Adaptation to life on land at high O₂ via transition from ferredoxin-to NADH-dependent redox balance

S. B. Gould¹, S. G. Garg¹, M. Handrich¹, S. Nelson-Sathi², N. Gruenheit¹, A. G. M. Tielens³,⁴ and W. F. Martin¹

¹Institute for Molecular Evolution, Heinrich Heine University Düsseldorf, 40225 Düsseldorf, Germany
²Interdisciplinary Biology, Computational Biology Laboratory, Rajiv Gandhi Centre for Biotechnology, Thrissur, Kerala, India
³Department of Biochemistry and Cell Biology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands
⁴Department of Medical Microbiology and Infectious Diseases, Erasmus University Medical Center, Rotterdam, The Netherlands

Pyruvate : ferredoxin oxidoreductase (PFO) and iron only hydrogenase ([Fe]-HYD) are common enzymes among eukaryotic microbes that inhabit anaerobic niches. Their function is to maintain redox balance by donating electrons from food oxidation via ferredoxin (Fd) to protons, generating H₂ as a waste product. Operating in series, they constitute a soluble electron transport chain of one-electron transfers between FeS clusters. They fulfil the same function—redox balance—served by two electron-transfers in the NADH- and O₂-dependent respiratory chains of mitochondria. Although they possess O₂-sensitive FeS clusters, PFO, Fd and [Fe]-HYD are also present among numerous algae that produce O₂. The evolutionary persistence of these enzymes among eukaryotic aerobes is traditionally explained as adaptation to facultative anaerobic growth. Here, we show that algae express enzymes of anaerobic energy metabolism at ambient O₂ levels (21% v/v), Chlamydomonas reinhardtii expresses them with diurnal regulation. High O₂ environments arose on Earth only approximately 450 million years ago. Gene presence/absence and gene expression data indicate that during the transition to high O₂ environments and terrestrialization, diverse algal lineages retained enzymes of Fd-dependent one-electron-based redox balance, while the land plant and land animal lineages underwent irreversible specialization to redox balance involving the O₂-insensitive two-electron carrier NADH.

1. Introduction

Molecular oxygen (O₂) had a far-reaching impact on evolution. From about 2.7–2.5 billion years ago onwards, cyanobacteria started using H₂O as the electron donor for a photosynthetic electron transport chain consisting of two photosystems connected in series [1,2], generating O₂ as a waste product of primary production. Before that, all life was anaerobic [3,4]. However, oxygenation of the planet did not occur quickly, as atmospheric oxygen concentrations remained low for almost 2 billion years [5,6] (figure 1).

Current findings have shown that the monophyletic origin of land plants, which occurred some 450 Ma [12,13], boosted O₂ accumulation to modern levels through massive carbon burial [9,10]. Eukaryotes arose roughly 1.8 billion years ago [14,15], from which it follows that the first 1.3 billion years of eukaryote evolution took place in low oxygen conditions [7] at atmospheric and marine O₂ levels comprising only a fraction—0.001–10%—of today’s O₂.

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levels [5,6,9,10]. Because eukaryotes arose and diversified over a billion years before atmospheric O2 reached the current value of 21% [v/v], it is hardly surprising that all major lineages (or supergroups) of eukaryotes possess enzymes of anaerobic energy metabolism (figure 2) [7]. In diverse eukaryotic lineages, these enzymes afford redox balance during ATP synthesis in mitochondria, anaerobic mitochondria [17], hydrogenosomes [18,19] and the cytosol [20] without requiring the presence of O2 as the terminal acceptor [7,21].

The enzymatic backbone of redox balance in anaerobic energy metabolism in unicellular eukaryotes is pyruvate:ferredoxin oxidoreductase (PFO) and [Fe-Fe] hydrogenase ([Fe]-HYD), which were first described for eukaryotes in studies of carbon and energy metabolism in trichomonad hydrogenosomes [18]. The ecophysiological function of these enzymes, together with the larger suite of proteins widely distributed across eukaryotes (figure 2), is generally interpreted as affording growth without oxygen. Hence, they are typically designated as enzymes of anaerobic metabolism. Like the pyruvate dehydrogenase complex of human or yeast mitochondria, PFO performs oxidative decarboxylation of pyruvate, generating acetyl-CoA and transferring electrons to the 4Fe4S cluster of the one-electron carrier ferredoxin (Fd). To maintain redox balance from growth substrate oxidation, reduced Fd (Fdred) is reoxidized by [Fe]-HYD, which donates the electrons to protons, generating H2 gas that leaves the cell as a waste product. Fdred generated by PFO is a low potential one-electron carrier (Fdred/Fe3+ = −420 mV) that can readily transfer a single electron to O2 generating the superoxide radical, O2[2,23] and reactive oxygen species (ROS). ROS are potent cytotoxins, a reason why organisms that employ the soluble PFO-Fd-[Fe]-HYD electron transport chain avoid high O2 environments. In addition, PFO and [Fe]-HYD are irreversibly inactivated by O2. Accordingly, eukaryotes that employ PFO and [Fe]-HYD in energy metabolism typically inhabit low oxygen environments, with their possession of these enzymes being interpreted as niche specialization [20,24,25].

