oxygen species (ROS) produced during inflammation can modulate ion channels through signaling pathways such as cyclic nucleotide, phosphoinositide, and mitogen-activated protein kinase (MAPK) signaling. Additionally, ion channels may be directly modified by molecules elevated in inflammation such as nitric oxide (NO), adenosine triphosphate (ATP), or protons. Inflammation can also disrupt the cytoskeleton in neuronal and epithelial cells, which can further alter ion channel function [75].

It has been shown that the inflammasome [101], a multiprotein intracellular complex that forms in response to stress, is a key regulator of the cellular inflammatory response. It is activated in response to stressors, such as pathogenic microorganisms and non-infectious triggers, and leads to the release of the pro-inflammatory cytokines IL-1β and IL-18 [101]. Changes in potassium and chlorine levels and an influx of calcium affect the activation of the NLRP3 inflammasome [102, 120]. One known activator of NLRP3, ATP, reduces potassium levels and alters other ionic contents within cells [120], which is necessary for inflammasome activation in monocytes and macrophages [222]. Inflammasome activation can also impact of potassium, chlorine, and calcium homeostasis within cells, leading to the release of cytokines in cells of the innate immune system [102, 221]. NRLP3 activation has been linked to chronic inflammation, Alzheimer's disease, and metabolic disorders such as Type 2 diabetes [120].

Meta- analyses indicate that calcium-channel blockers have been shown to have a potential protective effect against the development of Parkinson's disease, with some studies suggesting a

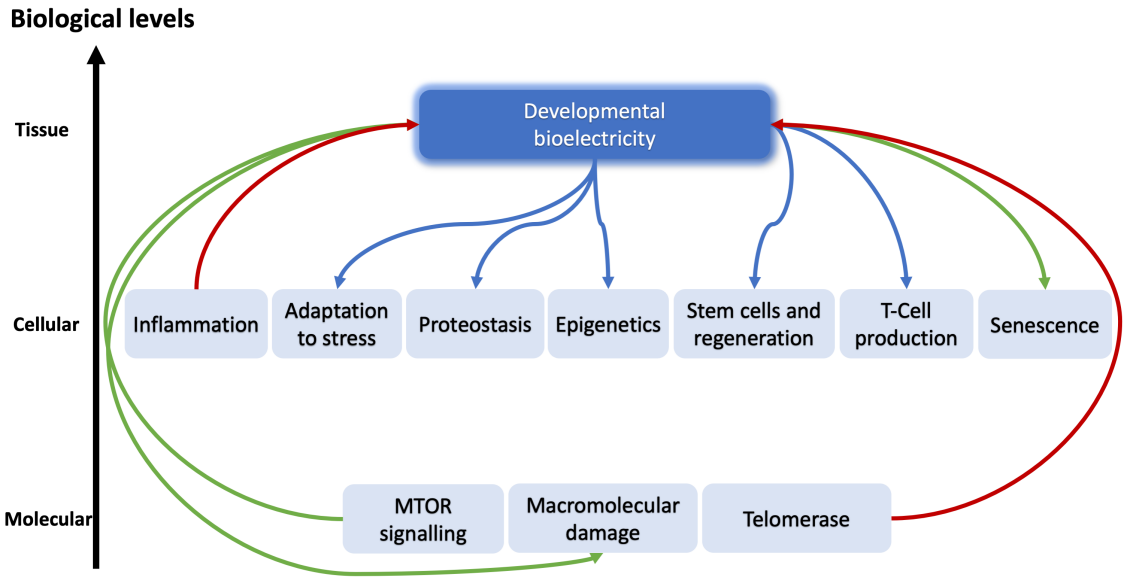

Figure 4: Bioelectricity and the hallmarks of aging. This graph represents links between bioelectricity and cell- or molecular-level hallmarks of aging. Blue arrows show top-down unidirectional links, green arrows indicate bidirectional links and red arrows indicate bottom-up directional links from hallmarks of aging towards bioelectricity.

reduction in risk of up to 30% [137]. Additionally, blockers of the specific calcium channel, CaV1.3, are being studied as a potential treatment for Parkinson's due to their ability to reduce mitochondrial impairment and neuroinflammation [288]. These blockers are currently in phase III clinical trials.

3.3 Bioelectricity and the hallmarks of aging

In this section, we review the links between bioelectricity and the remaining hallmarks of aging [164].

In the previous section bioelectricity, we described links between bioelectricity and 7 of them: disabled macroautophagy, deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, altered intercellular communication, and inflammaging. In this section, we refine the links with bioelectricity by analysing the 5 remaining: stem cell exhaustion, loss of protostasis, telomere attrition, genomic instability and dysbiosis.

Stem cells and large-scale regeneration are controlled by developmental bioelectricity (reviewed in [103, 146, 180, 311]). One mechanism linking bioelectric state to tissue outcomes is via epigenetic chromatin modifications [296]. In addition to its role in tail regeneration, voltage control of epigenetic state also plays a role in left-right asymmetry of development via the rightward electrophoretic transport of serotonin, which acts as a cofactor for histone deacetylases (HDACs) [36]. Furthermore, potassium chloride-induced membrane depolarization can trigger the demethylation of the BDNF promoter and subsequent dissociation of the MeCP2 transcription repressor complex, which facilitates BDNF expression [45, 173]. Interestingly, HDACs have also been shown to be involved in regulation of gene expression and its impact on various biological processes, including aging. For instance, sirtuin is an HDAC that has been linked to aging as it promotes longevity in yeast by deacetylating histone H4K16Ac [62].

