Chapter 5

FOLLOW THE OXYGEN: COMPARATIVE HISTORIES OF PLANETARY OXYGENATION AND OPPORTUNITIES FOR LIFE

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Abstract

Aerobic respiration—the ability to couple the reduction of molecular oxygen (O_2) to the oxidation of reduced compounds while conserving energy to drive cellular processes—is the most widespread and bioenergetically favorable metabolism on Earth today. Aerobic respiration is essential for the development of complex life, and so the presence of abundant molecular oxygen is a crucial component of planetary habitability. O_2 on Earth is supplied by oxygenic photosynthesis, but it is becoming more widely understood that other, abiotic processes may supply small amounts of O_2 on other worlds. It is not yet clear however whether these sources are sufficient for aerobic organisms. Observations of the modern Martian atmosphere and analysis of the Martian rock record suggest a history of relatively high O_2 as a result of photochemical processes, potentially overlapping with the range of oxygen concentrations used by biology. Europa, meanwhile, may have accumulated high oxygen concentrations in its subsurface ocean due to the radiolysis of ices at its surface. Recent modeling efforts have suggested that coexisting water and oxygen may be common on exoplanets, with confirmation from measurements of exoplanet atmospheres expected in the near future. In all of these cases, O_2 is thought to have accumulated through abiotic processes, independent of photosynthesis. If correct, this would have far-reaching implications for interpretations of the habitability of these

worlds, potentially allowing the development of highly-energetic aerobic respiration, and even the complex multicellular life which depends on it, without first evolving oxygenic photosynthesis, a biochemically-complex metabolism which has evolved only once in Earth history. This suggests a new approach to investigating planetary habitability: “Follow the Oxygen”, in which environments with opportunities for energetic metabolisms such as aerobic respiration are targeted for investigation and life detection.

Introduction

Aerobic respiration—the ability to couple the reduction of O_2 to the oxidation of reduced compounds such as organic carbon, ferrous iron, reduced sulfur compounds, or molecular hydrogen while conserving energy to drive cellular processes—is the most widespread and bioenergetically favorable metabolism on Earth today. Aerobic respiration is also viewed as essential for the development of complex multicellular life (Catling et al. 2005), and so the presence of abundant molecular oxygen is a crucial component of planetary habitability (Cockell et al. 2016). O_2 on Earth is almost entirely supplied by oxygenic photosynthesis—a sophisticated metabolism involving the photochemical oxidation of water (Fischer et al. 2016)—but it is becoming more widely appreciated that other, abiotic processes may supply small amounts of O_2 and related compounds such as H_2O_2 (e.g. Borda et al. 2001, Liang et al. 2006, Lu et al. 2014). While it appears that these abiotic oxidant fluxes are insufficient to supply biologically useful oxygen concentrations on the Earth (Pavlov et al. 2001), differing histories and conditions on other planets could lead to sufficient accumulation of oxygen to concentrations that would be relevant to biology. Observations of such abiotically produced oxygen oxygenation have been made on Mars, and are predicted for Europa and certain classes of exoplanets, potentially to be confirmed by upcoming observations.

Classically, the Pasteur Point—the O_2 threshold at which brewer's yeast transitions from respiration to fermentation (~3 μM)—has long defined the assumed lower oxygen limit for the viability of aerobic respiration. However, recent experiments involving diverse organisms under extremely low oxygen concentrations have demonstrated aerobic respiration and growth at far lower oxygen concentrations, expanding the viable range for aerobic life downward. Laboratory experiments with marine sponges have demonstrated that low-micromolar oxygen concentrations are sufficient to support the metabolisms of these animals (Mills et al. 2014), while similar experiments with *Escherichia coli* demonstrate growth at oxygen levels three orders of magnitude lower (Stolper et al. 2010). Meanwhile aerobic organisms including invertebrates (Sperling et al. 2013b) and microbes (e.g. Morris and Schmidt 2013) have been observed in environments that have previously been considered anoxic, indicating that this micro-aerobic respiration is ecologically meaningful in the natural environment. This suggests that more environments could be suitable for aerobic life than previously recognized. Discussion of the expanded range of environments habitable by aerobes has primarily been discussed in the context of Earth history (e.g., Zhang et al. 2016, Fischer 2016) and low oxygen environments on the modern Earth (e.g. Ulloa et al. 2012), but it also has broad relevance to a range of planetary environments, including those on Mars, Europa, and elsewhere.

O_2 is a major component of Mars' atmosphere today, where it is the fourth most abundant gas (Mahaffy et al. 2013). Though the absolute abundance of O_2 is relatively low on modern Mars due to the thin atmosphere, recent discoveries of manganese enrichments in Martian sedimentary rocks imply the existence of relatively high oxygen levels in aqueous environments sometime in Mars' past (Lanza et al 2014, Arvidson et al. 2016). These data, together with other studies (e.g. Halevy et al. 2011, Farquhar et al. 1998, Shaheen et al. 2015, and Hurowitz et al. 2010) provide support for the hypothesis that O_2 was once more abundant in Mars' atmosphere. Combined with improved understanding of the lower oxygen limits for aerobic respiration, these observations suggest that ancient Mars may have possessed sufficient oxygen to support aerobic organisms. If correct, this would have far-reaching implications for interpretations of the habitability of Mars and other worlds.

