

(150 K) and radius of the plume (200 km) should be regarded as lower and upper bounds, respectively, because the finite sizes of both the Fresnel zones of the converted phases and the patches used for stacking tend to smooth lateral variations in discontinuity depths (see Supplementary Information). These values nonetheless provide strong support for models of a hot and narrow plume penetrating the mantle transition zone beneath Iceland, and are similarly strong evidence against models in which the plume is broader (radius > 300 km) and has less excess temperature ( $\Delta T \approx 70 \text{ K}$ )<sup>3</sup>. □

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Supplementary information is available on Nature's World-Wide Web site (<http://www.nature.com>) or as paper copy from the London editorial office of Nature.

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## Microbiological evidence for Fe(III) reduction on early Earth

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It is generally considered<sup>1</sup> that sulphur reduction was one of the earliest forms of microbial respiration, because the known microorganisms that are most closely related to the last common ancestor of modern life are primarily anaerobic, sulphur-reducing hyperthermophiles<sup>2–4</sup>. However, geochemical evidence indicates that Fe(III) is more likely than sulphur to have been the first external electron acceptor of global significance in microbial metabolism<sup>5–7</sup>. Here we show that Archaea and Bacteria that are most closely related to the last common ancestor can reduce Fe(III) to Fe(II) and conserve energy to support growth from this respiration. Surprisingly, even *Thermotoga maritima*, previously considered to have only a fermentative metabolism, could grow as a respiratory organism when Fe(III) was provided as an electron acceptor. These results provide microbiological evidence that Fe(III) reduction could have been an important process on early Earth and suggest that microorganisms might contribute to Fe(III) reduction in modern hot biospheres. Furthermore, our discovery that hyperthermophiles that had previously been thought to require sulphur for cultivation can instead be grown without the production of toxic and corrosive sulphide, should aid biochemical investigations of these poorly understood organisms.

Our understanding of the geochemical conditions at the time when respiratory systems were first evolving suggests that the number of potential electron acceptors for microbial respiration was much more limited than at present. Important modern electron acceptors such as oxygen, nitrate and sulphate are unlikely to have been present in quantities sufficient to support globally significant rates of respiration<sup>8,9</sup>. However, Fe(III), derived from photochemical oxidation of Fe(II) in Archaean seas and from hydrothermal vent fluids, is thought to have been abundant on early Earth<sup>5–7,10</sup>. To examine whether Fe(III) reduction might have been a metabolic feature of ancient anaerobic microorganisms, various hyperthermophilic microorganisms were screened for their capacity for Fe(III) reduction. The rationale for this was that geochemical and microbiological evidence indicates that early life might have evolved in or near hydrothermal zones and thus extant hyperthermophilic microorganisms that are most closely related to the last common ancestor (LCA) may serve as models for early forms of microbial respiration<sup>2,5,11,12</sup>. Although a capacity for Fe(III) reduction has been found in a variety of microorganisms, including some moderate thermophiles<sup>13–18</sup>, none of these is closely related to the LCA. Furthermore, the phylogenetic placement of these organisms in small, discrete groups does not provide convincing evidence for Fe(III) being an early metabolic characteristic<sup>13</sup>.

All of the hyperthermophiles were found to reduce Fe(III) (Fig. 1). In each case, Fe(III) reduction was enzymatic as there was no Fe(III) reduction in controls without cells, or when cells were incubated at 35°C, a temperature too low for hyperthermophilic enzymatic activity. The capacity for Fe(III) reduction was constitutive, as these organisms reduced Fe(III) even though they had been grown with other electron acceptors. This is similar to mesophilic Fe(III)-reducing bacteria, which also constitutively produce Fe(III) reductase when grown anaerobically with electron acceptors other than Fe(III)<sup>14</sup>.

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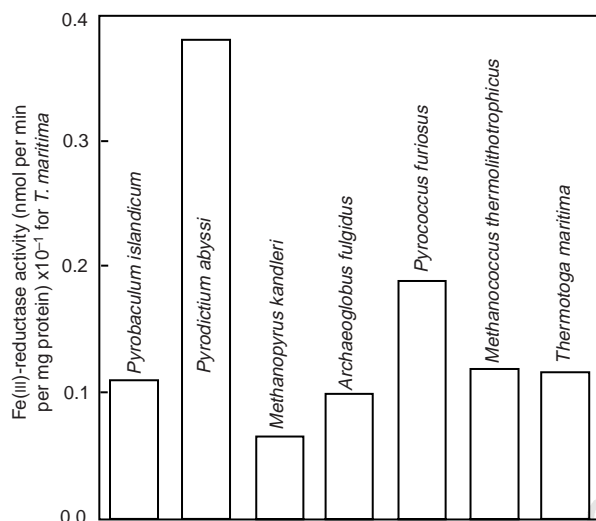


Figure 1 Rates of Fe(III) reduction in cell suspensions of hyperthermophiles.