However, PFO, [Fe]-HYD and a larger suite of enzymes associated with anaerobic energy metabolism are also present in algae [7,26–28], phototrophic eukaryotes with plastids that generate O2. Their presence in algae is known to enable facultative anaerobic growth in low oxygen environments [7,28], and their expression is observed to be upregulated in response to anoxia in algae [29,30], in the same way that fermentation enzymes are hypoxia-induced in higher plants [31]. However, the expression in O2-producing algae of enzymes associated with anaerobic redox balance has not been studied under normoxic conditions. Here, we investigated gene expression data from eukaryotic algae grown at ambient O2 levels (21% v/v) to better understand the physiology, function and evolutionary persistence of Fd-dependent enzymes for one-electron-based redox balance in algae.

2. Distribution of enzymes for anaerobic metabolism in eukaryotes

The distribution of 47 genes for enzymes involved in anaerobic energy metabolism [7] in 56 eukaryotes spanning the
The diversity of known lineages is summarized in figure 2. The absence of each enzyme in eukaryotes scored as a dark blue square. An additional BLAST-based search (at least 30% identity and e-value of less than 10^{-7}) identifies additional homologues (shown in magenta) that are not represented in the eukaryote–prokaryote clusters (EPCs) from Ku et al. [16] that is based, for example, on 40% global sequence identity for eukaryote proteins including BLAST hits for K. nitens, which was not included the original analysis [16]. Enzymes of anaerobic metabolism are present among all eukaryotic supergroups recognized, including all groups of algae, that is those carrying plastids of primary (e.g. C. reinhardtii, Cyanaphora paradoxa, V. carteri) and secondary origin (e.g. B. natans). For the enzymes that are identified as EPCs, phylogenetic trees (see the electronic supplementary material) indicate that 36 out of 43 (80%) of the genes show a single origin that traces to the eukaryotic common ancestor. Eukaryote monophyly as observed in phylogenetic trees constructed from protein sequences present in each cluster is shown with a dark red square (far right column), while orange indicates trees where the eukaryotes are non-monophyletic. For an extended presence-absence pattern including prokaryotes, see the electronic supplementary material, figure S1.

Figure 2. The presence–absence pattern of enzymes associated with anaerobic metabolism across the eukaryotic tree of life. The presence of each enzyme in eukaryotes scored as a dark blue square. An additional BLAST-based search (at least 30% identity and e-value of less than 10^{-7}) identifies additional homologues (shown in magenta) that are not represented in the eukaryote–prokaryote clusters (EPCs) from Ku et al. [16] that is based, for example, on 40% global sequence identity for eukaryote proteins including BLAST hits for K. nitens, which was not included the original analysis [16]. Enzymes of anaerobic metabolism are present among all eukaryotic supergroups recognized, including all groups of algae, that is those carrying plastids of primary (e.g. C. reinhardtii, Cyanaphora paradoxa, V. carteri) and secondary origin (e.g. B. natans). For the enzymes that are identified as EPCs, phylogenetic trees (see the electronic supplementary material) indicate that 36 out of 43 (80%) of the genes show a single origin that traces to the eukaryotic common ancestor. Eukaryote monophyly as observed in phylogenetic trees constructed from protein sequences present in each cluster is shown with a dark red square (far right column), while orange indicates trees where the eukaryotes are non-monophyletic. For an extended presence-absence pattern including prokaryotes, see the electronic supplementary material, figure S1.

