

Left/right, up/down: the role of endogenous electrical fields as directional signals in development, repair and invasion

Kenneth R. Robinson^{1*} and Mark A. Messerli²

Summary

A fundamental aspect of biological systems is their spatial organization. In development, regeneration and repair, directional signals are necessary for the proper placement of the components of the organism. Likewise, pathogens that invade other organisms rely on directional signals to target vulnerable areas. It is widely understood that chemical gradients are important directional signals in living systems. Less well recognized are electrical fields, which can also provide directional information. Small, steady electrical fields can directly guide cell movement and growth and can generate chemical gradients of charged macromolecules against the leveling action of diffusion. At the site of a lesion in an ion-transporting epithelium, for example, a substantial electrical field is instantly generated and may extend over many cell diameters. There are numerous other situations in which relatively long-range electrical fields have been shown to exist naturally. Recently, there has been substantial progress in identifying specific processes that are controlled, to some extent, by these endogenous electrical fields. This review highlights these recent data and discusses possible mechanisms by which the fields might affect biological processes. *BioEssays* 25:759–766, 2003. © 2003 Wiley Periodicals, Inc.

Introduction

There is a substantial body of data showing that many cell types, when cultured in vitro, respond directionally to an applied D. C. electrical field. It is relatively easy to carry out such experiments. All that is required is to connect the medium in which the cells are grown to a current source via appropriate electrodes and buffers (usually agar bridges) to prevent the appearance of electrode products in the culture medium.

Current flow in a conductive medium and an electrical field distributed through the medium are two aspects of the same phenomenon; one cannot exist without the other. The two aspects are quantitatively related through Ohm's law.

The responses of the cells can be monitored by time-lapse recording or by some trace that the cells leave of their behavior. For example, perhaps the most-extensively-studied cells in this regard are the embryonic spinal neurons of *Xenopus*. As the growth cone advances, a record of its trajectory is left in the form of the neurite that connects the growth cone to the cell body. It has been well documented that the neurites grow toward the negative (cathodal) electrode in an applied field and do so at remarkably small field strengths.^(1–3) The growth cones can detect and respond to fields as small as 10 mV mm^{-1} , which corresponds to a voltage difference across the growth cones of about $500 \mu\text{V}$. Among other cells that have been shown to respond to small electrical fields (i.e., $<100 \text{ mV mm}^{-1}$) are neural crest cells^(4–6) (cathodal migration), fish keratocytes⁽⁷⁾ (cathodal migration), pollen tubes⁽⁸⁾ (anodal growth), bone cells⁽⁹⁾ (osteoclasts: anodal migration, osteoblasts: cathodal migration), and chick sensory neurons.⁽¹⁰⁾

The sensitivity of many migrating and growing cells to small, steady electrical fields has led to the numerous suggestions that electrical fields may be involved in development and repair.⁽¹¹⁾ Despite earlier demonstrations of the existence of natural electrical fields of $10\text{--}100 \text{ mV mm}^{-1}$ near wounds to adult mammalian skin⁽¹²⁾ and in developing embryos,^(13,14) and evidence that disruption of the fields produces developmental abnormalities along the entire embryonic axis,^(14,15) this idea has not gained general acceptance. In part, this is because a well-defined target of the endogenous electrical fields has not been identified in the examples cited above, and the effect of disrupting the field on such a well-defined target has not been assessed. Recently, there have been a number of published studies that do identify specific targets of endogenous fields and that clearly show that the fields are a necessary part of a surprising array of biological phenomena. Furthermore, the mechanisms of generation of the fields show unexpected diversity. Below we review these new findings in an effort to make the results accessible to a wider audience of cell and developmental biologists.

¹Department of Biological Sciences, Purdue University, West Lafayette.

²BioCurrents Research Center, Marine Biological Laboratory, Woods Hole, MA.

*Correspondence to: Kenneth Robinson, Department of Biological Sciences, Purdue University, West Lafayette.

E-mail: ken@video.bio.purdue.edu

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Development of left/right asymmetry

The establishment of left/right asymmetry in vertebrates has been the subject of intense study recently. As a result, a cascade of sided gene expression that precedes and directs morphological asymmetry has been revealed. In the chick, *Sonic hedgehog* (*Shh*) is initially symmetrically expressed around Hensen's node. At stage 4, the expression becomes restricted to the left side of the node, which in turn is responsible for the later left-sided expression of the TGF β -family member, *Nodal*, in the left lateral plate mesoderm.⁽¹⁶⁾ It was inferred that the right-sided activity of an activin-like molecule repressed the expression of *Shh* and was thus responsible for the observed *Shh* asymmetry.⁽¹⁷⁾ A number of other genes exhibit left/right asymmetry in their expressions in the chick embryo and variations on this pattern are seen in other vertebrates.^(18,19) The asymmetries of gene expression occur on a multicellular scale, suggesting that relatively long-range communication is necessary for the establishment of left/right asymmetry.

The first evidence of the physiological control mechanism underlying the generation of the asymmetry was the finding that gap junctional communication is necessary for proper left/right asymmetry formation in *Xenopus*.⁽²⁰⁾ Levin and Mercola⁽²¹⁾ then extended this result to the formation of left/right asymmetry in chick. Inhibition of gap junctional communication by pharmacological agents, antibodies or antisense oligonucleotides led to the symmetrical expression of *Shh* and *Nodal*. In addition, they found that mechanical interruption of embryo continuity by radial slits remote from the primitive streak abolished proper asymmetric expression of *Shh* and *Nodal*. These data strongly suggest that the normal development of left/right asymmetry requires the biased movement of a small molecule or molecules through gap junctions, and that this movement depends on a circumferentially intact communication pathway.

