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PROBLEMS & PARADIGMS

Prospects & Overviews

How mitochondria showcase evolutionary mechanisms and the importance of oxygen

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Abstract

Darwinian evolution can be simply stated: natural selection of inherited variations increasing differential reproduction. However, formulated thus, links with biochemistry, cell biology, ecology, and population dynamics remain unclear. To understand interactive contributions of chance and selection, higher levels of biological organization (e.g., endosymbiosis), complexities of competing selection forces, and emerging biological novelties (such as eukaryotes or meiotic sex), we must analyze actual examples. Focusing on mitochondria, I will illuminate how biology makes sense of life's evolution, and the concepts involved. First, looking at the bacterium – mitochondrion transition: merging with an archaeon, it lost its independence, but played a decisive role in eukaryogenesis, as an extremely efficient aerobic ATP generator *and* internal ROS source. Second, surveying later mitochondrion adaptations and diversifications illustrates concepts such as constructive neutral evolution, dynamic interactions between endosymbionts and hosts, the contingency of life histories, and metabolic reprogramming. Without oxygen, mitochondria disappear; with (intermittent) oxygen diversification occurs in highly complex ways, especially upon (temporary) phototrophic substrate supply. These expositions show the Darwinian model to be a highly fruitful paradigm.

INTRODUCTION

In a quite serendipitous way, this article came about as a consequence of what I can only call the ongoing online creationist disinformation campaigns (which I will return to briefly at the end of the article, in the acknowledgements). An anonymous writer claimed that if all eukaryotes are the descendants of the singular merger leading to the last eukaryotic ancestor (LECA) this would imply that all mitochondria should not differ fundamentally. But, as they are surprisingly different, it is maintained that we are facing "yet another disaster for the coherence of evolutionary theory". What seems to be argued here is that during the huge eukaryotic diversification over more than 1.8 billion years since the days of LECA, everything diversified, but mitochondria should have basically stayed the same in all later lineages. Rephrasing it thus, answers the "argument", I think. However, for all its inanity, the argument got me thinking about how mitochondria perfectly illustrate evolutionary mechanisms. In this context, we can consider both their development from engulfed bacteria[1–3] to the mitochondria present in LECA[1,4,5] as well as their later myriad diversifications.[1,6,7] In the following article I will highlight how these evolutionary trajectories elucidate many of the intricacies of evolutionary models, clarifying "how nature works".

Abbreviations: FAO, fatty acid oxidation; FECA, first eukaryotic ancestor; ISC, iron-Sulphur cluster; LECA, last eukaryotic ancestor; LGT, lateral gene transfer; MROs, mitochondrion-related organelles; OXPHOS, oxidative phosphorylation; ROS, reactive oxygen species.

