Unmiraculous? Yes. Ancient? Probably not. (response to DOI 10.1002/bies.201700041)

Dave Speijer

In his commentary [1] regarding my recent article proposing that the original symbiosis at the basis of the eukaryotes was based on exchange of intermediate carbohydrate metabolites [2], instead of hydrogen [3], William Martin makes a few assumptions that (alas!) are incorrect. Let me first say that I always find his insights highly important and, in the overwhelming majority of cases, enlightening. I also thank him for this reaction, because it allows me to further highlight some crucial aspects of my proposal.

So what is the real argument here? It is this: Are the examples of hydrogen production we find in quite a few different eukaryotic lineages “ancient” in that they always retained these genes for the synthesis of hydrogen, or “derived,” in that aerobic eukaryotes picked up the necessary genes at a (much) later date as they encountered hypoxic/anoxic environments (in many cases as part of a parasitic lifestyle)? William Martin thinks that at least some of them represent “the genuine article” (i.e., ancient adaptations), whereas I doubt this, and recent analyses do not make it likely either [4, 5]. Martin sees the existence of such organisms as representative of a primordial state in which the alphaproteobacterium (the future mitochondrion) alternated between using oxygen, producing water, and producing H2, which could be used between using oxygen, producing water, and aerobic metabolism cannot exist within the same organism.” Of course I contend no such thing, and am well aware of the existence of facultatively anaerobic (proteo) bacterial species. Martin goes on to say: “In particular he argues that anaerobic eukaryotes that switch their overall metabolism and use only “a pinch of O2” as well. Both of these descriptions have been borrowed from the beautiful overview of anaerobic energy metabolism by Tielens, Martin and co-workers in Microbiology and Molecular Biology Reviews [6], cited by Martin above, which I also consulted.

So what are these specific pronouncements regarding my position based on? On a very narrow, incorrect, interpretation of this quote from my article: “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?”, without taking into account what directly follows. Let me highlight a few points. The wording “future endosymbiont” shows that in this context I am talking about relatively long periods of time, with many generations (though the evolution up till LECA seems to have been rapid, we are of course still talking about a really long period, as reflected in the term “prolonged”). The text directly following the passage quoted, reads: “On the other hand, as soon as O2 is present, the ETC of the archaeon would be damaged (in particular, unadjusted iron-sulphur clusters would be vulnerable to oxidation [7]). Indeed, upon prolonged exposure to O2 this archaeal ETC did completely disappear (it is absent in LECA).”

But let us assume that the alphaproteobacterium in question indeed not only started out as an organism that also could produce hydrogen, but that it retained this capacity after disappearance of the archaeal ETC and subsequent (non-phagocytotic) uptake. In the oxygen-containing environment that was the crucible for its development toward LECA, for how long would the new entity retain this oxygen sensitive pathway? – The question is even more relevant given that H2 itself must then have become “useless” without consumption by the host. – This extended crucial period of momentous cellular changes has oxygen stamped all over it. To name but a few of the “marks” O2 left on all eukaryotes: it was absolutely essential for the generation of the necessarily large amounts of ATP to “pay” for the eukaryotic inventions [8], using oxygen as the final electron acceptor; the myriads of adaptations in response to endogenous ROS formation, including a new oxidative organelle, the peroxisome [9] – the absence of which in anaerobic eukaryotes, mentioned by Martin, I understand to be secondary loss; the pathways that use ROS as a cellular signal in all eukaryotes, new instances of which are popping up almost daily [10].

The presence of a hydrogenase pathway does not seem likely when LECA emerged at the end of this intense...
period of change, initiated and permitted by oxygen (mirroring the archaean ETC that was also lost). Thus, all eukaryotic instances of hydrogen production are probably later adaptive acquisitions in response to anoxia. In this respect they may play a role in this discussion, resembling eukaryotes without mitochondria (“archezoa”), which also used to be seen as the ancient state before uptake (even considered phagocytic!) of an alphaproteobacterial organism destined to become the mitochondrion. These “archezoa” are now known to represent derived states following adaptations to extreme environments [11]. In this context Martin’s example of *Chlamydomonas* as a well-studied organism capable of producing hydrogen while retaining a functional oxidative respiratory chain is enlightening. As I indicated, I do not find its existence miraculous. But does it reflect the primordial state? LECA did not have chloroplasts. *Chlamydomonas* possesses a hydrogenase (HydA1), which is coupled to the photosynthetic electron transport chain via photosynthetic ferredoxin (PetF) [12], so again this clearly was a later acquisition.

