Unmiraculous facultative anaerobes

(Comment on DOI 10.1002/bies.201600174)

William F. Martin

In a recent paper in these pages, Speijer [1] contends that anaerobic, aerobic metabolism cannot exist within the same organism. In particular he argues that anaerobic, aerobic metabolism cannot exist within the same eukaryote. He also contends that this argument constitutes evidence against the view that mitochondria are ancestrally facultatively anaerobic. However, he neglects the literature on prokaryotic, eukaryotic facultative anaerobes, so a short reply is in order.

The strongest argument that Speijer can muster against the view that the ancestral mitochondrion (or ancestral eukaryotes) were facultative anaerobes is found in his section entitled "Problems with assuming hydrogen exchange." There he writes "But how could the future endosymbiont have retained its complex multi-subunit aerobic respiratory chain under prolonged anoxic conditions? Do not prokaryotes rapidly lose what they do not use?" (emphasis from the original). According to that argument, no facultative anaerobes, either prokaryotic or eukaryotic, should exist at all. *Chlamydomonas reinhardtii* should not exist, because it is a facultative anaerobe [2], nor should *Euglena gracilis* exist, because it is also a facultative anaerobe [3, 4], nor should other biochemically well-studied eukaryotes exist that use their mitochondria to survive with or without oxygen [5, 6]. Indeed, according to Speijer's strongest argument, *Escherichia coli*, which is an excellent facultative anaerobe [7], should not exist either, nor should alpha-proteobacteria in general, most of which are in fact facultative anaerobes [8], nor should thousands of facultatively anaerobic proteobacterial species, as a look in the pages of The Prokaryotes [9] will attest. That facultative anaerobes are very common in nature is obvious and well known. In fact, everything alive that is not an obligate aerobe or an obligate anaerobe is in some way a facultative anaerobe. In lakes, soil and marine environments, facultative anaerobes are quite common, if not the rule. Facultative anaerobes retain anaerobic pathways and a respiratory chain. They are not miraculous. They are altogether unmiraculous.

Speijer rightly argues [1] that discovery of organisms capable of producing hydrogen while having retained a functional oxygen respiratory chain would be a boost to the hydrogen hypothesis, but he does not mention that such organisms were discovered long ago. There is of course the hydrogenosome with a genome encoding components of the canonical mitochondrial respiratory chain [10], but there are other examples. Some eukaryotes, such as *Chlamydomonas reinhardtii*, when grown in the light, produce oxygen in plastids and respire oxygen using a fully functional mitochondrial electron transport chain [11]; when transferred to the dark, and given fermentable substrate under anaerobic conditions, *Chlamydomonas* expresses enzymes of anaerobic energy metabolism and produces copious amounts of hydrogen [12, 13]. *Chlamydomonas* is a model organism for oxygenic photosynthesis while at the same time being a model organism for strictly anaerobic biological hydrogen production. That oxygen-producing algae can produce hydrogen anaerobically is nothing special, it is just facultatively anaerobic energy metabolism. *Chlamydomonas* is a well-studied example of an organism capable of producing hydrogen [12, 13] while having retained [1] a functional oxidative respiratory chain.

Many eukaryotes can respire oxygen when it is around and can perform anaerobic fermentations that produce hydrogen anaerobically. The literature on eukaryote anaerobes has been reviewed briefly [14], extensively [5], and specifically for eukaryotes that produce oxygen in plastids [6]. The adage that prokaryotes rapidly lose what they do not use is probably correct, and it is also probably correct that eukaryotes rapidly lose what they do not use. It has escaped many that most oxygen respiring prokaryotes, and many oxygen respiring eukaryotes (in particular marine species and those that, like *Chlamydomonas*, typically live below the soil line), have not completely lost their anaerobic capabilities at all. On the contrary, they have retained genes for enzymes of aerobic and anaerobic energy metabolism [5, 6, 14], so they apparently use them.

Speijer has made very important contributions to our understanding of peroxisomes and oxygen [15, 16]. Anaerobic energy metabolism in eukaryotes does not, however, require peroxisomes [5, 17, 18]. Some eukaryotes with facultative anaerobic mitochondria, *Euglena gracilis* for example, effectively run β-oxidation, a typical peroxisomal pathway, in reverse for energy conservation. But the pathway is localized to mitochondria [5, 6, 14].

