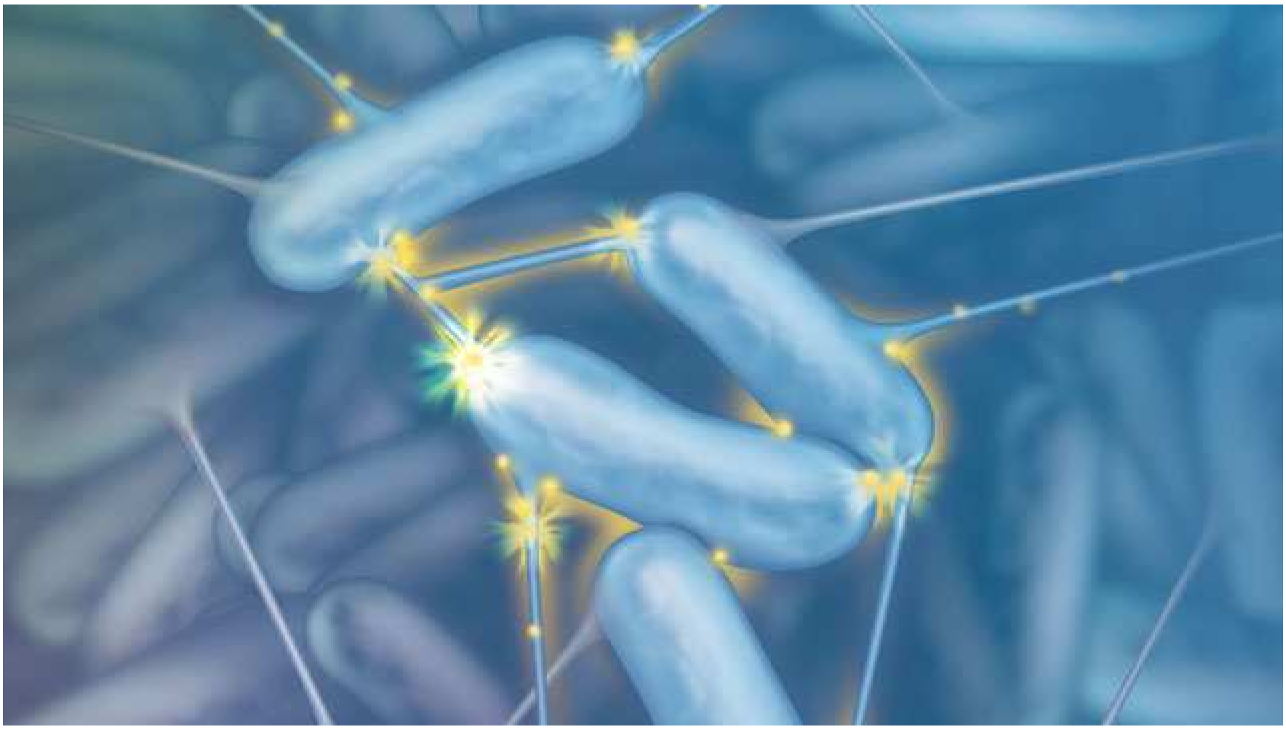
Live Wires

Discoveries of microbial communities that transfer electrons between cells and across relatively long distances are launching a new field of microbiology.

By Mohamed Y. El-Naggar and Steven E. Finkel | May 1, 2013



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Today's information age rests on a basic understanding of how electrons move. The remarkable success of computers, cell phones, and other devices, such as solar cells, depends on our ability to mediate the flow of electrons through the semiconductors and microchips that control the function of these machines and give them their intelligence. But the importance of electron flow is by no means limited to these man-made systems; electron transfer is also central to energy storage and conversion in living cells.

Organisms depend on the flow of electrons for key energy-generating cellular processes. Continuous electron flow is necessary for the formation of the electrochemical gradients that enable the synthesis of adenosine triphosphate (ATP), life's energy currency. In eukaryotes, including animals, this power generation is the specialty of mitochondria. But the same process is also at play in domains of life that lack internal organelles, namely archaea and bacteria, from which mitochondria evolved.

