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Review

OXPHOS gene expression and control in mitochondrial disorders

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ABSTRACT

The cellular consequences of deficiencies of the mitochondrial OXPHOS system include a variety of direct and secondary changes in metabolite homeostasis, such as ROS, Ca^{2+}, ADP/ATP, and NAD/NADH. The adaptive responses to these changes include the transcriptional responses of nuclear and mitochondrial genes that are mediated by these metabolites, control of the mitochondria permeability transition pore, and a great variety of secondary signalling elements. Among the transcriptional responses reported over more than a decade using material harboring mtDNA mutations, deletions, or depletions, nuclear and mitochondrial DNA OXPHOS genes have mostly been up-regulated. However, it is evident from the limited data in a variety of disease models that expression responses are highly diverse and inconsistent. In this article, the mechanisms and controlling elements of these transcriptional responses are reviewed. In addition, the elements that need to be evaluated, in order to gain an improved perspective of the manner in which OXPHOS genes respond and impact on mitochondrial disease expression, are highlighted.

1. Introduction

The mitochondrial oxidative phosphorylation (OXPHOS) system, which produces the majority of cellular energy in the form of ATP, is controlled on the genetic level by two distinct genomes: the circular mitochondrial genome (mtDNA) and the nuclear genome. The circular mitochondrial genome of ~16.6 kb encodes thirteen structural subunits of complex I, III, IV, and V 22 tRNA and two ribosomal RNA genes used for RNA translation [1]. The nuclear genome encodes the additional genes required for mtDNA maintenance, replication, transcription, translation, post-translational modification, transport, and assembly exclusively. In addition, the nuclear genome controls all other aspects of mitochondrial biosynthesis and function. Nuclear–mitochondrial communication is a highly complex process dominated by the nucleus [2].

A deficiency in mitochondrial function is caused by a dysfunction of one (or more) of hundreds of nuclear- or mitochondrial-encoded proteins. Over the past two decades, it has become clear that the interplay between the mitochondrion and nuclear genome affects mitochondrial disease expression, as evident in diseases that result from mutations in genes involved in the mtDNA replication machinery and in nucleotide metabolism. This impacts qualitatively and/or quantitatively on mtDNA, such as progressive external ophthalmoplegia (PEO), mitochondrial DNA depletion syndrome, and mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) [3].

Mitochondrial interplay with the nuclear genome is also evident in the disorders of nuclear- and mtDNA-encoded subunits of the OXPHOS complexes. Primary deficiencies of the OXPHOS system impact directly on mitochondrial function and result in several disease phenotypes [4]. With recent advances in systems biology for investigating gene expression and function, key aspects of nuclear–mitochondrial communication in these deficiencies have been revealed. As with these deficiencies in which the mtDNA replication machinery has been primarily compromised, differential expression of mtDNA and nuclear OXPHOS genes occurs in cells with mtDNA or nuclear mutations of structural subunits of the OXPHOS system. Differential expression of OXPHOS and related genes has a significant impact on disease expression because of the importance of these genes in energy metabolism, which is compromised in these disorders. This article highlights these observations and investigates the underlying cellular mechanisms that control mitochondrial and nuclear OXPHOS gene expression.

2. Cellular biochemical consequences of OXPHOS deficiencies

Oxidative phosphorylation and deficiencies thereof involve and modulate a great number of cellular functions and metabolic processes upstream and downstream of the five enzyme complexes. Moreover, in considering the effect of OXPHOS deficiencies, it is essential to recognize that deficiencies of the individual enzyme complexes may result in varied biochemical responses. This is evident from existing (but limited) reports of biochemical and gene expression responses to various deficiencies of OXPHOS as discussed in this article.

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Table 1
Summarized findings of OXPHOS and other gene expression investigations of human mitochondrial disorders.

