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Author for correspondence:
Emma U. Hammarlund
e-mail: emma.hammarlund@med.lu.se

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Harnessing hypoxia as an evolutionary driver of complex multicellularity

Emma U. Hammarlund[1,2]

[1]Department of Laboratory Medicine, Translational Cancer Research, Lund University, Scheelevägen 2, Medicon Village Building 404, 223 81 Lund, Sweden
[2]Department of Geology, Lund University, Sölvegatan 12, 223 62 Lund, Sweden

EUH, 0000-0001-7625-4793

Animal tissue requires low-oxygen conditions for its maintenance. The need for low-oxygen conditions contrasts with the idea of an evolutionary leap in animal diversity as a result of expanding oxic conditions. To accommodate tissue renewal at oxic conditions, however, vertebrate animals and vascular plants demonstrate abilities to access hypoxia. Here, I argue that multicellular organisms sustain oxic conditions first after internalizing hypoxic conditions. The 'harnessing' of hypoxia has allowed multicellular evolution to leave niches that were stable in terms of oxygen concentrations for those where oxygen fluctuates. Since oxygen fluctuates in most settings on Earth's surface, the ancestral niche would have been a deep marine setting. The hypothesis that 'large life' depends on harnessing hypoxia is illustrated in the context of conditions that promote the immature cell phenotype (stemness) in animal physiology and tumour biology and offers one explanation for the general rarity of diverse multicellularity over most of Earth's history.

1. Introduction

Animal tissues are usually thought of as growing and differentiating effectively when given *sufficient* access to nutrients and oxygen. However, the view that tissue growth and oxic conditions are easily compatible could be a fallacy based on observer bias. Although humans effortlessly live and grow at oxic conditions, the cells that fundamentally drive the formation of our tissues lose their function at oxygen exposure. An oxygen-sensitive basis of tissue (multicellularity) could imply that oxic conditions posed an evolutionary hurdle for the diversification of multicellular life, which contrasts with our current view on events of oxygenation as both permissive and prompting animal diversification. The following investigation of the potential hardships for life to become big is based on a tissue perspective and, thus, on clues from cell behaviour in plants, animals and indeed also tumour multicellularity.

Animal tissues consist of cells with many different functions and fates. The establishment of cell fate, or so-called cell differentiation, is a delicate process during development and tissue renewal where parts of the DNA are made inaccessible, while other parts remain accessible. Exposing an animal cell to oxygen, however, can immediately condense DNA (heterochromatin) within the nuclei [1,2], which restricts much gene transcription and leaves the cell with a specific fate (differentiation state) (figure 1). In contrast to differentiated cells, cells that continuously regenerate tissue originate from self-renewing immature cells (stem cells) that maintain versatile access to DNA through either an open chromatin conformation (euchromatin) or molecular mechanisms that open it. Recent discoveries relate stem cell phenotype in animals and plants with very low (hypoxic) oxygen concentrations [7,8] or even no oxygen (anoxia) [9]. This oxygen imbalance leaves us with a paradox: aerobic metabolism sustains 'large life' but tissue renewal and maintenance rely on hypoxia.

The evolution of complex multicellularity (organ-grade) is generally regarded as the result of our eukaryotic ancestors acquiring the necessary genetic toolkit, regulatory networks and ecospace in the presence of sufficient free

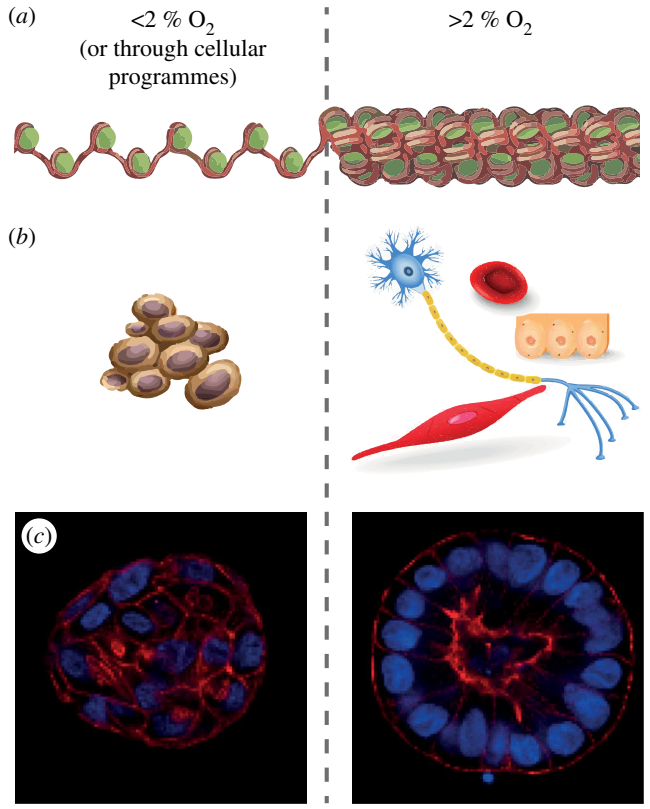

Figure 1. A critical threshold at approximately 2% O_2 separates (*a*) relaxed euchromatin from tight heterochromatin [1,2], (*b*) stem cells from differentiated cells [3–5] and (*c*) immature from differentiated cell behaviour within tissues [6].

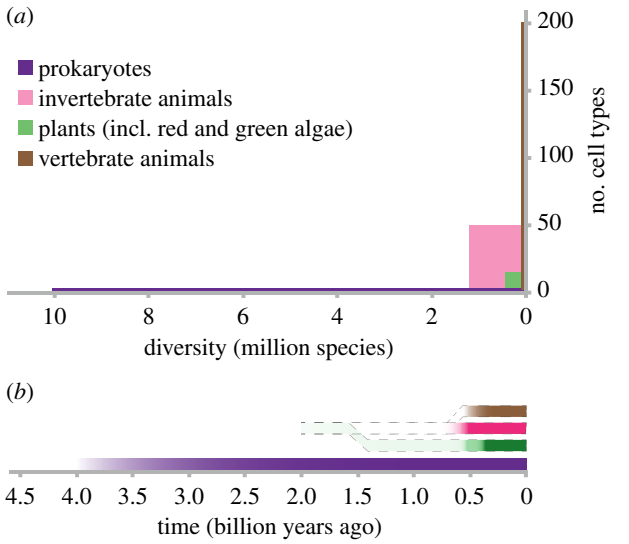

Figure 2. The rarity of complex multicellularity visualized as (*a*) the diversity of prokaryotes (purple) compared to the diversity of organisms with more than 10 different cell types: plants (green), invertebrate animals (pink) and vertebrate animals (brown); and (*b*) the late diversification of animals (brown and pink) and plants (green) in comparison to estimates of the ancestral roots and presence of the eukaryotic cell (dashed boxes) and prokaryotes (purple).

oxygen. Here, I propose that the success of 'large life' in oxygenated niches (above approx. 2% O_2) can be considered improbable, assigning an essential role to biological mechanisms that preserve hypoxia, its cellular responses, and cell fate plasticity in tissues (tissue competencies). Building on previous work [9,10] and animal tissue competencies in particular, I present a framework by which tweaking tissue competencies to 'harness' hypoxia would have solved the paradox of oxygen-sensitive tissue renewal and allowed complex multicellular lifeforms to decouple from their deep, ancestral and stably hypoxic niches.

Small, unicellular lifeforms dominate diversity on Earth today [11,12] and probably always have. By contrast, complex multicellularity is rare, as demonstrated by the described diversity of life forms with greater than 10 different cell types (figure 2). Animals are the most complex, with up to 50 (most invertebrates) and 200 (vertebrates) morphologically different cell types [13], but constitute a mere 8% of all described diversity [11] (whereof vertebrate animals constitute only 0.4%). Modern plants constitute the largest component of global biomass (80%) [14] and can grow over 100 m in height with less than 10 cell types, but their diversity constitutes only 3% of all known species; macroalgae and fungi even less (0.5%). Furthermore, 'large life' diversity appeared late in the history of life. The age of our last eukaryotic common ancestor (LECA) is debated. When the eukaryotic crown group diverges is also under discussion [15–17]. However, after up to possibly a billion years after the LECA, dramatic within-clade diversifications of Archaeplastida (plants including green and red algae) and of animals are noted to occur in close temporal proximity [18] (geologically speaking). While animals and plants today appear omnipresent and normal, an overall rarity and late appearance may indicate that the success of large life is the anomaly, and thus a remarkable biological achievement.

The lag between clade divergence and diversification is debated, the main hypotheses being that it took time for: (1) the necessary genetic traits and regulatory networks to gather, (2) ecological dynamics to fuel diversification, or (3) the environment to offer suitable chemical conditions [19,20]. Studies of genetic traits and regulatory networks now emphasize that biological refinement, such as the co-opting of ancestral genes, facilitated the transition between unicellular and multicellular holozoans [21], which eventually also led to a shift from temporal to spatio-temporal cell fate control [22]. Nevertheless, much effort has focused on the third hypothesis, particularly when and how sufficient free oxygen became available to animals in marine niches. Note that we as observers who experience high (21%) oxygen as necessary and normal may be prone to a bias towards the importance of oxygen and, then, the sufficiently *high* oxygen concentrations. Therefore, it is essential to define the thresholds and mechanisms relating to oxygen and the formation of multicellularity.

