Mitochondrial diseases: the contribution of organelle stress responses to pathology

Anu Suomalainen[1–3] and Brendan J. Battersby[4]

Abstract | Mitochondrial diseases affect one in 2,000 individuals; they can present at any age and they can manifest in any organ. How defects in mitochondria can cause such a diverse range of human diseases remains poorly understood. Insight into this diversity is emerging from recent research that investigated defects in mitochondrial protein synthesis and mitochondrial DNA maintenance, which showed that many cell-specific stress responses are induced in response to mitochondrial dysfunction. Studying the molecular regulation of these stress responses might increase our understanding of the pathogenesis and variability of human mitochondrial diseases.

Respiratory chain enzyme complexes
A set of four enzyme complexes that couple the transfer of electrons from carrier molecules, such as NADH or $FADH_2$, to a series of electron acceptors of increasing affinity and, ultimately, to molecular oxygen, which is coupled to the pumping of protons across the inner mitochondrial membrane to generate an electrochemical potential.

[1]University of Helsinki, Research Programs Unit, Molecular Neurology, Biomedicum Helsinki, 00290 Helsinki, Finland.
[2]Neuroscience Center, University of Helsinki, 00790 Helsinki, Finland.
[3]Helsinki University Hospital, Department of Neurology, 00290 Helsinki, Finland.
[4]Institute of Biotechnology, University of Helsinki, 00790 Helsinki, Finland.
anu.wartiovaara@helsinki.fi; brendan.battersby@helsinki.fi

doi:10.1038/nrm.2017.66
Published online 9 Aug 2017

Mitochondrial diseases are one of the most common types of inherited metabolic disorder, which show unprecedented variability in clinical presentation and can manifest at any age in any organ[1–3] (FIG. 1). Despite dramatic improvements in the genetic and metabolic diagnosis of these severe progressive diseases, there are still no curative treatments[4,5]. Unfortunately, our understanding of the molecular mechanisms that underlie the pathogenesis of these diseases has not kept pace with the diagnostic advances, and this poses a significant roadblock to developing therapeutic treatments.

The breakthrough of next-generation sequencing approaches for genetic diagnosis has revealed extensive genetic heterogeneity of the mitochondrial disease group. More than 1,300 genes that are encoded in the nucleus are synthesized as proteins in the cytosol and targeted to mitochondria[6]. Mitochondria also contain a small circular multicopy genome (referred to as mitochondrial DNA (mtDNA)) that is maternally inherited and contains only 37 genes. Mutations in any one of these nuclear or mitochondrial genes can lead to mitochondrial dysfunction by different modes of transmission: sporadic, maternal, autosomal recessive, autosomal dominant or X-linked[3]. Furthermore, mutations can arise *de novo* during embryogenesis and can thus be present in all cells of the embryo or can occur only in specific tissues. Thus, mitochondrial diseases can follow any inheritance model, and are probably one of the most heterogeneous conditions known in the whole field of human genetic disorders.

Genetic mutations that disrupt mitochondrial gene expression (mtDNA replication and transcription, and mRNA translation in mitochondria) appear to be the most common cause of mitochondrial diseases[3,7,8]. Defects in all of these processes lead to a failure in the assembly of the respiratory chain enzyme complexes and ATP synthase, which impinge upon mitochondrial oxidative phosphorylation. These enzymes are assembled in the mitochondrial inner membrane folds called cristae and use nutrient-derived reducing equivalents to generate an electrochemical potential across the inner membrane that is essential for organelle functions[2] (BOX 1).

Despite the fact that defects in oxidative phosphorylation are a typical outcome of genetic mutations that disrupt mitochondrial function, the clinical manifestations of mitochondrial disorders show remarkable variability (FIG. 1). This pleiotropy would suggest that a deficiency in mitochondrial production of ATP alone cannot account for the clinical range and tissue specificity of these disorders. Further support for this idea comes from severe inherited respiratory chain deficiencies, which do not typically compromise fetal development, but instead manifest as rapidly progressing diseases soon after birth, which are characterized by organ failure and lactic acidosis with a fatal outcome[1]. However, in some cases, infants can survive the critical early phase and recover[9,10], or they can develop another progressive disorder later in life[11]. Even though the demand for oxidative phosphorylation is high immediately after birth, the sensitivity of different organs to respiratory chain dysfunction appears to be influenced by age, thereby affecting the onset and manifestations of disease. These aspects indicate that: first, glycolytic, non-oxidative ATP production prevails during normal fetal growth; second, oxidative phosphorylation is essential immediately after birth for postnatal maintenance of tissues; and third, specific organs have age-dependent sensitivity to specific mitochondrial

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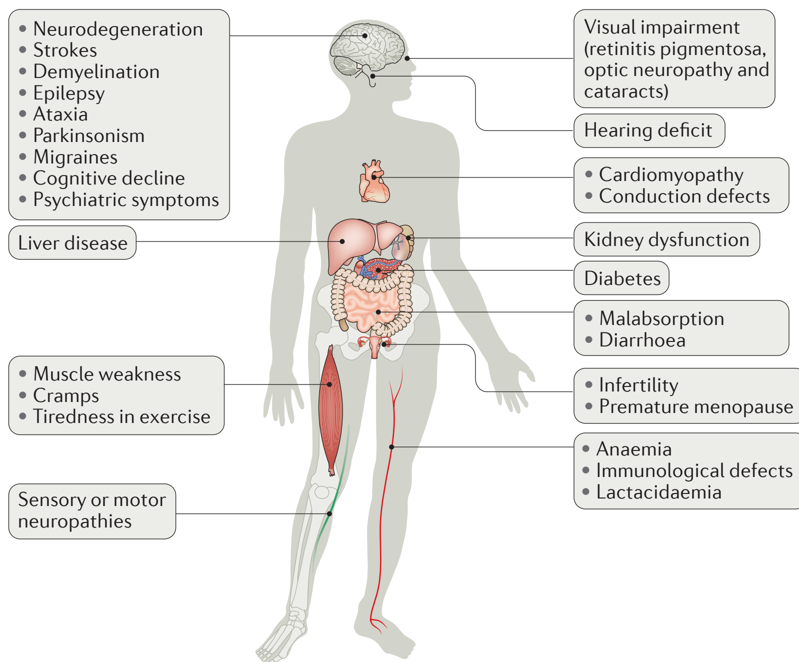

Figure 1 | **The variability of mitochondrial disease manifestations.** Mitochondrial diseases can manifest both in children and in adults, and can present in various organs, including in multiple organs that may have no apparent functional links to each other, such as the brain and liver, or pancreatic β-cells and the auditory system. Sometimes manifestations only affect one tissue, such as the heart or the optic nerve. Children may recover from one phenotype and later develop another — for example, in Pearson syndrome, the primary manifestation is exocrine pancreatic dysfunction and megaloblastic anaemia, and the survivors may later develop brain disease. Typically, these disorders are progressive.

ATP synthase
Rotary enzyme in the inner mitochondrial membrane that couples the proton motive force to the synthesis of ATP.

Oxidative phosphorylation
The process of coupling oxidation of nutrients and electron transfer to molecular oxygen with the proton motive force for ATP synthesis.

Reducing equivalents
The major electron acceptors NAD^+ and FAD, which are used in the breakdown of the carbon backbone of nutrients to produce the reduced molecules NADH and $FADH_2$.

Electrochemical potential
The $[H^+]$ gradient across the inner mitochondrial membrane that is generated through proton pumping by the respiratory chain complexes.

Lactic acidosis
Elevation of lactate, which is a product of glycolytic energy metabolism, in the blood of patients, resulting in life-threatening acidification.

dysfunctions. Little knowledge exists of mechanisms underlying such sensitivity, although they might be excellent targets of therapy.

Mitochondria respond to environmental stimuli, such as nutrient supply and exercise, to boost oxidative nutrient combustion or feed cellular biosynthesis reactions, generating 'retrograde signals', including metabolites, cofactors, nutrients, gasotransmitters and the overall redox balance of the organelle. These retrograde signals allow the modulation of cellular metabolism in response to environmental cues, and thus, have the capability to strongly influence cell function and behaviour. For example, reactive oxygen species (ROS) may modify cellular fates[12,13]. The response of mitochondria can be further differentially modulated depending on the cell type, developmental timing, age and daily feeding and fasting cycles[14]. According to recent evidence, these different signals are modified by mitochondrial dysfunction and may contribute to the clinical range or progression of human disease[2,15,16]. Surprisingly, the current understanding of these parameters in the progression of human mitochondrial disease is incomplete.

In this Review, we focus on the emerging paradigms that are common to the molecular pathophysiology of mitochondrial disease, and we place a special emphasis on mtDNA genome maintenance and mitochondrial protein synthesis, as these two disease groups make up the clear majority of mitochondrial disorders[3,7,8]. The pools of building blocks (for example, deoxynucleotides, ribonucleotides and amino acids) that are needed for mitochondrial gene expression are interconnected with the rest of the cell, and therefore it is perhaps not surprising that molecular defects in these mitochondrial pathways generate integrated signalling stress responses. Furthermore, they may also be relevant to the pathogenesis of other common human disorders, such as Parkinson disease, where mitochondrial dysfunction has been consistently implicated as a secondary event in progression of the disease.

mtDNA maintenance and translation

The machinery that is needed to maintain the mitochondrial genome is entirely encoded in the nucleus and appears to have a bacteriophage origin. By contrast, protein synthesis within the mitochondrial compartment occurs on unique ribosomes by mechanisms that share an ancestry with those in the Alphaproteobacteria[17,18]. Here, we summarize the key components and processes that are needed for the faithful expression of the mitochondrial genome (FIG. 2). More detailed reviews can be found elsewhere[19,20].

mtDNA maintenance machinery. The minimal replisome of mtDNA consists of DNA polymerase-γ (POLG; a heterodimer of the catalytic α-subunit and the accessory β-subunit), the replicative helicase Twinkle and mitochondrial single-stranded DNA-binding protein[21–23] (FIG. 2a). mtDNA maintenance also requires other factors, including the mitochondrial RNA polymerase (which produces replication primers[24]), mitochondrial transcription factor A (TFAM)[25] and various mtDNA-processing enzymes (see FIG. 3 for disease-linked genes and the associated processes that are related to mtDNA maintenance or expression; TABLE 1 and Supplementary information S1 (table) include information on diseases and disease-associated phenotypes). POLG replicates mtDNA with high fidelity compared with other polymerases, and inactivation of its proofreading exonuclease function in mice leads to highly increased mtDNA mutagenesis and progeric syndrome: hair greying, anaemia, loss of subcutaneous fat, osteoporosis and overall organ wasting[26,27]. Based on the extensive mtDNA mutagenesis, these mice were named 'mtDNA mutator' mice.