Europa is uniquely positioned in the outer solar system as a promising candidate for providing an environment habitable to aerobic life. Europa is thought to possess a liquid water ocean ~100 km deep, which is potentially oxygenated due to interactions with its icy shell, which accumulates O_2 from radiolysis of water ice at the surface (Hand et al. 2007, Hand and Brown 2013). Europa therefore provides an intriguing case study for habitability, with the interesting caveat that any Europan biosphere would be supported not by photosynthesis but instead driven by redox chemistry between oxidants produced at the surface and reductants sourced water-rock interactions at the base of the ocean (Chyba and Hand 2001). Metabolisms potentially supported by Europa include aerobic iron, sulfide, hydrogen, and methane oxidation. However, the rates of productivity and total biomass that these processes could support are not well constrained (e.g. Ward et al. 2017, Neubauer et al. 2002), making predictions of the extent of any Europan biosphere challenging.

In this paper, we contextualize the possibility of aerobic environments on other worlds within the framework of what is known about the history of oxygen on Earth and the role of oxygen in biology. The history and modern distribution of oxygen on Mars and Europa are used as test cases to begin examining the opportunities for aerobic respiration on other planets where the history of O_2 is not contingent on the evolution of oxygenic photosynthesis. This provides a basis for considering the more general case of exoplanets, which under a range of circumstances may support oxygenated atmospheres that may be detectable with upcoming investigations. The potential for substantial abiotically-sourced oxygen in various planetary environments expands the range of potentially habitable environments, provides the opportunity for highly energetic metabolisms, and may improve the likelihood of evolution of complex life by removing evolutionary bottlenecks contingent on challenging biochemical innovations related to oxygenic photosynthesis. The impact of oxidizing environments on the origin of life is unknown, however, and could introduce challenges to the origin of life where abiotic O_2 is abundant.

Oxygen on Earth

The history of molecular oxygen on Earth is closely tied to the history of photosynthesis. Atmospheric oxygen concentrations are constrained to well below $10^{-5.7}$ atm before the Great Oxygenation Event (GOE) about 2.35 Gya, as recorded by a range of proxies including the behavior of iron in paleosols and red beds (Holland 1984, Rye and Holland 1998), redox-sensitive detrital grains (Johnson et al. 2014), mass-independent fractionation of sulfur isotopes (Farquhar et al. 2000, Bekker et al. 2004, Johnson et al. 2013), and manganese deposits (Johnson et al. 2013, Maynard 2010). During the GOE, O_2 accumulated in the atmosphere to 1-10% present atmospheric levels (PAL) ($\sim 10^{-2}$-10^{-3} atm), and has remained a significant component of the fluid Earth ever since (Lyons et al. 2014). The oxygenation of Earth's atmosphere was caused by oxygenic photosynthesis by Cyanobacteria (e.g., Falkowski 2011, Shih 2015), and may have occurred rapidly following the evolution of this metabolism (Ward et al. 2016, Shih et al. 2016, Fischer et al. 2016). The history of molecular oxygen in Earth's atmosphere is therefore tightly coupled to the history of oxygenic photosynthesis by Cyanobacteria, making it an almost entirely biological history. The availability of O_2 ultimately paved the way for the evolution of aerobic respiration (Hemp et al. 2012), endosymbiosis and radiation of the eukaryotes (Katz 2012), and complex multicellularity (Catling et al. 2005, Knoll 2011).

From the perspective of thermodynamics, aerobic respiration is by far the most energetically favorable metabolism over a tremendous range in O_2 concentrations when compared to common anaerobic respiration processes (Figure 2). For example, it typically yields 4 times more energy per electron than sulfate reduction across more than six orders of magnitude in O_2 concentrations. While thermodynamics predicts that aerobic respiration should be viable down to vanishingly low concentrations of O_2, it has remained contentious whether life could make use of these lowest concentrations, or if kinetic constraints on the biochemistry of aerobic respiration set a threshold below which respiration is not physiologically viable. Historically, the Pasteur Point has been considered to mark the minimum oxygen concentration for aerobic respiration, below which organisms transition to fermentation (or other anaerobic metabolisms) to maintain energetic balance. The Pasteur Point corresponds to an oxygen concentration of about 1% of PAL dissolved in seawater, or about 3 μM. This is also thought to be the minimum oxygen concentration for aerobic respiration (e.g. Berkner and Marshall 1965), but a recent study demonstrated aerobic respiration by *Escherischia coli* at vastly lower oxygen concentrations, closer to 0.001% PAL (Stolper et al 2010). This greatly extends the potential range of oxygen concentrations under which aerobic respiration is a viable metabolism, and therefor the range of environments habitable by aerobic organisms, as confirmed by observations of obligately aerobic organisms in environments such as Oxygen Minimum Zones (Sperling et al. 2013b, Ulloa et al. 2013), freshwater sediment (Ettwig et al. 2010), and wastewater sludge (Yamada et al. 2006, Ward et al. 2015).