Prokaryotic [Fe]-HYD enzymes can be trimeric [37], with 24 and 51 kDa subunits associated with the catalytic 64 kDa subunit, which contains the H2-producing site, the H cluster. The 24 and 51 kDa subunits allow the enzyme to accept electrons simultaneously from both NADH and Fd via electron confurcation [37], affording redox balance for both Fd and NADH pools. Some eukaryotic [Fe]-HYD enzymes, including the one from Trichomonas hydrogenosomes, also possess the 24 and 51 kDa subunits [38], which are related to mitochondrial complex I subunits. They are thought to allow the eukaryotes in question to perform electron confurcation, facilitating redox balance via NADH-dependent H2 production [7], which would be thermodynamically unfavourable in the absence of Fdred [37,39].

Intermediate states in the evolutionary transition from Fd-dependent, one-electron-based redox balance to NADH-dependent redox balance are observed. In various eukaryotic lineages, PFO has become fused to an FAD–FMN–NAD binding domain that converts the ancestrally Fd-dependent enzyme (one-electron transport) into an NAD(P)+-dependent enzyme that transfers hydride (two-electron transport) to generate NADPH. This fusion, called PNO for pyruvate : NADP+ oxidoreductase [40], is now known to be widespread among eukaryotes (figure 2) [7,25], and represents an evolutionary intermediate in the transition from Fd-dependent to NADH-dependent redox balance, in that electrons from the FeS clusters of the PFO domain are channelled directly to NAD(P)H, bypassing the generation of soluble Fdred.
3. Algae express enzymes for anaerobic metabolism at ambient O2

The presence of the genes in representatives of the major algal groups (figure 2) raises the question of whether and when they are expressed. This is important, because genes for anaerobic energy metabolism have been retained in some eukaryotes with strictly O2-dependent energy metabolism [41]. To determine whether enzymes of anaerobic redox balance are expressed independent of anaerobic culturing conditions, we generated transcriptome data for several algal lineages with sequenced genomes: the red alga *Porphyridium purpureum*, the glaucophyte *Cyanophora paradoxa*, the chlorarachniophyte *Bigelowiella natans* with a plastid of secondary green origin and the cryptophyte *Guillardia theta* with a plastid of secondary red origin. All algae were grown under the same culturing conditions and at ambient O2 levels of 21% [v/v]. In all algae studied, including algae with secondary plastids (figure 3a), we were able to detect the expression of at least a subset of the corresponding genes. It is well known that other algae such as *Vitrella brasicaformis* and *Chromera velia* encode a set of anaerobic

![Figure 3](https://royalsocietypublishing.org/doi/10.1098/rspb.2019.1491)
enzymes that is as complete as that of C. reinhardtii [25]. We therefore screened available transcriptome data for aerobi-
cally grown Chr. velia [42,43], Volvox carteri [44], Chlorella
variabilis [45,46] and Thalassiosira pseudonana [47] and Klebsorm-
dium nitens [48], and find that, for example, the chlorophyte C. var
iabilis and the chromerid Chr. velia (carrying a secondary
plastid of red algal origin), express pyruvate formate lyase
(PFL), PNO, hydrogenase maturases A/F/G (HydA/F/G)
and bifunctional alcohol and aldehyde dehydrogenase
(ADE) in the same way as C. reinhardtii for which we
generated RNA-Seq data (figure 3a).
The high-resolution RNA-Seq data available for
C. reinhardtii [49] provided detailed insights into expression
of enzymes for redox balance over the time course of 24 h.
Chlamydomonas is among the algae that has preserved
the most complete repertoire of O2-sensitive enzymes involved
in redox balance among eukaryotes studied so far (figure 2);
it expresses them in the presence of 21% oxygen and in a diur
nal fashion (figure 3b). PFL is found to be constantly
expressed, but more so during the dark phase and in particular
towards the end of the night (consistent with our RNA-Seq
data). The same pattern is observed for its activating
enzyme, although at much lower levels, similar to what is
observed in prokaryotes [50]. Other genes in question, includ-
ing both genes for the [Fe]-HYD catalytic subunit, HydA1 and
HydA2, are upregulated with the onset of night (figure 3b).
Importantly, this induction is observed independent of
anaerobic culturing conditions, the standard method
employed to induce [Fe]-HYD expression, typically in the con-
text of biohydrogen applications [51–54]. The Chlamydomonas
relatives Chlorella and Volvox display similar induction of
enzymes involved in H2 production and dark fermentation
[55,56]; hence, anaerobiosis-independent expression is
conserved and Chlamydomonas is the rule, not an exception.
The main finding from figure 3 is that the expression of
the enzymes for anaerobic redox balance in eukaryotes
does not correspond to any form of adaptation to anaerobic
niches, as ambient O2 does not change during the 24 h
cycle. Instead, their expression in C. reinhardtii corresponds
to the onset and end of illumination, where electron flux to
and from the photosynthetic electron transport chain undergoes transient changes. In Chlamydomonas, PFO and
[Fe]-HYD are localized in the plastid [54], not the mitochon
drion or the cytosol, where they help to buffer electron flow
into and out of the thylakoid membrane. This function does
not preclude the existence of other functions under other con-
ditions. For example, the same genes are expressed in
C. reinhardtii during anaerobiosis [29,30]. Yet, for most of
the algae surveyed in figure 3a, extended anaerobic growth
phases are unknown, and the main habitat is the pho
tic zone, where daily diurnal light conditions are encountered.