Hypoxia remains a common denominator for tissue formation, even though its definitions are discipline-specific and easily confused. For example, geoscientists may seek *oxygenation* events at the time of animal diversification, but there is agreement that atmospheric oxygen concentrations were 25–50% [23,24] of present atmospheric levels. Such levels would correspond to approx. 5–10% O_2, which in the modern ocean would be regarded as hypoxic (depending on the definition [25]). In parallel, medical researchers seek abnormal tissue *deoxygenation* events, where maximum tissue oxygenation is 5–7% O_2 [26] and many tissues function well at, or even require, even lower levels [3]. Therefore, 'normality' in both settings spans a similar range of low oxygen concentrations although the nomenclature to describe it differs. Here, the present atmospheric oxygen concentration of 21% likely serves as a subtle reference point that makes us

unconsciously assume it as normal. Fact is that if animal divergence began in the Cryogenian [27] and atmospheric oxygen concentrations reached modern levels first in the Devonian [28], hypoxic conditions would have prevailed in most environmental settings and tissues for the earlier half of animal history. It is from this hypoxic context that adaptations to and dependencies on even higher oxygen would have developed.

In parallel, tissue built and renewed by stem cells highlights that an upper threshold of oxygen concentrations may hinder the diversification of complex multicellular life in oxic niches. While a lower oxygen threshold for animal diversity has been investigated [20,29,30], an upper threshold to 'higher oxygen' where adaptations would be needed has not. An upper threshold can be inferred from the chromatin tightening point (CTP). Since animal tissue unconditionally requires immature cells with broad access to chromatin and consequent cell fates, the CTP for vertebrate cells at 1–3% O_2 (for simplicity here set at approx. 2%) may represent a boundary above which tissue function requires new adaptations. The CTP has mostly been explored in vertebrate animal cells, and vertebrates appear to possess additional mechanisms to unfold chromatin. Nevertheless, with the information at hand it is fair to propose that environments with oxygen concentrations above the CTP (greater than 2%) would have been challenging for tissue renewal and thus also for big life. Irrespective of the status of global oxygenation, environments where oxygen concentrations reach above the CTP would have been most common in Earth's dynamic surface environments, where also the fuel for new biomass (i.e. the labile carbon) is produced. Consequently, the innovation of competencies that preserve hypoxia, its cellular responses, or in other ways promote versatile access to cell plasticity would offer a selective advantage by offering a way across the 'oxic boundary' and beyond the hypoxic cradle.

The formation of multicellularity can also be studied through tumours, which misuse the same cellular capacities as available to normal cells in the host. Tumour biology represents an evolutionary system, where new multicellularity (transformed tissue) arises within and beyond an established tissue environment and 'ecosystem'. The lifetime risk to develop cancer in an ageing human population is high (about 1 in 3) [31]. Still, the appearance (incidence) and diversification (metastasis) of tumours occur late and are statistically rare [32] in comparison to the evaluated risk by having many cells. Human cancer incidence increases dramatically after the age of sixty [33], i.e. after a vast number of normal tissue renewal cycles. For example, human intestinal crypt cells have a turnover time of approximately 3 days, which means the entire intestinal lining is renewed several thousand times before the age of 60 years. The statistical rarity of tumour multicellularity is also indicated when considering the total number of cells in the human body: an adult human contains 10^{12} [14] somatic cells and roughly the same number of new somatic cells are replenished through asymmetric stem cell renewal each year. Although errors in cell stemness thus comprise an evolutionary liability [34], cancer clones have to pass multiple evolutionary bottlenecks [32,35] before only a small proportion 'succeed' to become malignant [36]. The potential rarity of tissue transformation is interpreted as if the transition from cellular autonomy to collaboration [37] is rare, fragile and even improbable [38,39]. These observations suggest that transition to tumour multicellularity in the form of cancer is difficult. Although the necessary genetic toolkits and regulatory networks are available, the transition to the construction of this version of multicellularity (or perhaps the interruption of a functional ecosystem) remains improbable. The 'success' of tumours, on the other hand, critically involves cell stemness [40], self-renewal [41] and alterations in the so-called cellular hypoxia-response machinery [42].

Here, I combine insight from cell stemness studies, developmental evidence from both normal and transformed (tumour) tissue, and observations in the geological record to argue for the hypothesis that the 'harnessing' of hypoxia—either on demand or through controlled cellular mechanisms—shaped large life evolution. I summarize the main factors that give rise to the hypothesis and illustrate the potential impact of the harnessing hypoxia hypothesis. I describe a scenario that provides an explanation for the rare bursts in diversity of complex multicellularity and link evolving mechanisms that sustain access to cell fate plasticity to the development and distribution of early animals in hypoxic, stable and deep-water niches.

2. Solutions to the paradox of oxygen-sensitive tissue construction

The paradox, in the eyes of us as observers, that aerobic metabolism sustains 'large life' while maintenance of tissue in multicellular organisms requires hypoxia, compels us to explore biological solutions that have solved it. Tissue maintenance balances cell renewal with cell death, thereby defining the life expectancy of multicellular organisms. While tissue homeostasis more broadly includes balances of, for example, nutrients or gases, here, I focus particularly on cell replenishment in tissues. Vertebrates clearly *cope* in oxic conditions and thus serve as an exemplar of complex multicellular life that has overcome the paradox of oxygen-sensitive tissue renewal. Tissue renewal has been extensively studied during animal development but even more so in vertebrate tumours. Using examples of how animal tissue and its tumours circumvent the paradox, I infer three evolutionary strategies for tissue renewal under oxic conditions: (i) hypoxia on demand, (ii) pseudohypoxia and (iii) fast cell turnover or tissue plasticity.

2.1. Hypoxia on demand

Tissue niches that are particularly low in oxygen exist in both animals and plants. In vertebrates, the bone marrow, where the most basal and active hematopoietic stem cells (HSCs) reside, is one such niche. HSCs give rise to, for example, platelets, macrophages and red blood cells (RBCs) that live for approximately three months. Thus, HSCs replenish roughly 100 billion new RBCs every day [43], and the HSCs reside in a truly low-oxygen niche (figure 3*a*). Bone marrow oxygenation is notoriously difficult to determine without disturbing the microenvironment, but blood studies suggest that mitochondrial inactivity is central to HSC stemness [46,47]. Vascular plants also have pluripotent stem cells (meristem stem cells) that give rise to new leaves, and it was recently determined that these core stem cells also reside in a hypoxic niche [8] (figure 3*b*). This hypoxic niche is somewhat surprising since it is found within 100 µm of the cuticle, suggesting either an impermeable layer to the atmosphere or high oxygen

