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Eukaryotic cellular intricacies shape mitochondrial proteomic complexity

Michael Hammond[1,2] | **Richard G. Dorrell**[3] | **Dave Speijer**[4] | **Julius Lukeš**[1,2]

[1]Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice (Budweis), Czech Republic

[2]Faculty of Sciences, University of South Bohemia, České Budějovice (Budweis), Czech Republic

[3]Institut de Biologie de l'ENS (IBENS), Département de Biologie, École Normale Supérieure, CNRS, INSERM, Université PSL, Paris, France

[4]Medical Biochemistry, UMC, University of Amsterdam, Amsterdam, The Netherlands

Correspondence
Michael Hammond, Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice (Budweis), Czech Republic.
Email: michael.hammond@paru.cas.cz

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Abstract

Mitochondria have been fundamental to the eco-physiological success of eukaryotes since the last eukaryotic common ancestor (LECA). They contribute essential functions to eukaryotic cells, above and beyond classical respiration. Mitochondria interact with, and complement, metabolic pathways occurring in other organelles, notably diversifying the chloroplast metabolism of photosynthetic organisms. Here, we integrate existing literature to investigate how mitochondrial metabolism varies across the landscape of eukaryotic evolution. We illustrate the mitochondrial remodelling and proteomic changes undergone in conjunction with major evolutionary transitions. We explore how the mitochondrial complexity of the LECA has been remodelled in specific groups to support subsequent evolutionary transitions, such as the acquisition of chloroplasts in photosynthetic species and the emergence of multicellularity. We highlight the versatile and crucial roles played by mitochondria during eukaryotic evolution, extending from its huge contribution to the development of the LECA itself to the dynamic evolution of individual eukaryote groups, reflecting both their current ecologies and evolutionary histories.

KEYWORDS
mass spectrometry, mitoproteome, multicellularity, parasite, photosynthesis, plastid, protist

INTRODUCTION

Mitochondria are regarded as critical components for the process of eukaryogenesis,[1,2] providing the energy and metabolic partitioning necessary for a rapid development of a large contingent of structural and functional genes.[3] In turn this has enabled expansions of both eukaryotic metabolic rates as well as their maximal size constraints.[4–6] Accordingly, the eukaryote domain is an extremely diverse group in terms of size, ranging from unicellular organisms less than 1 μm in diameter, to colonial tree populations spread over 40 hectares.[7] Mitochondria contribute multiple essential functions, including oxidative phosphorylation resulting in ATP generation, using $NADH_2$ and $FADH_2$ generated through the Tricarboxylic Acid (TCA) cycle and fatty acid oxidation; fermentation, with some organisms also performing the lower half of glycolysis mitochondrially[8]; key steps in the synthesis, degradation and recycling of amino acids[9]; heme[10], fatty acid and iron-sulphur cluster syntheses[11]; and the maintenance of inter-organellar $NAD(P)H_2$ and ATP homeostasis.[12] In plants and other photosynthetic eukaryotes, mitochondrial respiration has been shown to be important for photosynthetic function [13].

This amazing diversity of eukaryotes springs from a single common ancestor, which is believed to have existed between one and two billion years ago.[14] The vast majority of eukaryote lineages comprise protists, which are in most cases, distantly related to plants, animals and fungi, and indeed to one another (Figure 1). Most eukaryotes are aerobes, but a significant minority are facultatively, or even obligatory, anaerobic, typically living either as commensals of other organisms or in deep benthic anoxic habitats.[15,16] Eukaryotes may derive their energy through autotrophy (photosynthesis) or heterotrophy (predation, saprotrophy, or parasitism). Many photosynthetic unicellular algae and a handful of (carnivorous) plant species also retain the ability

Abbreviations: LECA, last eukaryotic common ancestor; LOPIT, localisation of organelle proteins via isotope tagging; TCA, tricarboxylic acid; TOM/TIM, translocation of the outer membrane/inner membrane

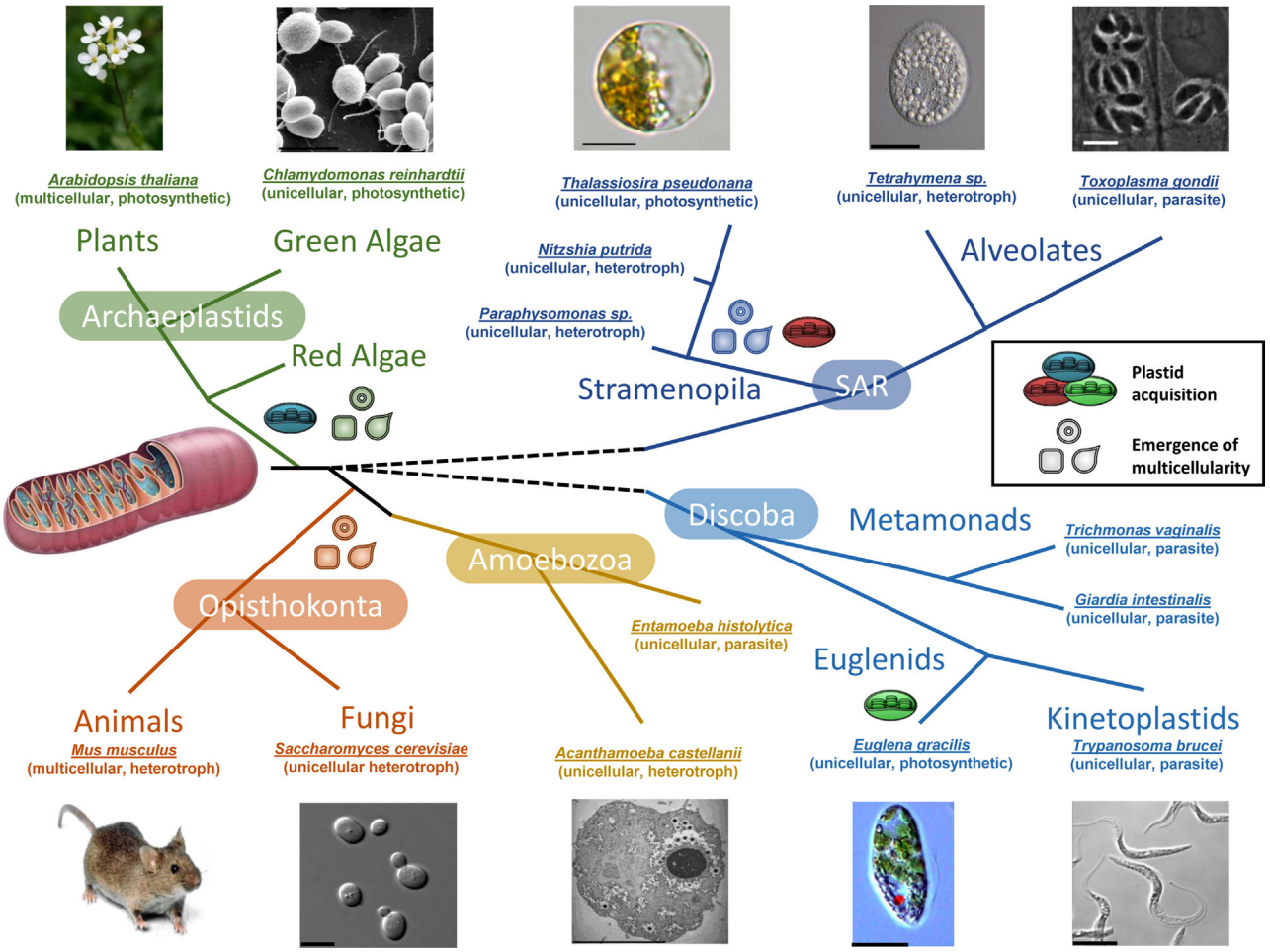

FIGURE 1 Simplified eukaryotic tree of life; organisms of interest with experimentally determined mitoproteomes indicated by representative images on border. Supergroups shown in white. Scale bars, where shown, are 10 μm; other images are to macro-scales. Images: public domain, except *E. gracilis* (Ellis O'Neill, Oxford University), *A. thaliana* (Marie-Lan Nguyen, Jardin des Plantes, Paris), *Tetrahymena sp.* (William Bourland, Boise State University) and *Toxoplasma gondii* (Luděk Kořený, Cambridge University)

to perform heterotrophy, either as phagotrophs or osmotrophs, in combination with photosynthesis (photo-mixotrophy).[17–19] Despite this diversity in morphology and lifestyle, the vast majority of eukaryotes have retained mitochondria, with even most obligately anaerobic groups retaining mitochondria-derived organelles, such as DNA-lacking hydrogenosomes or non-respiring mitosomes, excepting a small number of unicellular species.[20,21]

This all but ubiquitous presence of mitochondria across the eukaryotic tree speaks not only to their importance, but also to their evolutionary history. It is clear that mitochondria were present in the Last Eukaryotic Common Ancestor (LECA), originating from the endosymbiosis of an early-branching alpha-proteobacterium with an archaeon (related to the Asgard-like group of extant Archaea), eventually transitioning to a stable organelle.[22] It remains debated whether other bacteria have participated in this endosymbiotic event, for example, in a 'ménage à trois' or other complicated syntrophic scenario (e.g., a chimeric bacterial symbiont).[23,24] To complicate matters further, contemporary mitochondria are supported by a patchwork of proteins coming from the endosymbiont, archaeal host, and other bacteria.[25] Subsequent endosymbiotic events involving photosynthetic bacteria and eukaryotes have yielded a wealth of different plastid-carrying groups, spread across the eukaryotic tree, and like mitochondria, engaged in both fundamental bioenergetic and non-bioenergetic metabolism; although punctuated with frequent instances of secondary loss.[26,27]

One of the most useful indicators of an organism's mitochondrial capacity is the variety of proteins contained within its mitochondria, referred to as the mitochondrial proteome or 'mitoproteome'. Not surprisingly, animals and fungi have received most attention in regard to mitochondrial proteomic investigations. Comparatively little is known for the mitochondria of plants (with the exception of some model and crop representatives) and protists. In their case, this is due to both their extreme diversity and the relatively few, recent and well-resolved experimental mitoproteome datasets. In this review, we consider mitoproteomes across previously published datasets to gauge mitochondrial evolution (Figure 1).

We describe the mechanisms by which mitochondria have adapted to facilitate major transitions in later stages of eukaryotic evolution,

represented by the uptake of photosynthetic plastids, and the development of complex multicellularity. The versatility and biochemical range of mitochondria was vastly reduced in some cases. In contrast, further complexity in mitochondrial pathways also evolved, for instance influenced by photosynthetic metabolism, in many cases arising via organelle retargeting events.[28] The independent, repeated advent of multicellularity, both in plants and animals has been consistently supported by metabolic and proteomic partitioning. Overall, this development has reduced mitochondrial complexity in individual cell types, reflecting functional specialisation. Thus, our data illustrate how ancestral constraints have interplayed with novel complexities to give rise to the extensive divergence of mitochondrial metabolism across the eukaryotic tree.