Unfortunately, in contrast to our detailed knowledge of the electron flow in popular solid-state electronics, our understanding of biological electron transport remains limited, especially when the distances traveled far exceed the length of a cell. Much is known about the mechanisms that enable electron transfer reactions between nearby molecules in very small spaces, such as the inner mitochondrial membrane; but bacteria, the planet's oldest organisms, are able to transfer the charged particles to a variety of acceptors, including some at great distances. For instance, we now know that some anaerobic bacteria gain energy through electron transfer to inorganic minerals, and even to synthetic surfaces, hundreds of cell-body lengths away.

So how do they do it? This basic question lies at the heart of electromicrobiology, a new research discipline that seeks to understand the transmission of electrical signals between microbes. The emerging answers—which include startling molecular conductors built by bacteria and unique population architectures in which thousands of microbes act in concert as a multicellular unit—point to the interesting role that electron transport has played in the evolution of the planet and its inhabitants. Understanding how bacteria transmit those signals may also inform the development of technologies that extract power from these living systems, by transmitting signals from and to cells at hybrid living-synthetic interfaces.

Rock breathers

Cells of aerobic organisms generate energy by passing electrons derived from food along a series of molecules called electron carriers. The resulting energy is used to pump protons across a membrane, creating a proton gradient that powers ATP synthesis. This process requires oxygen to serve as the final electron acceptor, and most aerobic organisms, including humans, will die if their oxygen supply is cut off.

But bacteria thrived on Earth for more than 1.5 billion years, before the atmosphere had accumulated sufficient levels of molecular oxygen to support aerobic life. At that time, iron and other metals would have served as common electron acceptors for microbes. This form of "rock-breathing" metabolism, known as extracellular electron transfer, is also discussed as a possible extraterrestrial metabolism that may conceivably support the existence of microbes on other iron-rich planets, such as Mars. And on Earth, a class of anaerobic microbes, called dissimilatory metal-reducing bacteria (DMRB), still survives by transferring electrons to metal oxides, such as iron and manganese minerals.

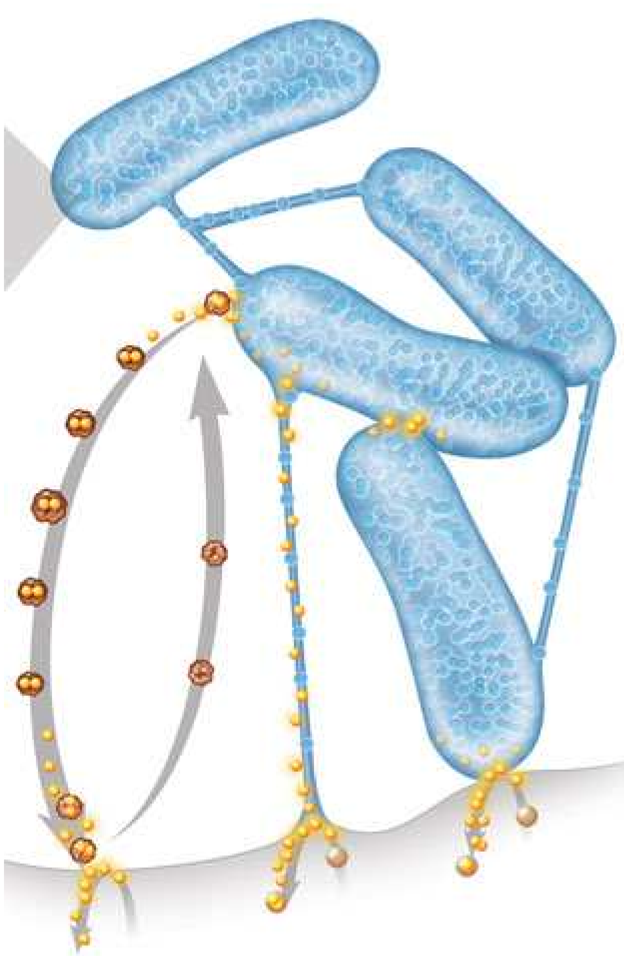
Scientists discovered the first metal-reducing bacteria, *Shewanella* and *Geobacter*, in the late 1980s. The DMRB metabolism, which couples biological electron transport chains to inorganic materials, gives us a unique opportunity to both study and harness such reduction-oxidation (redox) reactions at synthetic surfaces. In fact, if a synthetic electrode is poised at a favorable redox potential, it is possible to "trick" the metal-reducing bacteria into transferring their electrons to the electrode surface in the absence of any other electron acceptor. This not only provides a quantitative readout to study respiration in real time, it gives researchers precise control of the energetic redox conditions, thereby allowing them to direct the growth of the microbes, and even to culture some bacteria that may be difficult to grow in standard media.