The exact mechanisms that limit mtDNA copy number in different tissues are still unclear. Twinkle helicase licenses mtDNA for replication and thereby its levels correlate with mtDNA copy number[28]. TFAM increases the half-life of mtDNA by increasing its compactness[25,29,30] and thereby also engages in mtDNA copy number control. In addition to these proteins, mtDNA requires nucleotides for replication, and their deficiency challenges mtDNA maintenance. The mitochondrial nucleotide pools are closely linked to cytosolic nucleotide metabolism and, in non-dividing tissues, the cellular nucleotide pools mostly serve mtDNA replication[31]. As a consequence, quantitative changes or an imbalance in cellular nucleotide pools impair mtDNA replication

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Cofactors
Molecules required for the function of enzymes and/or the progression of metabolic pathways.

Alphaproteobacteria
A class of Gram-negative bacteria, including intracellular parasites, from which mitochondria are thought to have originated following an endosymbiotic event that gave rise to eukaryotes.

Replisome
Enzymes and proteins that function at DNA replication forks.

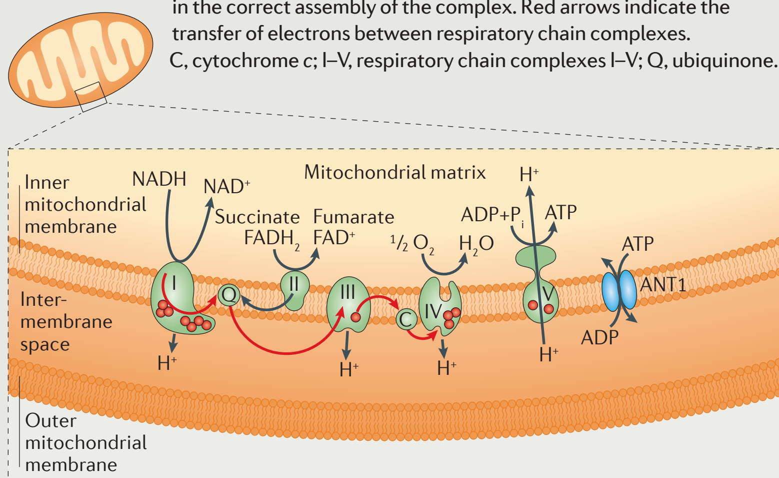
and result in mtDNA depletion or deletions, and the activation of metabolic stress responses (see below). Also, nucleotide analogues, which are used as drugs to target retroviruses, can block mtDNA replication by binding to POLG, leading to reversible mtDNA depletion[32]. This sensitivity is presumably a consequence of the shared evolutionary origin of the mitochondrial and the retroviral replication systems.

The major defects of mtDNA maintenance. Defective replication of the mitochondrial genome leads to a quantitative decrease in the abundance of mtDNA (depletion) or genome instability that generates mtDNA molecules with large-scale deletions. This can arise from genetic mutations in the core mtDNA replication machinery (POLG and Twinkle) or as a result of nucleotide synthesis defects that lead to imbalanced nucleotide pools[33] (FIGS 2,3; TABLE 1). Typically, mtDNA depletion is associated with severe, infantile-onset, highly tissue-specific manifestations. For example, mutations in mitochondrial thymidine kinase 2 (*TK2*) — an enzyme that is crucial for the mitochondrial pyrimidine salvage pathway and that phosphorylates thymidine, deoxycytidine and deoxyuridine — cause a rapidly progressive muscle weakness and degeneration in infants, whereas the effects of mutations that affect other nucleotide metabolism enzymes, such as mitochondrial deoxyguanosine kinase (*DGUOK*), first manifest in the brain and liver[33–36]. In adults, functional impairment of the mtDNA replication machinery manifests as progressive neurodegeneration (ataxia, parkinsonism and sensory polyneuropathy) or pure muscle disease, with subtle progressive accumulation of multiple mtDNA deletions in the muscle, heart and brain[33,37,38]. mtDNA instability can also arise as a secondary consequence of mutations, such as in enzymes that are involved in mitochondrial fusion. For example, mutations in the dynamin-related mitochondrial GTPase optic atrophy protein 1 (OPA1), which is a central regulator of mitochondrial inner membrane dynamics and ultrastructure, result in multiple mtDNA deletions and manifest as optic atrophy and neurodegenerative disease. These findings link the dynamics of mitochondrial inner membrane morphology to mtDNA maintenance[39–41] and may be related to the coupling of mtDNA replication and mitochondrial division[42].

Mitochondrial protein synthesis. Mitochondria contain their own unique protein synthesis machinery that is devoted to synthesizing only the 13 hydrophobic proteins that are encoded in mtDNA (FIG. 2b). The translation process has many similarities to that in bacteria, with some important differences: first, the mammalian mitoribosomes are protein rich and incorporate mt-tRNA^{Val} or mt-tRNA^{Phe} into the large subunit[43]; second, mitochondrial mRNAs do not have a 5′ untranslated region, cap or Shine–Dalgarno sequence, indicating that translation initiation is accomplished by a unique means that is still not properly understood[20]. Similarly to bacteria, translation initiation requires a formylated methionine tRNA, which distinguishes the initiator methionine from the methionine that is used during elongation, as mtDNA only encodes a single tRNA^{Met} (FIG. 2b). Defects in this formylation reaction cause severe progressive mitochondrial disease combined with respiratory chain deficiency[44]. The main steps of mitochondrial translation initiation, elongation and termination are depicted in FIG. 2b. Mitochondria also contain a unique set of 17 aminoacyl-tRNA synthetases that are dedicated to the aminoacylation of the mtDNAencoded tRNAs[45] (FIG. 3). The lysyl and glycyl aminoacyl-tRNA synthetases function in both the cytoplasmic and the mitochondrial compartments. The inability, to date, to reconstitute a functional *in vitro* translation system hampers the ability to develop a detailed molecular and biochemical understanding of mitochondrial protein synthesis. The advent of new methodologies, such as ribosome profiling[46], promises to transform our understanding of human mitochondrial protein synthesis.

Box 1 | **Oxidative phosphorylation and respiratory chain deficiency**

The oxidation steps of the tricarboxylic acid cycle break down nutrients to reduce NAD^+ and FAD to NADH and $FADH_2$, respectively. These carriers donate the electrons to the respiratory chain complexes, complex I (NADH) and complex II ($FADH_2$), and thereafter to ubiquinone (Q). The electrons are transferred from one respiratory chain complex to another by a series of reducing–oxidizing reactions, which drives the pumping of protons (H^+) by respiratory chain complexes I, III and IV to the intermembrane space and generates an electrochemical gradient across the mitochondrial inner membrane (see the figure). Complex IV donates the electrons to oxygen, which is the terminal electron acceptor, and is consequently reduced to water. The proton motive force (straight arrow across the inner mitochondrial membrane) is used by the ATP synthase (also known as complex V) to phosphorylate ADP to produce ATP (transferring inorganic phosphate (P_i) to ADP). ATP transport out of the mitochondria is carried out by ADP–ATP translocase 1 (ANT1; also known as SLC25A4). The respiratory chain and ATP synthase consist of a total of 89 different protein subunits, of which 76 are encoded by nuclear genes and 13 are encoded by mitochondrial DNA (mtDNA) (red circles in the figure denote the number of mtDNA-encoded subunits in individual complexes; complex II is entirely nucleus encoded). In addition, tens of nucleus-encoded assembly factors are required to assemble these multisubunit complexes. Deficiencies in the respiratory chain or oxidative phosphorylation can be caused by defects in the quality or quantity (deletions and/or depletion) of mtDNA, by mtDNA expression defects (tRNA mutations, mtRNA transcription or processing defects and disruption of the translation apparatus), by mutations in structural subunits, the complex assembly factors or factors involved in import of nuclear-encoded subunits, or by a failure of the quality control in the correct assembly of the complex. Red arrows indicate the transfer of electrons between respiratory chain complexes. C, cytochrome *c*; I–V, respiratory chain complexes I–V; Q, ubiquinone.



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Progeric syndrome
A group of genetic disorders that manifest with symptoms of premature ageing.

Shine–Dalgarno sequence
A ribosome binding site that is located upstream of the AUG start codon.

Aminoacyl-tRNA synthetases
Enzymes that catalyse the charging of individual tRNAs with their cognate amino acid.