Tissue	Phenotype/deficiency (genotype)	Technique	nDNA expression	mtDNA expression	Reference
Skeletal muscle	MERRF (mtDNA 8344), MELAS (mtDNA 3243), KSS (mtDNA del)	mRNA, Northern blot	•ATPsynβ, ANT1 ↑, GAPDH ↓ (MELAS, MERRF) •ATPsynβ, ANT, GAPDH ↓ (KSS)	•Transcripts ↑ •Processing (light/heavy strand transcript ratios) ↓	[32]
Skeletal muscle (cultured)	CIII deficiency (mtDNA cytb), MELAS (mtDNA 3243, *CI + IV*), MELAS + CM (mtDNA 3243, *CI + IV*), CPEO + PM (mtDNA del, *CI + IV*), KSS (mtDNA del, *CI+IV*), CII deficiency (nuclear), CIV deficiency (nuclear)	mRNA, Northern blot	•ATPsynβ, GAPDH ↑ (MELAS, MELAS + CM, CPEO, CII deficiency) •ATPsynβ, GAPDH ↓ (CIII deficiency)	•Transcripts ↑ (excl. CIII deficiency) •Transcripts ↓ (CIII deficiency) •mtDNA/nDNA ↓	[34]
Skeletal muscle, heart muscle, liver, kidney, brain	LHON (mtDNA 11,778), NARP (mtDNA 8993), MELAS (mtDNA 3243, *CI+IV*), MERRF (mtDNA 8344/9344), MDMD (mtDNA del/dup), CPEO (mtDNA del), FSHMD (nuclear)	mRNA, Northern blot	•↓ATPsynβ, ANT1/2 ↑ (excl. MDMD) •Glycolytic/bioenergetic genes generally ↑	•Transcripts ↑ •mtDNA/nDNA ↓ (most tissues for MELAS)	[33]
Skeletal muscle (cultured)	MELAS (mtDNA 3243), KSS (mtDNA del)	mRNA, Northern blot, competitive RT-PCR	•ATPsynβ ↓ (MELAS)	•ND2 ↑ •mtDNA/nDNA ↓ (MELAS) •mtDNA/nDNA ↑ (KSS)	[35]
Fibroblasts	mtDNA depletion (*CII + III*, *CIV*), RhoO (EtBr induced, *CII + III*, *CIV*)	Protein, Western blot	•COXVIc absent •COXVI, COXVa, SD30, SD70 ↓	Absent due to defect	[36]
Skeletal muscle	Myopathy (mtDNA del), PEO (mtDNA 3243), MELAS (mtDNA 3243) (for all groups, varying deficiencies of combined CI, CI + III, CII + III, and CIV are reported)	mRNA, microarray (Affymetrix HG U133A, 22 283 oligonucleotide targets)	•OXPHOS structural genes ↑ (mtDNA del) •Genes involved in urea cycle/arginine catabolism ↑ •CDKN1A, -1C (cell cycle G1 arrest, DNA repair mediators), and other cell cycle regulators ↑ •CFLAR (anti-apoptosis) ↑ •PEX6 (peroxisomal biogenesis), MAOA (neurotransmitter catabolism) ↓ •RNA Pol II regulation ↑ (MELAS) ↓ (PEO) •Neurobiological structures, fatty acid oxidation, detoxification of H_2O_2, cell signalling ↓ (PEO)	Not reported	[38]
Cybrids	LHON (mtDNA 11778 and 3460), mtDNA depletion	mRNA, microarray (Affymetrix U95Av2, 12 599 oligonucleotide targets)	•Respiratory chain genes, TCA and other aerobic bioenergetic pathways, Pol II promoter transcription and regulation, anti-apoptosis mostly ↑ (mtDNA depletion) •Aldose reductase (aldehyde reduction), integral membrane protein 2B (anti-apoptotic), H2A histone O (chromosome organization/biogenesis) ↑ (LHON cell line shared) •Scaffold protein TUBA (dynamin/actin regulatory), MTHFD (THF/purine metabolism), sialyltransferase 1 (sialic acid transfer/cell surface antigens/ determinants), Raf1 (signal transduction/proliferation/ differentiation/apoptosis), lipin 1, immunoglobin super family member 3 ↓ (LHON cell line shared)	Not reported	[42]
Lymphoblasts	mtDNA depletion	mRNA, microarray (Affymetrix HG U133A, 22 283 oligonucleotide targets)	•Lipid, amino acid metabolism, bioenergetics and transport, intracellular homeostasis, DNA/RNA binding, transcription, translation, redox balance, cell cycle control, growth arrest, signalling, apoptosis, DNA damage and oxidative stress protection ↑	Absent due to defect	[40]

