Bioelectricity and epimorphic regeneration

Scott Stewart,1 Agustín Rojas-Muñoz,1 and Juan Carlos Izpisúa Belmonte1,2*

Summary

All cells have electric potentials across their membranes, but is there really compelling evidence to think that such potentials are used as instructional cues in developmental biology? Numerous reports indicate that, in fact, steady, weak bioelectric fields are observed throughout biology and function during diverse biological processes, including development. Bioelectric fields, generated upon amputation, are also likely to play a key role during vertebrate regeneration by providing the instructive cues needed to direct migrating cells to form a wound epithelium, a structure unique to regenerating animals. However, mechanistic insight is still sorely lacking in the field. What are the genes required for bioelectric-dependent cell migration during regeneration? The power of genetics combined with the use of zebrafish offers the best opportunity for unbiased identification of the molecular players in bioelectricity. *BioEssays 29:1133–1137, 2007. © 2007 Wiley Periodicals, Inc.

Introduction

Living organisms may be viewed as biological batteries since their epithelial tissues function to maintain relatively high concentrations of electrical charge, in the form of ions, inside their bodies. This property endows epithelia with the remarkable property of being able to generate an electric field, or wound potential, when tissue damage leaks electrical charge (freely diffusible ions) from inside the body to the outside environment. Wound potentials are just one example of the many documented bio-generated electric fields that play roles in a surprisingly wide array of biological processes, including cell–cell communication in the nervous system, cell migration, differentiation, division, animal development and organ regeneration.1–6

In contrast to action potentials of the nervous system, now considered integral to the study of neurobiology, wound potentials and other relatively weak, steady, long-lasting bioelectric fields have been somewhat overlooked by scientists and clinicians alike. This is in spite of the fact that they were elegantly described decades ago. This most likely can be attributed to a lack of insight into the mechanism of action of bioelectricity at the molecular level. However, at the time these experiments were first performed, the wide array sophisticated molecular tools used today were not yet available to biologists and geneticists.

A number of years ago, investigators observed that bioelectric fields are generated by vertebrate tissues when they undergo two intimately related, but seemingly opposite biological processes, namely epimorphic regeneration and non-regenerative wound healing.1–6 However, the trail leading to the identification of the molecular mechanisms functioning downstream of bioelectricity during these two events has gone cold and many questions remain unanswered even though research in regenerative biology has increased exponentially in the last 25 years. We are ultimately left with the reality that little is still known about how cells and tissues derive information from these weak electrical cues and turn it into a biological outcome.

With the advent of modern molecular, cellular and genomic methodologies, scientists and clinicians alike now find themselves in an ideal position to revisit these pressing questions in a sorely neglected field. The most glaring of these is what are the factors that are responsible for sensing, interpreting and translating weak bioelectric signals into languages well understood by biologists, namely gene expression and signal transduction? Is there a difference between the magnitude, direction or duration of wound potentials or the cellular response to them by a regenerating animal when compared to a non-regenerating one? Might variations in bioelectricity-mediated signaling and physiology be factors that contribute to understanding why humans fail to undergo the mysterious process of epimorphic regeneration in response to organ loss and injury?

Epimorphic regeneration

Certain organs and tissues in vertebrates cope with everyday wear and tear by being continuously renewed. Such is the case for the skin, blood and the lining of the gut. This type of
self-renewal is mediated exclusively by stem cells. The cells that will repopulate the organ undergoing renewal are the progeny of resident or circulating progenitor (stem) cells, with varying degrees of plasticity. The cellular mechanisms that underlie the process of tissue turnover greatly depend on the specific tissue in question and may include cell migration, proliferation and differentiation. Although the end-result of this increased tissue turnover process is often referred to as tissue “regeneration”, the term “regeneration”, as it is used in this article, is reserved for an entirely different biological process altogether that is only observed in select vertebrate species, such as salamanders and zebrafish.

In contrast to stem-cell-mediated tissue restorative processes described above, bona fide vertebrate regeneration, technically referred to as epimorphic regeneration (Fig. 1A), involves several unique biological events, namely dedifferentiation of post-mitotic cells, activation of multipotent progenitor cells, cell proliferation, pattern formation and, in some cases, transdifferentiation of specialized cells to rebuild parts of the body plan after amputation or injury.7–10 The beauty and extraordinary complexity of regeneration has fascinated and attracted the interest of biologists for centuries and, indeed, it marked the beginning of the field of experimental developmental biology.

