A century of mitochondrial research, 1922–2022

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Abstract

Although recognized earlier as subcellular entities by microscopists, mitochondria have been the subject of functional studies since 1922, when their biochemical similarities with bacteria were first noted. In this overview I trace the history of research on mitochondria from that time up to the present day, focussing on the major milestones of the overlapping eras of mitochondrial biochemistry, genetics, pathology and cell biology, and its explosion into new areas in the past 25 years. Nowadays, mitochondria are considered to be fully integrated into cell physiology, rather than serving specific functions in isolation.

1. Foreword

Mitochondria were first recognized by microscopy as subcellular entities in the 19th century. However, it was only in 1922 that Wallin published a series of papers, e.g. [1], revealing that the chemical signatures of mitochondria and of aerobic bacteria were similar, from which he proposed the idea that mitochondria arose, in the course of evolution, by invasion of an ancient cell by organisms akin to modern-day bacteria. From this developed the theory of endosymbiosis, that such an invasion or engulfment led to a mutually beneficial collaboration within a single cell

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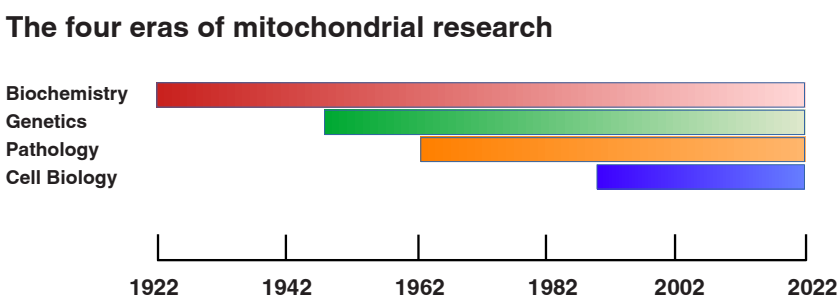

Fig. 1 The eras of mitochondrial research. Mitochondrial biochemistry began in the 1920s and continues until the present day, albeit at a lower intensity. Similarly, mitochondrial genetics extended from the late 1940s onwards. Mitochondrial pathology has its origins in the early 1960s and remains intensively investigated, whilst the explosion of interest in mitochondrial cell biology occurred in the 1990s and is ongoing.

that later evolved into a multicellular organism. In this article, I shall trace how we progressed from this ingenious concept, nowadays supported by a large body of data, to our present-day understanding of mitochondria and the physiological functions they perform. Importantly, space constraints prevent me from going into any depth in respect of any of the historical milestones I shall discuss. It is also not practical to cite every one of the thousands of published works to make up this catalogue of knowledge. Instead I cite only a handful of key references that underlie, illustrate or summarize the main advances, focusing, in the interests of open scholarship, on those that are freely available. In so doing, I apologize to all those works remain uncited, including many collaborators and friends. This article can be but a short overview of a nowadays enormous field, which can nevertheless be of value to those just entering it.

The past century of mitochondrial research can be considered to comprise four overlapping eras, those of mitochondrial biochemistry, genetics, pathology and cell biology (Fig. 1), which I shall now consider in that order, after reviewing Wallin's ideas of cellular evolution and where they have led us to.

2. Endosymbiosis

Wallin's work, and hence his ideas on cellular symbiosis as the origin of eukaryotes, were largely ignored for almost a half-century [2]. This was partly because they appeared to contradict tenets of Darwinian evolution, and partly because some of the experimental work on which they were

based was dubious, notably the claim of being able to grow mitochondria in vitro. However, his main presentation of the evidence for a bacterial affinity or origin of mitochondria seems sound. Not only do mitochondria resemble bacteria morphologically, they also multiply in cells by growth and division. Most importantly, some vital dyes for mitochondria, notably Janus Green B, understood later to be specific for the enzyme cytochrome *c* oxidase [3], also stained most aerobic bacteria, including those that were endosymbionts in the root tissue of leguminous plants [4].

Despite misgivings over his experimental work, Wallin's thesis began to be seriously reconsidered in the 1960s, most famously by Lynn Margulis (Sagan), who developed the concept into the wider idea that the eukaryotic cell was a mosaic of organelles derived from different bacterial ancestors [5,6]. Once again, the hypothesis was received with some skepticism, in particular as regards the origins of the cell nucleus and of the cytoskeleton, whilst the evidence in favour of an endosymbiotic origin for mitochondria and for plant chloroplasts gradually came to be accepted. Eventually, with the characterization of organelle DNAs in the 1980s by Mike Gray and others, the theory came to be more or less universally accepted, based on the fact that the ribosomal RNA genes of both mitochondrial and chloroplast DNAs were clearly much more closely related to those of bacteria than to their counterparts encoded by eukaryotic nuclear genomes [7]. In fact, DNA sequence analysis allowed the placement of organelles within specific bacterial taxa: chloroplast DNA with cyanobacteria, and mitochondrial DNA with alpha-proteobacteria, both with a monophyletic origin [8]. In a sense, I am already getting ahead of myself, since mitochondrial DNA was unknown in 1922, and its discovery will be covered in a later section.

3. The era of mitochondrial biochemistry

The discovery of the key enzymes of intermediary metabolism is the foundation stone of mitochondrial biochemistry, dating back to the 1920 and 1930s. This was also the heyday of the study of enzymes in general and, thus, it is fitting in a volume such as this to devote slightly more space to it. One name stands out from the crowd, that of Hans Krebs, whose findings allowed him to propose the cycle of enzymatic interconversions of metabolites [9] that bears his name, and which is still considered valid, even if a few 'bells and whistles' have been added on since it was first elaborated [10].

Krebs applied rather laborious chemical and enzymatic assays of metabolites to determine the precise reactions that were carried out in various animal tissues. In the first paper in his series [9], mitochondria were not even mentioned, and it was only a decade later that the mitochondrial localization of the Krebs cycle enzymes was firmly demonstrated by Lehninger and colleagues [10]. This latter work also demonstrated that the oxidation reactions carried out by mitochondria were coupled to the incorporation of radio-labelled inorganic phosphate into various types of macromolecules [11], a process which has been termed 'oxidative phosphorylation' (OXPHOS) ever since, although the specific link to the production of ATP was only proven later [12]. Nowadays, the term is applied to the entire pathway of mitochondrial respiration and ATP synthesis.

OXPHOS involves the reoxidation of the primary electron acceptors used by the cell in glycolysis and in the reactions of the TCA cycle. This is accomplished in a stepwise fashion in mitochondria, with electron transfer to carriers of decreasingly negative redox potential that constitute the respiratory (or electron-transport) chain. These reactions are catalyzed by large enzyme complexes embedded in the inner mitochondrial membrane, each containing many different polypeptide subunits (almost 100 in total). Their counterparts in aerobic bacteria are simpler, and there is a continuing debate as to what biochemical or physiological roles are performed by the so-called supernumerary subunits found in the mitochondrial versions of the OXPHOS enzyme complexes. The stepwise passage of electrons to oxygen involves three of these complexes, confusingly named as complexes I, III and IV – complex II (cII) being a component of the Krebs cycle (succinate dehydrogenase, more precisely termed as succinate:ubiquinone oxidoreductase) which serves as a side-branch of the main respiratory chain, feeding electrons to complex III via ubiquinol, alongside some other flavin-linked dehydrogenases. The elucidation of the structure and enzymatic mechanisms of this complex array was the result of the work of many laboratories, involving their purification and reconstitution, the determination of their subunit composition, the characterization of the genes that encode their subunits, their enzymatic mechanisms and eventually their atomic structures. This endeavour began in the 1950s [13,14] and extended into the first decades of the present century. There remains debate about the status of some remaining subunits which are loosely bound, sub-stoichiometric or taxon-/tissue-specific, and about the overall organization of the complexes in the respiratory membrane.

Complex I (cI), NADH:ubiquinone oxidoreductase, comprises over 40 different subunits in mammals, though rather fewer in filamentous fungi. In budding yeast (*Saccharomyces cerevisiae*) it is completely absent, being replaced by members of the Ndh2 family that are composed of a single type of polypeptide, and catalyze a similar chemistry (reduction of ubiquinone by NADH) but are non energy-conserving. After the purification of cI was refined in the 1970s [15], its structure at ~4 Å resolution was finally elucidated as recently as 2016 [16,17]. It is one of the largest and most complex multisubunit assemblies in biology, being approximately 1 MDa in molecular weight, i.e. comparable with the large subunit of the bacterial ribosome.

Complex III (cIII), ubiquinol:cytochrome *c* reductase, is the common entry point to the 'trunk section' of the respiratory chain, receiving electrons from cI, cII and other dehydrogenases that reduce ubiquinone. Structurally it is a simpler enzyme than cI, in mammals comprising only 11 subunits [18], although it catalyzes an equally or arguably even more intricate reaction, originally proposed by Peter Mitchell [19], known as the Q cycle. The complex was successfully purified and characterized in the 1960s and 1970s [20–22], but it was crystallized only in the 1980s [23] and its atomic-level structure was elucidated only in the 1990s [18].

The structure of mammalian mitochondrial respiratory complex IV (cIV, cytochrome [*c*] oxidase), now recognized to consist of 14 different polypeptides [24], was solved at < 3 Å resolution in the late 1990s [25], although how the 14th subunit was incorporated was only recently revealed [26].

There are now known to be dozens of other important metabolic enzymes in mitochondria for which there is no space to mention individually, including pathways shared with other organelles, for example, fatty acid oxidation (with peroxisomes), the urea cycle (with the cytosol), steroid and phospholipid synthesis (with the ER); plus the elaborate machineries of metabolite and protein import. However, a fifth enzymatic complex embedded in the inner mitochondrial membrane, ATP synthase or complex V (cV), is a vital part of the OXPHOS system.

Although the purification of the respiratory chain and OXPHOS complexes in their active state was considered a huge achievement of 20th century biochemistry, it has come to be regarded as a case of over-reductionism (redox pun not intended). This is because Hermann Schägger and colleagues, around the year 2000, applying gentle electrophoretic techniques, showed that the respiratory chain complexes could be recovered in their

native state in the form of 'supercomplexes', also called respirasomes, in which cI, cIII and cIV are physically associated in a variety of stoichiometries [27,28]. For a long time the existence of supercomplexes was controversial, and their biochemical and physiological significance are still debated [29–31]. Their molecular architecture remains a subject of ongoing studies, but their formation and/or reorganization appears to depend on specific assembly factors [28,29], as well as variant subunits that may be expressed tissue-specifically or subject to metabolic or developmental regulation.

A major advance came with Mitchell's chemiosmotic hypothesis [32], an ingenious concept that filled in the 'missing link' between the redox reactions of the respiratory chain and the recovery of the released energy in the form of ATP. The basic idea, now universally accepted despite the many controversies surrounding it in the early years [33], is that the release of free energy from the oxidation steps of the respiratory chain is directly coupled to the pumping of protons across the inner mitochondrial membrane, setting up a proton gradient against which this pumping action operates. The free energy thus stored is recaptured by the return of protons to the mitochondrial matrix through cV, where it drives the phosphorylation of ADP by inorganic phosphate. The proton gradient can also energize other transport processes directly. The molecular mechanism of the ATP synthase was elucidated in the 1990s by the determination of its structure, an effort led by John Walker [34]. This showed that cV was essentially built from two components: one that was essentially static in the inner mitochondrial membrane, and one that was free to rotate. The rotary movement is driven by the proton gradient and involves a series of conformational changes that energize the formation and release of ATP. The principle is very similar to that of an electric motor or dynamo and, like them, can operate also in reverse, creating a proton gradient and thus a membrane potential by hydrolysis of ATP, even if the respiratory chain itself is inoperative. The protonmotive nature of cI, cIII and cIV is thus an intrinsic aspect of the mechanistic biochemistry of these complexes. As was the case for cV, their structural elucidation and the study of specific mutants that disturb their structures in predictable and/or experimentally verifiable ways have been the key to understanding not only their primary redox chemistry but how electron transport between the redox-active centres is coupled to proton pumping. The molecular mechanism is different for each of the complexes. Nevertheless, each of them appears to be ancient within the bacterial lineage which gave rise to mitochondria and chloroplasts, including modern-day aerobic bacteria.

