Rust never sleeps: a new wave for neutral-pH Fe redox cycling

Eric Roden, Department of Geoscience, University of Wisconsin, Madison, WI, USA (Email: eroden@geology.wisc.edu)
David Emerson, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME, USA (Email: demerson@bigelow.org)

'It's better to burn out 'cause rust never sleeps', Neil Young, *Hey Hey, My My (Into the Black)* Rust Never Sleeps, 1979

For Neil Young cognoscenti this song signalled the onset of new forces in popular music that changed the face of Rock-n-Roll. These were forces of artistic vitality fighting against the corrosive effects of aging and obsolescence – in short, a round of Rustoleum. Here we consider a new wave of information and tools to expand established concepts of the structure and function of neutral-pH Fe redox cycling microbial communities. Looking out over this field is in many ways like looking back in time, given that the pathways of Fe redox metabolism involved are widely thought to be some of the most ancient in Earth's history. Could the past be the key to understanding the present here? We believe so, and invite you to gaze into our (t)rusty crystal ball to try to identify a few of the 'so many things [we] need to know' (Styx, *Crystal Ball*, 1976) about microbial Fe cycling.

What we need to know is the modus operandi of *extracellular* Fe redox metabolism in nature. From a biochemical and physiological perspective, Fe(III)-reducing bacteria (FeRB) and Fe(II)-oxidizing bacteria (FeOB) share the respective energetic challenge of donating electrons to a mineral (FeRB), or extracting electrons from a soluble compound that rapidly turns into a mineral (FeOB). What mechanisms do microorganisms use to drive these reactions? At what spatial and temporal scales are the reductive and oxidative sides of the cycle connected? We have a decent understanding of the large-scale linkages between Fe oxidation and reduction: Fe(II)-bearing primary minerals are weathered to Fe(III)-bearing secondary mineral phases (oxides, clays, etc.) on the terrestrial landscape, which in turn can be transferred to sedimentary environments (hydromorphic soils, aquatic sediments, and aquifers) where Fe(III) serves as an electron acceptor for anaerobic respiration. These mass transfers take place on time scales of years, decades, centuries . . . What about short-term, fine-scale interactions of Fe oxidation and reduction in the myriad of redox interfacial environments that are present in sediments? Is there a unique organization of oxidative and reductive communities in such environments, e.g. as is known for the carbon, nitrogen, and sulfur cycles? Are there quorum sensing or tactic modalities that link populations of FeRB and FeOB in a synergistic way? Recent work suggests that the answer is likely to be affirmative: in almost every redox-interfacial environment that has been examined, organisms responsible for both Fe oxidation and reduction have been identified, and in some cases direct evidence for their contribution to *in situ* Fe redox cycling has been documented. However, details remain sketchy, particularly in terms of physiological regulation and environmental conditions at μm-to-mm spatial scales across and within redox gradients.

Additional and more detailed microbial ecosystem-level analyses of the identity and abundance of FeRB and FeOB populations involved in Fe redox cycling in different types of redox environments are required as a first step toward tackling these questions. To go deeper will require a thoroughgoing search for the basic machinery of oxidative and reductive Fe transformations at the cell surface. Hints as to the possible modularity of such metabolic pathways are emerging in the context of outer membrane cytochromes as a conduit for electron flow to and from cells and their local environment (Hartshorne *et al*., 2009). Speaking of extracellular electron flow, we cannot forget about the elusive bacterial nanowire, which continues to rear its nm-sized head, apparently able to deliver to a shock on spatial scales equal to or greater than a single cell (El-Naggar *et al*., 2010; Nielsen *et al*., 2010). What's needed here are both broad (across taxa) and deep (within each taxa) comparative genomic and biochemical analyses, to explicate common patterns and mechanisms of cell surface-mediated electron transfer. Recent analysis of multiple whole genomes of the dissimilatory Fe(III)-reducing taxa *Shewanella* (Konstantinidis *et al*., 2009) and *Geobacter* (Butler *et al*., 2010) provide examples of such an approach, and insight into the complex tapestry of cytochrome-based mechanisms these FeRB utilize to solve a common problem. This work illustrates the challenges for comprehensive identification (and, ergo, detection) of genes and gene products associated with the final flip of the (extracellular electron transfer) switch. Analysis of aerobic acidophilic FeOB and archaea, as well as anoxygenic phototrophic FeOB, is also revealing a vision (as yet still hazy) of involvement of multiple families of cytochromes playing key roles in acquisition of electrons from Fe(II). To date no such comparative genomic analyses are available for neutral-pH microaerophilic, or nitrate-reducing Fe(II)-oxidizers. Based on these studies, our crystal ball suggests microbes have evolved a myriad of theme-based solutions to the opportunity of extracting energy from iron through extracellular electron transfer. It is quite likely these systems are finely tuned to local redox conditions (Denef *et al*., 2010), and probably other environmental factors as well, and that while there may be complicity in means, it is unlikely there will be simplicity in mechanism.

