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Review

The fate of mitochondrial respiratory complexes in aging

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While mitochondrial dysfunction is one of the canonical hallmarks of aging, it remains only vaguely defined. Its core feature embraces defects in energy-producing molecular machinery, the mitochondrial respiratory complexes (MRCs). The causes and consequences of these defects hold research attention. In this review, we assess the lifecycle of respiratory complexes, from biogenesis to degradation, and look closely at the mechanisms that could underpin their dysfunction in aged cells. We discuss how these processes could be altered by aging and expand on the fate of MRCs in age-associated pathologies. Given the complexity behind MRC maintenance and functionality, several traits could contribute to the phenomenon known as age-associated mitochondrial dysfunction. New advances will help us better understand the fate of this machinery in aging and age-related diseases.

Highlights

Mitochondrial dysfunction is a key hallmark of aging, with a core feature embracing the defects in the oxidative phosphorylation (OXPHOS) system.

The origins and consequences of age-associated OXPHOS alteration are the focus of research.

The lifecycle of the OXPHOS system could be vulnerable to aging, with some traits (such as mtDNA mutations) playing a primary role.

Adjustments in OXPHOS functionality form part of the response to age-associated pathologies.

OXPHOS fate is heterogeneous across aged organs and cell types.

New methodological advances will accelerate development in the field and help to address outstanding questions.

Mitochondrial dysfunction as a hallmark of aging

Aging is a progressive decline in the ability to withstand stress and damage. Determining the mechanisms of aging presents a key challenge for medicine. The world's population is aging, and age constitutes the most significant risk factor for developing chronic diseases that diminish the quality of the final years of human life. The hallmarks of aging were proposed as a holistic framework to summarize the molecular and cellular mechanisms underlying age-associated decay, with mitochondrial dysfunction listed among them [1,2]. However, what is concealed under the broad term of 'age-related mitochondrial dysfunction' remains hazy.

Mitochondria are the multipotent energy-generating, biosynthetic, and signaling hubs of eukaryotic cells [3]. Many of these processes directly or indirectly involve the central metabolic machinery, **oxidative phosphorylation (OXPHOS)** system (see Glossary), formed by MRCs, ATP synthase, and additional elements (Box 1). It is not easy to separate its core function, energy production, from its other roles. To synthesize **ATP,** the OXPHOS system receives, converts, and discharges molecules implemented in global metabolism and signaling cascades [e.g., **electron carriers** and **reactive oxygen species (ROS)**]. It also shapes the chemical and physical features of mitochondria that dictate their functionality (e.g., **mitochondrial membrane potential; Δψ**). While energy generation is pivotal for tissues and organs with high energy demands, the extensive metabolism and signal transduction facilitated by this system are key to cell function and survival [3].

In this review, we closely examine the fate of the OXPHOS system during aging as an inherent part of age-associated mitochondrial dysfunction. Its primary attributes reported in the literature include reduced respiration, declined enzymatic activity of MRCs, decreased mitochondrial membrane potential, decreased ATP production, increased generation of ROS, and reduced expression of OXPHOS-related genes (Figure 1) [4]. All these features are integral to the formation, availability, and functionality of the same core machinery, **OXPHOS complexes** (Figure 2). The aforenamed manifestations are often counterintuitive (e.g., the relationship between low OXPHOS levels and increased ROS

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Box 1. Structure makes function

Mitochondrial bioenergetics is mediated by five protein complexes (OXPHOS complexes) and defined by three mutually interconnected terms: MRC, ETC and OXPHOS. Together, they describe the structure–function relationship between individual system components, their contribution to energy production, and their ability to wire electron-delivering metabolic pathways. The spatial distribution of OXPHOS components across mitochondrial subcompartments is an important determinant of the functionality of a system.

Structure

Each of the five OXPHOS complexes (CI–V) comprises multiple subunits with highly defined stoichiometric composition (Figure 2 in the main text). Subunits display modular organizations, with particular modules bearing specific functions. Protein components are complemented by highly reactive redox cofactors that warrant complex activity. Importantly, the entire machinery spans three highly separated mitochondrial subcompartments, with the major part embedded in the IMM and several parts protruding to the mitochondrial matrix and IMS, where they fulfill key biochemical functions.

How do we study the structure, composition, and dynamics of OXPHOS?

Cryo-electron microscopy can be used to determine the structure of a protein complex. Native gel-based analysis (dedicated BN-PAGE) traces the assembly, levels, and organization of individual subunits into complexes. Complexome profiling estimates the subunit composition of molecular assemblies. When combined with dynamic labeling methods, this provides insights into subunit turnover rates.

Function

The most recognized function of OXPHOS complexes is energy production. ATP is generated as an output of sequential and spatially organized events. Nutrients converted to electron carriers (as NADH) by various metabolic circuits [tricarboxylic acid (TCA) cycle, fatty acid oxidation, and amino acid and nucleotide metabolism] give rise to a stream of electrons wired through MRCs (CI–CIV). CII forms a critical connection with the TCA cycle. Electron relay via three of these complexes (CI, CIII, CIV) is coupled to proton pumping that builds the electrochemical gradient between two sides of highly impermeable membrane, ultimately fueling the ATP-generating motor (CV). Five complexes are not alone in this task and benefit from a unique architecture of mitochondrial membranes, two mobile electron carriers (ubiquinone and cytochrome c), and an additional set of dehydrogenases gathering electrons from consolidated pathways (e.g., ETFQOR, DHODH, and G3PDH).

How do we study the OXPHOS function?

Respirometry estimates the flow of electrons through the ETC to its final acceptor, oxygen. Changes in complex activities can be determined *in vitro* using colorimetric reactions in the presence of complex-specific substrates coupled with spectrophotometric or native gel-based readout.

production), further necessitating their careful evaluation. Here, we look at global OXPHOS biology, tracing step-by-step the hot spots in the biogenesis, maintenance, disposal, and functional adjustments of MRCs that could be impacted by aging or age-associated diseases. We also take the perspective of aging to pursue the heterogeneity of OXPHOS alterations in the human body. Understanding how progressing age impacts the OXPHOS system may reveal novel mechanisms that could help gain better control over aging.