As already indicated, the mitochondrial localization of the respiratory machinery and of the Krebs cycle enzymes was not immediately appreciated in the 1930s. Over the succeeding decades, the topological issue has become more complex, since the mitochondrial membrane system is now understood to divide the organelle into at least six compartments [35] – see Fig. 2. In addition to an outer membrane, the inner membrane is nowadays considered to comprise two separate compartments, the inner boundary membrane surrounding the matrix, and the cristal membrane in which the OXPHOS complexes and some accessory machinery are embedded. These cristae enclose a lumen that is separated by cristal junctions from the intermembrane space, which is located between the outer and boundary membranes. In addition, there are contact sites where the outer and boundary membranes are closely juxtaposed, and where transport of proteins into mitochondria is facilitated. Finally there are similar contact regions where the outer membrane is intimately associated with specific domains of the ER, the so-called MAMs (mitochondria-associated membranes), and where exchanges of calcium ions,

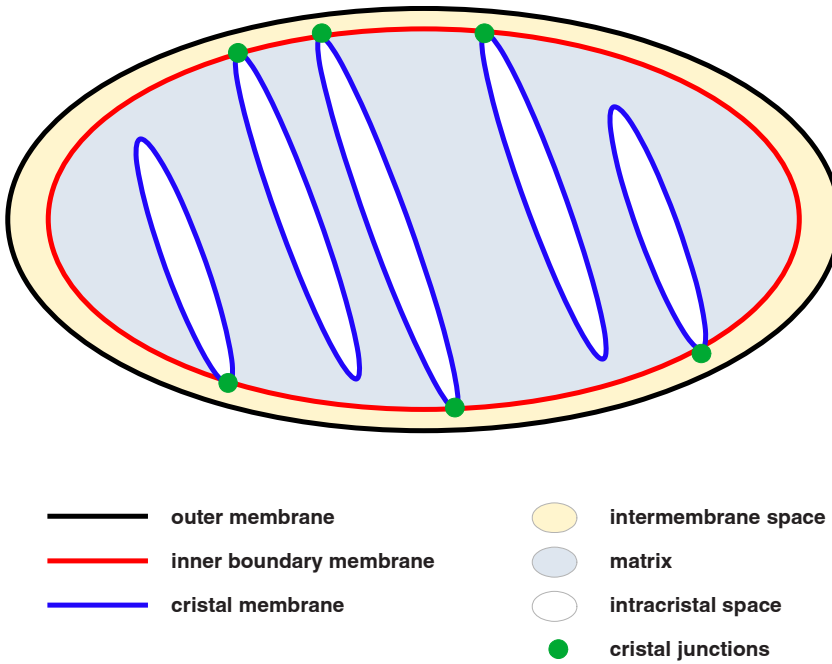

Fig. 2 The topological compartments of mitochondria. In addition to the external space of the cytosol, the mitochondrial membrane system defines three membrane compartments and three internal spaces, as shown.

phospholipids, sterols/steroids and their intermediates are believed to occur [36]. Specific protein complexes within this overall topology define boundaries, interconnections and transport machineries, such as those dedicated to protein import and sorting to the appropriate intramitochondrial compartment. These protein complexes have been discovered and characterized by the standard tools of biochemistry: fractionation (based on size, buoyant density, detergent action), co-immunoprecipitation, proximity labelling, mass spectrometry and the two-hybrid approach [37], combined with classical and reverse genetics and high-resolution imaging. These methods are under continuous refinement, and our current understanding of the mitochondrial proteome, and the submitochondrial localization and physiological functions of the ~1100 proteins that it comprises [38], is reflected in the huge number of recent studies on mitochondria reported in the literature and accessible on Pubmed: 24,600 in 2022, 3500 in 1992, just 360 in 1962. Although not all of these publications deal with biochemical issues, the structural and functional characterization of the mitochondrially localized proteins remains the object of the majority of these ongoing studies. Thus, the era of mitochondrial biochemistry is far from over.

I cannot conclude my survey of the era of mitochondrial biochemistry without acknowledging the visionary contribution of Otto Warburg, again dating largely from the 1920s and extending into the 1960s and beyond. As an early mentor of Krebs, he also didn't fully appreciate, until later, that he was working on mitochondria. His most important observation was that cancer cells rely mostly upon glycolysis for their energy supply, whilst normally differentiated cells in the body rely principally on respiration [39]. His 1931 Nobel prize citation was "for his discovery of the nature and mode of action of the respiratory enzyme".

Although, like many facets of cancer cells, Warburg's conclusions represent a broad generalization to which there are important caveats [40] and even exceptions, it has recently become central, once again, in thinking about the metabolic transformations that enable tumour cells to proliferate and invade, and to survive under hypoxic conditions [41].

4. The era of mitochondrial genetics

Before the actual discovery of mitochondrial DNA (mtDNA), Boris Ephrussi and Piotr Slonimski in the late 1940s into the 1960s uncovered genetic evidence that pointed to its existence [42]. They isolated mutants in

budding yeast that were unable to respire, but survived on fermentable substrates such as glucose. These mutant strains also formed smaller colonies. It soon became clear that most of these so-called *petite* mutant traits were inherited in a fashion that disobeyed the by-then well established rules of Mendelian genetics, and instead appeared to be inherited cytoplasmically [43], without meiotic segregation. With the discovery of mtDNA, initially in mammalian cells by Margit and Sylvan Nass [44,45] in 1963, and soon after in budding yeast by Gottfried Schatz and colleagues [46], it quickly became apparent that these extranuclear genetic determinants were carried on a separate DNA genome located inside the organelle.

A crucial advance, initiated already by Slonimski himself in the 1960s, was that not all mutants with the petite phenotype mapped to the mtDNA [42]. In fact, a much larger number of complementation groups generating the respiration-defective petite phenotype showed completely standard nuclear inheritance. These *pet* mutants were later catalogued and characterized systematically by Alex Tzagoloff and Carol Dieckmann [47], and found to number over 400. Their work implied that the vast majority of proteins required for the construction and maintenance of the OXPHOS system are encoded in the nuclear genome, with only a small minority in the mtDNA itself. This supported the idea that, in the course of evolution, most of the genes of the bacterial ancestor of mitochondria were either transferred to the nucleus or substituted by pre-existing genes contributed by the original host. Some may also have evolved *de novo* since the endosymbiotic partnership began, while others may have been transferred horizontally from elsewhere, or were simply lost because they were no longer useful. It has been argued that these gene transfer processes confer an energetic and hence selective advantage, such that gene loss from the endosymbiont is essentially unidirectional [48].

Throughout the remainder of the 1960s and into the 1980s, the characterization of mtDNA and its gene products became possible due to the rapid advances in analytical methods, notably the development of dideoxy DNA sequencing by Fred Sanger and colleagues, for whom human mtDNA became only the second replicon to be fully analyzed [49]. Combined with classical genetics, molecular methods led Slonimski and colleagues to the discovery of introns in yeast mtDNA [42], at about the same time as they were being found by others in viral genomes and in eukaryotic nuclear genes. However, the introns of organelle DNAs turned out to be of a special type, categorized as Group I or Group II, and capable of self-splicing in vitro. Although relatively common in bacteria and their viruses, as well as organelles, they are very rare in eukaryotic

nuclear DNAs. Albeit self-splicing in vitro, their removal from primary transcripts in vivo was found to depend on maturases actually encoded with the introns, as well as on a variety of nuclear-coded proteins. The laboratories of Bernard Dujon [50] and Ron Butow [51] then proceeded to identify another extraordinary property of these introns in that they were also transposable elements 'homing' to specific sites in target genomes, by mechanisms again involving intron-encoded proteins.

The sequencing of mtDNA enabled the identification of the mitochondrially encoded gene products. In the case of humans, these comprised 13 polypeptides plus the RNA components of a separate translation system, namely two rRNAs and a minimal set of 22 tRNAs. The latter were shown to employ expanded codon recognition and a modified genetic code [49]. The existence of this translation system had been noted already in the 1970s, but it was not until the turn of the millennium that the constituent polypeptides of mitoribosomes, as well as its associated factors were fully identified, involving a number of laboratories, notably those of Linda Spremulli [52], Tom O'Brien [53] and Tsutomo Suzuki together with Kimitsuna Watanabe [54]. In fact, it was not until 2015 that the atomic-level structure of the mammalian mitoribosome was elucidated, by Venki Ramakrishnan and colleagues [55]. Even the precise structure of the full set of human mitochondrial tRNAs with their many base-modifications was published only recently by the Suzuki lab [56]. In animals, all of the mitoribosomal proteins are nuclear-coded, although mtDNA encodes one (Var1) in yeast, several in plants and tens in some protists.

At about the same time as the full human mtDNA sequence was being determined, Julio Montoya and Deanna Ojala in the group of Giuseppe Attardi mapped its transcription products, identifying the full genome-length precursor transcripts, the 11 mature mRNAs, two of them bicistronic, plus the rRNAs, tRNAs and various intermediates of RNA processing [57]. The genome organization and inferred RNA processing pathway gave rise to the so-called punctuation model [58], wherein the processing steps that create the 5′ and 3′ termini of the tRNAs, which are interspersed amongst the other coding segments of the genome, also generate the corresponding termini of the upstream and downstream transcripts, be they mRNAs, rRNAs or other tRNAs. The genome organization of human mtDNA was thus found to be extremely economical, with almost no non-coding information except for the extended non-coding region in which were embedded the signals governing transcription and DNA replication [59].

During the following decade, the mtDNAs from many other animal species were sequenced and characterized, revealing that most of the above features as well as the overall order of protein-coding and rRNA genes were highly conserved. Only a small number of inversions and transpositions appear to have occurred since the metazoan radiation, except in some specific taxa where many rearrangements have taken place, such as in nematodes [60] and some molluscs [61].

As more mtDNAs from diverse taxa were sequenced, some specific anomalies were revealed that appear to be taxon specific. These include the arrangement of most of the tRNA genes in a single cluster in echinoderms and in yeast, putatively self-splicing introns in cnidaria, trans-splicing in plants and diplonemids and repetitive sequence elements in some nematodes. Across phylogeny, variant genetic codes and noncanonical tRNA structures were frequently observed, along with cases of massive and even complete loss of tRNA genes, and the co-existence of linear and multipartite genomes rather than the 'standard' monomeric circle. RNA editing was first described in the late 1980s (in mitochondria) in the laboratories of Rob Benne, Larry Simpson and Ken Stuart [62,63], and led to the discovery of cryptogenes in the mtDNA of kinetoplastids, some requiring very extensive RNA pan-editing to generate functional mRNAs [64]. In turn, editing in this taxon is dependent on a large number of extragenomic elements – the minicircles, each encoding a guide RNA with a specific target site in the editing programme. The types of mitochondrial RNA editing and gene fragmentation in different taxa are extremely diverse – see, e.g. [65]. All of these cases suggest that mitochondrial genome evolution has proceeded as a kind of arms race, with lineage-specific encryption mechanisms resisting (or in some cases harnessing) invasive genetic elements.

The discovery of mitoribosomes and of poly(A)-tailed transcripts containing the protein-coding sequences of mammalian mtDNAs prompted the question as to what functions are performed by the polypeptides encoded by the 13 open-reading frames of the mammalian mitochondrial genome. Several of their counterparts in yeast had already been identified biochemically, enabling five of them to be annotated immediately as crucial redox-active proteins of the OXPHOS system: cIV subunits COX1, 2 and 3, associated with cytochromes *a* and a_3 plus the copper centres of cIV, apocytochrome *b*, belonging to cIII, and subunit 6 or *a* of cV, into which protons are fed by the rotating ring of *c* subunits for transit across the membrane to energize the cycle of conformational changes that drive ATP synthesis. The remaining open reading-frames were finally shown by Paolo

Mariottini and Anne Chomyn in the Attardi lab, and John Walker and colleagues, to encode other OXPHOS polypeptides, namely subunit 8 (or A6L) of cV [66,67] and seven subunits of cI [68].

The mechanism(s) of mtDNA replication remains incompletely understood, and various models have been put forward, none of which is supported by comprehensive data. The prevalent view is based around the ingenious model originally proposed in the 1970s by Jerome Vinograd and David Clayton, in which the two strands of the circular mtDNA are replicated asynchronously from different origins, leaving large expanses of the molecule partially single-stranded during much of the replication cycle [69]. The main points of this model are still valid except that, in most molecules, the displaced strand appears to be provisionally hybridized to RNA over most of its length [70,71]. However, fully dsDNA replication intermediates are also detected, implying that a proportion of molecules replicate by a more conventional, strand-coupled mechanism [71]. There is also evidence for rolling-circle replication and recombination-driven replication in some tissues and organisms. Given this confusing multiplicity of inferred mechanisms, probably indicative of genetic redundancy in a biologically essential process, working out the precise mechanistic details and enzymology is still a work in progress.