In order to establish asymmetric movement of morphogens across the midline, some means of biasing the diffusion of the morphogen through gap junctions must exist. It has recently been shown that a sharp asymmetry in epithelial membrane potential exists across the midline of the stage-2 to -4 chick embryo (Figs. 1A, 2).⁽²²⁾ These voltage gradients, which were measured using a fluorescent dye that accumulates in cells in a membrane potential-dependent manner, are as large as 20 mV, with the cells on the left side of the midline being depolarized with respect to those on the right side and the rest of the epiblast. The chick embryo is an especially favorable system for such investigation because of the planar nature of early development. Inhibitor studies showed that the membrane potential asymmetry depended on H⁺/K⁺ ATPase function. Furthermore, inhibition of the H⁺/K⁺ ATPase randomized both the expression of normally sided genes and the distribution of internal organs (a condition known as heterotaxia). It was also shown that the function of the H⁺/K⁺ ATPase

plays a similar critical role in left/right asymmetry formation in *Xenopus* embryos.

The picture that emerges from these studies is one of long-range lateral connectivity among the cells on the surface of the early vertebrate embryo. The cells, which are extensively coupled via gap junctions, must communicate across the dorsal–ventral midline in order to establish normal left/right asymmetry. There is an electrical component to the communication that requires a voltage gradient (an electrical field) between the future left and right sides. It appears that an electrical component is involved upstream of the establishment of *Nodal* expression asymmetry in a mammal. A knock-out of the ion channel, polycystin-2, results in the loss of proper left/right development in mouse.⁽²³⁾ The implication of these results is that a small, charged molecule (or molecules) is being asymmetrically distributed across the midline, and the molecule directs the downstream events of asymmetric gene expression. Clearly, however, some earlier event must produce the asymmetries in ion transport that create and maintain the voltage gradients. Thus, the electric-field-dependent gap junctional communication should be viewed as just one step, albeit an early one, in a series of positive feedback loops that amplify a faint asymmetry into the final asymmetrical positioning of the internal organs. The specific molecules that act as morphogens and connect the electrical field to the later gene expression pattern are still unknown. These molecules must be small enough to pass through gap junctions and charged under the physiological conditions of the intracellular environment. Possibilities include Ca²⁺, inositol phosphates, cyclic nucleotides and neurotransmitters.

Repair and regeneration in the cornea

The foregoing discussion of intercellular electrical fields within the epithelial cells of the early embryo emphasized the role of gap junctions and differences in the membrane potentials between blocs of cells as a means of generating electrical fields that span many cell diameters. The functional units of the system are pairs of cells connected by gap junctions that are each electrically polarized so that the movement of charged molecules between the cells is biased in one direction. There is another anatomical specialization that leads to a quite different kind of long-range electrical field in organisms. From the earliest stages, the outer cells of the developing vertebrate embryo are organized into a protective barrier, an epithelium, that separates the developing embryo from the surrounding fluid. An essential part of that organ is the array of tight junctions that form between the cells near the outward-facing, apical side of the epithelium. The tight junctions act to provide a seal between the cells that greatly reduces the electrical conductivity of the paracellular space; thus, ion transport across the epithelium is limited to, and regulated by, the plasma membranes of the cells. The ion transport properties of the apical and basolateral faces of the epithelial cells are typically