Informatic and experimental strategies to identify mitoproteins

The vast majority of mitochondria are supported by their own genomes, reflecting their bacterial origin. However, these genomes only encode a handful of proteins,[29] ranging from two in apicomplexans within the alveolates (Figure 1)[30] to 65 in jakobids within the discobans (Figure 1).[31] These numbers are vastly inferior to the thousands of proteins identified in free-living bacteria, or indeed the hundreds to thousands of proteins documented in different mitochondrial lineages. Hence, the overwhelming majority of mitochondrial proteins (mitoproteins) are encoded by nuclear genes,[32] entailing that only limited proteomic knowledge can be acquired by studying these highly reduced mitochondrial genomes (mitogenomes). The mitoproteins encoded by the nuclear genes are typically post-translationally imported into the mitochondria, using dedicated protein import complexes, for example, Translocation of the Outer Membrane/Inner Membrane (TOM/TIM) that recognise semi-conserved hydrophilic N-terminal targeting sequences.

Different strategies exist for defining the likely mitoprotein content of diverse cells and tissues. In silico targeting predictors such as Mitofates[33] and TargetP,[34] that have been trained on the hydrophilic N-terminal targeting motifs recognised by mitochondrial import complexes, may be used to identify mitochondria-targeted proteins directly from genome or transcriptome sequences. The sensitivity and specificity of in silico mitoprotein prediction significantly varies amongst species and depends on having well-annotated genome or transcriptome data, as well as experimentally validated sequences for calibration. Of note, these may not be able to predict mitoproteins imported using non-conventional targeting pathways (e.g., with ambiguous or dual-targeted sequences; or imported using TOM/TIM-independent pathways, as in the case of many mitochondrial outer membrane proteins). In addition, in silico targeting predictors cannot estimate mitoproteome content variations in multicellular organisms (e.g., in different tissues or life stages) except in very specific cases (e.g., where different gene splice-variants or alleles are associated with different samples).

Comprehensive tagging studies of organisms possessing well-annotated genomes represent ongoing projects still in their infancy,[35,36] though such efforts continue to demonstrate hundreds of mitochondrial proteins that evade software-based identification, in spite of recent improvements in target peptide detection sensitivity using machine learning methods.[37,38]

Mass spectrometric proteomic surveys remain the gold standard to determine mitoproteomes, though such experimental datasets are still costly and labour-intensive, requiring the specific optimisation of purification protocols for individual species. Mitochondrial purification may be particularly complicated in unicellular species by the difficulty of obtaining pure mitochondrial fractions from cell lysate, either as a result of small cell sizes, sensitivity of cells to mechanical disruption and co-association of the mitochondria with other organelles, such as peroxisomes and chloroplasts.[39] Such issues are being increasingly resolved through the application of next-generation proteomic strategies such as Localisation of Organelle Proteins via Isotope Tagging (LOPIT), which enables the algorithmic identification of probable organelle proteomes from semi-pure organelle fractions.[40,41] Nonetheless, even the most sensitive experimental reconstructions of mitoproteome content are still likely to miss low abundance proteins below equipment detection thresholds that may be identifiable through in silico prediction and green fluorescent protein localisation, such as mitochondrial-targeted RNA polymerases.[42]

Such complementary strengths and limitations afforded by in silico and proteomic approaches may ultimately necessitate hybrid approaches, as seen in a variety of model organisms that have been investigated in such a manner (Table 1). These should ideally be grounded in well-resolved experimental proteomes in individual model species and given phylogenomic context through targeted computational reconstructions in others: affording higher levels of insight for tissue-specific and low-abundant mitoproteins, respectively. The recent increase in experimental mitoproteome resolution across eukaryotes has allowed critical insights into the genomic diversity of understudied but important protist lineages,[43] which has in turn provided unprecedented glimpses into mitoproteome diversity across the eukaryotes, warranting a timely review of knowledge obtained so far.

An elaborate mitoproteome in early eukaryotes?

The first unicellular mitoproteome to be sequenced was that of the fungus *Saccharomyces cerevisiae* and initially established 332 proteins,[56] which was subsequently elevated to 749,[57] and then to 851[58] (Figure 2). However, as a model for unicellular mitochondrial investigation, *S. cerevisiae* represents a non-ideal organism, having descended from a multicellular ancestor itself, while additionally flocculating under appropriate stimuli. Further experimental mitoproteomes have been completed for protist lineages much more distantly related to animals or fungi, including the alveolates *Tetrahymena thermophila*[59] and *Toxoplasma gondii*,[60] the green alga *Chlamydomonas reinhardtii*,[61] the amoebozoan *Acanthamoeba castellanii*,[46] and the stramenopile

TABLE 1 Model organisms with mitoproteomes predicted in silico as well as experimentally determined within the last decade

Species	Metabolic lifestyle	Experimental mitoproteome	Predicted mitoproteome
Saccharomyces cerevisiae **(Baker's yeast)**	Unicellular, Heterotroph	901	1187
Acanthamoeba castellanii	Unicellular, Heterotroph	709	1033
Trypanosoma brucei	Unicellular, Parasite	1120	1527
Arabidopsis thaliana **(Thale cress)**	Multicellular, Photosynthetic	1005	2585
Solanum tuberosum **(Irish potato)**	Multicellular, Heterotroph (Root cells)	1060	1250
Euglena gracilis	Unicellular, Photosynthetic	1756	2523
Thalassiosira pseudonana	Unicellular, Photosynthetic	325	2387
Homo sapiens	Multicellular, Heterotroph	1357	1626
Mus musculus **(House mouse)**	Multicellular, Heterotroph	1204	1591

Species listed include *Saccharomyces cerevisiae*,[44,45] *Acanthamoeba castellanii*,[46] *Trypanosoma brucei*,[47–49] *Arabidopsis thaliana*,[50,51] *Solanum tuberosum*,[52] *Euglena gracilis*,[53] *Thalassiosira pseudonana*,[39] *Homo sapiens*[54] and *Mus musculus*.[54,55]

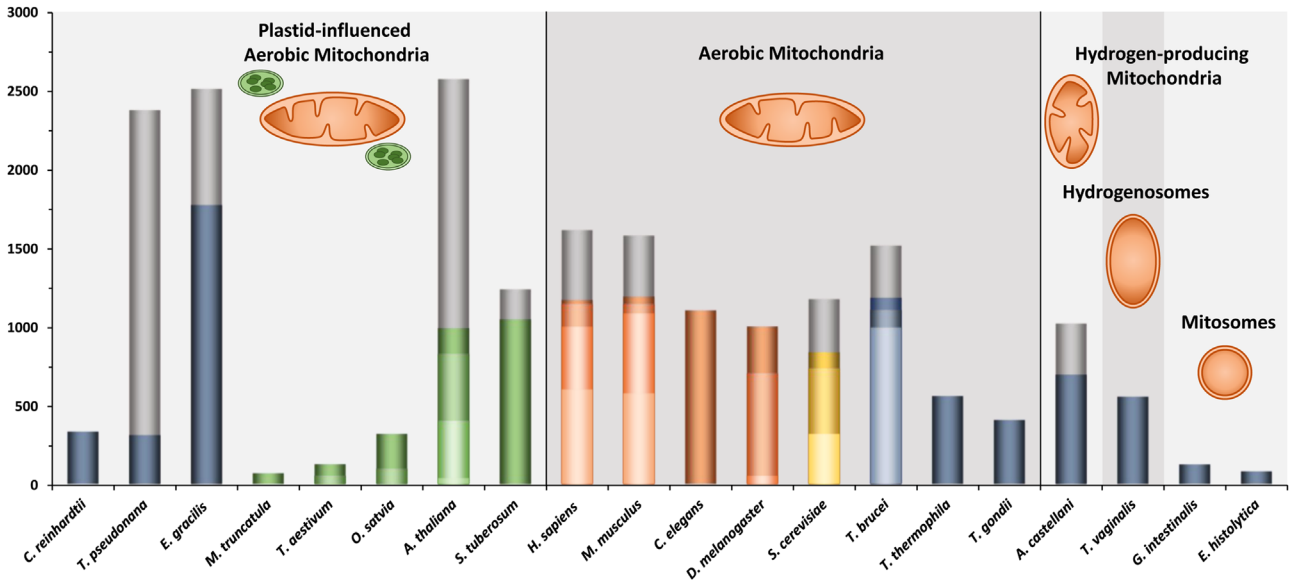

FIGURE 2 Mitoproteomes (≥50 proteins) of surveyed organisms including plants (green), animals (orange), fungi (gold) and protists (blue) grouped by mitochondrial form. Grey regions indicate in silico predictions based on molecular data and experimental yields. Different proteomic studies (listed in Supporting Information S1) are represented by colour shaded regions. Species include: *T. pseudonana*,[39] *Chlamydomonas reinhardtii*,[61] *Euglena gracilis*,[53] *Medicago truncatula*,[62] *Triticum aestivum*,[63,64], *Oryza sativa*,[65,66] *A. thaliana*,[50,51,67–69] *S. tuberosum*,[52] *H. sapiens*[54,70–72] *M. musculus*[54,71–73] *Caenorhabditis elegans*,[74] *Drosophila melanogaster*,[75–77] *S. cerevisiae*,[44,45,56–58] *T. brucei*,[47–49] *T. thermophila*,[59] *Toxoplasma gondii*,[41,60] *T. vaginalis*,[78] *G. intestinalis*[79] and *E. histolytica*[80]

Thalassiosira pseudonana,[39] resolving between 325 and 709 mitoproteins each (Figures 1 and 2). These findings must be seen in the light of the caveat that many of these organisms still lack the proteomic reanalysis applied to *S. cerevisiae* and other multicellular organisms, which typically improves on previous efforts through increased equipment sensitivity (Figure 2).

Comparisons of the mitoproteomes of these distantly related unicellular eukaryotes can provide clues about the mitochondrial complexity of the LECA. While it is difficult to be precise about the kind of mitochondrion that was part of the LECA, given that this organism existed between one and two billion years ago, the picture afforded by considering the proteomes of modern mitochondria is one of surprising complexity. The LECA mitoproteome presumably contained traits that are almost universally represented in its descendants, for example, oxidative phosphorylation and the TCA cycle, primary metabolic pathways such as iron-sulphur cluster formation, and core biogenesis processes including protein import through the TOM/TIM complexes, mitochondrial division and functional expression of the mitogenome. Moreover, several traits that could have been lost from the mitochondria of most eukaryotes are occasionally detected in the organelles of distantly related and understudied unicellular lineages,[81] such as the bacterial secretion system[82] and the signal recognition

particle-based machinery.[83] The sporadic distribution in separate protist lineages might imply that they were indeed present in the LECA mitochondrion, although alternative explanations (e.g., later instances of horizontal gene transfer to unicellular recipients[16]) also need to be considered.

Reduction, but not disappearance, of the mitoproteome in parasitic protists

Parasitism as a life strategy has evolved multiple times across the eukaryotes, including in several plant lineages,[84] and is frequently associated with a shift towards anaerobic metabolism. The streamlining effects on the genomes of parasites are well known, and while most cases represent the effects of neutral evolution, some examples of loss, particularly those in eukaryote parasites, have been shown to provide adaptive advantages, such as energy conservation and spatial efficiency.[85] Mitochondria can likewise be reduced in complexity, particularly in parasites inhabiting anaerobic environments, which leads to simplified organelles of mitochondrial origin in the form of hydrogenosomes and mitosomes.[86] Their proteomes in turn reflect the extremes of mitochondrial content across unicellular eukaryotes, with fewer than 150 proteins recovered from the non-respiring mitosomes of the discoban *Giardia intestinalis* and the amoebozoan *Entamoeba histolytica*.[79,87] Hydrogenosomes, which can be seen as energy producing intermediates between mitochondria and mitosomes,[88] yielded an appropriately intermediate number of proteins (569) within the discoban *Trichomonas vaginalis*[78] (Figure 2).