Lysosomal function plays a crucial role in regulating proteostasis, which is in turn modulated by ion channels [301]. Various stress-regulated ion channels have been identified [132, 235], and damage to macromolecules may affect ion channels, leading to the disruption of the bioelectrical pattern necessary for maintaining the organism's structure. Ion channels and transporters are involved in regulating membrane excitability, Ca2+ homeostasis, mitochondrial and endolysosomal function, and the transduction of sensory signals, which in turn affect aging and longevity [301].

Telomerase has also been associated with ion channel defects in cardio-vascular diseases and genomic imbalances in key ion channel genes [12] and telomere shortening have been observed in sudden cardiac death victims [11].

We didn't find direct links between bioelectricity and genomic instability and dysbiosis.

Overall, a picture emerges where bioelectricity can affect a large number of the hallmarks of aging. But the opposite is also true, stress, cellular senescence, mTOR signaling and different macromolecular damage can affect the bioelectrical pattern. Developmental bioelectricity seems to stand between damage-based and programmatic theories [63, 64, 131]. As the bioelectrical pattern can control development and regeneration, it may control morphostasis during the aging process. A disruption of cellular information processing capabilities may lead to a drift in morphospace, either by reducing the capacity of cells to follow the bioelectrical pattern (or by being not able to maintain the appropriate Vmem) or by corrupting the bioelectrical pattern itself in charge of the morphostasis, both resulting in aging or in other words a drifting in morphospace.

Therefore, in addition to investigating the mechanistic links between ion channel dysfunction and aging, it is crucial to explore the changes in bioelectric prepatterns during aging. Furthermore, it is important to study the aging process in species such as planaria, which are apparently immortal, to gain insights into their remarkable regenerative capacity, cancer resistance, and lack of aging. The strong tissue-level morphogenetic control mechanisms in these species may be linked to the control of bioelectric patterns.

4 Broader implications: homologies between development, aging, cancer and regeneration

4.1 Same facets of the same problem: from anatomical homeostasis to morphological homeorhesis

Development and regeneration are both error-minimization scheme working towards reaching a target morphology. The concept of anatomical homeostasis during regenerative repair can be extended to the broader concept of morphological homeorhesis, in which developmental progression is a collection of regenerative repairs [225]. This concept suggests that each stage of development is essentially a "birth defect" from the viewpoint of the subsequent stage, and is repaired by a process of regulative development that minimizes system-level stress and moves toward a desired target morphology dictated by the bioelectric pattern [150]. Thus, development can be viewed as a collection of regenerative repairs towards an evolving target morphology [225]. This perspective generates the hypothesis that the bioelectric target pattern changes more quickly than the transcriptional and anatomical patterns, which are pulled along by stress-minimizing loops that operating on metrics larger than individual cell stress states [320]. As development progresses, maturation and adulthood occur when the bioelectric and biochemical prepatterns stop changing, and the anatomy catches up. From this point on, in the absence of injury or pathology, any subsequent changes are small-scale

maintenance to preserve the already-achieved target anatomy, or in other words, morphostasis. The electric face [5, 300] is an example of such an instructive prepattern.

In this view, aging and cancer are both morphostasis defects, failures to maintain the target anatomy and function during adulthood and following morphological homeorhesis, they are both regenerative/developmental defects. Morphostasis is a key aspect of development, regeneration, and cancer suppression. Tissues regenerate, cells are replaced, molecules turn over rapidly, but the body/anatomy stays the same during the healthspan. Morphostasis may be seen as a smaller-scale anatomy changes compared to regeneration (of limbs for example) and development and as such it corresponds to a smaller deviations from the trajectory in morphospace (see Figure 8).

This viewpoint provides a research trajectory to investigate aging. If aging is a morphostasis defect, a developmental/regenerative defect like cancer and lack of regenerative ability, the model systems, concepts, and approaches of regenerative biology should play a central role in the fight against aging.

4.2 Bioelectric signaling, regeneration and cancer resistance

4.2.1 Tumors as a morphostasis defect

The concept that cancer is a morphostasis/developmental defect has been around for a long time [230, 247]. Needham and Waddington proposed the idea that tumors are a result of cells breaking free from the constraints of the morphogenetic field [193,308]. According to this perspective, cancer develops when cells no longer follow the normal patterns of the body, leading to an "inexorable process in which the organism falls behind in its ceaseless effort to maintain order" [247]. This view emphasizes the role of the environment in shaping cell behavior and has gained renewed attention as an alternative to the mainstream gene-centric paradigm, which sees irreversible changes in DNA sequence or gene expression profiles as the driving force behind cancer stem cells. While some focus on the microenvironment [21, 55, 166], others have focused on long-range order on the scale of the entire organism [32, 49].

An escape from the morphogenetic field can also be conceptualized as a shift in the computational boundary within the body, with goal states (specific regions of physiological or anatomical state space which cellular collectives normally seek to occupy) dropping from the scale of an entire organ to that of a single cell. This results in cells reverting to their primitive unicellular behavior and regarding the surrounding tissues as an external environment [59, 147, 247]. According to this perspective, cancer is caused by a disruption in the computational mechanism that usually coordinates cells cooperatively to work towards achieving organ-level morphogenetic objectives [145]. This dysregulation of the "computational glue" that binds individual cells to a common navigation policy in anatomical morphospace [149] can lead to uncontrolled growth and spread of abnormal cells, which is characteristic of cancer. Accumulating evidence suggests that bioelectric signaling is a key component of this cooperative computational mechanism [147].

