control littermates, aceruloplasminemic mice sustained significantly greater liver injury in this ischemia model, and the extent of liver damage was substantially reversed by administration of exogenous NO_2^-.

Copper is no stranger to NO_2^- biology. In denitrifying organisms, NO_2^- reduction to NO is accomplished by copper-containing nitrite reductases, and in mammalian mitochondria cytochrome *c* oxidase can generate NO in a copper- and nitrite-dependent reaction critical to hypoxic gene induction[7]. Nevertheless, the current findings address an important gap in our rapidly evolving knowledge of NO_2^- biology by providing convincing evidence of an enzymatic mechanism for NO_2^- production in the plasma. The authors did not address NO_2^- production outside the plasma, but given the abundance of GPI-linked ceruloplasmin in glial cells and macrophages, analysis of the role of ceruloplasmin and NO_2^- in neuronal function and host defense will surely follow. Indeed, ceruloplasmin is synthesized in a wide variety of tissues and cell types not directly implicated in iron homeostasis[8], which raises the possibility that they are sites of NO_2^- production.

This discovery reveals an intersection of the pathways of copper and NO homeostasis and raises questions relevant to the study of both pathways (**Fig. 1**). Could the pathogenesis of tissue injury in iron-overload syndromes be dependent on NO_2^-? Is copper homeostasis regulated by NO_2^- pathways in tissues? What is the relationship between the microbiomes of the oral and stomach cavities, in which nitrite is produced by nitrate-reducing bacteria, and how is this relationship affected by alterations in copper and NO_2^- content in the diet? The studies of Shiva *et al.* remind us that the pathways comprising the inorganic chemistry of living organisms do not exist in isolation, having evolved through opportunity and selection over millions of years to permit the complex and precise interplay necessary to regulate our physiology.

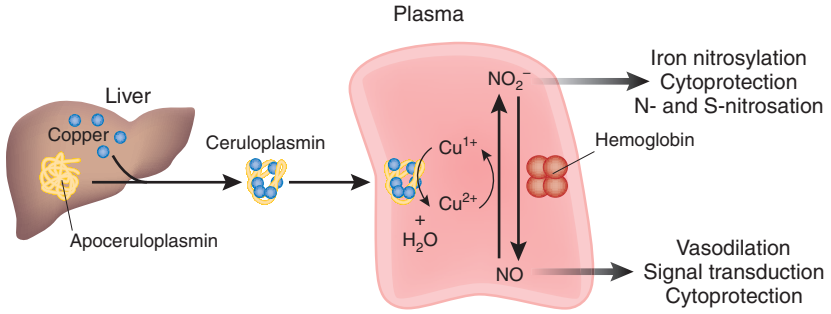

Figure 1 Ceruloplasmin is a multicopper oxidase that is synthesized and secreted by the liver into the plasma. Copper is incorporated into ceruloplasmin during synthesis and is essential for oxidase activity. Within the plasma, ceruloplasmin catalyzes the oxidation of the signaling molecule NO concomitantly with cupric (Cu^{2+}) to cuprous (Cu^{1+}) reduction. Nitrite (NO_2^-) ions can therefore be used as a sink for NO production through reduction by deoxyhemoglobin, which allows for the mobilization of NO as a signaling molecule involved in hypoxic vasodilation and ischemia-reperfusion cytoprotection. In addition, nitrite acts independently as a signaling molecule necessary for cytoprotection and post-translational modifications such as iron nitrosylation and N- and S-nitrosation.

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Charging the batteries to heal wounds through PI3K

Bart Vanhaesebroeck

Endogenous electric fields in wounds have been documented for centuries, but they have received little attention from the scientific community. A new study shows that manipulation of these electric fields affects wound healing *in vivo* and identifies the phosphoinositide 3-kinase signaling pathway as a key component of cell migration in response to electric cues.

Our daily routines would be impossible without the wonders of electricity. This also applies to the cells in our bodies, each of which generates internal mini-currents to carry out its functions. It is less well known that cells in tissues (for example in our skin) can team up to form 'biological batteries' that can generate current upon wounding of the tissue. Zhao *et al.*[1] now present evidence that this extracellular electric field is the driving force in wound healing, and they uncover signaling pathways that control this biological phenomenon.