The major defects of mtDNA expression. The implementation of next-generation sequencing approaches for the identification of genetic mutations in human mitochondrial disorders has revealed that mutations in the nucleus-encoded proteins that are needed for the faithful synthesis of mitochondrial proteins are a major cause of mitochondrial diseases[8,45,47] (FIG. 3; TABLE 1). The tissue-specific manifestations of mitochondrial diseases vary from infantile cardiomyopathies to progressive children's diseases of the nervous system, adult-onset muscle weakness, stroke-like episodes, large benign tumours of the brown adipose tissue, cardiac or kidney dysfunction, diabetes or hearing loss[7]. These heterogeneous clinical presentations are not strictly compatible with a loss of the synthesis of the 13 mtDNA-encoded proteins, but they indicate that the cellular consequences of defects in tRNA functions, tRNA aminoacylation, ribosome assembly, polypeptide synthesis and protein

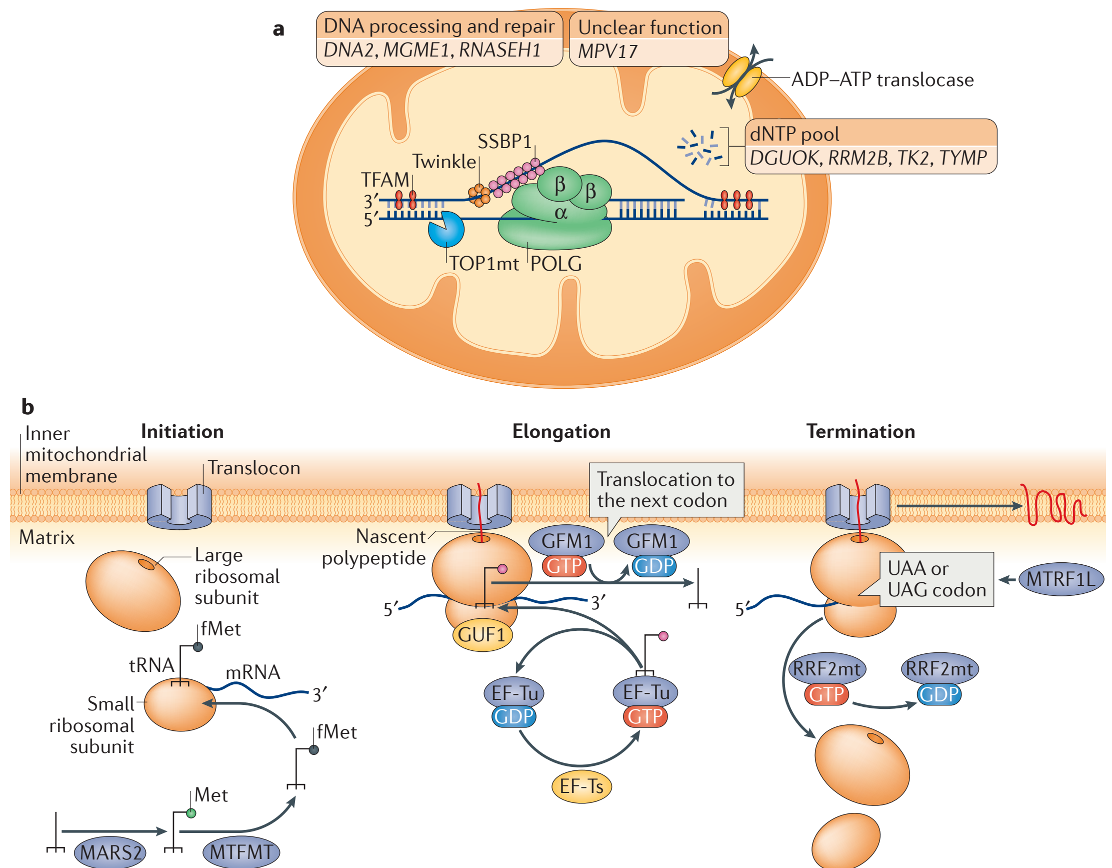

Figure 2 | **Mechanisms of mtDNA replication and translation highlighting factors implicated in human disease.** **a** | The mitochondrial DNA (mtDNA) replisome includes DNA polymerase-γ (POLG), which comprises a catalytic α-subunit and a processive β-subunit, as well as the replicative helicase Twinkle and mitochondrial single-stranded DNA-binding protein (SSBP1). Mitochondrial transcription factor A (TFAM) has a histone-like function and contributes to mtDNA organization, whereas the mitochondrial DNA topoisomerase I (TOP1mt) cleaves and rejoins one strand of the DNA duplex to prevent over- and underwinding of the DNA strands during mtDNA processing. Replication also requires nucleotide pools, which are regulated separately in the mitochondria and the cytoplasm, although the pools influence each other. **b** | The steps of protein synthesis on mitochondrial ribosomes. The initiator methionine is formylated (fMet) by mitochondrial methionyl-tRNA formyltransferase (MTFMT). The recognition of codons is mediated by a ternary complex of the amino acid-charged tRNA, mitochondrial elongation factor Tu (EF-Tu) and GTP; the hydrolysis of GTP releases the tRNA, which can then bind to the ribosome. The ribosome then catalyses the formation of the peptide bond. The energy released from the hydrolysis of GTP that is bound to the mitochondrial elongation factor G (GFM1) allows the translocation of the ribosome to the new codon; GUF1 is proposed to be involved in a one-codon back-translocation event at improperly translocated ribosomes. Recycling of EF-Tu is regulated by mitochondrial elongation factor Ts (EF-Ts). The growing polypeptide is translocated by inner mitochondrial membrane proteins. Mitochondrial translation terminates at the standard UAA and UAG stop codons, which are recognized by the mitochondrial peptide chain release factor 1-like (MTRF1L)[125–127]. Finally, hydrolysis of GTP that is bound to mitochondrial ribosome-releasing factor 2 (RRF2mt) catalyses ribosomal disassembly and polypeptide release. *ANT1*, adenine nucleotide translocator 1; *DGUOK*, deguanosylkinase, mitochondrial; *DNA2*, DNA replication ATP-dependent helicase 2; MARS2, mitochondrial methionine–tRNA ligase 2; *MGME1*, mitochondrial genome maintenance exonuclease 1; MPV17, mitochondrial inner membrane protein 17; *RNASEH1*, ribonuclease H1; RRM2B, ribonucleotide-diphosphate reductase subunit M2B; *TK2*, thymidine kinase 2; *TYMP*, thymidine phosphorylase. See also FIG. 3 and TABLE 1.

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quality control, are different. Antibiotics that inhibit bacterial translation, such as aminoglycosides, tetracycline derivatives, chloramphenicol and actinonin, also inhibit mitochondrial protein synthesis, and have differential effects on cell fitness and human health[48–52].

The fact that many human disorders arise from defects in the assembly of the mitoribosome is not surprising, given that it is a complex structure that comprises 80 individual proteins (all are encoded by the nuclear genome), two mtDNA-encoded ribosomal RNAs (rRNAs) and a tRNA, and that its assembly involves a large number of factors that are needed for RNA modifications and for coordination of the assembly of the small and large ribosomal subunits[53]. It is surprising that there are so few pathogenic mutations in nucleus-encoded genes that have been reported to affect this process (FIG. 3; TABLE 1). To date, loss-of-function mutations in five genes that encode mitoribosomal proteins (MRPs) of the mitochondrial large subunit (termed MRPLs) and the mitochondrial small subunit (termed MRPSs) have been reported that affect the steady-state stability of the ribosomal subunits (FIG. 3; TABLE 1). Mutations in *MRPL3*, *MRPL12*, *MRPS16* and *MRPS22* decrease mitochondrial protein synthesis; however, the exception to this pattern is *MRPL44* — a mutation in *MRPL44* was found to disrupt the assembly of the large ribosomal subunit, but no translation defect was apparent in cultured cells[54]. Instead, newly synthesized cytochrome *c* oxidase subunit 1 (MT-CO1) of complex IV of the respiratory chain was unstable. MRPL44 is located on the surface of the large subunit near MRPL45, which interacts or docks with the membrane[55], suggesting a role for MRPL44 in coordinating the transport of the nascent MT-CO1 polypeptide that is emerging from the ribosomal exit tunnel. This example aptly demonstrates the value of detailed analysis of human disease mutations and their underlying causes for understanding mitoribosome functions and their dysfunction in pathology.

Diseases that are associated with mitochondrial protein synthesis defects most commonly arise from disruption of the decoding of mRNA messages during translation elongation on mitoribosomes (FIGS 2b,3). These defects arise from mutations in the mitochondrial tRNAs (affecting tRNA stability, base modifications); proteins that are required for the charging and delivery of tRNAs to the ribosome; and protein factors that recycle these components to sustain translation elongation (FIG. 3; TABLE 1). The disorders associated with mutations in genes that encode aminoacyl-tRNA synthetases have emerged as an especially important cause of human disease[45] (TABLE 1). Despite the fact that they are all crucial to maintain translation of the 13 mtDNA-encoded proteins, their dysfunction causes highly variable phenotypes, which range from cardiomyopathies to anaemia, ovarian dysfunction and disorders of the brain[45] (TABLE 1). Depletion of amino acid-charged tRNAs in the ribosome leads to pausing or stalled translation elongation. This event can either lead to premature termination of the nascent polypeptide or disturb the temporal regulation of mtDNA expression, both of which lead to a failure to assemble the respiratory chain complexes.

Ribosomal release factors
Release factors terminate translation elongation by catalysing cleavage of the ester bond of the polypeptidyl-tRNA to release the nascent chain from the ribosome.

Of the putative ribosomal release factors, pathogenic loss-of-function mutations have only been described for *C12ORF65*, which is a codon-independent release factor (FIG. 3; TABLE 1). All of the reported *C12ORF65* mutations are premature stop codons. A loss of function of this protein does not affect mitoribosome assembly, but it does lead to impaired synthesis of mitochondrial proteins[56,57]. This raises the intriguing possibility that the loss of a codon-independent termination factor signals to the pool of translating ribosomes, impairing translation. Whether the presence of this release factor is required for the translation of specific messages or all 13 mitochondrially encoded transcripts, and whether the factor functions only in specific situations or functions constitutively during translation elongation, are open questions, but new techniques, such as ribosome profiling, should begin to address them.