Table 1 *(continued)*

Tissue	Phenotype/deficiency (genotype)	Technique	nDNA expression	mtDNA expression	Reference
143B cells (osteosarcoma)	mtDNA depletion	Protein, 2-DE/MS	•Respiratory chain complexes (excl. CII and CV) ↓ (not uniformly) •Mitochondrial translation apparatus ↓ •Mitochondrial transport systems ↓ •Catabolic energy metabolism ↓ •Hax-1 (anti-apoptotic), Smac protein (pro-apoptotic), rhodanese, hydroxysteroid dehydrogenase ↓	Absent due to defect	[43]
Fibroblasts	CV deficiency (mtDNA 9205 and nuclear uncharacterized)	mRNA, microarray (custom-made, 1632 oligonucleotide targets)	•OXPHOS structural genes for complex IV and V, cell growth, differentiation and transduction ↓ (CV nDNA defects) •Cell cycle regulation, Krebs cycle and gluconeogenesis, mitochondrial transcription regulation (TFAM, TFB1M), CytC, NFκB (apoptosis) ↓ (CV mtDNA 9205) •Branched chain amino acid and fatty acid oxidation, complex I structural genes and apoptosis ↑ (CV nDNA defects)	•MTATP6, MTATP8, MTCOX2 ↓ (CV mtDNA 9205) •ND1, ND2, ND4, ND4L ↑ (CV nDNA defects)	[41]
Fibroblasts (differentially cultured)	CI deficiencies (nuclear): LLD (NDUFS4, NDUFS7, NDUFS8), HCE (NDUFS2), HPEM (NFUFV1)	mRNA, microarray (custom-made, 618 cDNA targets)	•Metallothioneins (ROS scavenging, heavy metal regulation), ATP1G1, heat shock proteins ↑ •Pro-apoptotic protein (BNIP3), pyruvate dehydrogenase de-activation (PDK1) ↓	•Transcripts ↓ (selected cell lines)	[37]

Respiratory chain enzyme deficiencies are shown in italics where reported. The following abbreviations are used: LHON (Leber's hereditary optic neuropathy); NARP (neurogenic muscle weakness, ataxia, and retinitis pigmentosa); CPEO (chronic progressive external ophthalmoplegia); KSS (Kearns–Sayre syndrome); MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes); MERRF (myoclonic epilepsy and ragged red fibers); MDMD (maternally transmitted diabetes mellitus and deafness); FSHMD (facio-scapulohumeral muscular dystrophy); LLD (Leigh-like disease); HCE (hypertrophic cardiomyopathy and encephalomyopathy); HPEM (highly progressive encephalomyopathy); 2-DE/MS (Two-dimensional electrophoresis/mass spectrometry).

An initiator of the immediate and downstream consequences of OXPHOS deficiencies is the production of superoxide. Mitochondrial superoxide production can originate from the ineffective transfer of electrons through the various subunits of the electron transport chain (ETC; complexes I–IV) and the ineffective transfer of carriers (ubiquinone, cytochrome *c*) through the inner mitochondrial membrane. This can lead to the accumulation of electrons and excessive leaking to oxygen to produce reactive oxygen species (ROS), particularly when there is an increased supply of reducing equivalents to the ETC. Complex I (at the bound flavine on the matrix side) and complex III (at the ubiquinol oxidation side) are generally regarded as the main sources of superoxide radicals originating from the ETC [5,6]. The percentage of oxygen converted in this way to ROS under steady state conditions is considered to be much less than the previously estimated 1 to 2% [7] and, in case of respiratory chain deficiencies, would quantitatively be dependent on the amount of electron transfer through the chain and the site of a deficiency within the chain. In fact, a significant part of mitochondrial superoxide production may also originate from the tricarboxylic acid cycle enzyme, α-ketoglutarate dehydrogenase [8], and through a deficiency of the complex II subunit SdhB [9]. Superoxide can result in the generation of other ROS and nitrogen species (RNS), if not dismutated by superoxide dismutases on either side of the mitochondrial inner membrane (Mn and Cu/Zn) or by the radical scavenging effects of antioxidants (vitamins E and C), metallothioneins, or quinone reductase [10,11]. Hydrogen peroxide, which is formed by SOD, can be converted to water by catalase and glutathione peroxidase but can alternatively be converted to hydroxyl radicals by means of the Fenton reaction. The damaging effects of ROS, RNS, and particularly hydroxyl radicals on macromolecules have been extensively documented [12,13] and, through oxidation of these molecules, have been shown to have a direct impact on the viability of genetic and functional molecules inside the mitochondrion and elsewhere in the cell. However, ROS and RNS also act as key messengers in signalling mechanisms that lead to the induction of genes often involved in maintenance and restoration of the cellular redox balance [14,15]. They can also act more directly by altering protein function, such as the activation of uncoupling proteins in brown adipose tissue and the subsequent shift from mitochondrial coupling towards thermogenesis [16].