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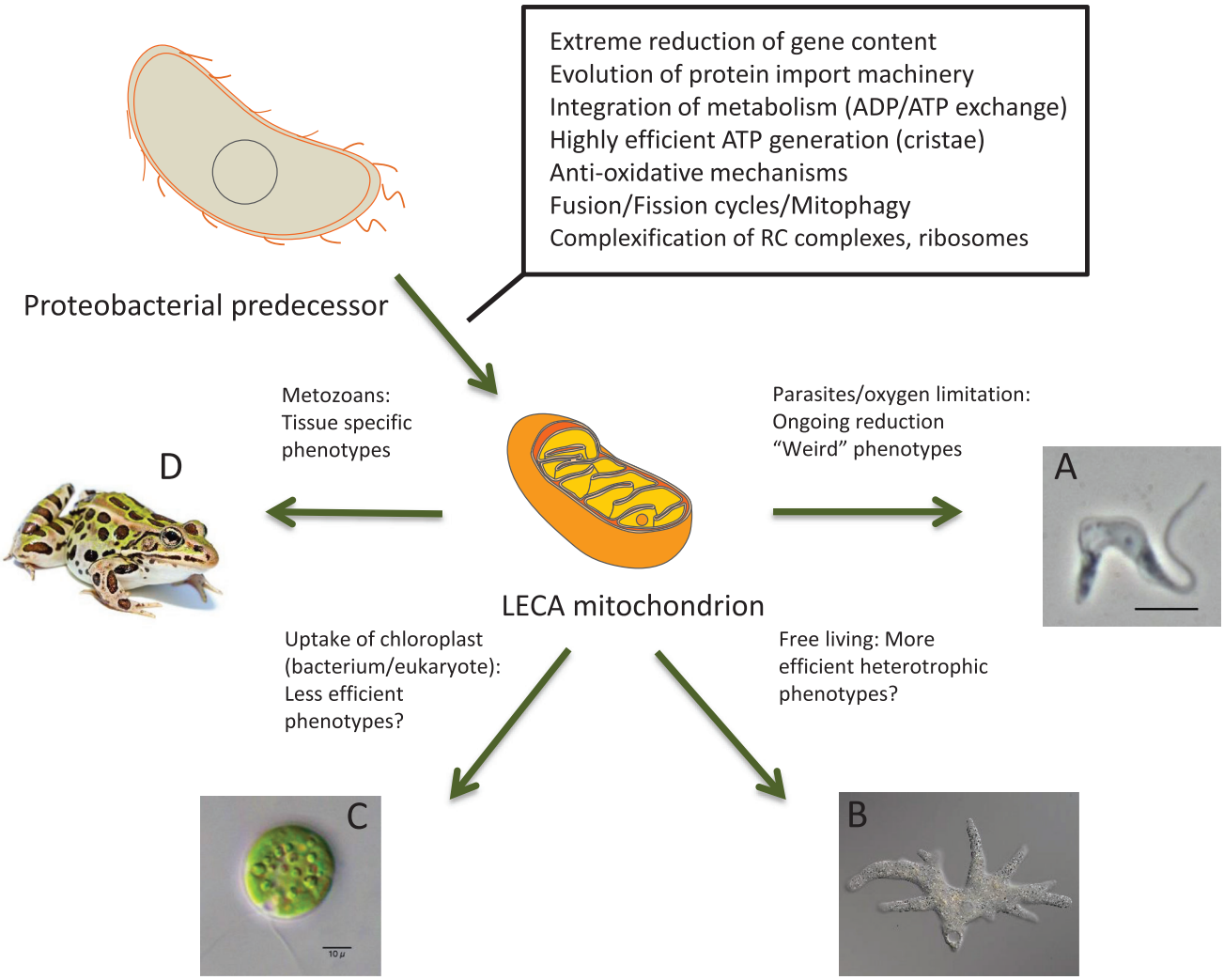

FIGURE 1 A highly schematic overview of the conversion of a free-living alpha-proteo (like) bacterium into the mitochondrion of the last eukaryotic common ancestor (LECA) and some of the main later developments in the different lineages arising from it. Of note, many eukaryotes have complex life histories involving primary and secondary uptake, as well as loss, of photosynthetic organisms. For further information see the main text. RC = respiratory chain. Clockwise: (A) *Trypanosoma brucei* (one large mitochondrion); (B) *Amoeba proteus* (up to 2 mm long, ~ $3.4e^5$ mitochondria); (C) *Chlamydomonas globosa* (many mitochondria, dynamic system, about 1%–3% of total cell volume); (D) *Lithobates pipiens* (Northern Leopard Frog, about 10 cm in length, dynamic content also depending on tissue, average ~ e^3). Black bars indicate 10 μm. All photos taken from Wikipedia pages (open domain).

I will delve into the dynamic of chance and necessity during mitochondrial development, using concepts such as constructive neutral evolution (CNE) and selection. The somewhat contentious issue of whether the arrival of the ancestor of mitochondria explains the evolution of one the eukaryotes most complex, but highly conserved, characteristics, meiotic sex, will also be touched on. Next, the sculpting influence of population genetics, so crucial in many instances of eukaryotic evolution, again, is nicely illustrated by mitochondria. With the introduction of population genetics an important aspect of evolution has to be mentioned. Darwin studied (macroscopic) organisms, which is reflected in the way he formulated his theory. However, nowadays, a multi-level generalization considers any biological unit displaying heritable variation to be evolving. Thus, for example, cells, chromosomes, genes, multicellular organisms, organelles, populations, species, and viruses are all evolving, albeit at different rates. I will try to integrate all these viewpoints into a coherent picture of the inherent complexities of evolutionary developments.