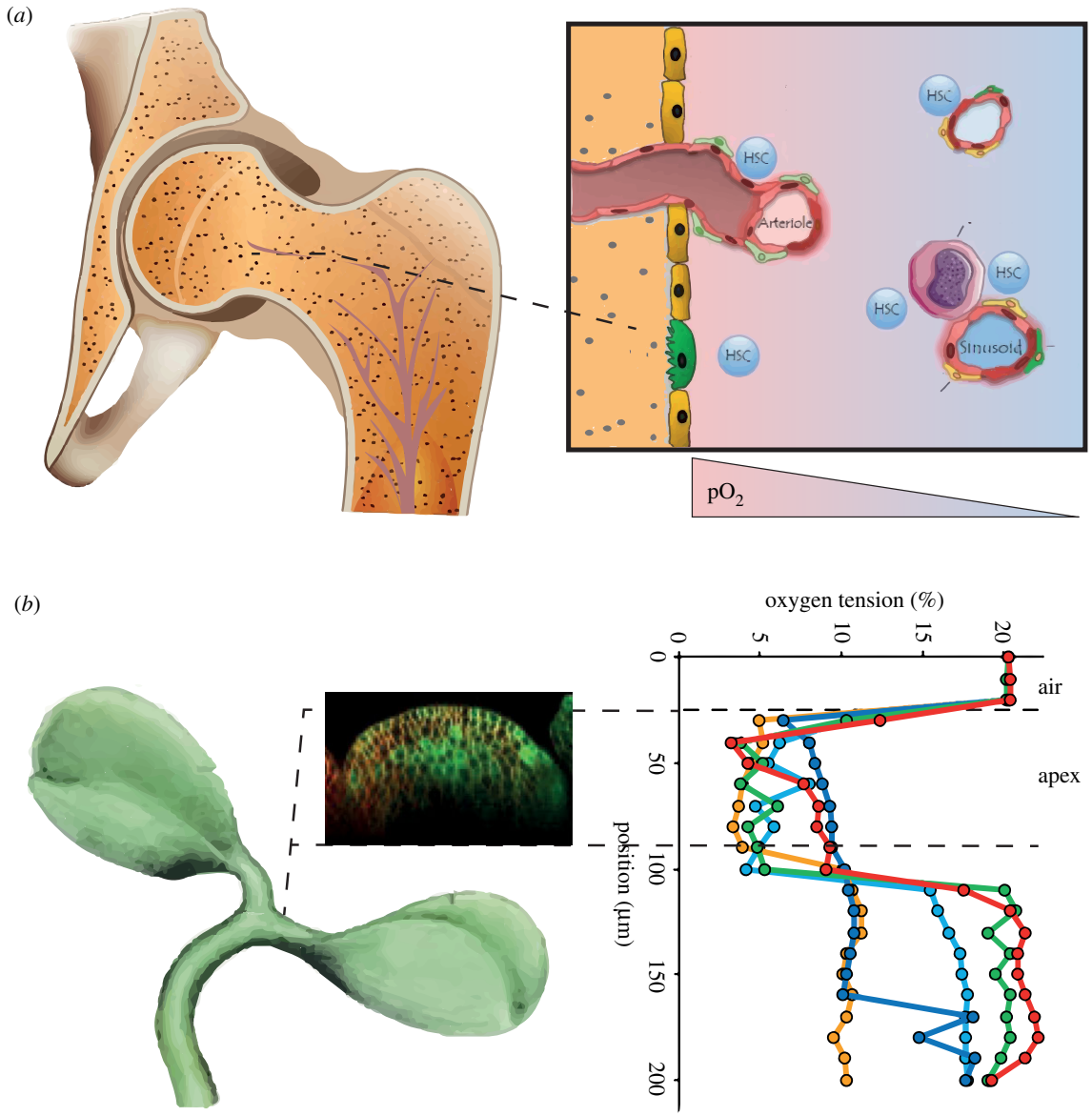

Figure 3. Hypoxic stem cell niches in (*a*) human bone marrow (modified from Wielockx *et al*. [44]) and (*b*) the vascular plant *Arabidopsis thaliana* (modified from Weits *et al*. [45]).

consumption. Overall, for both mammals and vascular plants, access to a permanently hypoxic niche is deemed vital.

Oxygen is also particularly low during mammalian embryogenesis, at peri-implantation, during the first trimester, and for some placental cells throughout gestation [48–50]. Oxygen tension in the uteruses of monkeys and humans is less than 2% [49,50], and gaseous exchange between the maternal and fetal blood only becomes fully developed towards the end of the first trimester [49]. During the first trimester, cells migrate, find their fate and build organs, which argues for an association between the narrow range of low oxygen concentrations that can promote both cell stemness and differentiation.

Hypoxic phases in other animals are less well documented, but numerous observations suggest that invertebrates control not only access of oxygen but also its restriction. For example, insect larvae live in hypoxic habitats like mud or trunks [51] while their shorter-lived adult versions hold breathing canals predominantly closed [52] unless flying. Blue mussels are closed when not filtering [53,54], while sponges and annelids arrest ventilation in their canals and burrow for extended periods of time (minutes to days), such that hypoxic or anoxic conditions follow repeatedly [55,56]. Anoxia is associated with crab and lobster moulting [57,58], while coral tissue anoxia at night when symbiotic algae respire is hypothesized to contribute to successful nocturnal skeletal extension [59]. At the cellular level, hypoxia does not promote higher animal cell death rates than oxic conditions [13]. Hypoxia is associated, however, with the reversal, or de-differentiation, of previously specialized cells [60]. By inference, hypoxic phases may offer a simple solution to reboot stem cell capacity in invertebrates.

One known adaptation to fluctuations in oxygen concentrations is the synthesis of hypoxia-inducible factors such as HIF-1α. While mainly known from the cancer field and indeed its discovery awarded the 2019 Nobel Prize in Physiology or Medicine, HIF-1α is expressed by all animals but sponges and ctenophores [10,61,62]. HIF-1α is commonly described as a necessary response system to low oxygen conditions, but it primarily assists in handling of *fluctuations* by, for example, switching from glycolysis and muscle angiogenesis at hypoxia to aerobic metabolism and seized construction of blood vessels at conditions with higher oxygen concentrations (in a context-specific manner). Indeed, HIF proteins

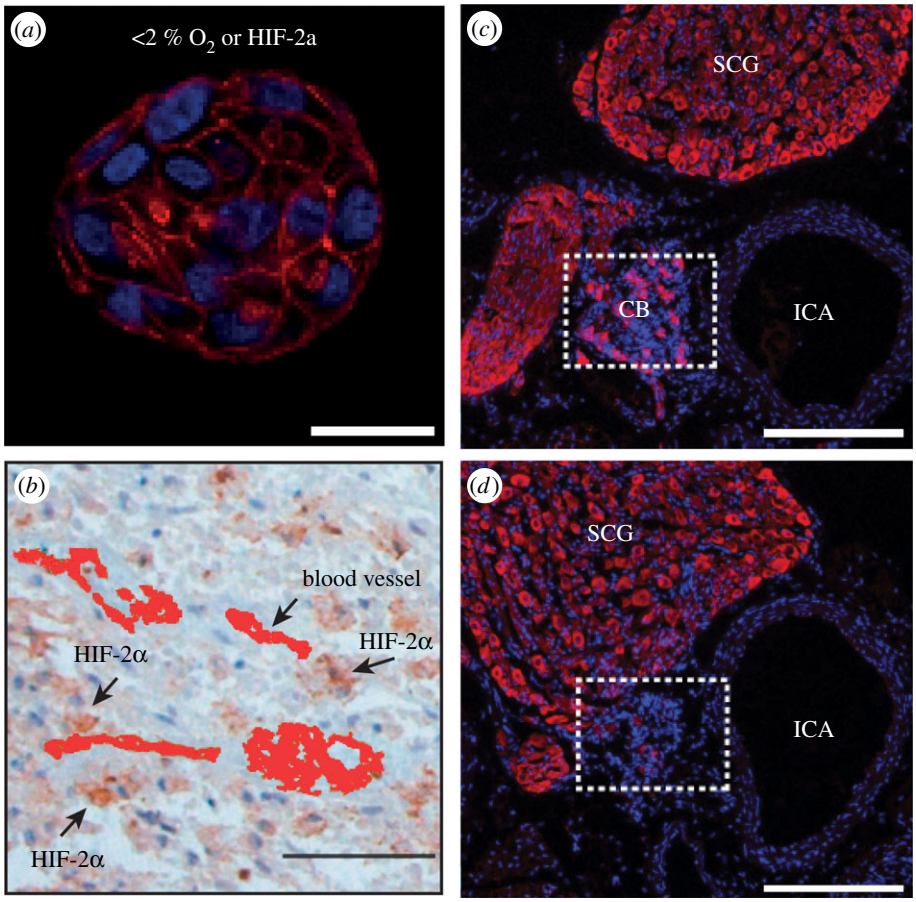

Figure 4. The immature cell phenotype (*a*) depicted as promoted by HIF-2-driven pseudohypoxia (modified from Vaapil *et al*. [16]), (*b*) in tumour tissue (nuclear HIF-2α; brown) and next to blood (red) (modified from Holmqvist-Mengelbier *et al*. [68]), (*c*) in the oxygen-sensitive glomus cells (red) in the carotid body that are (*d*) lost when *EPAS1/HIF2* is knocked out (Macias *et al*. [69]). Scale bars 20 µm (*a*), 100 µm (*b*) and 200 µm (*c*,*d*).

are omnipresent in the cell also under oxic conditions but then continuously degraded through ubiquitination and, in essence, transcriptionally silenced. Degrading HIFs under oxic conditions suggests that their stabilization under hypoxia (approx. 1% in the case of HIF-1*α*) was the early and ancient mode of function, while their silencing under oxic conditions the adaptation [9]. Since biological innovations must first be expressed in order for their silencing to undergo selection [9], the silencing of HIFs at oxic conditions represents an adaption. Therefore, HIFs are better regarded as means for organisms to adapt to oxic and conditions with fluctuating oxygen rather than, as it is commonly described by us today, assisting animals to acutely adapt to low oxygen conditions [62].

We must, therefore, consider the difference between multicellular organisms in a low O_2 setting that first lack and then possess critical control of the hypoxia machinery through mechanisms such as HIFs. HIF-1*α* likely provides several 'services' to bilaterians and cnidarians (also recently called *Hifozoa*) [62], such as regulating aerobic glycolysis [63], innate immune responses [64], or tolerance to sulfide [65], and are suggested to be a new or co-opted transcription family [21] that allowed dramatic diversification of primarily bilateral animals [10]. By contrast, organisms without HIF-1*α* would depend on a predictable external environment for predictable cell fates (e.g. hypoxia and stem cells at the core of the organism with more oxic and differentiated cells at its edges). Thus, organisms without HIF-1*α* (or similar) would have depended on a stable external environment, which is rare on the Earth's surface. One of the more stable environments on Earth is the deep ocean (here defined as below the storm wave base), where oceanographic factors like wind, currents, primary production and carbon remineralization change at much longer intervals than at the surface.

In conclusion, hypoxia is fundamental for vertebrate stem cells and mammalian embryogenesis and can also be inferred to be common to invertebrate animals where, for example, HIFs provide a means to adapt to fluctuating oxygen conditions such as those common in shallow marine settings.