Aerobic respiration has played a critical role in the development of complex life. Aerobic respiration was a major factor in the development of eukaryotes (Katz 2012) and ultimately animals (Knoll 2011), as large, complex organisms have inherently high energetic demands that can be met by aerobic respiration but not by anaerobic metabolisms (Catling et al. 2005). While it has historically been assumed that animals required high oxygen concentrations to evolve due to inherently high O_2 demand of multicellularity (e.g. Nursall 1957), these critical oxygen concentrations have never been rigorously quantified, though they have been estimated based largely on diffusion limitations (e.g. Runnegar 1991). Recent efforts to understand the minimum oxygen concentrations necessary to support sponges and other basal animals have demonstrated survival and growth under surprisingly low O_2 concentrations, down to about 1% PAL (Mills et al 2014, Sperling et al. 2013a). While these values are still much higher than the minimum oxygen concentrations for bacterial respiration (Stolper et al 2010), they greatly extend the spatial and temporal range of environments habitable by animals (e.g. oxygen minimum zones and oceans during the Proterozoic, e.g. Sperling et al. 2013b, Zhang et al. 2016, Fischer 2016) and are an important new constraint in thinking about oxygen as a requirement and biosignature of complex life on exoplanets

Oxygen on Mars

The history of O_2 on Mars is a curious contrast to that on Earth. While terrestrial O_2 is tied to the biological process of oxygenic photosynthesis, Mars appears to have a history of O_2 despite no apparent biological source. It is widely appreciated that the surface of Mars is, and perhaps has been since its early history, “oxidizing” (e.g., Hunten, 1979; Zent and McKay, 1994; Christensen et al., 2001; Tosca et al., 2005; McLennan et al., 2005; Goetz et al., 2005; Bibring et al., 2006; Zahnle et al., 2008; Hecht et al., 2009; Hurowitz et al. 2010; Leshin et al., 2013). Though it is the fourth most abundant species in the atmosphere, Mars currently has low absolute concentrations of O_2—a mixing ratio of 1.45×10^{-3} (Mahaffy et al. 2013). For comparison, these levels of O_2 are approximately equivalent to upper levels of O_2 allowed by geochemical proxy data on the Archean Earth (Farquhar et al. 2000; Johnson et al. 2014). However, a range of proxies suggest that oxygen concentrations may have been higher in Mars’ past, perhaps when the atmosphere was thicker (e.g. Brain and

Jakosky 1998, Manga et al. 2012, Kite et al. 2014). This notion is supported by the presence of enriched manganese deposits (Lanza et al. 2014), multiple oxygen isotope ratio data collected from shallow, low-temperature carbonates preserved in the Martian meteorite ALH84001 (Farquhar et al. 1998, Halevy et al. 2011, Shaheen et al. 2015), and the presence of high concentration of oxidized salts on the surface of Mars thought to be derived from reactions involving ozone and other oxygen-derived species (e.g. Zahnle et al. 2008, Catling et al. 2010).

These results offer an interesting perspective on the habitability of Mars now and in the past. While free oxygen is essentially a trace gas in the modern Martian atmosphere, saturation of O_2 in liquid water under Martian surface conditions today would be approximately 10 nM—quite low, but within the range of oxygen concentrations shown to be utilized by *E. coli* (Stolper et al. 2010) (Figure 2). If liquid water is present on Mars (as now appears to be the case, at least transiently, e.g. Ojha et al. 2015), it could present a viable environment for aerobic microbes.

The ancient atmosphere of Mars is thought to have been much thicker than it is today, perhaps on the order of 1-2 bar (Kite et al. 2014). If the mixing ratio of O_2 were similar in this thick early atmosphere to that of modern Mars, saturation of O_2 would be on the order of ~4 μM, sufficient not only for aerobic bacteria, but in fact above the Pasteur Point and in line with the oxygen requirements of basal animals like sponges (Sperling et al. 2013a, Mills et al. 2014) (Figure 2). This is much higher than O_2 concentrations on the early Earth, perhaps reflecting relatively higher rates of hydrogen escape due to Mars' lower gravity (e.g. Lammer et al. 2013). Of course, this value is determined utilizing an overly simple model, with a more nuanced view arising from careful analysis of paleooxygen proxies.

While many of the trace metal geochemical proxies utilized for understanding the history of oxygen on Earth either have not or cannot be applied to Mars, recent descriptions of concentrated manganese deposits in Martian rocks can help constrain ancient O_2 concentrations on Mars (Lanza et al. 2014). Manganese is the third most abundant transition metal in the crusts of Earth and Mars, where it is present only as Mn(II) and substitutes for ferrous iron in a wide variety of primary igneous minerals (Turekian and Wedepohl 1961, Taylor and McLennan, 2009). Chemical weathering of basalt, therefore, provides a substantial flux of Mn^{2+} to surface and ground waters. Mn^{2+} is soluble unless oxidized to Mn(III) or Mn(IV), which subsequently undergoes hydrolysis and forms insoluble oxide phases that rapidly sediment (Stumm and Morgan 1996). Compared to iron or sulfur, however, manganese requires uniquely high potential oxidants >500 mV (O_2 or species derived from O_2 like O_3) to undergo redox cycling and become concentrated in sedimentary rocks. In addition, the oxidation of Mn(II) is comparatively sluggish even in environments with abundant O_2, particularly in the absence of biological catalysts (Luther 2010; Morgan 2005). Due to this, Mn-rich sedimentary rocks do not appear on the Earth until after the evolution of photosynthesis and rise of oxygen (Johnson et al. 2013, Maynard 2010).

A wide range of Mn-rich materials have been discovered in sedimentary rock targets in the first 360 sols of the Curiosity rover's traverse across sedimentary rocks deposited in Gale crater (Lanza et al. 2014); these rocks contain Mn abundances that are 1–2 orders of magnitude higher than previously observed on Mars and require redox cycling of Mn under highly oxidizing aqueous conditions. Subsequent observations made by Curiosity's instrument payload revealed that at least some of these Mn-rich phases include Mn-oxides that precipitated in an ancient groundwater aquifer (Lanza et al. 2016). These results are important because unlike the prior widespread observations of iron oxides and sulfates, Mn oxides tell us that molecular oxygen has left its mark on the early Mars geological record at a time and place in which liquid water was also present.