Building energy-producing machines in the aging context

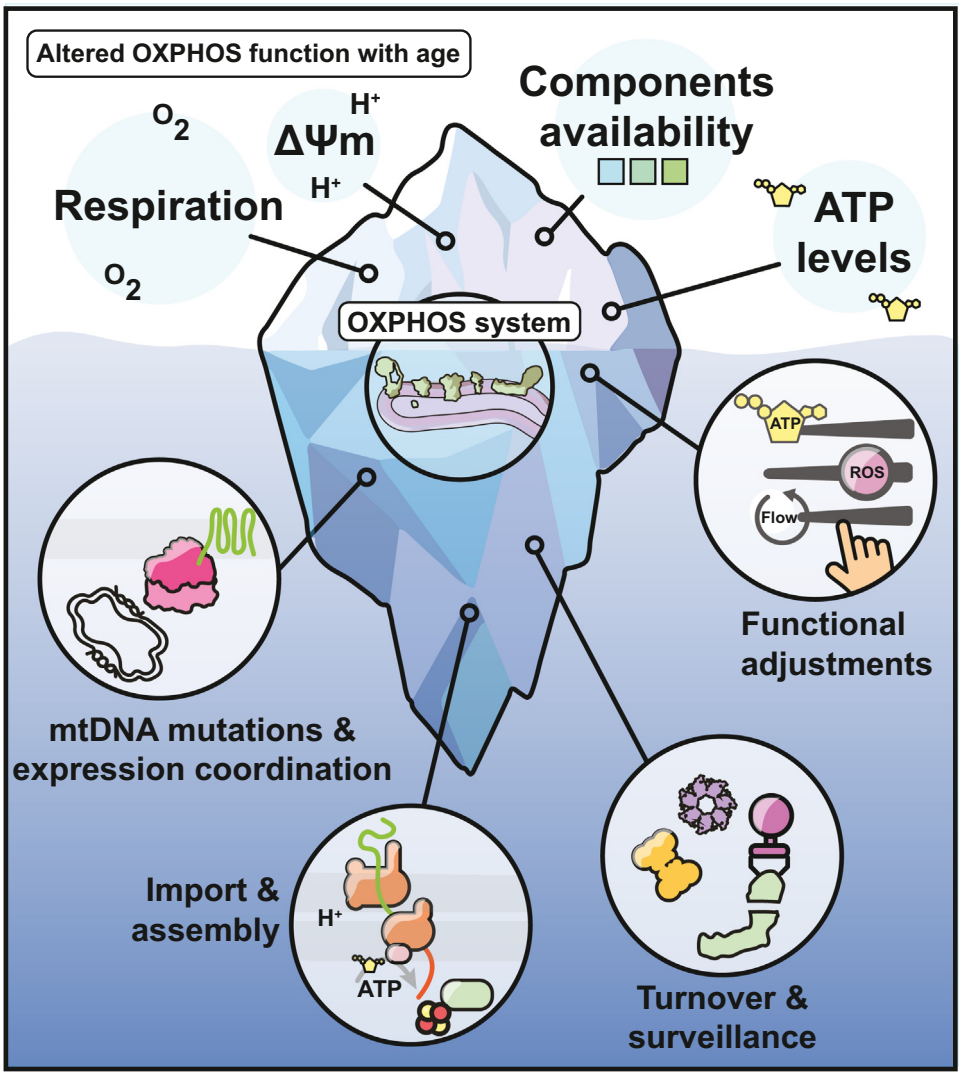
The molecular machinery that powers eukaryotic cells is a highly complex and multifunctional biological system. Why is OXPHOS complexity important in the context of aging? The high level of structural and spatial organization necessitates precise coordination of multiple processes, rendering this system theoretically prone to disruption by stochastic errors that accumulate during aging. Here, we describe and discuss several hot spots in the production of OXPHOS that are currently the focus of research and were proposed to be implicated in aging or age-related pathologies (Figures 1 and 3).

Coordination of mitonuclear gene expression

The first hot spot for plausible OXPHOS disruption emerges at the origin. The biogenesis of OXPHOS is unique in the world of protein biosynthesis because it requires a coordinated gene expression from two genomes, **nuclear (nDNA)** and **mitochondrial (mt)DNA** (Figure 2 and Box 1). This means that mitochondria maintain separate machinery to replicate, transcribe, and

Glossary

ATP: primary energy carrier in cells. Released energy supports many cellular functions, including protein degradation and folding.
Cofactors: nonprotein compounds that assist enzymes to catalyze reactions.
Cristae: IMM invaginations and the primary location of the OXPHOS system.
Electron carriers: molecules that enable electron transport through metabolic pathways and MRCs using redox reactions [including Coenzyme Q (CoQ), cytochrome C, and NAD^+/NADH].
Electron transport chain (ETC): involves the MRCs (CI–IV) and essential electron carriers. It delivers electrons to the terminal acceptor (O_2) and establishes the proton gradient across the IMM.
Heteroplasmy: presence of more than one type of mtDNA within a defined pool.
Inner mitochondrial membrane (IMM): encloses the mitochondrial matrix; it is where MRCs are located A physical determinant of membrane potential.
Intermembrane space (IMS): space between the inner and outer mitochondrial membranes.
Matrix: inner compartment of mitochondria surrounded by the IMM; contains multiple metabolic pathways, mtDNA, and mitoribosomes.
Mitochondrial biogenesis: self-renewal mechanism by which new mitochondria are generated from already-existing ones.
Mitochondrial integrated stress response (ISRmt): global multilayer program activated by mitochondrial deficiency.
Mitochondrial membrane potential (Δψ): the difference in electrical potential between two sides of the IMM and a form of energy store built up by proton-pumping MRCs and transformed to ATP by CV. It underpins the regulation of mitochondrial dynamics, protein import, and apoptosis.
Mitochondrial (mt)DNA: integral mitochondrial genetic information encoding several protein components of OXPHOS and the complete set of RNAs required by the mitochondrial gene expression system.
Mitohormesis: response to mild mitochondrial stress that augments resistance to subsequent disturbance.



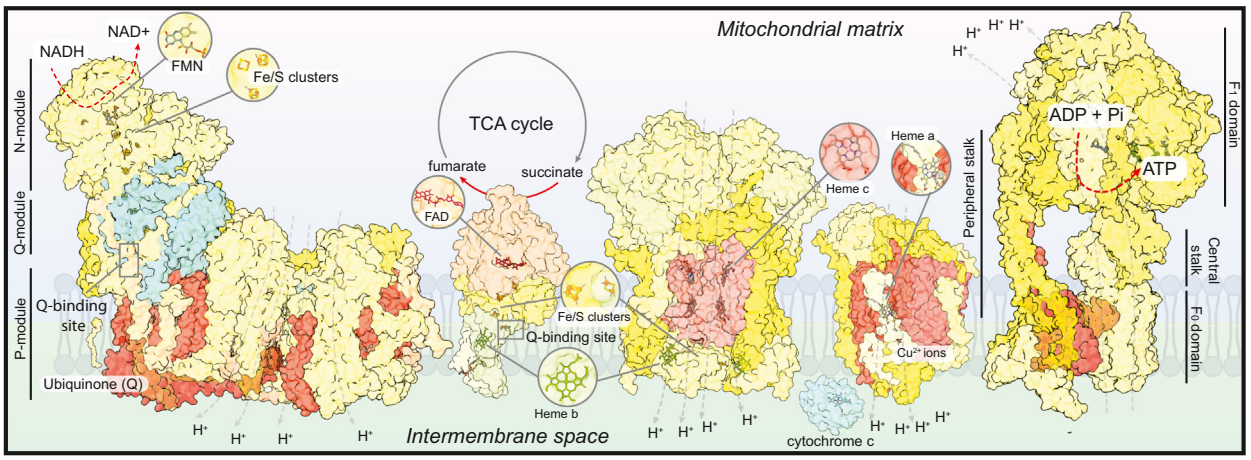
Mitoribosomes: unique species of ribosomes specific and integral to mitochondria.
Nuclear (n)DNA: nuclear genome that encodes 99% of the mitochondrial proteome.
O_2 tension: level of oxygen in a particular microenvironment. **Hypoxia** refers to oxygen levels lower than normal.
Outer mitochondrial membrane (OMM): membrane that separates mitochondria from the cytoplasm.
Oxidative phosphorylation (OXPHOS): energy production driven by the proton-motive force (membrane potential) generated by the ETC, culminating in ATP synthesis via CV.
OXPHOS complexes: five major protein complexes (CI–V) contributing to OXPHOS.
Proteostatic responses: dedicated biological programs used to restore disrupted protein folding.
Quality control: mechanisms that survey and ensure the integrity and proper functioning of macromolecules and biological systems.
Reactive oxygen species (ROS): heterogenous group of species with diverse properties, activities, and biological functions.
Reverse electron transfer (RET): catalytic state of Complex I when electrons are forced back from the CoQ pool to flavin moiety.
Turnover: process of continuous degradation and synthesis of cellular components.