What is very clear is that all of the enzymes required for mtDNA replication, as well as for transcription and RNA processing are exclusively nuclear-coded, except in jakobids [72], which are considered to be an anciently diverged group of eukaryotes. In animals and fungi, the major DNA polymerase found in mitochondria (Pol γ), assumed to be the mtDNA replicase, is related to the single-subunit DNA polymerases of T-odd bacteriophages [73,74]. However, its processivity-enhancing partner protein is not the chromosomally encoded thioredoxin as in bacteria, but a novel tRNA ligase-related polypeptide, at least in animals [75], with no counterpart at all in yeast. Other phage-related enzymes are part of the machinery of mtDNA maintenance, notably the major mitochondrial primase-helicase, related to phage T7 gp4 which, in metazoans, has lost primase activity [76]. Another example is the single-subunit RNA polymerase [77] which, like Pol γ, has acquired accessory factors that facilitate initiation, most notably the transiently interacting factor Mtf1 (in yeast) or TFB2M (in metazoans), as well as a DNA-bending, minor-groove DNA-binding protein (TFAM in metazoans). Intriguingly, the jakobids have a bacterial-like multisubunit RNA polymerase encoded in their mtDNA [72].

Amongst the hundreds of PET genes are those that contribute to the uniquely mitochondrial apparatus of protein import, the so-called TOM

and TIM complexes that act as import and sorting complexes located, respectively, in the outer and inner mitochondrial membranes [78]. These were originally identified genetically in yeast, starting in the 1980s, by the laboratories of Walter Neupert and Gottfried Schatz, but appear to be essentially the same in all animals, fungi and plants, with the essential components present in all eukaryotes [79]. This led to the assumption that a necessary step in 'organellogenesis' after endosymbiosis was the evolution of a machinery for importing (nuclear-coded) proteins from the cytosol, and that protein transport was a one-way process. Such a view has been challenged by the recent finding of remnants of a bacterial type-2 secretion system in jakobids and other excavates [80], suggesting that proteins may originally have been exchanged in both directions. Protein export from mitochondria to enable 'bacterial-like' functions to be performed elsewhere in the cell would have become redundant once the relevant genes had all been transferred to the nucleus, but this process appears not to have reached completion in all eukaryote lineages.

Mitochondrial DNA in animals, most plants and many fungi shows uniparental inheritance. This makes sense in highly anisogamic species – essentially all metazoans, where the amount of cytoplasm contributed to the zygote by the maternal gamete (oocyte) is vastly greater than that originating from the male gamete. However, as revealed by mtDNA studies beginning in the 1980s, there are curious exceptions [81]. One is evident in some molluscs, which maintain two distinct isotypes of mtDNA inherited through opposite parents [82]. Interspecific hybrids of mice and some other species also show evidence of paternal transmission of mtDNA [83], which is believed to represent a breakdown in the normal mechanisms by which paternal mitochondria are recognized and destroyed in early embryonic development, as documented in both flies [84] and mammals [85], and involving targeted ubiquitination as well as active degradation of sperm mtDNA [86].

One of the most intriguing aspects of (animal) mitochondrial genetics is the fact that within a single individual and in all of their cells, all mtDNA molecules are identical by sequence. How this condition, known as homoplasmy (genetic *homo*geneity of the cyto*plasm*), is established and maintained is still debated, but appears to involve a drastic reduction in mtDNA copy number from thousands per cell to tens. Combined with multiple rounds of cell division during germ-cell development, this leads to mitotic segregation possibly followed by selective mtDNA amplification during oogenesis, resulting in homoplasmic oocytes [87,88]. Some

instances of 'benign' heteroplasmy have been observed, alongside the intriguing observation that shifts to homoplasmy can occur over very few maternal generations [89]. Low level heteroplasmy across the mitochondrial genome is now considered the norm [90]. The studies of Jim Stewart and Nils-Göran Larsson have provided evidence for purifying selection to eliminate deleterious mtDNA mutants during oogenesis [91]. However, not all potentially harmful variants are eliminated, with tRNA mutants especially prone to escape this selective process, resulting in persistent heteroplasmy. This will be discussed further in the following section.

5. The era of mitochondrial pathology

The centrality of mitochondrial metabolic processes for life, coupled with the highly unorthodox genetics of mtDNA and the involvement of over hundreds of nuclear genes in the building and maintenance of the OXPHOS system, strongly suggest that there must be many mutations that lead to impaired mitochondrial function and hence to disease. However, for a long time this was overlooked, most likely for two reasons. First, the very necessity of these metabolic pathways and gene products suggested that almost all such mutations would be lethal. Second, that any nonlethal OXPHOS mutants would affect physiological functions in a similar way, producing a stereotypical phenotype, akin to the uniform petite phenotype of the many hundreds of OXPHOS-defective mutants in yeast. Both assumptions proved incorrect.

The first reported case of a clinical OXPHOS defect came in the early 1960s, when Rolf Luft and colleagues observed a patient showing signs of severe hypermetabolism and concomitant thermogenesis, due to disruption of the normally tight coupling of ATP production to respiration in mitochondria [92]. Over the following decades, clusters of patients with varying neuromuscular symptoms were identified, notably by the groups of John Morgan-Hughes and Salvatore DiMauro, with mitochondrial dysfunction as a primary underlying feature [93,94]. However, it was the discovery of the role of mtDNA in disease in the late 1980s that was the major breakthrough that opened up the field. First, Ian Holt and coworkers reported the association between heteroplasmic, large deletions of mtDNA and many cases of neuromuscular disease [95]. Soon after, the groups of Doug Wallace and of Yu-ichi Goto, Ikuya Nonaka and Satoshi Horai identified inherited point mutations in mitochondrial tRNA genes

[96,97], associated with syndromic disorders affecting the major organs, notably skeletal muscle, the CNS and heart. These first-described mutations were heteroplasmic, supporting the idea that wild-type mtDNA was indispensable for life. However, homoplasmic mutations were soon added to the list by the Wallace group, which identified point mutations in mitochondrial genes for subunits of cI as the cause of LHON [98], a type of optic-nerve atrophy, and by the group of Nathan Fischel-Ghodsian who found that a homoplasmic mutation in the gene for mitochondrial large-subunit rRNA predisposed to aminoglycoside-induced deafness [99].

Very soon after the discovery of mtDNA deletions as a cause of disease, patients with multiple deletions of mtDNA, but showing autosomal inheritance, came to light in studies by the group of DiMauro and others [100,101]. Other cases, with different clinical features, were found to be associated with depletion of mtDNA content in specific tissues [102]. Painstaking genetic mapping and mutational analyses eventually led to the identification of some of the key genes that were responsible, notably those coding for the major DNA polymerase [103] and replicative helicase [104] of mitochondria, and some other products involved in nucleotide transport and metabolism.

The 1990s saw an explosion of data, connecting mutations not only in mtDNA but also in many hundreds of nuclear genes coding for mitochondrial components with an increasingly broad and, to some extent baffling, heterogeneity of clinical conditions. With the advent of techniques for interrogating the sequence of the entire genome, the list of 'mitochondrial disease genes' continues to grow.

More surprising, but also in a way obvious, has been the increasing realization that an even wider disease spectrum than these classic 'mitochondriopathies' is associated with mitochondrial dysfunction, which may be at the root of diverse pathological processes or constitute an instrumental step therein [105,106]. Much of this additional disease burden is attributable to the broader cellular role of mitochondria [107], to which I now turn.

6. The era of mitochondrial cell biology

In the 1990s, in parallel to the exploration of the involvement of mitochondria in disease, an entirely new field opened up with the realization that mitochondria are not simply biological fuel cells converting chemical into electrical energy. Nor are they to be regarded merely as metabolic 'clearing houses' with essential roles in the biosynthesis of lipids,

purines and pyrimidines, iron-sulphur clusters, heme and one-carbon compounds [108]. They are also now recognized as essential components of cell signalling networks [109], operating to maintain biological homeostasis both within individual cells, as well as in tissues, organs and even the entire body. Moreover, mitochondria have been shown to be dynamic entities, undergoing fusion, fragmentation, movement and the exchange of macromolecules with other organelles. These findings have fundamentally altered our view not only of mitochondria but of the whole cell. Organelles were once thought of as structurally distinct and functionally independent cellular compartments. However, the current view which has emerged since 1990 is that organelles in general, and mitochondria in particular, are highly interactive, share many essential tasks upon which cellular integrity and function depend, and communicate continuously with each other and with the rest of the cell, as well as with the extracellular environment. I shall briefly review some of the key findings that led to this revolution in thinking.

The work of Stan Korsmeyer and others in the early 1990s demonstrated that mitochondria operate as platforms for the integration of the signals instructing the cell to remain alive or instead enter the programmed suicide pathway of apoptosis and its variants [110]. Under appropriate conditions, signals emanating from the cell surface and from inside the mitochondria themselves converge at the outer mitochondrial membrane to generate the irreversible opening of channels through which holocytochrome *c*, as well as some other small proteins normally confined within the intermembrane space, are exported to the cytosol, where they activate members of the caspase protease family [111]. These then propagate the death message throughout the cell, and disassemble and degrade its components.

However, it soon became apparent that mitochondria are also the source of several other classes of signalling molecules that influence diverse physiological processes in the rest of the cell (Fig. 3). These include calcium ions, hydrogen peroxide and other reactive oxygen species (ROS), plus a number of Krebs cycle intermediates that are essential for epigenetic processes acting in the nucleus and their regulation. These various small molecules, as well as some proteins whose mitochondrial import can become blocked, then signal to the rest of the cell the metabolic state of mitochondria, including the various stress conditions to which they are prone, such as proteotoxic or oxidative damage or infection by a virus or other pathogen. In the first instance, such signals then regulate metabolic enzymes residing elsewhere in the cell, as well as their expression, or

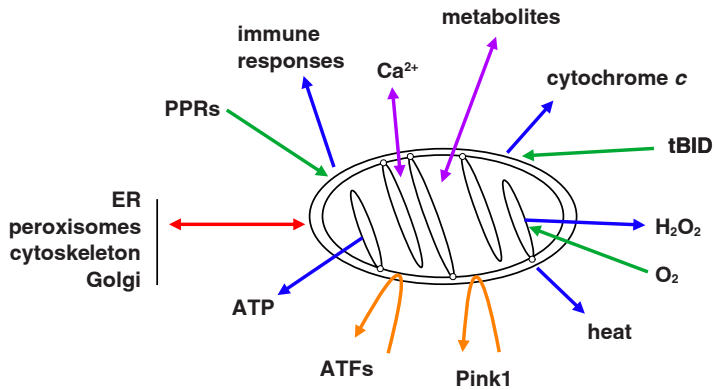

Fig. 3 Mitochondria are cell signalling hubs. A summary of some of the major signals received and emitted by mitochondria. Incoming biochemical signals (green arrows) include ligand-bound pathogen pattern receptors (PPRs), pro-apoptotic molecules such as tBID, and oxygen, whose levels are effectively monitored by mitochondria in crucial oxygen-sensing tissues. These signals elicit various responses (blue arrows), respectively the activation of immune pathways, e.g. via the inflammasome or the MAVS signalosome, the release of cytochrome c to initiate apoptosis, and hydrogen peroxide to activate hypoxia and oxidative stress signalling. Other emitted signals from mitochondria include heat and ATP itself, which in turn modulates many protein kinases, being also a primary substrate thereof. A large number of metabolites, as well as calcium and other ions, are taken up or exchanged by mitochondria (purple arrows), regulating many of cellular enzymes and also providing substrates for the creation of epigenetic marks in the nucleus. Mitochondrial proteostasis is monitored by the failure to uptake reporter proteins (orange arrows) such as the ATF transcription factors or Pink1 kinase, respectively activating mitochondrial unfolded protein responses or mitophagy. In addition, mitochondria communicate biochemically and biogenetically with other organelles and cellular systems (red arrow) via physical connections and vesicle transit.

facilitate the delivery of stress-response proteins to restore intramitochondrial homeostasis. If these measures do not correct the stress, more drastic outcomes follow. Defective mitochondria can be eliminated by a limited mobilization of the cell's autophagic machinery, 'mitophagy'. If damage persists the last resort is apoptosis.