Mitochondrial stress and disease

Abnormalities in mtDNA expression, including aberrant mtDNA maintenance and translation, induce various stresses, which include protein stress (resulting from defects in translation or unbalanced translation of respiratory chain components that are encoded by mitochondrial and nuclear genomes), replication and transcription stress as well as stress that is associated with mtDNA depletion as a consequence of insufficient and/or unbalanced deoxyribonucleoside triphosphate (dNTP) pools[58]. A considerable body of evidence indicates that mitochondrial stress responses that are triggered by a primary molecular defect in the organelle, and not the defects in oxidative phosphorylation per se, are the major contributing factor to mitochondrial disorders.

Protein synthesis defects, protein stress and mitochondrial disorders. Disorders of mtDNA maintenance and translation ultimately impede the assembly of the oxidative phosphorylation complexes. The cytoplasmic ribosomes synthesize 85% of the ~90 structural subunits of the respiratory chain complexes and the ATP synthase, as well as all of the required assembly factors. The assembly process has multiple points of regulation, including protein quality control that precedes the integration of the proteins into the inner membrane, and during and after translation or import into mitochondria[59]. By contrast, all 13 mitochondrial proteins are co-translationally inserted directly into the inner membrane. This polypeptide pool is labile: in human cultured fibroblasts, up to 75% of *de novo* mitochondrial translation products are turned over by quality control pathways[60], which are homologous to those in the Alphaproteobacteria[61]. The two gene expression machineries, the cytoplasmic and mitochondrial, must also be temporally and spatially coordinated, and are coupled with mitochondrial membrane dynamics. Disrupted coordination of any of these processes results in the loss of protein homeostasis and, as a consequence, protein stress and organelle membrane stress. The mitochondrial inner membrane is one of the most proteinaceous membranes in cells, so quality control of

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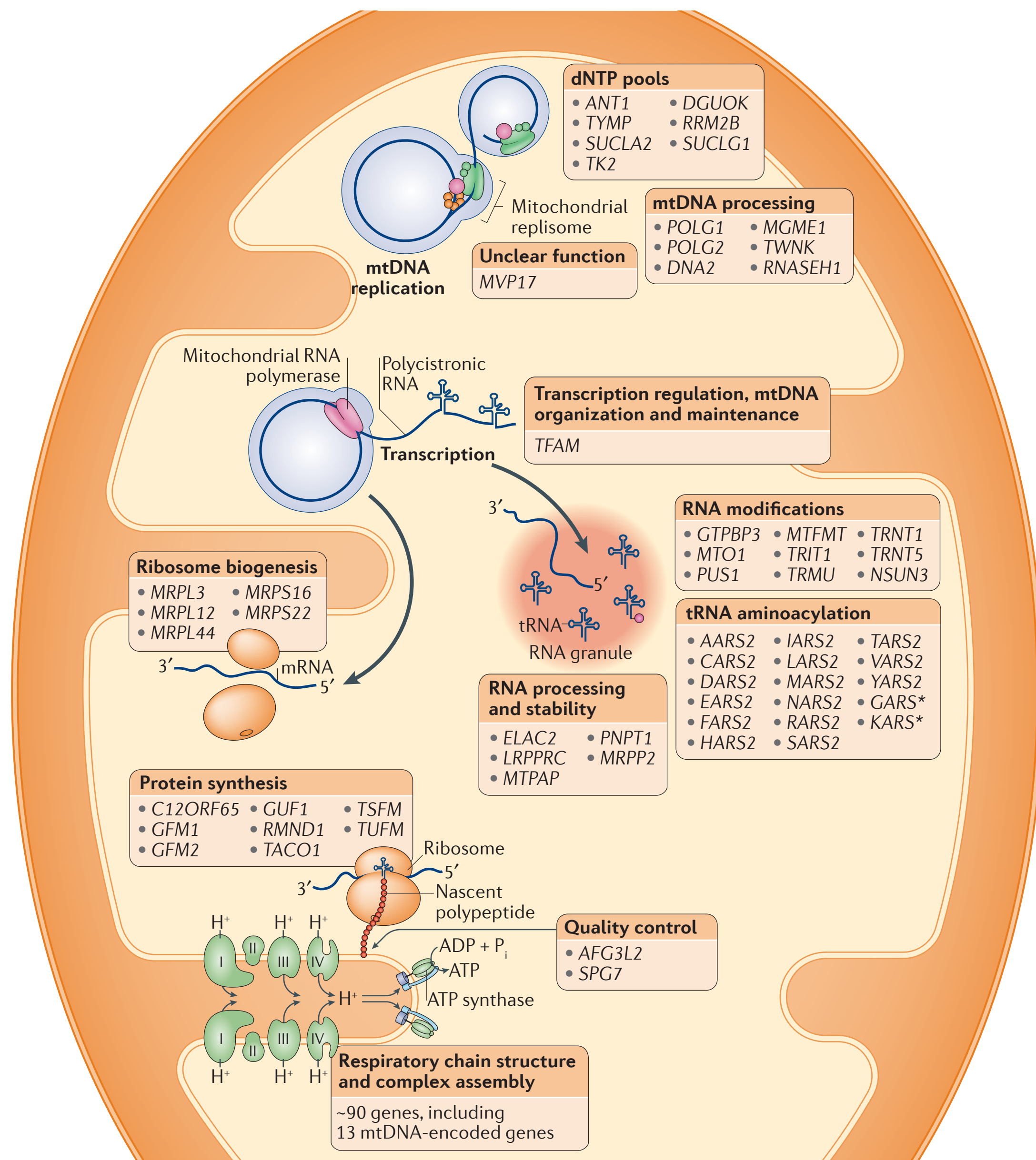

Figure 3 | **Genes that encode components of the mtDNA maintenance and expression machineries and that are associated with human mitochondrial disorders.** See also TABLE 1 for details of disease genes and their associated phenotypes. Mutations in mitochondrial DNA (mtDNA) maintenance proteins (that is, enzymes that are involved in mtDNA replication and repair, or nucleotide synthesis) cause either mtDNA depletion (which is associated with severe disorders that typically manifest in childhood) or multiple mtDNA deletions that accumulate in postmitotic tissues (which is characteristic of progressive neurological diseases). The deletions are assumed to lead to an imbalance of functional tRNAs, which disrupts mitochondrial translation and leads to decreased production of mtDNA-encoded proteins. Defects in mitochondrial tRNAs or in factors that affect the processing of the polycistronic mtDNA transcript to the individual tRNAs or mRNAs, RNA stability or modifying RNA species, impair translation efficiency and cause a decrease in the expression of mtDNA-encoded proteins. Dysfunction in the assembly of the mitochondrial ribosome, which comprises mitoribosomal proteins (MRPs) of the large (MRPL) or small (MRPS) subunits (all are nucleus encoded), as well as 12S and 16S ribosomal RNAs (rRNAs) (mtDNA encoded), or in factors that mediate polypeptide biosynthesis during translation, leads to inefficient translation of mtDNA-encoded proteins. Other defects in translation, such as the incorporation of aberrant amino acids, lead to unfinished and/or aberrant (for example, misfolded) protein products. These aberrant proteins may cause membrane stress, which can be resolved by quality control proteases (members of the ATPases associated with diverse cellular activities (AAA)-protease family, paraplegin (encoded by *SPG7*) and AFG3-like protein 2 (AFG3L2)); defects in these proteases affect the nervous system in particular. In all these cases, efficient generation of mitochondrial subunits of the respiratory chain complexes (I–IV) is perturbed, which leads to defects in mitochondrial function that manifest most notably as a decrease in oxidative production of ATP. The asterisks denote proteins that are localized in both the mitochondria and the cytoplasm. Please see Supplementary information S2 (box) for abbreviations used in FIG. 3.

Polycistronic
A product of transcription that generates RNA molecules that encode more than one protein.

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MELAS
(Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes). A mitochondrial disease that is most commonly caused by a mutation (m.3243A > G) in the mitochondrial DNA-encoded tRNA$^{Leu(UUR)}$.

MERRF
(Myoclonic epilepsy with ragged red fibres). A mitochondrial disease with variable symptoms, which affects predominantly the nervous system and the muscles.

the incorporated proteins is paramount for maintaining homeostasis of the organelle. Overaccumulation of newly synthesized proteins by mitochondrial ribosomes can dissipate the mitochondrial membrane potential and trigger remodelling of mitochondrial morphology, resulting in mitochondrial fragmentation[57]. Recent evidence suggests that mitochondrial responses to this and similar stresses contribute to mitochondrial disease manifestations, and could account for the tissue-specific characteristics of mitochondrial disorders.

For example, mitochondrial protein synthesis defects are associated with two common pathogenic mutations in mitochondrial tRNAs in humans: a mutation at position 3243 in the tRNA^{Leu} that recognizes the UUR (where R is A or G) codon (m.3243A>G tRNA$^{Leu(UUR)}$)[62,63] and m.8344A>G tRNA$^{Lys(AAR)}$ (REF. 64), which are associated with the disorders MELAS and MERRF, respectively. Interestingly, despite both disorders resulting from tRNA mutations, the clinical manifestations of these disorders are different[62,63,64]. In the mitochondrial genome of humans, the abundance of the UUR (Leu) or AAR (Lys) codon is similar (approximately 2.5%), yet mutations in the cognate tRNAs produce fundamentally distinct molecular defects during mitochondrial translation elongation[65] (FIG. 4a,b). To rescue the defects in mitochondrial protein synthesis that arise from these tRNA mutations, distinct molecular processes, explained below, are required, which may have different importance across cell types. Thus, there could be cell type-specific stress responses because of the inherent ability to rectify the disruptions to mitochondrial protein synthesis that are associated with these tRNA mutations.