Figure 1. Oxidative phosphorylation (OXPHOS) alterations with aging: what is concealed beneath the iceberg. The iceberg tip represents the phenotypical manifestation of OXPHOS defects detected in older individuals. The underwater part of the iceberg indicates the hot spots in OXPHOS production, maintenance, and functionality that might be compromised or influenced by aging and age-associated diseases. Abbreviations: MRC, mitochondrial respiratory complex; mtDNA, mitochondrial DNA; ROS, reactive oxygen species.

translate the concise but critical subset of OXPHOS subunits. MRC biogenesis involves more than 200 dedicated factors, including components of mitochondrial ribosomes (**mitoribosomes**), which are distinct from their cytoplasmic counterparts and exploit discrete molecular mechanisms. Together with the cytoplasmic gene expression system, this gives rise to a complete OXPHOS apparatus. The ultimate balance between the two systems can be achieved by combined effects of coordinated gene transcription, protein synthesis, and post-translational adjustments in protein quantities [5,6].

The major challenge lies in parameters describing mitochondrial and nuclear gene expression systems (Figure 3). The two genomes have different modes of transcription, with mtDNA genes

(A)



(B)

	Complex I NADH:ubiquinone oxidoreductase	Complex II Succinate dehydrogenase	Complex III$_2$ Ubiquinol:cytochrome c oxidoreductase	Complex IV Cytochrome c oxidase	Complex V ATP synthase
Subunits	44 unique (45 total) subunits mtDNA encoded subunits (7)	4 subunits all nuclear-encoded subunits	Obligate homodimer of 11 subunits each mtDNA encoded subunits (1/monomer)	14 subunits mtDNA encoded subunits (3)	17 unique subunits (28 total), forms dimers mtDNA encoded subunits (2)
Assembly factors	19 assembly factors	4 assembly factors	8 assembly factors	47 assembly factors	5 assembly factors
Reactions	Oxidation of NADH followed by reduction of ubiquinone (Q)	Oxidation of succinate to fumarate and reduction of Q to ubiquinol QH_2	Oxidation of QH_2 coupled to reduction of cytochrome c	Oxidation of cytochrome c and reduction of oxygen molecules into water.	Phosphorylation of ADP into ATP using proton motive force generated by CI, III and IV
Cofactors	Flavin mononucleotide (FMN), chain of eight iron-sulfur (Fe/S) clusters (4Fe-4S and 2Fe-2S)	Flavin adenine dinucleotide (FAD), Fe/S clusters (2Fe-2S, 4Fe-4S, 3Fe-4S), b-type heme is catalytically inactive	Heme b_L, heme b_H, heme c_1 and Fe/S cluster (2Fe-2S)	Copper centre (Cu_B), Mg^{2+}, Zn^{2+} and heme a/a_3	n/a
Membrane potential generators	Translocates 4 protons/2 é from NADH transferred to Q (1 QH_2)	Does not translocate protons.	Translocates 4 protons/Q cycle (2 QH_2)	Translocates 4 protons/1 O_2 to form 2 H_2O molecules	Uses proton gradient to make ATP; may act in a reverse mode to translocate protons
Molecular weight	~1 MaD	~140 kDa	~500 kDa	~200 kDa	~600 kDa

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Figure 2. Oxidative phosphorylation (OXPHOS) system. (A) OXPHOS complexes are modular multi-subunit protein assemblies (structural representation) accompanied by cofactors (encircled) and embedded in the inner mitochondrial membrane (IMM). Complex I initiates the electron transport chain (ETC) by transferring electrons from NADH to ubiquinone (Q). This is coupled with the pumping of protons (H^+) from the mitochondrial matrix to the intermembrane space (IMS). Complex II transfers electrons from succinate to ubiquinone, further fueling the ubiquinol pool in the IMM. It does not pump protons but couples the Krebs cycle [tricarboxylic acid (TCA) cycle] to the ETC. Complex III transfers electrons from ubiquinol to cytochrome c via the so-called 'Q cycle' and allows the translocation of additional protons into the IMS. Complex IV transfers electrons from cytochrome c to oxygen, the final electron acceptor, reducing it to water and pumping additional protons across the membrane. To complete OXPHOS, protons flow back into the matrix through the ATP synthase. This generates rotational force, which catalyzes ATP production. Structural representations were generated using the following Protein Data Bank (PDB) IDs: CI, 5XTD; CII, 8GS8; CIII, 5XTE; CIV, 5Z62; CV, 6J6I; cytochrome c, 3ZCF. Structures were visualized with ChimeraX-1.8 and adjusted for selected aesthetic parameters. Mitochondrially encoded subunits are labeled in red. (B) Summary of the origin of OXPHOS subunits, number of assembly factors, types of enzymatic reaction, cofactors, and molecular mass of respective complexes.

co-transcribed in polycistronic units and nDNA genes scattered throughout chromosomes and controlled by individual promoters. Moreover, each stage of RNA life (synthesis, processing, ribosome association, translation, and degradation) displays distinct kinetics between the cytosol and mitochondria and is carried out by separate machinery [6]. The expression of the mitochondrial proteome can be synchronized in frames of a global transcriptional program known as **mitochondrial biogenesis**, which is stimulated in response to particular needs or stresses. Balancing subunit production could create an obstacle for cells with age-compromised homeostasis. Distinct processes within this pipeline were found to be changed or disrupted by aging [7–10]. Reduced cytoplasmic translation, depletion/stalling/collision of ribosomes, and loss of transcriptome–proteome correlation emerge as important aging hallmarks, posing a potential challenge to the dually