Oxygen on Europa

Another interesting example of worlds with a significant role played by abiotic oxygen is that of icy moons such as Europa, which may host a subsurface ocean with substantial oxygen sourced from ice radiolysis (Hand et al. 2007, Hand et al. 2009). It is generally accepted that Europa possesses an extensive subsurface ocean (Carr et al. 1998, Stevenson 2000, Hand et al. 2007), potentially containing 2-3 times the volume of water on Earth (Pappallardo et al. 1999, Stevenson 2000, Chyba and Phillips 2002). This ocean is though to be salty based on measurements of magnetism (Khurana et al., 1998; Kivelson et al., 2000), potentially dominated by magnesium sulfate (Zolotov and Shock 2001). The base of Europa's ocean is thought to be in contact with the rocky core, where water/rock interactions can provide a source of hydrogen and other reduced compounds through serpentinization-like reactions (Vance et al. 2007). These observations, as well as that of putative subduction of the icy shell (Kattenhorn and Prockter 2014), suggest a geologically active world with sources of both reduced and oxidized compounds that could support a biosphere in the ice-covered ocean.

Observations of a tenuous oxygen atmosphere of ~10^{-11} bar were first made by Hubble Space Telescope observations (Hall et al. 1995), with the dominant source of molecular oxygen and other oxidants in the Europa system being bombardment of water and CO_2 ice on the surface by energetic electrons and ions driven by Jupiter's magnetic field (Johnson et al. 2004). As these radiolytically sourced oxidants can be trapped in ice and eventually subducted or otherwise brought into contact with Europa's ocean, this can ultimately serve as a substantial source of abiotic O_2 to any biosphere that may exist on Europa. The potential for biologically relevant oxygen accumulation in the subsurface ocean has been described previously (e.g. Chyba 2000, Hand et al. 2007). While the rate of oxygen production at the surface of Europa can be reasonably well estimated (e.g. Chyba and Phillips 2001), the rate of resurfacing and of admixture of oxygen into the subsurface ocean is not known, but will ultimately determine the flux of O_2. Estimates of resurfacing rates have been made based on crater counts and inference of subduction rates, suggesting a turnover time of <90 Ma (Zahnle et al. 2003, Kattenhorn and Prockter 2014), potentially resulting in a flux of O_2 >10^9 moles/year (Hand et al. 2007).

Depending on the rate at which Europa's ice shell is mixed with the ocean, as well as the rates at which this oxygen is consumed, oxygen may accumulate to concentrations higher than those in Earth's oceans (Hand et al. 2007). Oxygen concentrations in these environments might therefore be sufficiently high to not impose any limitations on aerobic respiration, though any net global productivity would instead likely be due to limited fluxes of reducing power from water/rock interactions at the base of the subsurface ocean (McCollom 1999, Ward et al. 2017). Improved understanding of the oxygen requirements and metabolic efficiencies of aerobic chemolithotrophic metabolisms could allow improved predictions of biomass, which could potentially be supported on icy moons like Europa.

Role of biology in oxygen

The discovery of proxy evidence for even trace oxygen in Earth history is frequently interpreted as evidence for the presence of oxygenic Cyanobacteria (e.g. Rosing and Frei 2002, Anbar et al. 2007, Crowe et al. 2013, Planavsky et al. 2014). Meanwhile, by any number of these proxies, Mars would appear now and in the past to have been well oxygenated. However, this Martian history is certainly not interpreted as evidence for the presence of Cyanobacteria. This double standard in interpretation should serve as a reminder to be careful in assumptions about the source of oxidizing potential in the past. Oxygen need not always be biologically sourced to make an appearance in the rock record—photochemical processes can also produce oxygen and other oxidants, on the Earth as well as Mars (e.g. Haqq-Misra et al. 2011). Care must therefore be taken when interpreting evidence of oxygen, as Mars provides clear evidence that significant oxygen can be the result of purely abiotic processes. This caution must extend to the possible future interpretation of oxygen in expolanet atmospheres. Oxygen, by supporting aerobic respiration, is thought to be essential for the evolution of multicellularity and complex life, and so is an important target for detection in exoplanet atmospheres (Leger et al. 2011, McKay 2014). While its discovery would be important for interpreting the habitability of a body, it may not necessarily reflect biological sources.

Role of oxygen in biology

While aerobic respiration is generally considered in the context of organoheterotrophy (i.e. organisms that consume fixed organic matter as both a carbon and energy source), oxygen is also utilized for biosynthesis, activation of recalcitrant metabolic substrates, as well as for aerobic respiration coupled to lithoautotrophy (i.e. organisms that oxidize inorganic compounds as an energy source to drive carbon fixation).

Oxygen is utilized in several important biosynthetic pathways, including the synthesis of sterols by eukaryotes and collagen by animals (Towe 1981). While these processes may seem minor, these and other biosynthetic pathways may account for up to 10% of O_2 consumption by mammals (Rolfe and Brown 1997). O_2 concentrations required for biosynthesis can be incredibly low, with sterol synthesis requiring O_2 at only nanomolar concentrations for the epoxidation of squalene and later oxidative demethylations of intermediates like lanosterol, but nonetheless cannot proceed under fully anoxic conditions (Waldbauer et al. 2011). The utilization of oxygen in biosynthetic processes appears to be a relatively late innovation, after the radiation of the three domains of life (Raymond and Segre 2006). This suggests that while molecular oxygen may not be necessary for the origin of life, it does provide an opportunity for significant diversification of biochemical processes and may play a role in the development of complex life beyond just serving as a highly energetic electron acceptor (Raymond and Segre 2006, Falkowski 2006).