Some might view C. reinhardtii as an extreme case among
algae, as it appears to mimic true anaerobic protists such as
Trichomonas or soil-dwelling anaerobic bacteria when experi-
encing hypoxia. But Chlamydomonas can only endure
anaerobic conditions for a limited amount of time, not
thrive under them. To produce H2 in a biofuels context, the
typical procedure is to let Chlamydomonas cells assimilate
CO2 normally, then expose them to anoxic conditions while
blocking photosystem II (PSII) to induce H2-generating
assimilate fermentation [29]. Low-level H2 production by
Chlamydomonas for up to two weeks can, however, be
achieved under low-light conditions without PSII inhibition
[57]. This indicates that photosynthetic redox balance and
one-electron-based redox balance conferred by the soluble
PFO-Fd-[Fe]-HYD electron transport chain can operate indepen-
dent of anaerobiosis. Finally, C. reinhardtii is not the
only alga encoding such a complete set of anaerobic enzymes
[25,28], but the only one that has been extensively studied in
this respect.

4. Discussion
The retention and anaerobiosis-independent expression of
Fd-dependent enzymes in algae, together with their locali-
ization to plastids in cases studied to date, indicates that the
enzymes have been retained during algal evolution as the
result of selection for redox balance in cells with one-electron
transport. In terms of gene distribution (figure 2) and phylo-
genetic (electronic supplementary material, figure S1), the
enzymes of anaerobic energy metabolism in eukaryotes
trace to the eukaryote common ancestor [17,26,28] (figure 2);
hence, the archaeoplastidan founder lineage that acquired the
cyanobacterial ancestor of plastids already possessed them.
Eukaryotic enzymes involved in Fd-based redox balance
have been the subject of many evolutionary investigations.
There are two alternative hypotheses to account for their
presence in eukaryotes. One has it that the Fd-dependent
enzymes were present in the eukaryote common ancestor,
which was a facultative anaerobe that was able to survive
with or without O2 as terminal acceptor, and were involved
in its energy metabolism and redox balance [7,17,20,58].
The alternative lateral gene transfer (LGT) hypothesis has it
that the ancestral eukaryote was a strict aerobe, unable to
survive under anaerobic conditions, the presence of the
Fd-dependent enzymes in eukaryotes resulting from multiple
LGTs during eukaryote evolution to confer the ability to
colonize anaerobic niches [25,59,60]. Directly at odds with
the LGT theory is the observation that the archaeoplastidan
ancestor, whose PFL and PFL-activating enzyme are of
monophyletic origin [59], did not adapt to an anaerobic
niche, rather it acquired a cyanobacterial endosymbiont that
became an O2-producing plastid. The archaeoplastidal lineage
diversified into three main groups, representatives of which
have retained the enzymes [25,28] (figure 2).