Abundant evidence of increased superoxide production in OXPHOS deficiencies exists. However, in several reports increased superoxide production is not detected, or an increased superoxide level has no detectable effect on parameters associated with oxidative damage or changes in metabolic homeostasis. Moreover, the origin of superoxide production is not clearly established; for example, ROS production is reported to occur in cell lines harboring mtDNA mutations [17], as well as nuclear mutations of complex I [18,19]. However, ROS production was not detected in a pathogenic mutation of the NDUFS4 subunit of complex I [20] or in HeLa cells containing no mtDNA (ρ°) [21]. ROS production in OXPHOS deficiencies is therefore not a generalized occurrence and depends on several factors, including the position and severity of the dysfunction, the source of production, and the mechanisms that protect the cell against its possible harmful effects [19,22–24].

Deficiencies of OXPHOS also result in other immediate and downstream metabolic, structural, and functional effects. These effects are closely associated with mitochondrial dysfunction, and are briefly described here. The nicotinamide dinucleotide (NAD) redox balance, which is converted to the reduced state in OXPHOS deficiencies, is a fundamental mediator of several biological processes, such as energy metabolism, calcium homeostasis, cellular redox balance, immunological function, and gene expression [25,26]. Not surprisingly, ATP production, and subsequently ATP/ADP homeostasis, is disturbed in OXPHOS deficiencies [7]. Cellular calcium handling also becomes disturbed during an increased oxidative state, with an influx of Ca^{2+} into the cytoplasm, nucleus, and mitochondria [27]. This has an effect on cellular signalling events, where Ca^{2+} is often a key messenger, and more specifically mitochondrial Ca^{2+} loading, which is compounded by ROS, opens the mitochondrial transition pore, disrupts the inner membrane potential ($\Delta\Psi$), and increases cell death through apoptosis

[28,29]. In complex I deficient fibroblasts, the depolarization of $\Delta\Psi$ itself and the subsequent reduced supply of ATP to Ca^{2+}-ATPases leads to reduced cellular Ca^{2+} stores [30].

The varied biochemical changes that occur in cases of OXPHOS deficiencies have a direct effect on cellular functions. Yet, they are also key underlying mediators of the (retrograde) communication between the mitochondrion and the nucleus, which results in specific gene expression of both nuclear and mitochondrial genomes.

3. Differential expression of mitochondrial and nuclear genes in human OXPHOS deficiencies

The biochemical and structural changes that occur because of deficiencies of the OXPHOS system involve the nuclear and mitochondrial genomes. Differential expression of nuclear and mitochondrial genes has been reported for various in vivo and in vitro OXPHOS deficiency models. Initial reports using targeted investigations of RNA and protein expression have revealed the interaction between the nuclear and mitochondrial genome. However, the development of system biology tools over the past decade has rapidly expanded the number of cellular processes that are affected when a deficiency of the OXPHOS system occurs. In addition, these tools have shown that energy metabolism plays a major role in several related diseases that are not discussed in this article [31]. Table 1 summarizes the main findings of several studies investigating gene expression in the presence of mitochondrial disorders and highlights the expression of nuclear and mitochondrial OXPHOS and related genes. The diversity of the disease models used is evident; thus, except for perhaps the data on muscle in patients harboring common mtDNA mutations and deletions, these profiles cannot be directly compared with confidence. Several factors that greatly affect gene expression are significantly different among these reports, including the type of cell lines/tissues, phenotypes, mutations, experimental designs, and genetic background. In addition, key information on OXPHOS enzyme activities, which is necessary for making a comparison based on enzyme deficiencies, is mostly not present or inconclusive.