However, parasitism is not inherently reductive for mitochondria, as illustrated by the discoban parasite *Trypanosoma brucei*, from the organelle of which 1008 proteins were initially recovered.[48] This number was subsequently extended using comparative knockdown studies, establishing 1120 proteins differentially present upon ablation of the mitochondrion-specific import apparatus[47] (Figure 2). The dual-host lifestyle of *T. brucei* was assumed to explain such organellar complexity, with an intricate, aerobically respiring mitochondrion required in the tsetse fly host midgut, followed by a simplified organelle present in a life stage occupying the bloodstream of the mammalian host. However, profiling both mitoproteomes showed relative parity in proteins found in the insect and mammalian life stages (1197 vs. 956).[49] Further investigations into this organism's mitochondrion have established expanded respiratory complexes,[71] extensive RNA editing,[89] a complex protein import machinery[90] and protein-rich ribosomes[91] with a complex assembly pathway.[92]

Complex evolutionary histories of mitoproteomes in photosynthetic protists

The specific changes undergone by mitochondria in response to the uptake of photosynthetic plastids, and plastids in general, remain a mostly underexplored area. Chloroplasts have been acquired many times across the eukaryotic tree through either the two recorded endosymbiotic uptakes of photosynthetic bacteria (primary endosymbiosis) or the numerous uptakes of eukaryotic algae (secondary, tertiary, etc. endosymbioses).[26,93] This acquisition of a photosynthetic organelle, with its associated metabolism, may dramatically impact the ways in which mitochondria subsequently function. Accordingly, photosynthetic eukaryotes may have highly complex mitoproteomes. These include over 1000 verified proteins in the mitochondria of the plants *Arabidopsis thaliana* and *Solanum tuberosum*, and in silico predictions of as many as 3000 in *A. thaliana*[69] (Figure 2). An unprecedented complexity of 1756 proteins resolved experimentally in the mitoproteome of the photosynthetic discoban *Euglena gracilis*[53] further challenges naïve assumptions about organellar composition within 'simple' unicellular organisms. Future exploration of the mitoproteomes of photosynthetic eukaryotes with large and highly diversified genomes, for example, dinoflagellates of the alveolates, may set further records in mitoproteome complexity.

Comparisons of the mitoproteomes of photosynthetic and non-photosynthetic eukaryotes reveal an intricate dance of mitoprotein gain, loss and replacement upon the acquisition of the chloroplast. Certain mitoproteins that may be widespread across non-photosynthetic species seem to be lost in photosynthetic lineages as they become redundant to chloroplast functions. For example, heme biosynthesis typically occurs in the mitochondria of non-photosynthetic eukaryotes via the condensation of glycine and succinyl-CoA to produce δ-aminolevulinic acid. In contrast, in photosynthetic eukaryotes δ-aminolevulinic acid is typically synthesised in the chloroplast via glutamyl-tRNA, although exceptions are known.[94]

Other endogenous mitoproteins may be replaced by equivalents of chloroplast origin. This has frequently occurred in the case of organellar aminoacyl-tRNA synthetases, which in photosynthetic organisms are typically represented as single-copy enzymes that are dual-targeted to the chloroplast and mitochondria, and which may derive from either the mitochondrial or chloroplast ancestor[95–97] (Figure 3). The outsourcing of chloroplast metabolic pathways may particularly contribute to the bloated *E. gracilis* mitoproteome, which includes sulphate assimilation and cysteine synthesis pathways of plastid origin,[98] as well as at least 185 proteins that are co-enriched from the *E. gracilis* plastid proteome,[99] suggesting their dual-localisation.

Organelle-organelle crosstalk shape mitoproteome complexity

The metabolic and physiological interactions between chloroplasts and mitochondria may further necessitate elaborations of mitochondrial metabolism in plastid-containing eukaryotes. Typically, mitochondria form close physical associations with chloroplasts,[100,101] even in the case of non-photosynthetic plastids, for example, in apicomplexans.[102] In photosynthetic species, mitochondria perform elaborate metabolic pathways not known in non-photosynthetic relatives that are directly essential in supporting photosynthetic activity, particularly the recycling of photosynthesis waste products. Key examples include photo-respiration, in which 2-phospho-glycolate

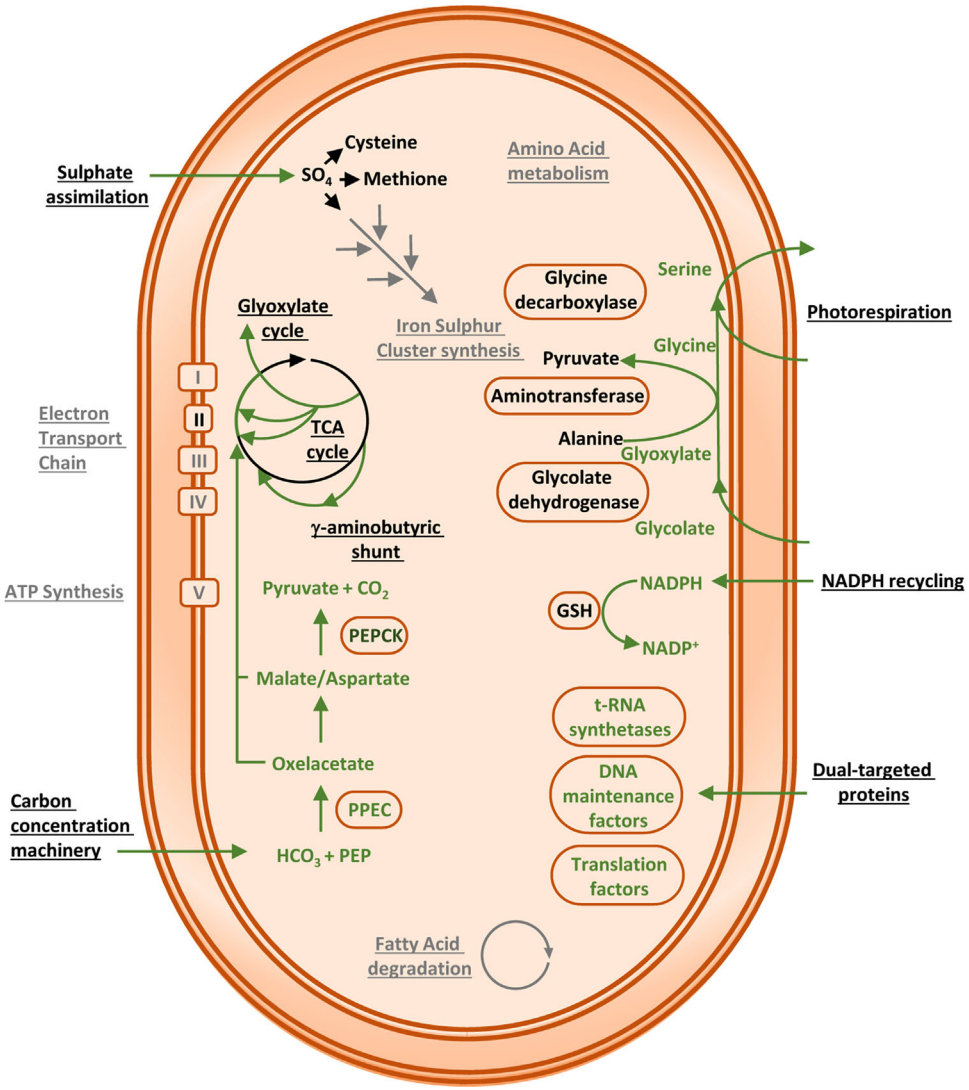

FIGURE 3 Schematics of pathways and proteins found in the mitochondria of various photosynthetic organisms. Metabolites, proteins and pathways of chloroplast origin indicated in green. Core mitochondrial pathways indirectly affected by chloroplast influence indicated in grey

generated through the wasteful oxidase activity of RuBisCo is recycled through mitochondrial glycine and serine intermediates[103,104] (Figure 3), as well as the presence of the γ-aminobutyric shunt in the TCA cycle of the *E. gracilis* mitochondrion[53] (Figure 3), which in other organisms enables metabolic conditions more amenable to photosynthesis.[105,106] An interesting example of chloroplast-mitochondrion dynamics, most extensively described in diatoms but potentially widespread amongst photosynthetic algae, is the dissipation of excess photosynthetic reducing agents (e.g., NADPH) via mitochondrial oxidative respiration [12,107] (Figure 3). The exact mechanisms by which this interaction occurs remain poorly resolved, but likely include amino acid (e.g., aspartate and alanine) shunts[108] (Figure 3), and potentially the diversion of succinyl-CoA into lipid synthesis.[109] This latter interaction depends on the formation of a metabolic intermediate, propionyl-CoA, by methylmalonyl-CoA mutase (which uses vitamin B_{12} as a co-factor), giving rise to an additional auxotrophy in the mitochondria of photosynthetic species.

Mitochondria may even play roles in the efficient generation of primary metabolites in photosynthetic species. This involves the management of storage compounds, or the supplementation of the carbohydrate stores generated via photosynthesis and the glyoxylate cycle, employed within specialised peroxisomes (termed glyoxysomes) of photo-mixotrophic plants. By contrast, *E. gracilis* directly employs a glyoxylate cycle within its mitochondrion for this purpose[53] (Figure 3). Furthermore, mitochondria may have indirect roles in facilitating photosynthetic carbon assimilation, for example, via the presence of mitochondria-targeted carbon concentrating machinery enzymes[110] or C4-type photosynthetic metabolism in some photosynthetic algae,[111] although the exact functions of these enzymes remain to be experimentally confirmed (Figure 3).