FROM BACTERIA TO MITOCHONDRIA

If we consider the bacterium that ended up as the mitochondrion as a "run-off-the-mill" proteobacterium, quite a lot of partly sequential, profound, changes occurred from the moment of its engulfment (by an unknown mechanism which still is the subject of debate[8] until LECA was formed (some of the most important alterations are indicated in Figure 1). Let's take a, by no means complete, inventory here. (i) Integration of metabolism (e.g., ADP/ATP exchange; import of substrates

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for oxidation; crucial, central role of the Krebs cycle; iron-Sulphur cluster (ISC) synthesis, and many others);[9,10] (ii) Evolution of protein import machineries;[11,12] (iii) Extreme reduction of gene content;[13–15] (iv) Highly efficient ATP generation of many different substrates (using molecular oxygen as terminal electron-acceptor combined with the evolution of cristae);[16–18] (v) Anti-oxidation damage mechanisms (e.g., using the permeability transition pore;[19]) (vi) Fusion/Fission cycles, linked to removal of oxidatively damaged parts by mitophagy and/or replication;[20–23] (vii) Increase of protein numbers of the complexes involved in oxidative phosphorylation and ribosomes;[2,3] (viii) delegation of part of the breakdown of fatty acids to newly-formed peroxisomes.[24,25] Much of the challenging pioneering work to characterize many of these facets of mitochondrial evolution was performed by the groups of Attardi, Neupert, and Schatz.[11,26–28]

Before I go into more detail about some of these aspects, I want to stress some of the fundamental, singular, aspects of eukaryogenesis, the combination of processes by which LECA evolved. The earliest ideas about engulfed bacteria being the progenitors of organelles (giving rise to mitochondria and chloroplasts) are to be found with Mereschkowsky and Wallin, later rediscovered by Margulis,[29–31] while Margaret Dayhoff's pioneering phylogenetic tree reconstructions supported (endo) symbiosis at the origin of the eukaryotes.[32,33] Further painstaking phylogenetic analyses by Bonen et al.,[34] sealed the deal for many. Extensive genome comparisons by Gray, Burger & Lang, again showed proteobacterial ancestry, but raised questions regarding the evolutionary sources of the mitochondrial proteome, which are still being debated.[35–37] Though the importance of the merger with the mitochondrial ancestor for eukaryogenesis is now more broadly recognized, most of those working in the field still envisage a quite complex archaeal "pre-eukaryote" taking up an alpha (like?[38]) proteobacterium, in the form of either prey or parasite.[1] Such a model was challenged with the advent of the so-called "hydrogen hypothesis" of Martin and Muller,[39,40] in which all the main eukaryotic characteristics evolve as a result of mutual adaptations of the prokaryotes involved: symbiogenesis. Though I think Margulis was correct in stressing the aerobic nature of the pre-mitochondrion,[31] instead of having it produce hydrogen, the importance of the extension of a radical symbiogenic framework cannot be overstated[41–43]; also, much of the possible metabolic transitions from the first eukaryotic ancestor (FECA) to the LECA remains completely unknown. In the remainder of the article I will interpret many of the mitochondrial developments from the standpoint of symbiogenesis, with O_2 as the highly efficient terminal electron-acceptor for ATP generation, as well as the source of destructive reactive oxygen species (ROS), being crucial from the start; see[10,18] and references therein. One more point of note: above I spoke of "…the merger with the mitochondrial ancestor…" instead of "…the acquisition of the mitochondrial ancestor…", because LECA has archaeal, bacterial and (quite a lot of) de novo eukaryotic characteristics that seem to have come about because of the merger itself. Regarding this event, as I mentioned, most theories still revolve around uptake by a form of phagocytosis (either of prey, or the cell being misled, of a parasite),[1,6] while not taking into account possible pre-adaptations, more favorable for a successful integration of the respective metabolisms, stemming from a pre-existing symbiosis.[8,10]