These bacteria are also being heavily investigated as practical biological catalysts in renewable energy technologies that now attract millions of dollars annually in government and industry funding. Microbial fuel cells and bacterial batteries, for example, are constructed with microbes that oxidize diverse organic fuels—including waste products such as raw sewage—then route the resulting electrons to fuel-cell anodes, where the flow is converted into electricity. Another emerging technology is microbial electrosynthesis, which essentially runs the process in reverse by supplying microbes with renewable (e.g., solar) electrical energy in order to drive reductive microbial metabolisms for the synthesis of biofuels and other high-value chemicals. Both these technologies—fuel-to-electricity and electricity-to-fuel—rely on the ability of microbes to donate and accept electrons at synthetic surfaces.

Shuttles and wires

The curious aspect of extracellular electron transfer (EET) is that microbes are transferring electrons from the inside of the cell to the outside. In essence, these microbes are controlling the transfer of electrons outside their own cell bodies. One clue to how they do it has come from observations that metal-reducing bacteria localize electron transfer proteins called cytochromes to the outer cell membrane.[1] Cytochromes perform electron transfer reactions when the iron ions in their heme groups switch redox states, from reduced Fe^{2+} to oxidized Fe^{3+}, or vice versa. However, while most gram-negative bacteria and mitochondria confine these electron-transfer reactions to the inner membrane of the cell or organelle, Shewanella and Geobacter locate some of their heme-containing cytochromes on the outer membrane, where the molecules can access external, solid electron acceptors, such as a mineral surface. (See illustration here.)

In addition to this direct cell interaction with the surface, it has also been proposed that *Shewanella* secretes small redox-active molecules called flavins to act as electron shuttles from the cell to more distant electron receptors.[2] Flavins essentially swim laps, picking up electrons from the cell, dropping them off at a solid electron acceptor, and coming back to the cell for more—a cyclic process that is driven by diffusion. The distinction between these direct and indirect strategies is perhaps not as rigid as it once seemed, since we now also know that *Shewanella*'s outer-membrane cytochromes are necessary for the reduction of flavins and that they contain flavin binding sites.



ELECTRON SHUFFLE: *Shewanella* bacteria generate energy for survival by transporting electrons to nearby mineral surfaces. Cytochromes (structure depicted above) on the bacterial outer membranes contain a number of heme groups that accept and donate electrons, allowing the charges to flow along the membrane. Cytochromes also line cellular appendages known as pili that can conduct charges down their length to other microbes or to the mineral substrate. Additionally, the bacteria employ flavin molecules to work as electron shuttles, collecting electrons at the cell surface and carrying them to a nearby electron acceptor.
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In 2005 and 2006, microbiologists Gemma Reguera and Yuri Gorby, now at Michigan State University and Rensselaer Polytechnic Institute, respectively, and colleagues reported on another intriguing EET pathway found in both *Geobacter* and *Shewanella*.[3,4] Using atomic force and scanning tunneling microscopy techniques, they found that some of the bacteria's extracellular protein nanofilaments, called pili, are electrically conductive. Specifically, they showed that electrons could travel a few nanometers across the width of the pili, now commonly referred to as bacterial nanowires. In 2010, one of our own laboratories (Mohamed El-Naggar's) at the University of Southern California found that the nanowires are also conductive along their lengths, over micrometer-long distances. We used nanoscale metal electrodes to measure the conductivity of individual *Shewanella* nanowires, and found it to be on par with silicon conductivity, such that one nanowire can sustain the respiration rate of a single cell (estimated to require the transport of around 106 electrons per second). Meanwhile, we also found that mutant *Shewanella* cells incapable of making two types of outer-membrane cytochromes produced filaments that were not conductive, suggesting that the cytochromes are responsible for the nanowires' conductivity.[5]

The discovery of bacterial nanowires attracted the attention of many theoretical physicists and chemists. Proteins typically have low electron mobility—a measure of the ability of the electron to move in response to an electric field—and are therefore regarded as insulators rather than conductors over micrometer-long distances. How were these bacteria so efficiently conducting electrons along these protein-based appendages? Early insight came from Duke University molecular biophysicist David Beratan and his colleagues, who proposed that, based on the measured electron transport rates in bacterial nanowires, there must be a multistep mechanism in which electrons "hop" between charge carriers in a manner somewhat analogous to the movement of water in a bucket brigade. The carriers, such as the hemes within cytochromes, must be separated by less than 1 nm, they calculated.[6]