What might the acquisition of a commanding set of comparative genomic analyses for Fe reducers and oxidizers lead to in terms of understanding *in situ* Fe redox transformation? We hypothesize that it should eventually become possible to delineate (and eventually study, in an experimental manner) what is likely to be a tractable array of fundamental evolutionary 'inventions' of biochemical machinery involved in extracellular Fe redox metabolism. It seems intuitive that there must be a few basic structural and physiochemical properties of the proteins involved in this process, which will likely involve, one way or another, multi-haem cytochromes as prominent players. Once such properties are understood, they can be used to design clever miniaturized devices (e.g. Nagaraj *et al*., 2010) that in principle should be able to detect the presence and activity of such proteins across a range of scales, including the μm-scale upon which rapid Fe redox cycling is likely to take place in nature (Emerson *et al*., 2010). A key feature of the proteins referred to here is their presumed exposure at the cell surface, which means that they may be interrogated in a whole-cell manner, i.e. without the need to analyse intracellular components. The introduction of 'microbiosensors' for analysis of cell surface Fe cycling proteins would open the way to leverage traditional and emerging techniques for microscale chemical profiling of redox-active species (e.g. Luther *et al*., 2008) for identifying the spatial structure Fe cycling communities at redox interfaces. Along the way, bulk and (eventually) microscale detection of extracellular signalling compounds that one might speculate, as recently suggested for coupled carbon/oxygen/sulfur cycling in modern marine stromatolites (Decho *et al*., 2010), to play a critical role in the physical and biochemical organization of oxidizing and reducing microbial communities. Of course one must know what to look for, which brings us back to comparative genomics, where new insights into the role of signalling mechanisms in Fe cycling (Dietrich *et al*., 2008; Tran *et al*., 2008; Wang *et al*., 2010) is emerging.

Here at the end of our little journal we find ourselves back where we started: understanding of the evolutionary adaptations involved in extracellular Fe redox transformation will lead a new generation of tools for understanding how things actually work in nature. In turn, understanding modern Fe cycling environments (including high and low temperature settings, both near and far beneath the Earth's surface) could unlock ancient secrets about how extracellular metabolism of the fourth most abundant element in the crust may have been involved in the origin and early proliferation of life on Earth (Lovley, 2004). Recent documentation of sedimentological, geochemical and microfossil evidence for truly ancient ($\geq$ *c*. 2 billion year old) layered Fe cycling microbial communities (Planavsky *et al*., 2009; Schopf *et al*., 2010) provides clear motivation for unravelling the way these communities operate at the Earth's surface today.

References

Butler, J.E., Young, N.D., and Lovley, D.R. (2010) Evolution of electron transfer out of the cell: comparative genomics of six *Geobacter* genomes. *BMC Genomics* **11:** 40.

Decho, A.W., Norman, R.S., and Visscher, P.T. (2010) Quorum sensing in natural environments: emerging views from microbial mats. *Trends Microbiol* **18:** 73–80.

Denef, V.J., Mueller, R.S., and Banfield, J.F. (2010) AMD biofilms: using model communities to study microbial evolution and ecological complexity in nature. *Isme J* **4:** 599–610.

