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Mitochondrial DNA Mutation, Diseases, and Nutrient-Regulated Mitophagy

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Abstract

A wide spectrum of human diseases, including cancer, neurodegenerative diseases, and metabolic disorders, have been shown to be associated with mitochondrial dysfunction through multiple molecular mechanisms. Mitochondria are particularly susceptible to nutrient deficiencies, and nutritional intervention is an essential way to maintain mitochondrial homeostasis. Recent advances in genetic manipulation and next-generation sequencing reveal the crucial roles of mitochondrial DNA (mtDNA) in various pathophysiological conditions. Mitophagy, a term coined to describe autophagy that targets dysfunctional mitochondria, has emerged as an important cellular process to maintain mitochondrial homeostasis and has been shown to be regulated by various nutrients and nutritional stresses. Given the high prevalence of mtDNA mutations in humans and their impact on mitochondrial function, it is important to investigate the mechanisms that regulate mtDNA mutation. Here, we discuss mitochondrial genetics and mtDNA mutations and their implications for human diseases. We also examine the role of mitophagy as a therapeutic target, highlighting how nutrients may eliminate mtDNA mutations through mitophagy.

Contents

1. INTRODUCTION

The mitochondrion, an important and vulnerable organelle, lies at the interface between environmental energetic supplies and organismal energetic demands. Mitochondria are double-membraned organelles that exist in eukaryotic cells. The emergence of mitochondria through symbiosis approximately 2–4 billion years ago has been proposed as a pivotal step in the evolution of multicellularity (34). Due to their symbiotic origin, mitochondria host their own genome, composed of mitochondrial DNA (mtDNA), an important feature that is unique among all other organelles in animals. The primary function of mitochondria is to produce adenosine triphosphate (ATP), which supplies more than 90% of cellular energy (166). Mitochondria are also responsible for a series of diverse cellular processes, including calcium signaling, iron homeostasis, steroid synthesis, heme biosynthesis, reactive oxygen species (ROS) production, and programmed cell death (144). Recently, mitochondria have also been implicated as being a master regulator of epigenetics and inflammasome assembly (18, 159). Despite the importance of mitochondria, mtDNA, most of which encodes critical components for mitochondrial function, mutates at a rate 10–70-fold higher than the nuclear genome (55). Understanding this paradox, that is, the important function of the organelle coupled with the high mutation rate of its genome, could enable a deeper understanding of disease origins and treatment.

mtDNA mutations have important implications for diseases. Well-established mitochondrial diseases—such as Leber hereditary optic neuropathy (LHON) and mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS)—occur at a rate of roughly one in several thousand newborns, and they are mostly caused by inherited mtDNA mutations (9, 81, 99). Mitochondrial diseases are heterogeneous and often multisystemic. Tissues with high energy demands are the most vulnerable to energy shortages resulting from inherited mtDNA mutations, including the brain, muscle, heart, and the endocrine system. It has also been shown recently that more prevalent children's diseases, such as autism, have associations with mtDNA mutations (163). A growing spectrum of common human metabolic diseases, including diabetes, obesity, cardiovascular disease, and cancer, has also been found to be associated with mitochondrial functional decline (121). The importance of mitochondrial function, in combination with the high somatic mutation rate of mtDNA, makes the mitochondrion a likely mediator of these diseases.

Mitophagy is a cellular process that has been evolutionarily conserved from yeast to humans, and it plays an important role in removing defective mitochondria. Thus, it is important in eliminating mitochondria with mutated mtDNA and maintaining mitochondrial homeostasis (117).

Understandably, mitophagy impairment is associated with aging and a plethora of pathological conditions, such as neurodegenerative diseases, myopathies, metabolic disorders, inflammation, and cancer (116). Both essential and nonessential nutrients participate in improving mitochondrial function and regulating intermediate metabolism for diverse human systems. Understanding nutrient-induced mitophagy could lead to therapeutic intervention strategies targeting mitochondrial-associated pathologies. Identifying mitophagy modulators and understanding their mechanisms will provide critical insights with broad relevance for human health and quality of life.

We start with a brief overview of mtDNA genetics and mutations and their possible roles in disease. Subsequently, we summarize the function of various nutrients in mitophagy regulation, with a focus on illustrating how these nutrients could help eliminate mtDNA mutations through mitophagy. It is important to emphasize that by focusing on mtDNA mutations in this review, the authors are not devaluing the importance of the fact that mitochondrial functions, including the mtDNA mutational process itself, can be affected by many genes encoded by the nuclear genome. By discussing the possible link between various nutrients and mitophagy and their functional mechanisms, we also recognize that it is not always healthy to increase mitophagy by applying nutritional intervention strategies, especially when the mechanisms underlying the relationships between various nutrients and mitophagy are still not well understood. Due to the extensive research on these topics and limited space in this review, the authors apologize for not reviewing certain aspects of the literature.

2. mtDNA MUTATIONS AND DISEASES

2.1. Mitochondrial Genetics and DNA Mutations

Compelling evidence suggests that mitochondria were once primitive bacterial cells and were acquired by the host through an endosymbiotic event (1). During endosymbiosis, the bacteria became double-membrane organelles and gradually transferred genes to their symbiotic host cell nucleus, with only a few genetic materials retained as mtDNA (154, 167). Human mtDNA is a circular double-stranded molecule comprising 16,569 base pairs. The two strands are distinguished by their molecular weight: a guanine-rich heavy (H) strand and a cytosine-rich light (L) strand. mtDNA encodes 13 peptides, which serve as core subunits for 4 of the 5 enzyme complexes (I, III, IV, and V) in the oxidative phosphorylation system. mtDNA also encodes 2 ribosomal RNAs and 22 transfer RNAs, which are essential for intramitochondrial protein synthesis (**Figure 1**).

Unlike human nuclear DNA (nDNA), mtDNA has a high gene density. About 93% of its entire length encodes genes. The 13 protein-coding genes are separated either by transfer RNAs or 1–2 noncoding bases. The mtDNA noncoding region is mainly located in the displacement loop (D-loop), which plays important regulatory roles by hosting the mtDNA replication initiation site and two H-strand transcription promoters. Because of this functionally dense organization, nucleotide substitutions in mtDNA are more likely to cause functional outcomes than mutations in nDNA are to cause such outcomes.

There are hundreds to thousands of copies of mtDNA in a cell depending on the tissue in contrast to only two copies of nDNA contained in a single cell. As a result, a mutation can be present in all copies of mtDNA (homoplasmy) or only a proportion of them (heteroplasmy), as illustrated in **Figure 2**. The proportion of mutant copies is referred to as the heteroplasmy frequency, variations in which can have different impacts on cellular functions (122). Heteroplasmy frequency is critical to determining the phenotypic effect of a specific mutation, a phenomenon called the phenotypic threshold effect. At low heteroplasmy frequencies, the deleterious effect of mutant mtDNA is mostly masked by coexisting wild-type copies, but once it exceeds a threshold