Mitochondria are even influenced by chloroplast metabolism even after the loss of photosynthesis, for example, in amino acid recycling and breakdown.[9,109] This mostly under-explored aspect is brought to light through the study of the mitoproteomes of secondarily non-

photosynthetic algae, which may retain plastids that perform essential, but photosynthesis-independent, metabolic pathways. For example, the recently completed genome of the non-photosynthetic stramenopile *Nitzschia putrida* notably encodes complete mitochondria-targeted urea and glyoxylate cycles, and a mitochondrial glutamate shunt, which may be linked to the retention of most of its plastidial amino acid synthesis pathways.[112,113] In a further example, the secondarily non-photosynthetic stramenopile *Paraphysomonas* has retained at least 10 amino-acyl tRNA synthetases of apparent plastidial evolutionary origin, despite the complete loss of plastid DNA from this species. These tRNA-synthetases, which were presumably ancestrally dual-targeted to the *Paraphysomonas* chloroplast and mitochondria, have now been relocalised to uniquely support the mitoproteome.[96]

Multicellularity reveals mitochondrial heterogeneity through mass spectrometry

In terms of major evolutionary developments affecting the eukaryote landscape, the acquisition of further endosymbiotic organelles is joined by the development of true multicellular lineages. Multicellularity is known to have arisen at least nine times independently in organisms as diverse as animals, plants, fungi, seaweed and slime moulds[114] (Figure 1). Truly multicellular organisms have been distinguished by their protein content, but also through diversification of cell types (i.e., into different tissues and the partitioning of germline and somatic cells). Much is known about the mitoproteome content of model multicellular plants (*A. thaliana*) and animals (*Homo sapiens*, *Mus musculus*), for which multiple proteomic surveys have been conducted.[51,54]

Mass spectrometry studies have identified 1005 mitoproteins in *A. thaliana* [51], and 1204 and 1330 mitoproteins in mouse and human cells, respectively[54,55] (Figure 2). In silico predictions for animals estimate approximately 1500 mitoproteins to be present in animals[54] against 3000 for certain plants.[69] It remains to be determined which of these plant mitochondrial innovations differ from those of unicellular photosynthetic algae,[115], that is, those which may be connected specifically to multicellularity, alongside general photosynthesis-associated mitoproteome innovations, such as the presence of glycine decarboxylase and thioredoxins involved in photorespiration.[116]

Since their inception, mass spectrometry studies have validated that mitochondria of multicellular organisms vary dramatically depending on tissue type in plants[117,118] and in animals.[73,119] Mitochondria can even vary within the same cell type, with striated muscles in mammals exhibiting two distinct forms of the organelle, characterised by differences in structure, function and proteome,[120,121] similar to the heteroplastidy observed in certain plants, whose proteomically distinct chloroplasts enable C4 photosynthesis to be performed in a single cell.[122]

Tissue-specific mitoproteomes may arise via multiple mechanisms. For example, some mitoproteins (e.g., glutaminase in humans) undergo alternative splicing, with different splicing variants accumulating in different cells.[123] Some of these alternative splicing variants may show tissue-specific localisation (e.g., glutamine synthetase, which is uniquely targeted to mitochondria in liver tissue).[124] Certain mitoproteins, such as cytochrome *c* in many animals, may be encoded by multiple paralogous genes, with specific isoforms expressed in certain tissues only.[125,126] Most dramatically, entire mitochondrial metabolic functions may be confined to a given tissue, as is the case of tobacco root cells, which are capable of reducing nitric oxide, a trait absent from leaf cell mitochondria,[127] or the first steps of the urea cycle occurring only in liver and kidney mitochondria.[72] An important part of this specialisation may be determined by different isoforms of complexes involved in mitochondrial protein import, as 'gatekeepers' of the entire mitoproteome. This can be achieved via differential accumulation of common mitochondrial protein import subunits in different tissues over entire developmental cycles (as observed, e.g., in *A. thaliana*[128]). Alternatively, expression of tissue-specific isoforms of core mitochondrial import complex subunits, or tissue-specific protein import motifs, as has been observed with plant chloroplasts,[129,130] may also play roles.

Recent investigations have illuminated which tissue types, generally, show the greatest contrast in mitoproteome contents. MitoCarta, the most comprehensive organelle proteomics study at a tissue-specific level, surveyed the mitochondria of 14 tissues and organs, categorising 1158 proteins across both human and mouse.[72] Furthermore, a Mito-tag screen in mouse showed significant enrichment of 511 proteins in hepatocytes,[55] while additional Mito-tag lines allowed generation of separate mitoproteomes of Purkinje cells, astrocytes and granule cells, with 5-fold enrichment of 18, 114 and 51 mitoproteins, respectively.[131]

Reanalysing these mitoproteomes beyond enrichment, we can also highlight proteins absent from each tissue. While it is expected that some low abundance peptides may manifest as 'absent' proteins, and will eventually be detected in future investigations, it remains notable that at present, fewer than one third of the mouse MitoCarta proteins (380/1158) were recorded in all 14 tissues, with a variety of proteins unique to, or uniquely absent from, each tissue[72] (Figure 4A). These include 20 mitoproteins confined to testes, pointing to specific mitochondrial requirements in male gametes such as increased energy generation, and nearly 30 uniquely absent from the heart compared to all other tissue types (Figure 4A).

Such tissue-specific trends are additionally observed at a cellular level. Mito-tagged cerebral tissue reveals 41, 56 and 7 Mito–Carta verified proteins that were absent in Purkinje, granule and astrocyte cells, respectively[131] (Figure 4B). Mito-tag-based analysis of mouse hepatocytes additionally shows 177 proteins recovered solely from their mitochondria, while 58 'typical' mitoproteins were absent[55] (Figure 4C). It remains to be determined which of them are amongst the key drivers in the differentiation of specific mouse tissues.

One of the manifest advantages arising from multicellularity has been the specialisation of cell types to best suit their designated function. The corollary observation is that such an important organelle as the mitochondrion has to follow a similar trajectory in multicellular organisms. This is due to the fact that the key mitochondrial advantages, such as efficient ATP generation and pathway redistribution, as

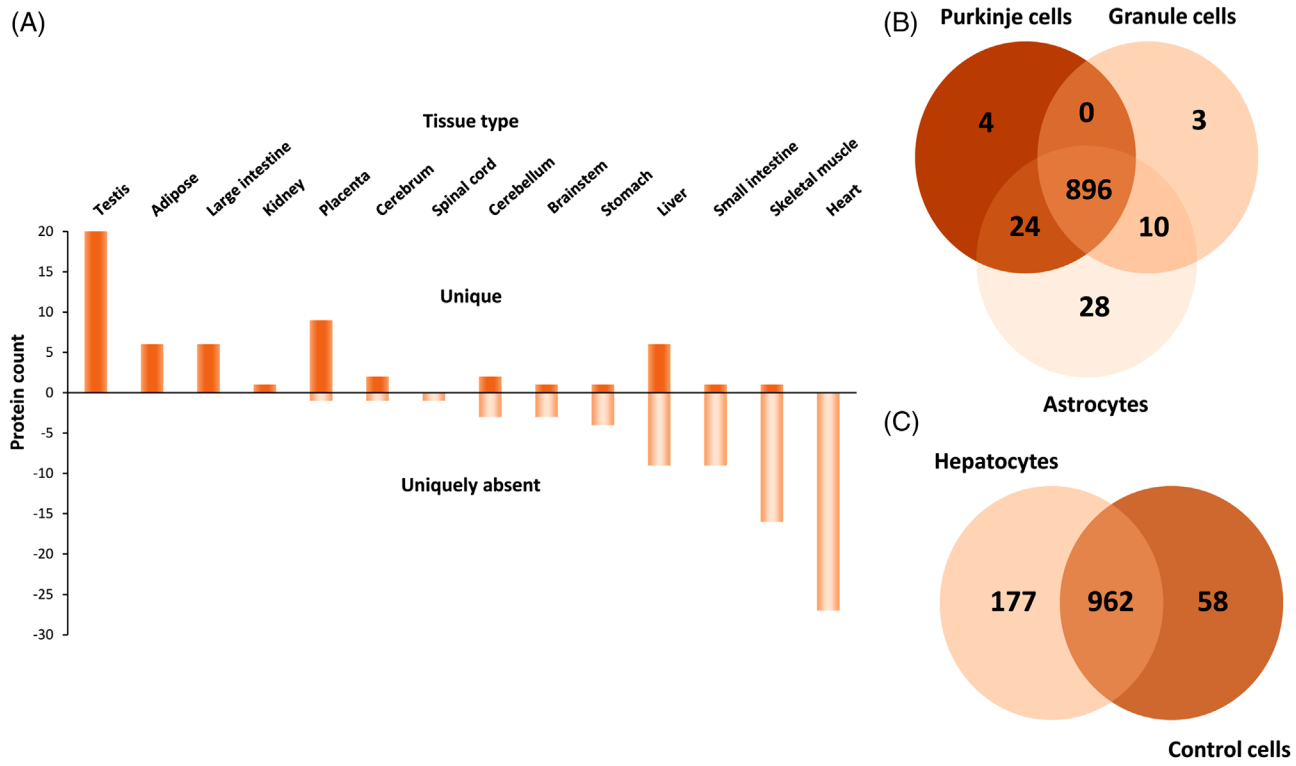

FIGURE 4 Mitochondrial protein datasets generated from MitoCarta2.0,[72] showing number of proteins unique to one tissue or uniquely absent (A). Comparison of mitochondrial proteins in Mito-tagged mouse cerebral tissues[131] (B) and in mouse hepatocytes versus controls[55] (C)

well as sequestration of toxic metabolic by-products, are hard-wired in eukaryotic cells. In the case of multicellular model organisms, we indeed observe the specialisation of mitochondria through a reduction of protein complexity. Proteomic variance has equally been observed in the plastids of different plant tissues.[132] We thus expect this trend to be observed beyond key model organisms, in the full multitude of multicellular lineages. As is so often the case, and again as illustrated above, the tissue diversity of mitoproteomes has been much more extensively studied in animals than in plants. However, an interesting overview of the variation in plant mitochondrial composition observed for different tissues, especially in root mitochondria challenged by adverse soil conditions can be found in Ref.[133]

Mitochondrial modifications and evolutionary transitions

While the mitochondrion's most important evolutionary contribution might have been to enable the development of the LECA, our survey stresses subsequent, somewhat neglected evolutionary developments (at least from a mitochondrial perspective). Indeed, plastid acquisitions (whether following primary or secondary endosymbiosis) on the one hand, and the transition to multicellularity on the other, have been supported by further organelle remodelling and protein redistribution.

In protists, mitochondria appear to have responded to several dynamic factors. Certain environmental niches and survival strategies strongly shift resources, enabling some organisms to simplify their mitochondria, or to even lose them entirely, as seen in some anaerobic protists.[134] However, the reasons for the apparent loss of complexity in mitochondria of multicellular eukaryotes (Figure 2) remain to be determined. If the LECA mitochondrion was indeed complex, as seems highly likely, one might speculate that the mitoproteome 'splits up' across different tissues. Equally, a certain level of mitochondrial functionality is presumably necessary for the evolution of multicellularity, such as aerobic metabolism to allow sufficient ATP production to support complex tissues, although its long-term retention in multicellular eukaryotes turns out to be non-universal.[135] Moreover, mitochondria of multicellular organisms presumably should have developed more functions in signalling and stress responses than their unicellular predecessors. It remains to be determined what kind of mitochondrial incarnation, with regard to protein complexity, capacity for ATP production and signalling, is best suited to make the jump towards tissue-specific mitochondrial function in multicellular species.

Finally, we note that similar 'power sharing' relationships as documented between mitochondria and chloroplast proteomes have also occurred with other cellular organelles, including ones probably present in the LECA. For example, the mitochondrial fatty acid degradation pathway (β-oxidation) has been relocated to peroxisomes either partially (e.g., only the breakdown of very long-chain fatty acids in mammals) or completely, as in many fungal species and in plants (in their guise of glyoxysomes).[136,137] This might be related to overall radical oxygen species (ROS) control.[138] Further dynamic connections link the mitochondria to the nucleus (e.g., iron-sulphur cluster biosynthesis and ROS signalling) and endomembrane system (e.g.,

glycoprotein import and fatty acid synthesis) in eukaryotic lineages. Probing the diversity of interactions between mitochondria and other organelles across the eukaryote tree may give insights as to how different eco-physiological constraints impact their co-evolution and provide new levels of understanding regarding the synthetic potential of organelle proteomes working in partnership.