The outer mitochondrial membrane, which was previously believed to serve only a topological function defining the intermembrane space, has gradually emerged as a crucial decision-making 'platform'. Not only does it house the machinery that initiates the cell-death programme. It also serves as a platform on which signals indicative of infection by a pathogen are assembled and transduced to the nucleus, cell surface and ribosomes, so as to mount an intra- and inter-cellular immune response.

Following the discovery of these various phenomena, much effort has been expended since 1990 to identify the molecular machineries responsible, elucidate the mechanisms by which they act, investigate their roles in disease and, where appropriate, target them pharmacologically, aiming to correct and eventually treat the various pathologies in which they are involved. The field has become vast, so I shall here just cite a few examples from each of these areas of 'mitochondrial cell biology'.

One of the first identified protein families with roles in the mitochondrial regulation of apoptosis is the one related to the oncoprotein Bcl-2, whose inappropriate overexpression in lymphocytes is a common initiation step in follicular lymphoma [110]. The Bcl-2 family of proteins comprises both pro- and anti-apoptotic members. The former can form homo- and/or heterodimers which directly operate as channels through which cytochrome *c* can be exported to the cytosol. The anti-apoptotic members of the family, including Bcl-2 itself, generally antagonize this process by forming inactive heterodimers with pro-apoptotic family members, thus titrating out the membrane permeabilization activity that would otherwise initiate the cascade of events leading to cell death [111].

The machinery involved in mitophagy, both in regard to its signalling and implementation, remain somewhat controversial. The general consensus is that mitophagy is, in part, a constitutive process in which organelles are continuously turned over, so as to prevent the accumulation of defective mitochondria and ensure their functional quality maintenance [112]. However, there appears also to be an overlapping machinery whereby mitochondria that have been subjected to irreversible damage can signal their plight and undergo mitophagy. In regard to the latter, a specific E3 ubiquitin ligase, parkin, implicated in the etiology of a familial form of early-onset Parkinson's Disease, is accumulated at the surface of mitochondria that have lost their membrane potential and are no longer able to import cytosolically synthesized proteins. The crucial accessory player in the pathway is the protein kinase Pink1, which phosphorylates parkin and probably other targets involved in the induction of mitophagy, when its import is prevented [113].

In most cells, mitochondria exist in long filamentous structures that are continuously split and reformed in a cycle of fusion and fission that is essential for mitochondrial quality maintenance [114]. Mitochondria must be fragmented, so as to isolate defective parts of the 'mitochondrial network' and target them for mitophagy. However, various physiological stresses can lead either to fragmentation or hyperfusion of the

mitochondria, and these processes are poorly understood, despite years of painstaking research to identify the molecular machinery involved, which remains incomplete. In addition to their endogenous or 'homotypic' fusion-fission cycle, mitochondria are also in communication with other membrane-bound organelles, notably peroxisomes, for the delivery of specific peroxisomal components via mitochondrial-derived vesicles [115], endosomes for the recycling of iron [116], Golgi-derived vesicles for the acquisition of proteins required for mitochondrial fission [117] and most extensively of all, with the endoplasmic reticulum for diverse molecular exchanges required for metabolic homeostasis. The latter include, for example, lipid biosynthesis, Calcium flux, endocrine signalling, redox maintenance in the secretory system and some aspects of ROS handling [118,119]. Mitochondria-derived vesicles are also believed to act as an additional pathway for mitochondrial quality control [120].

As well as the dynamic aspects of their own structure, mitochondria have been shown to be motile within cells [121], which has generally been interpreted as their being guided by metabolite gradients to where they are needed in the cell, in order to supply ATP, deliver waste products, be replenished with substrates or collect newly synthesized macromolecules. These processes have been thought most critical in developing neurons, where mitochondrial energy should be important in synaptic processes, but where the major supplies of new proteins and substrates are far away in the cell body. However, there may be other reasons for mitochondrial motility, which can vary even within different compartments of a single cell [122]. Mitochondria may not even be the primary source of ATP for synaptic transmission [122]. Once again, the identification of the molecular machinery of mitochondrial motility, notably involving the microtubule network and the motor proteins that move cargo along it, have been the major achievements of this area of research [121]. A proper understanding of the physiological reasons for which mitochondria move within cells, and the role of its malfunction in the pathology of neurodegeneration and other diseases, remain incomplete.

Mitochondria were once thought of as sinks for excess cytosolic calcium, a process highly dependent on the mitochondrial calcium uniporter (MCU). However, calcium currents clearly play multiple roles in mitochondria [123], as highlighted by the regulatory complexity of the MCU itself [124]. The buffering of cellular calcium is, indeed, an important role of mitochondria in many cell-types, where it fine-tunes a great variety of physiological process, notably the contractile cycle of cardiomyocytes and of skeletal muscle, the

activation of T-lymphocytes and the excitability of neurons. But calcium also regulates many of the metabolic enzymes of mitochondria, although the main avenue of regulation appears to be indirect, via the modulation of pyruvate uptake by cytosolic calcium [125]. Genetic ablation of the MCU has surprisingly mild physiological effects [126], suggesting that it is operationally redundant to other pathways.

Major interest has centred around the role of mitochondria in signalling oxidative stress, arising from overload or inhibition of the OXPHOS system, e.g. due to toxins, anoxia or physicochemical damage. When respiratory chain function is limited, and its various electron carriers accumulate in the reduced form, electron flow can eventually be reversed through complex I [127], leading to massive overproduction of superoxide and other reactive oxygen species (ROS). ROS release from mitochondria, usually in the metastable form of hydrogen peroxide, leads to a broad response targeted at stress mitigation. Although this response has been largely construed as a 'cry for help', promoting the increased synthesis and transport to mitochondria of polypeptides with functions in OXPHOS or in ROS detoxification, it undoubtedly has a broader role in remodelling cell metabolism and gene expression so as to resist or accommodate actual or incipient stress. It also has a more specific role in the signalling of hypoxia in oxygen sensing organs and tissues, such as the carotid body and the vasculature of the lung [128,129].

Recently, it has become clear that the mitochondria are in constant communication with the cell nucleus, to signal aspects of their own functionality. A variety of metabolic stresses induce translocation to the nucleus of transcription factors that reprogramme gene expression to correct or mitigate the inducing stress. A notable stress condition is the proteotoxicity that can result from the accumulation of misfolded proteins due to redox imbalance, errors in protein synthesis, or the lack of coordination of mitochondrial and cytosolic translation, leading to the presence of unincorporated subunits of the OXPHOS complexes. Although commonly recognized as 'the' mitochondrial unfolded protein response (UPR^{mt}), it is modulated in fine detail according to the nature of the problem, the tissue in which it has occurred, and other physiological abnormalities that may accompany it, related to its underlying cause. This implies that is its better regarded as a complex tapestry of signals and responses induced by different types of mitochondrial stress [130]. In addition to stress mitigation, mitochondrial biogenesis is adjusted to fit physiological and developmental needs by a number of nuclear transcription factors and co-factors [131], often in response to endocrine signals [132] that may also have more direct effects on mitochondrial metabolism [133].

The outer mitochondrial membrane as a platform for the detection of pathogens and their biological signatures, is especially important for the response to RNA viruses [134]. Although the relevant molecular mechanisms have not yet been fully elucidated, a key player is the mitochondrial anti-viral signalling protein MAVS, which coordinates the activation of interferon-linked pathways and autophagy. Pathogen pattern receptors such as the Toll-like, NOD-like, RIG1-like and cGAS receptor families interact with and are regulated by mitochondria in diverse ways, leading to the activation of large protein arrays such as the inflammasome and the MAVS signalosome, that are essential for pathogen clearance and the production of endocrine signals to alert neighbouring cells to the threat of infection. The physiological rationale for mitochondrial involvement in innate immunity has been extensively discussed. Whilst local ATP delivery under metabolically challenged conditions appears to be essential, many of the same pathways are involved in responding to mitochondrial damage, which may also be a sign of infection, i.e. indicating that mitochondria could be an ideal place for viruses to remain hidden. In addition, many viruses manipulate mitochondrial functions as part of their own life cycle, or to disable host immunity [135].

The overall lesson from the era of 'mitochondrial cell biology' is that mitochondria are not merely metabolic machines harbouring the enzymes needed to process substrates and release bioenergy, but serve also as hubs of cellular decision-making, interacting with many other cellular components and integrating and transducing many of the signals that ensure cellular and organismal survival. The earlier view of organelles as a manifestation of the division of cellular labour has given way to a more holistic picture of the cell in which its component parts, most centrally the mitochondria, are seen as a dynamic community of interactive elements.

7. The future of mitochondrial enzymology

The discovery of the mitochondrial dimension in human pathology has spurred a broad effort to correct or mitigate the effects of the underlying genetic defect, so as to arrest disease progression, prevent pathological manifestations or transmission, and even reverse the more egregious symptoms. Bound up with this is the intriguing but disappointing failure of many laboratories to achieve a convincing genetic transformation of metazoan mtDNA, even at the level of cultured cells. One potential avenue that yet to be pursued is to manipulate and mobilize genetic

elements that can replicate inside mitochondria, such as endogenous retroviruses, mitoviruses, or other classes of virus, some components of which appear to access mitochondria or shuttle promiscuously between mitochondria and other cellular compartments (e.g. see Ref. [136,137]).

The allotopic expression of mitochondrial genes in the nucleus [138] is advancing, but has not yet achieved consistent success. Strategies to reduce the burden of heteroplasmy by destroying mutant mtDNA molecules with targeted nucleases [139], by metabolic or nutritional therapies that impose selection at the cellular or organismal level for mitochondrial fitness [140], or by drugs that mitigate oxidative stress [141], all show promise. The replacement of mutant mtDNA in oocytes, even when homoplasmic, has been proposed as a viable technology for prevention [142], although technical issues remain [143]. Other ideas, such as the introduction of alternative respiratory chain enzymes [144] or the use of viral vehicles for replacement gene therapy [145] are actively being explored.

Mitochondria are increasingly regarded as a viable target for therapeutic intervention in a whole host of disease conditions where mitochondrial dysfunction may not be the underlying cause, but is nevertheless an essential step in the pathological process. Examples include the targeting of mitochondrial oxidative stress in heart disease [146], metabolic interventions in cancer treatment [147] and therapies designed to bolster failing mitochondrial functions in neurodegeneration [148]. Unique metabolic features of the mitochondria of diverse parasites can be targeted by drugs that are essentially benign to humans [144,149]. Research on the evolutionary peculiarities of the mitochondrial genetic system in many protists, such as apicomplexa [150] and kinetoplastida [151,152] is leading towards new therapeutic concepts,

A huge literature is accumulating, regarding the application of mtDNA sequence data in species identification and population biology, including deep roots as well as recent events. Although collected for purposes distinct from understanding the specific biochemistry of mitochondria, these data nevertheless represent a valuable resource for understanding not only the evolution of mtDNA, but also the processes governing the co-evolution of the nuclear and mitochondrially encoded components of the OXPHOS enzyme complexes, or that of the enzymes responsible for mitochondrial genome maintenance and expression with the mtDNA itself, and its transcripts. There is much still to learn about the functions of these basic elements of metabolism and genetics.

Sequence information from mtDNA continues to be applied in forensics and archaeology as well, although its use has been largely supplanted

by nuclear DNA data, which provides much finer resolution. However, mtDNA-derived data is still crucial where samples are very degraded or minimal in quantity. Once again, the application has little or nothing to add to knowledge of the biology or biochemistry of mitochondria, but has served a totally different purpose, namely to nourish the popularization of mito-science. Stories such as the evidence for Mitochondrial Eve [153], the identification of the remains of the family of the last Tsar of Russia [154] or of England's King Richard III [155] have fired the popular imagination and brought the topic of mitochondria to wide public attention. Public support for any area of research is essential if it is to continue to be funded. Keeping mitochondria it at the forefront of public attention is vital for ensuring that it continues to be supported through the inevitable 'lean' periods when major discoveries are few.