*Pyrobaculum islandicum* and *Thermotoga maritima* were chosen for further study because of the generally recognized ease in culturing these organisms and because they provided examples of Archaea and Bacteria that were closely related to the LCA. Both hyperthermophiles grew in medium with H<sub>2</sub> as the electron donor and Fe(III) as the electron acceptor (Figs 2 and 3). There was no growth when either H<sub>2</sub> (Figs 2 and 3) or Fe(III) (data not shown) was omitted. Although these studies were done using soluble Fe(III), both *P. islandicum* and *T. maritima* grew readily in medium in which insoluble Fe(III) oxide was the electron acceptor, converting the Fe(III) oxide to magnetite in a manner similar to that observed previously in cultures of mesophilic Fe(III) reducers<sup>19,20</sup>. This is significant because magnetite accumulations that might be attributed to Fe(III)-reducing microorganisms have been used as evidence that Fe(III) reduction was the first globally significant respiratory process on Earth<sup>6</sup>, as well as evidence for early life on Mars<sup>21</sup>, and for a hot deep terrestrial biosphere<sup>22</sup>.

The finding that a diversity of microorganisms most closely related to the LCA have the ability to reduce Fe(III) suggests that the LCA was an Fe(III)-reducing microorganism. For organisms most closely related to the LCA, Fe(III) reduction seems to be a more

universal metabolic characteristic than elemental sulphur (S<sup>0</sup>) reduction, because hyperthermophiles such as *Archaeoglobus fulgidus* that do not reduce S<sup>0</sup> do reduce Fe(III). More significant is the finding that with Fe(III) as the electron acceptor, *T. maritima* can grow as a respiratory organism, whereas it was previously considered to have only a fermentative metabolism as it does not conserve energy from S<sup>0</sup> or thiosulphate reduction<sup>3,4,23,24</sup>. This suggests that Fe(III) reduction is more closely associated with central metabolism in some hyperthermophiles than S<sup>0</sup> reduction. However, the role of Fe(III) reduction in the metabolism of all of the hyperthermophiles found to have the capacity for Fe(III) reduction is not yet known. For example, the methanogens continued to produce methane during Fe(III) reduction, which makes it difficult to determine whether energy to support growth is conserved during Fe(III) reduction by these organisms. In addition to investigating Fe(III) reduction by hyperthermophiles, it will be interesting to determine whether the as-yet uncultured Archaea, whose 16S ribosomal DNA sequences have been found in cooler environments such as soils and aquatic sediments<sup>12</sup>, can reduce Fe(III) and whether isolation with medium containing Fe(III) as the electron acceptor may be a productive strategy for culturing these organisms.

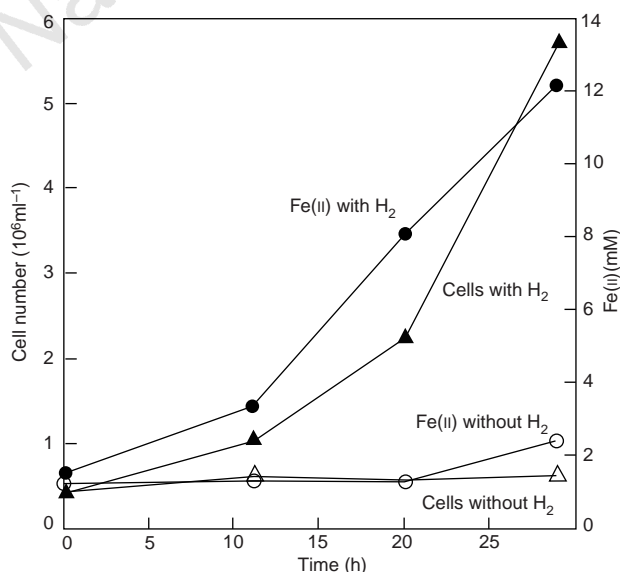


Figure 2 Growth of *Pyrobaculum islandicum* with H<sub>2</sub> as the electron donor and Fe(III) as the electron acceptor.

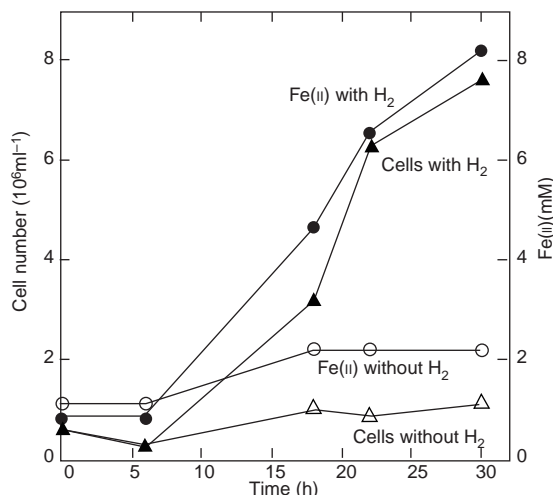


Figure 3 Growth of *Thermotoga maritima* with H<sub>2</sub> as the electron donor and Fe(III) as the electron acceptor.