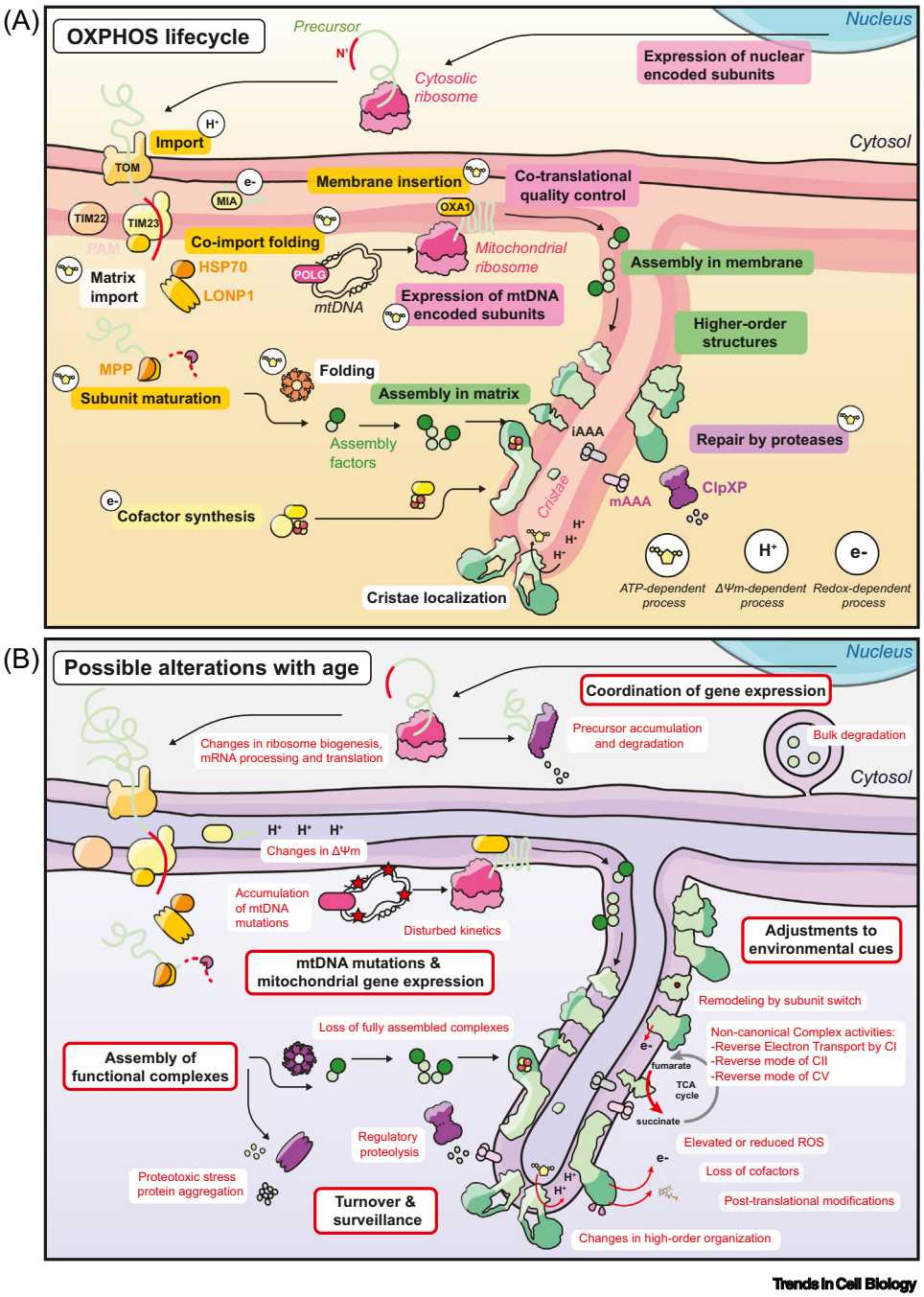

Figure 3. The mitochondrial respiratory complex (MRC) production pipeline, service, and functional adjustments as hot spots for aging-associated challenges. (A) Oxidative phosphorylation (OXPHOS) production begins with the expression of nuclear and mitochondrial genomes. Nuclear-encoded subunits and assembly factors are synthesized in the cytoplasm and imported into mitochondria via Δψ-dependent translocases. ATP hydrolysis pulls precursors into the matrix. The redox-dependent mitochondrial intermembrane space import and assembly (MIA) pathway mediates oxidative folding in the IMS. Precursors mature by processing peptidases and fold with the help of ATP-dependent chaperones. Mitochondrial (mt)DNA-encoded subunits are translated by mitoribosomes, inserted into the membrane by OXA1L, and monitored by quality control systems. Assembly factors and proteases aid complex assembly

(Figure legend continued at the bottom of the next page.)

regulated OXPHOS system [10–13]. Existing literature suggests that intramitochondrial translation could be reduced in some age-related conditions, including cellular senescence [11,14]. By contrast, dysregulated transcription and stochastic transcriptional noise have not been denoted as universal aging characteristics, although mitochondria-dedicated transcriptional programs could still be altered in a tissue-specific context, as observed for other gene groups [15]. The important questions to address are: how much do the possible changes in nuclear gene expression caused by aging interfere with the mitochondrial gene expression? How resilient is mitochondrial gene expression? And what is the physiological threshold that has to be exceeded to trigger discoordination of two programs?

The third coordination layer is defined by post-translational refinement of subunit pools. The proteins that build complexes are often governed by complex-dedicated rules. For example, they become clearly stabilized within the complex, while non-incorporated subunits (so-called 'orphan subunits') undergo more rapid degradation, buffering the proteostatic landscape of the complex [5]. Similar modes of post-translational regulation may apply to mitochondrial complexes. It has been shown that multiple components of MRCs and mitoribosomes are produced in excess under normal conditions [16–18]. This indicates the high basal buffering capacity of mitochondria for disproportionate subunits. In agreement with this, matrix chaperones are the most abundant components of the mitochondrial proteome across various tissues [19]. Several lines of evidence suggest that disproportionate subunit provision is important for cost-effective repair or efficient assembly of multimeric protein complexes [17,18]. This suggests that the 'desynchronized' nuclear expression and presence of disproportional subunits in mitochondria is part of a natural physiological phenomenon with pivotal regulatory implications and does not necessarily need to trigger stress responses.

It remains to be determined whether the proteostatic capacity of mitochondria is just enough or exceeded during aging. Disruption of OXPHOS causes activation of global stress responses aiming to restore mitochondrial homeostasis, known as the **mitochondrial integrated stress response (ISRmt)** [20]. The proteostatic component of this broad program, the mitochondrial unfolded protein response (UPRmt), has been widely characterized in lower organisms [1], but its independence from ISRmt in mammals and exact mechanisms remain debated [21,22]. OXPHOS defects were shown to increase longevity in yeast, flies, and worms by activating protective UPRmt [1]. Yet, only in rare cases is the same true for mammals, and MRC defects usually cause severe disease manifestation and reduced lifespan. Moreover, prolongevity effects in lower eukaryotes are mediated by **mitohormesis** mechanisms and are enabled only when the disruption occurs early in life. This raises the recurrent question in aging research of how confidently the mechanisms observed in model organisms, from cell cultures through worms to mice, can be extrapolated to humans. Another challenge behind understanding the mechanisms involved in **proteostatic responses** is the remarkable multifunctionality of mitochondrial proteases. These are involved in numerous key mitochondrial processes, including the regulation of gene expression and metabolic circuits [23]. Therefore, discriminating between their regulatory and purely **quality**

and repair. Fully assembled complexes organize into higher-order structures. They perform their function and can be degraded when damaged or redundant. (B) Aging imposes a risk for the OXPHOS lifecycle, and several vulnerabilities might be considered from the perspective of age-related pathologies. The biogenesis of complex components could decelerate or become discoordinated. Newly produced subunits could contain point mutations that destabilize or inactivate the complexes. Inefficient import could limit the provision of new OXPHOS subunits. Changes in post-import subunit processing could impede the formation of functional complexes. The removal of damaged or redundant complexes might become restrained or exaggerated. Functionality of complexes could be altered by microenvironmental cues or changing metabolic demands. MtDNA mutations with replicative origin are commonly detected in aged individuals, and there is substantial evidence supporting their role in the OXPHOS decline. Some other processes are currently investigated in the context of age-related pathologies, and their direct relevance to OXPHOS defects awaits determination.

control functions can be difficult. Although the coordination of two expression programs as a cornerstone for one biological system represents a plausible Achilles heel for MRCs, our understanding of this process in mammalian aging is fragmentary and could be context dependent.