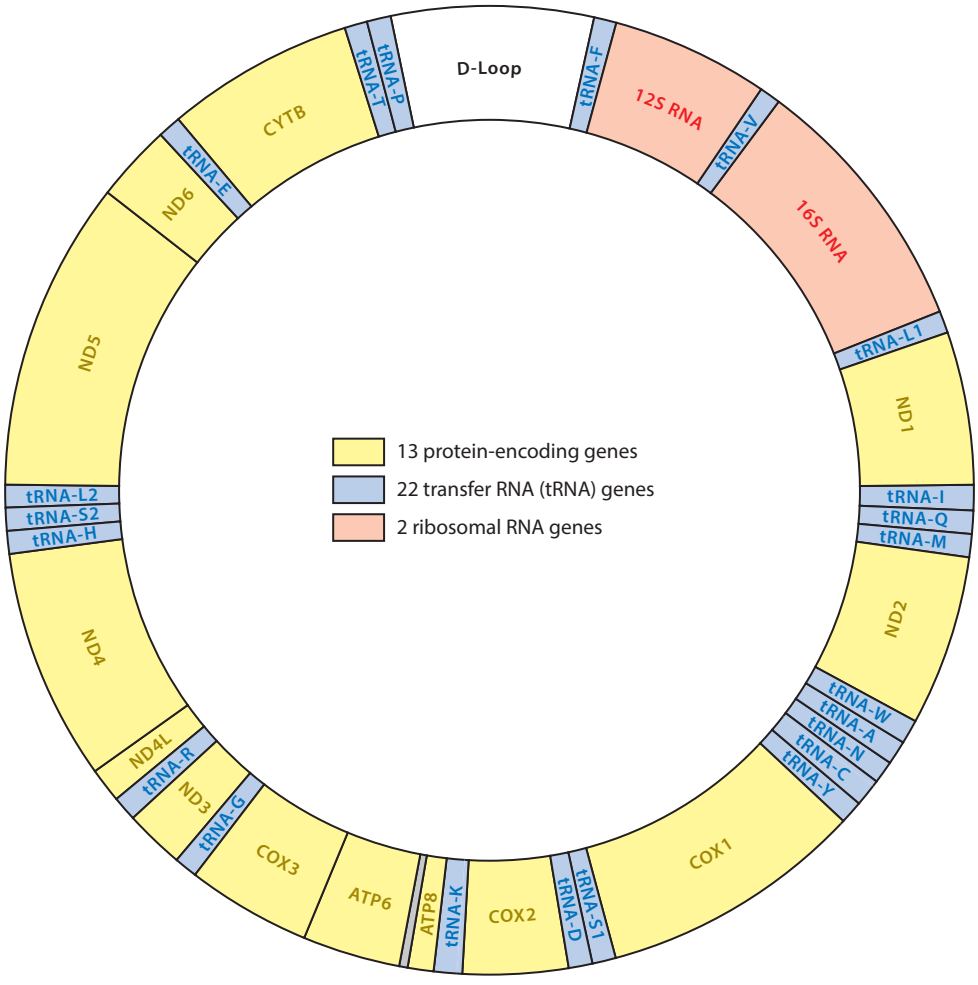

Figure 1

Schematic of the human mitochondrial genome. Human mitochondrial DNA is a 16,569 base-pair double-stranded circular DNA molecule. The figure was generated using mtviz (**http://pacosy.informatik.uni-leipzig.de/mtviz**).

value (typically 60–80%), mutant mtDNA will result in an altered phenotype (19, 80, 134) (**Figure 2**). This frequency threshold varies across mutations and tissues (154).

mtDNA mutations can be inherited or somatic. Several studies have taken advantage of recent advances in next-generation sequencing technology to demonstrate that most individuals, if not all, have heteroplasmy in their mitochondrial genomes (185). Heteroplasmic mutations may be inherited from maternal mtDNA, or they may be de novo mutations that arise during embryonic development. Unlike the nuclear genome, which is transmitted by sexual reproduction, the human mitochondrial genome is strictly maternally transmitted. Although inherited from a single parent, extensive differences in mtDNA heteroplasmy frequency have been observed between mothers and offspring and among siblings (50, 80). mtDNA is replicated constantly throughout the lifetime, and replication is independent of the cell cycle. Moreover, mtDNA replication and repair systems are less accurate than those in nDNA (57). Therefore, both dividing cells and postmitotic

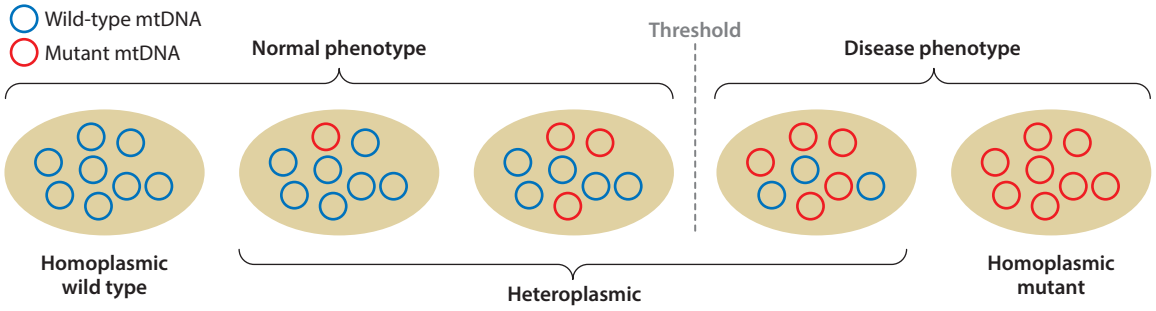

Figure 2

Mitochondrial DNA (mtDNA) heteroplasmy and threshold effects. All of the mtDNA within a cell may be identical (homoplasmic) or it may be a mixture of wild type and mutant (heteroplasmic). The cells can contain different proportions of mutated and wild-type mtDNA (referred as the heteroplasmy frequency). The heteroplasmy frequency is critical to determining the pathogenicity of a mutation. If the heteroplasmy frequency is below a certain threshold, the cell can maintain a normal phenotype. Once the frequency exceeds the threshold, the cell will show signs of mitochondrial dysfunction.

cells can accumulate somatic mtDNA mutations, especially heteroplasmic mutations, over time. A newly introduced mutation in a single mtDNA molecule may clonally expand to a higher frequency in a subpopulation of cells, and it may even reach the phenotypic threshold, as a result of only random effects during cell division or internal mitochondrial turnover inside cells, or both. Computational models suggest that mtDNA mutations arising early in life have sufficient time to reach the phenotypic threshold and to cause mitochondrial dysfunction at the level of the individual cell (37).

2.2. The Implications of mtDNA Mutations in Disease

Mitochondrial dysfunction is implicated in a broad spectrum of human diseases. A conceptual appreciation of the mtDNA mutational process in the evolutionary context is critical for understanding the origins of disease and potential treatments. Purifying the selection of mtDNA mutations in the germline in every generation is critical due to the high mutation rate and functional importance of the organelle. A sloppy process during this stage could lead to the survival of pathogenic mutations in the next generation, a devastating scenario that can quickly lead to species extinction during evolution. Nevertheless, if inefficient purifying selection of mtDNA mutations during egg production occurs in certain individuals, it can result in the survival of certain pathogenic mtDNA mutations, which could underlie the etiology of some childhood diseases, including classic mitochondrial diseases. However, due to the multicopy nature of mtDNA and functional redundancy among the copies, low levels of inherited pathogenic mtDNA mutations and somatic mutations early in life might not be able to affect reproduction, therefore avoiding natural selection during evolution. Nevertheless, these mutations are likely to accumulate to a high frequency later in life in a subpopulation of cells, due simply to random drift during cell division or mtDNA turnover, or both, and this could play an important role in the functional decline of cellular mitochondria and associated diseases later in life.

The term classic, or primary, mitochondrial disease refers to a group of diseases caused by defects in the oxidative phosphorylation system, which are the result of mutations in nDNA- or mtDNA-encoded mitochondrial genes. Some mtDNA mutations can contribute to several different mtDNA diseases. The most common disease-causing mutation is 3243A>G, and it is associated with chronic progressive external ophthalmoplegia, MELAS, and maternally inherited

diabetes and deafness (109). In contrast, a specific mitochondrial disease can be caused by a set of mutations. To date, mutations located in more than 75 genes (both mitochondrial and nuclear) have been identified as being involved in Leigh syndrome (79). The mtDNA mutations 3460G>A, 11778G>A, and 14484T>C were found in both homoplasmic and heteroplasmic states in families with LHON (23). Advances in next-generation sequencing technology have helped elucidate the genetic basis of mitochondrial diseases and their diagnosis, but treating these diseases remains a challenge.