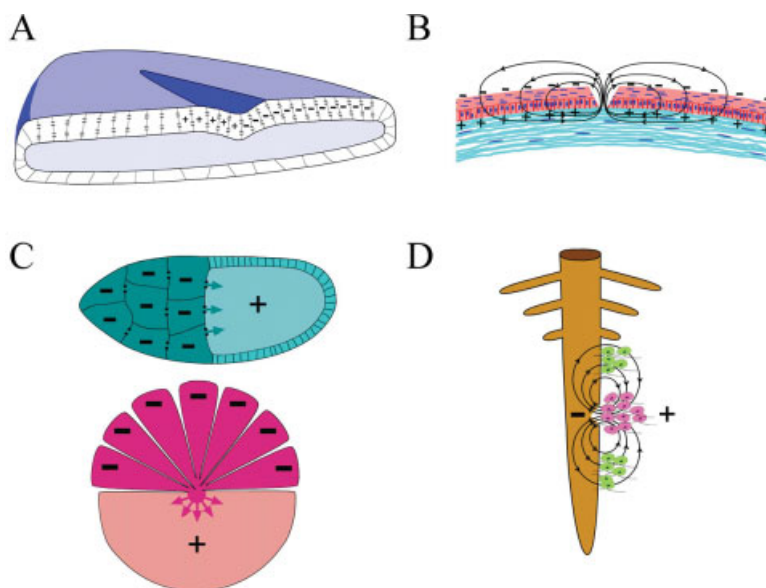


Figure 1. Diagrams of the four situations discussed in this review where endogenous electrical fields have been shown to regulate biological processes. **A:** An early chick embryo with a region of depolarized cells on the left side of the midline of the embryo. These cells are extensively coupled via gap junctions and the electrical gradient is proposed to control the asymmetric distribution of some important left/right determinant. Also see Fig. 2. **B:** The wounded mammalian cornea. Unlike the chick embryo, the relevant potentials are extracellular. Here epithelial cells are joined by tight junctions and carry out electrogenic transport of ions, resulting in a TEP of tens of millivolts. If the integrity of the epithelium is compromised by a wound, as shown in the diagram, the TEP collapses locally and the epithelium drives current as shown. This results in an electrical field as shown. Note that, in a conductive medium, the electric field lines and the current are coincident and indistinguishable. This pattern of electrical field is an inevitable consequence of the physiology of the epithelium and the wound. The electric field generated by the wound was found to stimulate and polarize epithelial cell division, reestablish damaged neurons and increase the rate of wound closure. **C:** The fly (above) and the moth (below) follicles. Only eight of the 15 nurse cells are shown in the *Drosophila* follicle. In both cases, the electrical potential of the oocytes is positive with respect to the follicle cells, and both exogenous and endogenous macromolecules exhibit a polarized distribution across the cytoplasmic bridges connecting the nurse cells to the oocytes. A variety of manipulations show that the asymmetric distribution is a function of the potential difference and the net charge on the molecules. **D:** The accumulations of *P. palmivora* zoospores (green) and *P. aphanidermatum* zoospores (magenta) in the vicinity of a wound in a root. *P. palmivora* zoospores are anodotactic while *P. aphanidermatum* zoospores are cathodotactic when the zoospores are subjected to an applied electrical field. The distribution of the zoospores in the wounded root correlates with the *in vitro* behavior. Also see Fig. 3.

polarized so that net ion transport across the epithelium can be accomplished. The apical surface of frog skin epithelium contains specialized epithelial sodium channels while the basolateral surface contains the Na^+/K^+ ATPase, with the result that there is net transport of Na^+ into the embryo. One result of this ATP-dependent charge separation is the formation of a transepithelial potential (TEP), with the interior of the embryo being electrically positive with respect to the outside fluid. If an electrically conductive pathway is made between the two sides of the frog outer epithelium, it can drive large electrical currents; that is, the polarized epithelium can act as a battery. This capacity to generate a TEP and drive currents through leaks (either developmentally programmed or artificially made) arises very early in the development of the frog embryo^(24–26) and other vertebrate embryos.

The ability of epithelia to create electrical differences between two compartments extends to organs other than the integument; for example it is essential for kidney function. Another interesting case is the cornea of the eye. It is well established that the cornea maintains a TEP,⁽²⁷⁾ with the interior being electrically positive with respect to the tear side. This potential is maintained by the active outward transport of Na^+ and K^+ and the inward transport of Cl^- . If a wound is made in the bovine cornea, large currents leave the wounded area and return in the space just under the epithelium, producing measurable electrical fields parallel to the surface of the epithelium that are greater than 40 mV mm^{-1} .⁽²⁸⁾ It is important to emphasize that these fields exist in the extracellular spaces of the cornea, and any cell near the wound will experience the field (see Fig. 1B). It has been shown that the normal rate of epithelization of the wound can be inhibited if the epithelial

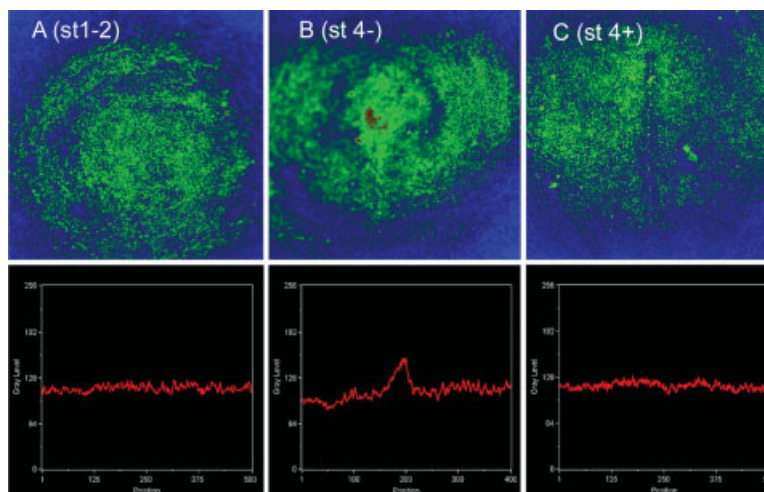


Figure 2. Images of the membrane potentials of a chick embryo at three different stages: (A) stage 1–2, (B) stage 4–, (C) stage 4+. Below each image is a linescan of the gray level across the embryo. The embryos were incubated in the anionic fluorescent dye, DiBAC₄, which accumulates in cells in a membrane potential-dependent fashion. Relatively depolarized cells accumulate more dye and appear brighter, shown in the pseudocolored images here as toward red. A zone of depolarized cells can be clearly seen on the left side of the midline in B, but not in A or C. This asymmetry of membrane potential was eliminated when the embryos were treated with BaCl₂ or omeprazole (images not shown), treatments that cause a substantial loss of normal left/right asymmetry in chick embryos.⁽²²⁾ Images supplied by Dr. Thorleif Thorlin of the Institute for Clinical Neuroscience, Sahlgrens University Hospital, Gothenburg, Sweden.

sodium channels are blocked and the normal rate of epithelization could be enhanced if the natural field was increased by an exogenous current source.⁽²⁹⁾

