

tants and react faster than expected on the basis of bulk solution concentrations of the reactants. Thus, CDOM is a microreactor that enhances the reactivity of a molecule by bringing it into close association with a very reactive intermediate.

Latch and McNeill now demonstrate that hydrophobic molecules bound to CDOM encounter much higher concentrations of singlet oxygen produced photochemically from CDOM by light in the solar spectrum. A probe that is essentially completely bound to CDOM reports a  $^1\text{O}_2$  activity that is more than 100 times the average concentration encountered by hydrophilic furfuryl alcohol. The environmental significance of this finding is that a hydrophobic molecule that is only 10% bound to CDOM will still encounter a  $^1\text{O}_2$  activity that is 10 times the average concentration in the whole solution. Therefore, the potential for degradation of compounds susceptible to reaction with  $^1\text{O}_2$  is much greater than expected on the basis of experiments with furfuryl alcohol. Latch and McNeill introduce a model for distribution of  $^1\text{O}_2$  and find that it is limited to the CDOM matrix and to a "corona" that extends out a few nanometers from each CDOM molecule. Thus, it appears that most of the solution contains essentially no  $^1\text{O}_2$ . The concept of a corona predicts enhanced reactivity not only of hydrophobic compounds but also of cations such as protonated amines attracted to the net negative charge of DOM. Blough (3) in fact

observed this to be the case for cationic nitroxides reacting with photochemically produced radicals.

The localized nature of these reactions leads to quantitative differences from results expected for a homogeneous solution. Hydrophilic scavengers of  $^1\text{O}_2$  or other reactive intermediates have little affinity for the DOM matrix and therefore have a low probability of being present where  $^1\text{O}_2$  is formed. Thus, they are much less effective at interfering with the reaction of a hydrophobic molecule than would be expected in a homogeneous water solution. Latch and McNeill demonstrate this for azide ion, which has little quenching effect on the intra-DOM reaction, and also for  $\text{D}_2\text{O}$ , which promotes reaction in homogeneous solution by stabilizing  $^1\text{O}_2$  but has no promotional effect on the intra-DOM reaction. Conversely, a hydrophobic scavenger ( $\beta$ -carotene in this case) is much more effective than expected. However, molar concentrations of hydrophobic compounds in natural waters tend to be much lower than the concentrations of DOM, leaving most of the DOM molecules unoccupied. Consequently, substantial quenching of intra-DOM reactions by hydrophilic or hydrophobic scavengers is unlikely under normal environmental conditions.

Latch and McNeill use custom-made probe molecules that are ideal for these types of studies. Hydrophobic molecules are difficult to work with in water solution because of their low solu-

bility and tendency to attach to surfaces. By measuring the chemiluminescence of the reaction product, the authors are able to achieve very low detection limits on small subsamples. This helps to minimize spurious results from molecules attached to the container wall that might be included if the entire solution was extracted. They are also able to observe production of a product rather than disappearance of a reactant, which greatly aids monitoring slow reactions that can still be important in the environment.

Intra-DOM reactions have received little attention, but as the present work demonstrates, they may be important photochemical mechanisms for transformation of organic chemicals in the environment. This has recently been shown to be true for intra-DOM reaction of the insecticide mirex in Lake Ontario (5), and is likely to be so for other compounds and other systems as well.

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## EVOLUTION

# Tracing Oxygen's Imprint on Earth's Metabolic Evolution

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Life on Earth created, and is dependent on, nonequilibrium cycles of electron transfers involving primarily five elements: hydrogen, carbon, nitrogen, oxygen, and sulfur (1). Although biophysical and biochemical reactions catalyze specific electron transfers at a local, molecular level, the metabolic consequences are global. Through opportunity and selection, metabolic pathways evolved to form an interdependent, planetary "electron market" where reductants and oxidants are traded across the globe. The exchanges are made on a planetary scale because gases, produced by all organisms, can be transported around Earth's surface by the ocean and atmosphere. Exactly how these five elements came to form an electron market

place remains largely unresolved, however. On page 1764 of this issue, Raymond and Segrè (2) use an ingenious bioinformatics approach to reveal the evolution of metabolic pathways. Their analysis elegantly reveals not only the profound role that molecular oxygen ( $\text{O}_2$ ) has played in shaping the electron market place, but also the evolutionary constraints on, and trajectories of, the ensemble of electron traders.