For many years, it has been known that tumors exhibit abnormal electrical properties, such as depolarized bioelectric signatures and disrupted electrical connectivity [1, 46, 162, 295], along with excessive sodium accumulation compared to normal tissue [114, 140, 234]. Tumorigenesis can start with the disconnection of cells from the larger morphogenetic network of the body [124, 184]. In fact, metastatic melanoma can be triggered simply by disrupting the bioelectric signaling between two cell types [23, 161], without the presence of carcinogens or oncogenic mutations. As noted above, cancer has been described by some researchers as a sub-type of channelopathy called onco-channelopathies [28, 69, 231]. However, genetic changes are not required to induce abnormal

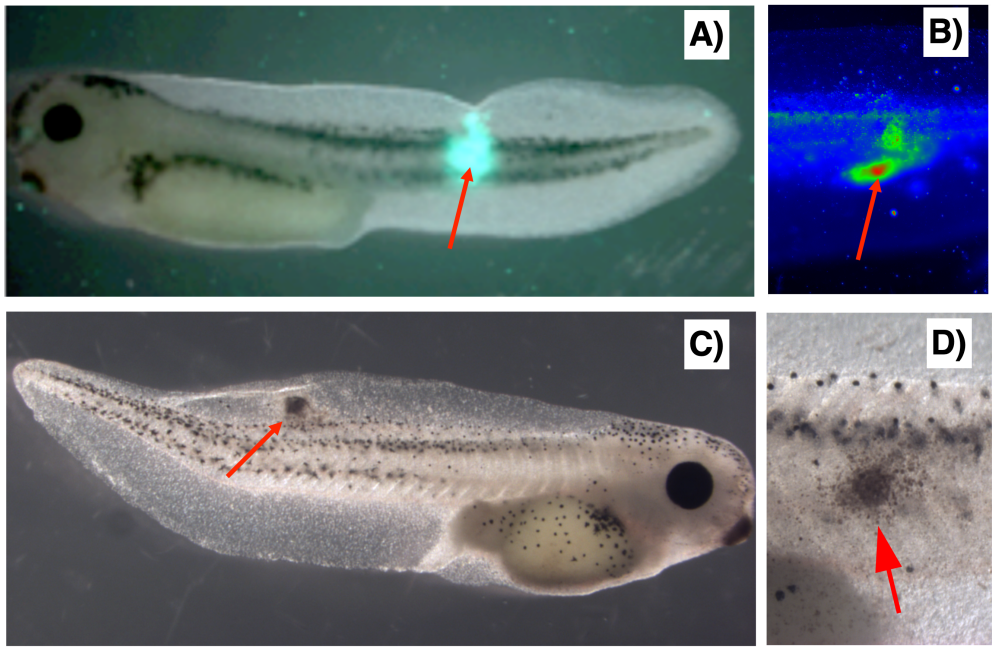

Figure 5: Abnormal electrical signatures of tumors. **(A-B)** Depolarization of cancer cells can be observed in tadpoles using voltage-sensitive fluorescent dyes. **(C-D)** They reveal the locations of tumors. Figure used with permission from [147]

bioelectric states: channel regulation is highly dynamic and can be altered by a range of physiological events that open or close channels post-translationally [181].

In this view, cancer is not a special case of disease by accumulation of genetic damage as in the aging as a software design flaw theory [64] but a bioelectric signaling disease that extract the cells from the morphogenetic field of the body. The comparison of tumors to wounds that fail to heal supports this idea that they are areas of disorganized cell growth lacking a proper goal state for patterning [226, 245] or a default of morphogenetic field. This concept is also supported by molecular profiling studies that have shown similarities between the molecular signatures of tissue repair and carcinoma in the kidneys [245].

4.2.2 Normalization of tumors during regeneration: the morphogenetic field as a suppressor of morphostasis defects

The hypothesis that cancer arises at the level of multicellular organization as a disruption of the morphogenetic field of the body is supported by experimental data. For instance, cells in dispersed monolayer culture are much more susceptible to chemical carcinogenesis compared to organized tissues in vivo [215]. Furthermore, cells with malignant potential can be found in normal primary mammalian cells that are placed in culture. The development of tumors is not commonly seen in salamander species with high regenerative capabilities such as newts and axolotls, as reported by various studies [297] and in addition tumors are normalized during the regenerative process [297, 298].

While exposure to carcinogens may lead to the formation of tumors in such species, it occurs only at high doses and over extended periods of time. Studies have also shown that regenerating tissues, like limbs, are particularly resistant to the development of tumors [297, 298]. In fact, malignant

growths in these areas often regress, become incorporated into the regenerating tissue, or lead to the formation of duplicate body parts, rather than persisting as tumors in the regenerated structure. This is surprising as the process of regeneration shares many similarities with tumor development, including decreased activity of tumor suppressor genes (e.g., p53) [325, 326], increased activity of oncogenes (e.g., c-myc) [169], and increased cell proliferation [277]. Similarly, newt regeneration blastemas exposed to carcinogenic chemicals or ultraviolet radiation produce ectopic, but normal limbs or lenses, rather than tumors [29, 33]. Similarly, in the mammalian liver, one of the most regenerative tissues in mammals [78, 291], regeneration has been observed to counteract cancer. Early nodules induced by carcinogens were transformed into normal liver tissue [305]. Quantitatively, in carcinogen-induced tumors, more than 95% of these tumors were transformed into normal tissue by the liver [78, 291].