CONCLUSIONS

In particular, the further investigation of protists holds promise to better understand the evolutionary dynamics discussed in this review. The relative ease of isolating mitochondria from *E. gracilis* recommends algal protists as a potentially superior alternative for the study of photosynthetic trends within mitochondria, contrasting with the traditionally much less tractable plant mitochondria (Figure 2). The advent of next generation proteomics tools such as LOPIT holds the potential to reliably separate dual-targeted proteins from mere contaminants. Genome-wide localisation studies through tagging, while unwieldy and expensive, are suited to investigations of unicellular organisms to identify low abundance proteins currently not detected via mass spectrometry. While tissue-specific investigations serve to catalogue multicellular specialisations, accounting for life cycle-specific mitochondrial changes is underexplored amongst unicellular organisms.[49] Indeed, determination of mitoproteomes in different protist life stages will allow us to functionally characterise mitoproteins that undergo changes. Finally, a broader resolution of the eukaryote domain will serve to highlight features likely possessed by the LECA and provide a backdrop to better understand the mitochondrial specialisations coinciding with multicellularity. We hope that the data and questions presented in this study will encourage future endeavours within this broad space of the eukaryote domain, representing, by some accounts, the large majority of its diversity.[139] This will deepen understanding of this absolutely crucial organelle and its evolutionary history, as influenced by cell differentiation according to body plan and organellar crosstalk.

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AUTHORS' CONTRIBUTIONS

Julius Lukeš, Dave Speijer and Michael Hammond conceived the manuscript. All authors provided ideas and topics of interest. Michael Hammond and Richard G. Dorrell performed the research and wrote the manuscript. Michael Hammond generated figures. Julius Lukeš and Dave Speijer edited the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests in writing this manuscript.

REFERENCES

1. Speijer, D. (2015). Birth of the eukaryotes by a set of reactive innovations: New insights force us to relinquish gradual models. *Bioessays*, *37*(12), 1268–1276.
2. Roger, A. J., Muñoz-Gómez, S. A., & Kamikawa, R. (2017). The origin and diversification of mitochondria. *Current Biology*, *27*(21), R1177-R1192.
3. Martin, W. (2017). Symbiogenesis, gradualism, and mitochondrial energy in eukaryote evolution. *Periodicum Biologorum*, *119*(3), 141–158.
4. Delong, J. P., Okie, J. G., Moses, M. E., Sibly, R. M., & Brown, J. H. (2010). Shifts in metabolic scaling, production, and efficiency across major evolutionary transitions of life. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(29), 12941–12945.
5. Okie, J. G. (2013). General models for the spectra of surface area scaling strategies of cells and organisms: Fractality, geometric dissimilitude, and internalization. *American Naturalist*, *181*(3), 421–439.
6. Okie, J. G., Smith, V. H., & Martin-Cereceda, M. (2016). Major evolutionary transitions of life, metabolic scaling and the number and size of mitochondria and chloroplasts. *Proceedings of the Royal Society B-Biological Sciences*, *283*(1831), 20160611.
7. Palenik, B., Grimwood, J., Aerts A., Rouzé, P., Salamov, A., Putnam, N., Dupont, C., Jorgensen, R., Derelle, E., Rombauts, S., Zhou, K., Otillar, R., Merchant, S. S., Podell, S., Gaasterland, T., Napoli, C., Gendler, K., Manuell, A., Tai, V., … & Grigoriev, I. V. (2007). The tiny eukaryote*Ostreococcus*provides genomic insights into the paradox of plankton speciation *Proceedings of the National Academy of Sciences*, *104*(18), 7705–7710.
8. Río Bártulos, C., Rogers, M. B., Williams, T. A., Gentekaki, E., Brinkmann, H., Cerff, R., Liaud, M. F., Hehl, A. B., Yarlett, N. R., Gruber, A., Kroth, P. G., & Van Der Giezen, M. (2018). Mitochondrial glycolysis in a major lineage of eukaryotes. *Genome Biology and Evolution*, *10*(9), 2310–2325.
9. Allen, A. E., Dupont, C. L., Oborník, M., Horák, A., Nunes-Nesi, A., Mccrow, J. P., Zheng, H., Johnson, D. A., Hu, H., Fernie, A. R., & Bowler, C. (2011). Evolution and metabolic significance of the urea cycle in photosynthetic diatoms. *Nature*, *473*(7346), 203–207.
10. Kořený, L., Oborník, M., Horáková, E., Waller, R. F. & Lukeš, J. (2021). The convoluted history of haem biosynthesis. *Biological Reviews*, *97*(1), 141–162.
11. Lill, R. (2020). From the discovery to molecular understanding of cellular iron-sulfur protein biogenesis. *Biological Chemistry*, *401*(6-7), 855–876.
12. Bailleul, B., Berne, N., Murik, O., Petroutsos, D., Prihoda, J., Tanaka, A., Villanova, V., Bligny, R., Flori, S., Falconet, D., Krieger-Liszkay, A., Santabarbara, S., Rappaport, F., Joliot, P., Tirichine, L., Falkowski, P G., Cardol, P., Bowler, C., & Finazzi, G. (2015). Energetic coupling between plastids and mitochondria drives CO2 assimilation in diatoms. *Nature*, *524*(7565), 366–369.
13. Godaux, D., Bailleul, B., Berne, N., & Cardol, P. (2015). Induction of photosynthetic carbon fixation in anoxia relies on hydrogenase activity and proton-gradient regulation-like1-mediated cyclic electron flow in *Chlamydomonas reinhardtii*. *Plant Physiology*, *168*(2), 648–658.
14. Parfrey, L. W., Lahr, D. J. G., Knoll, A. H., & Katz, L. A. (2011). Estimating the timing of early eukaryotic diversification with multigene molecular clocks. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(33), 13624–13629.
15. Park, J. S., Kolisko, M., Heiss, A. A., & Simpson, A. G. B. (2009). Light microscopic observations, ultrastructure, and molecular phylogeny of *Hicanonectes teleskopos* n. g., n. sp., a deep-branching relative of diplomonads. *Journal of Eukaryotic Microbiology*, *56*(4), 373–384.
16. Stairs, C. W., Leger, M. M., & Roger, A. J. (2015). Diversity and origins of anaerobic metabolism in mitochondria and related organelles.