2.2. Pseudohypoxia

Vertebrate cell stemness under oxic conditions (greater than 2%) would be fundamentally impossible, since oxygen promotes differentiation [6] and chromatin condensation without mechanisms to stop differentiation [9] or induce stemness [10]. Still, cell stemness can clearly occur next to oxygenated blood. For example, multipotent stem cells (adult stem cells) in the basal skin stem cell layer reside just above vascularization that shuttles oxygenated blood. In tumours, immature cells also maintain their immature phenotype next to vessels, which represent areas of maximum oxygenation. In the tumoural setting, the main regulatory mechanism to induce cell stemness despite oxic conditions is called pseudohypoxia [66,67]. Cancer stem cells maintain their stemness properties at physiologically oxic conditions through HIF-2*α* [66] (figure 4*a*,*b*). The association between HIF-2*α* and stemness is further evidenced by how constitutional HIF-2*α* expression

(gain of function mutation in *EPAS1*) is associated with heritable types of cancers [66,70]. Since these cancer stem cells demonstrate hypoxic responses under physiologically oxic conditions, the phenotype is called *pseudohypoxia* [67]. HIF-2α is vertebrate-specific [10,61] and—although a term derived from tumour biology—the pseudohypoxic phenotype is postulated to control stemness to allow animals to fully enter oxic niches [10]. One argument supporting pseudohypoxia being necessary for vertebrate evolution is that the ability to produce RBCs, which efficiently transport oxygen, appears to have evolved after HIF-2α [10]. Other and possibly older mechanisms for inducing cell stemness in vertebrates independently of HIF, through lactate or energy sensing are also recently demonstrated [71–73]. The recent discoveries highlight that much likely remains to be discovered of how pseudohypoxia can be induced by HIF-2α or parallel mechanisms. Nevertheless, investigations of cellular mechanisms that sense oxygen, induce stemness, and their evolutionary sequence are pertinent in order for us to decipher their role for multicellularity to adapt to life in oxic environments (i.e. O_2 concentrations above CTP, or greater than 2%).

There is only indirect evidence of pseudohypoxia regulated by HIF-2α or other key molecules maintaining adult stem cells in normal vertebrate tissues. Indeed, whether stemness in adult stem cells is an induced or a hardwired property has been intensely explored within different tissues. In the small intestine, for example, epithelial cells have a lifetime of approximately 3 days and must be continuously replenished. The cell fate plasticity of these cells is thought to reflect an induced, rather than hardwired, property [74]. However, HIF-2α has also been observed in the basal stem cell layer of skin [75] and is essential for the function and development of the carotid body (which regulates vertebrate heart rate and ventilation) [69]. HIF-2α is also transiently expressed during normal rodent and mammalian embryogenesis and, particularly, during the development of the sympathetic nervous system [76–78]. While the ability to manage stemness is complex, it is likely that pseudohypoxia played a role in the way large life solved the paradox of an oxygen-sensitive core of tissue renewal. It might even be argued that HIF-2-driven pseudohypoxia assists vertebrates to generally reach larger size, higher complexity (organs), and longer lifespan in oxic conditions than invertebrate animals [10]. However, the ability to promote stemness under oxic conditions must be balanced against the risk of developing stemness-related disease like cancer. Tumour growth is noted in all multicellular life, but it is primarily in the vertebrates that tumours are observed to invade and metastasize [79].

2.3. Tissue plasticity

Some modes of tissue renewal appear to bypass the need of oxygen-sensitive stem cells and thus the need of harnessing hypoxia. For example, large life can consist of tissue where *all* cells are pluripotent (stem cells of sorts) such as in macroalgae, fungi or sponges. Any cell in the tissue of brown algae can give rise to a new individual. Sponge cells, on the other hand, continuously transdifferentiate between a stem cell state (archaeocyte) and a more differentiated state (e.g. choanocyte) [80], a metastable state that may be as short as four hours [81]. Cnidaria also demonstrate remarkable regenerative capacity. For example, the fresh water polyp (*Hydra*) can regenerate from dissociated cells or half of the original organism through cellular de- and transdifferentiation [82]. This capacity to 'toggle' between cell fates is suggested to be an ancestral capacity of primitive multicellularity and even unicellular holozoans [21,22,81,83]. When cell fate alternation is fast, as in the sponge example, this biologically driven plasticity could be regarded as a microcosm where signals derived from the surrounding environment are kept at bay. In this microcosm, tissue renewal bypasses the need of both oxygen-sensitive stem cells and fully differentiated cells. However, the ability of cells to maintain plasticity could also come with limitations since it has been suggested to restrict the number of possible cell fates, and thus cell types [84]. This would mean that cell differentiation states in, for example, sponges and cnidarians are non-terminal and that reversion to an immature phenotype is possible at the cost of a small wardrobe of cell fates. This is supported by the observation that Cnidaria possess similar genes to bilateral animals for muscle formation but lack the typical terminal differentiation proteins [82]. However, several modes of tissue renewal occur across the animal kingdom [85,86] and even the human liver can regenerate through transdifferentiation [87] (although proliferation is more common) [88]. Mammals during development and also adult lizards demonstrate remarkable scar-free tissue regeneration [89,90] that involves de- and transdifferentiation [91]. The ultimate indication that all cells may hold the potential capacity for transdifferentiation is experimental, such as when fully differentiated human keratinocytes (skin cells) can be induced to acquire stemness properties [92]. Although complex, solutions for tissue plasticity that involves fast fate turnover likely matter for how life decoupled from the environment and these solutions may be independent of those involving hypoxia.

3. Primordial coexistence of oxic and hypoxic niches

Conventionally, we think of atmospheric oxygen concentrations to have increased when animals diversified on Earth [19,29]. The presence, evidence and timing of oxygenation events are also actively explored within the geoscientific community. However, this view mainly considers animal diversity depending on a *lower* oxygen threshold for respiration [20,29,30], when the oxygen-sensitive core of tissue renewal also clearly highlights an *upper* oxygen threshold (approx. 2%) [2,4] in the absence of additional competencies [9,10]. Also, the geological record demonstrates that oxic niches (greater than 2%) were present long before the diversification of animals and possibly for long periods of time [93,94].

Reconstructions of atmospheric oxygen are always indirect and primarily estimate the extent of anoxia (which is more clearly evidenced by, for example, redox-sensitive geochemistry). However, oxic niches at the very least have been present at the microscale since the rise of oxidative photosynthesis [95] some three billion years ago (Ga) [96]. Thus, in the vicinity of cyanobacterial mats in the photic zone (down to approx. 80 m), oxygen would have been present approximately 2 billion years before diversification of any large life. Oxygenation on the larger scale is also noted well before the Phanerozoic. Mesoproterozoic rocks yield point observations of oxidative weathering [97] and oxic deep-water conditions [98]; studies that still result in either no or much debate [99–102]. Indeed, debates pertaining to Precambrian events

of both oxygenations and macrofossil occurrences may be particularly fierce since our current model relies heavily on the assumption that Precambrian oxygen concentrations were insufficiently low for large life. For example, the so-called great oxidation event (GOE), noted at 2.45 Ga [103] and during the Lomagundi carbon isotope excursion at 2.3 to 2.1 Ga [104], reflects two things. First, that oxygen built up in the atmosphere, possibly to concentrations as today [104], and, secondly, our choice of the term 'event'. The oxygenation occurred or re-occurred during some three hundred million years or more [104], which is as long as the time during which the Phanerozoic was fully oxygenated [24]. The term 'event' is thus slightly misleading. Also, between putative eukaryotic macrofossils at 1.6 Ga [105] or 1.2 Ga [106], up to a billion years pass (with likely some time periods of oxygenation [97]) before visible diversification of large life appears to have occurred. The time needed for evolution to exploit new conditions, such as increased oxygen, remains unknown, but the million years of 'oxic' conditions in the Mesoproterozoic or Neoproterozoic clearly did not lead to large life diversification. I do not claim that oxic niches are unimportant for animal evolution; clearly, modern animal diversity and their ability to biomineralize is reduced at low oxygen concentrations (less than 2 ml l^{-1}, which corresponds to less than 25% of full modern saturation or less than 5% O_2) [25,30]. I do claim, however, that oxic and hypoxic niches have coexisted for most of Earth's history without leading to large life diversification (figure 5). This megaevolutionary stasis [15] could reflect that oxic niches were inaccessible to tissue-grade organisms.

Oxic, hypoxic, and anoxic niches are hypothesized to have existed at the microscale since the dawn of photosynthesis and in the atmosphere since 2.4 Ga (figure 5). During this coexistence, both animal ancestors [107] and animals have evolved within the hypoxic niche [9], restricted from the oxic niche by the CTP threshold. First, after the development and selection of adaptations to oxic conditions, large life could colonize the oxic niche [10]. Once there, the development of energy-intensive organs made vertebrate animals wholly dependent on oxic conditions. Other eukaryotic multicellular organisms may also be limited by how oxygen induces chromatin condensation, but their solutions for sensing hypoxia and oxygen and accessing cell stemness are yet much less well understood. However, if the competencies that curb the oxygen-sensitivity of tissue maintenance are as central as claimed here, some of their evolution must have been shared by organisms entering the oxic niches around the same time. This hypothesis requires further testing.