This demonstrates that that in actively patterning tissues, regeneration can suppress tumorigenesis, suggesting that the induction of cancer and large-scale patterning disorganization are two points on a single axis. These findings support the notion that cancer is a result of the breakdown of multicellular organization, highlighting the importance of understanding the interactions between cells and their environment. These findings have ramifications at the bioelectrical level that are borne out in experimental findings. Co-injection of a hyperpolarizing ion channel significantly reduces tumor formation, even in the presence of potent human oncogenes such as Xrel3 or KRAS [47, 49]. It is noteworthy that hyperpolarization still has therapeutic potential in cases where tumors have already formed or when induced at a distance through a gap-junction-dependent mechanism [48, 49]. Similarly, a recent study by Mathews et al. demonstrated that ion-channel drugs can inhibit the cancer phenotype in glioblastoma cell lines [176].

Therefore, the morphogenetic field can be manipulated by bioelectricity and it disconnection can lead to cancer or normalization of tumorigenesis. This opens new avenues for aging viewed as a morphostasis defect as cancer.

4.3 Bioelectricity, regeneration and longevity: the morphogenetic field as a regulator of morphostasis ?

4.3.1 Regeneration and longevity are intrinsically linked

Given the connections between large-scale anatomical order in regeneration, and its small-scale counterpart in cancer suppression and aging, it is interesting to consider animal model systems with increased regenerative abilities and/or long lifespans. Several organisms, including salamanders, the naked mole rat and the Brand's bat, show high longevity associated with high regenerative/morphostatic capabilities [38, 249, 258]. These examples contradict the Gompertz-Makeham law of mortality, which posits that death risk increases exponentially with age [87, 99, 168].

The urodele species do not exhibit the typical signs of deterioration associated with aging in mammals and are therefore considered to have negligible senescence [38, 86]. One of the key characteristics of salamander regeneration is its robustness. This regenerative ability does not deteriorate over time and is not affected by frequent regeneration events [324]. A study by Eguchi et al. tracked the process of lens regeneration in Japanese newts over a period of 16 years, during which the lens was removed and regenerated 18 times [74]. The resulting lenses were found to be structurally identical to the original ones and expressed similar levels of lens-specific genes, indicating that the regenerative capacity of the newts remained unchanged. Additionally, the study found that the transcriptomes of young and old (19-times regenerated) lenses were nearly identical, demonstrating the robustness of newt lens regeneration [272]. The specimens used in the study

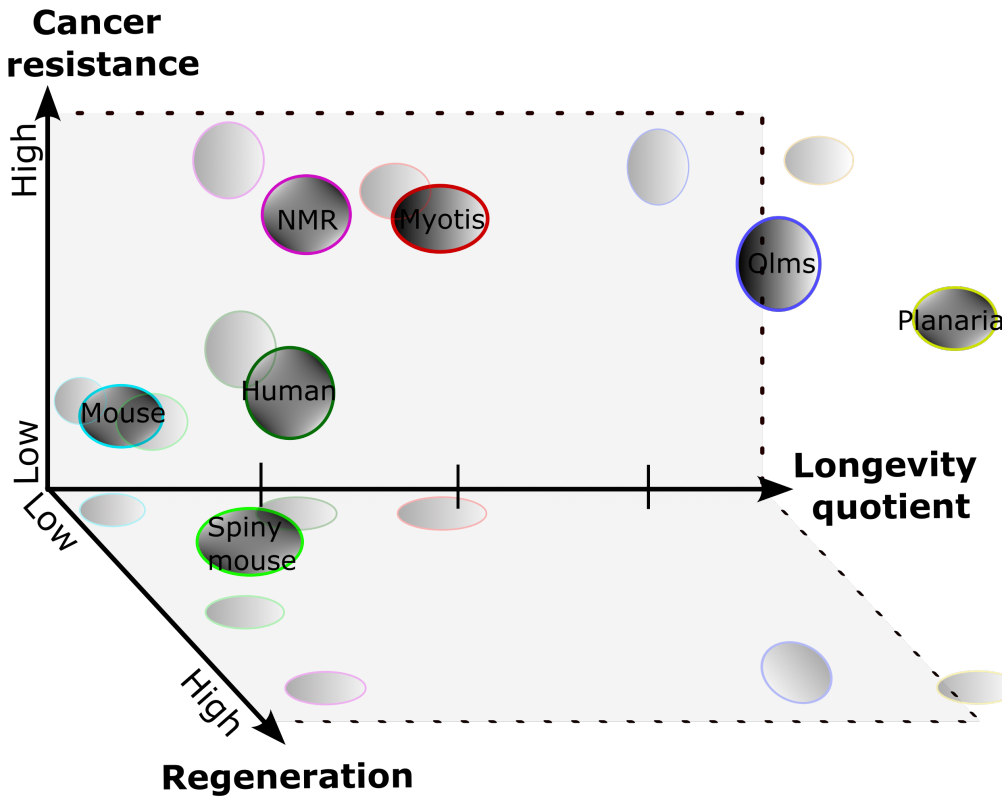

Figure 6: Graph representing several organisms according to their lifespan, cancer resistance and regenerative capacities. The organisms with high regenerative capabilities form a cluster with long lifespan. Planaria is a complete outlier as it is immortal. Longevity quotients have been retrieved from [8, 110, 313].

were at least 30 years old, an advanced age for this species, further highlighting the exceptional regenerative abilities of salamanders.