Colin McCaig and his colleagues at the University of Aberdeen have extended these results and have explored the role of the wound-generated fields on the complex healing process that repairs the wound in the human and rat cornea. They first showed that both individual cells and cell aggregates in culture responded to applied electrical fields by migrating toward the cathode.⁽³⁰⁾ They subsequently showed, also in culture, that the plane of division of corneal cells was oriented perpendicular to the electric field vector.⁽³¹⁾ More recently, they have taken advantage of the fact that many features of wound healing occur in isolated, intact corneas. Under those conditions, the responses of the cells in situ can be monitored. In addition, because the physiological basis of the TEP is well understood, the endogenous electrical fields can be modulated in a known way by a variety of pharmacological treatments. They examined three aspects of wound healing: (1) the orientation of cell division, (2) the rate of cell division and (3) the rate of closure of the wound as the endogenous fields were modulated.⁽³²⁾

With regard to the orientation of the axis of cell division, they found that the cleavage plane of dividing cells near the wound tended to be perpendicular to the direction of the endogenous wound-induced electrical field; that is, the cleavage plane was parallel to the margin of the wound. This effect was strongest on cells residing within 200 μm of the edge of the wound and,

by 600 μm from the wound, the orientation of cell division was random. If the TEP (and thus the endogenous electrical field) was increased by a factor of 3–4 by the addition of prostaglandin E₂ or aminophylline, the degree of orientation of cell division was increased at all distances from the wound up to 600 μm . In contrast, treatment with ouabain to reduce the TEP to 10% of control levels randomized the plane of cell division at all distances. Interestingly, the rate of cell division was also influenced by the magnitude of the endogenous electrical field. The maximum rate of cell division was found at 600 μm from the wound and increasing or decreasing the TEP significantly increased or decreased the rate of cell division, respectively. Finally, they assessed the effect of changing the endogenous field on the rate of closure of a circular wound to the cornea. Again, the rate of healing was increased by increasing the TEP and decreased by decreasing the TEP.

Repair in the cornea does not involve only epithelial cells. The cornea is richly innervated and neurons must reestablish themselves following a wound. As discussed in the Introduction, it is well established that cultured neurons, including sensory neurons, tend to grow toward the cathode in an applied electrical field. The corneal system offers an ideal situation for testing the effect of endogenous electrical fields on neuronal growth, because the endogenous field can be manipulated in a known way and also because the optical properties of the cornea favor observation and recording of neuronal growth. McCaig and colleagues⁽³⁾ have found that neuronal growth is stimulated by a wound to the cornea and

that the orientation of the growing neurites is strikingly parallel to the endogenous electrical field (perpendicular to the margin of the wound). Collapsing the TEP with ouabain dramatically reduced the number of new neurites and their organization.

Transport of charged molecules into the insect oocyte

The developing follicles of many insects consists of a syncytium that results from the incomplete cleavage of the cystoblast. All but one of the daughter cells become polyploid nurse cells and nourish the remaining cell, which becomes the growing oocyte. Woodruff and Telfer⁽³³⁾ some years ago showed that cytoplasm of the oocytes of the moth, *Hyalophora cecropia*, are about 5–10 mV positive with respect to cytoplasm of the nurse cells (Fig. 1C). This difference in potential is focused entirely across the cytoplasmic bridges that connect the oocyte to the nurse cells. The bridges are about 30 μm in diameter and perhaps 50 μm long, so the electrical field in the bridges is some 200 mV mm^{-1} . In a channel of these dimensions, it is unlikely that there is any structural selectivity or barrier to the free diffusion of molecules, including proteins and mRNA, and the electrical field across the barrier would be expected to inevitably affect the movement of charged macromolecules.

They tested this idea by injecting a positively charged, fluorescently labeled protein, lysozyme (Fly) and the negatively charged methyl-carboxylated lysozyme (McFly).⁽³⁴⁾ When microinjected into the nurse cells, Fly did not cross the bridges into the oocyte but, when microinjected into the oocyte, Fly diffused throughout the oocyte and crossed the bridges into the nurse cells. The behavior of McFly was precisely the opposite. It could cross the bridges from nurse cells to oocyte, but not from oocyte to nurse cells. These data provide strong evidence that endogenous electrical fields can markedly affect the distribution of proteins in vivo, and that the distribution is determined by the charge on the protein.

In order to address the question of whether electrical polarization of the cytoplasmic bridges was a general phenomenon, Woodruff and colleagues studied the 16-cell syncytium of *Drosophila melanogaster*. They found that the potential difference between the oocyte and nurse cells averaged 2.3 mV, with the oocytes being positive with respect to the nurse cells, and again the voltage drop was focused within the intracellular bridges.⁽³⁵⁾ This difference was highly reproducible and statistically significant at the 0.1% level. They also found that Fly and McFly had the same restricted diffusion as in *H. cecropia*, based on their respective charge, when microinjected into nurse cells and oocytes of *Drosophila* follicles. Further refinement showed that medium osmolarity was an important component of the culture method and, when it was carefully matched to the measured osmolarity of adult *Drosophila* hemolymph, a stable potential difference of 2.5 mV between nurse cells and oocytes was recorded, with the