The m.3243A>G tRNA$^{Leu(UUR)}$ mutation disrupts modification of the wobble position (the third, less crucial position in the codon–anticodon pair) by taurine in this tRNA. It was shown that the lack of taurine at this position enables the tRNA to decode all UUX codons (where X is any base), leading to Leu misincorporation[66], but with no apparent adverse effects on the rate of translation elongation[65]. As a result, unstable proteins are synthesized[65,67]. These aberrant polypeptides are then inserted into the inner membrane and need to be recognized by chaperones for extraction from the membrane, and are then degraded by proteases. The m.8344A>G tRNA^{Lys} mutation affects tRNA stability, aminoacylation and also the RNA modification at the wobble position. The mutant tRNA appears to have a substantial inability to decode lysine during translation elongation[68,69], which leads to a severe stalling of the mitochondrial ribosome during translation elongation. Rescue of the failure to synthesize a full-length polypeptide requires a stepwise rescue pathway to recover the stalled ribosomes: endoribonuclease activity to cleave the mRNA, a release factor to catalyse the cleavage of the ester bond of the peptidyl-tRNA, and chaperones and proteases to recognize and degrade the aberrant polypeptide. Even though both tRNA mutations decrease the steady-state abundance of the assembled respiratory chain complexes and the ATP synthase, different molecular processes are required to resolve the faulty mitochondrial protein synthesis. A more detailed understanding of the specific quality control factors and the regulation, and misregulation, of these molecular mechanisms is needed to shed more light on the different clinical manifestations of these tRNA mutations in humans.

Disruption of mitochondrial protein quality control can contribute to disease. A key quality control complex required for the turnover of mitochondrially synthesized proteins is the mAAA (matrix ATPases associated with diverse cellular activities) protease and chaperone complex. This AAA complex is a membrane-anchored hexamer that faces the mitochondrial matrix, and is composed of homo-oligomers of AFG3L2 (AFG3-like matrix AAA peptidase subunit 2) subunits or a hetero-oligomer with paraplegin (encoded by *SPG7*). Mutations in both subunits are linked to progressive neurological disorders, which affect the anterograde trafficking of mitochondria in the long neuronal axons[70–72]. The chaperone function of the AAA complex is required for the assembly of the two mitochondrially encoded subunits of the ATP synthase[73]. The absence of the chaperone function of AFG3L2 activates the metalloendopeptidase OMA1. This protease cleaves the membrane-anchored isoforms of the dynamin-related GTPase OPA1, thereby releasing them from the inner membrane, which leads to remodelling of the mitochondrial membrane morphology, producing a fragmented state[41,50,57,60,74]. In neurons, mitochondrial fragmentation due to OPA1 processing impairs axonal traffic, which leads to neurodegeneration[70]. Recent work demonstrates that the trigger for this stress response arises from failed quality control of *de novo* synthesized proteins that exit the mitoribosomes, leading to over-accumulation of polypeptides in the membrane[57].

Activation of an integrated stress response in mitochondrial disease. The induction of mitochondrial stress responses and the activation of quality control pathways initiates retrograde signals in mitochondria, which then activate nuclear genetic programmes for organelle maintenance and enhanced quality control. A robust nuclear transcriptional stress response has been found to be induced in patients, mice and cell lines with mtDNA maintenance and translation defects[50,58,75–77]. This response involves the upregulation of genes that carry a conserved amino acid response element (AARE) in their upstream regulatory region[76]. The AARE is the binding site of activating transcription factors (ATFs), different isoforms of which have been linked to the unfolded protein response in the endoplasmic reticulum[78] and the mitochondrial unfolded protein response (UPRmt)[79]. UPRmt was first described in cultured mammalian cells that overexpressed an unstable mutant mitochondrial matrix protein[79], and was then further characterized in the nematode *Caenorhabditis elegans*[80]. In the worm, the orthologue of the mammalian ATF proteins, activating transcription factor associated with stress 1 (ATFS-1), is constitutively targeted to mitochondria but, in response to mitochondrial dysfunction and decreased mitochondrial protein import, a nuclear

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Table 1 | **Nuclear genes that cause disorders of mitochondrial DNA maintenance or expression**

Mechanism	Gene	Manifestation	Onset
mtDNA maintenance			
mtDNA replication	*TWNK* (also known as *PEO1* and *C10ORF2*)	Alpers–Huttenlocher syndrome and hepatocerebral MDS	Infant
		Infantile-onset spinocerebellar ataxia, and hepatocerebral MDS	1–2 years
		PEO, mitochondrial myopathy and multiple mtDNA deletions	Adult
	POLG1	Alpers–Huttenlocher syndrome and hepatocerebral MDS	Infant
		Spinocerebellar ataxia, valproate liver toxicity, peripheral neuropathy (MIRAS, SANDO and SCA-E) and mtDNA deletions and depletion	Juvenile to adult
		PEO, mitochondrial myopathy, neuropathy, parkinsonism, primary ovarian failure and multiple mtDNA deletions	Adult
	POLG2	PEO, mitochondrial myopathy and mtDNA deletions	Adult
	*DNA2**	Mitochondrial myopathy, ptosis and mtDNA deletions	Adult
	*RNASEH1**	PEO, exercise intolerance, spinocerebellar ataxia and mtDNA deletions	Adult
	*MGME1**	PEO, emaciation, cardiac arrhythmia, gastrointestinal disturbances, renal dysfunction and MDS	Juvenile
	*TFAM**	Intrauterine growth retardation, hypoglycaemia and liver disease	Prenatal
Mitochondrial dNTP pools	*ANT1*	Respiratory distress, lactacidosis and MDS	Neonate
		Muscle weakness, hypertrophic cardiomyopathy and mtDNA depletion	Juvenile
		PEO and multiple mtDNA deletions	Adult
	DGUOK	Hepatocerebral MDS	Infant
		PEO and mtDNA deletions	Adult
	SUCLA2	Encephalomyopathic MDS with or without methylmalonic acid	Infant to child
	TK2	Myopathic MDS	Infant
		Myopathy and MDS	Adult
		PEO with mtDNA deletions	Adult
Cytosolic dNTP pools	*RRM2B*	Encephalomyopathy with renal tubulopathy, and MDS	Neonate
		PEO and multiple mtDNA deletions	Adult
	TYMP	Mitochondrial neurogastrointestinal encephalomyopathy, mtDNA deletions and MDS	Child to adult
Unknown function	*MPV17*	Hepatocerebral MDS and peripheral neuropathy	Infant
Mitochondrial protein synthesis			
RNA processing and stability	*ELAC2*	Hypertrophic cardiomyopathy	Infant
	LRPPRC	Leigh syndrome	Infant
	MTPAP	Progressive cerebellar ataxia, spastic paraparesis, dysarthria and optic atrophy	Child
	PNPT1	Encephalopathy and Leigh syndrome	Neonate
		Deafness	Child
RNA modifications	*GTPBP3*	Leigh syndrome	Infant
	MTFMT	Leigh syndrome	Infant
	MTO1	Hypertrophic cardiomyopathy	Prenatal
	NSUN3	Progressive encephalopathy, muscle weakness and lactacidosis	Infant
	PUS1	Myopathy, lactacidosis and sideroblastic anaemia	Child
	TRIT1	Neurodevelopmental delay and seizures	Infant
	TRMU	Acute transient infantile liver failure	Infant
	TRNT1	Retinitis pigmentosa and erythrocytic microcytosis	Juvenile
	TRMT5	Hypertrophic cardiomyopathy and exercise intolerance	Child

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Table 1 (cont.) | **Nuclear genes that cause disorders of mitochondrial DNA maintenance or expression**

Mechanism	Gene	Manifestation	Onset
Mitochondrial protein synthesis (cont.)			
tRNA aminoacylation	*AARS2*	Hypertrophic cardiomyopathy	Prenatal
		Progressive leukoencephalopathy with ovarian failure	Infant
	CARS2	Progressive myoclonic epilepsy, tetraparesis and hearing and visual impairment	Child
	DARS2	Leukoencephalopathy with brain stem and spinal cord involvement, and lactate elevation	Child
	EARS2	Leukoencephalopathy with thalamus and brain stem involvement, and lactate elevation	Infant
	FARS2	Alpers–Huttenlocher syndrome	Infant
		Spastic paraplegia	Infant
	HARS2	Perrault syndrome, ovarian dysgenesis, sensorineural hearing loss, with or without SCA-like encephalopathy	Juvenile
	IARS2	Cataracts, growth hormone deficiency, sensory neuropathy, sensorineural hearing loss and skeletal dysplasia	Adult
	LARS2	Perrault syndrome, ovarian dysgenesis and sensorineural hearing loss	Juvenile
	MARS2	Autosomal recessive spastic ataxia with leukoencephalopathy	Child to adult
		Developmental delay and sensorineural hearing loss	Infant
	NARS2	Epilepsy, muscle weakness and intellectual disability	Child
	RARS2	Pontocerebellar hypoplasia and encephalopathy	Infant
	SARS2	Hyperuricaemia, pulmonary hypertension, renal failure and alkalosis	Infant
	TARS2	Developmental delay, facial dysmorphism and myoclonic epilepsy	Neonate
	VARS2	Axial hypotonia, lactacidosis, and developmental delay	Neonate
	YARS2	Myopathy, lactacidosis and sideroblastic anaemia 2	Adult
	GARS	Charcot–Marie–Tooth disease, type 2D	Juvenile to adult
	KARS	Charcot–Marie–Tooth disease (intermediate B), developmental delay, self-abusive behaviour and dysmorphic features	Child to adult
Ribosome biogenesis	*MRPL3*	Hypertrophic cardiomyopathy	Infant
	MRPL12	Growth retardation, epilepsy and failure to thrive	Neonate
	MRPL44	Cardiomyopathy	Infant
	MRPS16	Agenesis of corpus callosum, dysmorphism and lactacidosis	Neonate
	MRPS22	Hypertrophic cardiomyopathy and tubulopathy	Prenatal
Translation elongation	*C12ORF65*	Leigh syndrome	Infant
		Spastic paraplegia, neuropathy and optic atrophy	Child
	GFM1	Progressive hepatoencephalopathy	Neonate
	GFM2	Leigh syndrome with arthrogryposis multiplex congenita	Infant
	GUF1	Epileptic encephalopathy	Infant
	RMND1	Encephalopathy, seizures and lactacidosis	Neonate
	TACO1	Leigh syndrome	Child
	TSFM	Hypertrophic cardiomyopathy	Infant
		Leigh syndrome	Child
	TUFM	Macrocystic leukoencephalopathy and microgyria	Neonate
Protein quality control			
Degradation of misfolded proteins	*AFG3L2*	Spinocerebellar ataxia 28	Juvenile to adult
		Spastic ataxia, neuropathy, ptosis, oculomotor apraxia, dystonia and progressive myoclonic epilepsy	Infant
	SPG7	Spastic paraplegia	Adult

*Denotes diseases with only a few reports. The full version of the table, which includes references to Online Mendelian Inheritance in Man (OMIM) and PubMed databases, is supplied as Supplementary information S1 (table). Please see Supplementary information S3 (box) for abbreviations used in TABLE 1.