4. Hypothetical framework for conquering oxic niches

If multicellular organisms solved the paradox of oxygen-sensitive tissue maintenance by accessing hypoxia or cell plasticity, this allows us to sketch the hypothetical steps taken to improve the control of cell stemness and, thus, their ability to live in oxic environments. The evolutionary steps that eventually led to animals can be argued to have included stemness control through (a) oxygen gradients, (b) fast cell fate turnover, (c) the hypoxia-response machinery (HIF-1 or similar) and (d) pseudohypoxia (HIF-2 or similar) (table 1).

Each of these hypothetical steps has different implications. (a) If primitive multicellular organisms (e.g. Ediacaran

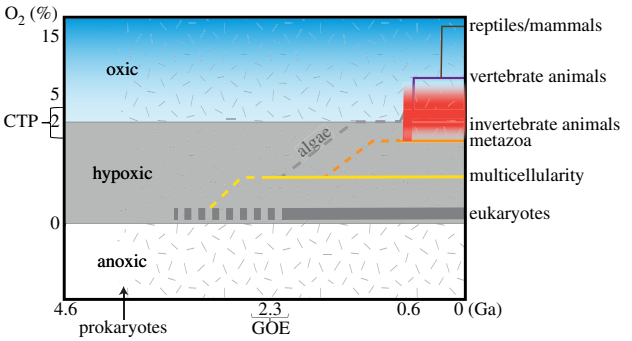

Figure 5. Schematic depiction of the coexistence of niches (also on microscale) during Earth's evolution, with the appearance of complex multicellularity deliberately placed before the GOE in the hypoxic niche, and the later diversification of those organisms with solutions to uphold hypoxia (or fast cell fate turnover) into the oxic niche (separated from the other niches by the chromatin condensation point, CTP).

rangeomorphs [108]) possessed spatial cell stemness as a function of oxygen gradients within the tissue, they were vulnerable to fluctuations in environmental oxygen concentrations. This vulnerability would make them unlikely to survive in Earth's shallow marine niches, where oxygen concentrations continuously fluctuate as a result of wind, temperature and primary production (figure 6). One geological observation of proto-animals are the Ediacaran organisms found in deep marine settings [108,110]. Their occurrence is biased by preservation, but a deep marine setting [111] would be consistent with the prediction that primitive stemness control demands an environment with stable oxygen concentrations. (b) Organisms that possess cell stemness as a function of fast cell fate turnover create an independent environment in which fast-paced tissue maintenance may outcompete, or delay, signalling from conditions (like oxygen) in the environment. Continuous cellular transdifferentiation would create sovereignty but maybe at the price of a limited range of cell fates and complexity or other mechanisms by which the chromatin is remodelled. (c) For invertebrate animals, HIF-1α (or similar) allows versatile access to hypoxic cell responses (the hypoxia response machinery) which allow them to *cope* for longer periods in oxic conditions. In comparison to organisms that require stable conditions for stemness via oxygen gradients, these organisms (e.g. annelid worms or blue mussels) would through HIF regulation (e.g. of cellular metabolism) be able to cope with a life that ventures into oxic conditions. However, their limited stemness control would require continuous access to true hypoxia on demand. This implies that invertebrate animals remain coupled to truly hypoxic conditions for their livelihood. Studies of the earliest preserved animals also suggest that both the Chengjiang and Sirius biotas were established in very low oxygen conditions that would have been particularly stable [112,113]. Indeed, two subsequent animal diversification events in the Phanerozoic are also associated with, and hypothesized driven by, phases of dominantly anoxic water column conditions [114]. (d) Vertebrate animals would, with their ability for HIF-2-driven pseudohypoxia, excel in cell stemness under oxic conditions and thus be mostly decoupled from true hypoxia (but still exploit it on demand, such as in the bone marrow). Their ability for spatio-temporal stemness control and organ-grade complexity (such as the brain or kidneys) would, however, later have developed into oxygen dependency and,

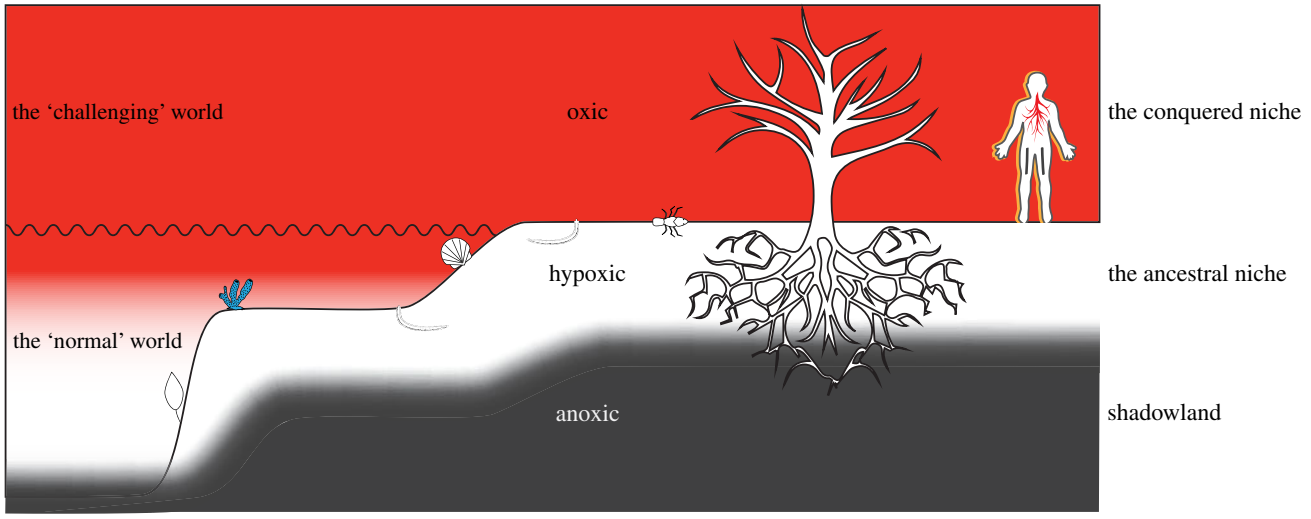

Figure 6. The framework of niches on Earth, of which the hypoxic niche represents the ancestral, stable, deep and 'normal' world for large life, from which it conquered the challenging, unstable, oxic and more surficial niche. Solutions to conquering the oxic niche couple to the harnessing of hypoxia (white) and temporal anoxia (dark grey), promotion of pseudohypoxia (yellow) or fast cell fate turnover (blue). Although not always anoxic (black), the deep biosphere or shadowland remains Earth's most ancient biosphere and, until the rise of high plant biomass, largest reservoir of living cells (carbon mass) (McMahon & Parnell [109]).

Table 1. Cell stemness is inferred to be regulated through oxygen gradients, cell fate plasticity, controlling (CTRL) the hypoxia response machinery and pseudohypoxia, which resulted in varying capacities, dependencies and implications for the large lifeforms that acquired these solutions.

cell stemness option				dependency on the external environment	implications	model organism
X				high (vulnerable to high O_2)	struggle to colonize the oxic niche	rangeomorphs
X	X			medium (microcosms)	limited cell fates	sponges, algae, fungi
X	X	X		low (cope in fluctuating conditions)	requires true hypoxia for stemness	insects, vascular plants
X	X	X	X	high (vulnerable to low O_2)	increased cancer risk	vertebrate animals
O_2 gradients	cell fate plasticity	CTRL hypoxia (e.g. HIF-1α)	pseudohypoxia (e.g. HIF-2α)			

thus, vulnerability to oxygen deficiency (which has since affected our evaluation of the importance of high oxygen). Their ability to control stemness would also increase the risk of stemness-related disease such as metastasizing cancer. While vertebrates are hypoxic organisms since end-capillary pressures rarely exceed 7% [26], their solutions for stemness control emphasize that their harnessing of hypoxia and its responses (spatio-temporal cell fate control) reached a new level. I suggest that the potential of these solutions is reflected in the vast number of cell types observed in vertebrates, which is not observed within other multicellular organisms.

This hypothetical framework has several unknowns. There is no comprehensive overview of the mechanisms in multicellular organisms that curb the paradox of oxygen-sensitive tissue renewal. This hypothetical framework also does not address how such solutions could have been shared or evolved in parallel, while diversification within clades (animals, plants and algae) and in oxic niches occurred within a short geological timespan. Rather, we have until now not asked or determined whether invertebrates maintain dependency on truly hypoxic conditions. We do know, however, that oxygen above a certain threshold challenges cells in tissues, that oxygenation events through Earth's history can be argued as being uncoupled from the diversification of animals and plants, and that implications of a model in which solutions involve hypoxic responses or cell plasticity are consistent with observations within the geological record and in developmental biology. This view of tissue competencies that solve the paradox of oxygen-sensitive renewal holds potential for rethinking the diversification of large life via integrated studies of geology, evolutionary principles and developmental biology.

Data accessibility. This article has no additional data.

Competing interests. I declare I have no competing interests.