Though various formulations of the LGT hypothesis for
enzymes of anaerobic redox energy metabolism in eukar-
yotes differ with respect to the number, nature and direc-
tion of LGTs [25], the underlying evolutionary rationale
of the LGT hypothesis has remained constant: the lateral
acquisition of Fd-dependent enzymes supposedly allowed
eukaryotes to colonize oxygen-poor niches [61]. Notwith-
standing the circumstance that the majority of eukaryote
evolution occurred in oxygen-poor environments [5,7,9,20]
(figure 1), the diurnal expression of Fd-dependent enzymes
in algae at 21% [v/v] O2 (figure 3) and independent of
anaerobic growth conditions is incompatible with the view
that the presence of these genes has anything to do with lat-
eral acquisitions for adaptation to anaerobiosis. Rather,
the data indicate that the genes for Fd-dependent redox balance
were present in the eukaryote common ancestor, lost in
some lineages during specialization to permanently oxic
habitats (electronic supplementary material, figure S2) and
retained in lineages that did not undergo the irreversible
adaptation to complete O2 dependence and NADH-
dependent redox balance (figure 1).
Evolutionary responses to redox balance in eukaryotes can include recompartmentalization of pathways [62] to the cytosol, to plastids [63], to glycosomes [64] or to mitochondria [65]. Based upon their presence in the eukaryote common ancestor and their current localization within plastids in algae studied to date, the Fd-dependent enzymes PFO and [Fe]-HYD were recompartmentalized to the plastid during algal evolution. In the plastid, they assumed essential roles in light-dependent redox balance in an organelle that, upon contact with light, has no choice but to commence photosystem I (PSI)-dependent Fd reduction, rapidly depleting the available Fdred pool. In land plants, Fdred is mainly reoxidized by ferredoxin : NADP+ oxidoreductase (FNR), NADPH being reoxidized in turn by NADP+-dependent glyceraldehyde 3-phosphate dehydrogenase (GAPDH) [66] in the Calvin cycle. In aquatic environments, CO2 is more limiting than in air, for which reason algae have evolved diverse CO2 concentrating mechanisms [67]. Algae thus require a means in addition to CO2 fixation for redox balance at the onset of illumination, Fd-dependent enzymes of anaerobic energy metabolism fulfil that role. That functional aspect, redox balance in the plastid rather than anaerobiosis, accounts for diurnal expression and retention of enzymes for anaerobic redox balance among many independent algal lineages (figure 1). The expression of ferredoxin-dependent enzymes thus enables redox balance in the presence of O2 in plastids and in the absence of O2 as it occurs in C. reinhardtii (299) and many lineages of anaerobic protists that arose and diversified before the origin of plastids [7,20].

The transition to life on land approximately 450 Ma marked the advent of life in very high O2 conditions [9,10]. Plants were the first major colonizers of land [68]. Massive carbon burial by land plants precipitated the high O2 environment into which the first land animals followed (electronic supplementary material, figure S2). The colonization of land was, physiologically, an adaptation to high O2 air. That adaptation to high O2 witnessed the loss of Fd-dependent redox balance independently in both the land plant and land animal lineages (electronic supplementary material, figure S2) in response to the O2 sensitivity of FeS clusters in PFO and [Fe]-HYD and in response to the ROS generating potential PFO of Fdred. Once on land, both the plant and animal lineages were subsequently confronted again with hypoxic environments in adaptations to aquatic environments. The corresponding adaptations did not, however, involve gene acquisitions via LGT, merely novel expression regulation for NADH-dependent enzymes involved in redox balance during hypoxic response. In plants, these responses include mainly ethanol fermentations in waterlogged roots [31,69,70]. In animals, the evolutionary responses include various pathways regulated by the hypoxia-induced factor HIF [71,72], and secondary adaptations to the aquatic lifestyle among various vertebrates [73,74]. In addition, many marine and soil-dwelling invertebrates independently evolved their own specialized strategies for redox balance [7], from opine accumulation in mussels [75] to rhodoquinone dependent short chain fatty acid excretion in worms [76]. The land plant and land animal anaerobiosis adaptation pathways are, however, always NADH-dependent.

The retention of the chloroplast encoded NADH dehydrogenase complex (cpNDH) specifically in the land plant lineage (figure 1) is noteworthy. The functional cpNDH complex is localized close to complex I in thylakoids, both in the cyanobacterium Synechocystis [77] and in land plants, where it supports the cyclic flow of electrons essential for PSI to properly perform photosynthesis [78,79]. Among genes in plastid DNA, the cpNDH genes have undergone the highest number of independent losses [80]. Their retention in the plastid was probably a prerequisite for the transition to life on land [48,68], because they have been retained by the plastid in all plant lineages, indicating a strong functional constraint for maintaining redox balance in the organelle [81]. Land plants have recruited a cytosolic NADH-dependent GAPDH [82] and a cytosolic malate dehydrogenase [83] to plastids for NADH-based redox balance. Even the origin of photosorperesis, a process central to NAD(P)H-dependent redox balance, can be understood as an evolutionary response to high O2 in the transition to life on land [84]. Land plant thylakoids cannot, however, relinquish Fd-dependent one-electron transport altogether, because the structure and function of PSI strictly require a steady flow of single electrons from the FeS clusters of PSI to generate soluble Fdred, the stromal levels of which are monitored in some photosynthetic lineages by the flavodiiron (FLV) proteins [85]. Our findings indicate that in the plant and animal lineages, terrestrialization entailed an irreversible physiological transition away from one-electron-based Fd-dependent redox balance towards NAD(P)H-dependent redox balance involving two-electron transfers. The underlying evolutionary mechanisms were gene expression changes, enzyme recompartmentalization and gene loss in adaptation to high O2 levels. Algae retained the Fd-dependent pathway for Fd-dependent, one-electron-based redox balance in plastids, not for anaerobic growth.