oocyte positive with respect to the nurse cell.⁽³⁶⁾ It was also found that raising medium osmolarity to greater than optimal levels reversed the electrical polarity of the nurse cell–oocyte potential difference. It should be pointed out that the cytoplasmic bridges are shorter in the considerably smaller *Drosophila* follicle, compared to the *H. cecropia* follicle, so the electrical field across the bridges in the *Drosophila* follicle is of a comparable magnitude. These studies appear to have convincingly resolved the controversy⁽³⁷⁾ about the existence of a measurable nurse cell–oocyte potential difference in *Drosophila* in favor of its existence. This phenomenon is not limited to insects. Emanuelsson and Arlock⁽³⁸⁾ measured the voltage difference between the single nurse cell and the oocyte of a polychaete, *Ophryotrocha labronica*, and found that the oocyte was about 30 mV positive with respect to the nurse cell. This robust voltage gradient across the 3 μm wide cytoplasmic bridge between oocyte and nurse cell indicates a much larger electrical field in the polychaete bridge, compared to that in the insect.

As compelling as the evidence is for electrophoresis of exogenous proteins in the insect follicle, it remained to be shown that endogenous proteins were electrophoretically distributed. Recently, the distribution of endogenous proteins in the *Drosophila* follicle was studied.⁽³⁹⁾ Using two-dimensional gel electrophoresis, they identified twelve soluble acidic proteins and seven soluble basic proteins that were present in both oocytes and nurse cells. When the polarity of the nurse cell–oocyte transbridge electrical field was reversed by raising external osmolarity, the concentration of seven of the acidic proteins in the oocyte decreased while the concentration of all twelve acidic proteins in the nurse cells increased. Reversal of the electrical field also caused the concentration of all seven of the basic proteins in the oocytes to increase and the concentration of four of the seven basic proteins to decrease in the nurse cells. These results clearly demonstrated that the distribution of soluble proteins in the complex could be modulated both by the charge on the particular protein and the electrical polarity of the bridge.

Electrical guidance of motile plant pathogens at the root surface

In the three cases considered above, the electrical fields act within the organism in which they are generated. However, at the interface of an organism with its environment, surface-generated electrical currents will produce electrical fields in the environment to which other organisms may respond. A well-studied case is the interaction of oomycete plant pathogens of the genera *Phytophthora* and *Pythium* with roots of their hosts. Zoospores of plant pathogens, *Phytophthora palmivora* and of *Pythium aphanidermatum*, exhibit opposite responses to applied electrical fields in vitro, anodal and cathodal, respectively.⁽⁴⁰⁾ They are quite sensitive in this regard and produce a measurable directional response at fields as low as

0.2 mV mm⁻¹. Their ability to sense an electric field seems to be related to a charge dipole across the body of the zoospore and the stimulation of zoospore turning by electric fields.⁽⁴⁰⁾

It is well established that growing roots of higher plants produce currents that are detectable in the surrounding medium.⁽⁴¹⁾ The pattern of the currents varies, depending on growth conditions and species. However, a wound to the root always results in inward current at the site of the wound⁽⁴²⁾ and, because of the high resistivity of the soil water, fields as large as 50 mV mm⁻¹ are generated in the soil water near the wound.⁽⁴³⁾ This suggests that the distribution of zoospores on the surface of roots may be modulated by the endogenous growth and wound currents. This possibility has recently been directly tested by Neil Gow and his colleagues,⁽⁴³⁾ taking advantage of the fact that the zoospores of *P. palmivora* and *P. aphanidermatum* exhibit different accumulation profiles on growing and wounded roots. They found a striking correlation between the sites of accumulation and the *in vitro* electrostatic responses of the zoospores. For example, *P. aphanidermatum* zoospores showed a massive accumulation at the site of the wound in a rye grass root while *Phytophthora palmivora* did not accumulate at the wound but rather in the adjacent regions. As the wound is a current sink, the cathodally galvanotactic *P. aphanidermatum* zoospores would be expected to be attracted to the wound while the anodally galvanotactic *P. palmivora* zoospores would be expected to be excluded from the wound but attracted to the adjacent areas. By GFP labeling of *P. palmivora*, they were able to distinguish the species in a mixed population of *P. palmivora* and *P. aphanidermatum* zoospores, and they found that the initially homogeneous mixed population segregated spatially at the surface of a wounded root according to the respective galvanotactic properties (Figs. 1D, 3). The accumulation of *P. aphanidermatum* zoospores at the site of a wound could be reversed by placing an artificial current sink, in the form of a glass microelectrode, adjacent to a part of the root surface that was normally repellent for these zoospores. These experiments and others in which the endogenous fields were modulated led to the conclusion “that electrical signals can augment or override chemical ones in mediating short-range tactic responses of oomycete zoospores at root surfaces”.⁽⁴³⁾

Concluding discussion

The four examples discussed above make a compelling case for the participation of endogenous electrical fields as directional signals in a variety of important biological processes. A common feature of these endogenous fields is that they are all generated by the combined action of groups of cells acting as current sources in parallel, with the currents passing through a localized, relatively high resistance pathway in order to generate substantial electrical fields. While many polarized cells drive large currents through themselves, there is scant evidence of significant voltage differences within the cytoplasm of a