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References

1. Melvin A, Rocha S. 2012 Chromatin as an oxygen sensor and active player in the hypoxia response. *Cell. Signal.* **24**, 35–43. (doi:10.1016/j.cellsig.2011.08.019)
2. Gaspar-Maia A, Alajem A, Meshorer E, Ramalho-Santos M. 2011 Open chromatin in pluripotency and reprogramming. *Nat. Rev. Mol. Cell Biol.* **12**, 36–47. (doi:10.1038/nrm3036)
3. Ivanovic Z. 2009 Hypoxia or in situ normoxia: the stem cell paradigm. *J. Cell. Physiol.* **219**, 271–275. (doi:10.1002/jcp.21690)
4. Zhou X, Sun H, Chen H, Zavadil J, Kluz T, Arita A, Costa M. 2010 Hypoxia induces trimethylated H3 lysine 4 by inhibition of JARID1A demethylase. *Cancer Res.* **70**, 4214–4221. (doi:10.1158/0008-5472.CAN-09-2942)
5. Buravkova LB, Andreeva ER, Gogvadze V, Zhivotovsky B. 2014 Mesenchymal stem cells and hypoxia: where are we? *Mitochondrion* **19**(Part A), 105–112. (doi:10.1016/j.mito.2014.07.005)
6. Vaapil M, Helczynska K, Villadsen R, Petersen OW, Johansson E, Beckman S, Larsson C, Påhlman S, Jögi A. 2012 Hypoxic conditions induce a cancer-like phenotype in human breast epithelial cells. *PLoS ONE* **7**, e46543. (doi:10.1371/journal.pone.0046543)
7. Mantel CR *et al.* 2015 Enhancing hematopoietic stem cell transplantation efficacy by mitigating oxygen shock. *Cell* **161**, 1553–1565. (doi:10.1016/j.cell.2015.04.054)
8. Weits DA *et al.* 2019 An apical hypoxic niche sets the pace of shoot meristem activity. *Nature* **569**, 714–717. (doi:10.1038/s41586-019-1203-6)
9. Ivanovic Z, Vlaski-Lafarge M. 2015 *Anaerobiosis and stemness: an evolutionary paradigm for therapeutic applications*. New York, NY: Academic Press.
10. Hammarlund E, Stedingk K, Påhlman S. 2018 Refined control of cell stemness allowed animal evolution in the oxic realm. *Nat. Ecol. Evol.* **2**, 220–228. (doi:10.1038/s41559-017-0410-5)
11. IUCN. 2014 The World Conservation Union. Red List of Threatened Species 2014.3. Summary Statistics for Globally Threatened Species. Table 1: Numbers of threatened species by major groups of organisms (1996–2014).
12. Whitman WB, Coleman DC, Wiebe WJ. 1998 Prokaryotes: the unseen majority. *Proc. Natl Acad. Sci. USA* **95**, 6578–6583. (doi:10.1073/pnas.95.12.6578)
13. Valentine JW, Collins AG, Meyer CP. 1994 Morphological complexity increase in metazoans. *Paleobiology* **20**, 131–142. (doi:10.2307/2401015)
14. Bar-On YM, Phillips R, Milo R. 2018 The biomass distribution on Earth. *Proc. Natl Acad. Sci. USA* **115**, 6506–6511. (doi:10.1073/pnas.1711842115)
15. Cavalier-Smith T. 2010 Deep phylogeny, ancestral groups and the four ages of life. *Phil. Trans. R. Soc. B* **365**, 111–132, (doi:10.1098/rstb.2009.0161)
16. Eme L, Sharpe SC, Brown MW, Roger AJ. 2014 On the age of eukaryotes: evaluating evidence from fossils and molecular clocks. *Cold Spring Harbor Perspect. Biol.* **6**, a016139. (doi:10.1101/cshperspect.a016139)
17. Sánchez-Baracaldo P, Raven JA, Pisani D, Knoll AH. 2017 Early photosynthetic eukaryotes inhabited low-salinity habitats. *Proc. Natl Acad. Sci. USA* **114**, E7737–E7745. (doi:10.1073/pnas.1620089114)
18. Porter SM. 2004 The fossil record of early eukaryotic diversification. *Paleontological Soc. Papers* **10**, 35–50. (doi:10.1017/S1089332600002321)
19. Nursall JR. 1959 Oxygen as a prerequisite to the origin of the metazoa. *Nature* **183**, 1170–1172. (doi:10.1038/1831170b0)
20. Berkner LV, Marshall LC. 1965 On the origin and rise of oxygen concentration in the Earth's atmosphere. *J. Atmos. Sci.* **22**, 225–261. (doi:10.1175/1520-0469(1965)022<0225:OTOARO>2.0.CO;2)
21. Sebé-Pedrós A, Degnan BM, Ruiz-Trillo I. 2017 The origin of Metazoa: a unicellular perspective. *Nat. Rev. Genet.* **18**, 498–512. (doi:10.1038/nrg.2017.21)
22. Mikhailov KV *et al.* 2009 The origin of Metazoa: a transition from temporal to spatial cell differentiation. *Bioessays* **31**, 758–768. (doi:10.1002/bies.200800214)
23. Bergman NM, Lenton TM, Watson AJ. 2004 COPSE: a new model of biogeochemical cycling over Phanerozoic time. *Am. J. Sci.* **304**, 397–437. (doi:10.2475/ajs.304.5.397)
24. Krause AJ, Mills BJW, Zhang S, Planavsky NJ, Lenton TM, Poulton SW. 2018 Stepwise oxygenation of the Paleozoic atmosphere. *Nat. Commun.* **9**, 4081. (doi:10.1038/s41467-018-06383-y)
25. Sperling EA, Knoll AH, Girguis PR. 2015 The ecological physiology of earth's second oxygen revolution. *Annu. Rev. Ecol. Evol. Syst.* **46**, 215–235. (doi:10.1146/annurev-ecolsys-110512-135808)
26. McKeown SR. 2014 Defining normoxia, physoxia and hypoxia in tumours—implications for treatment response. *Br. J. Radiol.* **87**, 20130676. (doi:10.1259/bjr.20130676)
27. Zumberge JA, Love GD, Cárdenas P, Sperling EA, Gunasekera S, Rohrssen M, Grosjean E, Grotzinger JP, Summons RE. 2018 Demosponge steroid biomarker 26-methylstigmastane provides evidence for Neoproterozoic animals. *Nat. Ecol. Evol.* **2**, 1709–1714. (doi:10.1038/s41559-018-0676-2)
28. Lenton TM, Dahl TW, Daines SJ, Mills BJW, Ozaki K, Saltzman MR, Porada P. 2016 Earliest land plants created modern levels of atmospheric oxygen. *Proc. Natl Acad. Sci. USA* **113**, 9704–9709. (doi:10.1073/pnas.1604787113)
29. Cloud P. 1968 Pre-metazoan evolution and the origins of the Metazoa. In *Evolution and environment* (ed. ET Drake), pp. 1–72. New Haven, CT: Yale University Press.
30. Rhoads DC, Morse JW. 1971 Evolutionary and ecologic significance of oxygen-deficient marine basins. *Lethaia* **4**, 413–428. (doi:10.1111/j.1502-3931.1971.tb01864.x)
31. American Cancer Society. 2020 Cancer facts & figures. Atlanta, GA: American Cancer Society.
32. Greaves M. 2010 Cancer stem cells: back to Darwin? *Semin. Cancer Biol.* **20**, 65–70. (doi:10.1016/j.semcancer.2010.03.002)
33. DePinho RA. 2000 The age of cancer. *Nature* **408**, 248–254. (doi:10.1038/35041694)
34. Cairns J. 1975 Mutation selection and the natural history of cancer. *Nature* **255**, 197–200. (doi:10.1038/255197a0)
35. Nowell PC. 1976 The clonal evolution of tumor cell populations. *Science* **194**, 23–28. (doi:10.1126/science.959840)
36. Gillies RJ, Verduzco D, Gatenby RA. 2012 Evolutionary dynamics of carcinogenesis and why targeted therapy does not work. *Nat. Rev. Cancer* **12**, 487–493. (doi:10.1038/nrc3298)
37. Maynard Smith J, Szathmáry E. 1995 *The major transitions in evolution*. Oxford, UK: Freeman.
38. Hardin G. 1968 The Tragedy of the Commons. *Science* **162**, 1243–1248. (doi:10.1126/science.162.3859.1243)
39. Archetti M, Pienta KJ. 2019 Cooperation among cancer cells: applying game theory to cancer. *Nat. Rev. Cancer* **19**, 110–117. (doi:10.1038/s41568-018-0083-7)
40. Bonnet D, Dick JE. 1997 Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat. Med.* **3**, 730–737. (doi:10.1038/nm0797-730)
41. Kreso A, Dick JE. 2014 Evolution of the cancer stem cell model. *Cell Stem Cell* **14**, 275–291. (doi:10.1016/j.stem.2014.02.006)
42. Semenza GL. 2014 Oxygen sensing, hypoxia-inducible factors, and disease pathophysiology. *Annu. Rev. Pathol. Mech. Disease* **9**, 47–71. (doi:10.1146/annurev-pathol-012513-104720)
43. Boulais PE, Frenette PS. 2015 Making sense of hematopoietic stem cell niches. *Blood* **125**, 2621–2629. (doi:10.1182/blood-2014-09-570192)
44. Wielockx B, Grinenko T, Mirtschink P, Chavakis T. 2019 Hypoxia pathway proteins in normal and malignant hematopoiesis. *Cells* **8**, 155. (doi:10.3390/cells8020155)

