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Review

Bioelectric mechanisms in regeneration: Unique aspects and future perspectives

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ABSTRACT

Regenerative biology has focused largely on chemical factors and transcriptional networks. However, endogenous ion flows serve as key epigenetic regulators of cell behavior. Bioelectric signaling involves feedback loops, long-range communication, polarity, and information transfer over multiple size scales. Understanding the roles of endogenous voltage gradients, ion flows, and electric fields will contribute to the basic understanding of numerous morphogenetic processes and the means by which they can robustly restore pattern after perturbation. By learning to modulate the bioelectrical signals that control cell proliferation, migration, and differentiation, we gain a powerful set of new techniques with which to manipulate growth and patterning in biomedical contexts. This chapter reviews the unique properties of bioelectric signaling, surveys molecular strategies and reagents for its investigation, and discusses the opportunities made available for regenerative medicine.

Contents

1. Introduction

Bioelectrical signals are mediated by the steady-state electrical properties of cells and tissues. Despite much fascinating data on the role of endogenous bioelectric signals controlling limb and spinal cord regeneration [1–3], cell and embryonic polarity [4–6], growth control [7,8], and migration guidance of numerous cell types [9], the field as a whole is unfamiliar to several generations of modern cell and developmental biologists. However, some well-known processes, such as the fast, electrical polyspermy block [10,11], are in fact good examples of such signaling.

This chapter discusses the roles of ion-based physiological processes in guiding cell activity during regeneration, and more broadly, pattern formation. Functional experiments throughout the last decades showed that some bioelectric events were not merely physiological correlates of housekeeping processes, but rather provided specific instructive signals regulating cell behavior during embryonic development and regenerative repair [12,13]. Roles for endogenous currents and fields were found in numerous systems (Table 1), and in several cases, spatially instructive signaling was demonstrated [14–19]. Here, I discuss bioelectric controls of morphogenesis in the larger context of pattern formation, outlining controls of individual cell behavior and the unique properties of electrical processes that may underlie the orchestration of higher-order patterning. Specifically excluded in this review are action potentials in neurons, and electromagnetic radiations

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Nomenclature

Allometric scaling remodeling of tissue during changes of cell number or type that maintains correct proportions between organ dimensions (an example of control of non-local, large-scale structure)

Bioelectrical signals information transmitted via spatio-temporal properties of membrane voltage, ion flux, or electrical fields. These are produced by ion channels or pumps functioning in an individual cell or in cell sheets (e.g., epithelial cells arranged in parallel to maximize current) and sensed by the cell itself, neighboring cells, or distant cells. Some are instructive—they carry specific morphogenetic cues used to determine position, differentiation, or proliferation/apoptosis decisions by cells

Epigenetic morphogenesis induced by mechanisms other than changes in DNA sequence or transcription. Bioelectric signals are often epigenetic because these physiological processes can accomplish much patterning via post-translational and physical (e.g., electrophoresis) events not relying on transcription or translation. Ultimately, bioelectric events do induce changes in gene expression

Gavanotaxis ability of cells to utilize field lines and voltage gradients as migratory cues, moving towards the anode or cathode (depending on cell type)

Morphostasis maintenance, throughout life, of large-scale pattern despite death or injury of individual cells or cell groups

Second anatomy coding (in terms of positional, gene expression, or signaling factor gradients) of the components of any system. Roughly, this is the molecular identity by which the embryo or regenerating field spatially addresses (maps) its different parts

State space the set of all possible states of a dynamical system. When applied to cell properties, this is a multi-dimensional theoretical construct where each orthogonal dimension reflects a specific parameter such as voltage, pH, potassium content, etc. Current modeling efforts often make use of the X, Y, Z, t, g space where cells occupy a given point in this space corresponding to their three-dimensional position, gene expression, etc. We propose a physiological state space that instead groups cells by their bioelectrical properties

and biophotons generated by cells. The review concludes with a discussion of the molecular mechanisms transducing bioelectrical events into genetic cascades, and the opportunities provided for the field of regenerative medicine by state-of-the-art molecular tools for the study and manipulation of bioelectric cues.