Although the LCA was likely to have been a metabolically sophisticated respiratory organism<sup>2,5,12</sup>, earlier more primitive microorganisms might also have had the capacity to transfer electrons to extracellular Fe(III). The evolution of this metabolic capability would have provided organisms with an energetic advantage over early fermentative microorganisms by greatly expanding the range and extent of potential electron-donor oxidation. Once reduced, iron can readily transfer electrons to other electron acceptors, and this may have led to the intracellular incorporation of Fe(III)/Fe(II) redox couples that are common in more complex, modern electron-transport pathways. □

**Methods**

Organisms were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). For studies with cell suspensions, cells were grown in the medium suggested by the DSMZ for their cultivation. Cells were collected by centrifugation under N<sub>2</sub>-CO<sub>2</sub> (80:20), and washed cell suspensions were prepared in anaerobic bicarbonate buffer (30 mM; pH 6.8) to provide ~0.1 mg of cell protein in 5 ml bicarbonate buffer under N<sub>2</sub>-CO<sub>2</sub>. H<sub>2</sub> (101 kPa) was added as the electron donor and Fe(III) citrate (5 mM) was added as the electron acceptor. For studies on the growth of *P. islandicum* with Fe(III), cells were grown in DSMZ medium 390 in which thiosulphate and sodium sulphide had been replaced by Fe(III) citrate (15 mM) as the electron acceptor and 0.25 mM cysteine as a sulphur source, with 0.02% yeast extract added. H<sub>2</sub> was provided at 101 kPa; the incubation was done at 90 °C. For studies on the growth of *T. maritima* with Fe(III), cells were grown in TB medium<sup>25</sup> modified by substituting PIPES buffer with bicarbonate (30 mM), decreasing the cysteine to 0.5 mM, adding 0.05% yeast extract, and supplying H<sub>2</sub> (101 kPa) and Fe(III) citrate (15 mM); incubation was at 80 °C. For growth on Fe(III) oxide, organisms were grown on the media described but with Fe(III) citrate replaced with 100 mM Fe(III) oxide<sup>26</sup>. Cell numbers were monitored with acridine orange staining and epifluorescent microscopy<sup>27</sup>. Production of Fe(II) was quantified with ferrozine<sup>26</sup>.

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## The role of nonlinear dynamics of the syrinx in the vocalizations of a songbird

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Birdsong is characterized by the modulation of sound properties over a wide range of timescales<sup>1</sup>. Understanding the mechanisms by which the brain organizes this complex temporal behaviour is a central motivation in the study of the song control and learning system<sup>2–8</sup>. Here we present evidence that, in addition to central neural control, a further level of temporal organization is provided by nonlinear oscillatory dynamics that are intrinsic to the avian vocal organ. A detailed temporal and spectral examination of song of the zebra finch (*Taeniopygia guttata*) reveals a class of rapid song modulations that are consistent with transitions in the dynamical state of the syrinx. Furthermore, *in vitro* experiments show that the syrinx can produce a sequence of oscillatory states that are both spectrally and temporally complex in response to the slow variation of respiratory or syringeal parameters. As a consequence, simple variations in a small number of neural signals can result in a complex acoustic sequence.

Zebra-finch songs are organized into short segments of sound, referred to as syllables, that often contain rapid sequences of whistled notes, harmonic stacks and aperiodic signals<sup>9,10</sup>. Sounds within a syllable are produced contiguously with no silent interval during the transitions between distinct signal types (Fig. 1a). This continuity allows one to observe changes in the oscillatory state of the syrinx during modulations of the acoustic signal. We recorded the directed song of male zebra finches (*n* = 12) in a sound-isolation chamber. Close examination of the song spectra and acoustic waveforms shows sudden transitions from periodic to aperiodic or chaotic dynamics, period doubling, and mode-locking transitions. We believe that these features arise because the syrinx behaves as a low-dimensional nonlinear dynamical system.

Most (10 of 12) of the songs we recorded contained syllables exhibiting rapid (<10 ms) transitions from periodic to chaotic, or noisy, signals. Figure 1b shows two examples of this behaviour at an expanded timescale. These transitions sometimes occur in less than 1 ms, and often appear to jump back and forth between the periodic and chaotic oscillation. Another characteristic of nonlinear dynamical systems, period doubling, is characterized by a change in oscillation frequency such that spectral components appear at half the original frequency spacing. An example of period doubling in zebra-finch song is shown in Fig. 1c; at the time marked with the asterisk (\*), note the rapid onset of interspersed harmonic components as described above. Period-doubling transitions were seen in three of the zebra-finch songs examined.

Zebra-finch song syllables can also exhibit modulations that suggest the presence of mode locking in the syringeal dynamics.