Dietrich, L.E.P., Teal, T.K., Price-Whelan, A., and Newman, D.K. (2008) Redox-active antibiotics control gene expression and community behavior in divergent bacteria. *Science* **321:** 1203–1206.

El-Naggar, M.Y., Wanger, G., Leung, K.M., Yuzvinsky, T.D., Southam, G., Yang, J., *et al*. (2010) Electrical transport along bacterial nanowires from *Shewanella oneidensis* MR-1. *Proc Natl Acad Sci USA* **107:** 18127–18131.

Emerson, D., Fleming, E.J., and McBeth, J.M. (2010) Iron-oxidizing bacteria: an environmental and genomic perspective. *Annu Rev Microbiol* **64:** 561–583.

Hartshorne, R.S., Reardon, C.L., Ross, D., Nuester, J., Clarke, T.A., Gates, A.J., *et al*. (2009) Characterization of an electron conduit between bacteria and the extracellular environment. *Proc Natl Acad Sci USA* **106:** 22169–22174.

Konstantinidis, K.T., Serres, M.H., Romine, M.F., Rodrigues, J.L.M., Auchtung, J., McCue, L.A., *et al*. (2009) Comparative systems biology across an evolutionary gradient within the *Shewanella* genus. *Proc Natl Acad Sci USA* **106:** 15909–15914.

Lovley, D.R. (2004) Potential role of dissimilatory iron reduction in the early evolution of microbial respiration. In *Origins, Evolution and Biodiversity of Microbial Life*. Seckbach, J. (ed.). Amsterdam, the Netherlands: Kluwer, pp. 301–313.

Luther, G.W., Glazer, B.T., Ma, S.F., Trouwborst, R.E., Moore, T.S., Metzger, E., *et al*. (2008) Use of voltammetric solid-state (micro)electrodes for studying biogeochemical processes: laboratory measurements to real time measurements with an *in situ* electrochemical analyzer (ISEA). *Mar Chem* **108:** 221–235.

Nagaraj, V.J., Aithal, S., Eaton, S., Bothara, M., Wiktor, P., and Prasad, S. (2010) Nano Monitor: a miniature electronic biosensor for glycan biomarker detection. *Nanomedicine* **5:** 369–378.

Nielsen, L.P., Risgaard-Petersen, N., Fossing, H., Christensen, P.B., and Sayama, M. (2010) Electric currents couple spatially separated biogeochemical processes in marine sediment. *Nature* **463:** 1071–1074.

Planavsky, N., Rouxel, O., Bekker, A., Shapiro, R., Fralick, P., and Knudsen, A. (2009) Iron-oxidizing microbial ecosystems thrived in late Paleoproterozoic redox-stratified oceans. *Earth Planet Sci Lett* **286:** 230–242.

Schopf, J.W., Kudryavtsev, A.B., Sugitani, K., and Walter, M.R. (2010) Precambrian microbe-like pseudofossils: a promising solution to the problem. *Precambrian Res* **179:** 191–205.

Tran, H.T., Krushkal, J., Antommattei, F.M., Lovley, D.R., and Weis, R.M. (2008) Comparative genomics of *Geobacter* chemotaxis genes reveals diverse signaling function. *BMC Genomics* **9:** 471.

Wang, Y., Kern, S.E., and Newman, D.K. (2010) Endogenous phenazine antibiotics promote anaerobic survival of *Pseudomonas aeruginosa* via extracellular electron transfer. *J Bacteriol* **192:** 365–369.