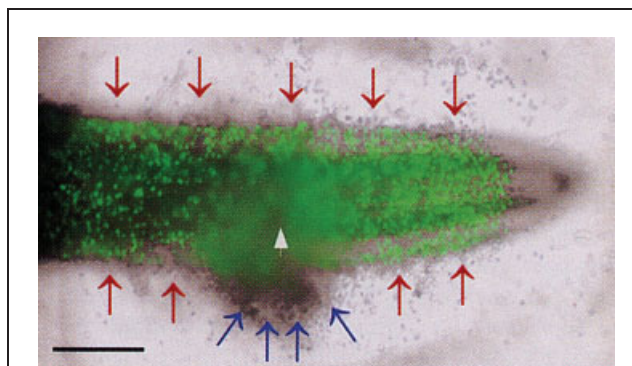


Figure 3. The pattern of accumulation of zoospores near a wound in a rye root. GFP-labeled zoospores of *P. palmivora* were added to an initially unwounded root and they accumulated around the distal tip of the root (red arrows), which is cathodic in the intact organ. The root was then wounded (white arrow), creating an anodic site, and unlabeled zoospores of *P. aphanidermatum* were added. The root was photographed 10 minutes later. The intense accumulation of non-GFP *P. aphanidermatum* zoospores near the wound site is marked with blue arrows. Reprinted with permission from van West P, Morris BM, Reid B, Appiah AA, Osborne MC, Campbell TA, Shepherd SJ, Gow NAR. Oomycete plant pathogens use electric fields to target roots. *Mol Plant Microbe Interact* 2002;15:790–798.

single cell. The growing pollen tube, for example, produces large currents that enter the growing tip and leave the more distal regions.^(44,45) Despite extensive effort, attempts to measure cytoplasmic voltage gradients along the pollen tube axis have yielded negative results (K.R.R., unpublished results). Thus, it would seem that the earlier suggestion of intracellular “self-electrophoresis”⁽⁴⁶⁾ does not yet have experimental support. In one case, however, there is evidence of a significant voltage gradient associated with a single cell. The plasma membrane of the *Drosophila* egg drives a large current that enters the anterior end and leaves the posterior end. The extracellular leg of this current passes through the restricted space between the plasma membrane and the vitelline layer, and generates measured voltage differences of about 5 mV between the two poles of the egg.⁽⁴⁷⁾ This corresponds to a field of more than 10 mV/mm in the perivitelline space, which could affect the distribution of soluble proteins in the space as well as the distribution of transmembrane proteins in the plasma membrane. Altogether, the general idea of endogenous currents as control elements in the spatial organization of living organisms^(48–50) has amassed considerable experimental support.

The focal point of the endogenous fields is the locus of the main high resistance element in the circuit and is different in each of the four cited examples. In left/right asymmetry, it appears to be the gap junctions coupling embryonic cells; in corneal wound healing, it is the restricted space just beneath

the electrogenic epithelium; in insect follicle growth, it is the cytoplasmic bridges between nurse cells and the oocyte; in roots, it is the very dilute surrounding water. The electrical circuitry acts to channel currents that are generated by a large surface of ion-transporting membranes into a restricted space or a low-conductivity space where fields are generated that are sufficiently large to affect the distribution of molecules or the direction of movement or growth of cells.

In two of the examples, embryonic left/right asymmetry and the developing insect oocyte, cell growth or movement is not directly involved. Some molecule small enough to move through gap junctions is implicated in left/right asymmetry. If two coupled cells differ in their membrane potentials by only a few millivolts, the electrical field in the gap junction will be of the order of 10^5 mV mm⁻¹, which is certainly large enough to affect the distribution of small charged molecules. The estimated field in the cytoplasmic bridges of the insect follicle is of the order of 100 mV mm⁻¹. On theoretical grounds, it can be shown that a field as large as that can significantly affect the distribution of a typical charged protein. Bovine serum albumin (BSA), a well-characterized protein, has a diffusion coefficient (D) of 6×10^{-7} cm² s⁻¹ and an electrophoretic mobility (μ) of -1.0×10^{-4} cm² s⁻¹ V⁻¹ at pH 7. It can be shown that a field of 20 mV mm⁻¹ will produce a twofold difference in concentration over a distance of 200 μ m when electrophoretic movement is in steady state with back diffusion.⁽⁵¹⁾ The size of the gradient will depend on the field as well as the ratio of μ to D. The movement of fluorescent BSA in an agarose gel under the influence of an electrical field has been studied. Measurable asymmetry in the diffusion of a bolus of BSA injected into a slab of agarose gel could be induced by a field as small as 25 mV mm⁻¹.⁽⁵²⁾ When fluorescent BSA was injected into the flank of a *Xenopus* embryo, where endogenous fields of up to 40 mV mm⁻¹ have been measured,⁽¹⁴⁾ asymmetric movement along the electric field vector was observed.⁽⁵²⁾ Thus, there is ample theoretical and experimental evidence to conclude that electrical fields of the magnitude regularly measured in or near living organisms (10–100 mV mm⁻¹) can produce asymmetries in the diffusion of charged macromolecules against the leveling action of diffusion. In this view, endogenous electrical fields can be seen as modulators of diffusive gradients that are already recognized as important factors in establishing morphogenetic gradients.^(53,54)

In order to extend these ideas, it will be necessary identify specific molecules that are actually asymmetrically distributed by electric fields in a developing or regenerating system, and to show that the electrically mediated asymmetry is causally related to a developmental asymmetry. The study of left/right asymmetry holds particular promise in this regard. The involvement of gap junctions restricts the range of possible molecules to about 1 kDa or less, and the electrically mediated events are quite restricted temporally, so the search for specific molecules can be narrowed considerably.