High-frequency pathological mtDNA mutations can lead to primary mitochondrial diseases, which might nevertheless represent only one extreme of a continuous phenotypic spectrum. It remains unclear how mtDNA mutations with less pathogenicity or those with low frequency but high pathogenicity might contribute to childhood diseases that are not traditionally considered classic mitochondrial diseases because, theoretically, these should be more prevalent than the mutations underlying primary mitochondrial diseases. For example, autism spectrum disorder, which usually affects prepubescent children, is also associated with mitochondrial dysfunction. Our research indicates that heteroplasmic mtDNA mutations could contribute to the etiology of certain cases of this disorder (171). Efforts to systematically investigate the frequency of mtDNA mutations in newborns and their association with various diseases might be able to determine the contribution of mtDNA mutations to other childhood diseases.

Aging is a degenerative process, with a gradual impairment of physiological function that eventually leads to the deterioration of cellular function, disease, and death (73). During the past several decades, multiple lines of evidence have shown that impaired mitochondrial function is implicated in aging and age-associated disease (78). The accumulation of mutations in mtDNA over time can lead to severe impairment of cellular energy production and to mitochondrial dysfunction. In humans, the accumulation of mtDNA mutations has been observed in both dividing cells and nondividing (postmitotic) cells, for example, in the brain, muscle, and colon (22, 54, 133). The first experimental evidence for the causative link between the accumulation of mtDNA mutations and aging came from the mutator mouse model. Mutator mice have deficiencies in the proofreading function of mtDNA polymerase-γ that lead to an accumulation of extensive mtDNA mutations. These mice have a reduced lifespan and premature onset of aging-related phenotypes, such as weight loss, hair loss, and reduced fertility (78, 162).

It has been proposed for decades that ROS generated during metabolism can damage mtDNA, while the resultant mtDNA mutations would lead to further disruption of the electron transport chain, which then produces more ROS, creating a vicious cycle. Recent studies have suggested that the majority of the accumulated age-associated mtDNA mutations arise not from ROS damage, but rather from spontaneous errors during mtDNA replication. These replication errors occur as low-frequency heteroplasmy, and the potential subsequent clonal expansion of these heteroplasmic mutations may disturb mitochondrial function, especially at the level of the individual cell (154). Therefore, managing the expansion of these mtDNA mutations could be critical for reducing age-related degeneration.

3. MITOPHAGY AND NUTRIENT-REGULATED MITOCHONDRIAL QUALITY CONTROL

3.1. An Overview of Mitophagy

Mitophagy may be the most efficient way to eliminate mtDNA mutations, especially in aging tissues where mtDNA mutations may have extensive cellular heterogeneity. In mammalian cells, the process of mitophagy was first observed in electron microscopy studies, which showed increased

mitochondrial enrichment in lysosomes after glucagon stimulation in hepatocytes (55). Similar to autophagy, mitophagy reuses mitochondrial components during nutrient deprivation, but other important roles for mitophagy are to maintain mitochondrial quality control, avoid the accumulation of mutant mtDNA, and decrease the occurrence of mitochondrial damage–induced diseases (186).

There are multiple mitophagy regulatory pathways, which can be generally classified as ubiquitin-dependent or -independent (summarized in **Figure 3**). The phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (PINK1)/parkin pathway regulates ubiquitin-dependent mitophagy, the most well-known pathway for regulating mitochondrial genome integrity. PINK1 is a mitochondrial serine/threonine kinase, which resides in the cytoplasm and is barely detectable in healthy mitochondria (65). Under steady-state conditions, PINK1 can be rapidly degraded by the E3 ubiquitin ligases UBR1, UBR2, and UBR4 to protect mitochondria from degradation (184). In damaged mitochondria, following the loss of mitochondrial membrane potential (designated as $\Delta\Psi m$), PINK1 aggregates on the outer mitochondrial membrane (OMM) of depolarized mitochondria and phosphorylates ubiquitin, triggering the recruitment of parkin to mitochondria and activation of its E3 ligase activity to further recruit mitophagy receptors (49, 146). PINK1 mutation–bearing cells lack the ability to remove damaged mitochondria, which causes more mitochondrial defects, and thus can directly induce several mitochondria-related diseases (118).

Parkin is an E3 ubiquitin ligase and resides in the cytoplasm in an inactive state (172). Cytoplasmic parkin is phosphorylated and activated by the stabilization of PINK1 on the OMM at residue serine 65 in its ubiquitin-like domain, thereby sustaining and amplifying the mitophagy signals (13).

It remains unclear whether PINK1 and parkin are essential for bulk basal mitophagy (82). In addition to the parkin-dependent pathway, there are three main parkin-independent mitophagy pathways in mammalian cells: receptor mediated, lipid mediated, and ubiquitination mediated (165) (**Figure 3**). Receptor-mediated mitophagy is accomplished by several microtubule-associated protein 1A/1B light chain 3 (LC3)-interacting regions (LIRs) containing autophagic receptors, including BCL2-interacting protein 3 (BNIP3), BCL2-interacting protein 3-like (BNIP3L), FUN14 domain–containing protein 1 (FUNDC1), FK506-binding protein 8 (FKBP8), and BCL2-like protein 13 (BCL2L13) (165), all of which are located at the OMM, and prohibitin 2 (PHB2) (174), which is located at the inner mitochondrial membrane (IMM). Their LIR motifs bind directly with LC3 proteins and gamma-aminobutyric acid receptor–associated protein (GABARAP) autophagosomal membrane proteins, linking the autophagic vesicle to the targeted mitochondria directly, without the involvement of the PINK1/parkin pathway (91).

Phosphorylation of BNIP3L serine 81 is essential for BNIP3L-mediated mitophagy. Inactivation of BNIP3 can also increase PINK1 proteolytic processing and suppress PINK1-parkin-mediated mitophagy (192). BNIP3L and parkin (also known as PRKN) might regulate mitophagy independently because *Bnip3l* and *Prkn* double-knockout mice show a synergistic mitophagy deficiency (187). FUNDC1 interacts with both fission and fusion machinery components, and it regulates mitochondrial dynamics. Knockdown of *FUNDC1* significantly decreases LC3 recruitment to mitochondria, which leads to a reduction in mitophagy (90). FKBP8 promotes mitophagy by LIR-dependent recruitment of LC3A (15). BCL2L13 binds to LC3 through the WXXI motif and induces mitophagy in HEK293 cells. Knockdown of *Bcl2l13* reduces damage-induced fragmentation in mitochondria and mitophagy and promotes the accumulation of mutant mtDNA (105). The LIR domain is predicted to lie between the IMM and OMM in PHB2 (3). PHB2 forms a ternary protein complex with sequestosome 1 (SQSTM1, or ubiquitin-binding protein P62) and LC3, loading LC3 onto damaged mitochondria (180). Although overexpression