MtDNA mutations

Accumulation of somatic mutations is a well-recognized aging effect. MtDNA mutations, both single nucleotide substitutions and deletions, are commonly detected in samples from older donors [24], and their source, lifetime dynamics, and contribution to aging have been investigated for a long time. The mutational signatures determined in aged tissues with ultra-sensitive sequencing techniques link their origin to replication errors rather than to historically postulated oxidative DNA damage [24,25]. In line, mouse models with boosted mtDNA mutagenesis due to proofreading-deficient replication, so-called 'mtDNA mutators', recapitulate multiple aging features and display progressive progeria [26,27], supporting the causative role of somatic mtDNA mutations with replicative origin in aging. An alternative hypothesis has also been proposed linking the progeroid phenotype of mutator mice with nucleotide imbalance and nuclear genome instability, especially in stem and progenitor cells [28]. It suggests that mitochondrial alterations impose secondary effects on other hallmarks of aging [2].

Despite the limited coding capacity of the mitochondrial genome, mtDNA mutations can severely impair energy production and tissue homeostasis due to defective OXPHOS [29]. As evident in patients with heritable mitochondrial diseases, the mutations in protein-coding genes can compromise the stability and function of particular MRCs. Meanwhile, alterations in other mtDNA regions exert more global effects by hampering mitochondrial gene expression. Recent advances in mtDNA engineering demonstrated that premature STOP-codon-causing mtDNA mutations in protein-coding genes can lead to MRC destabilization [30], opening doors for new experimental evidence and discoveries. The critical determinant of mtDNA mutation phenotypic manifestation is its prevalence in the total pool of cellular mtDNA molecules, known as the **heteroplasmy** level [29]. The 'effects' of mutation can only be visible when it is sufficiently enriched in the mtDNA pool. This enrichment (clonal expansion) occurs as a result of the ability of mtDNA to replicate independently of the cell cycle (relaxed replication), allowing somatic mtDNA mutations to expand in postmitotic tissues over a lifetime. The absolute mtDNA copy number could also modulate the pathological manifestation of mutation [31]. Heteroplasmy is dictated by multiple factors, including drift, selection, and the mutation type itself. Recent findings suggest that the interaction of mtDNA mutation with tissue, environment, and nuclear contexts is also important in shaping heteroplasmy dynamics [32,33]. Follow-up studies are necessary to clarify if and how changes caused by aging contribute to the selection or propagation of particular mtDNA variants in defined tissues. Notably, the accumulation of somatic mtDNA mutations with replicative origin remains the best evidentially supported concept explaining the age-progressing decline of OXPHOS machinery. As such, it largely defines the fate of OXPHOS during normal aging.

Import of nuclear-encoded subunits

Mitochondrial protein import constitutes the next hot spot in OXPHOS biogenesis (Box 1). Import inhibition at the entry gate activates global rescue programs, including reduced cytosolic protein translation and enhanced proteasome-dependent degradation, emphasizing the weight of the stress it can cause the cell [34]. The efficient delivery of proteins is linked, at least partially, to the functional OXPHOS/**electron transport chain (ETC)** system. There is substantial crosstalk between protein import and the OXPHOS machinery [34], and mitochondrial dysfunction can lead to import delay [35]. Nuclear-encoded components of the OXPHOS machinery are synthesized on cytoplasmic ribosomes and enter mitochondria as unfolded precursors via dedicated import pathways servicing distinct mitochondrial subcompartments (Figure 3). Precursors are recognized explicitly by import machinery of the outer (TOM) and inner (TIM) membranes, often (but

not always) with the help of an N-terminal targeting sequence. The efficient translocation via the **inner mitochondrial membrane (IMM)** depends on Δψ and requires ATP hydrolysis to pull protein precursors into the **matrix**. Δψ dependence also appears to be required for the import of many IMM integral subunits. It is believed that Δψ enhances the translocation of positively charged presequences via an electrophoretic mechanism. Furthermore, the import of MRC components is tightly coupled with post-import protein processing and complex assembly pipeline [36] and requires firm cooperation between matrix and **intermembrane space (IMS)** machinery [37]. The latter is defined by the mitochondrial IMS import and assembly (MIA) pathway, which folds the cysteine-rich proteins in a redox-dependent manner and uses ETC components for electron exchange. The entire process is supported by proteases on both sides of the IMM, including critical role of membrane proteases i-AAA and m-AAA.

Overall, the efficient import of OXPHOS components is an energy-consuming and membrane potential-dependent process that requires an intact redox balance and availability of electron acceptors. Its role in aging was recently accentuated by the identification of mutations in components of protein import and sorting machinery associated with progeroid syndromes [38,39]. Nevertheless, whether age-related loss of function within OXPHOS can self-perpetuate due to the perturbed import of its components remains an open question. Aged cells are known for reduced mitochondrial respiration interconnected with a decrease in Δψ [4]. However, OXPHOS conceals an efficient coping mechanism that enables mitochondria to sustain Δψ even upon severe MRC defects [40], although at a high energy cost. This is mediated by the ATP-consuming reverse CV mode, which drives proton extrusion to the IMS and restores the gradient. Cells tend to safeguard Δψ because it is important for many critical mitochondrial functions. Therefore, the estimation and interpretation of Δψ changes, as with many other mitochondrial features, should be done with a degree of caution.

Post-import subunit processing

Precursors that have made it into the matrix are proteolytically processed by a subset of mitochondrial peptidases (including MPP) to remove presequences and then to be folded with the aid of ATP-dependent proteostatic factors (Figure 3). mtHSP70/LONP1 has a key role in facilitating co-import protein folding, and LONP1 loss, similar to disruption of presequence processing, leads to matrix aggregation of newly imported proteins [41,42]. Reciprocally, protein aggregation in mitochondria was proposed to reduce import efficiency [43] and the former is a hallmark of both aging and neurodegenerative disease [2]. However, whether OXPHOS components aggregate or misfold in mitochondria of aged cells has not yet been addressed. The remarkable abundance of major matrix chaperonins [19] suggests that mitochondria have high basal protein misfolding resilience.

Assembly of functional complexes

The subunits successfully delivered to mitochondria must undergo stepwise incorporation into fully assembled MRCs (Figure 3). This highly organized manufacturing process does not occur spontaneously but is mediated by dedicated factors that are not complex parts themselves. Individual protein subunits and **cofactors** are precrafted into modules with the help of assembly factors and then integrated into the final MRC. Notably, the number of assembly factors assisting OXPHOS production can equal or even exceed the number of its structural subunits (Figure 2). Finally, the OXPHOS manufacturing pipeline is physically coupled to the translation of mtDNA-encoded subunits and assisted by a protein quality control machinery [23,44,45], completing the global picture of OXPHOS production. This step involves IMM proteases (m-AAA, i-AAA) and other factors (including OXAL1 and prohibitins).