5. Material and methods

(a) Identification of homologous proteins

As part of a larger study [16], sequences from 55 eukaryotes and 1981 prokaryotes (1847 bacteria and 134 archaea) were clustered into protein families in order to identify eukaryotic proteins with prokaryotic homologues. This approach resulted in 2585 disjunct clusters that contain at least two eukaryotes and no less than five prokaryotes. Within these 2585 eukaryote–prokaryote clusters (EPCs) using existing annotations, we identified 42 clusters containing proteins involved in anaerobic energy metabolism, which were relevant for the current analysis (electronic supplementary material, table S1). Phylogenetic trees and results from the tests on eukaryote monophyly were taken directly from [16] (shown in the electronic supplementary material, table S1). For proteins that did not have an EPC, the same dataset was used to perform a BLAST search and only hits with an identity of greater than 10 were considered and provided in the electronic supplementary material, table S2. All the sequences that were used to identify the EPCs and perform the BLAST search are provided in the electronic supplementary material, file S1 along with the BLAST hits.

(b) Cultivation of algae, RNA isolation and transcriptomics

All algae were grown in their respective media (see SAG Göttingen or ncma.bigelow.org) in aerated flasks under controlled conditions at 22°C and illuminated with 50 µE under a
frames (ORFs) were identified using TransDecoder (v. 3.0.1) (https://github.com/TransDecoder/TransDecoder/wiki). These ORFs were used for a BLAST with an identity cut-off of 30% against the genome of the respective organisms to verify their presence in the genome and to remove possible contaminations. Transcriptomes are available via the Sequence Read Archive of NCBI (https://www.ncbi.nlm.nih.gov/sra) with the accession number PRJNA509798.

Data accessibility. All data have been made available in the form of electronic supplementary material or are publicly available.

Author’s contributions. S.B.G., S.G.G. and W.F.M. conceived the idea for the manuscript. S.G.G., M.H., and N.G. constructed the presence-absence table and compiled the expression data. All authors contributed to the writing and illustration of the manuscript.

Competing interests. We declare we have no competing interests.

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References

1. Allen JF. 2007 A redox switch hypothesis for the origin of two light reactions in photosynthesis. FEBS Lett. 579, 963–968. (doi:10.1016/j.febslet.2005.01.015)

2. Fischer WW, Hemp J, Johnson JE. 2016 Evolution of aerobic photosynthesis. Annu. Rev. Earth Planet. Sci. 44, 647–683. (doi:10.1146/annurev-earth-060313-054810)

3. Bekker A, Holland HD, Wang PL, Rumble 3rd D, Stein HI, Hannah JL, Gerteez LE, Beukes NJ. 2004 Dating the rise of atmospheric oxygen. Nature 427, 117–120. (doi:10.1038/nature02260)

4. Rasmussen B, Bekker A, Fletcher IR. 2013 Correlation of paleoproteoroclastic glaciations based on U-Pb zircon ages for tuff beds in the transvaal and huronian supergroups. Earth Planet. Sci. Lett. 382, 173–180. (doi:10.1016/j.epsl.2013.08.037)

5. Lyons TW, Reinhard CT, Planavsky NJ. 2014 The rise of oxygen in Earth’s early ocean and atmosphere. Nature 506, 307–315. (doi:10.1038/nature13068)

6. Allen JF, Thake B, Martin WF. In press. Nitrogenase inhibition limited oxygenation of Earth’s Proterozoic atmosphere. Trends Plant Sci.