O_2 is furthermore essential for the activation of otherwise inaccessible electron donors. Compounds such as methane and ammonia must first be activated utilizing O_2 via a monooxygenase enzyme in a non-energy conserving step before they can subsequently be further oxidized to conserve energy (Hanson and Hanson 1996). While these processes have an absolute requirement for oxygen, they can make use of exceedingly low oxygen concentrations. Half saturation constants for ammonia oxidation are on the order of ~300 nM O_2 (Bristow et al. 2016), and both methane and ammonia oxidation has been observed in environments with much less than 1 μM O_2 (Hatzenpichler 2012, Kits et al. 2015, Bristow et al. 2016).

Finally, oxygen is utilized as an electron acceptor in many lithotrophic metabolisms. Oxygen reduction can be bioenergetically coupled to the oxidation of reduced sulfur compounds (such as sulfide and elemental sulfur), ferrous iron, molecular hydrogen, and reduced nitrogen compounds (ammonium and nitrite). These processes are carried out by diverse bacteria and archaea, and are essential for much of the biogeochemical nutrient cycles on Earth. The reduction of oxygen in these processes is through a combination of respiratory electron transfer through the HCOs and *bd* oxidase in addition to utilization of molecular oxygen to activate recalcitrant substrates (such as the activation of ammonium to hydroxylamine by ammonia monooxygenase). These various enzymes may have different oxygen requirements; further characterization of the oxygen requirements of the oxygenase enzymes in the context of lithoautotrophs will help to constrain what if any of these metabolic couples may be viable in modern or ancient Martian environments.

Because the oxidation of reduced compounds by O_2 is often a competition between the spontaneous abiotic reaction and microbes performing this process for energy conservation, microbes have adapted to microaerobic conditions where the abiotic rate is sufficiently sluggish. Iron oxidizing bacteria, for instance, typically grow under microaerobic conditions below 50 μM O_2, and have been demonstrated to grow at O_2 concentrations as low as 3 μM (Chan et al. 2016). Whether this is an ecological lower limit or a biochemical one is unclear, as the thermodynamics of iron oxidation remain favorable even under much lower O_2 concentrations (Emerson et al. 2010). Furthermore, the yield of carbon fixed by these metabolisms is poorly constrained, but appears to be significantly less efficient than ideal stoichiometries (e.g. Neubauer et al. 2002, Ward et al. 2017), adding additional uncertainty to the potential extent of aerobic lithotrophic metabolisms on other worlds.

Potentially viable metabolisms on Mars and other worlds

The mounting evidence for liquid water throughout Mars' past as well as potentially on the modern surface (Martin-Torres et al. 2015, Grotzinger et al. 2015, Ojha et al. 2015), combined with the apparent availability of free oxygen, suggests a history of Martian environments that are not only habitable but also present a variety of thermodynamically favorable metabolic couples. For instance, the putative discovery of methane on Mars (Formisano et al 2004, Mumma et al. 2009, Webster et al. 2015) along with metabolically useful oxygen presents the opportunity for aerobic methanotrophy, the metabolic oxidation of methane to CO_2 using O_2 while conserving energy, a process driven by diverse bacteria on Earth (e.g. Hanson and Hanson 1996). This metabolism requires molecular oxygen for the activation of methane, and typically couples this process to aerobic respiration (Hanson and Hanson 1996). Recently, however, it has been discovered that the terminal electron accepting process for this metabolism is more versatile than previously realized, with the potential in some bacteria to couple aerobic methanotrophy to nitrate reduction when oxygen concentrations are low (e.g. Kits et al. 2015, Skennerton et al. 2015). The recent discovery of nitrate in Martian sediment (Stern et al. 2015) therefore supports the potential viability of this metabolism in some Martian environments as well.

Other potentially viable metabolisms on the Martian surface include the aerobic oxidation of ferrous iron. Iron oxidizing bacteria are typically microaerobic, with preferred oxygen concentrations on the order of ~1-100 μM O_2, but likely functioning down to much lower concentrations (Chan et al. 2016). As the metabolic byproduct of iron oxidation is solid ferric iron oxides, iron oxidizing bacteria must shed ferric minerals, typically in diagnostic, preservable morphologies such as sheathes and stalks (e.g. Chan et al. 2011). If similar microbes exist on Mars, iron biominerals could be observable biosignatures. The yield of carbon fixed per iron oxidized by iron oxidizing bacteria is poorly constrained: the ideal electron-balanced stoichiometry of this metabolism is 4 Fe: 1C, yet experimental yields are much poorer, on the order of 40 Fe:

1C (Neubauer et al. 2002), with similarly poor yields in natural systems (e.g. Ward et al. 2017). As a result, the size of biosphere and amount of productivity this metabolism could support is poorly constrained.

Meanwhile, Europa is predicted to have extensive water-rock interactions at the base of the ocean, potentially supporting ecosystems analogous to hydrothermal vents on Earth. The geochemical makeup of these systems on Europa is not well constrained, but likely supply a flux of reduced compounds such as molecular hydrogen, ferrous iron, and/or sulfide. These compounds can all be aerobically oxidized by lithoautotrophs, potentially supporting primary productivity entirely detached from photosynthesis.