One of the changes occurring during the transition of the FECA to the LECA I want to discuss is the increased complexity of the five molecular machines involved in oxidative phosphorylation (OXPHOS) and the conversion of bacterial ribosomes into more intricate mitoribosomes. The starting point for this discussion is the deeply probing study by van der Sluis et al., entitled "Parallel Structural Evolution of Mitochondrial Ribosomes and OXPHOS Complexes".[2] Here, the authors document the extensive elaboration of these molecular machines during the bacterium to mitochondrion transition, with, for example, total mitochondrial ribosomal protein mass ranging from about 1.3 to 2.3 MDa, as opposed to the 0.8 MDa of their bacterial counterparts, and OXPHOS complexes recruiting a large number of mitochondrion-specific proteins, known as "accessory" or "supernumerary" proteins. They then convincingly show that the new, nuclear encoded, proteins allow structural redress for mutationally destabilized mitochondrially encoded components, whether rRNAs or proteins involved in OXPHOS. Though the newly enlisted proteins seem to bring selective advantages due to their compensation mechanism, the authors stress the fact that these mutations, requiring their subsequent recruitment, were non-adaptive (or even slightly detrimental) in nature.[2] Two related evolutionary mechanisms have been described to explain how (slightly) deleterious mutations can lead to the recruitment of novel proteins, or even complete, overly elaborate, "repair mechanisms" such as mitochondrial RNA editing in kinetoplastids.[44,45] The kindred mechanisms are known as "random genetic drift followed by secondary selection"[46] and "constructive neutral evolution (CNE)".[44,47,48] Van der Sluis and co-workers take pains to point out that the presence of such "neutral" innovations "may have opened evolutionary pathways toward novel mitochondrion-specific functions",[2] a point occasionally too easily dismissed by CNE proponents.[49] I will come back to these mechanisms below the following paragraph.

Another important finding of this study of the mitochondrial complex machinery is to be found in the comparison with developments in the other primary endosymbiont: the chloroplast (the descendant of cyanobacteria) in the Archaeplastida. Though this also was an ancient uptake, giving phototrophic capabilities to its recipient, it involved a host with a mitochondrion, distinguishing it from the merger involving the mitochondrial progenitor.[50] Despite this, also in this instance large scale bacterial genome reduction and migration to the nucleus has occurred, only lagging behind that of the mitochondrial ancestor to a minor degree.[51] However, in a surprising contrast, hardly any complexification of the ribosome has occurred, marking it out as a "normal" bacterial ribosome,[52,53] with the chloroplast ATPase and its electron transport complexes being quite stable as well.[54] One way to explain this difference: the archaeon/proteo-bacterium merger gave rise to a large increase in endogenous ROS formation which led to many of the (e.g., anti-oxidant) eukaryotic inventions, while the chloroplast resulted from an uptake with less internal ROS formation occurring to begin with, and many anti-oxidant adaptations in place; for further details see.[3]

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CHANCE AND NECESSITY: CNE, RANDOM GENETIC DRIFT AND THE INTERPLAY WITH SELECTION

It might be useful to ponder the relative contributions of selection and neutral processes to evolutionary developments in somewhat more detail here. We can loosely interpret Monod's famous book title about "chance and necessity",[55] as meaning that all of evolution can be seen as the dynamic interplay between chance (e.g., random mutations) and necessity (e.g., the positive selection of a mutation giving a gene and the organism in which it resides a replicative advantage). Though it is probably a fool's game to try to separate the relative contributions of the two components because of their constant interaction, it is still perfectly natural to try to do so. In the case of a random mutation, for example, leading to a higher binding affinity for a rare, high value, substrate it is easy. However, in the case of the big increase of complex I, NADH dehydrogenase, which might have started of as the consequence of a neutral, outsized, "repair mechanism", possible selective advantage might only have accrued later on. For instance, I recently argued that a fatty acid oxidation (FAO) enzyme, ACAD9, is involved in chaperoning complex I biogenesis, to quickly counteract oxidative damage in complex I resulting from FAO.[56] It is not hard to imagine that this mechanism relies on the increased complexity of complex I biogenesis,[57] allowing much more fine-tuning and speed in responses to metabolic events.