7. Müller M, Mentel M, van Hellemond JJ, Henke Z, Woehle C, Gould SB, Yu R-Y, van der Gezen M, Tielens AGM, Martin WF. 2012 Biochemistry and evolution of anaerobic energy metabolism in eukaryotes. Microbiol. Mol. Biol. Rev. 76, 444–495. (doi:10.1128/MMBR.00524-11)

8. Zimorski V, Mentel M, Tielens AGM, Martin WF. In press. Energy metabolism in anaerobic eukaryotes and Earth’s late oxygenation. Free Radic. Biol. Med. (doi:10.1016/j.freeradbiomed.2019.03.030)

9. 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.1004787113)

10. Stolper DA, Keller CB. 2018 A record of deep-ocean dissolved O2 from the oxidation state of iron in submarine basalts. Nature 533, 323–327. (doi:10.1038/nature25090)

11. Fenchel T, Finlay BJ. 1995 Ecology and evolution in anoxic worlds. Oxford, UK: Oxford University Press.

12. Wickett NJ et al. 2014 Phylogenetic transcriptomic analysis of the origin and early diversification of land plants. Proc. Natl Acad. Sci. USA 111, E4859–E4868. (doi:10.1073/pnas.1323926111)

13. de Vries J, Stanton A, Archibald JM, Gould SB. 2016 Streptophyte terrestrialization in light of plastid evolution. Trends Plant Sci. 21, 467–476. (doi:10.1016/j.tplants.2016.01.021)

14. Parfrey LW, Lahr D, Knoll AH, Katz LA. 2011 Estimating the timing of early eukaryotic diversification with multigene molecular clocks. Proc. Natl Acad. Sci. USA 108, 13 624–13 629. (doi:10.1073/pnas.1110633108)

15. Betts HC, Puttick MN, Clark JW, Williams TA, Donoghue PCJ, Pisani D. 2018 Integrated genomic and fossil evidence illuminates life’s early evolution and eukaryote origin. Nat. Ecol. Evol. 2, 1556–1562. (doi:10.1038/s41559-018-0644-x)

16. Ku C, Nelson-Sathi S, Roettger M, Sousa FL, Lockhart PJ, Bryant D, Hazkani-Covo E, Mcinnerny JO, Landan G, Martin WF. 2015 Endosymbiotic origin and differential loss of eukaryotic genes. Nature 524, 427–432. (doi:10.1038/nature14963)

17. Tielens AGM, Rotte C, van Hellemond JJ, Martin W. 2002 Mitochondria as we don’t know them. Trends Biochem. Sci. 27, 564–572. (doi:10.1016/S0968-0004(02)02193-X)

18. Lindmark DG, Müller M. 1973 Hydrogenosome, a cytoplasmic organelle of the anaerobic flagellate Trichomonas foetus, and its role in pyruvate metabolism. J. Biol. Chem. 248, 7724–7728.

19. Boxma B et al. 2004 The anaerobic chytridiomyctere fungus Piramyces sp. E2 produces ethanol via pyruvate : formate lyase and an alcohol dehydrogenase E. Mol. Microbiol. 51, 1389–1399. (doi:10.1046/j.1365-2958.2003.03912.x)

20. Martin WF, Müller M. 1998 The hydrogen hypothesis for the first eukaryote. Nature 392, 37–41. (doi:10.1038/32096)

21. Mentel M, Martin WF. 2008 Energy metabolism among eukaryotic anaerobes in light of Proterozoic ocean chemistry. Phil. Trans. R. Soc. B 363, 2717–2729. (doi:10.1098/rstb.2008.0031)

22. Misra, HP, Fridovich, I. 1971 The generation of superoxide radical during the autoxidation of ferredoxins. J. Biol. Chem. 246, 6886–6890.

23. Allen JF. 1975 A two-step mechanism for the photosynthetic reduction of oxygen by ferredoxin. Biochem. Biophys. Res. Comm. 66, 36–43. (doi:10.1016/S0006-291X(75)80291-9)

24. Hug LA, Stechmann A, Roger AJ. 2010 Phylogenetic distributions and histories of proteins involved in anaerobic pyruvate metabolism in eukaryotes. Mol. Biol. Evol. 27, 311–324. (doi:10.1093/molbev/msp037)

25. Stairs CW, Leger MM, Roger AJ. 2015 Diversity and origins of anaerobic metabolism in mitochondria and related organelles. Phil. Trans. R. Soc. B 370, 20140326. (doi:10.1098/rstb.2014.0326)