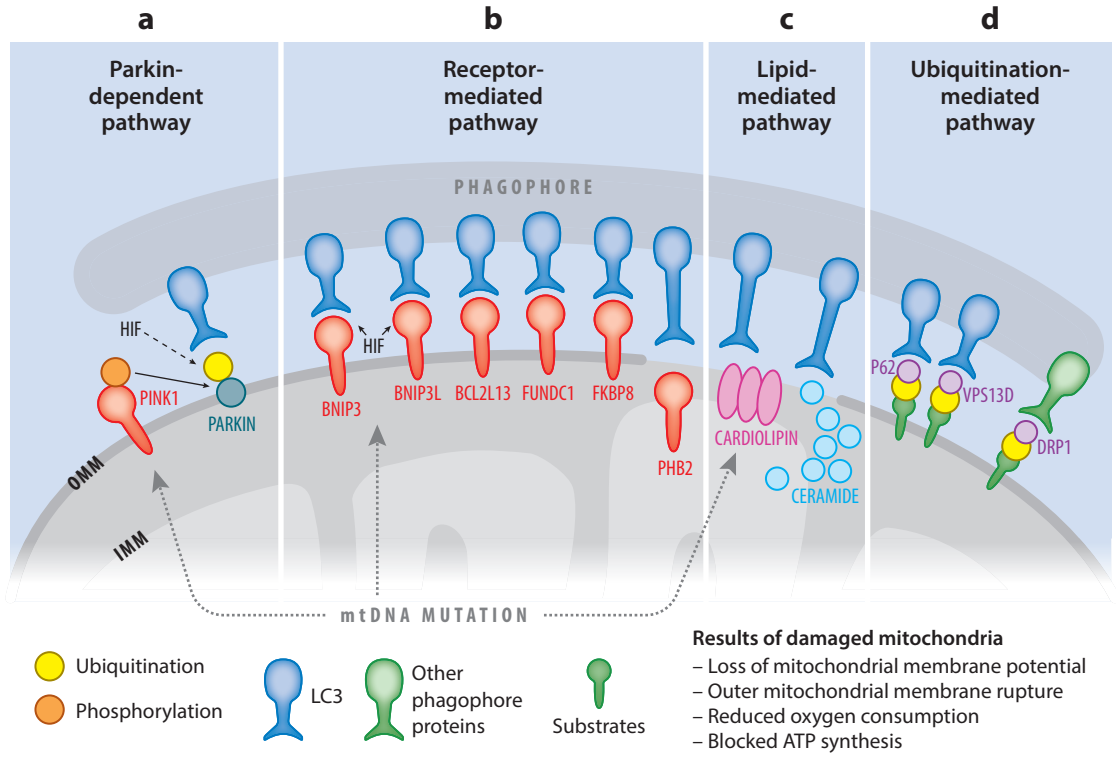

Figure 3

Four known mitophagy regulatory pathways in damaged mitochondria. (*a*) Parkin-dependent pathway: phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (PINK1) is a mitochondrial serine/threonine kinase that aggregates on the outer mitochondrial membrane (OMM) of depolarized mitochondria and phosphorylates ubiquitin, triggering the recruitment of parkin to mitochondria and activation of its E3 ligase activity to further recruit mitophagy receptors. (*b*) Receptor-mediated pathway: BCL2-interacting protein 3 (BNIP3), BCL2-interacting protein 3-like (BNIP3L), BCL2-like protein 13 (BCL2L13), FUN14 domain–containing protein 1 (FUNDC1), FK506-binding protein 8 (FKBP8), and prohibitin 2 (PHB2) are mitochondrial surface receptors containing a light chain 3 (LC3)-interacting region (LIR) domain that binds directly to microtubule-associated protein 1A/1B LC3, linking the phagophore directly to the targeted mitochondria and leading to degradation of mitochondria. (Not all receptors are shown.) (*c*) Lipid-mediated pathway: Following the rupture of the OMM, the cardiolipin located in the inner mitochondrial membrane (IMM) is exposed to the cytoplasm and directly binds to the phagophore to initiate mitophagy. Ceramide is actively and passively released from the mitochondrial matrix into the cytoplasm. Ceramide–LC3B binding facilitates the binding of mitochondria to the phagophore. (*d*) Ubiquitination-mediated pathway: Several ubiquitin ligases mediate mitophagy other than through the parkin-dependent pathway. Ubiquitin-binding protein P62 is absent in a proportion of parkin-positive mitochondria; P62 directly binds to ubiquitinated protein aggregates via its ubiquitin binding–associated domain, and it binds to LC3 via its LIR motif. Vacuolar protein sorting 13 homolog D (VPS13D), dynamin-related protein 1 (DRP1) and other ubiquitin ligases also have domains that bind to ubiquitin chains, facilitating the subsequent assembly of branched ubiquitin chains and directing mitochondria to the proteasome and phagophore. Hypoxia-inducible factor (HIF) can induce multiple receptors, including BNIP3 and BNIP3L, and the PINK1/parkin pathway to regulate mitophagy. Mutations in mitochondrial DNA (mtDNA) stimulate the receptor-mediated pathway and the PINK1/parkin-mediated pathway through the loss of mitochondrial membrane potential and inhibition of the mammalian target of rapamycin proliferative pathway. The synthesis of cardiolipin and lipids can be altered by mtDNA mutations. The accumulation of mutant mtDNA can also influence mitochondrial–nuclear communications, which mediate mitophagy. (Not all receptors are shown.) Solid arrows represent direct interactions; the dotted arrows represent indirect interactions.

of these receptors, such as BNIP3 at the OMM, can promote mitophagy, the key underlying the mobilization of these different receptors to induce mitophagy remains unclear (61).

In addition to receptor-mediated mitophagy, some lipids can also play important roles in mitophagy. Cardiolipin (CL) is an essential lipid for the IMM and regulates mitochondrial fission and fusion, morphology, the respiratory chain, and mitochondrial quality control (35). P53 and sirtuin 6 (SIRT6) bind to cytidine diphosphate diacylglycerol synthase promoters to regulate the de novo biosynthesis of CL (87). Lack of CL biogenesis can cause problems in respiratory complex assembly and a reduction in respiration, which has been observed in various diseases, such as cardiovascular diseases (53), and in Barth syndrome patients (153). After CL is exposed to the cytoplasm as a result of a fractured OMM occurring in mitochondrial damage, the lipid directly binds to autophagosomes to initiate mitophagy (6). Exposed CL also binds to α-synuclein, which increases the recruitment of LC3B to mitochondria and promotes mitophagy in dysfunctional nerve cells (137). Ceramide, another major lipid in mitochondria, suppresses the electron transport chain and induces ROS generation (77). Ceramide generation is controlled by ceramide synthase 1 (CerS1), located at the endoplasmic reticulum (ER) membrane, the overexpression of which promotes ceramide–LC3B binding at the site of the isoleucine 35 and phenylalanine 52 residues and facilitates the binding of mitochondria to autophagosomes (147). *LC3B* knockdown inhibits CerS1-ceramide-dependent mitophagy and promotes tumorigenesis in vivo (147).

Ubiquitination is essential for regulating mitochondrial structure and mitophagy. The PINK1/parkin pathway is not the only critical pathway in ubiquitination-mediated mitophagy (82). Several other ubiquitin ligases have been identified as important players in removing damaged mitochondria. P62 (SQSTM1) is localized to mitochondria in nonstressed conditions. P62 deficiency interrupts the supply of nicotine adenine dinucleotide (NADH) to the electron transport chain and activates the pentose phosphate pathway, leading to mitochondrial dysfunction (10, 145). P62 is absent in a proportion of parkin-positive mitochondria, indicating its independence from the parkin-regulated pathway (178). In P62-mediated mitophagy, P62 directly binds to ubiquitinated protein aggregates via its ubiquitin-binding associated domain, and it binds to LC3B via its LIR motif (85). Upon oxidative stress, the nuclear factor erythroid 2–related factor 2 (NRF2) binds directly to an antioxidant response element in the P62 promoter to induce its expression (62). P62 then recruits two subunits of a cullin-RING ubiquitin E3 ligase complex, Keap1 and Rbx1, during mitophagy induction (183). Dynamin-related protein 1 (DRP1) (70) and vacuolar protein sorting 13 homolog D (VPS13D) (1) are other ubiquitin ligases with important roles in mitophagy. Their ubiquitin-binding domains bind to K63 ubiquitin chains, facilitating the subsequent assembly of K48–K63 branched ubiquitin chains and directing mitochondria to the phagophore (112). Other ubiquitin ligases, including MUL1, ARIH1, and SIAH1, can also associate with damaged mitochondria to ubiquitinate OMM proteins, which then bind to optineurin (OPTN) and NDP52 and induce mitophagy in a parkin-independent manner (165).