The amazing capacity of urodele amphibians, such as the newt and axolotl, to regenerate limbs, tail, jaws, heart or lens, has made these animals the preferred subject for research on vertebrate regeneration. This process is commonly divided into four sequential steps: (1) formation of a wound epidermis, in which the amputation site is covered by epithelial cells, (2) disorganization and dedifferentiation of mesenchymal tissue near the wound, (3) formation of a mass of undifferentiated cells, known as the blastema, primarily by dedifferentiation of cells in the surrounding tissue and (4) proliferation of the dedifferentiated cells concomitantly with re-development, in which the correct pattern is formed in the blastema resulting in the regeneration of the amputated portions of the organ (Fig. 1). Importantly, the first two steps outlined above do not require cell proliferation and, therefore, depend on the properties inherent to the existing cells. Working models of regeneration using various experimental systems, suggest that the re-development stages of regeneration are recapitulations of early development. The same signaling molecules and mechanisms, under the control of highly complex gene regulatory mechanisms, appear to function in regeneration as they do in early embryonic development.7–10

What then are the factors that initiate this complicated biological program of organ regeneration? This is perhaps the

Figure 1. Epimorphic regeneration. A diagram of regeneration in newt limb. Formation of the wound epithelium is one of the first cytologically discernable steps in regeneration and proceeds blastema formation and cell proliferation. Adapted from Essential Developmental Biology, Slack J. W. Second Edition. Blackwell Publishing.
most-compelling question in the field of regeneration. If observed from a temporal perspective, the first steps of regeneration that redeploys developmental signaling processes can be thought of as truly “regeneration specific” phenomena whose molecular basis is completely unknown. Insight into these processes would yield clues as to the identity of the driving force behind the regenerative response. Therefore, elucidation of the mechanisms underlying the early regenerative response is likely to be crucial in explaining the huge disparities between regenerating and non-regenerating organisms. Due to the extremely rapid and dramatic nature of the early regenerative response, one cannot help sense that something fundamentally different between these two types of organisms exists, yet continues to be overlooked.

Wound potentials
Sometime before 1849, the German physiologist Emil DuBois-Reymond found that, when he made a cut in one of his fingers, he could cause a deflection of a galvanometer by putting the injured finger and a contralateral unwounded finger into the circuit. This was the first documented experimental evidence of endogenous wound electric fields, also known as wound potentials. We now know that such electric fields are generated when the epithelial layer is cut and the lesion short-circuits the trans-epithelial potential (TEP). The TEP arises because specialized epithelial cells have evolved mechanisms to selectively transport ions from the extracellular space to the inside of the body.

Let us consider the example of frog skin, which contains epithelial sodium channels on its apical surface and Na⁺/K⁺ ATPase on its basolateral surface. In this setting, Na⁺ channels and Na⁺/K⁺ pumps act in concert to maintain a charge differential on the inside of the animal with respect to the outside environment. This charge separation, combined with the high electrical resistance of the plasma membrane and the presence of tight junctions between epithelial cells, results in the formation of a TEP with the inside of the animal being electrically positive (the anode) with respect to the outside environment of consisting of pond water (the cathode). Upon damage to the tissue, ion (charge) flux to the extracellular space ensues driven by an electrochemical gradient. This is, of course, an electrical current with ions, rather than electrons, being the carriers of charge and this creates an electrical field with a magnitude on the order of...
tens of millivolts. The phenomena of current flow and the presence of an electric field are two sides of the same coin; they are one and the same and you cannot have one without the other. The two are, in fact, quantitatively related through Ohm’s law. The phenomena of TEPs can be applied to all ion-transporting epithelia, including mammalian skin.

Bioelectricity in regeneration: directed cell migration

During the initial stages of epimorphic regeneration in salamanders, a strong outward-directed electric current driven through the stump is generated by the injury. Furthermore, the current generated upon wounding is necessary for normal regeneration to proceed. Unlike wound healing in a non-regenerating species, the intensity and direction of the electric current generated during regeneration is maintained for several days after the formation of the wound epithelium, further suggesting that the electric current may play a role in later processes of epimorphic regeneration. Based on these observations, one can envision that the generation of a steady, weak electric field upon wounding may well be a regenerating animal’s first response to such trauma. This can be attributed to the fact that the extremely rapid leakage of ions from the body to the outside is driven by electrochemical forces, as opposed to the relatively slow interactions between biological macromolecules. This crucial piece of information, we would argue, makes it imperative to decipher the role of bioelectricity during the early stages of regeneration.