Aberrant MRC formation resulting from compromised structural subunits and malfunction of assembly machinery have already been linked with age-related pathologies, including Parkinson's

and Alzheimer's diseases [46,47]. Several pieces of evidence suggest that MRC assembly is susceptible to tunning by aging and related alterations. The mtDNA point mutations can impair respiratory complex formation by affecting the structure and stability of mitochondrially-encoded subunits. The cofactor provision and complex stability can be modulated by tissue oxygenation and aging-related post-translation protein modifications [48–50], with reduced CI methylation associated with premature aging in flies (Box 2). By contrast, age-related stress may induce complex composition remodeling, leading to the assembly of context-optimized MRCs. One example is inflammatory signals, commonly aggravated by aging, which may stimulate the expression of alternative CIV subunit isoforms, which become incorporated into the complex to support the response of cells to inflammation [51]. Similar switches in MRC subunit isoforms were also observed for environmental cues, such as hypoxia, or in adaptations to tissue-specific contexts [52]. This suggests that aging shapes MRCs at the single-subunit level and indicates that MRC biogenesis is controlled at multiple layers, starting from subunit provision through their folding and cofactor incorporation to the availability of assembly machinery and post-translational modifications.

Higher-order organization

The organization of MRCs into larger entities, known as supercomplexes (SCs), confers an additional level of complexity. While the most recognized is a respirasome (CI/$CIII_2$/CIV), SCs can exist with varying stoichiometries and usually form disc-like structures occupying **cristae** membranes [53]. Although the existence of SCs is well accepted, their actual role and the selective advantage

Box 2. OXPHOS under the weight of age-related stresses

During aging, our bodies undergo systemic changes, including cardiovascular decay, transitions in body composition and metabolism, and progressive tissue deterioration. Many of these changes directly impact OXPHOS, influencing its abundance and functionality. The communication is reciprocal, with OXPHOS alterations aggravating pre-existing disease phenotypes. By contrast, changes in OXPHOS can also be protective against some age-related pathologies. Here, we briefly summarize selected co-dependencies.

Compromised tissue oxygenation

Oxygen is a final ETC acceptor and a key determinant of OXPHOS functionality. **O_2 tension** can also modulate survival of cells and animals with OXPHOS defects with protective role of hypoxia [48]. Fluctuations in tissue oxygenation directly influence OXPHOS activity and are associated with local bursts of oxidative damage [73]. Furthermore, exploiting RET-mediated ROS signaling programs [74,75] could mediate changes in cell properties and fate. The most recognized example is ischemia-reperfusion injury underlying heart infarction and ischemic stroke. However, many other age-prevalent conditions impact oxygen delivery at the systemic or organ-specific level, including chronic obstructive pulmonary disease, diabetes, obesity, and anemia.

Chronic inflammation

Chronic inflammation is a hallmark of aging manifested locally in various tissues and systemically by circulating inflammatory factors ('inflammaging'). OXPHOS controls inflammatory phenotypes in a range of physiological and pathological processes. In cellular senescence, inflammatory phenotypes are skewed by OXPHOS inhibition, exploiting changes in the NAD+/NADH ratio [89]. Activation of immune cells depends on adjustments in MRC function, particularly ROS generation by CI or CIII or epigenetic regulation by OXPHOS-dependent metabolites [74,75,90]. The decline in immune function (immunosenescence) is associated with abnormal mitochondrial responses, leading to increased susceptibility to bacterial and viral infections with age and unconstrained pathogen-stimulated inflammation [91]. Furthermore, OXPHOS dysfunction and the inability to buffer redox status underlie several forms of inflammatory cell death implicated in age-associated diseases.

Age-related lifestyle changes

While aging, humans decrease physical activity due to changes in behavior or acquired injuries. Lack of activity may reduce mitochondrial biogenesis, limiting the renewal of OXPHOS machinery in muscles and compromising the bioenergetics in the body. The caloric surplus resulting from a sedentary lifestyle directs the body toward lipid storage rather than OXPHOS-dependent catabolism, leading to changes in organ adiposity. These events may modify how particular tissues with defined metabolic programs use OXPHOS. Intriguingly, rewiring ETC activity by restrained mitochondrial gene expression can ameliorate obesity and metabolic syndrome [92], showing that repurposing OXPHOS functionality can be a health-promoting intervention.

of having MRCs packed in higher-order structures remain enigmatic. Several SC functions have been counterargued over recent years, including substrate channeling [54]. Others, such as modulation of complex assembly, ROS production, and metabolic activity, are intensively debated. For example, SCs have been observed to reformulate depending on the metabolic status of the cell [55], leaving an open question for the relevance of this in terms of aging. Noteworthy, the tight interaction between MRCs is not a prerequisite for normal bioenergetic function and or physiology in mice [56]. The alternative hypothesis, that SCs formation prevents protein destabilization and aggregation in highly dense mitochondrial membranes, hints at an interesting link with age-associated phenotypes [56]. Nevertheless, whether it is the biological function or simply the specificity of the mitochondrial membrane microenvironment that triggers the arrangement of MRCs into higher-order structures, remains an open question.

The data addressing the role of SCs in animal aging indicate altered SC proportions in aged organs but are scarce [57]. Moreover, their correlative nature hampers definitive conclusions. Recently, SCs were studied in the context of exercising, a known anti-aging intervention. Although higher levels of SCs were linked to cardiorespiratory fitness [58], high-intensity training alone failed to reorganize SCs, albeit clearly boosting mitochondrial respiration [59]. Despite multiple hypotheses proposed, the biological meaning of SCs and their role in age-related pathologies await determination.

Another distinct category of higher-order structures includes OXPHOS homo-oligomers. These are represented by CV dimers, which contribute to shaping mitochondrial ultrastructure by promoting cristae architecture. Mitochondrial ultrastructure can be compromised in aged tissues, involving a feasible role for CV dimerization [60].

Respiratory complex turnover and surveillance

Compromised protein **turnover** is a common manifestation of aging. The mitochondrial proteome, with its double-membrane boundaries and tightly controlled unidirectional protein trafficking, remains highly separated from the milieu and escapes the canonical proteolytic guards of the cell. Instead, mitochondria have evolved a multilayered quality control system exploiting various strategies to survey, maintain, and alter their protein inventory.