Before the evolution of free  $\text{O}_2$  ~2.3 billion years ago (3, 4), there was a glut of reducing equivalents on Earth's surface. The first traders consumed electrons from the large populations of potential donors, including  $\text{H}_2$ ,  $\text{H}_2\text{S}$ , and  $\text{CH}_4$  (5). These electrons, extracted either with the release of energy or with the aid of low-energy (infrared) solar photons, were sold at relatively low energy prices to acceptors such as  $\text{CO}_2$  and, to a lesser extent,  $\text{SO}_4$ . Although there was a very large pool of an alternative electron acceptor,  $\text{N}_2$ , considerable metabolic energy is required to reduce the gas to  $\text{NH}_3$  at physiological tempera-

As oxygen built up in Earth's atmosphere during the Precambrian, organisms evolved more complex biochemical networks. This expansion made feasible oxygen-dependent metabolisms that coopted parts of preexisting anaerobic ones.

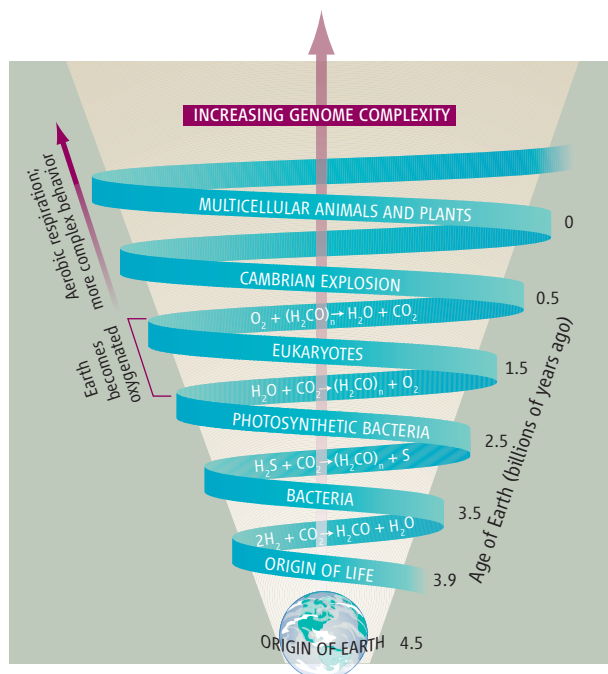
tures. Over the first 2 billion years of Earth's history, the electron market evolved to produce a well-structured set of metabolic pathways distributed among groups of interconnected anaerobic microbes, each selected to conduct one, or at most a small subset, of redox reactions. Because of the relatively large investment in energy to oxidize water, the biggest electron-donor pool,  $\text{H}_2\text{O}$ , remained biologically inaccessible.

When and how the first photosynthetic organisms evolved that were capable of oxidizing water to oxygen remains one of the great mysteries in the evolution of life on Earth (6). However, between ~3.2 and 2.4 billion years ago, either through progressive gene duplication events and selection, or by massive lateral transfer of genes, or both (7, 8), an organism emerged that was capable of extracting four electrons from two molecules of water to form free  $\text{O}_2$  as a metabolic waste product. This waste product proved not only highly useful as an electron acceptor, but also potentially damaging to the intricate metabolic

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networks that had evolved over the first 2 billion years of Earth's history. The oxidation of water requires 1.2 V per electron. The only viable source of energy to sustain this reaction is higher energy (visible) solar radiation (9). Hence, oxygenic photosynthesis was confined to a thin film of sunlight on the surface of the planet. Over a relatively short period of geological time (~100 million years), O<sub>2</sub> built up in Earth's atmosphere, such that by 2.2 billion years ago, the old electron market place, which had operated so smoothly for so long, was in danger of becoming a historical relic, to be supplanted by a new, high-powered machine that used the latest, most complex, energy-transducing process (10). The core machinery for this process evolved only once on Earth, and because of protein-protein, protein-cofactor, and protein-lipid interactions, the fundamental design became an immutable, "frozen" metabolic accident, encoded by a set of highly conserved genes (11). But with the appearance of this new technology, the electron traders, which had evolved under anaerobic conditions, had to accommodate the new oxidant, go into hiding, or become extinct.

