

CRISPR defense against any known phages. The authors identify a multiprotein complex called CRISPR-associated complex for antiviral defense (Cascade), consisting of five Cas proteins (CasA to CasE), and show that it processes a long primary CRISPR transcript to 57-nucleotide fragments, each containing a unique spacer and bits of the flanking repeat sequences (see the figure). By cloning all five *cas* genes in different combinations into an *E. coli* strain lacking CRISPR sequences entirely, Brouns *et al.* show that only CasE is required for cleavage of the primary CRISPR transcript. Cascade processed primary CRISPR RNA, but not CRISPR RNA (with a different CRISPR repeat) from a different *E. coli* species, and could be copurified with the 57-nucleotide RNAs, indicating the formation of a ribonucleoprotein complex. Importantly, the authors could also construct a CRISPR defense against the bacteriophage lambda by engineering new spacers into the *E. coli* CRISPR locus, chosen from sites throughout the lambda genome. The artificial CRISPR array was efficient, reducing the ability of phage lambda to grow by a factor of 10,000,000. This effect depended on the pres-

ence of functional Cascade and expression of the *cas3* gene. In each case, the spacer sequences could be chosen from either the template or noncoding DNA strand of the phage genes, suggesting that the target of CRISPR is the phage DNA. This seems to be fundamentally different from the small inhibitory RNA strategy of eukaryotes, which suppresses viral gene expression by destroying corresponding messenger RNA.

The work by Brouns *et al.* has put at least the active defense aspect of the CRISPR system on track for thorough mechanistic and structural analysis. The ability to genetically and biochemically manipulate *E. coli* is far superior to other biological systems, and bacteriophage lambda is arguably the only biological entity for which we have nearly predictive understanding. It can be expected that rapid advances will be made in elucidating the molecular details for CRISPR gene expression, RNA processing, and the attack on the target phage.

Still obscure is how a bacterium acquires new spacer sequences. No one has reported a system for achieving this naturally, at efficiencies conducive to biochemical analysis.

Moreover, we should anticipate that phages have developed clever evasions of the CRISPR system just as they have done for restriction enzymes. Considering that the origin of modern molecular biology is grounded in the study of bacteriophage, it is puzzling that this particular weapon in the phage-bacteria war remained a secret for so long.

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Ironing Out Ocean Chemistry at the Dawn of Animal Life

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Few who study the evolution of Earth's early ocean and atmosphere would quibble with models that point to vanishingly low amounts of oxygen before the first major step in oxygenation about 2.4 billion years ago. Because iron is insoluble in O₂-containing waters, scientists have long linked this first step to the eventual disappearance of iron-rich oceans by 1.8 billion years ago; according to this model, iron-rich oceans only reappeared for brief curtain calls more than a billion years later, during the "snowball Earth" glaciations (see the figure). On page 949 of this issue, Canfield *et al.* (1) argue instead for a full repeat performance, with a global, long-lived return to dominantly iron-rich (ferruginous) conditions about 700 million years ago (see the figure). This surprising reappearance, with its implications for past and coeval ocean chemistry,

climate, and biological evolution, seems to have persisted to 540 million years ago and perhaps a little longer, overlapping with the second major step in atmospheric oxygenation and the concomitant rise of animals (2–4).

A decade ago, Canfield (5) challenged the prevailing paradigm for oxygenation of the ocean and the disappearance of banded iron formations (BIFs)—the layered, rusty, sulfur-poor smoking guns of the early, iron-rich ocean. The classic argument links the demise of BIFs to wholesale oxygenation of the deep ocean about 1.8 billion years ago, which overwhelmed the iron sources from that point forward and kept the ocean free of all but the tiniest amounts of iron. Canfield suggested instead that the deep ocean remained oxygen-free for another billion years or more. During that long period of deep-ocean anoxia, it was high concentrations of dissolved hydrogen sulfide (H₂S) that eventually drove out the iron, because H₂S and oxygen have a

New data change the picture of how the iron, oxygen, and sulfur contents of the ocean evolved.

similarly debilitating effect on iron solubility.

As the story goes, the still-small amounts of oxygen in the atmosphere after 2.4 billion years ago were consumed through decay of settling organic matter produced in the surface ocean, leaving the deep ocean oxygen-poor (5). But even a small increase of oxygen in the atmosphere meant greater oxidation of sulfur-containing minerals exposed on the continents. With that weathering came delivery of sulfate (SO₄²⁻) to the ocean by rivers. Increasing sulfate in seawater and the underlying sediments resulted in ubiquitous production of H₂S by bacteria that reduce sulfate in the absence of oxygen, and this H₂S reacted with the dissolved iron to form pyrite (FeS₂) (see the figure).

Today, large reservoirs of anoxic, H₂S-containing seawater are rare; the most voluminous is the Black Sea. In contrast, as sulfate began to accumulate in the still oxygen-poor earlier deep ocean (6), it is likely that H₂S-rich waters were far more common over the billion

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years following the disappearance of the BIFs about 1.8 billion years ago, before the reprise of ferruginous conditions around 700 million years ago (4, 7–10). Evidence for anything like a global Black Sea during this interval remains patchy, often indirect, and difficult to reconcile with the full range of data now available (4, 11), but H_2S must have been common in the deep waters and/or in the underlying sediments. And the capacity to form and bury pyrite in these oxygen-lean deep settings and thus remove sulfate from seawater was also sufficient to consume dissolved iron for millions of years. Canfield *et al.* now suggest that this delicate balance may have set the stage for the iron rebound 700 million years ago: A sudden dearth of sulfate overwhelmed the ocean's ability to lock up iron in pyrite, leaving more of the metal floating free once more.