One of the first cytologically distinguishable events during regeneration is cell migration. Migration of epidermal cells in amputated salamanders limbs and zebrafish caudal fins, covers the wounded surface and forms the wound epithelium that is essential for regeneration. Strikingly, this initial cell migration event in a regenerating animal, greatly contrasts with the response of a non-regenerating animal, which secrete an extracellular matrix to seal off the wound from the outside.

We propose that this crucial cell migration event, witnessed only in regenerating animals or in early stages of vertebrate development, is also mediated by bioelectrical cues. These recent experiments using in vitro models of non-regenerating wound healing suggest that cell migration during this process is mediated by bioelectric pathways. These studies indicate that wound-induced electric cues activate signaling pathways similar to those reported for chemotaxis. In both scenarios, the migratory response of cells depends on the opposing activity of two enzymes, phosphatidylinositol-3-OH kinase-γ (PI3Kγ) and the lipid phosphatase PTEN (phosphatase and tensin homolog). Overall, these observations indicate that, on one hand, PI3Kγ activity is essential for the ability of cells to respond to bioelectric fields and, on the other hand, loss of PTEN activity enhanced the migration of cells towards electrical cues. For instance, it would be interesting if epithelial cell migrate towards a wound in PTEN mutant mice. Additionally, the PI3 kinase inhibitor LY294002 can be administered to regenerating salamanders and fish to determine if cell migration is normally involved in wound epithelia.

In contrast, the observations of Donaldson and co-workers have suggested that migration of axolotl epidermal cells in vitro can occur without bioelectric cues, since the culture system used in these experiments is likely to short circuit any current generated. However, sensitive assays are required to measure possible weak electric fields in order to conclude that they play no role in cell migration. These observations can also be interpreted that it is possible to coax cells to migrate without bioelectric cues by in vitro culture. The multitudes of researchers who have derived cell lines from tumor samples on a tissue culture dish can readily attest to the existence of cell migration in in vitro setting where bioelectric cues are likely to be minimized. The question that needs to be addressed is not what can occur in vitro but what does occur in vivo during regeneration.

Unfortunately, at the present moment, researchers have not uncovered any mechanisms as to how electrical signals are received and converted into activation of cell signaling pathways. It is therefore essential to isolate the factors responsible for responding to bioelectrical signals as a first step in understanding this area of biology. One of the great advantages of the zebrafish as a model system is, of course, the ability to perform sophisticated genetic screens. We would argue that thorough unbiased genetic screens are likely to be one of the most-efficient and productive means of uncovering the genes required for the bioelectric response. As an experimental system, genetic screens in zebrafish should prove to be fruitful in identifying genes that function in bioelectric pathways. A straightforward approach along these lines is to screen mutagenized fish for cell migration defects in vivo upon caudal fin amputation using genetically encoded fluorescent reporter genes. These animals could then be crossed to fish engineered to express mutant alleles of PI3Kγ or PTEN to perform epistasis experiments. This type of assay has the advantage of being simple and quite rapid since it takes less than 24 hours for wound epithelial formation in the zebrafish caudal fin.

A pressing question is how is PI3Kγ activated by bioelectric signals? PI3Kγ has been shown to function mainly downstream of heterotrimeric G-proteins. Thus a logical guess would be that a G-protein-coupled receptor and its regulators may function in bioelectric signal transduction. This is an attractive hypothesis due to the fact that this large family of proteins has been extensively studied from biochemical and physiological standpoints and a large number of pharmacological agents that specifically target G-protein-coupled receptors are readily available, some in the form of FDA-approved drugs. Libraries derived from these small molecules could rapidly screen for effects on bioelectricity-mediated cell
migration in fish. Any "hits" from these types of screens would provide useful mechanistic information about bioelectric signaling and regeneration, as well as immediately raise the possibility of therapeutic intervention with antagonists/agonists of these receptors. For instance, the mouse, a non-regenerating animal, could be treated with pharmacological agents identified in zebrafish screens and cell migration could be tested after limb amputation. The use of ectoderm-specific fluorescent reporter line would provide an efficient assay to monitor cell migration in mice during wound healing.

The last decade has shed light on the basic cellular events that initiate a regenerative response. Yet, research has still not uncovered the mystery as to why only some animals can regenerate whereas humans cannot. Regeneration appears to proceed by a complex cascade of gene activity, but what initiates this cascade? Is bioelectricity the key to regeneration? It is too early to say, but it is clear that it is essential and should be accounted for by researchers in regenerative biology.

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References