Another possible, notorious, example of the complex interplay between random processes and selection advantage is the evolution of yet another essential eukaryotic characteristic: "meiotic sex".[58,59] Surprisingly, this characteristic might also have evolved during the FECA to LECA transition in response to enhanced endogenous ROS production by the mitochondrion-to-be. It could have evolved from archaeal DNA repair mechanisms, allowing the organism to cope with Muller's ratchet,[60] increasing in danger in view of the growth of both gene content and mutation rate upon the merger at the birth of the eukaryotes.[61–63] Many (interrelated) theories have been advanced to explain the evolutionary success of meiotic sex: the generation of variation for selection to work on (i.e., efficient probing of the combinatorial space), outrunning pathogens (the so-called red queen hypothesis), the break-up of unfavorable allele combinations, which would allow quick-fire adjustments under fluctuating selection, and, as mentioned, protecting the genome against mutational meltdown.[60] Interestingly, though all these explanations probably are to some extent correct, only the last one has been convincingly experimentally validated; see, for example.[64–66] Counteracting mutational meltdown, involving the higher level of animal sexual selection (operating both via male–male competition and female choice), was also beautifully demonstrated, using flour beetles.[67] The "emergent property" of sexual selection might thus be seen as the long-term result of repair mechanisms activated by internal ROS formation upon the merger at the basis of eukaryogenesis. This gives a dazzling illustration of a long, tortuous, sequence of chance interacting with selection. Of note, in eukaryotes, chance could be more of a factor, as random genetic drift followed by secondary selection is especially powerful with small population sizes, allowing neutral or slightly detrimental changes over longer periods of time. This might help explain the occurrence of other elaborate "repair systems", in their case seemingly without extra benefits, such as the kinetoplastid RNA editing system.[45] This brings us to the question of further mitochondrial diversification, in which population bottlenecks clearly have played important roles.

A MYRIAD OF "MITOCHONDRIA"

It is not my intention to give an extensive overview of mitochondrial phenotypes (or the huge diversity in morphology[68]) to be found in all of eukaryotic diversity, ranging from: those that still retain most of the likeness to their proteobacterial forebear, found in jakobids,[69] those present in a newly described class of unicellular predators,[70] the streamlined efficient mitochondria of some animals, such as ourselves; see, for example,[71] mitochondrion-related organelles (MROs), such as hydrogenosomes, using alternative terminal acceptors instead of O_2,[72,73] and mitosomes, which have not retained any role in ATP generation,[74] to examples of eukaryotes that have lost mitochondria all together.[75] Though not everybody agrees, most researchers think this reflects an overall decline from a fully integrated oxidative mitochondrion, as present in LECA. For much more detailed overviews, I refer to some excellent reviews.[1,76,77] Here I just want to highlight some of the most interesting new findings, as they elucidate the intricacies of evolutionary mechanisms.

As mentioned, an illuminating way of envisaging this evolutionary trajectory is by seeing it as representing stages, reflecting an overall tendency: to lose mitochondria, their functions and their genes (of note, the organelle can lose genes, without loss of function, for example, via gene transfer). One driving force at work might be just the cost difference in per-cell copy numbers of the mitochondrial (many) and nuclear genomes.[78] Interestingly, during the transition from FECA to LECA, the ancestors of LECA must often have been confronted with the scarcity of carbon, nitrogen and/or phosphorous in the "prokaryote world", possibly explaining the massive gene transfer and loss that took place, as going from many mitochondrial gene copies to only two nuclear ones (or, better still, completely losing them), would cut the amount of these elements needed.

So, what can descendants of LECA lose or transfer at any given time? As I alluded to above, the much smaller population sizes of eukaryotes allow them to wreak havoc with mitochondria. However, there are clearly also strong restraining factors, the most important one being ecological: does the organism rely heavily on the importance of efficient ATP generation by mitochondria, or not? At a minimum, this means that it is aerobic. Heterotrophs are of course also more dependent on constant efficiency, explaining why the arrival of a chloroplast is some lineages, had important effects on mitochondrial evolution; see, for example.[79] This also means that metazoans, especially those locked in prey-predator (muscle) evolution, retain streamlined, efficient mitochondria. Looking at unicellular eukaryotes, Jakobids (free-living, heterotrophic, and bi-flagellar), indeed retain the

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largest mitochondrial coding repertoire (~70 protein coding genes) with a (most likely ancestral) bacterial RNA polymerase.[80] This last protein seems to have been replaced by a "viral-type" nucleus encoded polymerase in all other known eukaryotes, which might help explain the larger amount of overall gene retention in this lineage. The newly discovered supergroup of unicellular eukaryotic predators, the "Provora" (separated into two ancient subgroups) are also illuminating here.[70] (i) Though ancient, and globally present, they have only very recently been discovered, because of their numerical rarity, a trait shared with top predators among metazoans; (ii) Though less proteins (> 50) are encoded by their mitochondria than is the case for Jakobids, they still contain more than double the amount found in metazoans, which might imply these organisms represent the most ancient predators, while metazoans and the related fungi started out as more passive consumers of organic matter, allowing more extensive transfer early on; (iii) The Provora mitochondrial diversity easily surpasses that of the metazoans, attesting to antiquity of the lineage and several population bottlenecks, with, for example, one of the subgroups still holding on to three complex II subunits, while the other one starts to resemble the metazoan configuration with migration of all three to the nucleus; (iv) As such, these organisms showcase the opposing effects of the importance of continuous efficient ATP production (hampering gene transfer) and small population sizes (allowing founder effects of further gene transfer).