Recent observations indicating that mammalian and even insect mitochondria are maintained in vivo at temperatures at least 15 °C hotter than the extracellular environment [156,157] have thrown a new spotlight on how heat is produced, retained and conducted from mitochondria, and how this affects all of the biological processes of the cell. Most obviously, the properties of almost all of the enzymes of mammalian mitochondria as well as enterobacteria have hitherto been studied at 37 °C, taking no account of the actual temperatures they may experience in vivo. These may vary drastically depending on their precise submitochondrial localization: the double membrane of mitochondria, as well as that of gram-negative bacteria (plus the bacterial cell wall) is implied to represent an insulating layer, limiting heat transfer but also enabling intraorganellar/intracellular temperature homeostasis to operate by still unknown mechanisms. Nevertheless, in many cases, especially of enzymes located in the inner mitochondrial membrane or mitochondrial matrix, 37 °C is far outside of the physiological range, and many earlier enzymological findings may need to be revisited and even, in some cases, overturned.

Finally, I dare to venture into the thorny subject of mitochondria and aging. At one time, the idea that oxidative damage arising from dysfunction of the mitochondrial OXPHOS enzymes was a or perhaps even 'the' key factor in the aging process [158] has given way to a broader concept, that the loss of turnover and repair capacity in the OXPHOS system and the lifetime accumulation of uncorrected mtDNA mutations in the soma *contribute* to the loss of cellular and organismal viability that we perceive as aging [159,160], but are not its sole cause. Moreover, it is the age-associated disruption of the mitochondrial signalling of oxidative stress rather

than the actual damage to biomolecules that has been postulated as the most important factor linking mitochondria to aging [161]. Artificially increasing the rate of mtDNA mutagenesis in model organisms does lead to phenotypes that resemble some aspects of aging [162–167], though to variable extents The exact reasons why this is so remain debated. Clearly these models do not precisely recapitulate every aspect of the aging process. Pinning down how loss of mitochondrial fitness impacts aging remains an elusive but important goal of current research.

References

[1] I.E. Wallin, Symbioticism and the Origin of Species, The Williams and Wilkins Company, Baltimore, 1927.
[2] M.A. O'Malley, Endosymbiosis and its implications for evolutionary theory, Proc. Natl. Acad. Sci. U. S. A. 112 (33) (2015) 10270–10277, https://doi.org/10.1073/pnas.1421389112
[3] A. Lazarow, S.J. Cooperstein, Studies on the enzymatic basis for the Janus green B staining reaction, J. Histochem. Cytochem. 1 (4) (1953) 234–241, https://doi.org/10.1177/1.4.234
[4] I.E. Wallin, A note on the morphology of bacteria symbiotic in the tissues of higher organisms, J. Bacteriol. 7 (5) (1922) 471–474, https://doi.org/10.1128/jb.7.5.471-474.1922
[5] L. Sagan, On the origin of mitosing cells, J. Theor. Biol. 14 (3) (1967) 255–274, https://doi.org/10.1016/0022-5193(67)90079-3
[6] L. Margulis, Symbiosis in Cell Evolution, WH Freeman, San Francisco, 1981.
[7] M.W. Gray, D. Sankoff, R.J. Cedergren, On the evolutionary descent of organisms and organelles: a global phylogeny based on a highly conserved structural core in small subunit ribosomal RNA, Nucleic Acids Res. 12 (14) (1984) 5837–5852, https://doi.org/10.1093/nar/12.14.5837
[8] M.W. Gray, Origin and evolution of organelle genomes, Curr. Opin. Genet. Dev. 3 (6) (1993) 884–890, https://doi.org/10.1016/0959-437x(93)90009-e
[9] H.A. Krebs, W.A. Johnson, Metabolism of ketonic acids in animal tissues, Biochem. J. 31 (4) (1937) 645–660, https://doi.org/10.1042/bj0310645
[10] P.K. Arnold, B.T. Jackson, K.I. Paras, J.S. Brunner, M.L. Hart, O.J. Newsom, et al., A non-canonical tricarboxylic acid cycle underlies cellular identity, Nature 603 (7901) (2022) 477–481, https://doi.org/10.1038/s41586-022-04475-w
[11] M. Friedkin, A.L. Lehninger, Oxidation-coupled incorporation of inorganic radiophosphate into phospholipide and nucleic acid in a cell-free system, J. Biol. Chem. 177 (2) (1949) 775–788.
[12] F.J. Hird, M.J. Weidemann, Oxidative phosphorylation accompanying oxidation of short-chain fatty acids by rat-liver mitochondria, Biochem. J. 98 (2) (1966) 378–388, https://doi.org/10.1042/bj0980378
[13] B. De Bernard, Studies on the terminal electron transport system. V. Extraction of a soluble DPNH-cytochrome c reductase from the electron transport particle, Biochim. Biophys. Acta 23 (3) (1957) 510–515, https://doi.org/10.1016/0006-3002(57)90370-0
[14] Y. Hatefi, A.G. Haavik, D.E. Griffiths, Reconstitution of the electron transport system. I. Preparation and properties of the interacting enzyme complexes, Biochem. Biophys. Res. Commun. 4 (1961) 441–446, https://doi.org/10.1016/0006-291x(61)90305-9

[15] Y. Hatefi, D.L. Stiggall, Preparation and properties of NADH: cytochrome c oxidoreductase (complex I–III), Methods Enzymol. 53 (1978) 5–10, https://doi.org/10.1016/s0076-6879(78)53005-x

[16] J. Zhu, K.R. Vinothkumar, J. Hirst, Structure of mammalian respiratory complex I, Nature 536 (7616) (2016) 354–358, https://doi.org/10.1038/nature19095

[17] K. Fiedorczuk, J.A. Letts, G. Degliesposti, K. Kaszuba, M. Skehel, L.A. Sazanov, Atomic structure of the entire mammalian mitochondrial complex I, Nature 538 (7625) (2016) 406–410, https://doi.org/10.1038/nature19794. Epub 2016 Sep 5.

[18] S. Iwata, J.W. Lee, K. Okada, J.K. Lee, M. Iwata, B. Rasmussen, et al., Complete structure of the 11-subunit bovine mitochondrial cytochrome bc1 complex, Science 281 (5373) (1998) 64–71, https://doi.org/10.1126/science.281.5373.64

[19] P. Mitchell, Possible molecular mechanisms of the protonmotive function of cytochrome systems, J. Theor. Biol. 62 (2) (1976) 327–367, https://doi.org/10.1016/0022-5193(76)90124-7

[20] Y. Hatefi, A.G. Haavik, JURTSHUKP. Studies on the electron transport system. XXX. DPNH-cytochrome c reductase I, Biochim. Biophys. Acta 52 (1961) 106–118, https://doi.org/10.1016/0006-3002(61)90908-8

[21] Y. Hatefi, A.G. Haavik, D.E. Griffiths, Studies on the electron transfer system. XLI. Reduced coenzyme Q (QH2)-cytochrome c reductase, J. Biol. Chem. 237 (1962) 1681–1685.

[22] C.M. Marres, E.C. Slater, Polypeptide composition of purified QH2:cytochrome c oxidoreductase from beef-heart mitochondria, Biochim. Biophys. Acta 462 (3) (1977) 531–548, https://doi.org/10.1016/0005-2728(77)90099-8

[23] T. Ozawa, M. Tanaka, Y. Shimomura, Crystallization of cytochrome bc1 complex, Proc. Natl. Acad. Sci. U. S. A. 80 (4) (1983) 921–925, https://doi.org/10.1073/pnas.80.4.921

[24] E. Balsa, R. Marco, E. Perales-Clemente, R. Szklarczyk, E. Calvo, M.O. Landázuri, et al., NDUFA4 is a subunit of complex IV of the mammalian electron transport chain, Cell Metab. 16 (3) (2012) 378–386, https://doi.org/10.1016/j.cmet.2012.07.015

[25] S. Yoshikawa, K. Shinzawa-Itoh, T. Tsukihara, Crystal structure of bovine heart cytochrome c oxidase at 2.8 A resolution, J. Bioenerg. Biomembr. 30 (1) (1998) 7–14, https://doi.org/10.1023/a:1020595108560

[26] S. Zong, M. Wu, J. Gu, T. Liu, R. Guo, M. Yang, Structure of the intact 14-subunit human cytochrome c oxidase, Cell Res. 28 (10) (2018) 1026–1034, https://doi.org/10.1038/s41422-018-0071-1. Epub 2018 Jul 20.

[27] H. Schägger, K. Pfeiffer, Supercomplexes in the respiratory chains of yeast and mammalian mitochondria, EMBO J. 19 (8) (2000) 1777–1783, https://doi.org/10.1093/emboj/19.8.1777

[28] S. Cogliati, E. Calvo, M. Loureiro, A.M. Guaras, R. Nieto-Arellano, C. Garcia-Poyatos, et al., Mechanism of super-assembly of respiratory complexes III and IV, Nature 539 (7630) (2016 24) 579–582, https://doi.org/10.1038/nature20157

[29] E. Calvo, S. Cogliati, P. Hernansanz-Agustín, M. Loureiro-López, A. Guarás, R.A. Casuso, et al., Functional role of respiratory supercomplexes in mice: SCAF1 relevance and segmentation of the Qpool, eaba7509, Sci. Adv. 6 (26) (2020), https://doi.org/10.1126/sciadv.aba7509

[30] G. Benegiamo, M. Bou Sleiman, M. Wohlwend, S. Rodríguez-López, L.J.E. Goeminne, P.P. Laurila, et al., COX7A2L genetic variants determine cardiorespiratory fitness in mice and human, Nat. Metab. 4 (10) (2022) 1336–1351, https://doi.org/10.1038/s42255-022-00655-0

[31] E. Fernández-Vizarra, S. López-Calcerrada, A. Sierra-Magro, R. Pérez-Pérez, L.E. Formosa, D.H. Hock, et al., Two independent respiratory chains adapt OXPHOS performance to glycolytic switch, Cell Metab. 34 (11) (2022) 1792–1808e6, https://doi.org/10.1016/j.cmet.2022.09.005

[32] P. Mitchell, Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism, Nature 191 (1961) 144–148, https://doi.org/10.1038/191144a0
[33] F.M. Harold, Gleanings of a chemiosmotic eye, Bioessays 23 (9) (2001) 848–855, https://doi.org/10.1002/bies
[34] D. Stock, A.G. Leslie, J.E. Walker, Molecular architecture of the rotary motor in ATP synthase, Science 286 (5445) (1999) 1700–1705, https://doi.org/10.1126/science.286.5445.1700
[35] J.C. Iovine, S.M. Claypool, N.N. Alder, Mitochondrial compartmentalization: emerging themes in structure and function, Trends Biochem. Sci. 46 (11) (2021) 902–917, https://doi.org/10.1016/j.tibs.2021.06.003
[36] L. Barazzuol, F. Giamogante, T. Calì, Mitochondria Associated Membranes (MAMs): Architecture and physiopathological role, Cell Calcium 94 (2021) 102343, https://doi.org/10.1016/j.ceca.2020.102343
[37] S. Fields, O. Song, A novel genetic system to detect protein-protein interactions, Nature 340 (6230) (1989) 245–246, https://doi.org/10.1038/340245a0
[38] S. Rath, R. Sharma, R. Gupta, T. Ast, C. Chan, T.J. Durham, et al., MitoCarta3.0: an updated mitochondrial proteome now with sub-organelle localization and pathway annotations, Nucleic Acids Res. 49 (D1) (2021) D1541–D1547, https://doi.org/10.1093/nar/gkaa1011
[39] O. Warburg, On the origin of cancer cells, Science 123 (3191) (1956) 309–314, https://doi.org/10.1126/science.123.3191.309
[40] O. Warburg, F. Wind, E. Negelein, The metabolism of tumors in the body, J. Gen. Physiol. 8 (6) (1927) 519–530, https://doi.org/10.1085/jgp.8.6.519
[41] M. Martins Pinto, P. Paumard, C. Bouchez, S. Ransac, S. Duvezin-Caubet, J.P. Mazat, et al., The Warburg effect and mitochondrial oxidative phosphorylation: friends or foes? Biochim. Biophys. Acta Bioenerg. 1864 (1) (2023) 148931, https://doi.org/10.1016/j.bbabio.2022.148931. Epub 2022 Oct 29.
[42] P.P. Slonimski, T.G. Cooper, R.C. von Borstel, P. Piotr, Slonimski—The Warrior Pope: the discovery of mitochondrial (petite) mutants and split genes, fow004, FEMS Yeast Res. 16 (2) (2016), https://doi.org/10.1093/femsyr/fow004
[43] S. Chen, B. Ephrussi, H. Hottinguer, Nature génétique des mutants à déficience respiratoire de la souche B-11 de la le levure de boulangerie [Genetic nature of mutants lacking respiratory enzymes in the B-11 strain of baker's yeast] (Undetermined Language), Heredity (Edinb.) 4 (3) (1950) 337–351, https://doi.org/10.1038/hdy.1950.26
[44] M.M. Nass, S. Nass, Intramitochondrial fibers with dna characteristics. I. Fixation and electron staining reactions, J. Cell Biol. 19 (3) (1963) 593–611, https://doi.org/10.1083/jcb.19.3.593
[45] S. Nass, M.M. Nass, Intramitochondrial fibers with dna characteristics. II. Enzymatic and other hydrolytic treatments, J. Cell Biol. 19 (3) (1963) 613–629, https://doi.org/10.1083/jcb.19.3.613
[46] G. Schatz, E. Haslbrunner, H. Tuppy, Deoxyribonucleic acid associated with yeast mitochondria, Biochem. Biophys. Res. Commun. 15 (2) (1964) 127–132, https://doi.org/10.1016/0006-291x(64)90311-0
[47] A. Tzagoloff, C.L. Dieckmann, PET genes of Saccharomyces cerevisiae, Microbiol. Rev. 54 (3) (1990) 211–225, https://doi.org/10.1128/mr.54.3.211-225.1990
[48] S. Kelly, The economics of organellar gene loss and endosymbiotic gene transfer, Genome Biol. 22 (1) (2021) 345, https://doi.org/10.1186/s13059-021-02567-w
[49] S. Anderson, A.T. Bankier, B.G. Barrell, M.H. de Bruijn, A.R. Coulson, J. Drouin, et al., Sequence and organization of the human mitochondrial genome, Nature 290 (5806) (1981) 457–465, https://doi.org/10.1038/290457a0