The interaction of reduced compounds from water/rock interactions at Europa's core and oxygen sourced from the icy shell could support aerobic chemoautotrophic metabolisms, such as hydrogen, sulfur, or ferrous iron oxidation. Biomass estimates have been derived for Europa (e.g. Chyba and Phillips 2001, Zolotov and Shock 2003, McCollom 1999, Hand et al. 2009); these estimates depend in part on the limits of aerobic respiration, and the yield of lithotrophic metabolisms. Both of these factors are only poorly constrained (e.g. Chan et al. 2016, Ward et al. 2017, Neubauer et al. 2002). As a result, the potential for the origin and survival of a substantial biosphere is contested (e.g. Gaidos et al. 1999). However, the depth of rock fracturing and therefore water-rock interactions on Europa may be much deeper than on Earth (Vance et al. 2007), potentially increasing the flux of reduced compounds available for biological productivity.

Oxygen fluxes into Europa's ocean may be quite high, particularly given recent evidence for subduction indicating resurfacing at <90 Ma timescales (Kattenhorn and

Prockter 2014). The resulting rates of delivery of oxygen from radiolysis on the surface into the ocean may even result in dissolved O_2 in Europa's ocean on the order of 10^{-3} moles/liter, nearly an order of magnitude higher than saturation at the surface of Earth's ocean today (Hand et al. 2007) (Figure 2). Recent analysis of the redox balance of Europa suggests that hydrogen fluxes from serpentinization-like reactions are comparable to oxygen delivery from the surface (Vance et al. 2016). Europa could therefore support a biosphere at a stable redoxicline at some region between the base and top of the ocean—the exact location and extent of this productive redoxicline could have significant bearing on life detection on Europa, and whether this could be viable from a plume-sampling flyby or if direct sampling of the Europan ocean would be necessary.

Evolutionary history of respiration

One caveat to the viability of aerobic respiration on Mars today is that while aerobic respiration continues down to vanishingly low oxygen concentrations, it may not be sufficient for supporting growth. Aerobic respiration is accomplished using a complex electron transport chain, which culminates in the enzymatic reduction of O_2 to H_2O, a step that can be accomplished using any one of several proteins with disparate evolutionary histories. These include the heme copper O_2 reductase superfamily (A-, B-, and C-families, which are all related), and the *bd* oxidases, which evolved independently (Borisov et al 2011). The A-family heme copper O_2 reductases (HCO) are the most widespread in nature (including in eukaryotic mitochondria), and require the highest O_2 levels to be effective, but also conserve the most energy (Han et al 2011). The other families (including the *bd* oxidases) conserve less energy but are effective at lower O_2

concentrations (Morris and Schmidt 2013). While it is unclear whether this inverse correlation between energy conservation and O_2 affinity is the result of biochemical optimization or evolutionary contingency, it must be considered that respiration under relatively low oxygen concentrations, such as those that may have existed on early Mars, may not be capable of supporting the same amount of biomass as respiration at higher oxygen concentrations. Further investigation into the relationship of O_2 concentrations, energy conservation, and the evolutionary history of aerobic respiration can help to resolve this uncertainty.

Oxygen and the evolution of complex life

The possibility of oxygen accumulating to substantial concentrations through purely abiotic processes has exciting implications for the evolution of life on other planets. Oxygenic photosynthesis is an evolutionary singularity, and has evolved only once in Earth history through the complicated coupling of two independent photosystems (Falkowski 2011). This innovation was essential for the eventual evolution of complex life on Earth, but is the unlikely result of evolutionary contingency. The evolution of complex multicellularity, on the other hand, has occurred independently several times on Earth (e.g. in the animals, fungi, plants, and red algae)(Knoll et al. 2011), and simple forms of multicellularity have even been artificially evolved over short timescales in the laboratory (Ratcliff et al. 2012). The evolution of multicellularity may therefore be "easier", though it has only occurred on Earth after the rise of oxygen. It is therefore possible that on other worlds where oxygen accumulates through abiotic processes, multicellularity could evolve more quickly and easily, as it does not require waiting for the “hard step” of oxygenic photosynthesis (Figure 3).

A potential caveat to abiotic oxygen improving the odds of evolution of complex life is the impact of oxidizing conditions on the origin of life. While conditions necessary for the origin of life are not known, most proposed environments for the origin of life, such as hydrothermal vents, are characterized by reducing conditions (e.g. Baross and Hoffman 1985, Martin et al. 2008). It is therefore possible that oxidizing conditions as a result of abiotic oxygen as proposed here could inhibit the origin of life, or at least restrict the environments in which it could occur. Abiotic oxygen could therefore be an evolutionary Catch 22: it can jumpstart the evolution of complex life, but prevents life from originating to begin with. This impasse could be solved by panspermia, in which life may originate on a world more favorable for its origin and then be transferred to one more conducive to its growth and evolution. While panspermia is usually discussed in the context of life originating elsewhere and subsequently seeding Earth (e.g. Kirschvink and Weiss 2002), this process could occur in either direction (e.g. Melosh 1988). As a result, life may have the opportunity to originate in one environment and subsequently thrive in another, at least in solar systems such as our own with a range of planetary environments.