The coexistence of aerobic and anaerobic metabolism within the same organism is neither unusual nor is it complicated. Eukaryotes underwent the majority of their evolution under low oxygen conditions [5, 19, 20]. Newer data even have it that the high levels of atmospheric oxygen arose only a few hundred million years ago [21], with very recent findings indicating that modern oxygen levels were attained following the origin of land plants, only some 400 million years ago [22]. By contrast, eukaryotes arose roughly 1.6 billion years ago [23]. Putting those

DOI 10.1002/bies.201700041

Institute for Molecular Evolution, University of Düsseldorf, Düsseldorf, Germany

Corresponding author:
William F. Martin
E-mail: bill@hhu.de

two simple observations together, it is evident that both the origin of eukaryotes and the diversification of their lineages occurred in low oxygen environments [5, 19, 20, 24]. Anaerobic energy metabolism in eukaryotes is not a problem, it is natural.

In mammals (obligate aerobes), redox balance and ROS are closely intertwined [15, 16]. Anaerobes have fewer issues with ROS, but have to maintain redox balance under anaerobic conditions nonetheless [25]. Even strictly anaerobic prokaryotes like *Clostridia* have to maintain redox balance [26]. The eukaryotes that produce H_2 in hydrogenosomes maintain redox balance [27] but for energy metabolic reasons, not to avoid the production of ROS. In oxygen respiring eukaryotes, peroxisomes have a very important role in keeping ROS levels in check [15, 16]. However, anaerobic energy metabolism in eukaryotes does not require peroxisomes at all. The origins of anaerobic energy metabolism [5, 14], peroxisomes [18, 28], the eukaryotic endomembrane system [29], and even the majority of eukaryotic genes [30] are all associated with the origin of mitochondria, not with the accumulation of oxygen in the Earth's atmosphere [19, 31]. The lack of peroxisomes in eukaryotic anaerobes [5, 32] and the existence of facultative anaerobes [5], both prokaryotic and eukaryotic, are important when it comes to understanding mitochondrial origin.

Acknowledgments

I thank Marek Mentel, Sriram G. Garg, and Verena Zimorski for discussions and the ERC (666053) for funding.

The author has declared no conflicts of interest.