All cells maintain a voltage gradient across their outer membrane (**Fig. 1**). Controlled changes in the ion permeability of the membrane lead to rapid alterations in voltage across the membrane, thereby generating action potentials. These trigger nerve cells to transmit signals, muscle cells to contract and gland cells to secrete hormones. Efforts from electrophysiologists and others have provided us with a deep understanding of the molecular details of these events.

In contrast, the existence of steady, extracellular voltage gradients in tissues has received far less attention, even though their discovery in the nineteenth century predates that of cellular action potentials[2]. The potential difference across the layers of an epithelium (such as skin) derives from an asymmetric transport

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of ions across the layers. When the skin is cut, this transepithelial potential difference is short-circuited, inducing current to flow out of the lesion from underneath the wounded epithelium and giving rise to a steady electric field at the wound edge[2] (**Fig. 1**).

Zhao *et al.* have now confirmed the presence of endogenous wound electric fields in a wide diversity of model systems, including organ cultures of stratified epithelia such as skin and cornea. These electric fields persist for several hours, with the flow of positive charge directed toward the wound center (**Fig. 1**).

The authors explored the ways electric fields control wound healing, first by using an assay in which a small area of a monolayer of epithelial cells is scratched. Over time, the neighboring cells then migrate and fill the damaged area. Application of an exogenous electric field, depending on polarity, led to faster wound closure or, notably, made cells move away from the wound. Similar responses were observed in the cornea whole-organ culture model. These new results support the notion that the electrical signal overrides other, coexisting closure-promoting signals such as the release of growth factors and loss of contact inhibition. Thus healing rates can be controlled by electrical cues.

An important question is, How does electrotaxis relate to chemotaxis, the movement of cells according to gradients of chemical cues in their environment? Much like chemotaxing cells[3], electrotaxing cells change shape and become elongated and polarized. Could the electric field lead to a buildup of chemical gradients in the extracellular milieu that is then sensed by the cells via chemotactic receptors on their surfaces? This may not be a key controlling factor, as Zhao *et al.* found that electrotaxis was unaffected when fresh culture medium was flushed perpendicularly to the electrical vector. They also observed that a mutant strain of a slime mold that can no longer chemotax (owing to the absence of a Gβ subunit, a signaling protein linked to receptors for chemoattractants) retained the capacity to electrotax.

Despite these differences, the authors found that chemotaxis and electrotaxis share a dependence on similar intracellular signaling molecules, most notably the phosphoinositide 3-kinase (PI3K) enzymes. The lipids generated by these kinases in response to receptor stimulation are asymmetrically distributed in the membranes of chemotaxing cells[4]. Lipid phosphatases such as phosphatase and tensin homolog (PTEN) are key in establishing this intracellular lipid gradient. The authors found that PI3K-generated lipids become polarized inside cells under the influence of an electric field, to a degree similar to that observed in chemotaxing cells. They further present genetic and pharmacological evidence supporting a role for PI3K in electrotaxis: a small-molecule inhibitor of PI3K blocked electrotaxis, as did deletion of p110γ, one of the isoforms of PI3K. Deletion of PTEN, a negative regulator of PI3K, enhanced electrotaxis.

Several important questions remain to be answered. Although key biochemical components controlling electrotaxis have now been identified, the ways in which these molecules become polarized in an electric field are not clear. For example, what determines the polarization of PI3K lipids in the electrotaxing cells? Could this simply be an electric field-induced asymmetric redistribution of cellular components, be it receptors, second messengers or signaling proteins? Evidence supporting this hypothesis has been presented[1,5,6]. It will also be interesting to apply the power of genetic screens in a variety of model systems to uncover additional genes involved in this phenomenon.

Last but not least, the p110γ isoform of PI3K, found by Zhao *et al.*[1] to be a key player in wound healing responses, is currently very actively pursued by the pharmaceutical industry as a new anti-inflammatory target[7]. It will therefore be of interest to test whether wounds heal slower in p110γ-null mice, and to test the effect of p110γ pharmacological inhibitors on wound healing.