[50] A. Jacquier, B. Dujon, An intron-encoded protein is active in a gene conversion process that spreads an intron into a mitochondrial gene, Cell 41 (2) (1985) 383–394, https://doi.org/10.1016/s0092-8674(85)80011-8

[51] I.G. Macreadie, R.M. Scott, A.R. Zinn, R.A. Butow, Transposition of an intron in yeast mitochondria requires a protein encoded by that intron, Cell 41 (2) (1985) 395–402, https://doi.org/10.1016/s0092-8674(85)80012-x

[52] E. Cavdar Koc, W. Burkhart, K. Blackburn, A. Moseley, L.L. Spremulli, The small subunit of the mammalian mitochondrial ribosome. Identification of the full complement of ribosomal proteins present, J. Biol. Chem. 276 (22) (2001) 19363–19374, https://doi.org/10.1074/jbc.M100727200

[53] H.R. Graack, M.L. Bryant, T.W. O'Brien, Identification of mammalian mitochondrial ribosomal proteins (MRPs) by N-terminal sequencing of purified bovine MRPs and comparison to data bank sequences: the large subribosomal particle, Biochemistry 38 (50) (1999) 16569–16577, https://doi.org/10.1021/bi991543s

[54] T. Suzuki, M. Terasaki, C. Takemoto-Hori, T. Hanada, T. Ueda, A. Wada, et al., Structural compensation for the deficit of rRNA with proteins in the mammalian mitochondrial ribosome. Systematic analysis of protein components of the large ribosomal subunit from mammalian mitochondria, J. Biol. Chem. 276 (24) (2001) 21724–21736, https://doi.org/10.1074/jbc.M100432200

[55] A. Amunts, A. Brown, J. Toots, S.H.W. Scheres, V. Ramakrishnan, Ribosome. The structure of the human mitochondrial ribosome, Science 348 (6230) (2015) 95–98, https://doi.org/10.1126/science.aaa1193

[56] T. Suzuki, Y. Yashiro, I. Kikuchi, Y. Ishigami, H. Saito, I. Matsuzawa, et al., Complete chemical structures of human mitochondrial tRNAs, Nat. Commun. 11 (1) (2020) 4269, https://doi.org/10.1038/s41467-020-18068-6

[57] D. Ojala, J. Montoya, G. Attardi, tRNA punctuation model of RNA processing in human mitochondria, Nature 290 (5806) (1981) 470–474, https://doi.org/10.1038/290470a0

[58] J. Montoya, D. Ojala, G. Attardi, Distinctive features of the 5'-terminal sequences of the human mitochondrial mRNAs, Nature 290 (5806) (1981) 465–470, https://doi.org/10.1038/290465a0

[59] J. Montoya, T. Christianson, D. Levens, M. Rabinowitz, G. Attardi, Identification of initiation sites for heavy-strand and light-strand transcription in human mitochondrial DNA, Proc. Natl. Acad. Sci. U. S. A. 79 (23) (1982) 7195–7199, https://doi.org/10.1073/pnas.79.23.7195

[60] R. Okimoto, H.M. Chamberlin, J.L. Macfarlane, D.R. Wolstenholme, Repeated sequence sets in mitochondrial DNA molecules of root knot nematodes (Meloidogyne): nucleotide sequences, genome location and potential for host-race identification, Nucleic Acids Res. 19 (7) (1991) 1619–1626, https://doi.org/10.1093/nar/19.7.1619

[61] R.J. Hoffmann, J.L. Boore, W.M. Brown, A novel mitochondrial genome organization for the blue mussel, Mytilus edulis, Genetics 131 (2) (1992) 397–412, https://doi.org/10.1093/genetics/131.2.397

[62] R. Benne, J. Van den Burg, J.P. Brakenhoff, P. Sloof, J.H. Van Boom, M.C. Tromp, Major transcript of the frameshifted coxII gene from trypanosome mitochondria contains four nucleotides that are not encoded in the DNA, Cell 46 (6) (1986) 819–826, https://doi.org/10.1016/0092-8674(86)90063-2

[63] J.M. Shaw, J.E. Feagin, K. Stuart, L. Simpson, Editing of kinetoplastid mitochondrial mRNAs by uridine addition and deletion generates conserved amino acid sequences and AUG initiation codons, Cell 53 (3) (1988) 401–411, https://doi.org/10.1016/0092-8674(88)90160-2

[64] J.E. Feagin, J.M. Abraham, K. Stuart, Extensive editing of the cytochrome c oxidase III transcript in Trypanosoma brucei, Cell 53 (3) (1988) 413–422, https://doi.org/10.1016/0092-8674(88)90161-4

[65] B. Kaur, K. Záhonová, M. Valach, D. Faktorová, G. Prokopchuk, G. Burger, et al., Gene fragmentation and RNA editing without borders: eccentric mitochondrial genomes of diplonemids, Nucleic Acids Res. 48 (5) (2020) 2694–2708, https://doi.org/10.1093/nar/gkz1215

[66] P. Mariottini, A. Chomyn, G. Attardi, D. Trovato, D.D. Strong, R.F. Doolittle, Antibodies against synthetic peptides reveal that the unidentified reading frame A6L, overlapping the ATPase 6 gene, is expressed in human mitochondria, Cell 32 (4) (1983) 1269–1277, https://doi.org/10.1016/0092-8674(83)90308-2

[67] I.M. Fearnley, J.E. Walker, Two overlapping genes in bovine mitochondrial DNA encode membrane components of ATP synthase, EMBO J. 5 (8) (1986) 2003–2008, https://doi.org/10.1002/j.1460-2075.1986.tb04456.x

[68] G. Attardi, A. Chomyn, R.F. Doolittle, P. Mariottini, C.I. Ragan, Seven unidentified reading frames of human mitochondrial DNA encode subunits of the respiratory chain NADH dehydrogenase, Cold Spring Harb. Symp. Quant. Biol. 51 (Pt 1) (1986) 103–114, https://doi.org/10.1101/sqb.1986.051.01.013

[69] D.A. Clayton, Replication of animal mitochondrial DNA, Cell 28 (4) (1982) 693–705, https://doi.org/10.1016/0092-8674(82)90049-6

[70] J.L. Pohjoismäki, J.B. Holmes, S.R. Wood, M.Y. Yang, T. Yasukawa, A. Reyes, et al., Mammalian mitochondrial DNA replication intermediates are essentially duplex but contain extensive tracts of RNA/DNA hybrid, J. Mol. Biol. 397 (5) (2010) 1144–1155, https://doi.org/10.1016/j.jmb.2010.02.029

[71] T. Yasukawa, D. Kang, An overview of mammalian mitochondrial DNA replication mechanisms, J. Biochem. 164 (3) (2018) 183–193, https://doi.org/10.1093/jb/mvy058

[72] G. Burger, M.W. Gray, L. Forget, B.F. Lang, Strikingly bacteria-like and gene-rich mitochondrial genomes throughout jakobid protists, Genome Biol. Evol. 5 (2) (2013) 418–438, https://doi.org/10.1093/gbe/evt008

[73] N. Lecrenier, P. Van Der Bruggen, F. Foury, D.N.A. Mitochondrial, polymerases from yeast to man: a new family of polymerases, Gene 185 (1) (1997) 147–152, https://doi.org/10.1016/s0378-1119(96)00663-4

[74] M.T. Oliveira, J. Haukka, L.S. Kaguni, Evolution of the metazoan mitochondrial replicase, Genome Biol. Evol. 7 (4) (2015) 943–959, https://doi.org/10.1093/gbe/evv042

[75] L. Fan, S. Kim, C.L. Farr, K.T. Schaefer, K.M. Randolph, J.A. Tainer, et al., A novel processive mechanism for DNA synthesis revealed by structure, modeling and mutagenesis of the accessory subunit of human mitochondrial DNA polymerase, J. Mol. Biol. 358 (5) (2006) 1229–1243, https://doi.org/10.1016/j.jmb.2006.02.073

[76] T.E. Shutt, M.W.T. Gray, the mitochondrial replicative DNA helicase, is widespread in the eukaryotic radiation and may also be the mitochondrial DNA primase in most eukaryotes, J. Mol. Evol. 62 (5) (2006) 588–599, https://doi.org/10.1007/s00239-005-0162-8

[77] J.J. Arnold, E.D. Smidansky, I.M. Moustafa, C.E. Cameron, Human mitochondrial RNA polymerase: structure-function, mechanism and inhibition, Biochim. Biophys. Acta 1819 (9–10) (2012) 948–960, https://doi.org/10.1016/j.bbagrm.2012.04.002

[78] W. Neupert, J.M. Herrmann, Translocation of proteins into mitochondria, Annu. Rev. Biochem. 76 (2007) 723–749, https://doi.org/10.1146/annurev.biochem.76.052705.163409

[79] A.J. Roger, S.A. Muñoz-Gómez, R. Kamikawa, The Origin and Diversification of Mitochondria, Curr. Biol. 27 (21) (2017) R1177–R1192, https://doi.org/10.1016/j.cub.2017.09.015

[80] L. Horváthová, V. Žárský, T. Pánek, R. Derelle, J. Pyrih, A. Motyčková, et al., Analysis of diverse eukaryotes suggests the existence of an ancestral mitochondrial apparatus derived from the bacterial type II secretion system, Nat. Commun. 12 (1) (2021) 2947, https://doi.org/10.1038/s41467-021-23046-7

[81] S. Breton, D.T. Stewart, Atypical mitochondrial inheritance patterns in eukaryotes, Genome 58 (10) (2015) 423–431, https://doi.org/10.1139/gen-2015-0090

[82] E. Zouros, A. Oberhauser Ball, C. Saavedra, K.R. Freeman, An unusual type of mitochondrial DNA inheritance in the blue mussel Mytilus, Proc. Natl. Acad. Sci. U. S. A. 91 (16) (1994) 7463–7467, https://doi.org/10.1073/pnas.91.16.7463

[83] H. Kaneda, J. Hayashi, S. Takahama, C. Taya, K.F. Lindahl, H. Yonekawa, Elimination of paternal mitochondrial DNA in intraspecific crosses during early mouse embryogenesis, Proc. Natl. Acad. Sci. U. S. A. 92 (10) (1995) 4542–4546, https://doi.org/10.1073/pnas.92.10.4542