As a salamander grows older, various changes occur in its tissues; however these do not affect regenerative potential, nor are they recognizable as signs of aging. These changes have been documented in the axolotl, and include an increase in overall size, a gradual replacement of cartilage in the skeleton with bone, a decrease in mobility, and a thickening of the dermal layer [302]. It is important to note that the changes observed in axolotls as they age are probably specific to the species and the natural development of the organism, rather than being solely a result of aging. For instance, the growth in size is a feature that is specific to the axolotl, and therefore the replacement of cartilage with bone, the thickening of the dermal layer, and the decrease in mobility are all likely outcomes of the organism's maturation process rather than aging itself. Additionally, species of salamanders that do not grow much as adults, such as Notophthalmus viridescens, do not exhibit a decline in their ability to regenerate as they age, thus continued growth is not required for maintained regenerative capacity. While an aging-related decrease in fertility has been reported in axolotls, this was based on anecdotal evidence and has not been observed in other salamander species or in other amphibian species [122].

In addition to their impressive regenerative capabilities, salamanders also possess exceptional longevity, living much longer than other animals of similar size [271]. Salamanders break the rule of larger animals living longer, as they have a lifespan that is much longer than other animals of similar size. For example, axolotls, which have an average mass of 60-110g, live over 20 years [309], while P. walt newts, with an average mass of 25g, live up to 20 years in the wild [309]. Japanese newts with an average mass of 8g have a lifespan of 25 years [272], while Spotted salamanders with an average mass of 13g can reach 30 years of age [306]. Cave olms, with an average mass of 17g, is the more extreme outlier, and can live more than 100 years [289]. This exceptional lifespan, despite their small size, makes salamanders outliers (see Figure 6) and interesting subjects for studying aging and longevity. They match and in some cases exceed the longevity quotient found in other well-known outliers such as the naked mole rat [249] and Brandt's bat [258].

However, despite the urodele's unflagging capacity for regeneration and their marked longevity, they do age. Other species, like the planaria and the jellyfish Turritopsis dohrnii, seem to be immortal [158, 252].

4.3.2 The case of immortal species: the role of bioelectrical signaling in the remarkable features of planaria – regeneration, resistance to cancer, immortality

Planarians are the kings of regeneration. This organism can be cut into t least 279 fragments and will regenerate as 279 identical worms [185]. Planarians not only exhibit remarkable regenerative abilities, but they also appear to be able to indefinitely avoid aging [39, 252]. Interestingly, because planaria often reproduce by fission, any mutation that does not kill the stem cell is carried into the next generation and expanded. These animals are therefore for some of them mixoploid chimeras (not every cell has the same chromosome number) with a very messy genome [153]. Despite accumulating a high percentage of changes, including large numbers of mutations, planarians can regenerate with 100% anatomical fidelity. Mutations have been found in protein-coding genes, including amino acid substitutions and non-synonymous SNPs, both within and outside gene-coding regions. Planaria can accumulate up to 74% mutations in protein-coding genes [195] Furthermore, the genome of the planarian Schmidtea mediterranea appears to lack many essential genes, including components of core pathways for cell division, DNA repair, and metabolism [100]. However, under normal

conditions, planarians can regenerate perfect anatomical structures each time, despite their messy genomes. How can these animals maintain accurate morphological control and escape aging despite their messy genomes and the accumulation of somatic mutations over hundreds of millions of years? It has been suggested [261] that this is due to an evolutionary ratchet that progressively emphasizes the capacity to create a normal animal despite hardware defects at the molecular level. Part of that algorithm may be the bioelectrical control system and the error-correcting codes that it implements in cellular collectives [72, 84].

During the regeneration process in planaria, bioelectric gradients can be observed in fragments using fluorescent voltage reporter dyes, as reported by several studies [3, 4, 205]. By manipulating the ion conductances responsible for these gradients through pharmacological or RNAi methods, researchers can induce predictable changes in the anatomical structure of the regenerated planaria, resulting in forms with two heads or no heads that are still viable [16]. This works because the bioelectric pattern is natively used by cells to store the large-scale target morphology information about the number of heads [73, 223] ; moreover, it also stores information about the shape of the heads – manipulation can force a genetically wild-type worm to make head and brain shapes belonging to other species of flatworm 100-150 million years in evolutionary distance [77, 278]. The anatomical setpoint for the regenerative process is stored bioelectrically, and is a true memory because it persists – 2-headed animals that are re-cut in plain water continue to demonstrate the 2-headed phenotype in perpetuity [204].

Studies of the bioelectric prepatterns that keep information necessary for large-scale order in vivo are a primary target for aging research, as are the mechanisms that enable cells to detect deviation from those patterns, and mechanisms that enable them to implement the changes needed to reduce error with respect to them.