With regard to the discussion about oxygen levels and the rise of the eukaryotes, the *overall* levels at the time of their evolution must indeed have been (much) lower, but:

1. Even really low amounts of oxygen inhibit and occasionally irreversibly damage sensitive enzymes such as the ones involved in hydrogen production.
2. Overall oxygen levels are less important than the fact that after the GOE, oxygen containing environments started to become more common.
3. Recently, selenium geochemical data indicating the expansion of suboxic (>0.4 μM O₂) habitats in the shallow oceans as early as between 2.3 and 2.1 billion years ago, about 500 million years before the rise of the eukaryotes, were published [13]. However I do not want to overstate the importance of these kind of data because we do not know what the specific environment in which early eukaryotic evolution occurred was like. But we do know that oxygen levels fluctuated over geological timescales (a decrease in surface oxygenation followed the period described) [13].

If we consider the metabolic organizations of anaerobic eukaryotes to be later adaptations to anoxic environments, the original symbiosis between archaeeon and bacterium is “up for grabs,” and one can find the rationale for my model in the original article. The logical evolution of the metabolic rewiring starting with the exchange of intermediate carbohydrate metabolites and extensive ROS signalling nicely fits with what we find in present-day eukaryotes [2].

I want to end by saying that I appreciated the remark “Speijer has made very important contributions to our understanding of peroxisomes and oxygen.”, while referring to [9, 14], very much. It shows that, though he clearly deeply disagrees with my position regarding the symbiosis that “started” the eukaryotes, Professor Martin made the effort to be balanced in his approach. In view of such balance I have to stress again that the ideas I presented were heavily influenced by the work of Martin and colleagues, such as the recent stunning insight of Gould, Garg, and Martin that the complete eukaryotic endomembrane system might have come from bacterial vesicles released by the mitochondrial ancestor into the cytoplasm, which also nicely explains the disappearance of the archaeal host membrane lipids [15]. My article is of course a direct result of the hydrogen hypothesis [3]. This is the article that launched symbiogenetic theory: the revolutionary idea that the defining eukaryotic characteristics evolved due to *mutual adaptations* of two merging *prokaryotes*. It constituted a paradigm shift [16]. This general framework is of more value than the specific embodiment chosen. To put it differently: the idea of the “give and take” itself as a driving force is more important than what is specifically exchanged at the beginning of the eukaryotic lineage, even if it is so passionately debated here.

References

1. Martin W. 2017. Unmiraculous facultative anaerobes. *BioEssays* 39: 1700041.
2. Speijer D. 2017. Alternating terminal electron-acceptors at the basis of symbiogenesis: how oxygen ignited eukaryotic evolution. *BioEssays* 39: 1600174.
3. Martin W, Muller M. 1998. The hydrogen hypothesis for the first eukaryote. *Nature* 392: 37–41.
4. Yarlett N, Hackstein JHP. 2005. Hydrogenosomes: one organelle, multiple origins. *BioScience* 55: 657–68.
5. Stairs CW, Leger MM, Roger AJ. 2015. Diversity and origins of anaerobic metabolism in mitochondria and related organelles. *Philos Trans R Soc Lond B Biol Sci* 370: 20140326.
6. Muller M, Mentei M, van Hellemont JJ, Henze K et al. 2012. Biochemistry and evolution of anaerobic energy metabolism in eukaryotes. *Microbiol Mol Biol Rev* 76: 444–95.
7. Imlay JA. 2006. Iron-sulphur clusters and the problem with oxygen. *Mol Microbiol* 59: 1073–82.
8. Lane N, Martin W. 2010. The energetics of genome complexity. *Nature* 467: 929–34.
9. Speijer D. 2011. Oxygen radicals shaping evolution: why fatty acid catabolism leads to peroxisomes while neurons do without it. *BioEssays* 33: 88–94.
10. Speijer D. 2015. Birth of the eukaryotes by a set of reactive innovations: new insights force us to relinquish gradual models. *BioEssays* 37: 1268–70.
11. Martin WF, Garg S, Zimorski V. 2015. Endosymbiotic theories for eukaryote origin. *Philos Trans R Soc Lond B Biol Sci* 370: 20140330.
12. Winkler M, Kuglerst S, Hippler M, Happe T. 2009. Characterization of the key step for light-driven hydrogen evolution in green algae. *J Biol Chem* 284: 36620–7.
13. Kipp MA, Stueken EE, Bekker A, Buick R. 2017. Selenium isotopes record extensive marine suboxia during the Great Oxidation Event. *Proc Natl Acad Sci USA* 114: 875–80.
14. 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.
15. Gould SB, Garg SG, Martin WF. 2016. Bacterial vesicle secretion and the evolutionary origin of the eukaryotic endomembrane system. *Trends Microbiol* 24: 525–34.
16. Doolittle WF. 1998. A paradigm gets shifted. *Nature* 392: 15–6.