[84] Y. Politi, L. Gal, Y. Kalifa, L. Ravid, Z. Elazar, E. Arama, Paternal mitochondrial destruction after fertilization is mediated by a common endocytic and autophagic pathway in Drosophila, Dev. Cell 29 (3) (2014) 305–320, https://doi.org/10.1016/j.devcel.2014.04.005

[85] P. Sutovsky, R.D. Moreno, J. Ramalho-Santos, T. Dominko, C. Simerly, G. Schatten, Ubiquitinated sperm mitochondria, selective proteolysis, and the regulation of mitochondrial inheritance in mammalian embryos, Biol. Reprod. 63 (2) (2000) 582–590, https://doi.org/10.1095/biolreprod63.2.582

[86] Y. Nishimura, T. Yoshinari, K. Naruse, T. Yamada, K. Sumi, H. Mitani, et al., Active digestion of sperm mitochondrial DNA in single living sperm revealed by optical tweezers, Proc. Natl. Acad. Sci. U. S. A. 103 (5) (2006) 1382–1387, https://doi.org/10.1073/pnas.0506911103

[87] L.M. Cree, D.C. Samuels, S.C. De Sousa Lopes, H.K. Rajasimha, P. Wonnapinij, J.R. Mann, et al., A reduction of mitochondrial DNA molecules during embryogenesis explains the rapid segregation of genotypes, Nat. Genet. 40 (2) (2008) 249–254, https://doi.org/10.1038/ng.2007.63

[88] T. Wai, D. Teoli, E.A. Shoubridge, The mitochondrial DNA genetic bottleneck results from replication of a subpopulation of genomes, Nat. Genet. 40 (12) (2008) 1484–1488, https://doi.org/10.1038/ng.258

[89] M.V. Ashley, P.J. Laipis, W.W. Hauswirth, Rapid segregation of heteroplasmic bovine mitochondria, Nucleic Acids Res. 17 (18) (1989) 7325–7331, https://doi.org/10.1093/nar/17.18.7325

[90] M. Ancora, M. Orsini, A. Colosimo, V. Russo, M. Marcacci, M. De Santo, et al., Mitochondrial heteroplasmy profiling in single human oocytes by next-generation sequencing, Mitochondrial DNA B Resour. 2 (2) (2017) 542–543, https://doi.org/10.1080/23802359.2017.1365634

[91] J.B. Stewart, C. Freyer, J.L. Elson, A. Wredenberg, Z. Cansu, A. Trifunovic, et al., Strong purifying selection in transmission of mammalian mitochondrial DNA, PLoS Biol. 6 (1) (2008) e10, https://doi.org/10.1371/journal.pbio.0060010

[92] R. Luft, D. Ikkos, G. Palmieri, L. Ernster, B. Afzelius, A case of severe hypermetabolism of nonthyroid origin with a defect in the maintenance of mitochondrial respiratory control: a correlated clinical, biochemical, and morphological study, J. Clin. Invest. 41 (9) (1962) 1776–1804, https://doi.org/10.1172/JCI104637

[93] J.B. Clark, D.J. Hayes, J.A. Morgan-Hughes, E. Byrne, Mitochondrial myopathies: disorders of the respiratory chain and oxidative phosphorylation, J. Inherit. Metab. Dis. 7 (Suppl. 1) (1984) 62–68, https://doi.org/10.1007/BF03047377

[94] S. DiMauro, E. Bonilla, M. Zeviani, M. Nakagawa, D.C. DeVivo, Mitochondrial myopathies, Ann. Neurol. 17 (6) (1985) 521–538, https://doi.org/10.1002/ana.410170602

[95] I.J. Holt, A.E. Harding, J.A. Morgan-Hughes, Deletions of muscle mitochondrial DNA in patients with mitochondrial myopathies, Nature 331 (6158) (1988) 717–719, https://doi.org/10.1038/331717a0

[96] J.M. Shoffner, M.T. Lott, A.M. Lezza, P. Seibel, S.W. Ballinger, D.C. Wallace, Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA tRNA(Lys) mutation, Cell 61 (6) (1990) 931–937, https://doi.org/10.1016/0092-8674(90)90059-n

[97] Y. Goto, I. Nonaka, S. Horai, A mutation in the tRNA(Leu)(UUR) gene associated with the MELAS subgroup of mitochondrial encephalomyopathies, Nature 348 (6302) (1990) 651–653, https://doi.org/10.1038/348651a0

[98] D.C. Wallace, G. Singh, M.T. Lott, J.A. Hodge, T.G. Schurr, A.M. Lezza, et al., Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy, Science 242 (4884) (1988) 1427–1430, https://doi.org/10.1126/science.3201231

[99] T.R. Prezant, J.V. Agapian, M.C. Bohlman, X. Bu, S. Oztas, W.Q. Qiu, et al., Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness, Nat. Genet. 4 (3) (1993) 289–294, https://doi.org/10.1038/ng0793-289

[100] M. Zeviani, N. Bresolin, C. Gellera, A. Bordoni, M. Pannacci, P. Amati, et al., Nucleus-driven multiple large-scale deletions of the human mitochondrial genome: a new autosomal dominant disease, Am. J. Hum. Genet. 47 (6) (1990) 904–914.

[101] A. Suomalainen, A. Majander, M. Haltia, H. Somer, J. Lönnqvist, M.L. Savontaus, et al., Multiple deletions of mitochondrial DNA in several tissues of a patient with severe retarded depression and familial progressive external ophthalmoplegia, J. Clin. Invest. 90 (1) (1992) 61–66, https://doi.org/10.1172/JCI115856

[102] E. Ricci, C.T. Moraes, S. Servidei, P. Tonali, E. Bonilla, S. DiMauro, Disorders associated with depletion of mitochondrial DNA, Brain Pathol. 2 (2) (1992) 141–147, https://doi.org/10.1111/j.1750-3639.1992.tb00682.x

[103] G. Van Goethem, B. Dermaut, A. Löfgren, J.J. Martin, C. Van Broeckhoven, Mutation of POLG is associated with progressive external ophthalmoplegia characterized by mtDNA deletions, Nat. Genet. 28 (3) (2001) 211–212, https://doi.org/10.1038/90034

[104] J.N. Spelbrink, F.Y. Li, V. Tiranti, K. Nikali, Q.P. Yuan, M. Tariq, et al., Human mitochondrial DNA deletions associated with mutations in the gene encoding Twinkle, a phage T7 gene 4-like protein localized in mitochondria, Nat. Genet. 28 (3) (2001) 223–231, https://doi.org/10.1038/90058

[105] A. Diaz-Vegas, P. Sanchez-Aguilera, J.R. Krycer, P.E. Morales, M. Monsalves-Alvarez, M. Cifuentes, et al., Is mitochondrial dysfunction a common root of non-communicable chronic diseases? bnaa005, Endocr. Rev. 41 (3) (2020), https://doi.org/10.1210/endrev/bnaa005

[106] D.A. Chistiakov, T.P. Shkurat, A.A. Melnichenko, A.V. Grechko, A.N. Orekhov, The role of mitochondrial dysfunction in cardiovascular disease: a brief review, Ann. Med. 50 (2) (2018) 121–127, https://doi.org/10.1080/07853890.2017.1417631

[107] J.J. Collier, M. Oláhová, T.G. McWilliams, R.W. Taylor, Mitochondrial signalling and homeostasis: from cell biology to neurological disease, Trends Neurosci. 46 (2) (2023) 137–152, https://doi.org/10.1016/j.tins.2022.12.001

[108] J.B. Spinelli, M.C. Haigis, The multifaceted contributions of mitochondria to cellular metabolism, Nat. Cell Biol. 20 (7) (2018) 745–754, https://doi.org/10.1038/s41556-018-0124-1

[109] N.S. Chandel, Mitochondria as signaling organelles, BMC Biol. 12 (2014) 34, https://doi.org/10.1186/1741-7007-12-34

[110] D. Hockenbery, G. Nuñez, C. Milliman, R.D. Schreiber, S.J. Korsmeyer, Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death, Nature 348 (6299) (1990) 334–336, https://doi.org/10.1038/348334a0

[111] A. Gross, J.M. McDonnell, S.J. Korsmeyer, BCL-2 family members and the mitochondria in apoptosis, Genes. Dev. 13 (15) (1999) 1899–1911, https://doi.org/10.1101/gad.13.15.1899

[112] M.J. Munson, B.J. Mathai, M.Y.W. Ng, L. Trachsel-Moncho, L.R. De La Ballina, A. Simonsen, GAK and PRKCD kinases regulate basal mitophagy, Autophagy 18 (2) (2022) 467–469, https://doi.org/10.1080/15548627.2021.2015154

[113] T.M. Durcan, E.A. Fon, The three 'P's of mitophagy: PARKIN, PINK1, and post-translational modifications, Genes. Dev. 29 (10) (2015) 989–999, https://doi.org/10.1101/gad.262758.115

[114] G. Twig, B. Hyde, O.S. Shirihai, Mitochondrial fusion, fission and autophagy as a quality control axis: the bioenergetic view, Biochim. Biophys. Acta 1777 (9) (2008) 1092–1097, https://doi.org/10.1016/j.bbabio.2008.05.001

[115] M. Neuspiel, A.C. Schauss, E. Braschi, R. Zunino, P. Rippstein, R.A. Rachubinski, et al., Cargo-selected transport from the mitochondria to peroxisomes is mediated by vesicular carriers, Curr. Biol. 18 (2) (2008) 102–108, https://doi.org/10.1016/j.cub.2007.12.038

[116] A. Das, S. Nag, A.B. Mason, M.M. Barroso, Endosome-mitochondria interactions are modulated by iron release from transferrin, J. Cell Biol. 214 (7) (2016) 831–845, https://doi.org/10.1083/jcb.201602069

[117] S. Nagashima, L.C. Tábara, L. Tilokani, V. Paupe, H. Anand, J.H. Pogson, et al., Golgi-derived PI**(4)**P-containing vesicles drive late steps of mitochondrial division, Science 367 (6484) (2020) 1366–1371, https://doi.org/10.1126/science.aax6089

[118] T. Hayashi, R. Rizzuto, G. Hajnoczky, T.P. Su, MAM: more than just a housekeeper, Trends Cell Biol. 19 (2) (2009) 81–88, https://doi.org/10.1016/j.tcb.2008.12.002

[119] Y. Oku, M. Kariya, T. Fujimura, J. Hoseki, Y. Sakai, Homeostasis of the ER redox state subsequent to proteasome inhibition, Sci. Rep. 11 (1) (2021) 8655, https://doi.org/10.1038/s41598-021-87944-y

[120] A. Sugiura, G.L. McLelland, E.A. Fon, H.M. McBride, A new pathway for mitochondrial quality control: mitochondrial-derived vesicles, EMBO J. 33 (19) (2014) 2142–2156, https://doi.org/10.15252/embj.201488104

[121] R.L. Frederick, J.M. Shaw, Moving mitochondria: establishing distribution of an essential organelle, Traffic 8 (12) (2007) 1668–1675, https://doi.org/10.1111/j.1600-0854.2007.00644.x

[122] A. Lee, Y. Hirabayashi, S.K. Kwon, T.L. Lewis Jr, F. Polleux, Emerging roles of mitochondria in synaptic transmission and neurodegeneration, Curr. Opin. Physiol. 3 (2018) 82–93, https://doi.org/10.1016/j.cophys.2018.03.009

[123] T.E. Gunter, L. Buntinas, G. Sparagna, R. Eliseev, K. Gunter, Mitochondrial calcium transport: mechanisms and functions, Cell Calcium 28 (5–6) (2000) 285–296, https://doi.org/10.1054/ceca.2000.0168

[124] S. Feno, R. Rizzuto, A. Raffaello, Vecellio, D. Reane, The molecular complexity of the mitochondrial calcium uniporter, Cell Calcium 93 (2021) 102322, https://doi.org/10.1016/j.ceca.2020.102322

[125] M. Szibor, Z. Gizatullina, T. Gainutdinov, T. Endres, G. Debska-Vielhaber, M. Kunz, et al., but not matrix, calcium is essential for adjustment of mitochondrial pyruvate supply, J. Biol. Chem. 295 (14) (2020) 4383–4397, https://doi.org/10.1074/jbc.RA119.011902

[126] X. Pan, J. Liu, T. Nguyen, C. Liu, J. Sun, Y. Teng, et al., The physiological role of mitochondrial calcium revealed by mice lacking the mitochondrial calcium uniporter, Nat. Cell Biol. 15 (12) (2013) 1464–1472, https://doi.org/10.1038/ncb2868

[127] K.R. Pryde, J. Hirst, Superoxide is produced by the reduced flavin in mitochondrial complex I: a single, unified mechanism that applies during both forward and reverse

electron transfer, J. Biol. Chem. 286 (20) (2011) 18056–18065, https://doi.org/10.1074/jbc.M110.186841

[128] B. Jiménez-Gómez, P. Ortega-Sáenz, L. Gao, P. González-Rodríguez, P. García-Flores, N. Chandel, et al., Transgenic NADH dehydrogenase restores oxygen regulation of breathing in mitochondrial complex I-deficient mice, Nat. Commun. 14 (1) (2023) 1172, https://doi.org/10.1038/s41467-023-36894-2

[129] N. Sommer, N. Alebrahimdehkordi, O. Pak, F. Knoepp, I. Strielkov, S. Scheibe, et al., Bypassing mitochondrial complex III using alternative oxidase inhibits acute pulmonary oxygen sensing, Sci. Adv. 6 (16) (2020) eaba0694.