Conclusions

The history of oxygen on Earth is, fundamentally, a biological one. O_2 is produced in staggering quantities as a result of oxygenic photosynthesis by Cyanobacteria, and is rapidly recycled as a result of aerobic respiration. This state of affairs has continued for the past 2.3 Ga, and has supported the radiation of many microbial groups as well as the origin and diversification of eukaryotes, and, eventually, complex multicellular organisms like plants, animals, and fungi. Before the rise of oxygenic photosynthesis, the Earth was essentially anoxic and supported only very limited productivity, and all life was microbial. The development of complex life therefore required multiple preconditions, some of which appear to have been very challenging for biology to evolve. Among these, oxygenic photosynthesis may be one of the hardest steps. This metabolism is biochemically complicated, required evolutionary contingencies to diverge and recombine two photosystems, and has evolved only a single time in Earth history. In comparison, aerobic respiration has multiple independent origins (heme copper oxidases, alternative oxidases, and *bd* oxidases), and multicellularity has even more (e.g. animals, plants, fungi, brown algae and red algae). The relatively late evolution of oxygenic photosynthesis may therefore have delayed the origin of complex life on Earth for a billion years or more. If oxygen can be produced abiotically in large quantities on other planets, then aerobic respiration –and the energetic, complex organisms that depend on it—may be capable of evolving more quickly. The presence of oxygen in a planet’s atmosphere or oceans may therefore be an important indicator of habitability, as even abiotic oxygen may provide a path to jumpstarting the emergence of complex life. We therefore propose a strategy of “Follow the Oxygen”, in which environments with opportunities for energetic metabolisms such as aerobic respiration are targeted for investigation and life detection. If life has evolved in these environments, it may be more likely to have developed into more complex forms than on Earth-like worlds where oxygenic photosynthesis was a necessary precondition for highly energetic organisms.

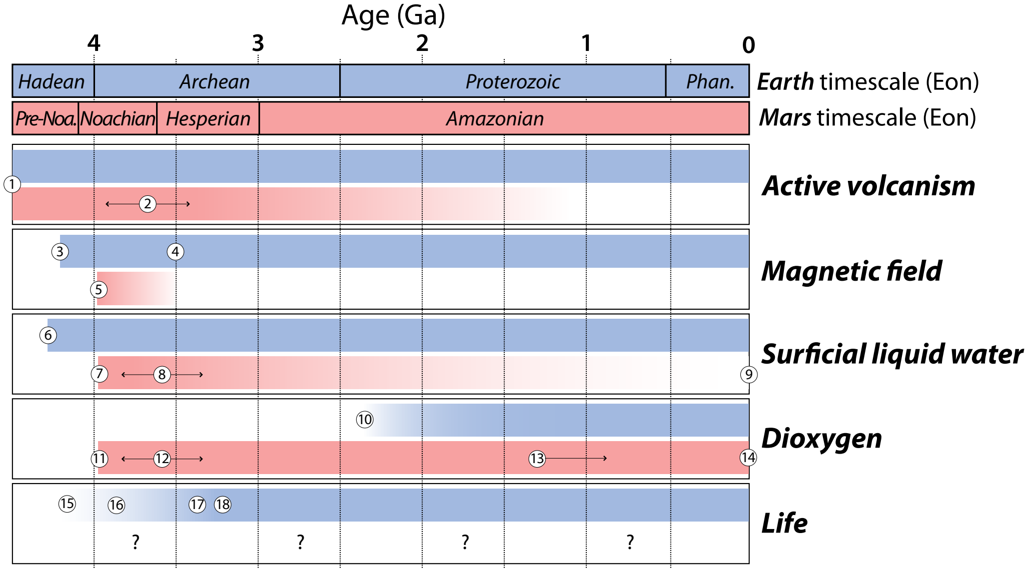

Figure 1: Comparative timelines of Earth and Mars. While the surfaces of Earth and Mars are now quite different, their early histories were more similar, with early Mars having once had a magnetic field, surficial water, active volcanism, and a dense atmosphere similar to Earth. Mars, however, appears to have had significant O_2 and ozone early in its history, in contrast to Earth.

1 - Active volcanism assumed due to the thermal status of both planets early in their history.

2 - Tharsis volcanic province (Bouley et al. 2016).

3 - Paleomagnetic data from Jack Hills zircons (Tarduno et al. 2015).

4 - Paleomagnetic data from early Archean greenstone belts (Tarduno et al. 2010, Biggin et al. 2011)

5 - Magnetic studies of ALH84001 (Weiss et al. 2002).

6 - Elevated O isotope ratios in Jack Hills zircons (Valley et al. 2002).

7 - Clumped isotope ratios of carbonates in ALH84001 (Halevy et al. 2011).

8 - Fluvio-deltaic strata in Gale Crater (Grotzinger et al. 2015) and Aeolis Dorsa (DiBiase et al. 2013).

9 - Hydrated salts associated with recurring slope lineae (Ojha et al. 2015).

10 - Great oxygenation event (Fischer et al. 2016).

11 - 17O mass anomalies in low-temperature carbonate salts in ALH84001 (Farquhar et al. 1998).

12 - Mn enrichments (Lanza et al. 2014) and high valent Cl-bearing salts (Leshin et al. 2013, Farley et al. 2016) in fluvio-deltaic strata deposited in Gale Crater.

13 - 17O mass anomalies in carbonate and sulfate salts of Nakhla and Lafayette (Farquhar et al. 2000).

14 - O2 mixing ratio of 1.45(+/-0.09)x10^-3 (by volume) in modern Mars atmosphere in Gale crater from Curiosity's quadrupole mass spectrometer (Mahaffy et al. 2013).

15 - Earliest speculative evidence for life from graphitic carbon inclusion in Jack Hills zircons (Bell et al. 2015).

16 - Promising but non-unique evidence for life graphite-bearing turbidite metasedimentary rocks from the Isua Supracrustal Belt (Rosing 1997).