The authors found evidence for this dramatic, long-lived return to iron-rich conditions by analyzing sediments from around the world, spanning from 760 to 530 million years in age. Their well-tested method delineates iron present as pyrite and as oxide and carbonate phases that form pyrite on geologically short time scales if exposed to H_2S . Broad temporal compilations often blur important details, but here the authors find that a clear first-order trend prevails: enrichments in iron minerals, with oxides and carbonates typically swamping the pyrite. Much of the unpyritized iron occurs as the same minerals that dominate BIFs deposited before 1.8 billion years ago, when the ocean was anoxic and iron-rich. These findings suggest that the deep sea was similarly oxygen-free and iron-rich starting 700 million years ago—which also implies that it was severely lacking in both H_2S and sulfate.

This lack of sulfate is surprising, considering that by then the atmosphere contained more than enough oxygen to foster sulfate delivery to the ocean by oxidative weathering on the continents. Part of the explanation stems from a global deep freeze that started about that same time. In most models for this snowball Earth event, during which ice shrouded much if not most of the continents and oceans, lower extents of chemical weathering on icy continents would have limited sulfate delivery to the ocean, and

loss of sulfate would be exacerbated by its bacterial conversion to H_2S and subsequent pyrite formation in an oxygen-free, ice-covered ocean (12).

But what is most striking about the data of Canfield *et al.* is that both anoxia and high-iron, low- H_2S conditions seem to dominate for many millions of years beyond the known effects of the snowball glaciations. The most important factor in the dearth of sulfate may thus be a long history of pyrite formation in the deep ocean and underlying sediments. Its burial and the eventual subduction of the pyrite-bearing seafloor below overriding tectonic plates utterly stripped the ocean of sulfate by shutting down its potential return by weathering (13). Independent of ice cover, oxygen in the atmosphere (and thus in seawater) was still too low to overcome the compensating effects of respiratory consumption in the ocean. In short, pyrite may have driven the ocean to anemia 1.8 billion years ago, but a billion years of pyrite burial may have shifted the balance back to iron.

Sulfur isotope data from similarly aged rocks in Oman capture the same extremes in sulfate deficiency (2), yet other sulfur isotope data spanning roughly the same time suggest bacterial cycling under higher sulfate concentrations (2, 14). Consistent with the latter, gypsum—a calcium sulfate mineral—is known to have formed from evaporation of seawater of the same age (15).

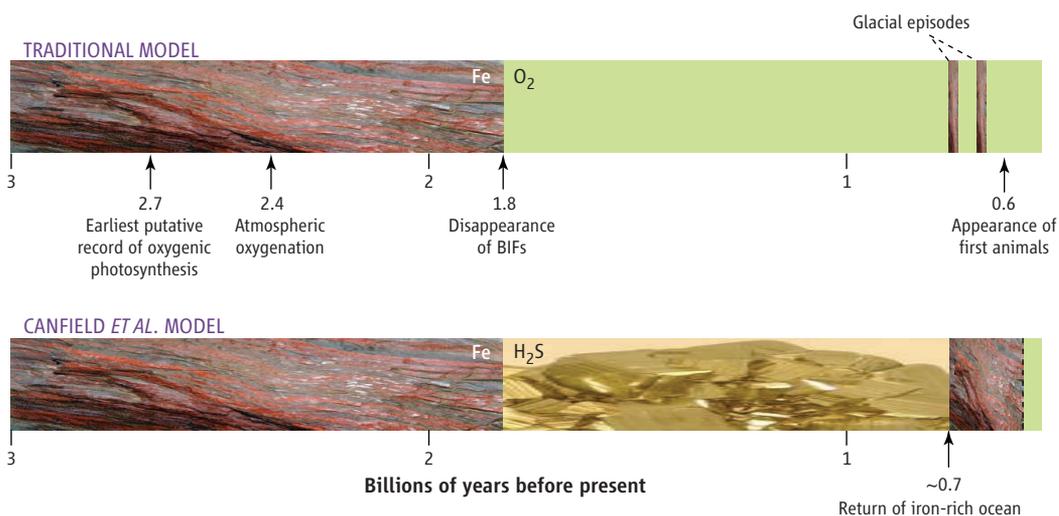
Other vexing inconsistencies and gaps remain. We still do not know much about H_2S and iron in the deep sea during the billion years that preceded the hypothesized iron ocean, although Canfield *et al.* give us a reason

to look harder. Snowball Earth proponents may have to remove a ferruginous ocean from their supporting arguments. Given that iron is a bioessential metal, evolutionary scientists (16) will have to reexplore eukaryotic evolution in light of a deep ocean that was persistently oxygen-poor but may have transitioned from iron-rich to iron-poor back to iron-rich. And, as always, global-scale extrapolations from local environments should be viewed with a squint. But once all this is ironed out, Canfield *et al.* will have guided many of the emerging views on biospheric oxygenation.

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The evolving deep ocean. In the traditional model, oxygenation of the ocean occurred 1.8 billion years ago, with only brief recurrence of ferruginous conditions during later global glaciations (11). Canfield *et al.* now suggest that widespread deep-ocean anoxia lasted to 540 million years ago and perhaps a little longer. Instead of early oxygenation, after 1.8 billion years ago the deep ocean was dominated by H_2S , followed by a repeat of widespread iron conditions (1).