26. Ginger ML, Fritz-Laylin LK, Fulton C, Candie WZ, Dawson SC. 2010 Intermediary metabolism in protists: a sequence-based view of facultative anaerobic metabolism in evolutionarily diverse eukaryotes. Protist 161, 642–671. (doi:10.1016/j.protis.2010.09.001)
27. Kruse O, Hankamer B. 2010 Microalgal hydrogen production. Curr. Opin. Biotechnol. Curr. Opin. Biotechnol. 21, 238–243. (doi:10.1016/j.copbio.2010.03.012)

28. Atteia A, van Lis R, Tielens AGM, Martin WF. 2013 Anaerobic energy metabolism in unicellular photosynthetic eukaryotes. Biochim. Biophys. Acta 1827, 210–223. (doi:10.1016/j.jbabio.2012.08.002)

29. Hemschmeer A, Happe T. 2005 The exceptional photofermentative hydrogen metabolism of the green alga Chlamydomonas reinhardtii. Biochem. Soc. Trans. 33, 39–41. (doi:10.1042/BST0330039)

30. Nguyen AT, Thomas-Hall SR, Malniet A, Timmins M, Müssnug HH, Rupprecht J, Kruse O, Hankamer B, Schenk PM. 2008 Transcriptome for photobiological hydrogen production induced by sulfur deprivation in the green alga Chlamydomonas reinhardtii. Eukaryot. Cell 7, 1965–1979. (doi:10.1128/EC.00418-07)

31. Loreti E, Valeri MC, Novi G, Perata P. 2018 Gene regulation and survival under hypoxia requires starch availability and metabolism. Plant Phys. 176, 1286–1298. (doi:10.1111/ppl.01002)

32. Huang J, Song D, Flores A, Zhao Q, Mooney SM, Shaw LM, Lee FS. 2007 IOP1, a novel hydrogenase-like protein that modulates hypoxia-inducible factor–fetal alcohol. Biochem. J. 401, 341–352. (doi:10.1042/BJ20060635)

33. Song D, Lee FS. 2011 Mouse knock-out of IOP1 protein reveals its essential role in mammalian cytosolic iron-sulfur protein biogenesis. J. Biol. Chem. 286, 15 797–15 805. (doi:10.1074/jbc.M110.157971)

34. Seki M, Takeda Y, Iwai K, Tanaka K. 2013 IOP1 protein is an external component of the human cytosolic iron-sulfur cluster assembly (CIA) machinery and functions in the MMO19 protein-dependent CIA pathway. J. Biol. Chem. 288, 16 680–16 689. (doi:10.1074/jbc.M111.241662)

35. Barth C, Weiss MC, Roettger M, Martin WF, Under G. 2018 Origin and phylogenetic relationships of the [FeFe]-hydrogenase with apicomplexa-related Chromera velia. Genome Biol. Evol. 3, 1220–1230. (doi:10.1093/gbe/evx100)

36. Bexkens ML, Zimorski V, Sarink MJ, Wienk H, Van Etten JL, Blanc G. 2014 Global analysis of the multicellular green alga Volvox carteri: photosynthetic eukaryotes. PLoS ONE 9, e99988. (doi:10.1371/journal.pone.0099988)

37. Cecchin M, Bentafou S, Grigario F, Mori A, Cazzaniga S, Vitulo N, Delledonne M, Ballottari M. 2018 Molecular basis of autotrophic vs mixotrophic growth in Chlorella sorokiniana. Sci. Rep. 8, 4645. (doi:10.1038/s41598-018-24979-8)

38. Domeij S et al. 2014 GOLLUM [FeFe]-hydrogenase-like proteins are essential for plant development in normoxic conditions and modulate energy metabolism. Plant Cell Environ. 37, 54–69. (doi:10.1111/pec.12128)

39. Schütz GI, Adams MW. 2009 The iron-hydrogenase of Thermotoga maritima utilizes ferredoxin and NADH synergistically: a new perspective on anaerobic hydrogen production. J. Bacteriol. 191, 4451–4457. (doi:10.1128/JB.01582-08)

40. Hrdý J, Foster PG, Tachezy J, Hirt RP, Dolezal P, Bardousi L, Embley T. 2004 Trichomes hydrogenosomes contain the NADH dehydrogenase module of mitochondrial complex I. Nature 432, 618–622. (doi:10.1038/nature03149)

41. Buckel W, Theurer RK. 2018 Flavin-based electron bifurcation, a new mechanism of biological energy coupling. Chem. Rev. 118, 3862–3886. (doi:10.1021/acs.chemrev.7b00707)