3.2. Mitophagy, mtDNA Mutations, and Disease

Despite the emergence of mitophagy as a key process for mitochondrial quality control, the relationship between pathogenic mtDNA mutations and mitophagy is not well studied (32). Deleterious mtDNA mutations can replicate in cells along with wild-type genomes in a state of heteroplasmy and can increase the proportion of damaged mitochondria. The accumulation of mutant mtDNA leads to progressive respiratory chain dysfunction, a premature aging phenotype, and decreases lifespan (162). Mitophagy as a mitochondrial quality control system plays an important role in clearing dysfunctional mitochondria, and this could be essential for preventing

and treating age-related diseases, such as cancer, neurodegeneration, muscle atrophy, diabetes, and aging in general (152).

The impact of mtDNA mutations on mitophagy has been examined in several studies. The mouse model with a proofreading-deficient form of the mtDNA polymerase-γ (*POLG*) indicates that mtDNA mutations lead to an increased level of hepatic mitophagy in vivo (158). Regarding mtDNA mutation–induced mitophagy, it has been argued that both the loss of $\Delta\Psi m$ and inhibition of the mammalian target of rapamycin (mTOR) proliferative pathway are essential for inducing mtDNA mutation–derived mitophagy (32). In mTOR-regulated mitophagy, rapamycin activates macroautophagy by inhibiting the mTOR-mediated proliferative kinase cascade, which initiates widespread mitophagy in cells with mtDNA-derived genetic loss (32). Inhibition of mTOR simulates BCL2 family members and the PINK1/parkin-mediated pathway and, subsequently, induces mitochondrial degradation (97). Moreover, mtDNA mutation induces the alteration of CL, and changes the IMM structure and mitochondrial dynamics (120). The accumulation of mutant mtDNA can also influence mitochondrial–nuclear communications (136), which mediate mitophagy through anterograde regulation (signaling from the nucleus to the mitochondria).

Changes in mitophagy can also alter the mtDNA mutation spectra. An accumulation of pathogenic mtDNA mutations was found in *Prkn*$^{-/-}$ mice (124). Moreover, enhanced mitophagy is an important means to selectively remove mitochondria with mtDNA mutations (157). In one study, the overexpression of parkin reduced the amount of mitochondria with pathogenic *COX1* mutations (157). In this context, it is interesting to see that mitophagy activators might be useful to inhibit tumorigenesis (20). Furthermore, due to the high selectivity of mitophagy, mutated mtDNA can be significantly degraded without affecting the overall mtDNA content, thus maintaining the overall energy supply (64). The relationship between mitophagy and mtDNA mutations could be more complicated and may include the involvement of the immune system. A recent paper has shown that PINK1/parkin-regulated mitophagy influences the cGAS/STING pathway (149), which acts to recognize and degrade cell-free mtDNA released from damaged mitochondria to ensure that harmful mtDNA molecules do not accumulate in the cytoplasm (24). It is worth noting that the cGAS/STING pathway is frequently suppressed during aging and in cancer cells (88). Further investigation is warranted of the biological significance of mitophagy in the immune system in the context of cell-free mtDNA in different diseases and during aging.

The mitophagy pathway was discovered through its connection with diseases. Indeed, mutations in *PINK1* and parkin were first connected to Parkinson's disease (31) and later to aberrant metabolism in cancer (188). Other genes involved in mitochondrial quality control also have implications for disease. Mutations in *Drp1* lead to mitochondrial fission-induced Alzheimer's disease (5) and Parkinson's disease (42). Mutation of the mitofusin 2 gene (*Mfn2*) disrupts the PINK1–parkin connection, which in turn blocks Mfn2 ubiquitination, an important signal for recognizing damaged mitochondria (26), resulting in the accumulation of damaged mitochondria and the pathogenesis of neurodegenerative diseases, metabolic disorders, cardiomyopathies, and cancer (41). It is noteworthy that in the progression of diseases, the loss of mitophagy can induce more damage to cells following their pathological development under a toxic environment, and this can further challenge mitochondrial functions (93). Due to the important role of mtDNA mutation in disease, it is easy to appreciate that defects in mitophagic mitochondrial quality control may play critical roles in various diseases.

3.3. Regulation of Mitophagy

The connections among mtDNA mutations, mitophagy, and disease suggest that during disease development, the orderly, controlled activation of mitophagy, without excessive mitochondrial

clearance, could play a positive role in preventing or treating diseases. However, it is important to emphasize that although mitophagy impairment perturbs mitochondrial function and causes the progressive accumulation of defective organelles, the transient activation of mitophagy might also bring disastrous consequences to cells (29, 104). Therefore, understanding the relationship between mitophagy activation and disease is critical. Here, we briefly discuss the role of nutrients in the quality control of mitochondria through mitophagy, and we summarize this information in **Supplemental Table 1**. It is important to emphasize that mitochondrial functions are interconnected. Most essential and nonessential nutrients are important for various aspects of mitochondrial function, but their involvement in mitophagy is yet to be demonstrated, which highlights an interesting direction for future research.

Supplemental Material >

3.3.1. Tools for mitophagy research. It is essential for the field to develop proper tools to investigate physiological mitophagy in vivo. Traditional methods, such as electron microscopy, which can be used to recognize mitochondria in autophagosomes, are difficult to use for mitophagy quantification. mt-Keima (158) and *mito*-QC (100) are promising tools to analyze how mitophagy is regulated quantitatively in vivo. mt-Keima is a protein derived from coral that has both the properties of pH-dependent excitation and resistance to lysosomal proteases (158). mt-Keima was inserted into the mitochondrial matrix by using a mitochondrial targeting sequence from COX8. During mitophagy, the lysosome wraps around the mitochondria, and the acidic environment inside the lysosome leads to mitochondrial acidification and degradation (2). The pH-dependent properties of mt-Keima allow for rapid identification of its location: either in mitochondria (pH 8.0, green) or in lysosomes (pH 4.5, red); and the ratio of 561-nm laser excitation to 458-nm laser excitation fluorescence reflects the level of mitophagy. Use of the tool has generated interesting observations. For example, even though various mitophagy regulatory pathways had been identified, the mechanisms underlying the tissue-specific occurrence of physiological mitophagy were largely unknown. Using the mt-Keima model, heterogeneous levels of mitophagy in different tissues have been observed, such as a low rate of mitophagy in the thymus and a high rate in the heart. Interestingly, mitophagy in the brain was reduced by 70% in 21-month-old mice in comparison to young mice, an observation prompting the authors to speculate that the reduction in mitophagy could underlie the increase in mtDNA mutations during aging (158).

mito-QC is constructed with a tandem mCherry–green fluorescent protein (GFP) tag with mitochondrial targeting sequence from an OMM protein, mitochondrial fission protein 1 (FIS1) (100). In healthy mitochondria, the mitochondrial network fluoresces with both mCherry (red) and GFP (green). During mitophagy, mitochondria are delivered to lysosomes, where GFP fluorescence becomes quenched by the acidification process but mCherry fluorescence remains stable, and, thus, the green-only and red-only sections can be used to quantify mitophagy. The application of *mito*-QC indicates that mitophagy is more active in tissues with low cell division, such as the kidney, than in tissues with larger populations of stem cells, such as the liver, and that the degree of mitophagy is proportional to the mitochondrial mass in normal cells (100). These tools will be critical for future research seeking to elucidate the mechanisms underlying physiological mitophagy and the role of nutrients in mitophagy regulation.

