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Evolutionary Biology: A New Home for the Powerhouse?

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Metagenomic assemblies of oceanic datasets have unearthed novel and diverse alphaproteobacterial groups. Sophisticated phylogenetic analyses based on these metagenomes suggest that mitochondria do not descend from within Alphaproteobacteria, as typically thought, but from a still undiscovered sister lineage.

Over one billion years ago, a bacterium (or bacterial population) was engulfed and retained by a cell related to the Asgard lineage of Archaea [1]. Ultimately, that bacterial endosymbiont became the mitochondrion: an inextricably integrated eukaryotic organelle that plays critical roles in cellular processes as diverse as cell suicide (apoptosis), fatty acid metabolism, and synthesis of adenosine triphosphate (ATP) [2].

Fortunately for biologists, genes encoded in the relic bacterial genome (mtDNA) of diverse mitochondria have revealed important clues about their evolutionary origins: time and again, mitochondria have been shown to originate from within the Alphaproteobacteria [3]. But the antiquity of the symbiosis has made it difficult to determine the precise identity of the alphaproteobacterial group most closely related to mitochondria. And, the biological basis of symbiosis has been further obscured by the considerable ecological and metabolic variety within Alphaproteobacteria.

Many phylogenetic analyses suggest a mitochondrial affinity to Rickettsiales [4,5], an attractive possibility given that both are obligately intracellular. Others posit that the affiliation of mitochondria and Rickettsiales is a phylogenetic artifact, and that the protomitochondrion was a metabolically complex mitochondrial ancestor, more akin to *Rhodospirillum* [6]. Regardless, there has been overwhelming consensus that mitochondria trace their roots to Alphaproteobacteria. However, in a recent publication in *Nature*, Martijn *et al.* [7] present the first compelling phylogenetic evidence that mitochondria may not have emerged from within Alphaproteobacteria, but from an enigmatic sister lineage.

Martijn *et al.* [7] harvested metagenomic data collected from various oceanic locations and depths by the Tara Oceans consortium [8]. By taking read abundance, tetranucleotide frequency, and read-pair linkage data into account, 45 metagenome-assembled genomes (MAGs) were generated, representing 12 distinct alphaproteobacterial lineages — several of which are novel — along with a putative alphaproteobacterial sister group.

Before attempting to resolve the origin of mitochondria, phylogenomic analyses were carried out using 72 highly conserved proteins to better resolve the phylogenetic relationships between novel and established alphaproteobacterial groups. Employing sophisticated phylogenetic models, the authors recovered associations between Rickettsiales, Pelagibacteraceae, alphaproteobacterium HIMB59, and other marine alphaproteobacteria, with maximum statistical support. These groups, however, have compositionally biased and fast evolving genomes, which signifies the potential for phylogenetic artifacts.

Reconstructing ancient phylogenetic relationships accurately is exceedingly

difficult because mutational saturation, genomic compositional differences, and uneven evolutionary rates accrue with immense time spans. This makes specifying a realistic evolutionary model problematic. Modern phylogenetic models attempt to alleviate such issues, for instance by permitting different evolutionary constraints at different positions in multiple alignments; but they aren't perfect. Martijn *et al.* [7] used numerous approaches to decrease the effects that compositional heterogeneity have on phylogenetic inference. These methods include recoding amino acid data from a 20-character to a 4-character state — i.e., 4 classes of similar amino acids — that is less complex, but relatively information-poor, and employing a conservative stationary-based trimmer to remove the most heterogeneous sites. Phylogenetic analyses of the trimmed alignments resulted in the aforementioned fast evolving groups being split up, and Bayesian posterior predictive tests demonstrate that analyses of the recoded/trimmed datasets were closer to accounting for the level of compositional heterogeneity.

Turning their attention to mitochondrial origins, Martijn *et al.* [7] analyzed their original untrimmed dataset, along with either 24 proteins from gene-rich mitochondrial genomes, or 29 nucleus-encoded — but mitochondrion-derived — proteins. In both cases, mitochondria affiliated with fast-evolving taxa, indicating the potential for artifacts. But as with the datasets lacking mitochondrial proteins, data recoding and removal of the most heterogeneous sites apparently reduced the influence of phylogenetic artifacts on the alphaproteobacterial groups, and placed mitochondria outside of Alphaproteobacteria. This surprising finding suggests that mitochondria arose from an ancient sister group of Alphaproteobacteria, and that mitochondria and Rickettsiales originated via distinct endosymbiotic events (Figure 1).

It is important to note, however, that distinct phylogenetic analyses employing similar datasets can produce conflicting, yet highly supported tree topologies (as seen above). The analyses of Martijn *et al.* [7] are comprehensive and technically excellent, but it is difficult to be certain how 'noise reduction' approaches, like data recoding, affect the outcome. And in the case of mitochondrial origins, there are few (if any) firmly established and similarly complex 'positive controls' to show that we're getting to the right answer.