5 Towards morphoceuticals for aging

Most molecular medicine currently approaches treatment of diseases by targeting the underlying biological hardware and attempting to push it into the desired biochemical state. This is typically done by making changes at the genetic level or by targeting specific protein activities - in other words, using a bottom-up approach. However, the anatomical homeostatic perspective we emphasize in this article suggests that it may be possible to achieve therapeutic outcomes by working top-down: rewriting the anatomical target or setpoint without altering the underlying morphogenetic machinery [175]. This approach is analogous to the seismic shift that occurred in information technology in the 1950's and 60's, where the focus moved from hardware-based control to controls that exploit the reprogrammability of software and modular, top-down control. We suspect that learning to control complex morphogenesis top-down, without making individual molecular changes, can be similarly transformative for biomedicine [229] with targets ranging from the learning abilities [22, 56, 310] and novel problem-solving capacities of gene-regulatory networks and pathways, to bioelectric large-scale circuits that coordinate configurations in anatomical space [149]. Improving the ability of cellular collectives to work towards the correct target morphology at all scales, rather than trying to control a potentially very large set of molecular states, could represent a much more efficient way to achieve true shifts toward health that are maintained long after the intervention is removed.

Inspired by this perspective, a novel class of therapeutics called morphoceuticals has been developed to leverage the error-correcting capabilities of tissues rather than micromanaging molecular-level pathways [229]. These compounds are designed to target the high-level morphogenetic

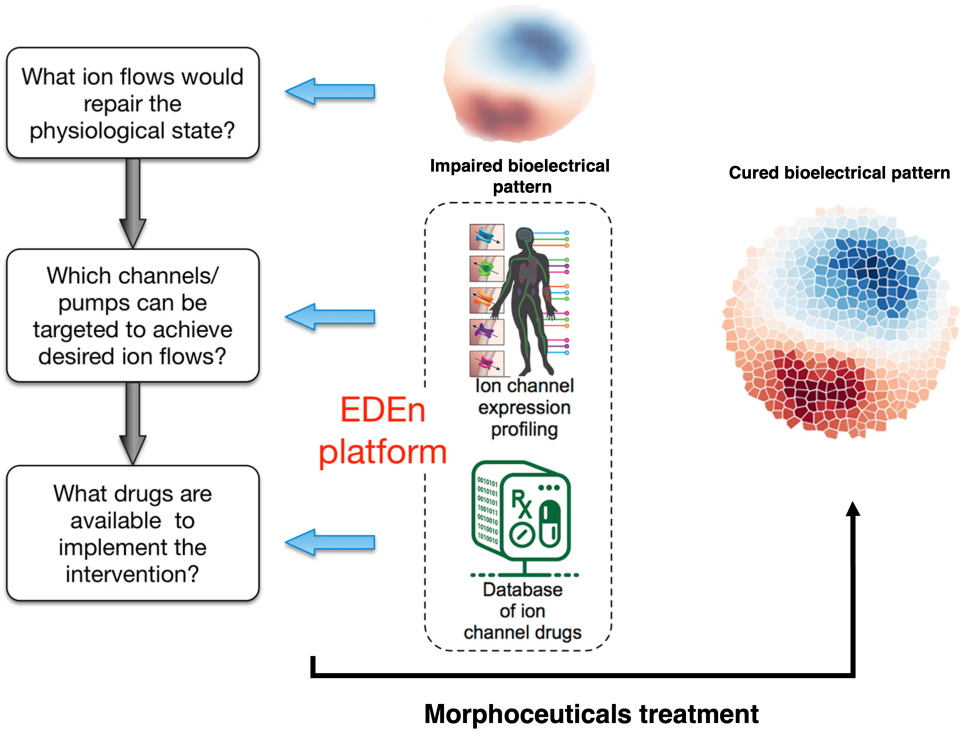

Figure 7: Morphoceuticals discovery for aging. One possible approach to altering the bioelectrical patterns associated with diseases involves utilizing transcriptomic databases to identify potential ion channel targets within a particular tissue. This information can then be utilized as an input into a bioelectrical model to simulate the resulting bioelectrical pattern after intervention, which can be further analyzed to determine candidate drugs capable of moving the current bioelectrical state towards the desired pattern. Figure reproduced and adapted with permission from [51].

control machinery, and can be repurposed from existing drugs or can be newly discovered ones. Morphoceutical's unique feature is that they aim to target computational mechanisms that assess and modify growth and form [224, 225].

The concept of top-down control of biological modules and the allostatic capabilities of living systems [58] suggests that it may not be necessary to micromanage dosage and timing to address the defects that result in aging and cancer. Recent examples of inducing organ and appendage regeneration in vertebrate models have demonstrated that organ type, composition, shape, and size can be induced without precise timing or dosage regimes [71, 191]. This approach leverages the organism's modular, regulative control structure to achieve complex outcomes without knowing the molecular details. Morphoceuticals are designed on top-down control mechanisms [224, 225] and can be discovered through computational [51, 229], pharmacological [279, 280] or genetic screening [96, 220].

Morphoceuticals have significant potential for addressing aging. Pai et al. demonstrated that the brain developmental defects resulting from the mutation of neurogenesis genes, such as Notch, can be rescued by activating or misexpressing HCN2 (hyperpolarization-activated cyclic nucleotide-gated) channels [210]. HCN2 channels are voltage-regulated ion channels that enhance contrast, sharpening the differences in membrane voltage potential (Vmem) across compartment boundaries. They are context-sensitive and increase hyperpolarization in slightly polarized cells, but have no effect on depolarized cells. This makes them effective "contrast enhancers" that emphasize order in a fuzzy image, similar to how a Sharpen filter functions.