LESS, AND LESS, MITOCHONDRIAL FUNCTIONS

So far, I discussed gene loss (migration), but in this paragraph the focus will be on loss of mitochondrial functions. Here, everything seems to be about the availability of molecular oxygen. What happens when that disappears over stretches of time (much) longer than the lifetimes of the organisms involved? Many of the possible adaptations in that case, for example, losing complex III and IV and using fumarate as the final acceptor, or getting rid of all of the OXPHOS complexes as a whole and using protons as the final acceptor, generating hydrogen, are extensively described in.[76,77] A highly interesting "snapshot" of a eukaryote in the earliest stages of mitochondrial changes induced by decreased oxygen in its environment has been found in a Rhizarian.[81] Not everybody is convinced about this interpretation of its mitochondrial metabolism.[76] However, the interpretation of a transitional organelle combining mitochondrial and hydrogenosomal attributes (after acquiring the requisite genes from other organisms in its micro-aerobic environment) seems accurate. Aerobic metabolism is degenerating, with loss of cytochrome-based electron transport (i.e., complexes III and IV). Surprisingly, the nucleus-encoded alpha subunit of complex V is fragmented into four separate pieces, each now having targeting signals, which might provide for a more efficient import of the protein into the mitochondrion, exemplifying complex adaptations during O_2 loss.[68] Last, but not least, in transmission electron micrographs, only an isolated indication for a crista remnant can be seen.[81] This indicates that the (important) analyses of highly efficient mitochondrial bio-enenergetics by Lane and Martin,[16] should have stressed both the enormous increase in internal membrane surface and the use of O_2 as the final electron-acceptor.[18]

It has been known for quite some time that even when ATP generation has disappeared altogether, mitochondrial ghosts, in the form of mitosomes, remain. In most cases this is due to another part of the metabolic integration of the mitochondrion: its crucial role in ISC biosynthesis.[82,83] In another surprising example of mitochondrial plasticity enabled by lateral gene transfer in micro-aerobes, anaerobes and parasites, Stairs and colleagues found an example of an MRO with a reduced respiratory chain and a metabolic mosaic of ancestral and more recently acquired pathways. In this instance, even the bacterial ISC biosynthesis was replaced by an archaeal sulfur mobilization apparatus obtained by lateral gene transfer (LGT).[84] When ISC biosynthesis is lost from mitochondria and all traces of normal mitochondrial function are gone, we are closing in on the natural endpoint of reduction: eukaryotes that have lost vestiges of mitochondria all together, which we find in the oxymonad *Monocercomonoides exilis*.[75] But this story has a few more twists. In a closely related oxymonad (which has to be polyxenically cultured, making analysis much more difficult), an MRO without ISC biosynthesis was retained. Using a new, highly sensitive, proteomic technique to specifically analyse organelle proteins by isotope tagging, Zitek and co-workers were able to discover why: they contain the glycine-cleavage system, linked to essential one-carbon metabolism.[85,86] In another recent discovery, Burki and colleagues analysed the MRO of *Paramikrocytos canceri*, a representative of the endoparasitic Ascetosporea.[87] By assembling its nuclear genome (no mitochondrial genome being left), studying its transcriptome, and using heterologous gene expression in yeast as a control, they found a completely new, and surprising, mix of functions for this remnant. Retention of ISC synthesis goes together with predicted ATP production via a partial glycolytic pathway and even some de novo phospholipid synthesis, the details of which can be found in.[87]

So, what is the main message from all these idiosyncratic MROs? First of all: when molecular oxygen disappears over extended time periods, all bets are off. Without the restraining factor of still using them as efficient aerobic ATP generators, which was an essential part of what the mitochondrial bacterial ancestors brought into the merger at the basis of the eukaryotes, mitochondrial genomes and functions start to go. All the different incarnations of the highly derived states described should, of course, never be mistaken for ancient eukaryotic forms on the way to having a full aerobic endosymbiont.[88] After much debate, the important role of LGT from organisms already adapted to the micro-aerobic or anaerobic environments that recipient eukaryotes are entering, seems sufficiently established to me as well.[89,90]

MAKING SENSE OF CONVOLUTED ECOLOGICAL HISTORIES: MORE, AND MORE, MITOCHONDRIAL FUNCTIONS?