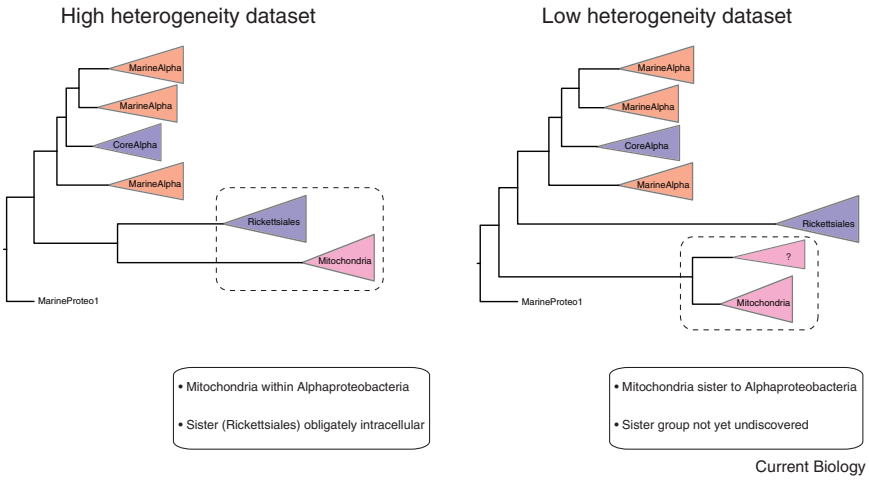

Figure 1. Dataset composition impacts interpretations of mitochondrial origins.
Schematized phylogenetic trees based on Martijn *et al.* [7] are shown. Phylogenetic reconstructions based on a highly heterogeneous dataset recover mitochondria as sister to Rickettsiales, an obligately intracellular group. Conversely, reduction of dataset heterogeneity via amino acid recoding and/or trimming places mitochondria as a sister group to Alphaproteobacteria.

The efforts of Martijn *et al.* [7] have on the one hand improved the representation of alphaproteobacterial diversity, and perhaps the phylogenetic position of the mitochondrial ancestor in the bacterial tree. On the other hand, we currently lack any representatives of the alphaproteobacterial sister lineage now proposed to have birthed mitochondria, and it is not clear if there are any still surviving. We are therefore left with more questions than answers regarding the genomic repertoire of the mitochondrial relatives, which could be decisive in resolving heated debates about the nature of the endosymbiosis that gave rise to mitochondria [9]. For example, studying the genomes and ecologies of mitochondrial sister species might tell us if the protomitochondrion was an energy parasite or free-living. Alternatively, it could help determine if the protomitochondrion was a facultative anaerobe, with the genomic capacity to transform into both aerobic mitochondria and anaerobic mitochondrion-related organelles (MROs) [10], or if it made its living aerobically, with MROs being shaped independently by gaining anaerobic metabolism via lateral gene transfers in eukaryotes [11].

The study presented here is certain to spur significant discussion, controversy and further research; but perhaps above all it highlights the power of species discovery in questioning evolutionary dogma. Our understanding of microbial life is heavily biased towards easily cultured organisms, and species associated with human health and disease. Yet, most microbes are refractory to laboratory culture, and exist in environments that are foreign to us. High throughput — and low input — sequencing technologies are dramatically enhancing our capacity to reconstruct high quality genomes from microbial communities [1] and single cells [12]. And in one stroke, Martijn et al. [7] have expanded a relatively well-studied class of prokaryotic life, and challenged a widely held view on mitochondrial origins. This work will not represent the final word on the provenance of mitochondria, but it does emphasize that mitochondria may have been forged in the undiscovered prokaryotic majority, of which there is much left to explore.

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Neuroscience: A 'Skin Warming' Circuit that Promotes Sleep and Body Cooling

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Skin and body warming help initiate sleep, but the underlying neural mechanisms remain unclear. New research in mice shows that skin warming recruits a previously unidentified hypothalamic circuit that functions to promote sleep and body cooling.

Many animals warm themselves before going to sleep. For example, humans warm themselves in beds and many land animals, including non-human primates, rodents and birds, retreat to warm environments such as nests or burrows. Certain types of fish even curl-up under the mud before entering a night of dormancy. Some biologists propose that animals engage in these 'pre-sleep' behaviours in order to warm themselves and that increases in skin, body and/or brain temperature may act as a natural mechanism to promote sleep [1–4]. This idea is supported by the fact that warm baths, hand and foot warming, and warming the hypothalamus itself can speed-up the transmission into natural sleep [1,5–7]. However, the neural circuits that engage sleep following skin, body or brain warming remained unidentified until now.

In this issue of *Current Biology*, Harding *et al.* [8] investigate how exposure to a warm environment promotes sleep in mice by identifying a brain circuit that responds to skin warming. Using an impressive range of genetic, behavioural and electrophysiological techniques they found that a warm ambient environment not only increased skin (and body) temperature, but it also switched on a specific group of neurons in the preoptic hypothalamus (Figure 1). Activation of these hypothalamic neurons rapidly triggered natural non-rapid-eye-movement (non-REM) sleep and the normal body cooling that occurs during sleep. These results are scientifically and biologically important because they identify a circuit mechanism that potentially explains how pre-sleep behaviors — such as skin/body warming — can initiate non-REM sleep and subsequent body cooling.

Harding *et al.*'s first observation was that mice prefer warm sleeping environments. To show this, they devised a straightforward but effective experiment in which mice were placed in cages that have both warm (32°C) and cool (22°C) areas [8]. They found that mice preferentially built their nests (where they sleep) and spent more time in the warmest part of their cage. Importantly, they also showed that a warm sleeping environment increases both skin and body temperature. These observations are important because many experimental biologists study sleep in mice that are housed in cool environments (~20–24°C), and Harding *et al.*'s observations not only suggest that mice prefer to nest (and presumably sleep) in a warm environment [9], but that