Philosophical Transactions of the Royal Society B-Biological Sciences, *370*(1678), 20140326.
17. Maruyama, S., & Kim, E. (2013). A modern descendant of early green algal phagotrophs. *Current Biology*, *23*(12), 1081–1084.
18. Burkholder, J. M., Glibert, P. M., & Skelton, H. M. (2008). Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. *Harmful Algae*, *8*(1), 77–93.
19. Graupner, N., Jensen, M., Bock, C., Marks, S., Rahmann, S., Beisser, D., & Boenigk, J. (2018). Evolution of heterotrophy in chrysophytes as reflected by comparative transcriptomics. *Fems Microbiology Ecology*, *94*(4), fiy039.
20. Müller, M., Mentel, M., Van Hellemond, J. J., Henze, K., Woehle, C., Gould, S. B., Yu, Re-Y, Van Der Giezen, M., Tielens, A. G. M., & Martin, W. F. (2012). Biochemistry and evolution of anaerobic energy metabolism in eukaryotes. *Microbiology and Molecular Biology Reviews*, *76*(2), 444–495.
21. Karnkowska, A., Vacek, V., Zubáčová, Z., Treitli, S. C., Petrželková, R., Eme, L., Novák, L., Žárský, V., Barlow, L. D., Herman, E. K., Soukal, P., Hroudová, M., Doležal, P., Stairs, C. W., Roger, A. J., Eliáš, M., Dacks, J. B., Vlček, Č., & Hampl, V. (2016). A eukaryote without a mitochondrial organelle. *Current Biology*, *26*(10), 1274–1284.
22. Imachi, H., Nobu, M. K., Nakahara, N., Morono, Y., Ogawara, M., Takaki, Y., Takano, Y., Uematsu, K., Ikuta, T., Ito, M., Matsui, Y., Miyazaki, M., Murata, K., Saito, Y., Sakai, S., Song, C., Tasumi, E., Yamanaka, Y., Yamaguchi, T., … Takai, K. (2020). Isolation of an archaeon at the prokaryote-eukaryote interface. *Nature*, *577*(7791), 519–525.
23. Facchinelli, F., Colleoni, C., Ball, S. G., & Weber, A P. M. (2013). Chlamydia, cyanobiont, or host: Who was on top in the menage a trois? *Trends in Plant Science*, *18*(12), 673–679.
24. Wu, F., Speth, D. R., Philosof, A., Crémière, A., Narayanan, A., Barco, R. A., Connon, S. A., Amend, J. P., Antoshechkin, I. A., & Orphan, V. J. (2022). Unique mobile elements and scalable gene flow at the prokaryote-eukaryote boundary revealed by circularized Asgard archaea genomes. *Nature Microbiology*, *7*, 200–212.
25. Gray, M. W. (2015). Mosaic nature of the mitochondrial proteome: Implications for the origin and evolution of mitochondria. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(33), 10133–10138.
26. Dorrell, R. G., & Smith, A. G. (2011). Do red and green make brown?: Perspectives on plastid acquisitions within chromalveolates, *Eukaryotic Cell*, *10*(7), 856–868.
27. Schön, M E., Zlatogursky, V. V., Singh, R. P., Poirier, C., Wilken, S., Mathur, V., Strassert, J. F. H., Pinhassi, J., Worden, A. Z., Keeling, P. J., Ettema, T. J. G., Wideman, J. G., & Burki, F. (2021). Single cell genomics reveals plastid-lacking Picozoa are close relatives of red algae. *Nature Communications*, *12*, 6651.
28. Carrie, C., & Small, I. (2013). A reevaluation of dual-targeting of proteins to mitochondria and chloroplasts. *Biochimica Et Biophysica Acta-Molecular Cell Research*, *1833*(2), 253–259.
29. Gray, M. W. (2012). Mitochondrial evolution. *Cold Spring Harbor Perspectives in Biology*, *4*(9), a011403–a011403.
30. Flegontov, P., Michálek, J., Janouškovec, J., Lai, De-H, Jirků, M., Hajdušková, E., Tomčala, A., Otto, T. D., Keeling, P. J., Pain, A., Oborník, M., & Lukeš, J. (2015). Divergent mitochondrial respiratory chains in phototrophic relatives of apicomplexan parasites. *Molecular Biology and Evolution*, *32*(5), 1115–1131.
31. Lang, B. F, Burger, G., O'kelly, C J., Cedergren, R., Golding, G. B, Lemieux, C., Sankoff, D., Turmel, M., & Gray, M. W. (1997). An ancestral mitochondrial DNA resembling a eubacterial genome in miniature. *Nature*, *387*(6632), 493–497.
32. Neupert, W., & Herrmann, J M. (2007). Translocation of proteins into mitochondria. *Annual Review of Biochemistry*, *76*, 723–749.
33. Fukasawa, Y., Tsuji, J., Fu, S. C., Tomii, K., Horton, P. & Imai, K. (2015). MitoFates: Improved prediction of mitochondrial targeting sequences and their cleavage sites. *Molecular & Cellular Proteomics*, *14*(4), 1113–1126.
34. Emanuelsson, O., Nielsen, H., Brunak, S., & Von Heijne, G. (2000). Predicting subcellular localization of proteins based on their N-terminal amino acid sequence. *Journal of Molecular Biology*, *300*(4), 1005–1016.
35. Weill, U., Yofe, I., Sass, E., Stynen, B., Davidi, D., Natarajan, J., Ben-Menachem, R., Avihou, Z., Goldman, O., Harpaz, N., Chuartzman, S., Kniazev, K., Knoblach, B., Laborenz, J., Boos, F., Kowarzyk, J., Ben-Dor, S., Zalckvar, E., Herrmann, J. M., … & Schuldiner, M. (2018). Genome-wide SWAp-Tag yeast libraries for proteome exploration. *Nature Methods*, *15*(8), 617–622.
36. Dean, S., Sunter, J. D., & Wheeler, R. J. (2017). TrypTag.org: A trypanosome genome-wide protein localisation resource. *Trends in Parasitology*, *33*(2), 80–82.
37. Almagro Armenteros, J. J., Salvatore, M., Emanuelsson, O., Winther, O., Von Heijne, G., Elofsson, A., & Nielsen, H. (2019). Detecting sequence signals in targeting peptides using deep learning. *Life Science Alliance*, *2*(5), e201900429.
38. Zhang, N., Rao, R. S. P., Salvato, F., Havelund, J. F., Møller, I. M., Thelen, J. J., & Xu, D. (2018). MU-LOC: A machine-learning method for predicting mitochondrially localized proteins in plants. *Frontiers in Plant Science*, *23*(9), 634.
39. Schober, A. F., Rï O Bï Rtulos, C., Bischoff, A., Lepetit, B., Gruber, A., & Kroth, P. G. (2019). Organelle studies and proteome analyses of mitochondria and plastids fractions from the diatom *Thalassiosira pseudonana*. *Plant and Cell Physiology*, *60*(8), 1811–1828.
40. Mulvey, C. M., Breckels, L. M., Geladaki, A., Britovšek, N. K., Nightingale, D. J. H., Christoforou, A., Elzek, M., Deery, M. J., Gatto, L., & Lilley, K. S. (2017). Using hyperLOPIT to perform high-resolution mapping of the spatial proteome. *Nature Protocols*, *12*(6), 1110–1135.
41. Barylyuk, K., Koreny, L., Ke, H., Butterworth, S., Crook, O. M., Lassadi, I., Gupta, V., Tromer, E., Mourier, T., Stevens, T. J., Breckels, L. M., Pain, A., Lilley, K. S., & Waller, R. F. (2020). A subcellular atlas of *Toxoplasma* reveals the functional context of the proteome. *Cell Host and Microbe*, *28*(5), 752–766.e9.
42. Liere, K., Weihe, A., & Börner, T. (2011). The transcription machineries of plant mitochondria and chloroplasts: Composition, function, and regulation. *Journal of Plant Physiology*, *168*(12), 1345–1360.
43. Lewin, H. A., Robinson, G. E., Kress, W. J., Baker, W. J., Coddington, J., Crandall, K. A., Durbin, R., Edwards, S. V., Forest, F., Gilbert, M. T. P., Goldstein, M. M., Grigoriev, I. V., Hackett, K. J., Haussler, D., Jarvis, E. D., Johnson, W. E., Patrinos, A., Richards, S., Castilla-Rubio, J. C., … & Zhang, G. (2018). Earth BioGenome Project: Sequencing life for the future of life. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(17), 4325–4333.
44. Cherry, J. M., Hong, E. L., Amundsen, C., Balakrishnan, R., Binkley, G., Chan, E. T., Christie, K. R., Costanzo, M. C., Dwight, S. S., Engel, S. R., Fisk, D. G., Hirschman, J. E., Hitz, B. C., Karra, K., Krieger, C. J., Miyasato, S. R., Nash, R. S., Park, J., Skrzypek, M. S., … & Wong, E. D. (2012). *Saccharomyces* genome database: The genomics resource of budding yeast. *Nucleic Acids Research*, *40*(D1), D700-D705.
45. Morgenstern, M., Stiller, S. B., Lübbert, P., Peikert, C. D., Dannenmaier, S., Drepper, F., Weill, U., Höß, P., Feuerstein, R., Gebert, M., Bohnert, M., Van Der Laan, M., Schuldiner, M., Schütze, C., Oeljeklaus, S., Pfanner, N., Wiedemann, N., & Warscheid, B. (2017). Definition of a high-confidence mitochondrial proteome at quantitative scale. *Cell Reports*, *19*(13), 2836–2852.
46. Gawryluk, R. M. R., Chisholm, K. A., Pinto, D. M., & Gray, M. W. (2014). Compositional complexity of the mitochondrial proteome of a unicellular eukaryote (*Acanthamoeba castellanii*, supergroup Amoebozoa) rivals that of animals, fungi, and plants. *Journal of Proteomics*, *109*, 400–416.
47. Peikert, C. D., Mani, J., Morgenstern, M., Käser, S., Knapp, B., Wenger, C., Harsman, A., Oeljeklaus, S., Schneider, A., & Warscheid, B. (2017).

Charting organellar importomes by quantitative mass spectrometry. *Nature Communications*, *8*, 15272.

48. Panigrahi, A K., Ogata, Y., Zíková, A., Anupama, A., Dalley, R A., Acestor, N., Myler, P. J., & Stuart, K. D. (2009). A comprehensive analysis of *Trypanosoma brucei* mitochondrial proteome. *Proteomics*, *9*(2), 434–450.
49. Zíková, A., Verner, Z., Nenarokova, A., Michels, P A. M., & Lukeš, J. (2017). A paradigm shift: The mitoproteomes of procyclic and bloodstream *Trypanosoma brucei* are comparably complex. *PLoS pathogens*, *13*(12), e1006679.
50. Cui, J., Liu, J., Li, Y., & Shi, T. (2011). Integrative identification of arabidopsis mitochondrial proteome and its function exploitation through protein interaction network. *PLoS One*, *6*(1), e16022.
51. Rao, R. S. P., Salvato, F., Thal, B., Eubel, H., Thelen, J. J., & Møller, I. M. (2017). The proteome of higher plant mitochondria. *Mitochondrion*, *33*, 22–37.
52. Salvato, F., Havelund, J. F., Chen, M., Rao, R. S P, Rogowska-Wrzesinska, A., Jensen, O. N., Gang, D. R., Thelen, J. J., & Møller, I. M. (2014). The potato tuber mitochondrial proteome. *Plant Physiology*, *164*(2), 637–653.
53. Hammond, M. J., Nenarokova, A., Butenko, A., Zoltner, M., Dobáková, E. L., Field, M. C., & Lukeš, J. (2020). A uniquely complex mitochondrial proteome from *Euglena gracilis*. *Molecular Biology and Evolution*, *37*(8), 2173–2191.
54. Smith, A. C., & Robinson, A. J. (2019). MitoMiner v4.0: An updated database of mitochondrial localization evidence, phenotypes and diseases. *Nucleic Acids Research*, *47*(D1), D1225-D1228.
55. Bayraktar, E. C., Baudrier, L., Özerdem, C., Lewis, C. A., Chan, S. H., Kunchok, T., Abu-Remaileh, M., Cangelosi, A. L., Sabatini, D. M., Birsoy, K., & Chen, W. W. (2019). MITO-Tag Mice enable rapid isolation and multimodal profiling of mitochondria from specific cell types in vivo *Proceedings of the National Academy of Sciences*, *116*(1), 303–312.
56. Kumar, A., Agarwal, S., Heyman, J. A., Matson, S., Heidtman, M., Piccirillo, S., Umansky, L., Drawid, A., Jansen, R., Liu, Y., Cheung, K. H., Mille, r P., Gerstein, M., Roeder, G. S., & Snyder, M. (2002). Subcellular localization of the yeast proteome. *Genes & Development*, *16*(6), 707–719.
57. Sickmann, A., Reinders, J., Wagner, Y., Joppich, C., Zahedi, R., Meyer, H E., Schönfisch, B., Perschil, I., Chacinska, A., Guiard, B., Rehling, P., Pfanner, N., & Meisinger, C. (2003). The proteome of *Saccharomyces cerevisiae* mitochondria. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(23), 13207–13212.
58. Reinders, J., Zahedi, R P., Pfanner, N., Meisinger, C., & Sickmann, A. (2006). The complete yeast mitochondrial proteome: Multidimensional separation techniques for mitochondrial proteomics. *Journal of Proteome Research*, *5*(7), 1543–1554.
59. Smith, D. G. S., Gawryluk, R. M. R., Spencer, D. F., Pearlman, R. E., Siu, K. W. M, & Gray, M. W. (2007). Exploring the mitochondrial proteome of the ciliate protozoon *Tetrahymena thermophila*: Direct analysis by tandem mass spectrometry. *Journal of Molecular Biology*, *374*(3), 837–863.
60. Seidi, A., Muellner-Wong, L. S., Rajendran, E., Tjhin, E. T., Dagley, L. F., Aw, V. Yt, Faou, P., Webb, A. I., Tonkin, C. J., & Van Dooren, G. G. (2018). Elucidating the mitochondrial proteome of *Toxoplasma gondii* reveals the presence of a divergent cytochrome c oxidase. *Elife*, *7*: e38131.
61. Atteia, A., Adrait, A., Brugiere, S., Tardif, M., Van Lis, R., Deusch, O., Dagan, T., Kuhn, L., Gontero, B., Martin, W., Garin, J., Joyard, J., & Rolland, N. (2009). A proteomic survey of *Chlamydomonas reinhardtii* mitochondria sheds new light on the metabolic plasticity of the organelle and on the nature of the alpha-proteobacterial mitochondrial ancestor. *Molecular Biology and Evolution*, *26*(7), 1533–1548.
62. Dubinin, J., Braun, H. P., Schmitz, U., & Colditz, F. (2011). The mitochondrial proteome of the model legume *Medicago truncatula*. *Biochimica Et Biophysica Acta-Proteins and Proteomics*, *1814*(12), 1658–1668.
63. Jacoby, R. P., Millar, A. H, & Taylor, N. L. (2010). Wheat mitochondrial proteomes provide new links between antioxidant defense and plant salinity tolerance. *Journal of Proteome Research*, *9*(12), 6595–6604.
64. Wang, S., Zhang, G., Zhang, Y., Song, Q., Chen, Z., Wang, J., Guo, J., Niu, Na, Wang, J., & Ma, S. (2015), Comparative studies of mitochondrial proteomics reveal an intimate protein network of male sterility in wheat (*Triticum aestivum L.*). *Journal of Experimental Botany*, *66*(20), 6191–6203.
65. Huang, S., Taylor, N. L., Narsai, R., Eubel, H., Whelan, J., & Millar, A. H. (2009). Experimental analysis of the rice mitochondrial proteome, its biogenesis, and heterogeneity. *Plant Physiology*, *149*(2), 719–734.
66. Heazlewood, J. L., Howell, K. A., Whelan, J., & Millar, A. H. (2003). Towards an analysis of the rice mitochondrial proteome. *Plant Physiology*, *132*(1), 230–242.
67. Kruft, V., Eubel, H., JäNsch, L., Werhahn, W., & Braun, H. P. (2001). Proteomic approach to identify novel mitochondrial proteins in Arabidopsis. *Plant Physiology*, *127*(4), 1694–1710.
68. Heazlewood, J. L., Tonti-Filippini, J. S., Gout, A. M., Day, D. A., Whelan, J., & Millar, A. H. (2004). Experimental analysis of the *Arabidopsis* mitochondrial proteome highlights signaling and regulatory components, provides assessment of targeting prediction programs, and indicates plant-specific mitochondrial proteins. *Plant Cell*, *16*(1), 241–256.
69. Lee, C. P., Taylor, N. L., & Millar, A. H. (2013). Recent advances in the composition and heterogeneity of the *Arabidopsis* mitochondrial proteome. *Frontiers in Plant Science*, *4*, 4.
70. Taylor, S. W., Fahy, E., Zhang, B., Glenn, G. M., Warnock, D. E., Wiley, S., Murphy, A. N., Gaucher, S. P., Capaldi, R. A., Gibson, B. W., & Ghosh, S. S. (2003). Characterization of the human heart mitochondrial proteome. *Nature Biotechnology*, *21*(3), 281–286.
71. Pagliarini, D. J., Calvo, S. E., Chang, B., Sheth, S. A., Vafai, S. B., Ong, S-En, Walford, G. A., Sugiana, C., Boneh, A., Chen, W. K., Hill, D. E., Vidal, M., Evans, J. G., Thorburn, D. R., Carr, S. A., & Mootha, V. K. (2008). A mitochondrial protein compendium elucidates complex I disease biology. *Cell*, *134*(1), 112–123.
72. Calvo, S. E., Clauser, K. R., & Mootha, V. K. (2016). MitoCarta2.0: An updated inventory of mammalian mitochondrial proteins. *Nucleic Acids Research*, *44*(D1), D1251-D1257.
73. Mootha, V. K., Bunkenborg, J., Olsen, J. V., Hjerrild, M., Wisniewski, J. R., Stahl, E., Bolouri, M. S., Ray, H. N., Sihag, S., Kamal, M., Patterson, N., Lander, E. S., & Mann, M. (2003). Integrated analysis of protein composition, tissue diversity, and gene regulation in mouse mitochondria. *Cell*, *115*(5), 629–640.
74. Li, J., Cai, T., Wu, P., Cui, Z., Chen, X., Hou, J., Xie, Z., Xue, P., Shi, L., Liu, P., Yates, J. R., & Yang, F. (2009). Proteomic analysis of mitochondria from *Caenorhabditis elegans*. *Proteomics*, *9*(19), 4539–4553.
75. Yin, S., Xue, J., Sun, H., Wen, Bo, Wang, Q., Perkins, G., Zhao, H. W., Ellisman, M. H., Hsiao, Y.-H., Yin, L., Xie, Y., Hou, G., Zi, J., Lin, L., Haddad, G. G., Zhou, D., & Liu, S. (2013). Quantitative evaluation of the mitochondrial proteomes of *Drosophila melanogaster* adapted to extreme oxygen conditions. *Plos One*, *8*(9), e74011.
76. Lotz, C., Lin, A. J., Black, C. M., Zhang, J., Lau, E., Deng, N., Wang, Y., Zong, N. C., Choi, J. H., Xu, T., Liem, D. A., Korge, P., Weiss, J. N., Hermjakob, H., Yates, J R., Apweiler, R., & Ping, P. (2014). Characterization, design, and function of the mitochondrial proteome: From organs to organisms. *Journal of Proteome Research*, *13*(2), 433–446.
77. Alonso, J., Rodriguez, J. M., Baena-López, L. A., & Santarén, J. F. (2005). Characterization of the *Drosophila melanogaster* mitochondrial proteome. *Journal of Proteome Research*, *4*(5), 1636–1645.
78. Schneider, R. E., Brown, M. T., Shiflett, A. M., Dyall, S D., Hayes, R. D., Xie, Y., Loo, J. A., & Johnson, P. J. (2011). The, *Trichomonas vaginalis* hydrogenosome proteome is highly reduced relative to mitochondria,