This view garnered support with the publication of the first crystal structure of MtrF, one of *Shewanella*'s cytochromes, showing edge-to-edge heme spacings of less than 0.7 nm.[7] More recently, we employed the multistep hopping model to directly calculate the current-voltage characteristics of a chain of hemes linking two electrodes and found the results to be in agreement with our conductivity measurements of bacterial nanowires, suggesting that hopping in redox chains is a viable strategy for this transport system.[8] This is an active area of research, and it is still unclear how cytochromes or other redox moieties are incorporated into bacterial pili to form functional bacterial nanowires. Progress in this area will benefit greatly from ongoing proteomics and structural biology approaches seeking to identify and resolve the organization of bacterial nanowire components.

Living power cables

An interesting aspect of this story is what happens when bacteria organize themselves in a 3-D biofilm community on an electron acceptor surface. This configuration creates an interesting dynamic between members of the biofilm community. Cells at the bottom of the biofilm have proximity to plenty of electron acceptors but have less access to chemical electron donors (i.e., food), which must diffuse through the dense biofilm from the surrounding growth medium. Cells exposed to the nutrient medium but farther away from the inorganic surface experience the opposite condition. The same situation can be set up with a biofilm sandwiched between two electrodes, one acting as an electron donor and the other as an acceptor. Do the cells on either side of the biofilm specialize in electron donation and uptake, respectively? This would require an actively respiring biofilm that is conductive enough to channel long-range electron flow between these two groups. In fact, a number of recent experimental reports are consistent with this notion.

In one pioneering study, US Naval Research Laboratory electrochemist Leonard Tender and his colleagues used microelectrodes to probe electron transport in *Geobacter sulfurreducens* biofilms.[9] The study demonstrated overall biofilm conductivity and captured the formation of a within-biofilm redox gradient that drives electron transport from electron-rich to electron-poor areas. Once again, redox components such as the hemes in cytochromes were implicated as the charge carriers, suggesting that electrons hop along electron transfer proteins that are associated with outer cell membranes, bacterial nanowires, and/or the extracellular matrix of the biofilm.

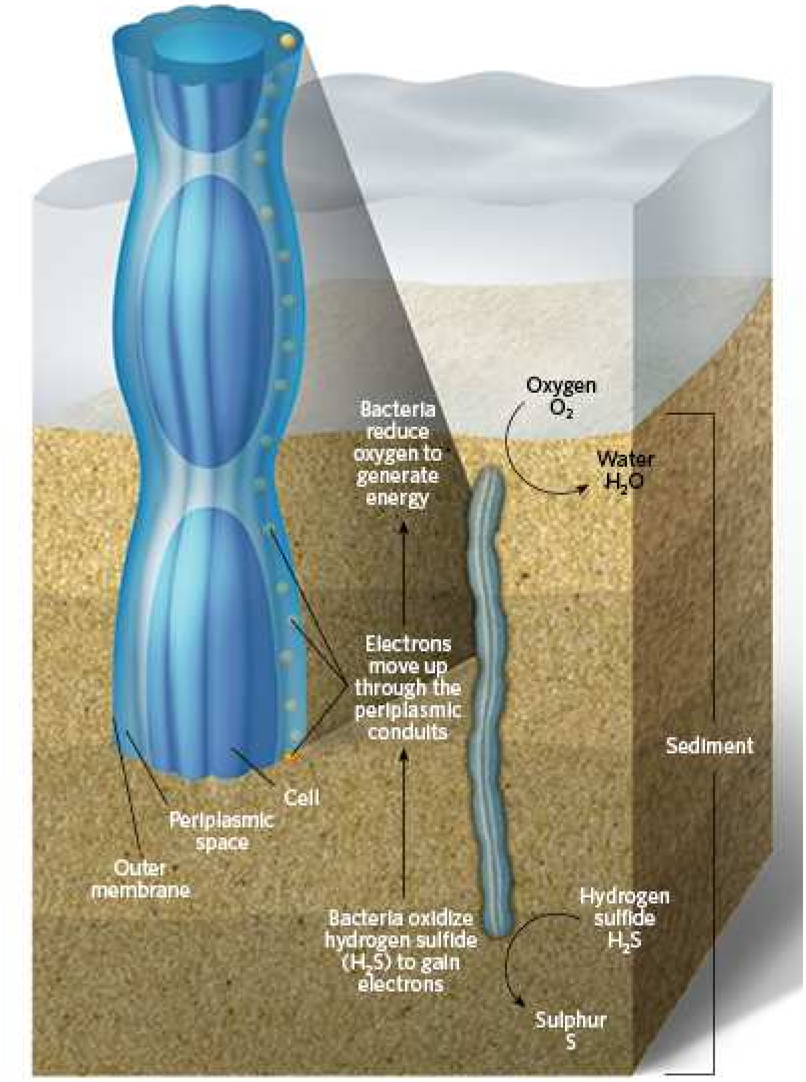
Furthermore, the phenomenon of long-distance biological electron flow is not restricted to metal-reducing bacteria. In 2010, microbiologist Lars Peter Nielsen at Aarhus University in Denmark and colleagues reported mysterious electron flow in marine sediments. They observed that the oxidation of hydrogen sulfide within the sediment was directly and quickly coupled to the reduction of oxygen at the sediment surface more than a centimeter away.[10] The rate of this process was much too fast for the molecular diffusion of any chemical species, so the authors reasoned that bacteria in the sediment must be actively transporting the electrons from bottom to top. Further research ruled out previously established long-distance electron transfer pathways, including bacterial nanowires, outer-membrane cytochromes, and conductive minerals in the sediment, but what the research team eventually found was even more remarkable.

