Eukaryotes really are special, and mitochondria are why

Booth and Doolittle (1) criticize three supposed flaws in our argument (2) that the energetic advantage of mitochondria enabled the prokaryote to eukaryote transition. Their critique, not our paper, is flawed. A reply is in order.

First, Booth and Doolittle (1) claim that our paper (2) argued that the energetic benefit of mitochondria is larger genomes. We clearly stated: The energetic cost of possessing many genes is trivial, the cost of expressing them as protein is not (2). This is because DNA synthesis consumes about 3% of a microbial cell's energy budget, whereas protein synthesis consumes about 75% (3). The energetic hurdle at eukaryote origin is gene expression, not genome size. Eukaryotes have four to five orders-of-magnitude more energy per gene than bacteria, meaning the number of proteins they can express increases by that much. Genome size is secondary.

Booth and Doolittle's (1) second point also criticizes something we did not say. They write "Second, an argument for why eukaryotes can, in principle, accomplish complex evolutionary feats that prokaryotes cannot should not rest on prokaryotic failures to do so in practice" (1). We made no such claim. It is an observation that prokaryotes have not become as complex as eukaryotic cells. Is there is a reason for that, or not? Booth and Doolittle (1) suggest no, eukaryotes just got lucky and any prokaryote can do it. We suggest that prokaryotes cannot become complex because they lack internalized membranes with associated bioenergetic genomes, which allowed eukaryotes to increase the number of proteins they can evolve and express (2).

Booth and Doolittle's (1) third criticism asserts that our paper (2) implies that anaerobic eukaryotes should lack sufficient energy to remain eukaryotic. Booth and Doolittle (1) write: "...there are many full-fledged anaerobic eukaryotes that do not derive energy from mitochondrial respiration. ... Therefore, high energy production is not, in principle, necessary to be a DNA-heavy and gene-rich eukaryote. We may find it difficult to imagine how such a cell could have evolved ... but invoking a transient aerobic intermediate does not reduce the difficulty." That critique is flawed in two ways.

Our argument (2) was that mitochondria, not oxygen, make the energetic difference that permitted eukaryotes to explore protein space by virtue of expressed genes. Booth and Doolittle (1) equate oxygen with increased energy; we clearly explained that oxygen is not the difference. Were oxygen crucial to becoming prokaryotic, *Escherichia coli* and other oxygen respiring prokaryotes would have become eukaryotic for the same reasons. True, oxygen won't make a prokaryote eukaryotic and anaerobic eukaryotes do not devolve into prokaryotes (4), but we made no such claim. The inference that only aerobes could remain complex is theirs, not ours. Many animal mitochondria are anaerobic (1, 4). Mitochondria lifted the energetic barrier to inventing the many protein-dependent traits that distinguish eukaryotes from prokaryotes (2). Once the inventing is over, maintaining those traits is energetically moot (5). By analogy, it takes far more energy to build a suspension bridge than it does to maintain it, once finished. Booth and Doolittle (1) criticize things we did not say, which readers need to know.

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