yet complex compared with mitosomes. *International Journal for Parasitology*, *41*(13-14), 1421–1434.
79. Jedelský, P. L., Doležal, P., Rada, P., Pyrih, J., Šmíd, O., Hrdý, I., Šedinová, M., Marcinčiková, M., Voleman, L., Perry, A. J., Beltrán, N. C., Lithgow, T., & Tachezy, J. (2011). The minimal proteome in the reduced mitochondrion of the parasitic protist *Giardia intestinalis*. *Plos One*, *6*(2), e17285.
80. Mi-Ichi, F., Makiuchi, T., Furukawa, A., Sato, D., & Nozaki, T. (2011). Sulfate activation in mitosomes plays an important role in the proliferation of *Entamoeba histolytica*. *Plos Neglected Tropical Diseases*, *5*(8), e1263.
81. Leger, M. M., Petrů, M., Žárský, V., Eme, L., Vlček, Č., Harding, T., Lang, B. F, Eliáš, M., Doležal, P., & Roger, A. J. (2015). An ancestral bacterial division system is widespread in eukaryotic mitochondria. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(33), 10239–10246.
82. Horváthová, L., Žárský, V., Pánek, T., Derelle, R., Pyrih, J., Motyčková, Alž, Klápšťová, V., Vinopalová, M., Marková, L., Voleman, L., Klimeš, V., Petrů, M., Vaitová, Z., Čepička, I., Hryzáková, K., Harant, K., Gray, M. W., Chami, M., Guilvout, I., … & Doležal, P. (2021). Analysis of diverse eukaryotes suggests the existence of an ancestral mitochondrial apparatus derived from the bacterial type II secretion system. *Nature Communications*, *12*, 2947.
83. Pyrih, J., Pánek, T., Durante, I. M., Rašková, V., Cimrhanzlová, K., Kriegová, E., Tsaousis, A. D., Eliáš, M., & Lukeš, J. (2021). Vestiges of the bacterial signal recognition particle-based protein targeting in mitochondria. *Molecular Biology and Evolution*, *38*, 3170–3187.
84. Fischer, K., Lachner, L. A. M., Olsen, S., Mulisch, M., & Krause, K. (2021). The enigma of interspecific plasmodesmata: Insight from parasitic plants. *Frontiers in Plant Science*, *12*, 641924.
85. Albalat, R., & Cañestro, C. (2016). Evolution by gene loss. *Nature Reviews Genetics*, *17*(7), 379–391.
86. Santos, H. J., Makiuchi, T., & Nozaki, T. (2018). Reinventing an organelle: The reduced mitochondrion in parasitic protists. *Trends in Parasitology*, *34*(12), 1038–1055.
87. Mi-Ichi, F., Yousuf, M. A., Nakada-Tsukui, K., & Nozaki, T. (2009). Mitosomes in *Entamoeba histolytica* contain a sulfate activation pathway. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(51), 21731–21736.
88. Maguire, F., & Richards, T. A. (2014). Organelle Evolution: A Mosaic of 'Mitochondrial' Functions. *Current Biology*, *24*(11), R518-R520.
89. Aphasizheva, I., Alfonzo, J., Carnes, J., Cestari, I., Cruz-Reyes, J., Göringer, H. U., Hajduk, S., Lukeš, J., Madison-Antenucci, S., Maslov, D. A., Mcdermott, S. M., Ochsenreiter, T., Read, L. K., Salavati, R., Schnaufer, A., Schneider, A., Simpson, L., Stuart, K., Yurchenko, V., … & Aphasizhev, R. (2020). Lexis and grammar of mitochondrial RNA processing in trypanosomes. *Trends in Parasitology*, *36*(4), 337–355.
90. Schneider, A. (2020). Evolution of mitochondrial protein import – Lessons from trypanosomes. *Biological Chemistry*, *401*(6-7), 663–676.
91. Tobiasson, V., Gahura, O., Aibara, S., Baradaran, R., Zíková, A., & Amunts, A. (2021). Interconnected assembly factors regulate the biogenesis of mitoribosomal large subunit. *Embo Journal*, *40*(6), e106292.
92. Ramrath, D. J. F., Niemann, M., Leibundgut, M., Bieri, P., Prange, C., Horn, E. K., Leitner, A., Boehringer, D., Schneider, A., & Ban, N. (2018). Evolutionary shift toward protein-based architecture in trypanosomal mitochondrial ribosomes. *Science*, *362*(6413), eaau7735.
93. Archibald, J. M. (2015). Endosymbiosis and eukaryotic cell evolution. *Current Biology*, *25*(19), R911-R921.
94. Kořený, L., Sobotka, R., Janouškovec, J., Keeling, P. J., & Oborník, M. (2011). Tetrapyrrole synthesis of photosynthetic chromerids is likely homologous to the unusual pathway of *Apicomplexan* Parasites. *Plant Cell*, *23*(9), 3454–3462.
95. Sharaf, A., Gruber, A., Jiroutová, K., & Oborník, M. (2019). Characterization of aminoacyl-tRNA synthetases in chromerids. *Genes*, *10*(8), 582.
96. Dorrell, R. G., Azuma, T., Nomura, M., Audren De Kerdrel, G., Paoli, L., Yang, S., Bowler, C., Ishii, K. I., Miyashita, H., Gile, G. H., & Kamikawa, R. (2019). Principles of plastid reductive evolution illuminated by nonphotosynthetic chrysophytes. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(14), 6914–6923.
97. Duchêne, A. M., Giritch, A., Hoffmann, B., Cognat, V., Lancelin, D., Peeters, N. M., Zaepfel, M., Maréchal-Drouard, L., & Small, I. D. (2005). Dual targeting is the rule for organellar aminoacyl-tRNA synthetases in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(45), 16484–16489.
98. Patron, N. J., Durnford, D. G., & Kopriva, S. (2008). Sulfate assimilation in eukaryotes: Fusions, relocations and lateral transfers. *BMC Evolutionary Biology*, *8*, 39.
99. Novák Vanclová, A. M. G., Zoltner, M., Kelly, S., Soukal, P., Záhonová, K., Füssy, Z., Ebenezer, T. E., Lacová Dobáková, E., Eliáš, M., Lukeš, J., Field, M C., & Hampl, V. (2019). Metabolic quirks and the colourful history of the *Euglena gracilis* secondary plastid. *New Phytologist*, *225*(4), 1578–1592.
100. Carraretto, L., Teardo, E., Checchetto, V., Finazzi, G., Uozumi, N., & Szabo, I. (2016). Ion channels in plant bioenergetic organelles, chloroplasts and mitochondria: From molecular identification to function. *Molecular Plant*, *9*(3), 371–395.
101. Dorrell, R. G. & Bowler, C. (2017). Secondary plastids of stramenopiles. *Secondary Endosymbioses*, *84*, 57–103.
102. Hopkins, J., Fowler, R., Krishna, S., Wilson, I., Mitchell, G., & Bannister, L. (1999). The plastid in *Plasmodium falciparum* asexual blood stages: A three-dimensional ultrastructural analysis. *Protist*, *150*(3), 283–295.
103. Kisaki, T., & Tolbert, N. E. (1969). Glycolate and glyoxylate metabolism by isolated peroxisomes of chloroplasts. *Plant Physiology*, *44*(2), 242–250.
104. Niessen, M., Krause, K., Horst, I., Staebler, N., Klaus, S., Gaertner, S., Kebeish, R., Araujo, W. L., Fernie, A. R., & Peterhansel, C. (2012). Two alanine aminotranferases link mitochondrial glycolate oxidation to the major photorespiratory pathway in *Arabidopsis* and rice. *Journal of Experimental Botany*, *63*(7), 2705–2716.
105. Nogales, J., Gudmundsson, S., Knight, E. M., Palsson, B. O., & Thiele, I. (2012). Detailing the optimality of photosynthesis in cyanobacteria through systems biology analysis. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(7), 2678–2683.
106. Che-Othman, M. H, Jacoby, R. P., Millar, A. H, & Taylor, N. L. (2020). Wheat mitochondrial respiration shifts from the tricarboxylic acid cycle to the GABA shunt under salt stress. *New Phytologist*, *225*(3), 1166–1180.
107. Dang, K. V., Plet, J., Tolleter, D., Jokel, M., Cuiné, S., Carrier, P., Auroy, P., Richaud, P., Johnson, X., Alric, J., Allahverdiyeva, Y., & Peltier, G. (2014). Combined increases in mitochondrial cooperation and oxygen photoreduction compensate for deficiency in cyclic electron flow in *Chlamydomonas reinhardtii*. *Plant Cell*, *26*(7), 3036–3050.
108. Broddrick, J. T., Du, N., Smith, S. R., Tsuji, Y., Jallet, D., Ware, M. A., Peers, G., Matsuda, Y., Dupont, C. L., Mitchell, B. G, Palsson, B. O., & Allen, A. E. (2019). Cross-compartment metabolic coupling enables flexible photoprotective mechanisms in the diatom *Phaeodactylum tricornutum*. *New Phytologist*, *222*(3), 1364–1379.
109. Ait-Mohamed, O., Novák Vanclová, A. M. G., Joli, N., Liang, Y., Zhao, X., Genovesio, A., Tirichine, L., Bowler, C., & Dorrell, R. G. (2020). PhaeoNet: A holistic RNAseq-based portrait of transcriptional coordination in the model diatom, *Phaeodactylum tricornutum*. *Frontiers in Plant Science*, *11*(1522), 590949.
110. Jensen, E. L., Maberly, S. C., & Gontero, B. (2020). Insights on the functions and ecophysiological relevance of the diverse carbonic anhy-