In 2012, Nielsen and an international team of physicists and biologists reported the discovery of centimeter-long multicellular bacterial chains, consisting of thousands of cells lined end-to-end within the marine sediments.[11] In a remarkable feat of coordinated respiration, cells at one end of each "bacterial cable" would oxidize hydrogen sulfide and supply electrons to the oxygen-consuming cells at the other end. Unlike nanowires, which serve to pass electrons between individual cells via external appendages, the cables serve to pass the electrons within a multicellular architecture. The bacteria, which belong to the family Desulfobulbaceae, share a common outer membrane, and the researchers found string-like structures running in the periplasmic space along the entire length of the cable just underneath this membrane. Electrostatic force microscopy confirmed that these strings had a high capacity for storing and conducting charge, hinting at a possible mechanism for transporting electrons along these conduits.

Within the span of a few years, observations of microbial electron transport jumped from nanometer- to centimeter-length scales! The implications for basic physiology are immense, as the findings suggest that the respiration activity of microbes can occur over distances as large as thousands of cell-lengths. And from an applied perspective, it now seems possible to create microbial fuel systems based on optimizing the performance of entire bacterial communities, instead of just individual cells.

A multicellular architecture?

The discovery of thousands of electrically conductive cells living together in biofilms or as single, filamentous cables raises the question of whether these communities of cells can be considered multicellular organisms. Indeed, examples of single-celled species behaving as multicellular groups can be found throughout the microbial world.[12-15] Many surface-attached bacterial communities exhibit a "division of labor" within the biofilm. When resources grow scarce, both myxobacteria and *Dictyostelium* amoebae can gather in species-specific aggregates that form fruiting bodies in which the top cells become spores to seed populations in new, hopefully more bountiful locales. And recently, researchers have observed the evolution of multicellular forms of yeast in laboratory culture.[16] This idea of genetically identical cells performing different activities, analogous to different cell types and organs within a higher organism, forms one of the driving principles in models of the evolution of multicellularity. (See "From Simple to Complex," *The Scientist*, January 2011.) To think that these electron-transferring bacteria are functionally multicellular is not such a stretch.



BACTERIAL CONDUIT: Desulfobulbaceae bacteria were recently discovered to form centimeter-long cables, containing thousands of cells that share an outer membrane. They live in the ocean floor where the deeper cells do not have access to the oxygen in the upper layers of sediment. The deeper cells do, however, have access to hydrogen sulfide, which the bacteria oxidize to gain electrons. The electrons then travel up through the shared periplasmic space of the bacterial cable where the cells closer to the sediment surface can use them to reduce oxygen molecules and generate energy.