First, dynamic fusion and fragmentation of the mitochondrial network in response to OXPHOS defects (e.g., $\Delta\psi$ decreases) allow for improving/adjusting mitochondrial functionality by mixing or separating their content. Once faulty mitochondria or their damaged subdomains are separated from their healthy counterparts [61,62], they can be subjected to bulk degradation or even exported outside the cell [63]. We define 'bulk' degradation as the disposal of heterogeneous cargo comprising membranes, nucleic acids, metabolites, and proteins in their native, often complex-associated, state. Such mitochondria degradation could operate in an autophagy-independent and -dependent manner, including mitochondria-dedicated mitophagy, mitochondria-derived vesicles (MDVs) and related disposal variants [mitochondria-derived compartments (MDCs) and structures positive for **outer mitochondrial membrane** (SPOTs)]. All these modes of mitochondrial removal are intensively explored in aging research, as reviewed recently [63,64]. Bulk mitochondrial turnover has been implicated in age-related neurodegeneration, as mutations in the core mitophagy players, PINK1 and Parkin, are associated with early-onset Parkinson's disease. Yet, research in mouse models failed to prove the causative relationship between mitophagy machinery and neuronal loss, instead linking the Parkinson disease phenotypes with MDVs and immune response [64]. Despite the significant interest in mitophagy, it remains controversial because of the highly nonphysiological stressors required to evoke this pathway. In line with this, the contribution of Parkin to healthy OXPHOS maintenance in aged

organs was recently questioned [65], suggesting the presence of alternative routes of OXPHOS surveillance.

A possible hint to these alternative routes is provided by studies on proteome dynamics, which show that the mitochondrial proteome is characterized by a highly heterogeneous turnover spanning three orders of magnitude, which corresponds to the protein half-lives ranging from hours to months [19]. Even the proteins belonging to the same complex display distinct degradation rates with certain exceptionally long-lived OXPHOS subunits detected in the brain, heart, and oocytes [66–68] (Box 3 and Figure 3). These are enriched within the membrane-embedded cristae, suggesting lifelong maintenance of certain mitochondrial structures [66,67]. This argues for the role of more precise modes of degradation, as only such modes could assure the preservation of complexes containing elements with varying lifespans. Interestingly, the turnover of several, but not all, OXPHOS components markedly accelerates in aging brains [69].

Asynchronous OXPHOS turnover can be executed by proteases, which, in contrast to bulk degradation, could operate in a highly selective manner, and represent a third layer of OXPHOS surveillance. While the cytosolic proteasome–ubiquitination system can dispose of mitochondria-undelivered subunits usually emerging following severe OXPHOS defects, a set of ancient ATP-dependent proteases mediates precise, often regulatory, degradation of OXPHOS subunits at their actual location [52]. Opposed to the long-lived proton-pumping membrane arm (Box 1), the matrix-exposed and highly redox-active CI input module undergoes independent degradation by ClpXP protease under both physiological and stress-induced conditions [18]. This maintenance-oriented salvage pathway preserves the activity of the complex by selective recognition and exchange of its damaged parts without a need for whole-complex disposal. It represents a fast and resource-saving way to repair large protein complexes. Furthermore, membrane hyperpolarization stimulates highly selective inactivation and degradation of CI by m-AAA protease [70],

Box 3. The most vulnerable (and smart) cells?

Altering OXPHOS levels, proportions, utilization, and activities can modulate cell fate and features. Some cell types, often highly specialized, hijack OXPHOS properties to change behavior or promote particular functions critical for the organism. A few examples from an aging-related context are described below.

Oocytes are formed before birth and can remain dormant in ovaries for up to 50 years in humans, awaiting the signal for further maturation. Even though undergoing developmental suspense, they stay surprisingly metabolically active. Early oocytes were shown to exploit an interesting adaptation of the OXPHOS system manifested as a limited abundance of CI in proportion to other respiratory complexes [93]. It was proposed that keeping CI suppressed protected long-lived cells from the harmful impact of ROS. However, it is unclear whether disabled mitochondrial ROS serves to keep signaling at bay or prevents macromolecule damage. The molecular mechanism of selective CI depletion in dormant oocytes also remains uncharted. Paradoxically, several OXPHOS subunits become exceptionally long-lived in oocytes, indicating their decreased turnover [68]. Another interesting layer was added by recent findings showing that advanced maternal age can promote purifying selection against deleterious mtDNA variants associated with CI defects and prevent their transmission to offspring [94]. This triggers the questions of when and how CI is restored during the life of an oocyte.

Keeping ETC function constrained due to redox signaling is a hallmark of many other quiescent cells, including neuronal and hematopoietic stem cells. However, the mechanisms might not always be uniform, and cells harness distinct strategies to modulate their fate via OXPHOS. For example, biased segregation of mitochondria during cell division can determine cell stemness by targeting daughter cells toward a quiescent or progenitor fate [78]. This, in turn, can influence the aging at the organismal level.

An interesting hypothesis was recently postulated by subjecting cells with distinct mtDNA mutations affecting respiratory CI to various environmental conditions [32]. Such mutations accumulate in aged tissues. Tracing cell fitness revealed that cells can favor and harness pre-existing OXPHOS alterations to adapt to new microenvironmental setups. Reciprocally, aging itself was suggested to create the environmental conditions that will select and propagate particular nonsynonymous MRC mutations. Such crosstalk should be highly relevant for cancer cells and the tumor microenvironment, possibly acting as a double-edged sword and preventing [95] or propagating metastasis to a defined tissue.

presenting another mechanism for on-site MRC removal beyond bulk degradation. The existence of similar regulatory pathways for other OXPHOS units and their relevance to age-related pathologies await investigation.

ROS-generating molecular machines and noncanonical electron flows

There are numerous reports describing the role of free radicals in aging, given historical interpretations of oxidative damage accumulation as a function of age-stipulated mitochondrial alterations, with ROS emerging as harmful by-products. This underpinned the mitochondrial free radical theory of aging, which is now largely debunked. Elevated ROS production is not always equal to the accumulation of oxidative damage, and free radicals can display both detrimental and beneficial effects depending on their type, source, and physiological context. Therefore, ROS production and the discrimination between damaging versus signaling roles of free radicals should be assessed and interpreted carefully [71].

Once built up, MRCs perform redox-heavy reaction series (Figure 2), which could make them potent ROS generators under certain circumstances. Two major OXPHOS sites contribute to ROS production: the matrix-exposed NADH-oxidizing module of CI and the IMS-proximal redox-active domain of CIII. Defects in MRCs or their decreased levels are frequently postulated as causes of augmented ROS production in aged cells. However, many of these findings are not backed by mechanistic explanations and often appear counterintuitive (e.g., the relationship between decreased levels of respiratory complexes and increased ROS generation). Nevertheless, as shown in models of drug-induced senescence [14,72], elevated ROS production is not a universal label for all aging-associated conditions and often simply reflects changes in the mitochondrial mass. Furthermore, considering the multifaceted role of ROS, it is not easy to predict which level of ROS will be helpful and which is harmful under certain circumstances. Rather than a result of dysfunction within the complex, elevated ROS generation often constitutes part of conserved metabolic programs, structural adaptations, and specialized signaling cascades. Accordingly, ROS-boosting **reverse electron transport (RET)** via CI not only contributes to outcomes of age-associated pathologies, such as ischemia-reperfusion injury, but also shapes cellular responses, such as inflammation [73–75].