45. Weits DA *et al.* 2019 An apical hypoxic niche sets the pace of shoot meristem activity. *Nature* **569**, 714–717. (doi:10.1038/s41586-019-1203-6)
46. Romero-Moya D, Bueno C, Montes R, Navarro-Montero O, Iborra FJ, Lopez LC, Martin M, Menendez P. 2013 Cord blood-derived $CD34_+$ hematopoietic cells with low mitochondrial mass are enriched in hematopoietic repopulating stem cell function. *Haematologica* **98**, 1022–1029. (doi:10.3324/haematol.2012.079244)
47. Takubo K *et al.* 2013 Regulation of glycolysis by Pdk functions as a metabolic checkpoint for cell cycle quiescence in hematopoietic stem cells. *Cell Stem Cell* **12**, 49–61. (doi:10.1016/j.stem.2012.10.011)
48. Dunwoodie SL. 2009 The role of hypoxia in development of the mammalian embryo. *Dev. Cell* **17**, 755–773. (doi:10.1016/j.devcel.2009.11.008)
49. Rodesch F, Simon P, Donner C, Jauniaux E. 1992 Oxygen measurements in endometrial and trophoblastic tissues during early pregnancy. *Obstet. Gynecol.* **80**, 283–285.
50. Fischer B, Bavister BD. 1993 Oxygen tension in the oviduct and uterus of rhesus monkeys, hamsters and rabbits. *J. Reprod. Fertil.* **99**, 673–679. (doi:10.1530/jrf.0.0990673)
51. Hoback WW, Stanley DW. 2001 Insects in hypoxia. *J. Insect Physiol.* **47**, 533–542. (doi:10.1016/S0022-1910(00)00153-0)
52. Punt A. 1950 The respiration of insect. *Physiol. Comp. Oecol.* **2**, 59–63.
53. Riisgård HU, Kittner C, Seerup DF. 2003 Regulation of opening state and filtration rate in filter-feeding bivalves (Cardium edule, Mytilus edulis, Mya arenaria) in response to low algal concentration. *J. Exp. Mar. Biol. Ecol.* **284**, 105–127. (doi:10.1016/S0022-0981(02)00496-3)
54. Tang B, Riisgård HU. 2016 Physiological regulation of valve-opening degree enables mussels mytilus edulis to overcome starvation periods by reducing the oxygen uptake. *Open J. Mar. Sci.* **6**, 341–352. (doi:10.4236/ojms.2016.63029)
55. Hoffmann F, Larsen O, Thiel V, Rapp HT, Pape T, Michaelis W, Reitner J. 2005 An anaerobic world in sponges. *Geomicrobiol. J.* **22**, 1–10. (doi:10.1080/01490450590922505)
56. Kristensen E. 1988 Benthic fauna and biogeochemical processes in marine sediments. In *Nitrogen cycling in coastal marine environments* (eds TH Blackburn, J Sörensen), pp. 275–300. Chichester, UK: John Wiley & Sons Ltd.
57. Clemens S, Massabuau J-C, Meyrand P, Simmers J. 1999 Changes in motor network expression related to moulting behaviour in lobster: role of moult-induced deep hypoxia. *J. Exp. Biol.* **202**, 817–827.
58. Chung JS, Dircksen H, Webster SG. 1999 A remarkable, precisely timed release of hyperglycemic hormone from endocrine cells in the gut is associated with ecdysis in the crab *Carcinus maenas*. *Proc. Natl Acad. Sci. USA* **96**, 13 103–13 107. (doi:10.1073/pnas.96.23.13103)
59. Wooldridge S. 2013 A new conceptual model of coral biomineralisation: hypoxia as the physiological driver of skeletal extension. *Biogeosciences* **10**, 2867–2884. (doi:10.5194/bg-10-2867-2013)
60. Helczynska K, Kronblad Å, Jögi A, Nilsson E, Beckman S, Landberg G, Påhlman S. 2003 Hypoxia promotes a dedifferentiated phenotype in ductal breast carcinoma in situ. *Cancer Res.* **63**, 1441–1444.
61. Loenarz C, Coleman ML, Boleininger A, Schierwater B, Holland PWH, Ratcliffe PJ, Schofield CJ. 2011 The hypoxia-inducible transcription factor pathway regulates oxygen sensing in the simplest animal, Trichoplax adhaerens. *EMBO Rep.* **12**, 63–70. (doi:10.1038/embor.2010.170)
62. Mills DB, Francis WR, Vargas S, Larsen M, Elemans CPH, Canfield DE, Wörheide G. 2018 The last common ancestor of animals lacked the HIF pathway and respired in low-oxygen environments. *eLife* **7**, e31176. (doi:10.7554/eLife.31176)
63. Li Y, Padmanabha D, Gentile LB, Dumur CI, Beckstead RB, Baker KD. 2013 HIF- and non-HIF-regulated hypoxic responses require the estrogen-related receptor in *Drosophila melanogaster*. *PLoS Genet.* **9**, e1003230. (doi:10.1371/journal.pgen.1003230)
64. Zuckerman B, Abergel Z, Zelmanovich V, Romero L, Abergel R, Livshits L, Smith Y, Gross E. 2017 Characterization of gene expression associated with the adaptation of the nematode *C. elegans* to hypoxia and reoxygenation stress reveals an unexpected function of the neuroglobin GLB-5 in innate immunity. *Free Radic. Biol. Med.* **108**, 858–873. (doi:10.1016/j.freeradbiomed.2017.05.007)
65. Livshits L, Chatterjee AK, Karbian N, Abergel R, Abergel Z, Gross E. 2017 Mechanisms of defense against products of cysteine catabolism in the nematode *Caenorhabditis elegans*. *Free Radic. Biol. Med.* **104**, 346–359. (doi:10.1016/j.freeradbiomed.2017.02.007)
66. Dahia PLM. 2014 Pheochromocytoma and paraganglioma pathogenesis: learning from genetic heterogeneity. *Nat. Rev. Cancer* **14**, 108–119. (doi:10.1038/nrc3648)
67. Mohlin S, Wigerup C, Jögi A, Påhlman S. 2017 Hypoxia, pseudohypoxia and cellular differentiation. *Exp. Cell Res.* **356**, 192–196. (doi:10.1016/j.yexcr.2017.03.007)
68. Holmqvist-Mengelbier L *et al.* 2006 Recruitment of HIF-1α and HIF-2α to common target genes is differentially regulated in neuroblastoma: HIF-2α promotes an aggressive phenotype. *Cancer Cell* **10**, 413–423. (doi:10.1016/j.ccr.2006.08.026)
69. Macias D, Cowburn AS, Torres-Torrelo H, Ortega-Sáenz P, López-Barneo J, Johnson RS. 2018 HIF-2α is essential for carotid body development and function. *eLife* **7**, e34681. (doi:10.7554/eLife.34681)
70. Jochmanová I, Yang C, Zhuang Z, Pacak K. 2013 Hypoxia-inducible factor signaling in pheochromocytoma: turning the rudder in the right direction. *J. Natl Cancer Inst.* **105**, 1270–1283. (doi:10.1093/jnci/djt201)
71. Zhang D *et al.* 2019 Metabolic regulation of gene expression by histone lactylation. *Nature* **574**, 575–580. (doi:10.1038/s41586-019-1678-1)
72. Chakraborty AA *et al.* 2019 Histone demethylase KDM6A directly senses oxygen to control chromatin and cell fate. *Science* **363**, 1217–1222. (doi:10.1126/science.aaw1026)
73. Batie M, Frost J, Frost M, Wilson JW, Schofield P, Rocha S. 2019 Hypoxia induces rapid changes to histone methylation and reprograms chromatin. *Science* **363**, 1222–1226. (doi:10.1126/science.aau5870)
74. Guiu J *et al.* 2019 Tracing the origin of adult intestinal stem cells. *Nature* **570**, 107–111. (doi:10.1038/s41586-019-1212-5)
75. Wong WJ, Richardson T, Seykora JT, Cotsarelis G, Simon MC. 2015 Hypoxia-inducible factors regulate filaggrin expression and epidermal barrier function. *J. Invest. Dermatol.* **135**, 454–461. (doi:10.1038/jid.2014.283)
76. Mohlin S, Hamidian A, Påhlman S. 2013 HIF2A and IGF2 expression correlates in human neuroblastoma cells and normal immature sympathetic neuroblasts. *Neoplasia* **15**, 328–338. (doi:10.1593/neo.121706)
77. Tian H, Hammer RE, Matsumoto AM, Russell DW, McKnight SL. 1998 The hypoxia-responsive transcription factor EPAS1 is essential for catecholamine homeostasis and protection against heart failure during embryonic development. *Genes Dev.* **12**, 3320–3324. (doi:10.1101/gad.12.21.3320)
78. Nilsson H, Jögi A, Beckman S, Harris AL, Poellinger L, Påhlman S. 2005 HIF-2α expression in human fetal paraganglia and neuroblastoma: relation to sympathetic differentiation, glucose deficiency, and hypoxia. *Exp. Cell Res.* **303**, 447–456. (doi:10.1016/j.yexcr.2004.10.003)
79. Aktipis AC, Boddy AM, Jansen G, Hibner U, Hochberg ME, Maley CC, Wilkinson GS, 2015 Cancer across the tree of life: cooperation and cheating in multicellularity. *Phil. Trans. R. Soc. B* **370**, 20140219. (doi:10.1098/rstb.2014.0219)
80. Fernàndez-Busquets X, Kuhns WJ, Simpson TL, Ho M, Gerosa D, Grob M, Burger MM. 2002 Cell adhesion-related proteins as specific markers of sponge cell types involved in allogeneic recognition. *Dev. Comp. Immunol.* **26**, 313–323. (doi:10.1016/S0145-305X(01)00079-9)
81. Sogabe S, Hatleberg WL, Kocot KM, Say TE, Stoupin D, Roper KE, Fernandez-Valverde SL, Degnan SM, Degnan BM. 2019 Pluripotency and the origin of animal multicellularity. *Nature* **570**, 519–522. (doi:10.1038/s41586-019-1290-4)
82. Leclere L, Röttinger E. 2017 Diversity of cnidarian muscles: function, anatomy, development and regeneration. *Front. Cell Dev. Biol.* **4**, 157. (doi:10.3389/fcell.2016.00157)
83. Young SL, Diolaiti D, Conacci-Sorrell M, Ruiz-Trillo I, Eisenman RN, King N. 2011 Premetazoan ancestry of the Myc–Max Network. *Mol. Biol. Evol.* **28**, 2961–2971. (doi:10.1093/molbev/msr132)
84. Rose SM. 1952 A hierarchy of self-limiting reactions as the basis of cellular differentiation and growth control. *Am. Nat.* **86**, 337–354. (doi:10.2307/2457985)