Bioelectric signals are generated by specific ion channels and pumps within cell membranes. The segregation of charges achieved by ion fluxes through such transporter proteins gives rise to a transmembrane voltage potential (usually on the order of −50 mV, inside negative). Ion channels and pumps are localized to distinct regions of some cell types; in particular, the apical-basal organization of epithelial cells results in a parallel arrangement of battery cells which in turn gives rise to a transepithelial potential [9,20]. Thus, all cells – not just excitable neurons and muscle – generate and receive steady-state bioelectrical signals. These transmembrane potentials, electric fields through tissue and surrounding fluids, iso-electric and iso-pH cell groups established by gap junctions [21], and fluxes

Table 1
Physiological data on endogenous bioelectric signal roles in morphogenesis.

Role	Species/system	References
Cellular polarization (anatomical asymmetry of cell or epithelium)	Alga *Fucus*	[13]
Patterning in gastrulation, neurulation, and organogenesis	Chick, axolotl, frog	[108,169,208,248]
Directional transport of maternal components into the oocyte	Moth, *Drosophila*	[249]
Growth control and size determination	Segmented worms	[250]
Neural differentiation	*Xenopus* embryo	[251]
Polarity during regeneration	Planaria and annelids	[15,18,19,27,28]

of individual ions, all carry information to the source cell as well as to its neighbors, and in some cases, to distant locations.

Early discoveries of "animal electricity" can be traced to Luigi Galvani in the late 1700s, and as early as 1903, it was found that hydroids have a specific electrical polarity [22]. However, the majority of the literature in this rich field has come from several subsequent major waves. Lund, through the 1920s and 1930s, focused on currents and showed that polarity was predicted by, and in some cases controlled by, the bioelectric polarity of ion flows *in vivo* [23]. Burr (1930s and 1940s) focused on measuring and correlating voltage gradients with future developmental pattern in a wide range of species and organs [24,25]; the measurements suggested that the voltage gradients are quantitatively predictive of morphology, and suggested that the measured fields carried patterning information (an example of Slack's "second anatomy" [26]). Some of the best early functional results were obtained by Marsh and Beams [15,27,28] who were able to specifically control anterior–posterior polarity in planarian regeneration by supplying bioelectrical signals to fragments. Enormously influential for the field was the work of Jaffe and co-workers including Nuccitelli, Robinson, and Borgens [12,13,20,29–37], who demonstrated that electrical properties of individual cells, epithelia, neural structures, and entire limbs were instructive for growth, pattern, and anatomical polarity.

The rise of molecular genetics has drawn attention away from a huge literature of not only descriptive, but also solid, well-controlled functional work using physiological techniques. However, in the last decade, state-of-the-art work has begun to identify proteins responsible for the well-characterized bioelectric signals, the genetic networks that shape them, and the mechanisms that allow cells to transduce the information into growth control decisions. Molecular and cell biology are now being applied to this problem in the areas of wound healing, neural guidance, and cell orientation responses to physiological electric fields [38–42], as well as the role of specific ion transporter activity in tail regeneration, left–right patterning, control of adult stem cells and regenerative polarity, and the switch between embryonic stem cell and neoplastic phenotypes [43–49].

Although many modern workers are unaware of this rich field, the connection between molecular-genetic pathways and bioelectric signaling is being forged by the data itself. A variety of relevant channelopathies has now been discovered by unbiased approaches [50,51], though ion transporters are usually de-prioritized for analysis when they show up on comparative microarray experiments because it is not yet second nature for cell and molecular biologists to think in terms of bioelectrical signaling. It is hoped that by highlighting the techniques and tools now available, and illustrating strategies for integrating bioelectrical signals with mainstream pathways, workers in multiple sub-fields will consider that modulation of ion flows, currents, and voltages may be at the root of their favorite patterning or mis-patterning problem when ion channels and pumps are identified in genetic screens or subtraction analy-