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One-carbon cycle
The major biosynthetic pathway of the cell, which is involved in the transfer of nutrient-derived one-carbon groups by one-carbon carriers, such as tetrahydrofolate, to feed methylation reactions and biosynthesis reactions, such as purine, phospholipid, creatine, amino acid and glutathione synthesis.

localization signal in the carboxyl terminus of the protein redirects ATFS-1 to the nucleus to activate a genetic programme of UPRmt, including upregulated expression of mitochondrial heat shock proteins (HSPs)[81,82] (FIG. 4c). In *C. elegans*, deficiency of the mitochondrial respiratory chain complex IV in neurons induces upregulation of HSP expression in the gut; it was suggested that this effect could be explained by humoral (that is, engaging body fluids) spreading of the stress response via a secreted 'mitokine' (REF. 83). The genomic AARE element in the worm is identical to that in mammals, suggesting that the response is probably highly conserved.

Despite the conservation in lower organisms and mammals of ATF–AARE induction in response to mitochondrial insults, the downstream targets in mammalian postmitotic tissues[76] are quite different from those in cultured cells or in worms. In addition, in mammals, there is little evidence of the existence of unfolded mitochondrial proteins. Furthermore, in tissues from patients with mitochondrial diseases there is only a subtle induction of the UPRmt-associated HSPs and the protease CLPP, which is a mitochondrial matrix protein that mediates the activation of UPRmt in the worm (FIG. 4c), and this response often coincides with induction of a mitochondrial biogenesis programme[15,16,77]. Instead, in mammals, defects in mtDNA maintenance and translation upregulate AARE transcripts that encode proteins that are involved in the regulation of lipid and glucose metabolism, as well as the anabolic one-carbon cycle[58,75,84] (BOX 2). These downstream targets can act both in a cell-autonomous manner (local effects within the affected cell) and in a non-cell-autonomous

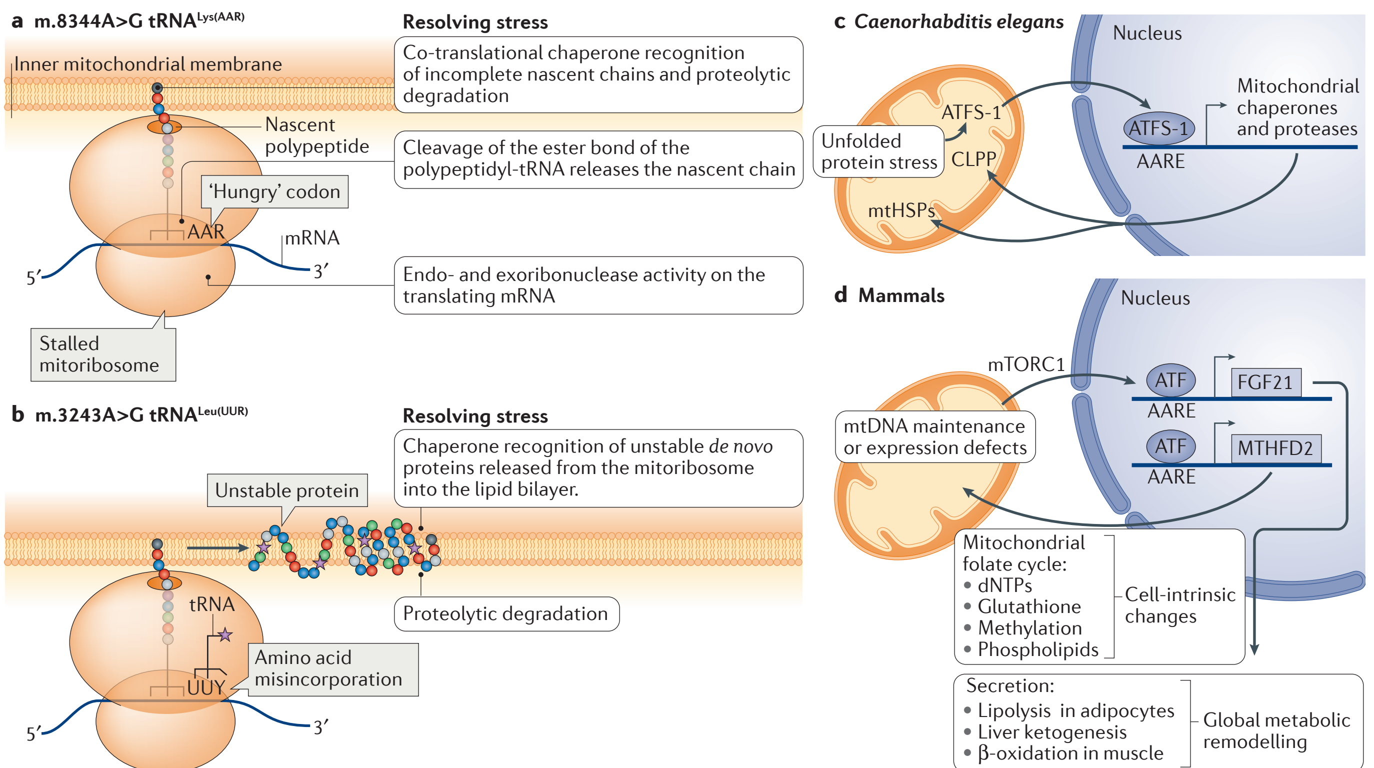

Figure 4 | **Stress responses in mitochondrial dysfunction.** **a** | A mitochondrial DNA (mtDNA) mutation in the tRNA for Lys that results in the substitution of adenine for guanine at position 8344 (m.8344A>G tRNA$^{Lys(AAR)}$ (where R can be guanine or adenine)) causes a reduction in the abundance of aminoacylated tRNA$^{Lys(AAR)}$ and defects in decoding Lys codons (this generates so-called 'hungry' codons). This defect results in the stalling of mitoribosomes and the production of incomplete polypeptides. Resolving this stress requires several coordinated molecular functions. **b** | The m.3243A>G tRNA$^{Leu(UUR)}$ mutation generates a decoding defect during translation elongation, which leads to amino acid misincorporation (star) and the generation of unstable proteins that can be inserted into mitochondrial membranes. This stress can be resolved by the coordinated action of chaperones and proteases. Y denotes U or C. **c,d** | Activating transcription factors (ATFS-1 in *Caenorhabditis elegans*, ATF3–5 in mammals) recognize amino acid response elements (AARE) in target genes and are involved in all the known mitochondrial stress responses, but they activate different downstream targets in different systems. **c** | In *C. elegans*, ATFS-1 is involved in the mitochondrial unfolded protein response (UPRmt). In response to mitochondrial dysfunction, ATFS-1 is stabilized and targeted to the nucleus, where it activates UPRmt genes, including mitochondrial heat shock proteins (mtHSPs) and mitochondrial proteases, such as CLPP[80–82]. **d** | In mammalian cells (cultured cells, mouse muscle and brain, and human muscle) that are affected by mtDNA expression defects, the ATFs drive a robust integrated mitochondrial stress response, with cell-intrinsic and cell-extrinsic effects on metabolism. The mitochondrial folate cycle is locally induced in the affected tissue, through the increased expression of mitochondrial bifunctional methylenetetrahydrofolate dehydrogenase/cyclohydrolase (MTHFD2), which impacts nucleotide synthesis, glutathione production as well as methylation reactions. ATFs also induce expression of fibroblast growth factor 21 (FGF21), which is a secretory signal that transmits the response from the affected tissue to the entire organism, having major consequences for the systemic energy metabolism[96]. In mammals, ATFs are at least partially under control of mechanistic target of rapamycin complex 1 (mTORC1), providing links between mitochondrial stress responses and growth control pathways.

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Paracrine
A form of cell-to-cell communication, in which a secreted factor affects the function of nearby cells.

Endocrine
A form of cell-to-cell communication, in which a secreted factor (for example, a hormone or cytokine) enters the bloodstream and affects the functions of distant cells or whole tissues.

Redox signalling
A process in which reactive oxygen species or other molecules that carry reducing equivalents (such as NADH, NAD^+; NADP and NADPH) change protein functions through reduction or oxidation reactions.

Mitochondrial folate cycle
The mitochondrial part of the one-carbon cycle, in which tetrahydrofolate-forms carry one-carbon units to mitochondrial formylation reactions, generates formate for purine synthesis, formyl-methionine for mitochondrial translation as well as NADPH.

Mechanistic target of rapamycin complex 1
(mTORC1). A kinase that is part of the mTOR complex, which is a master regulator of anabolic biosynthesis.

Liver ketogenesis
Hepatic synthesis of ketone bodies, which is typically induced by fasting and provides tissues with fuel in low-nutrient conditions.