Poor wound healing (for example, in diabetes) is an important health-care issue, and hence new wound healing therapies are needed. Could the observations of Zhao *et al.* be used to improve wound healing? Previous attempts to apply electric fields to treat nonhealing skin wounds by insertion of metal electrodes directly into wound beds have had mixed success[8,9]. The authors present evidence that there may be no need to resort to electrodes. Indeed, they find that manipulation of transepithelial ion transport in cornea wounds using agents known to alter ion transportation leads to changes in the transepithelial potential and results in augmented or decreased healing. Chemical intervention during wound healing with agents that affect ion transport may therefore

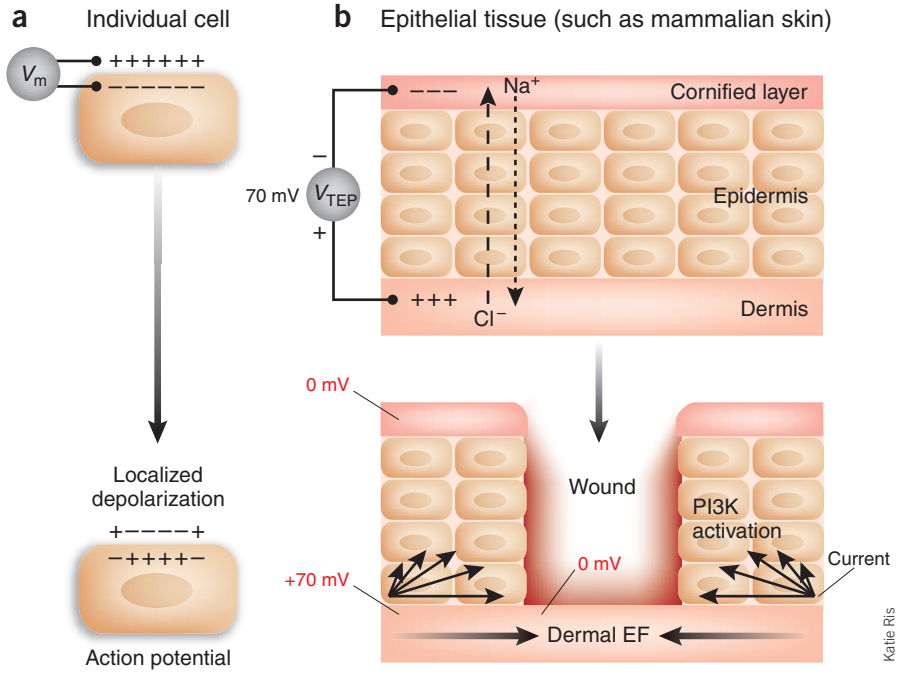

Figure 1 Action potential in individual cells and injury potential in tissues. (**a**) Individual cells maintain an electrical potential across the plasma membrane (V_m) as a result of the activity of membrane-bound ion channels. This results in a net negative charge on the inside of the cell relative to the outside. This resting membrane potential can be locally depolarized under the influence of cell stimuli, leading to an inward current (bottom). (**b**) Schematic representation of the generation of a transepithelial potential (V_{TEP}) in human skin (individual cells in cornified layer and dermis are not shown). Selective, directional ion transport across the intact epithelium gives rise to a V_{TEP} that can be measured directly across the epithelium (top; 70 mV in this case). Tight junctions between epithelial cells (not shown) create physical connections between cells, providing high electrical resistance to the epithelial sheet. Wounding of an epithelial sheet results in collapse of the V_{TEP} at the wound (to 0 mV) without affecting the V_{TEP} distally (70 mV). Na^+ leaks out of the wound, resulting in an injury current toward the cut (thin arrows) and a lateral voltage gradient oriented parallel to the epithelial sheet (EF, electric field; thick arrows at bottom). The wound site is the cathode of the electric field (bottom). Figure is based on refs. 1 and 2.

be a more practical avenue for enhancing wound healing.

The findings of Zhao *et al.* are provocative and will most likely be met with some skepticism, but they will hopefully also stimulate researchers to have a closer look at electrotaxis. It will be critical for the authors to make their technological expertise widely available so that others may embark on these investigations, not only in the interest of basic science but also in the interest of human therapy, which could benefit tremendously.

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One-way traffic control in replication termination

Karsten Theis

Tus proteins bound to multiple *ter* sequences on DNA determine the site of DNA replication termination in *Escherichia coli*. Biochemical and structural studies reveal how the Tus–*ter* complex arrests replication forks in a direction-sensitive manner.