Fluctuations in tissue oxygenation are commonly associated with age-related pathologies (Box 2). Oxygen is not only the final ETC acceptor, but also a critical modulator of OXPHOS. While hypoxia can be protective to MRCs [48,50], reoxygenation can damage it or even repurpose OXPHOS to initiate signaling cascades [73]. Hypoxia/reoxygenation associated with age-prevalent heart attack or ischemic stroke triggers the accumulation of reduced coenzyme Q (CoQ)in the presence of high membrane potential and stimulates RET, leading to ROS generation at the CI [73] (Figure 3). RET is a recognized initiator of proinflammatory programs [74,75], but its contribution to aging awaits exploration. To perform RET, CI needs to move from a deactive state imposed by hypoxia to an active state stimulated by reoxygenation. Biguanide drugs, characterized by broad anti-aging activity, such as metformin, bind CI and stabilize it at the deactive state [76]. This could prevent the deleterious consequences of RET resulting from reoxygenation. In contrast to reoxygenation, permanent upkeep of OXPHOS in hypoxic conditions was shown to activate alternative non-canonical electron flow through the system, with fumarate as an ultimate electron acceptor [77]. This route could be essential for tumors and other cells occupying low-oxygen microenvironments. Overall, oxygen fluctuations imposed by age-associated pathologies could serve as important modifiers of OXPHOS and contribute to its fate in aging.

Heterogeneity, age-related diseases, and OXPHOS alterations

OXPHOS function is not equally exploited by all the cells in the human body. There is high heterogeneity in OXPHOS dependency and how cells harness this machinery to address individual

needs. It acts at different organization levels, and substantial differences in OXPHOS utilization can be observed between various organs, across the specialized cell types within the same organ, or even between mitochondrial subpopulations within a single cell. Heterogeneity has also a time dimension, and cells undergoing differentiation display distinct OXPHOS reliance. Blood cell maturation provides a striking illustration of how time and space dictate mitochondrial aging. Quiescent stem cells are kept over the human lifetime in a safe low-oxygen microenvironment defined by a hematopoietic bone marrow niche. Following asymmetric division, these OXPHOS-inert cells replenish the blood population with young, mitochondrially active progenitors. Such division supports stem cell youth by segregating out older mitochondria into cells entering differentiation [78]. The partitioning of OXPHOS functionality within defined mitochondrial subpopulations is also observed in nonstem lineages [67,79], introducing an underexplored aspect of age-associated tissue deterioration.

Not surprisingly, age-related OXPHOS alterations are also not uniform across the body, with some tissues being more resilient than others [80,81]. Postmitotic organs with a high energy demand experience a particularly pronounced decline in OXPHOS function. Heart failure is a common aging manifestation with pathomechanisms underlain by changes in the fatty acids-linked bioenergetics of cardiomyocytes [82]. Age-associated muscle wasting also correlates with reduced OXPHOS availability, but can improve with increased physical activity [83]. Brain aging correlates with a higher risk of neurodegenerative disorders, such as Parkinson and Alzheimer diseases, which are linked with OXPHOS defects [46,47]. The brain showcases intraorgan heterogeneity in disease mechanisms, where loss of CI in neurons can lead to degeneration [46], while its inhibition in microglia may enhance neuroprotection and decrease neuroinflammation [75]. By contrast, MRC activity in microglia was shown to promote neuron remyelination after injury [84]. Similar diversification of OXPHOS utilization could also underpin age-associated kidney decline [85,86].

Recent concepts highlight the role of OXPHOS alterations in whole-body homeostasis. Hypermetabolism due to OXPHOS dysfunction emerges as a potential contributor to aging. It is a state of enhanced energy expenditure caused by the activation of costly stress responses and other energy-wasting processes [87], leading to aging traits, such as fatigue and physical inactivity [88]. All these studies illustrate the exceptional versatility of OXPHOS system in shaping human aging at the level of cells, tissue, organs, and the entire body.

Concluding remarks

Changes in OXPHOS availability and functionality are inherent to aging. Recent research has advanced our understanding of OXPHOS biogenesis and maintenance, laying a foundation for exploring how this system succumbs to aging and developing a global picture of OXPHOS alteration in age-associated pathologies. However, the consolidated view on how OXPHOS 'evolves' during aging in highly complex systems as the human body is not yet universally agreed upon. The ultimate consensus on whether OXPHOS alterations are a primary contributor to aging has not yet been reached. Identifying key vulnerabilities in OXPHOS and their effects on physiology across different tissues should provide insight. The above and other outstanding questions (see Outstanding questions) provide important directions for future research.

We are in exciting times for studying mitochondrial function in aging. High-throughput multi-omics approaches in single cell formats can reveal genetic changes (genomics and epigenomics), gene expression profiles (transcriptomics), proteome outputs (translatomics, proteomics, and post-translation modification-omics), and metabolic consequences (metabolomics) related to OXPHOS alterations throughout the body. Dynamic complexome profiling and cross-linking-

Outstanding questions

What is the fate and role of OXPHOS machinery during aging?

How does aging affect particular stages of the OXPHOS lifecycle? How do we define 'age-associated stress'? What is the aftermath effect of distinct co-occurring stresses? Could some of them cancel or amplify each other?

Are the stress-mimicking conditions applied *in vitro* to determine molecular mechanisms in aging relevant to physiological conditions in aged cells? How can we reconstitute the aging microenvironment more faithfully?

How reliably can we extrapolate the findings from various model systems to human aging?

How is the heterogeneity of age-related OXPHOS dysfunction established across different tissues? What are the key tissue-imposed vulnerabilities?

Do mechanisms and programs that respond to OXPHOS defects differ between the tissues? Once activated, do they change over time?

What is the threshold to activate responses to defective OXPHOS? What are the signals? Are adaptations to OXPHOS defects in aging always beneficial?

How much OXPHOS health relies on mitochondrial turnover? Is the mitochondrial proteostatic capacity enough or exceeded during aging?

Highly inflammatory senescent cells are commonly used as a model to explain the mechanisms underlying aging. Are senescent cells distinct from aged cells in terms of OXPHOS maintenance and functionality? Which OXPHOS alterations promote and which prevent inflammation?

What is the role of OXPHOS-derived ROS in age-related pathologies? How do we determine whether a certain level of ROS is harmful or helpful under particular circumstances? What are the underlying molecular mechanisms for ROS production in aged cells?

based native proteomics combined with time-resolved monitoring of mitochondrial complexes using *in situ* cryo-electron tomography and super-resolution microscopy, will enhance our understanding of the structural and spatial organization of OXPHOS in living cells. Innovations in real-time monitoring of metabolites, ROS, and physicochemical membrane properties in organs will also help assess OXPHOS functionality. Additionally, precise gene-editing techniques, including mitochondrial genome editing, will allow reliable validation of ideas with tailored experimental models. Integrating these data sets through advanced computational methods, machine learning, and artificial intelligence algorithms (including AlphaFold modeling of complex structures), can accelerate the prediction and validation of new hypotheses in this field.

The role of mitochondrial dysfunction in aging has been extensively debated, with several hypotheses validated or disproved. New concepts continue to emerge, awaiting careful testing. Combining these ideas with modern and classical methods will help address unresolved questions in the field.

Do transcriptomic and proteomic data always reflect the levels of fully assembled complexes? Can the gene expression profile or individual subunit abundance directly translate to the amount of functional OXPHOS?

How do OXPHOS defects influence other hallmarks of aging?

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Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Grammarly to check the grammar. After using this service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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