In the other two examples of endogenous electrical field effects discussed here, cornea wound healing and fungal zoospore–root interaction, the primary target of the fields appears to be the cells directly, not molecules in the extracellular space. In both cases, the responding cells have been shown to move or grow along the electric field vector in the absence of chemical gradients. The mechanism by which cells sense and respond to small electrical fields is not known and is an important area for future research. In addition, the possibility cannot be excluded that the epithelial cells and the neurons that respond to the endogenous field in the cornea are influenced by gradients of proteins (e.g., growth factors) that are also modulated by the endogenous field. The chemical gradients may augment or otherwise modulate the cells' intrinsic responses to the fields. In any case it must be recognized that endogenous electrical fields of 10–100 mV mm⁻¹ are a common feature of development, growth and wounding. The laws of physics insist that there will be consequences of the fields in the distribution of diffusible, charged molecules. The *in vitro* data clearly show that many cells respond directionally to such fields. Biologists can no longer elide these facts in their considerations of the mechanisms involved in establishing polarities in development, repair and host–pathogen interactions.

References

- Hinkle L, McCaig CD, Robinson KR. The direction of growth of differentiating neurones and myoblasts from frog embryos in an applied electric field. *J Physiol (Lond)* 1981;314:121–135.
- Patel N, Poo MM. Orientation of neurite growth by extracellular electric fields. *J NeuroSci* 1982;2:483–496.
- McCaig CD, Rajnicek AM, Song B, Zhao M. Has electrical growth cone guidance found its potential? *Trends NeuroSci* 2002;25:354–359.
- Stump RF, Robinson KR. *Xenopus* neural crest cell migration in an applied electrical field. *J Cell Biol* 1983;97:1226–1233.
- Cooper MS, Keller RE. Perpendicular orientation and directional migration of amphibian neural crest cells in DC electrical fields. *Proc Natl Acad Sci USA* 1984;81:160–164.
- Gruher H, Nuccitelli R. Neural crest cell galvanotaxis: new data and a novel approach to the analysis of both galvanotaxis and chemotaxis. *Cell Motil Cytoskeleton* 1991;19:121–133.
- Cooper MS, Schliwa M. Motility of cultured fish epidermal cells in the presence and absence of direct current electrical fields. *J Cell Biol* 1986; 102:1384–1399.
- Wang C, Rathore KS, Robinson KR. The responses of pollen to applied electrical fields. *Dev Biol* 1989;136:405–410.
- Ferrier J, Ross SM, Kanehisa J, Aubin JE. Osteoclasts and osteoblasts migrate in opposite directions in response to a constant electrical field. *J Cell Physiol* 1986;129:283–288.
- Jaffe LF, Poo MM. Neurites grow faster towards the cathode than the anode in a steady field. *J Exp Zool* 1979;209:115–128.
- Borgens RB, Robinson KR, Vanable JW, Jr., McGinnis ME. *Electrical Fields in Vertebrate Repair*. New York: Alan R. Liss, Inc. 1989. 310 pages.
- Barker AT, Jaffe LF, Vanable JW, Jr. The glabrous epidermis of cavies contains a powerful battery. *Am J Physiol* 1982;242:R358–R366.
- Hotary KB, Robinson KR. Endogenous electrical currents and the resultant voltage gradients in the chick embryo. *Dev Biol* 1990;140: 149–160.
- Hotary KB, Robinson KR. Endogenous electrical currents and voltage gradients in *Xenopus* embryos and the consequences of their disruption. *Dev Biol* 1994;166:789–800.