Before cell division, the circular genomic DNA in *E. coli* is duplicated by two replisomes starting at a single origin and moving in opposite directions (**Fig. 1**); each one takes ~40 min to replicate its half of the 3.9-million-base-pair genome. If a replisome dissociates prematurely (for example, because of DNA damage), incomplete DNA copies that are potentially lethal to the cell are released. Tight replisome-DNA interactions are therefore vital during replication. However, when the two replisomes meet again after 40 min of replication, they have to disassemble in a process called termination. In *E. coli*, termination is regulated by the Tus protein, which binds to multiple *ter* sites on the DNA. Each Tus–*ter* complex acts as a one-way sign for replication, letting replisomes pass in one direction but not the other (**Fig. 1**). Although the Tus–*ter* complex has been studied extensively[1], exactly how it establishes directionality has remained unclear. Now, Mulclair *et al.*[2] show that fork opening tightens Tus-*ter* interactions if and only if it occurs from the nonpermissive side, thereby resulting in a direction-sensitive roadblock that regulates the site of replication termination.

Replisomes are powerful molecular machines propelled by one helicase and two polymerase motors[3]. They have the highest priority in accessing DNA during fork progression, and they strip away any previously bound proteins.

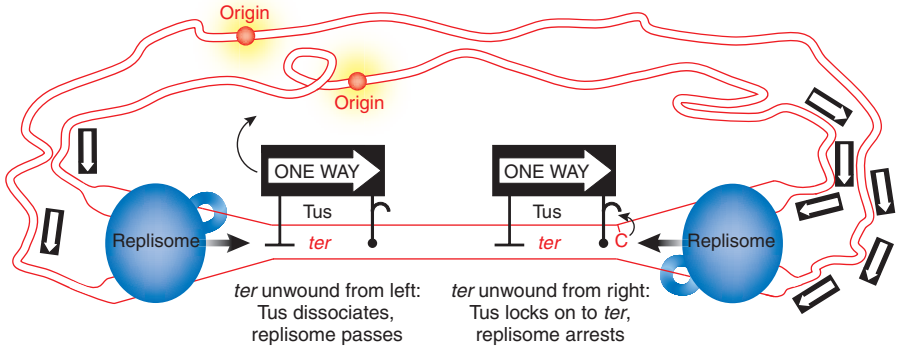

Figure 1 Traffic control for replisomes: two replisomes work in opposite directions to replicate circular genomic DNA (red lines) of *E. coli*. The region in which the two replisomes terminate is defined by multiple *ter* sites on the DNA, to which the Tus protein (one-way sign) binds. Mulcair *et al.*[2] show that Tus-*ter* interactions are substantially strengthened upon *ter* unwinding from the right; this effect is independent of Tus-replisome interactions and is attributed to a binding site on Tus for a conserved cytosine in the *ter* sequence that is flipped out of its DNA duplex orientation when unwound (see complex on the right). In the cartoon, the replisome on the right has reached its final destination and is arrested, whereas the one on the left still needs to pass two Tus–*ter* complexes.

Arresting replisomes artificially by a roadblock is lethal to cells and requires placing an array of binding proteins on the genome[4]. How does the Tus protein, a 36-kDa monomeric protein, arrest the replisome during replication, and moreover in a direction-sensitive manner? Two types of mechanisms have been proposed[1]: (i) protein-protein interactions between Tus and the helicase allosterically inhibit the helicase in one direction of replisome approach and lead to dissociation of Tus in the other, and (ii) fork opening and associated changes in DNA structure from one direction lead to Tus dissociation but do not affect Tus-*ter* binding in the other, thereby blocking the replisome.

To test the latter model, Mulcair *et al.*[2] studied the effect of fork opening on Tus binding by systematically varying the secondary structure of the DNA ligand. Binding to immobilized 21-mer *ter* duplexes was monitored via surface plasmon resonance, yielding binding kinetics and equilibrium constants. Sequence mismatches introduced on either end of the duplex to mimic fork opening had a considerable effect on binding. As predicted by the model, fork opening from the permissive side (**Fig. 1**, left) weakened binding. Surprisingly, fork opening from the nonpermissive side (**Fig. 1**, right) resulted in tighter binding, effectively locking Tus to the forked *ter* site. Point mutations of a cytosine that is unpaired in the locked complex and strictly conserved in all *ter* sites, together with further experiments with partially truncated *ter* duplexes, showed that

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