β-oxidation
Stepwise catabolic breakdown of the long carbon backbones of fatty acids in mitochondria, to generate fatty acyl-CoA that enters the Krebs cycle and eventually promotes ATP production.

Hypothalamus
A region in the brain that controls several homeostatic functions of the whole body, including feeding, water intake and the sleep–wake cycle.

manner (paracrine or endocrine effects) (FIG. 4d). As this response combines transcriptional components of UPRmt and also metabolite and redox signalling, we refer to it here as the 'integrated mitochondrial stress response' (ISRmt).

The integrated mitochondrial stress response, the one-carbon cycle and mtDNA defects. The cell-autonomous ISRmt involves early induction of the AARE–ATF-regulated mitochondrial folate cycle, which is an integral part of one-carbon metabolism[58,76] (BOX 2). This cycle provides formyl-methionine for mitochondrial translation initiation, as well as specific folate forms that carry one-carbon units to feed anabolic biosynthesis reactions in the cytoplasm[85]. The regulatory enzyme of the mitochondrial folate cycle, methylenetetrahydrofolate dehydrogenase 2 (MTHFD2), contains AAREs in its promoter[76]. The crucial role of the redox-regulated MTHFD2 and the mitochondrial folate cycle in cellular anabolic reactions and growth control was first identified in cancer cells[86]. Surprisingly, a similar response is induced in postmitotic skeletal muscle and heart tissues that have defective mtDNA expression[76]. In mitochondrial disease, however, the induction of MTHFD2 is a stress response, as the enzyme is not typically present in healthy tissues during postnatal life. This cell-autonomous metabolic stress response, which is driven by ATF–AARE, provides the affected cell with nucleotides for DNA maintenance, glutathione as a reducing agent and antioxidant, as well as phospholipids and NADPH for membrane synthesis[58,86–89]. Recent evidence indicates that the mechanistic target of rapamycin complex 1 (mTORC1) pathway is upstream of this response, and activates MTHFD2 induction, the mitochondrial folate cycle and purine synthesis, thereby providing a connection between the metabolic response and a major cellular growth regulatory pathway[90,91].

In mitochondrial myopathy, the primary mitochondrial defect was found to be the chronic induction of MTHFD2 and the mitochondrial branch of the folate-driven one-carbon cycle. This central biosynthetic pathway provides one-carbon units for purine and thymidine synthesis, as well as trans-sulfuration and methylation reactions that drive creatine and phospholipid synthesis[58] (BOX 2). The one-carbon metabolic imbalance in mitochondrial myopathy with multiple mtDNA deletions was found to lead to imbalanced dNTP pools, which was thereby suggested to contribute to mtDNA mutagenesis in mitochondrial myopathy[58,75]. The dNTP synthesis induction could be a response to mtDNA replication stalling that is caused by mutations in components of the replisome. Analogously low and imbalanced dNTP pools were observed in a mouse model of mtDNA depletion disease, which contained a mutant form of Twinkle[58]. A structural analysis of Twinkle suggested that this mutation modified the structure of the helicase so that dNTPs are hydrolysed even when Twinkle is not bound to mtDNA, thereby exhausting mitochondrial dNTP pools[58]. These examples illustrate the interconnection of mtDNA maintenance and dNTP pools, which is mediated by the one-carbon cycle. Furthermore, these findings suggest that one-carbon metabolic signalling contributes to adult-onset disorders with multiple mtDNA deletions, whereas mtDNA depletion is a consequence of a depleted dNTP pool. The fact that mtDNA maintenance defects modify dNTP pools also helps to explain why primary defects of dNTP metabolic enzymes lead to clinical consequences similar to those from mtDNA depletion: both affect cellular dNTP pools. This conclusion is further supported by the fact that genetic mutations that disrupt the function of the cytosolic enzyme ribonucleotide reductase subunit M2B (RRM2B), the main enzyme that synthesizes dNTPs, also cause mitochondrial depletion syndrome or disorders with mtDNA deletions[31]. These data fit with the recent suggestion of nucleoside supplementation as a therapy for mtDNA maintenance enzyme defects[92].

Systemic mediators of the integrated mitochondrial stress response. The ISRmt can also exert systemic effects. The non-cell-autonomous component of the ISRmt response is mediated by the metabolic cytokine fibroblast growth factor 21 (FGF21), which contains several AARE elements in its promoter[93]. FGF21 expression is induced and the cytokine is secreted into the blood in patients and mouse models that have translation or mtDNA defects[76,77,94,95]. FGF21 is considered to be a cytokine of the fasting response: in a healthy liver, FGF21 is induced after 12 hours of fasting in mice and 7 days of fasting in humans[96–98]. It has a multitude of metabolic effects, such as induction of liver ketogenesis and lipolysis in adipose tissue, as well as glucose and lipid uptake in tissues and induction of lipid β-oxidation[96–98]. Growth differentiation factor 15 (GDF15), a member of the transforming growth factor-β (TGFβ) family, is another cytokine that has metabolic and anti-inflammatory effects, which has been found to be upregulated in patients with mitochondrial disorders[99]. The metabolic effects of GDF15 upregulation mimic those of FGF21 upregulation[100,101]. GDF15 levels appear to be increased in a number of different severe human disorders, also without a clear connection to mitochondrial dysfunction. Nevertheless, these cytokines are good serum biomarkers for mitochondrial dysfunction in tissues[95].

The induction and secretion of FGF21 and GDF15 in response to mitochondrial dysfunction in the skeletal muscle indicate that metabolites and cytokines originating from a diseased tissue can modify metabolism and disease progression in distant organ systems. This non-cell-autonomous response mimics the proposed mitokine secretion that is part of the UPRmt in *C. elegans*[83] (see above), and is reminiscent of the effects of an unidentified secretory component that is protective in a mouse model of cardiac hypertrophy induced by knockout of a mitochondrial protease[102]. Whether FGF21 or GDF15 induction contributes to mitochondrial disease progression is still unclear. However, FGF21 is known to readily pass the blood–brain barrier in rodents and to affect various nutrition-related behaviours through its receptors β-klotho and FGFR1 in the hypothalamus[98,103]. Therefore, FGF21 that is expressed in peripheral tissues

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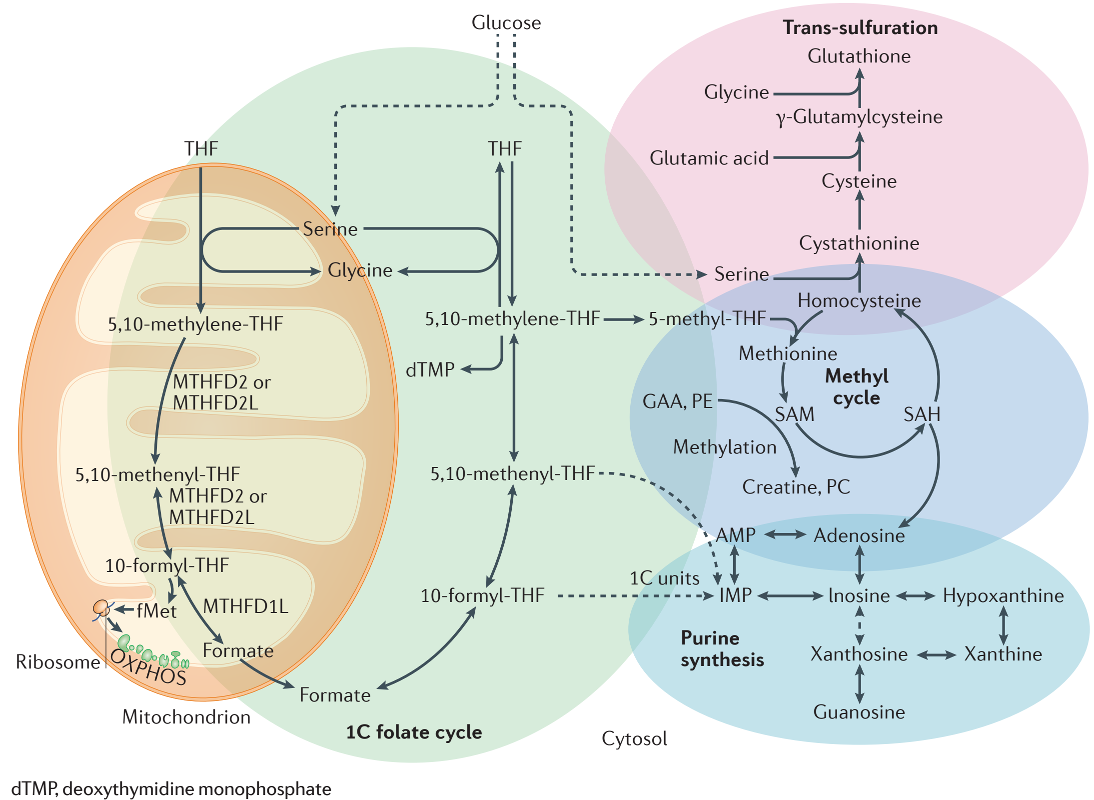
Box 2 | **One-carbon metabolism and the folate cycle**

Food-derived carbon units feed cellular anabolism. However, to be incorporated into biosynthetic reactions, these carbon units require specific carriers. Tetrahydrofolate (THF) is a versatile one-carbon (1C) carrier, and its different forms carry one-carbon units to feed growth pathways, including thymidylate and purine synthesis (through adenosine monophosphate (AMP) and inosine monophosphate (IMP) intermediates) and the methyl cycle. The methyl cycle involves the synthesis of *S*-adenosyl methionine (SAM), which can deposit methyl groups on substrates such as guanidinoacetate (GAA) or phosphatidylethanolamine (PE) to produce creatine or phosphatidylcholine (PC), respectively, as well as *S*-adenyl homocysteine (SAH), thereby being linked to glutathione synthesis (see the figure).The folate cycle connects nutrient intake (folate uptake and glucose-driven *de novo* serine biosynthesis) to growth pathways (DNA and phospholipid synthesis). The mitochondrial arm of one-carbon metabolism is known as the mitochondrial folate cycle. The mitochondrial folate cycle is important for mitochondrial translation as it generates formylmethionine (fMet), which initiates mitochondrial protein synthesis. Unperturbed mitochondrial translation is in turn important for the proper assembly of the respiratory chain complexes and oxidative phosphorylation (OXPHOS) (see the figure). Recent studies indicate the importance of the mitochondrial folate cycle for whole-cell biosynthetic pathways and disease manifestations[58,75,124]. The regulatory enzyme in the mitochondrial folate cycle is mitochondrial bifunctional methylenetetrahydrofolate dehydrogenase/cyclohydrolase (MTHFD2), which is highly induced in fetal development and in patients with primary mitochondrial DNA (mtDNA) expression defects[58,75]. The induction of this pathway in mtDNA maintenance diseases is robust and occurs as a stress response, but exactly how upregulation of the mitochondrial folate cycle contributes to disease progression is still unclear. The dashed arrows indicate a *de novo* serine biosynthesis pathway which originates from glucose.



dTMP, deoxythymidine monophosphate

can potentially modify the metabolism of the entire organism, including the metabolic regulation centres in the brain.