3.3.2. Induction of mitophagy by insufficient nutrients. Starvation usually induces a macroautophagy process, including mitophagy, and is a nonselective process that reuses cellular material for survival (110). During starvation, the loss of $\Delta\Psi m$ is common, and antiapoptotic proteins of the BCL2 family are expressed to prevent premature cell death (92). Most BCL2 family proteins are located in the OMM, making them sensitive to the nutrient status of the cytoplasm. In mammals, the autophagy-related protein 8 (ATG8) family, consisting of the LC3 and GABARAP

subfamilies, plays primary roles in starvation-related PINK1/parkin mitophagy (110). A study showed that inside the mitochondrial matrix, the loss of the nutrient sensors adenosine monophosphate-activated protein kinase (AMPK) or Unc-51-like autophagy activating kinase 1 (ULK1) results in abnormal accumulation of P62 and defective mitophagy (36). These findings revealed interesting biochemical mechanisms that sense mitochondrial nutrient status to induce mitophagy. Moreover, nutrient starvation also orchestrates mitochondrial–nuclear communication through anterograde regulation to regulate mitophagy (98), and this regulation plays important roles in the mitochondrial stress response, disease, and lifespan regulation (142).

An excess or deficient intake of individual nutrients, such as iron, vitamin C, or nitrite, can also induce the occurrence of hypoxia (160), which further induces mitophagy. Oxygen is essential for oxidative phosphorylation in mitochondria. Hypoxia leads to alterations in mitochondrial functions, including glucose metabolism, lipid metabolism, amino acid metabolism, and antioxidant activity (8). Brief hypoxia can induce mitochondrial biogenesis by regulating the AMPK pathway and peroxisome proliferator–activated receptor-γ coactivator 1-α (PGC-1a)-mediated energy metabolism (198). It can also promote cell survival by regulating transcription factors such as hypoxia-inducible factor 1 (HIF1) and Forkhead box O3 (FOXO3) to suppress oxygen consumption (63). HIF1 can induce mitophagy through different pathways, including the BNIP3 (7) and BNIP3L (190) receptors, and the PINK1/parkin pathway (128). In a *Pink1*$^{-/-}$ model, Pink1 deficiency triggers HIF1 stabilization in mouse embryonic fibroblasts and primary cortical neurons, and it promotes glycolysis to increase cell proliferation (128). In contrast, in a PINK1 overexpression model in vitro, PINK1 improved mitochondrial mass and alleviated myocardial hypoxia–reoxygenation injury (89). Moreover, FUNDC1 also plays an important role in hypoxia-induced mitophagy, and the loss of FUNDC1 leads to mitochondrial functional defects (193). The impact of hypoxia on mitophagy is different in different tissues, such as the liver, muscle, and heart. This tissue-specific effect of mitophagy in response to hypoxia could be caused by interactions among various biological functions—including different energy needs, mitochondrial biogenesis, and mitophagy—in different tissues (193).

3.3.3. Mitophagy and vitamins. Vitamins are essential for normal cell growth and metabolism. Although vitamins are needed in only small quantities, deficiencies may cause severe outcomes in mitochondrial metabolism. Some vitamins have been proven to regulate mitophagy in terms of maintaining the quality of mitochondria and the mtDNA genome. B vitamins are found in unprocessed foods and are essential for the metabolism of energy. A wide variety of B vitamins influences nuclear transcription and ER metabolite activity, and, ultimately, these effects are concentrated on mitochondrial function (33). B vitamin deficiency has many consequences, including appetite loss and uncharacteristic emotional sensitivity, and, ultimately, pathological changes in the nervous system (114). Vitamins C and D are also important for mitochondrial function. In this section, we briefly discuss the roles of vitamins in mitophagy. Although a tremendous amount of research has been done looking at the role of vitamins in general mitochondrial function, more research is needed to directly address the relationship between vitamins and mitophagy.

Mitochondria are the target organelles of vitamin B_1 (thiamine) (76). Thiamine deficiency (TD) leads to a reduced mitochondrial oxidative metabolism (74). Interestingly, TD induces the accumulation of autophagosomes in neurons by upregulating autophagic markers, such as LC3-II, ATG5 and Beclin 1. The expression of these autophagic markers is reversed when thiamine is reintroduced (74). However, TD inhibits the phosphorylation of p70S6 kinase and the mTOR/p70S6 kinase pathway in the cytoplasm, and this inhibition provides a prerequisite for the degradation of mitochondria (74). TD can also stimulate ER stress markers, such as heat shock protein family A (HSP70) member 5 (HSPA5), X-box-binding protein 1 (XBP-1), CHOP, activating transcription

factor 6 (ATF-6), phosphorylated eukaryotic initiation factor 2A (eIF2-a), and cleaved caspase 12, all of which can facilitate mitochondrial fission and mitophagy through ER–mitochondria interactions (44, 170).

The derivatives of vitamin B_2 (riboflavin), flavin mononucleotide and flavin adenine dinucleotide, are important for the complex I and II functions in the electron transport chain, thus playing important roles in ATP production (27). Riboflavin-responsive multiple acyl-coenzyme A dehydrogenation deficiency (RR-MADD) is associated with mutations in the gene coding for electron transfer flavoprotein–ubiquinone oxidoreductase. Patients with RR-MADD have defects in mitochondrial quality control, which can be treated with high doses of riboflavin (28). Moreover, riboflavin has been suggested to reduce mtDNA mutations, which might be particularly effective in people with non-H mtDNA haplotypes. This could be caused by the association between haplogroup H and an increased activity in complex I, which is a major target of riboflavin (21). However, direct evidence on the pathways underlying the relationship between riboflavin and mitophagy is yet to be revealed, but this could further elucidate the role of riboflavin in mitochondrial quality control.

The relationship between vitamin B_3 (nicotinamide) and mitophagy has been well studied (151). Nicotinamide supplementation promotes the activity of mitochondrial nicotinamide nucleotide transhydrogenase (NNT), which transfers electrons from NADH to NADP (nicotinamide adenine dinucleotide phosphate) and maintains the NADP:NADPH ratio that protects mitochondria from oxidative damage (151). Interestingly, NNT can also oxidize the NADP–NADPH pool and disrupt the antioxidant defense (107). For this reason, organs with high energy requirements, such as the brain, are particularly susceptible to nicotinamide deficiency (75). Nicotinamide can suppress mitochondrial permeability transition pore (mPTP) formation and induce mitophagy (151). Furthermore, nicotinamide treatment can induce the expression of genes involved in mitochondrial fission and fusion, such as *Fis1*, *Drp1*, and *Mfn1*, which may also lead to mitophagy (72). SIRT1 controls mitochondrial function and biogenesis, which weaken with aging. It has been suggested that nicotinamide could mimic the SIRT1 activator SRT1720 (197), thereby relieving defective mitophagy (39).

Pyridoxal is the active form of pyridoxine, vitamin B_6, in cells, which affects serine hydroxymethyltransferase (SHMT) through the regulation of pyridoxal 5′-phosphate (67). SHMT, especially its mitochondrial isoform SHMT2, plays an important role in mitochondrial serine metabolism (51) and influences the initiation of mitophagy (47). It has been shown that the fragmentation of the mitochondrial membrane structure is an important prerequisite for mitophagy during mitochondrial injury, and this fragmentation is promoted under serine deprivation (47).