15. Hotary KB, Robinson KR. Evidence of a role for endogenous electrical fields in chick embryo development. *Development* 1992;114:985–996.
16. Levin M, Johnson RL, Stern CD, Kuehn M, Tabin C. A molecular pathway determining left-right asymmetry in chick embryogenesis. *Cell* 1995;82:803–814.
17. Levin M, Pagan S, Roberts DJ, Cooke J, Kuehn MR, Tabin CJ. Left/right patterning signals and the independent regulation of different aspects of situs in the chick embryo. *Dev Biol* 1997;189:57–67.
18. Yost HJ. Establishment of left-right asymmetry. *Int Rev Cytol* 2001;203:357–381.
19. Mercola M, Levin M. Left-right asymmetry determination in vertebrates. *Annu Rev Cell Dev Biol* 2001;17:779–805.
20. Levin M, Mercola M. Gap junctions are involved in the early generation of left-right asymmetry. *Dev Biol* 1998;203:90–105.
21. Levin M, Mercola M. Gap junction-mediated transfer of left-right patterning signals in the early chick blastoderm is upstream of Shh asymmetry in the node. *Development* 1999;126:4703–4714.
22. Levin M, Thorlin T, Robinson KR, Nogi T, Mercola M. Asymmetries in H⁺/K⁺-ATPase and cell membrane potentials comprise a very early step in left-right patterning. *Cell* 2002;111:77–89.
23. Pennekamp P, Karcher C, Fischer A, Schweickert A, Skryabin B, Horst J, Blum M, Dworniczak B. The ion channel polycystin-2 is required for left-right axis determination in mice. *Curr Biol* 2002;12:938–943.
24. McCaig CD, Robinson KR. The ontogeny of the transepidermal potential difference in frog embryos. *Dev Biol* 1982;90:335–339.
25. Robinson KR, Stump RF. Self-generated electrical currents through *Xenopus* neurulae. *J Physiol (Lond)* 1984;352:339–352.
26. Rajniecek AM, Stump RF, Robinson KR. An endogenous sodium current may mediate wound healing in *Xenopus* neurulae. *Dev Biol* 1988;128:290–299.
27. Klyce SD. Electrical profiles in the corneal epithelium. *J Physiol (Lond)* 1972;226:407–429.
28. Chiang M, Robinson KR, Venable JW, Jr. Electrical fields in the vicinity of epithelial wounds in the isolated bovine eye. *Exp Eye Res* 1992;54:999–1003.
29. Sta. Iglesia DD, Venable JW, Jr. Endogenous lateral electric fields around bovine corneal lesions are necessary for and can enhance normal rates of wound healing. *Wound Repair Regen* 1998;6:531–542.
30. Zhao M, Agius-Fernandez A, Forrester J, McCaig C. Orientation and directed migration of cultured corneal epithelial cells in small electric fields are serum dependent. *J Cell Sci* 1996;109:1405–1414.
31. Zhao M, Forrester JV, McCaig CD. A small, physiological electric field orients cell division. *Proc Natl Acad Sci USA* 1999;96:4942–4946.
32. Song B, Zhao M, Forrester JV, McCaig CD. Electrical cues regulate the orientation and frequency of cell division and the rate of wound healing in vivo. *Proc Natl Acad Sci USA* 2002;99:13577–13582.
33. Woodruff RI, Telfer WH. Polarized intracellular bridges in ovarian follicles of the *Cecropia* moth. *J Cell Biol* 1973;58:172–188.
34. Woodruff RI, Telfer WH. Electrophoresis of proteins in intercellular bridges. *Nature* 1980;286:84–86.
35. Woodruff RI, Kulp JH, Lagaccia ED. Electrically mediated protein movement in *Drosophila* follicles. *Roux Arch Dev Biol* 1988;197:231–238.
36. Singleton K, Woodruff RI. The osmolality of adult *Drosophila* hemolymph and its effect on oocyte-nurse cell electrical polarity. *Dev Biol* 1994;161:154–167.
37. Sun YA, Wyman RJ. Lack of an oocyte to nurse cell potential difference in *Drosophila*. *Neuroscience* 1987;13:1139.
38. Emanuelsson H, Arlock P. Intracellular voltage gradient between oocyte and nurse cell in a polychaete. *Exp Cell Res* 1985;161:558–561.
39. Cole RW, Woodruff RI. Vitellogenic ovarian follicles of *Drosophila* exhibit a charge-dependent distribution of endogenous soluble proteins. *J Insect Physiol* 2000;46:1239–1248.
40. Morris BM, Gow NAR. Mechanism of electrotaxis of zoospores of phytopathogenic fungi. *Phytopathology* 1993;8:877–882.
41. Weisenseel MH, Dorn A, Jaffe LF. Natural H⁺ currents traverse growing roots and root hairs of barley (*Hordeum vulgare* L.). *Plant Physiol* 1979;64:512–518.
42. Hush JM, Newman IA, Overall RL. Utilization of the vibrating probe ion-selective microelectrode techniques to investigate the electrophysiological responses to wounding in pea roots. *J Exp Bot* 1992;176:56–64.
43. van West P, Morris BM, Reid B, Appiah AA, Osborne MC, Campbell TA, Shepherd SJ, Gow NAR. Oomycete plant pathogens use electric fields to target roots. *Mol Plant Microbe Interact* 2002;15:790–798.
44. Weisenseel MH, Nuccitelli R, Jaffe LF. Large electrical currents traverse growing pollen tubes. *J Cell Biol* 1975;66:556–567.
45. Messerli M, Robinson KR. Cytoplasmic acidification pulses and current influx follow growth pulses of *Lilium longiflorum* pollen tubes. *Plant J* 1998;16:87–93.
46. Jaffe LF, Robinson KR, Nuccitelli R. Local cation entry and self-electrophoresis as an intracellular localization mechanism. *Ann NY Acad Sci* 1974;238:372–389.
47. Overall R, Jaffe LF. Patterns of ionic current through *Drosophila* follicles and eggs. *Dev Biol* 1985;108:102–119.
48. Nuccitelli R. Ionic currents in morphogenesis. *Experientia* 1988;44:657–666.
49. Jaffe LF. Control of development by steady ionic currents. *Fed Proc* 1981;40:125–127.
50. Robinson KR. The responses of cells to electrical fields: a review. *J Cell Biol* 1985;101:2023–2027.
51. Robinson KR, Messerli MA. Electric embryos: the embryonic epithelium as a generator of developmental information. In: McCaig CD, editor. *Nerve Growth and Guidance*. London Portland Press, Ltd. 1996. p 131–150.
52. Messerli M, Robinson KR. Endogenous electrical fields affect the distribution of extracellular protein in *Xenopus* embryos. *Mol Biol Cell* 1997;8:1296.
53. Crick F. Diffusion in embryogenesis. *Nature* 1970;225:420–422.
54. McDowell N, Zorn AM, Crease DJ, Gurdon JB. Activin has a direct long-range signalling activity and can form a concentration gradient by diffusion. *Curr Biol* 1997;7:671–681.