The necessity of the division of labor becomes particularly acute when fundamental functions are incompatible or unable to occur simultaneously due to environmental factors. For example, in photosynthetic cyanobacterial filaments, carbon fixation must be physically separated from nitrogen fixation because the enzymes involved in nitrogen fixation are exquisitely sensitive to the presence of molecular oxygen, which is generated in the process of fixing carbon. In the conductive Desulfobulbaceae cables, the oxidation of sulfide in the anoxic sediment cannot occur in the same place as the reduction of oxygen because one environment is devoid of oxygen. However, long-range electron transport allows the coordination of these spatially separated metabolisms. In both examples, this required division of labor appears to be a key factor driving the development of the filamentous forms.

Another important phenomenon observed in multicellular organisms is intercellular communication. Many activities carried out within biofilms may be mediated by a form of cell-to-cell communication called quorum sensing. In quorum sensing, cells continually determine the density of their own population —"self" cells—by sensing the diffusion of chemical signaling molecules released by all "self" cells in the community. A variety of processes can only be performed in the presence of the requisite number of "self" cells, including bacterial light production, toxin secretion by pathogens, and the formation of biofilms.

Cell-to-cell electron transfer could additionally serve a similar function to quorum sensing: allowing cells to communicate with each other. For example, in both *Shewanella* biofilms and the Desulfobulbaceae cable system, the flow of electrons occurs in one direction: toward the terminal electron acceptor. This directionality

Within the span of a few years, observations of microbial electron allows cells downstream in the redox gradient to be directly "informed" of the oxidation activity of their respiration partners upstream in the donor-rich regions. In this way, each cell along the electron transport pipeline can tune its local gene expression in response to events occurring far away. Cells are communicating their metabolic state across the network.

transport jumped from nanometer- to centimeter-length scales.

This notion of electron transport driving information flow and communication in microbial communities is new, and as yet untested, but it has potentially transformative physiological and technological implications. Compared to the relatively slow diffusion of entire molecules, electron flow is a rapid process, allowing cells to more quickly sense and respond to environmental change. Such an electronic signaling network, in addition to regulating cell-cell interactions on the population level, could even form the backbone of new synthetic microbial networks designed as sensors to detect specific environmental conditions, such as harmful or desirable chemicals, or variations in light or pH. Eventually, researchers may even learn to interface these networks with solid-state microelectronics, using the extracellular electron transport pathways of metal-reducers such as Shewanella to perform functions from bioremediation to energy production. This vision of integrated microbial circuits was unimaginable 10 years ago. But as we unravel the molecular and biophysical basis of long-distance electron transport, these bacteria may one day become essential components of everyday technologies.

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Tags

redox, oxidation, microbiology, electrons, electron transfer, biofilm and bacteria

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Comments

Hank Roberts
Posts: 2

May 17, 2013

I've wondered for years -- after reading that raw crude oil self-organizes making complicated three-dimensional biofilm structures inside the first tier of pipes taking it away from wellheads -- how much pre-existing structure existed down in the rocks where the pores are chock full of bacteria and petrochemicals.

Perhaps we've been doing brain surgery with our fossil fuel drilling.

Look at e.g. http://2012.igem.org/Team:Lyon-INSA/microbialControl

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Alexandru
Posts: 86

May 29, 2013

I suppose that this is not exactly "live wires".

In my opinion, it is "live wireless" because the flow of electrons is generating by invisible bio-magnetic field only in the case of compatibility between two or more live creatures that contain bio-magnetic sensors, like mitochondria.

See also the wireless communication between sperm and ovum:

http://www.the-scientist.com/?articles.view/articleNo/28768/title/Sperm-motility-secrets-revealed/ (February 4, 2010)

Even it seems crazy for a non-specialist in wireless communication, this type of communication looks like mobile phone technology.

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Víctor Sánchez
Posts: 1

March 18, 2015

Great article on the truth of the life and the dimensions of all that exist. The new scientific research field of electromicrobiology opens the doors of a new point of view of the science.

Unfortunately, the mainstream of the scientific community does not like to recognize the huge implications of the current discoveries. At this point, I would like to ask you: Do we need a scientificists that are trying to cover the sun with one finger?, and what about of those that make fun of someone about these issues?

The illusion of the disconnected world is only a methodological strategy to learn" philosophical categories" in order to recognize the differences between the things and nonrelationships with our 5 senses .

Dr. Bruce Lipton studies on the cell biology and quantum physics shows us that genes and DNA do not control our biology. It seems that all is connected because all is energy, where electrons not only transfer energy between dimensions without consideration of the space and time.

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