Vitamin B_9 (folate) regulates the interaction between genetic risk variants and environmental factors through its important roles in one-carbon metabolism (43). Issues in folate-mediated one-carbon metabolism are associated with abnormal mtDNA methylation and several mitochondria-related diseases, including cancers, metabolic syndromes, and nervous system defects (155). Cerebral folate deficiency is a disease associated with a decrease in mitochondrial activity and, potentially, the occurrence of mtDNA mutations (60). There is more direct evidence that folate-appended methyl-β-cyclodextrin can induce mitophagy through the PINK1-mediated pathway and can enhance LC3 conversion (71, 115).

Vitamin C is known as an antioxidant, and it directly protects mitochondria by scavenging ROS (141). It plays an important role in reducing mitochondrial oxidative stress and increasing tolerance to mycotoxins by eliminating damaged mitochondria through PINK1/parkin-mediated mitophagy (17). Vitamin C supplementation increases the reprogramming efficiency of *Pink1* knockout mouse embryonic fibroblasts (164). This indicates that vitamin C might be able to increase mitophagy and function as an alternative means of maintaining mitochondrial quality

control when the PINK1/parkin pathway is blocked. Currently, there is no direct evidence that vitamin C is related to other pathways, such as receptor-mediated mitophagy.

Vitamin D (cholecalciferol) and its metabolites have important roles in mitochondrial function: Vitamin D is closely related to important mitochondrial functions, including oxidative phosphorylation, Ca^{2+} pump regulation, and ROS generation, and its deficiency has been suggested as an important environmental factor underlying the pathogenesis of many mitochondria-related diseases, such as declines in muscle capacity, cardiovascular disease, Alzheimer's disease, and cancer (14). Vitamin D also affects mitochondrial function by regulating autophagy, inflammation, and nuclear epigenetic changes (14). Deletion of the vitamin D receptor leads to defective general autophagy and impairment of mitochondrial integrity, which can lead to cell death (129, 177). Interestingly, in some studies, it was found that vitamin D deficiency may also induce mitophagy. In particular, vitamin D deficiency suppresses complex I of the electron transport chain, and the inhibition of complex I is a trigger to induce mitophagy (11). It will be important to elucidate the relationship between vitamin D deficiency and mitophagy and their important roles in disease.

3.3.4. Mitophagy and mineral nutrients. Mitochondria have evolved a highly integrated network of mechanisms for utilizing micronutrients to coordinate cellular energy metabolism, survival, and cell death (52). In this section, we focus on the trace mineral elements that are present at high levels in mitochondria, including calcium, zinc, iron, selenium, and manganese, and discuss their relationship with mitophagy. It is well known that certain trace elements, such as magnesium, are essential for mitochondrial functions (66, 103); however, research into their roles in mitophagy is lacking. Further studies are needed that illustrate these connections to aid in understanding nutrient-regulated mitophagy.

Calcium enters mitochondria through calcium channels, such as the mitochondrial calcium uniporter, to regulate apoptosis (139). Mitochondrial Ca^{2+} acts as an important secondary messenger, as either a cause or consequence of mitophagy induction (131). The loss of PINK1 reduces the activity of the mitochondrial sodium–calcium exchanger and leads to the accumulation of mitochondrial Ca^{2+} (46). The opening of the mPTP is activated by Ca^{2+}, indicating the important function of PINK1 in regulating mitochondrial activity under stress conditions. Interestingly, PINK1 has been suggested to be associated with Parkinson's disease through calcium-induced neuronal cell death (46). Moreover, calcium-induced mitophagy may also occur through receptor-mediated pathways. It is clear that BCL2 family members mediate Ca^{2+} release, thus protecting the cell from apoptosis (95). As mentioned earlier, BCL2L13 can induce mitophagy, and the regulation of BCL2L13 has the potential to be controlled by calcium channels in order to maintain mitochondrial homeostasis.

Zinc is an essential trace metal element, functioning as a cofactor for numerous enzymes and transcription factors. Cardiomyocytes are rich in zinc and contain the highest mitochondrial concentration in vivo. Cardiomyocytes produce large amounts of ROS, which makes cardiac tissue vulnerable to mitochondrial damage (106). Mitophagy in cardiomyocytes has been associated with the upregulation of the autophagy activator Beclin 1 and the opening of the mPTP (161). As an important trace element, zinc can induce the expression of Akt, extracellular signal-regulated kinase (ERK), and Beclin 1 (83), as well as the opening of the mPTP (16), thereby activating PINK1 in the hypoxia–reoxygenation model of cardiomyocytes (16), suggesting that zinc-mediated mitophagy is essential for maintaining the normal functioning of cardiomyocytes.

Iron is a trace element important for the biosynthesis of heme and iron–sulfur cluster-containing proteins in mitochondria, which are critical for a wide variety of cytoplasmic and nuclear functions, including oxidative phosphorylation, DNA replication, RNA transcription, protein translation, and many other cellular processes (130). Anemia caused by deficient iron

metabolism can lead to abnormal mitochondrial function (119). However, iron depletion can lead to reduced mitochondrial electron transport chain activity, which activates parkin-, PINK1-, and BNIP3-dependent mitophagy, and, interestingly, promotes lifespan extension in *Caenorhabditis elegans* (142). However, iron overload, especially in the central nervous system, can lead to oxidative stress, mitochondrial insufficiency, and impairment in mitophagy, common features of age-related central nervous system disorders (143). Metastatic tumor cells can sometimes accumulate excessive iron by altering iron metabolism and manipulating the mitophagy process (56). It has been hypothesized that inducing mitophagy through iron starvation might be an effective and novel strategy for treating cancer (148). Furthermore, the function of the ER–mitochondria junction is iron dependent, and it plays an important role in regulating mitochondrial metabolism (182). The lack of this connection can decrease parkin-dependent mitophagy (194).

Selenium is a cofactor of proteins that have important functions, such as glutathione peroxidase, which protects organisms from oxidative damage. Selenium deficiency induced by oxidative stress is one of the most important ways to activate mitophagy. Hallmarks of mitophagy—such as LC3 aggregation, mitochondrial protein degradation, and reduction in $\Delta\Psi$m—have been observed under conditions of selenium deficiency (59, 90). MUL1, the mitochondrial ubiquitin ligase activator of nuclear factor κB subunit 1 (NF-κB1), and ULK1 have been shown to play an important role following mitochondrial dysfunction in regulating selenite-induced mitophagy, providing a novel mechanism for the beneficial effects of selenium (86).

Manganese is essential for development, metabolism, and antioxidant functions. Excessive exposure to manganese increases the occurrence of nervous system diseases (135). The accumulation of manganese in mitochondria causes a decrease in $\Delta\Psi$m, opening of the mPTP, ROS generation, and apoptosis (191). Manganese has been shown to induce mitophagy by enhancing FOXO3 nuclear retention (150) and inducing the PINK1/parkin-mediated pathway in nerve cells (189). Moreover, manganese superoxide dismutase (MnSOD), encoded by a stress-responsive gene, is an antioxidant enzyme localized in mitochondria (175). MnSOD is expressed at a low level under normal conditions, and it is highly inducible by a wide range of agents, thus participating in mitochondrial–nuclear cross talk (181). MnSOD could interact with the activated mitochondrial cytochrome P450 1B1 to cause mitophagy (30).

3.3.5. Mitophagy, non-nutritive bioactive food components, and metabolites. In this section, we focus on some of the functional food components that have been shown to play important roles in mitophagy regulation. We discuss several naturally active components—such as taurine, *N*-acetyl-L-cysteine (NAC), resveratrol, spermidine, urolithin A (UA), and ammonia—and their roles in mitophagy regulation. Understanding the dietary regulation of mitophagy and its underlying mechanisms provides a promising direction for developing strategies to maintain mitochondrial genome integrity and mitochondrial health.