[130] E. Mick, D.V. Titov, O.S. Skinner, R. Sharma, A.A. Jourdain, V.K. Mootha, Distinct mitochondrial defects trigger the integrated stress response depending on the metabolic state of the cell, Elife 9 (2020) e49178, https://doi.org/10.7554/eLife.49178

[131] C.F. Bennett, P. Latorre-Muro, P. Puigserver, Mechanisms of mitochondrial respiratory adaptation, Nat. Rev. Mol. Cell Biol. 23 (12) (2022) 817–835, https://doi.org/10.1038/s41580-022-00506-6

[132] C.M. Klinge, Estrogenic control of mitochondrial function, Redox Biol. 31 (2020) 101435, https://doi.org/10.1016/j.redox.2020.101435

[133] F. Cioffi, A. Giacco, F. Goglia, E. Silvestri, Bioenergetic aspects of mitochondrial actions of thyroid hormones, Cells 11 (6) (2022) 997, https://doi.org/10.3390/cells11060997

[134] G. Refolo, T. Vescovo, M. Piacentini, G.M. Fimia, F. Ciccosanti, Mitochondrial interactome: a focus on antiviral signaling pathways, Front. Cell Dev. Biol. 8 (2020) 8, https://doi.org/10.3389/fcell.2020.00008

[135] M. Sorouri, T. Chang, D.C. Hancks, Mitochondria and viral infection: advances and emerging battlefronts, mBio 13 (1) (2022) e0209621, https://doi.org/10.1128/mbio.02096-21

[136] H.A. Saffran, J.M. Pare, J.A. Corcoran, S.K. Weller, J.R. Smiley, Herpes simplex virus eliminates host mitochondrial DNA, EMBO Rep. 8 (2) (2007) 188–193, https://doi.org/10.1038/sj.embor.7400878

[137] R. Ryback, A. Eirin, Mitochondria, a missing link in COVID-19 heart failure and arrest, Front. Cardiovasc. Med. 8 (2022) 830024, https://doi.org/10.3389/fcvm.2021.830024

[138] C. Bonnet, V. Kaltimbacher, S. Ellouze, S. Augustin, P. Bénit, V. Forster, et al., Allotopic mRNA localization to the mitochondrial surface rescues respiratory chain defects in fibroblasts harboring mitochondrial DNA mutations affecting complex I or v subunits, Rejuvenation Res. 10 (2) (2007) 127–144, https://doi.org/10.1089/rej.2006.0526

[139] U. Zekonyte, S.R. Bacman, J. Smith, W. Shoop, C.V. Pereira, G. Tomberlin, et al., Mitochondrial targeted meganuclease as a platform to eliminate mutant mtDNA in vivo, Nat. Commun. 12 (1) (2021) 3210, https://doi.org/10.1038/s41467-021-23561-7

[140] B. Pantic, D. Ives, M. Mennuni, D. Perez-Rodriguez, U. Fernandez-Pelayo, A. Lopez de Arbina, et al., 2-Deoxy-D-glucose couples mitochondrial DNA replication with mitochondrial fitness and promotes the selection of wild-type over mutant mitochondrial DNA, Nat. Commun. 12 (1) (2021) 6997, https://doi.org/10.1038/s41467-021-26829-0

[141] J. Smeitink, R. Van Maanen, L. de Boer, G. Ruiterkamp, H. Renkema, A randomised placebo-controlled, double-blind phase II study to explore the safety, efficacy, and pharmacokinetics of sonlicromanol in children with genetically confirmed mitochondrial disease and motor symptoms ("KHENERGYC"), BMC Neurol. 22 (1) (2022) 158, https://doi.org/10.1186/s12883-022-02685-3

[142] M. Herbert, D. Turnbull, Progress in mitochondrial replacement therapies, Nat. Rev. Mol. Cell Biol. 19 (2) (2018) 71–72, https://doi.org/10.1038/nrm.2018.3

[143] G. Hudson, Y. Takeda, M. Herbert, Reversion after replacement of mitochondrial DNA, Nature 574 (7778) (2019) E8–E11, https://doi.org/10.1038/s41586-019-1623-3
[144] M. Szibor, C. Schenkl, M.R.O. Barsottini, L. Young, A.L. Moore, Targeting the alternative oxidase (AOX) for human health and food security, a pharmaceutical and agrochemical target or a rescue mechanism? Biochem. J. 479 (12) (2022) 1337–1359, https://doi.org/10.1042/BCJ20180192
[145] A.R. Hanaford, Y.J. Cho, H. Nakai, AAV-vector based gene therapy for mitochondrial disease: progress and future perspectives, Orphanet J. Rare Dis. 17 (1) (2022) 217, https://doi.org/10.1186/s13023-022-02324-7
[146] J. Schwemmlein, C. Maack, E. Bertero, Mitochondria as therapeutic targets in heart failure, Curr. Heart Fail. Rep. 19 (2) (2022) 27–37, https://doi.org/10.1007/s11897-022-00539-0
[147] Y. Liu, Y. Shi, Mitochondria as a target in cancer treatment, MedComm l (2) (2020) 129–139, https://doi.org/10.1002/mco2.16
[148] J. Xu, W. Du, Y. Zhao, K. Lim, L. Lu, C. Zhang, et al., Mitochondria targeting drugs for neurodegenerative diseases—design, mechanism and application, Acta Pharm. Sin. B. 12 (6) (2022) 2778–2789, https://doi.org/10.1016/j.apsb.2022.03.001
[149] L. Monzote, L. Gille, Mitochondria as a promising antiparasitic target, Curr. Clin. Pharmacol. 5 (1) (2010) 55–60, https://doi.org/10.2174/157488410790410605
[150] L. Berná, N. Rego, M.E. Francia, The elusive mitochondrial genomes of apicomplexa: where are we now? Front. Microbiol. 12 (2021) 751775, https://doi.org/10.3389/fmicb.2021.751775
[151] C. Parrot, L. Moulinier, F. Bernard, Y. Hashem, D. Dupuy, M. Sissler, Peculiarities of aminoacyl-tRNA synthetases from trypanosomatids, J. Biol. Chem. 297 (2) (2021) 100913, https://doi.org/10.1016/j.jbc.2021.100913
[152] L. Rajappa-Titu, T. Suematsu, P. Munoz-Tello, M. Long, Ö. Demir, K.J. Cheng, et al., RNA Editing TUTase 1: structural foundation of substrate recognition, complex interactions and drug targeting, Nucleic Acids Res. 44 (22) (2016) 10862–10878, https://doi.org/10.1093/nar/gkw917
[153] R.L. Cann, M. Stoneking, A.C. Wilson, Mitochondrial DNA and human evolution, Nature 325 (6099) (1987) 31–36, https://doi.org/10.1038/325031a0
[154] R.W. Byard, The execution of the Romanov family at Yekatarinberg, Forensic Sci. Med. Pathol. 16 (3) (2020) 552–556, https://doi.org/10.1007/s12024-020-00218-3
[155] T.E. King, G.G. Fortes, P. Balaresque, M.G. Thomas, D. Balding, P. Maisano Delser, et al., Identification of the remains of King Richard III, Nat. Commun. 5 (2014) 5631, https://doi.org/10.1038/ncomms6631
[156] D. Chrétien, P. Bénit, H.H. Ha, S. Keipert, R. El-Khoury, Y.T. Chang, et al., Mitochondria are physiologically maintained at close to 50 °C, PLoS Biol. 16 (1) (2018) e2003992, https://doi.org/10.1371/journal.pbio.2003992
[157] M. Nakano, Y. Arai, I. Kotera, K. Okabe, Y. Kamei, T. Nagai, Genetically encoded ratiometric fluorescent thermometer with wide range and rapid response, PLoS One 12 (2) (2017) e0172344, https://doi.org/10.1371/journal.pone.0172344
[158] J. Miquel, A.C. Economos, J. Fleming, J.E. Johnson Jr., Mitochondrial role in cell aging, Exp. Gerontol. 15 (6) (1980) 575–591, https://doi.org/10.1016/0531-5565(80)90010-8
[159] Y. Guo, T. Guan, K. Shafiq, Q. Yu, X. Jiao, D. Na, et al., Mitochondrial dysfunction in aging, Ageing Res. Rev. 88 (2023) 101955, https://doi.org/10.1016/j.arr.2023.101955
[160] A. Picca, F. Guerra, R. Calvani, H.J. Coelho-Júnior, C. Leeuwenburgh, C. Bucci, et al., The contribution of mitochondrial DNA alterations to aging, cancer, and neurodegeneration, Exp. Gerontol. 178 (2023) 112203, https://doi.org/10.1016/j.exger.2023.112203

[161] B. Castejon-Vega, M.D. Cordero, A. Sanz, How the disruption of mitochondrial redox signalling contributes to ageing, Antioxidant (Basel) 12 (4) (2023) 831, https://doi.org/10.3390/antiox12040831

[162] A. Trifunovic, A. Wredenberg, M. Falkenberg, J.N. Spelbrink, A.T. Rovio, C.E. Bruder, et al., Premature ageing in mice expressing defective mitochondrial DNA polymerase, Nature 429 (6990) (2004) 417–423, https://doi.org/10.1038/nature02517

[163] T.E.S. Kauppila, A. Bratic, M.B. Jensen, F. Baggio, L. Partridge, H. Jasper, et al., Mutations of mitochondrial DNA are not major contributors to aging of fruit flies, Proc. Natl. Acad. Sci. U. S. A. 115 (41) (2018) E9620–E9629, https://doi.org/10.1073/pnas.1721683115

[164] C.L. Samstag, J.G. Hoekstra, C.H. Huang, M.J. Chaisson, R.J. Youle, S.R. Kennedy, et al., Deleterious mitochondrial DNA point mutations are overrepresented in Drosophila expressing a proofreading-defective DNA polymerase γ, PLoS Genet. 14 (11) (2018) e1007805, https://doi.org/10.1371/journal.pgen.1007805

[165] S. Andreazza, C.L. Samstag, A. Sanchez-Martinez, E. Fernandez-Vizarra, A. Gomez-Duran, J.J. Lee, et al., Mitochondrially-targeted APOBEC1 is a potent mtDNA mutator affecting mitochondrial function and organismal fitness in Drosophila, Nat. Commun. 10 (1) (2019) 3280, https://doi.org/10.1038/s41467-019-10857-y

[166] M. Vermulst, J. Wanagat, G.C. Kujoth, J.H. Bielas, P.S. Rabinovitch, T.A. Prolla, et al., DNA deletions and clonal mutations drive premature aging in mitochondrial mutator mice, Nat. Genet. 40 (4) (2008) 392–394, https://doi.org/10.1038/ng.95

[167] R.H. Hämäläinen, J.C. Landoni, K.J. Ahlqvist, S. Goffart, S. Ryytty, M.O. Rahman, et al., Defects in mtDNA replication challenge nuclear genome stability through nucleotide depletion and provide a unifying mechanism for mouse progerias, Nat. Metab. 1 (10) (2019) 958–965, https://doi.org/10.1038/s42255-019-0120-1