17 - Earliest likely microfossils in sedimentary chert of the Strelley Pool Formation (Sugitani et al. 2010).

18 - Oldest certain microfossils in fine-grained clastics of the Moodies Formation (Javaux et al. 2010).

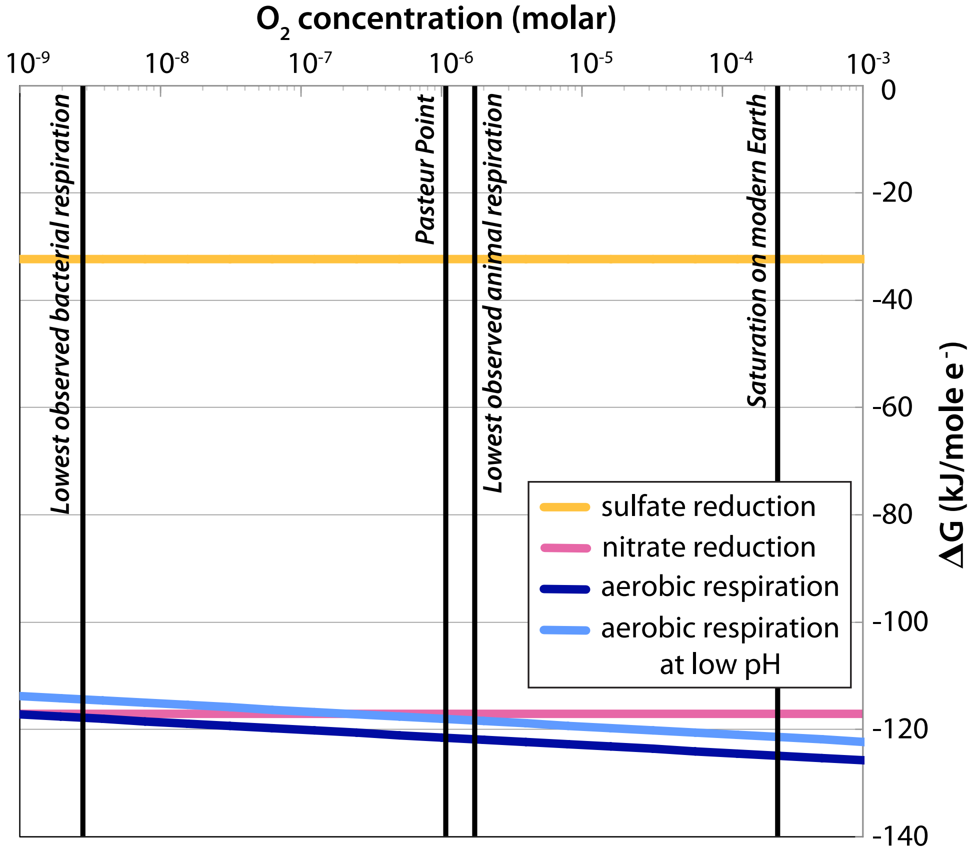

Figure 2: Thermodynamic favorability of microbial metabolisms as Gibbs free energy of reaction across a range of oxygen concentrations. Even at very low O_2 concentrations, aerobic respiration is still highly favorable ($\Delta G \ll 0$). Also plotted are lines denoting relevant oxygen concentrations, including known limits of respiration for bacteria (Stolper et al. 2010) and animals (Mills et al. 2014), as well as saturation under surface atmospheric conditions on modern Earth and Mars. Even the thin modern Martian atmosphere contains sufficient oxygen to support respiration by bacteria such as *E. coli*.

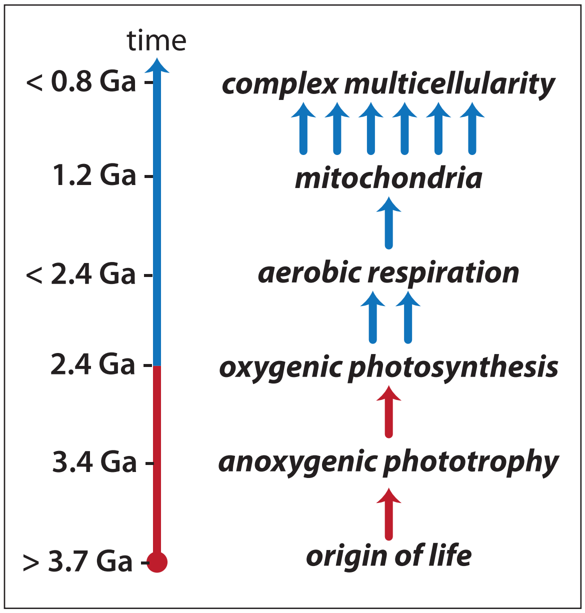

Figure 3: Evolutionary contingency and the origin of complex life. Several steps had to occur for complex life to evolve on Earth. Beginning with the origin of life, evolution of anoxygenic phototrophy, coupling in series of two reaction to perform oxygenic photosynthesis, evolution of aerobic respiration, evolution of eukaryotes and endosymbiosis of the mitochondrion, and finally the evolution of complex multicellularity. Of these, some steps have occurred independently multiple times, while others are evolutionary singularities. On a world with a significant abiotic O_2 flux, life may be able to skip the hard step of evolving oxygenic photosynthesis and jumpstart the evolution of complexity. Arvidson, RE et al. High concentrations of manganese and sulfur in deposits on Murray Ridge, Endeavour Crater, Mars. American Mineralogist, in press.

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