Unanticipated intra- and inter-kingdom cross-talk involving small molecules

Aswin S. N. Seshasayee, National Center for Biological Sciences, Bangalore, India (Email: aswin@ncbs.res.in)
M. Madan Babu, MRC Laboratory of Molecular Biology, Cambridge, UK (Email: madanm@mrc-lmb.cam.ac.uk)

Microbes are versatile organisms that do not live in isolation, but in competition and cooperation with other organisms and abiotic elements of the environment. Therefore, a comprehensive understanding of microbial behaviour will necessarily incorporate aspects of how they interact with their natural surroundings. The primary mechanism for controlling cellular functions in response to environmental cues is via signal transduction pathways. This often involves detecting alterations in the concentration of specific small molecules, ultimately leading to changes in the transcriptional state of the cell. Such small molecules may be naturally present in the environment, or may be part of the normal metabolism of the host organism that a microbe inhabits. We speculate that elucidation of unanticipated chemical cross-talk among microbes, and between microbes and eukaryotes, will be an area of significant interest in the immediate future. A better understanding of these signalling interactions is likely to yield fundamental insights into the emergence of pathogens, and the process of microbial pathogenesis and symbiosis.

Microorganisms produce a wide range of small molecules, a small fraction of which has been exploited medically as antibiotics. In the environment, however, antibiotics are present at sub-inhibitory concentrations, thus possibly ruling out a natural role as a growth inhibitor. In fact, some microbes that live in the human microflora are resistant to antibiotics (Sommer *et al*., 2009) and others even subsist on them (Dantas *et al*., 2008). Recent findings have shown that antibiotics, at sub-inhibitory concentrations, can act as signalling molecules. They regulate transcription of genes involved in diverse cellular processes spanning metabolic, adaptive and virulence functions (Yim *et al*., 2007). These effects might be linked to regulation through quorum sensing, partly because antibiotics may share structural similarity to known chemical mediators of microbial cell-to-cell communication. Given that antibiotic resistance and utilization genes are more common in bacteria than anticipated, regulatory functions of antibiotics may be of fundamental importance to bacterial ecology (Wright, 2007). From a medical standpoint, understanding the behaviour of resistant microorganisms, and even the host cells, in the presence of antibiotics might enable efficient intervention strategies that might minimize unintended side-effects of antibiotic treatment.

Microbes and their eukaryotic hosts can intercept each others' signals leading to 'inter-kingdom signaling' or 'inter-kingdom crosstalk' (Hughes and Sperandio, 2008). These signalling interactions include control of host function such as immune response by bacterial signalling molecules (Woodward *et al*., 2010), disruption of bacterial cell-to-cell communication by mammalian enzymes (Yang *et al*, 2005), and bacterial sensing of eukaryotic signalling compounds leading to the expression of several bacterial genes including virulence (Gotoh *et al*, 2010) and differentiation factors (Van de Velde *et al*, 2010). That such interactions exist is not surprising given the long history of mutuality and antagonistic interactions between eukaryotes and microbes. However, given that disruption of specific interactions might be a viable strategy for drug development (Gotoh *et al*, 2010), it is remarkable that emphasis on research in this field has been placed only recently. Moreover, one might envisage exploiting these signalling mechanisms in designing probiotics or in alleviating drug toxicity due to unanticipated cross-talk by inhibiting relevant enzymes in bacteria that inhabit the human microflora (Wallace *et al*, 2010).

Following from recent findings demonstrating extensive divergence in transcriptional responses to the same signal among related organisms across mammals, fungi and bacteria (Babu, 2010), we speculate that strains of a bacterial species and closely related microbes might differ in their transcriptional responses to the same small molecule signals. For example, in response to an antibiotic, a resistant strain might display a different transcriptional state than a sensitive strain even if the antibiotic is present at sub-inhibitory levels. Similarly, horizontally acquired genetic material which are unique to pathogenic strains of a species might be specifically regulated by factors that recognize particular host signals; further, antibiotic treatment might promote acquisition of foreign DNA (Yim *et al*., 2007). How such phenomena impact genome evolution and whether this has an effect on the bacterial phenotype (i.e. emergence of resistant and persister strains) are open questions.

Understanding the outcomes and the evolution of small molecule mediated microbe–environment interactions remain a challenge that can be tackled using genome-scale techniques. These include high throughput sequencing of nucleic acids and phenotypic analysis of deletion mutants in the presence of specific small molecules. A more challenging problem is identifying binding targets of small molecules on a genome-wide scale. In this direction, computational predictions based on information available