A computational model of the endogenous bioelectrical circuit that establishes the bioelectrical prepattern of the brain was developed by Pai et al [213]. This model employed HCN2 channels to reinforce voltage potential differences, which mark the boundaries of the developing brain. The model predicted that drugs that mimic the effect of HCN2 overexpression can rescue brain teratogenesis by repairing local and long-range bioelectric signals. This was tested experimentally using lamotrigine and gabapentin, which are approved for other human indications. Both drugs were found to rescue teratogen-induced defects in Xenopus embryos [209, 209, 211]. This approach relies on an inherent modularity of morphogenesis and the computational capacity of tissues to use resident bioelectrical pattern as triggers and respond with complex modules containing transcriptional and biomechanical implementation machinery that moves cells towards the appropriate morphological target, without individual manipulation of gene levels, stem cell behavior, and localization. This methodology holds potential for repairing complex organ defects by systemic application of repurposed channel activators or blockers. Similarly, if, as we propose here, aging corresponds to a corruption of the bioelectrical pattern over time (see Figure 8), this approach may correct it and help the cell regenerate and counteract the effects of aging on the bioelectrical pattern and subsequently on the body maintenance.

Many questions on the bioelectricity of aging remain to be solved, thus, the development of aging morphoceuticals will necessitate an iterative back and forth between computational modelling and experimentation. To identify potential targets for intervention in a specific tissue, transcriptomic databases such as EDEn [51] can be utilized (see Figure 7). The next generation of aging morphoceuticals will require the development of tools that can extract relevant information on ion channels. Dynamic computational modeling will also play a crucial role in predicting the bioelectrical pattern resulting from opening or closing specific targeted channels with existing drugs. The predicted results can then be used to suggest specific in vivo experiments to assess their potential for regeneration, repair or aging normalization.

A key question for the biomedical applications of morphoceuticals for aging is how the bioelectrical pattern is corrupted over time and if an enhancement/repair of it will lead to proper maintenance

or rejuvenation. While the study of endogenous bioelectric patterns (i.e., physiomic profiling) is important for specific regenerative therapeutics, especially given the increased availability of machine learning and data mining tools [229], a true anti-aging solution requires something different. A definitive aging intervention will not seek to micromanage specific bioelectric prepatterns, any more than it will try to enforce specific transcriptional profiles; rather, it will help cells maintain all of the multitude of required patterns and implement them despite the turnover of cells and materials over the lifespan.

6 Conclusion and perspectives

Here we have reviewed existing links between bioelectricity and aging, discussing how both current rejuvenation strategies and known molecular or clinical characteristics of age-related diseases have connections to bioelectric signaling. We highlighted unidirectional and bidirectional links between bioelectricity and the chief physiological and molecular hallmarks of aging. Accumulated evidence that bioelectricity provides the "computational glue" that links individual cells into larger-scale morphogenetic fields, enabling cooperative collective action needed to reach morphogenetic goals. This naturally suggests hypotheses about the nature of aging as increasing bioelectric dysregulation. Building on these, we introduced our concept that aging, along with its attendant diseases and dysfunctions, is a defect of morphostasis/regeneration, caused by disruption of the bioelectric patterns that connect individual cells into large-scale morphogenetic fields. Finally, we introduced the concept of morphoceuticals and discussed their potential power for treating aging. Many key questions remain open, providing a roadmap for future discovery in the bioelectricity of aging.

The bioelectrical pattern may become blurred over time (Figure 8) but there are other ways it may be degraded. A noisy bioelectrical pattern, like a pixelated image, may imply a depolarization of some clusters of cells, a disconnection of these cells with the morphogenetic field that could lead to cancer or progressive failure of maintenance of the body via an inappropriate information transmission. Of course, there are other possible large-scale modalities encoding information that may be important for resisting aging, including long-range biochemical, biomechanical, or even photonic patterns [40, 113, 307].

We presented the links between bioelectricity and the hallmarks of aging (see Figure 4). However, an open question remains as to whether the links can be bi-directional or not. In addition, the field of aging focused recently on the epigenetics markers of aging [64, 316]. It is still not clear what are the links between the bioelectrical pattern and the epigenetics and how the influence each other. Is bioelectricity an accurate marker of aging ? Can we measure this relationship quantitatively ?

We propose that both cancer and aging result from defects in or disconnection from the bioelectric mechanism that defines a morphogenetic field; however, the nature of that defect may be very different, as are the clinical manifestations of cancer and aging. Clinically, aging is more a gradual drift from anatomical homeostasis than cancer - an accumulation of small-scale maintenance failures over time. We don't yet know what bioelectric changes, if any, might be associated with aging. Bioelectricity as a control layer for anatomy may be a bioelectrical marker of aging. We hypothesize here that the bioelectrical pattern is corrupted over time (Figure 8) but it could lead to a progressive accumulation of damage still compatible with anatomical homeostasis. However, is this accumulation of damage the cause of a corruption of the bioelectrical pattern or is the corruption of the bioelectrical pattern the cause of this deficit of maintenance of the anatomy? In other words, do the cells lose the ability to follow the bioelectrical pattern or is it the corruption of the bioelectrical pattern that reduces their capability to maintain the body ?These are not mutually exclusive - the corruption

of the bioelectrical pattern may be also a cell-level problem where the cell can't maintain the appropriate Vmem pattern for example.