References

1. **Speijer D.** 2017**.** Alternating terminal electron-acceptors at the basis of symbiogenesis: how oxygen ignited eukaryotic evolution. *BioEssays* **39**:1600174.
2. **Atteia A**, **van Lis R**, **Gelius-Dietrich G**, **Adrait A**, et al**.** 2006. Pyruvate formate-lyase and a novel route of eukaryotic ATP synthesis in *Chlamydomonas* mitochondria. *J Biol Chem* **281**: 9909–18.
3. **Hoffmeister M**, **van der Klei A**, **Rotte C**, **van Grinsven KW**, et al**.** 2004. *Euglena gracilis* rhodoquinone:ubiquinone ratio and mitochondrial proteome differ under aerobic and anaerobic conditions. *J Biol Chem* **279**: 22422–9.
4. **Hoffmeister M**, **Piotrowski M**, **Nowitzki U**, **Martin W.** 2005**.** Mitochondrial trans-2-enoyl-CoA reductase of wax ester fermentation from *Euglena gracilis* defines a new family of enzymes involved in lipid synthesis. *J Biol Chem* **280**: 4329–38.
5. **Müller M**, **Mentel M**, **van Hellemond JJ**, **Henze K**, et al**.** 2012. Biochemistry and evolution of anaerobic energy metabolism in eukaryotes. *Microbiol Mol Biol Rev* **76**: 444–95.
6. **Atteia A**, **van Lis R**, **Tielens AGM**, **Martin WF.** 2013**.** Anaerobic energy metabolism in unicellular photosynthetic eukaryotes. *Biochim Biophys Acta* **1827**: 210–23.
7. **Hirsch CA**, **Rasminsky M**, **Davis BD**, **Lin ECC.** 1963**.** A fumarate reductase in *Escherichia coli* distinct from succinate dehydrogenase. *J Biol Chem* **238**: 3770–4.
8. **Degli Esposti M.** 2014**.** Bioenergetic evolution in proteobacteria and mitochondria. *Genome Biol Evol* **6**: 3238–51.
9. **Dworkin M**, **Falkow S**, **Rosenberg E**, **Schleifer K-H**, et al**.**, eds. 2006. *The Prokaryotes – A Handbook on the Biology of Bacteria*. New York: Springer.
10. **Boxma B**, **de Graaf RM**, **van der Staay GW**, **van Alen TA**, et al**.** 2005. An anaerobic mitochondrion that produces hydrogen. *Nature* **434**: 74–9.
11. **Merchant SS**, **Prochnik SE**, **Vallon O**, **Harris EH**, et al**.** 2007. The *Chlamydomonas* genome reveals the evolution of key animal and plant functions. *Science* **318**: 245–51.
12. **Melis A**, **Zhang L**, **Forestier M**, **Ghirardi ML**, et al**.** 2000. Sustained photobiological hydrogen gas production upon reversible inactivation of oxygen evolution in the green alga *Chlamydomonas reinhardtii*. *Plant Physiol* **122**: 127–35.
13. **Catalanotti C**, **Yan W**, **Posewitz MC**, **Grosman AR.** 2013**.** Fermentation metabolism and its evolution in algae. *Frontiers Plant Sci* **4**: 150.
14. **Tielens AG**, **Rotte C**, **van Hellemond JJ**, **Martin W.** 2002**.** Mitochondria as we don't know them. *Trends Biochem Sci* **27**: 564–72.
15. **Speijer D.** 2011**.** Oxygen radicals shaping evolution: why fatty acid catabolism leads to peroxisomes while neurons do without it. *Bioessays* **33**: 88–94.
16. **Speijer D.** 2014**.** How the mitochondrion was shaped by radical differences in substrates: what carnitine shuttles and uncoupling tell us about mitochondrial evolution in response to ROS. *Bioessays* **36**: 634–43.
17. **Lindmark DG**, **Müller M.** 1973**.** Hydrogenosome, a cytoplasmic organelle of the anaerobic flagellate, *Tritrichomonas foetus* and its role in pyruvate metabolism. *J Biol Chem* **248**: 7724–8.
18. **Bolte K**, **Rensing SA**, **Maier UG.** 2015**.** The evolution of eukaryotic cells from the perspective of peroxisomes: phylogenetic analyses of peroxisomal beta-oxidation enzymes support mitochondria-first models of eukaryotic cell evolution. *Bioessays* **37**: 195–203.
19. **Mentel M**, **Martin W.** 2008**.** Energy metabolism among eukaryotic anaerobes in light of Proterozoic ocean chemistry. *Philos Trans R Soc Lond B* **363**: 2717–29.
20. **Mentel M**, **Roettger M**, **Leys S**, **Tielens AGM**, et al**.** 2014. Of early animals, anaerobic mitochondria, and a modern sponge. *Bioessays* **36**: 924–32.
21. **Lyons TW**, **Reinhard CT**, **Planavsky NJ.** 2014**.** The rise of oxygen in Earth's early ocean and atmosphere. *Nature* **506**: 307–15.
22. **Lenton TM**, **Dahl TW**, **Daines SJ**, **Mills BJW**, et al**.** 2016. Earliest land plants created modern levels of atmospheric oxygen. *Proc Natl Acad Sci USA* **113**: 9704–9.
23. **Parfrey LW**, **Lahr DJG**, **Knoll AH**, **Katz LA.** 2011**.** Estimating the timing of early eukaryotic diversification with multigene molecular clocks. *Proc Natl Acad Sci USA* **108**: 13624–9.
24. **Theissen U**, **Hoffmeister M**, **Grieshaber M**, **Martin W.** 2003**.** Single eubacterial origin of eukaryotic sulfide:quinone oxidoreductase, a mitochondrial enzyme conserved from the early evolution of eukaryotes during anoxic and sulfidic times. *Mol Biol Evol* **20**: 1564–74.
25. **Shimizu T**, **Shen J**, **Fang M**, **Zhang Y**, et al**.** 2017. Sulfide-responsive transcriptional repressor SqrR functions as a master regulator of sulfide-dependent photosynthesis. *Proc Natl Acad Sci USA* **114**: 2355–60.
26. **Hua L**, **Huang H**, **Yuan H**, **Tao F**, et al**.** 2016. Rex in *Clostridium kluyveri* is a global redox-sensing transcriptional regulator. *J Biotechnol* **233**: 17–25.
27. **Martin W**, **Müller M.** 1998**.** The hydrogen hypothesis for the first eukaryote. *Nature* **392**: 37–41.
28. **Sugiura A**, **Mattie S**, **Prudent J**, **McBride HM.** 2017**.** Newly born peroxisomes are a hybrid of mitochondrial and ER-derived pre-peroxisomes. *Nature* **542**: 251–4.
29. **Gould SB**, **Garg SG**, **Martin WF.** 2016**.** Bacterial vesicle secretion and the evolutionary origin of the eukaryotic endomembrane system. *Trends Microbiol* **24**: 525–34.
30. **Ku C**, **Nelson-Sathi S**, **Roettger M**, **Sousa FL**, et al**.** 2015. Endosymbiotic origin and differential loss of eukaryotic genes. *Nature* **524**: 427–32.
31. **Lane N**, **Martin W.** 2010**.** The energetics of genome complexity. *Nature* **467**: 929–34.
32. **Lindmark DG**, **Müller M.** 1973**.** Hydrogenosome, a cytoplasmic organelle of the anaerobic flagellate *Tritrichomonas foetus*, and its role in pyruvate metabolism. *J Biol Chem* **25**: 7724–8.