In conclusion, the evidence indicates that the ISRmt resulting from organellar stress may contribute to the variable manifestations of mitochondrial disorders. First, the ISRmt is able to modify cellular one-carbon metabolism. As various tissues show differential requirements for the specific one-carbon metabolic pathway branches (BOX 2), such as skeletal muscle being the major user of creatine in adults[104], the specific consequences of the ISRmt may be different depending on the affected tissue. Second, defects in mtDNA maintenance and expression can elicit widespread remodelling of organismal metabolism through ISRmt. This remodelling of biosynthetic and whole-organism metabolism as a consequence of mitochondrial dysfunction is a novel concept in mitochondrial pathology and may have important implications for understanding tissue specificity and for developing novel therapies (for example, involving agents that target one-carbon pathways) for the treatment of patients suffering from mitochondrial diseases.

Other mitochondrial stress responses and quality control mechanisms, such as mitochondrially derived vesicles and mitophagy, also make important contributions to the manifestations of mtDNA maintenance and translation disorders. These responses have been recently reviewed elsewhere[105,106].

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Oxygen, ROS and mitochondrial disease

Of the various signals that are emitted by mitochondria, ROS are often associated with pathology, as oxygen radicals are known to damage membranes and DNA, if produced in large amounts. The primary mitochondrial form of ROS is the reactive superoxide radical, but hydrogen peroxide is the only species that can cross mitochondrial membranes. ROS are a major product of mitochondrial oxidative metabolism[107]. Therefore, defects in mitochondrial gene expression and the resulting defects in the assembly of the oxidative phosphorylation machinery could be expected to result in an increase in ROS production and oxidative damage[108]. However, such oxidative damage is rarely seen in animal models of mitochondrial disease, even in cases of severe respiratory chain deficiency or mtDNA mutagenesis (such as mtDNA mutator mice)[26,27]. Recent evidence indicates that the mitochondrial hydrogen peroxide may contribute to pathology in specific cell types by modifying cell behaviour and fate rather than by causing oxidative damage[109]; for example, hydrogen peroxide could promote proliferation and progenitor commitment of stem cells. Therefore, ROS may act as a rheostat in the cells, tuning their functions, and may contribute to pathology even in the absence of oxidative damage. This idea has been supported by studies of animal models of mtDNA maintenance defects, especially the mtDNA mutator mice, which present with a progeric syndrome that is associated with neuronal stem cell dysfunction as well as defects in the maturation of erythrocyte progenitors (erythroblasts), which showed abnormal iron loading[110]. Therefore, the explanation for the progeria was proposed to lie in the increased mtDNA mutation burden of the somatic stem cells and progenitor cells, leading to the loss of cell stemness[110,111]. As some of the defects could be rescued by antioxidants[111], this suggested a role of increased ROS and modified ROS signalling in this pathology. ROS have been described as important signalling molecules that also regulate stem cell proliferation and fate determination in non-mitochondrial models of progeria[13,112,113]. Thus, modified ROS signalling and the concomitant stem cell dysfunction could also contribute to the progression of mitochondrial diseases.

Considering the size of the global market for antioxidants, the physiological roles of ROS signalling are under-studied. In the mtDNA mutator mouse model, the antioxidants *N*-acetyl cysteine and mitoQ (a mitochondria-targeted ubiquinone) promoted stemness of somatic stem cells and rescued the iron-loading defect of erythroblasts[110,111]. However, the same dose of mitoQ that improved haematopoietic precursor function stalled neural stem cell proliferation and increased cell death in this cell type[12]. This evidence underscores the differential sensitivity of various stem cell types for ROS-modifying therapies and indicates the need for caution when developing potent antioxidants for therapeutic benefit.

A recent study indicated that oxygen itself may promote mitochondrial disease progression, as chronic hypoxia delayed the progression of clinical and morphological signs in a complex I-deficient mouse model of Leigh syndrome[114], which is a devastating, progressive infantile brain disease that manifests as necrotizing lesions in the brain stem[115]. Whether hypoxia pathway-directed interventions are relevant for the treatment of mtDNA expression disorders, and whether they are applicable for human therapy in general, remain to be studied.

Metabolism as a therapeutic target

The past 5 years have provided promise for the treatment of mitochondrial diseases, based on preclinical studies in mice. Most of these successful intervention strategies were based on boosting mitochondrial biogenesis and activating nutrient sensors using genetic and small-molecule approaches. The peroxisome proliferator-activated receptor (PPAR) agonist bezafibrate, a ketogenic diet and 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR; an agonist of AMP-activated kinase) all promoted mitochondrial biogenesis, induced lipid oxidation, and improved muscle metabolism in mitochondrial myopathy models[116–118]. In addition, application of vitamin B_3 (nicotinamide riboside) or inhibitors of the NAD^+-consuming enzyme PARP increased the ratio between oxidized NAD^+ and its reduced form (NADH), which reflects the redox state of the cell, induced sirtuins and PPARγ co-activator 1α (PGC1α) and promoted mitochondrial biogenesis in mitochondrial disease, and remarkably decreased the disease-related myopathy symptoms[15,16]. Rapamycin, an inhibitor of mTORC1, reduced brain manifestations in complex I-deficient mice[119]. However, it still remains to be clarified whether all tissues benefit in the long-term from these treatments, which aim to promote biogenesis and/or metabolic activity of the primary affected organelle.

Recent data demonstrating metabolic remodelling of the one-carbon cycle in mitochondrial diseases raises the possibility of applying specific metabolites that are involved in this metabolic pathway or B-group vitamins (which are the source of coenzymes in the one-carbon cycle) as therapeutic agents in these disorders. This possibility indicates how detailed molecular studies on genetically modified mice may point to unexpected and straightforward treatment options in humans. However, a study of a modified Atkins (ketogenic) diet in patients with mitochondrial myopathy reported sub-acute selective damage and lysis of the most affected muscle fibres, providing modest beneficial long-term effects on muscle strength[120], which differs from the dietary effects in mice, in which this diet showed a remarkable curative effect on mitochondrial disease markers, including the improvement of the structure and function of muscle and brown fat mitochondria, but no muscle damage[118]. These examples emphasize strongly the need for good animal models, strong preclinical data and pilot trials for testing the therapeutic benefit of any metabolic intervention.

Conclusions and perspectives

Recent evidence indicates that mitochondrial function is monitored at various levels in healthy and disease states, and that mitochondrial signals — in particular, those that are induced by stress responses — can have

Sirtuins
A group of NAD^+-dependent proteins that couple NAD^+ breakdown to the removal of acyl groups from other proteins.

PPARγ co-activator 1α
(PGC1α). A master transcriptional regulator of the expression of genes that are involved in energy metabolism.

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dramatic effects on oxidative and biosynthetic pathways in the cell, and can even affect whole-body metabolism. These effects of mitochondrial dysfunction appear to be intimately linked with nutrient-sensing pathways and the one-carbon cycle but, to date, these links are poorly understood, and more thorough analysis is required to improve our understanding of the metabolic remodelling that occurs in the context of mitochondrial diseases.

What has been revealed so far is that the highly conserved ATF transcription factors — which integrate nutrient availability (through the mTORC1 pathway) with ATP synthesis and folate-driven biosynthesis pathways — appear to serve as the mediators between mitochondrial dysfunction and metabolism. This finding underscores the physiological importance of the intricate crosstalk between mitochondria, cellular metabolism and the environment.

From a broader perspective, molecular insight into the mechanisms that lead to primary mitochondrial diseases may be valuable for the understanding of more common disorders, such as Parkinson disease. Clinical symptoms of parkinsonism are observed in some mtDNA maintenance disorders with multiple mtDNA deletions[121,122], whereas patients with idiopathic Parkinson disease (the most prevalent form of the disease) show respiratory chain deficiency and multiple mtDNA deletions in the affected neurons[123]. These observations raise the possibility that the mtDNA instability that is reported in Parkinson disease, or even during physiological ageing, might be a consequence of dNTP imbalance and changes in one-carbon metabolism. Therefore, results obtained from studying well-described, albeit rare, genetic mitochondrial disorders may provide important clues regarding the mechanisms and potential interventions for common polygenic human diseases.

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Acknowledgements
The authors would like to acknowledge the Academy of Finland, the University of Helsinki and the United Mitochondrial Disease Foundation USA (A.S. and B.B.), and the Sigrid Jusélius Foundation, the European Research Council and the Jane and Aatos Erkko Foundation (A.S.) for their support, and members of the FinMIT Centre of Excellence in Research on Mitochondria, Metabolism and Disease for their contributions and stimulating discussions.

Competing interests statement
The authors declare no competing interests.

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