Over the next 1.5 billion years, the oxidation of the atmosphere and, ultimately, oceans depleted Earth's surface of many of the reductants that were so abundant in the first half of the planet's history. Most of the anaerobic machinery in these organisms was corralled by the oxidizing conditions into relatively small, fragile, hypoxic or anaerobic reserves. However, some microbes broke free and either modified parts of their metabolic machinery, or formed symbiotic associations that permitted them to couple the oxidation of organic matter to the reduction of O<sub>2</sub> back to water. This supercharged respiratory pathway yielded four times as much energy per molecule of glucose oxidized than any of the ancient anaerobic pathways, but it came at a price. The triplet ground state of O<sub>2</sub> is highly reactive, and one misstep in an electron transfer can yield peroxides, superoxide, and/or hydroxyl anion radicals, collectively called reactive oxygen species. Reactive oxygen species can be highly destructive, oxidizing membrane lipids, ripping cofactors from proteins, and even oxidizing polymeric molecules such as proteins and nucleic acids (12). Rather than abandon O<sub>2</sub>, selection salvaged and remodeled parts of the old, anaerobic machinery. This gave rise to both enzymatic and nonenzymatic mechanisms to protect the genetic investment in the core metabolic pathways that drive the "water-water" cycle upon which most life on Earth would come to depend. The protective pathways evolved in turn from gene duplication and lateral gene-transfer processes to spread across all three domains of life, but were absolutely essential to the evolution of eukaryotic



organisms, from which all animals and plants descend (13, 14).

But the influence of O<sub>2</sub> did not stop there. Novel secondary gene products, including sterols (and their derivatives), indoles and alkaloids, several antibiotics (including penicillins), and some detoxifying pathways, evolved to become obligately oxygen dependent. Indeed, as Raymond and Segré enumerate in their meta-metabolomic analysis of 70 genomes [from the Kyoto Encyclopedia of Genes and Genomes (15)], O<sub>2</sub> is predicted to be either directly involved in, or indirectly associated with, more than 10<sup>3</sup> metabolic reactions not found in anaerobes. Hence, the evolution of O<sub>2</sub> not only created redox energy couples that would allow complex animal life to evolve in the Ediacarian and Cambrian periods (approximately 630 to 490 million years ago), but also precipitated an evolutionary explosion of unpredictable, alternative, as well as novel pathways that gave rise to a wide variety of secondary gene products that increased fitness through regulation of gene expression, modification of behavior, and protection against competitors. The metagenomic analysis reveals how the rise of O<sub>2</sub> forced a selection of parts of anaerobic metabolic pathways from ancestral genes and, through processes not yet fully understood, appropriated the salvaged bits and pieces to construct an expanding galaxy of interdependent, and increasingly complex, metabolic pathways that became dependent on O<sub>2</sub>. This is the biological equivalent of grabbing the most valuable possessions (including pictures of your ancestors) when your house is on fire, so that you may be able to start life anew after the catastrophe.

We still have a long way to go to understand how life evolved and how singular events (such as the rise of oxygen) altered evolutionary trajectories on Earth. The study by Raymond and Segré reinforces the fact that evolutionary processes are

### The evolution of genomic complexity and metabolic pathways during Earth's history.

The earliest origin of life is not known. However, assuming a single last universal common ancestor evolved in mid-Proterozoic, there is evidence of microbial life. When oxygenic photosynthesis evolved is not clear, but geochemical data suggest that between ~2.3 and 2.2 billion years ago, there was sufficient oxygen in the atmosphere to permit an ozone layer to form. That singular event appears to have precipitated a massive increase in genome and metabolic complexity, culminating in the rise of metazoans around 600 million years ago, and the rise of terrestrial plants around 430 million years ago. The feedbacks in the evolutionary trajectory have led to increasing genomic and metabolic complexity.

nonlinear and, over time, create "emergent" properties, which, by definition, cannot be predicted by reductive analyses. Ironically, bioinformatics itself is an emergent property of technology. The very tools used by Raymond and Segré to find the patterns of metabolic relationships were made possible by sequencing and annotation technologies developed primarily for the human genome project, by computer scientists searching for algorithms to organize and find relationships within the sea of data produced by genome sequencing, and by organismal microbial ecologists, who painstakingly isolate and grow individual organisms, study their metabolic pathways, and extract and purify their DNA so that the sequences obtained can be assembled into genomes. Although it is difficult to predict the emergent tools from present investments in science or technology, it is certain they will allow us to better understand the pathways that led to the connectivity and complexity in the electron market we observe on the planet today.

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