The causality likely flows in both directions - we have several bidirectional links between the different hallmarks of aging and bioelectricity (see Figure 4). And more may be found in the future. But maybe the corruption of the bioelectrical pattern is programmed as the programmatic theory of aging is stating it, or perhaps it is the accumulation of damage in cells that will impair it. The Planarian model system , given its immortality, cancer resistance, and regeneration, all despite somatic inheritance of mutations, is likely to be a critical part of the roadmap [153, 203, 252, 261].

One other key question is whether the aging morphoceuticals will simply allow better maintenance of existing tissues tissues (maintain status quo), or allow true rejuvenation? In other words, if the bioelectrical pattern represents the anatomy at age t once development is over, or if its enhancement/repair will allow a rejuvenation of the body or 'reverse' development [256]. The details of the relationship between bioelectricity and aging are currently unknown and represent an exciting avenue for investigation using existing and emerging tools. We think it likely that bioelectricity – the signaling layer that mediates global order in embryogenesis and large-scale repair – offers a clinically impactful roadmap for addressing the progressive deterioration of target morphology we call aging.

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Competing Interests Statement

M.L. is a scientific co-founder of Morphoceuticals Inc., which does research in the field of bioelectrically-induced organ regeneration. M.L. is also a scientific co-founder of Astonishing Labs, which does research in the field of aging.

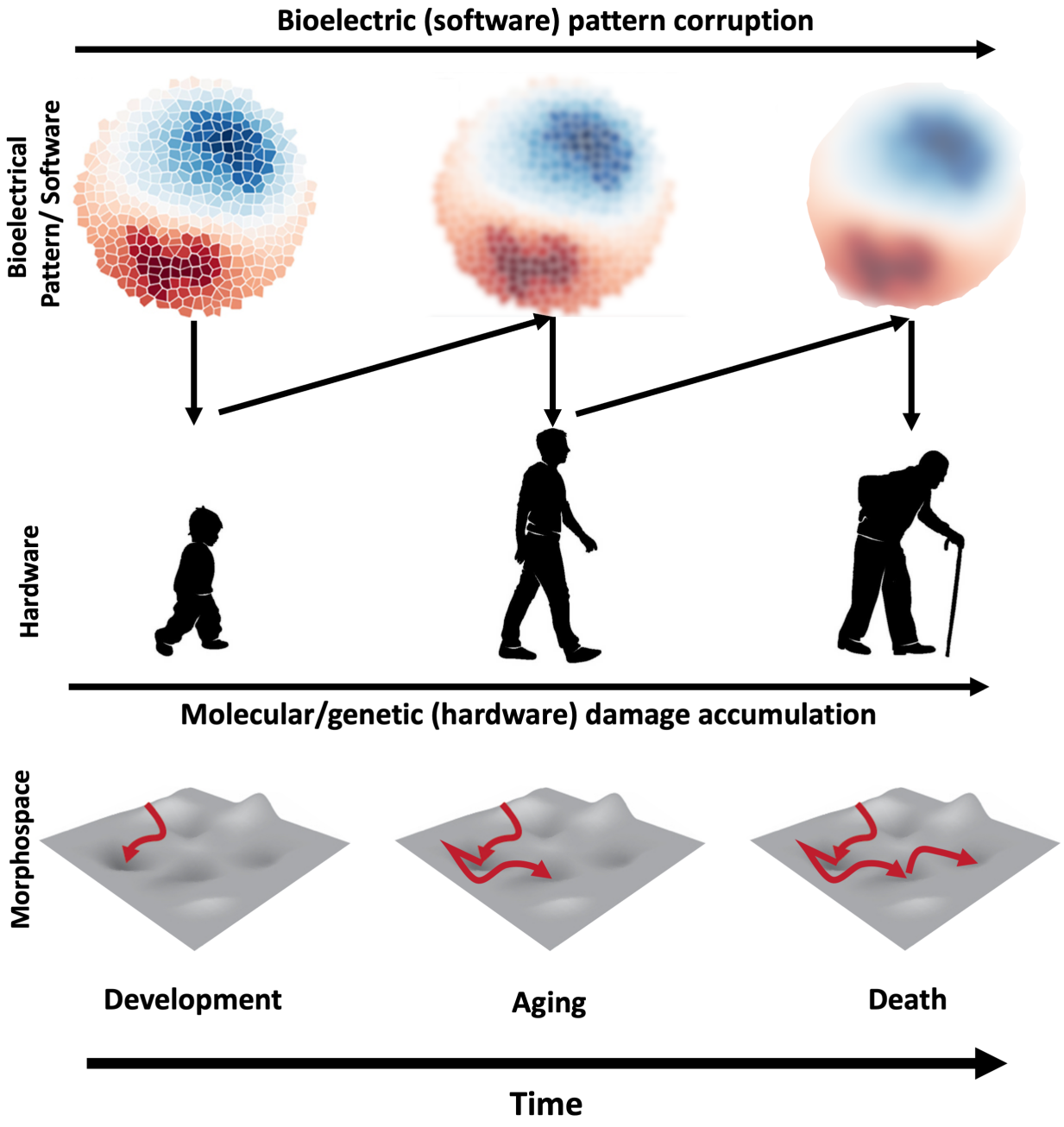

Figure 8: Aging as a drift in morphospace. By accumulation of molecular and cell damage, the bioelectrical pattern in charge of the maintenance of the anatomy is corrupted over time. The cell gradually loses the ability to follow the bioelectrical pattern, resulting in a gradual loss of anatomical homeostasis leading to aging and ultimately death.

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