85. Brockes JP, Kumar A. 2008 Comparative aspects of animal regeneration. *Annu. Rev. Cell. Dev. Biol.* **24**, 525–549. (doi:10.1146/annurev.cellbio.24.110707.175336)
86. Bely AE, Nyberg KG. 2010 Evolution of animal regeneration: re-emergence of a field. *Trends Ecol. Evol.* **25**, 161–170. (doi:10.1016/j.tree.2009.08.005)
87. Michalopoulos GK. 2011 Liver regeneration: alternative epithelial pathways. *Int. J. Biochem. Cell Biol.* **43**, 173–179. (doi:10.1016/j.biocel.2009.09.014)
88. Michalopoulos GK. 2013 Principles of liver regeneration and growth homeostasis. *Compr. Physiol.* **3**, 485–513. (doi:10.1002/cphy.c120014)
89. Singh NB, Koyano-Nakagawa N, Donaldson A, Weaver C, Garry M, Garry D. 2015 hedgehog signaling during appendage development and regeneration. *Genes* **6**, 417–435, (doi:10.3390/genes6020417)
90. Stocum DL, Crawford K. 2015 Regeneration of the urodele limb. *eLS*. (doi:10.1002/9780470015902.a0001100.pub4)
91. Brockes JP, Kumar A. 2002 Plasticity and reprogramming of differentiated cells in amphibian regeneration. *Nat. Rev. Mol. Cell Biol.* **3**, 566–574. (doi:10.1038/nrm881)
92. Yu J *et al.* 2007 Induced pluripotent stem cell lines derived from human somatic cells. *Science* **318**, 1917–1920. (doi:10.1126/science.1151526)
93. Bekker A, Holland HD. 2012 Oxygen overshoot and recovery during the early Paleoproterozoic. *Earth Planet. Sci. Lett.* **317–318**, 295–304. (doi:10.1016/j.epsl.2011.12.012)
94. Canfield DE. 2014 Proterozoic atmospheric oxygen. In *Treatise on geochemistry*, vol. 6, pp. 197–216. Oxford, UK: Elsevier Science. (doi:10.1016/B978-0-08-095975-7.01308-5)
95. Waldbauer JR, Newman DK, Summons RE. 2011 Microaerobic steroid biosynthesis and the molecular fossil record of Archean life. *Proc. Natl. Acad. Sci. USA* **108**, 13 409–13 414. (doi:10.1073/pnas.1104160108)
96. Crowe SA, Døssing LN, Beukes NJ, Bau M, Kruger SJ, Frei R, Canfield DE. 2013 Atmospheric oxygenation three billion years ago. *Nature* **501**, 535–538. (doi:10.1038/nature12426)
97. Gilleaudeau GJ, Frei R, Kaufman AJ, Kah LC, Azmy K, Bartley JK, Chernyavskiy P, Knoll AH. 2016 Oxygenation of the mid-Proterozoic atmosphere: clues from chromium isotopes in carbonates. *Geochem. Perspect. Lett.* **2**, 178–187. (doi:10.7185/geochemlet.1618)
98. Zhang S *et al.* 2016 Sufficient oxygen for animal respiration 1,400 million years ago. *Proc. Natl Acad. Sci. USA* **113**, 1731–1736. (doi:10.1073/pnas.1523449113)
99. Planavsky NJ, Cole DB, Reinhard CT, Diamond C, Love GD, Luo G, Zhang S, Konhauser KO, Lyons TW. 2016 No evidence for high atmospheric oxygen levels 1,400 million years ago. *Proc. Natl Acad. Sci. USA* **113**, E2550–E2551. (doi:10.1073/pnas.1601925113)
100. Zhang S, Wang X, Wang H, Bjerrum CJ, Hammarlund EU, Haxen ER, Wen H, Ye Y, Canfield DE. 2019 Paleoenvironmental proxies and what the Xiamaling formation tells us about the mid-Proterozoic ocean. *Geobiology* **17**, 225–246. (doi:10.1111/gbi.12337)
101. Diamond CW, Planavsky NJ, Wang C, Lyons TW. 2018 What the∼1.4 Ga Xiamaling formation can and cannot tell us about the mid-Proterozoic ocean. *Geobiology* **16**, 219–236. (doi:10.1111/gbi.12282)
102. Zhang S, Wang X, Wang H, Bjerrum CJ, Hammarlund EU, Dahl TW, Canfield DE. 2016 Reply to Planavsky *et al.*: strong evidence for high atmospheric oxygen levels 1,400 million years ago. *Proc. Natl Acad. Sci. USA* **113**, E2552–E2553. (doi:10.1073/pnas.1603982113)
103. Farquhar J, Bao HM, Thiemens M. 2000 Atmospheric influence of Earth's earliest sulfur cycle. *Science* **289**, 756–758. (doi:10.1126/science.289.5480.756)
104. Karhu JA, Holland HD. 1996 Carbon isotopes and the rise of atmospheric oxygen. *Geology* **24**, 867–870. (doi:10.1130/0091-7613(1996)024<0867:CIATRO>2.3.CO;2)
105. Bengtson S, Sallstedt T, Belivanova V, Whitehouse M. 2017 Three-dimensional preservation of cellular and subcellular structures suggests 1.6 billion-year-old crown-group red algae. *PLoS Biol.* **15**, e2000735. (doi:10.1371/journal.pbio.2000735)
106. Butterfield NJ. 2000 *Bangiomorpha pubescens* n. gen., n. sp.: implications for the evolution of sex, multicellularity, and the Mesoprotenterozoic/Neoprtoterozoic radiation of eukaryotes. *Paleobiology* **26**, 386–404. (doi:10.1666/0094-8373(2000)026<0386:BPNGNS>2.0.CO;2)
107. Müller M *et al.* 2012 Biochemistry and evolution of anaerobic energy metabolism in eukaryotes. *Microbiol. Mol. Biol. Rev. MMBR* **76**, 444–495. (doi:10.1128/MMBR.05024-11)
108. Narbonne GM, Laflamme M, Trusler PW, Dalrymple RW, Greentree C. 2015 Deep-water ediacaran fossils from northwestern Canada: taphonomy, ecology, and evolution. *J. Paleontol.* **88**, 207–223. (doi:10.1666/13-053)
109. McMahon S, Parnell J 2018 The deep history of Earth's biomass. *J. Geol. Soc.* **175**, 716–720. (doi:10.1144/jgs2018-061)
110. Boag TH, Stockey RG, Elder LE, Hull PM, Sperling E. 2018 Oxygen, temperature and the deep-marine stenothermal cradle of Ediacaran evolution. *Proc. R. Soc. B* **285**, 20181724. (doi:10.1098/rspb.2018.1724)
111. Fu D *et al.* 2019 The Qingjiang biota—a Burgess Shale-type fossil Lagerstätte from the early Cambrian of South China. *Science* **363**, 1338–1342. (doi:10.1126/science.aau8800)
112. Hammarlund EU, Smith MP, Rasmussen JA, Nielsen AT, Canfield DE, Harper DAT. 2019 The Sirius Passet Lagerstätte of North Greenland—a geochemical window on early Cambrian low-oxygen environments and ecosystems. *Geobiology* **17**, 12–26. (doi:10.1111/gbi.12315)
113. Hammarlund EU, Gaines RR, Prokopenko MG, Qi C, Hou X-G, Canfield DE. 2017 Early Cambrian oxygen minimum zone-like conditions at Chengjiang. *Earth Planet. Sci. Lett.* **475**, 160–168. (doi:10.1016/j.epsl.2017.06.054)
114. Wood R, Erwin DH. 2018 Innovation not recovery: dynamic redox promotes metazoan radiations. *Biol. Rev.* **93**, 863–873. (doi:10.1111/brv.12375)