Interestingly, in the case where aerobic function is (mostly) maintained, relaxations of required efficiency seem to matter a lot for further mitochondrial development. However, these resulting effects are much

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less straightforward. This is, for example, illustrated by the effects of the arrival of the chloroplast in the ancestor of the Archaeplastida, or the arrival, and subsequent loss, of a chloroplast in the ancestor of the sister groups Diplonemida (an understudied, abundant eukaryotic group, with mostly unknown lifestyles[91]) and Kinetoplastida, the latter group being mostly parasitic. Whenever energy-rich carbohydrates could be synthesized by an organism itself or easily appropriated from hosts or the local environment, mitochondrial evolution might have allowed more instances of run-away CNE examples, as exemplified by large mitochondrial DNA increases, or highly elaborate RNA editing schemes, involving a large array of dedicated proteins (cf. our own streamlined mitochondrial genome).[45]

In many of these examples, metabolic reprogramming of the cell, outside of mitochondria, played important roles as well. To give just one example, the peroxisome, involved in beta-oxidation in LECA, is found to have morphed into a glycosome (containing the enzymes of -part of- the glycolytic pathway and other core processes of carbon metabolism) in parasitic kinetoplastids,[92,93] possibly allowing them to metabolize glucose pilfered from their hosts at greater speed, for example, during anaerobic (!) growth in the bloodstream of the mammalian host, or, more likely, to adapt more quickly to changes in carbon sources (e.g., by autophagy of the organelle[94]). With aerobic growth, however, in insect hosts, the mitochondrion has to engage in "normal" OXPHOS for migration to the salivary glands[95] and the glycosomes are metabolically reprogrammed (reviewed in[96]). Similarly, the versatile, free-living diplonemid, *Diplonema papillatum*, can also survive in low-oxygen environments and dynamically restrict some carbon metabolism pathways to their glycosomes. Its metabolism is able to quickly adapt to changes in carbon availability (somewhat comparable to the effect of migrating from host to host by their parasitic relations?).[97] From the preceding we can conclude that glycosomes emerged before parasitism,[98] but may still have facilitated its development.[99]

Far from diminishing, step by step, towards oblivion upon sustained anoxia, mitochondria in these groups retain "normal" aerobic potential, but also have to alter their unicellular metabolism in highly diverse ways in response to abrupt changes in both nutrient and O_2 availability. If we further take into account that they often have to engage in very elaborate mechanisms (such as RNA pan-editing) to be able to express mitochondrial genes from complex networks, it is not surprising in the least, that such cells have enormously complex mitochondrial proteomes.[100,101] However, the mitochondrial proteome of a member of the third (free-living) sister group, which retained is phototrophic ability, *Euglena gracilis*, turns out to be surprisingly complex as well.[102] This indicates that mitochondrial proteome complexity was already present in the common ancestor of all three groups, and, just like glycosomes, arose independent of parasitism. All this illustrates how the convoluted ecological history of organisms, together with the population bottlenecks they encountered, determine later complex mitochondrial phenotypes. As Stephen Jay Gould so brilliantly explained to a broader public in "Wonderful Life": evolution is both highly complex and strongly contingent.[103]

CONCLUSIONS

In the development of mitochondria from a bacterium, evolution seems to have been dictated by efficient ATP generation and coping with endogenous ROS formation, such that a lot of the eukaryotic complexity allowed by aerobic mitochondria with hugely extended membrane surfaces (cristae), had to be used towards that goal. In later mitochondrial diversifications the availability of molecular oxygen was still paramount: without it, mitochondria get lost, but with alternating availabilities of O_2 and nutrients, they become all the more complex. I would like to make one final point. We often hear about flaws (and major improvements accompanying such claims) in Darwin's conceptualization of nature, but I think we can conclude life indeed basically evolves in a Darwinian fashion, by which I mean according to a framework in which myriads of lifeforms, living and extinct, are products of the interplay between chance and selection, along lines set out in his seminal theory, which has shown itself to be a very fruitful paradigm.[104] I hope to have illustrated that this interplay can give rise to complexities even he could not have envisaged.

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CONFLICT OF INTEREST STATEMENT

None

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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