drases in microalgae. *International Journal of Molecular Sciences*, *21*(8), 2922.
111. Tanaka, R., Kikutani, S., Mahardika, A., & Matsuda, Y. (2014). Localization of enzymes relating to C-4 organic acid metabolisms in the marine diatom, *Thalassiosira pseudonana*. *Photosynthesis Research*, *121*(2-3), 251–263.
112. Kamikawa, R., Moog, D., Zauner, S., Tanifuji, G., Ishida, K.-I., Miyashita, H., Mayama, S., Hashimoto, T., Maier, U. G., Archibald, J. M., & Inagaki, Y. (2017). A Non-photosynthetic diatom reveals early steps of reductive evolution in plastids. *Molecular Biology and Evolution*, *34*(9), 2355–2366.
113. Kamikawa, R., Mochizuki, T., Sakamoto, M., Tanizawa, Y., Nakayama, T., Onuma, R., Cenci, U., Moog, D., Speak, S., Sarkozi, K., Toseland, A., van Oosterhaut, C., Oyama, K., Kato, M., Kume, K., Kayama, M., Azuma, T., Ishii, K., Miyashita, H., Henrissat, B., Lombard, V., Win, J., Kamoun, S., Kashiyama, Y., Mayama, S., Miyagishima, S., Tanifuji, G., Mook, T., & Nakamura, Y. (2021). Genome evolution of a non-parasitic secondary heterotroph, the diatom *Nitzschia putrida*. *bioRxiv*, 2021.01.24.427197.
114. Brown, M. W., Kolisko, M., Silberman, J. D., & Roger, A . J. (2012). Aggregative multicellularity evolved independently in the eukaryotic supergroup *Rhizaria*. *Current Biology*, *22*(12), 1123–1127.
115. Millar, A. H., Heazlewood, J. L., Kristensen, B. K., Braun, H. P., & Møller, I. M. (2005). The plant mitochondrial proteome. *Trends in Plant Science*, *10*(1), 36–43.
116. Timm, S., & Hagemann, M. (2020). Photorespiration-how is it regulated and how does it regulate overall plant metabolism? *Journal of Experimental Botany*, *71*(14), 3955–3965.
117. Bardel, J., Louwagie, M., Jaquinod, M., Jourdain, A., Luche, S., Rabilloud, T. Macherel, D., Garin, J., & Bourguignon, J. (2002). A survey of the plant mitochondrial proteome in relation to development. *Proteomics*, *2*(7), 880–898.
118. Lee, C. P., Eubel, H., Solheim, C., & Millar, A. H (2012). Mitochondrial proteome heterogeneity between tissues from the vegetative and reproductive stages of *Arabidopsis thaliana* development. *Journal of Proteome Research*, *11*(6), 3326–3343.
119. Johnson, D. T, Harris, R. A., French, S., Blair, P. V., You, J., Bemis, K. G., Wang, Mu, & Balaban, R. S. (2007). Tissue heterogeneity of the mammalian mitochondrial proteome. *American Journal of Physiology-Cell Physiology*, *292*(2), C689-C697.
120. Ferreira, R., Vitorino, R., Alves, R. M. P., Appell, H. J., Powers, S. K., Duarte, J. A., & Amado, F. (2010). Subsarcolemmal and intermyofibrillar mitochondria proteome differences disclose functional specializations in skeletal muscle. *Proteomics*, *10*(17), 3142–3154.
121. Baseler, W. A., Dabkowski, E. R., Williamson, C. L., Croston, T. L., Thapa, D., Powell, M. J., Razunguzwa, T. T., & Hollander, J. M. (2011). Proteomic alterations of distinct mitochondrial subpopulations in the type 1 diabetic heart: Contribution of protein import dysfunction. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology*, *300*(2), R186-R200.
122. Offermann, S., Friso, G., Doroshenk, K. A., Sun, Qi, Sharpe, R. M., Okita, T. W., Wimmer, D., Edwards, G. E., & Van Wijk, K. J. (2015). Developmental and subcellular organization of single-cell C-4 photosynthesis in *Bienertia sinuspersici* determined by large-scale proteomics and cDNA assembly from 454 DNA sequencing. *Journal of Proteome Research*, *14*(5), 2090–2108.
123. Elgadi, K. M., Meguid, R. A., Qian, M., Souba, W. W., & Abcouwer, S. F. (1999). Cloning and analysis of unique human glutaminase isoforms generated by tissue-specific alternative splicing. *Physiological Genomics*, *1*(2), 51–62.
124. Matthews, G D., Gould, R M., & Vardimon, L. (2005). A single glutamine synthetase gene produces tissue-specific subcellular localization by alternative splicing. *Febs Letters*, *579*(25), 5527–5534.
125. Jelic, M., Soll, J., & Schleiff, E. (2003). Two Toc34 homologues with different properties. *Biochemistry*, *42*(19), 5906–5916.
126. Andrès, C., Agne, B., & Kessler, F. (2010). The TOC complex: Preprotein gateway to the chloroplast. *Biochimica Et Biophysica Acta-Molecular Cell Research*, *1803*(6), 715–723.
127. Gupta, K. J., Stoimenova, M., & Kaiser, W M. (2005). In higher plants, only root mitochondria, but not leaf mitochondria reduce nitrite to NO, in vitro and in situ. *Journal of Experimental Botany*, *56*(420), 2601–2609.
128. Lister, R., Chew, O., Lee, M. N., Heazlewood, J. L., Clifton, R., Parker, K L., Millar, A. H, & Whelan, J. (2004). A transcriptomic and proteomic characterization of the *Arabidopsis* mitochondrial protein import apparatus and its response to mitochondrial dysfunction. *Plant Physiology*, *134*(2), 777–789.
129. Yu, T. S., & Li, H. M. (2001). Chloroplast protein translocon, components atToc159 and atToc33 are not essential for chloroplast biogenesis in guard cells and root cells. *Plant Physiology*, *127*(1), 90–96.
130. Chu, C. C., Swamy, K., & Li, H. M. (2020). Tissue-Specific Regulation of Plastid Protein Import via Transit-Peptide Motifs. *Plant Cell*, *32*(4), 1204–1217.
131. Fecher, C., Trovò, L., Müller, S. A., Snaidero, N., Wettmarshausen, J., Heink, S., Ortiz, O., Wagner, I., Kühn, R., Hartmann, J., Karl, R. M., Konnerth, A., Korn, T., Wurst, W., Merkler, D., Lichtenthaler, S. F., Perocchi, F., & Misgeld, T. (2019). Cell-type-specific profiling of brain mitochondria reveals functional and molecular diversity. *Nature Neuroscience*, *22*(10), 1731–1742.
132. Yan, X., Khan, S., Hase, T., Emes, M J., & Bowsher, C G. (2006). Differential uptake of photosynthetic and non-photosynthetic proteins by pea root plastids. *Febs Letters*, *580*(27), 6509–6512.
133. Jacoby, R. P., Li, L., Huang, S., Lee, C. P., Millar, A. H., & Taylor, N. L. (2012). Mitochondrial Composition, Function and Stress Response in Plants. *Journal of Integrative Plant Biology*, *54*(11), 887–906.
134. Hampl, V., Čepička, I., & Eliáš, M. (2019). Was the mitochondrion necessary to start eukaryogenesis? *Trends in Microbiology*, *27*(2), 96–104.
135. Yahalomi, D., Atkinson, S D., Neuhof, M., Chang, E. S, Philippe, H., Cartwright, P., Bartholomew, J. L., & Huchon, D. (2020). A cnidarian parasite of salmon (Myxozoa: *Henneguya*) lacks a mitochondrial genome. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(10), 5358–5363.
136. Graham, I. A. (2008). Seed storage oil mobilization. *Annual Review of Plant Biology*, *59*, 115–142.
137. Speijer, D. (2017). Evolution of peroxisomes illustrates symbiogenesis. *Bioessays*, *39*(9), 1700050.
138. Speijer, D. (2011). Oxygen radicals shaping evolution: Why fatty acid catabolism leads to peroxisomes while neurons do without it. *Bioessays*, *33*(2), 88–94.
139. Pawlowski, J., Audic, S., Adl, S., Bass, D., Belbahri, L., Berney, C., Bowser, S. S., Cepicka, I., Decelle, J., Dunthorn, M., Fiore-Donno, A. M., Gile, G. H., Holzmann, M., Jahn, R., Jirků, M., Keeling, P J., Kostka, M., Kudryavtsev, A., Lara, E., … & De Vargas, C. (2012). CBOL Protist Working Group: Barcoding eukaryotic richness beyond the animal, plant, and fungal kingdoms. *Plos Biology 10*(11), e1001419.

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