Taurine, a sulfur-containing amino acid with important roles in fat metabolism, is abundant in organs enriched in mitochondria, including the heart, retina, skeletal muscle, and brain. It can protect the ultrastructure of neurons and enhance the antioxidant capacity and function of the mitochondrial respiratory chain complex (25). Decreases in taurine content can reduce the activity of respiratory chain complexes I and III (68) and alter energy metabolism and mitochondrial function (69). In a taurine transporter knockout heart, a defect in mitophagy limits the removal of damaged mitochondria (69). In enriched amounts, taurine can inhibit heat shock protein 90 (HSP90) (58) and induce mitophagy by maintaining PINK1 accumulation, increasing ubiquitin phosphorylation at serine 65, and upregulating parkin recruitment to mitochondria. The mitophagy associated with mitochondrial HSP90 inhibition is independent of mitochondrial membrane depolarization, and it plays an important role in mitochondrial quality control under stress conditions (40).

NAC is synthesized from the amino acid L-cysteine. NAC has been reported to lower endogenous oxidant levels and protect cells against a wide range of oxidative stress (38). The scavenging of ROS by NAC has been found to increase cell viability and substantially inhibit PINK1-dependent parkin translocation to mitochondria in response to the suppression of oxidative phosphorylation (179). The mitochondria-anchored receptors ATG32 (113) and peroxiredoxin 6 (94) are believed to be involved in NAC-induced mitophagy. Moreover, the combination of NAC, L-carnitine, and other antioxidants is considered to be an efficient strategy to induce mitophagy (173, 179).

Resveratrol (*trans*-3,5,4′-trihydroxystilbene), a naturally occurring polyphenolic phytoalexin, has been shown to be beneficial in human diseases, including cancer (12). Resveratrol treatment can increase mitochondrial biogenesis and improve insulin sensitivity (156). Resveratrol influences the mitochondrial permeability transition (195), SIRT1-mediated mitochondrial biogenesis (102), and MFN2-mediated mitochondrial fusion (132). It can directly enhance mitophagy by increasing acidic vesicular organelle numbers, the LC3-II:LC3-I ratio, parkin and Beclin 1 expression, and LC3 and translocase of outer mitochondrial membrane 20 (TOMM20) colocalization (168). Furthermore, resveratrol-induced mitophagy can attenuate inflammatory damage by regulating AMPK activation (176).

The natural hormone melatonin is present in mushrooms, cereals, germinated legumes, and seeds (101). Melatonin is a potent antioxidant because it can pass biobarriers easily due to being highly lipophilic and weakly hydrophilic. It scavenges free radicals and reduces oxidative damage in different tissues (169). Melatonin treatment can increase the expression of parkin and PINK1, and it can inhibit NLRP3 inflammasome activation (84). Interestingly, melatonin can also inhibit mitophagy by activating AMPKα in mitochondria and reducing DRP1-dependent mitochondrial fission (84). Melatonin-induced reduction of mitochondrial fission led to an interaction between voltage-dependent anion channel 1 (VDAC1) and hexokinase 2 (HK2), which further inhibited opening of the mPTP and PINK1/parkin activation (196).

UA is a metabolite produced from the transformation of ellagitannins by the gut bacteria, and it also exists in pomegranates and other fruits (48). It has been recently shown that UA can activate mitophagy in muscle and intestinal cell lines (138). Following UA induction, a signature sequence of events for mitophagy has been observed, including a loss of $\Delta\Psi$m, PINK1/parkin pathway activation, and P62 enrichment, and a significantly increased percentage of mitochondria were observed in the lysosome. Interestingly, UA-induced mitophagy had no impact on ROS production, and UA could prolong the lifespan in the presence of a potent antioxidant, *N*-acetylcysteine, indicating that UA's beneficial effects are independent of ROS status and functions (138). UA treatment also increased mitochondrial biogenesis and cellular respiration under both basal and uncoupled conditions, indicating that UA can improve both mitochondrial quality and quantity (138).

Spermidine is a natural polyamine, and in several model organisms supplementation with spermidine has been shown to improve epigenetic modifications, activate autophagy, and extend the lifespan (96). Spermidine induces mitophagy by increasing mitochondrial depolarization and activating the protein kinase ataxia–telangiectasia mutated (ATM). ATM regulates tuberous sclerosis complex 2 (TSC2) and HIF1α to modulate redox homeostasis, and it also promotes the PINK1/parkin pathway (127). Due to its ability to induce autophagy and mitophagy, spermidine is believed to be beneficial for several diseases, including type 2 diabetes (123).

Although ammonia is not a food component, dietary factors can affect the generation of ammonia in serum and urine (126). For a long time, ammonia was considered to be a cytotoxic factor, working through the mPTP and inducing apoptosis (111). An interesting study indicates that the ammonia/SIRT5 pathway plays an important role in regulating mitophagy. SIRT5 is a member of a family of NAD-dependent protein deacetylases that regulate metabolic homeostasis in the

IMM (140). SIRT5 has been implicated in regulating ammonia levels by deacetylation and activation of carbamoyl-phosphate synthetase 1, mitochondrial (CPS1), the rate-limiting enzyme of the urea cycle (108). Both pharmacological inhibition of SIRT5 and ammonia supplementation can stimulate mitophagy by inducing BNIP3 and the PINK1/parkin system (125) to clear damaged mitochondria.

4. CONCLUSIONS AND FUTURE DIRECTIONS

Mitochondrial dysfunction through mtDNA mutation is associated with the onset and progression of various diseases. It will be important to understand the processes through which mtDNA mutations occur and their functional consequences and to develop strategies, including dietary interventions and behavioral changes, to delay the occurrence of mtDNA mutations and remove mutations to delay the onset of and manage mitochondria-related diseases. Below are several research directions that the authors believe are critical in this endeavor.

1. Develop efficient methods to reveal mtDNA mutation patterns. It is important to take advantage of next-generation sequencing approaches to effectively reveal the existence of mtDNA mutations and establish their association with various diseases. Due to the likely heterogeneity of mtDNA mutations in different cells, it is critical to be able to conveniently see patterns of single-cell mtDNA mutations and establish their distribution in different disease contexts.
2. Investigate the origins of inherited mtDNA mutations. The process of eliminating germline mtDNA mutations is critical for species survival during evolution. It will be important to elucidate the mechanisms involved because disruptions to or inefficiencies in these could underlie important childhood diseases, including classic mitochondrial diseases and more common diseases, such as autism.
3. Create tools for the genetic manipulation of mtDNA. This is critical for elucidating the functional consequences of mtDNA mutations. Zinc-finger and transcription activator–like effector nucleases (or TALEN) tools have been developed to delete pathogenic mutations (4, 45). An efficient tool to create mtDNA mutations in cell lines and model organisms is essential to demonstrate the causal relationship between mtDNA mutations and diseases.
4. Develop better tools for targeted mitophagy. It is important to elucidate the unknown pathways in mitophagy, especially under normal physiological conditions, and come up with strategies that can take advantage of mitophagy to slow down and remove deleterious mtDNA mutations.
5. Identify functional food components, behavior changes, and combinations of these that can benefit mitochondrial functions, especially for targeting the removal of damaged mitochondria to reduce the accumulation of mtDNA mutations.

With the increasing awareness among the general population of the importance of disease prevention and increased understanding of the mechanisms underlying healthy dietary habits and food choices, foods and nutrition are receiving unprecedented attention in attempts to promote health. As the key organelles of cellular metabolism, mitochondria form a critical link between food and nutrition and diseases. Understanding mitochondrial functional homeostasis in the context of mtDNA mutations could mechanistically corroborate people's healthy choices in dietary behavior and provide further potential targets for developing efficient strategies to prevent and treat disease.

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