Initial investigations of the expression of targeted nuclear- and mitochondrial-encoded genes were conducted on the tissues of patients with mitochondrial DNA mutations, deletions, or depletion phenotypes [32–35]. Expression of nuclear genes involved in mitochondrial (*ATPsynβ* and *ANT1/2*) and glycolytic bioenergetics was often increased in muscle. However, many exceptions were observed, which included most of the various phenotypes and mutation types where expression of these genes was either decreased or similar to the controls [32–35]. Marusich et al. report decreased expression of nuclear genes encoding four OXPHOS subunits—*COXVI*, *COXVa*, *SD30*, and *SD70*—in mtDNA depleted fibroblasts [36]. In addition, mitochondrial gene transcripts were generally found to be increased in these patients, although exceptions in a CIII deficiency [34] and a MELAS and KSS patient have been reported [35]. Interestingly, among the cases of mtDNA mutations or deletions that mostly had a complex I and complex IV deficiency, a similar expression profile also occurred in a patient with complex II deficiency [34]. With the one exception of a KSS patient [35], mtDNA/nDNA ratios were generally found to be decreased [33–35], and reduced processing (light/heavy strand) of mtDNA transcripts was observed [32].

It was proposed from these early observations that the general increased expression of selected genes involved in ATP synthesis was due to a compensatory mechanism that increases transcription of genes involved in energy production. It was further suggested that this increased transcription only occurs when a certain threshold of reduced energy production has been reached [32,33]. This was evident from the study by Heddi et al., in which expression levels in different tissues of a patient identified with a MELAS mutation were measured [33]. They found increased expression of all selected nDNA-encoded genes involved in OXPHOS and the glycolysis pathway in all tissues. All tissues with more than 88% mutant mtDNA showed increased mtDNA transcripts, while kidney tissue with only 73% mutant mtDNA showed decreased transcripts of cyt *b*, ND5/6, COI, and COII; increased tRNA-Ser and -Asp; and unchanged 12S rRNA levels.

A more detailed overview of expression profiles in patients with OXPHOS deficiencies was obtained in recent times using micro-arrays [37–42]. For example, the differential expression of several genes in the muscle of patients with common mitochondrial DNA mutations (A3243G MELAS/PEO and 4977 bp deletion) that lead to varied combined deficiencies of OXPHOS enzymes, excluding complex II, are reported [38]. Many genes showed induced expression in all patients in the form of urea cycle/arginine metabolism; anti-apoptotic factor; CFLAR; and selected cell cycle regulators, including cyclin-dependent kinase inhibitor (CDKN), which is involved in G1 arrest and DNA repair. Only a few genes showed decreased expression in all patients. Significantly, it was shown that some genes were differently expressed in the MELAS and PEO patient subsets, which contained the same mutation but had varied levels of combined enzyme deficiencies, even within phenotype groups. These differently expressed genes include those involved in RNA polymerase II regulation, which were increased in the MELAS subset but decreased in the PEO subset, and genes involved in fatty acid oxidation, hydrogen peroxide detoxification, cell signalling, and the development of neurobiological structures. Increased expression of nuclear encoded OXPHOS genes were observed only in the mtDNA macro-deletion subset of patients, and it is striking to note that the enzyme deficiencies within this patient group were varied but similar to the other phenotypes. Although this is contrary to initial reports on similar patient tissues [32–34] in which general increased expression is reported for one nuclear OXPHOS gene, *ATPsynβ*, differential expression of OXPHOS genes was not associated to mtDNA mutations (LHON 11778 and 3460) in cybrids but rather strongly associated to mtDNA depletion [42]. In Danielson et al., the depletion process of mtDNA had a significant effect on genes involved in mitochondrial bioenergetics pathways, which included increased expression of seventeen genes involved in OXPHOS [42]. Supporting observations have been reported in mtDNA depleted (ρ°) lymphoblasts [40]. In 143B (osteosarcoma) cells, however, conflicting reports indicate either the decreased expression [43] or unaffected expression [44] of OXPHOS genes in mtDNA depleted cells. Differential expression of nuclear encoded structural OXPHOS genes is mostly not reported in micro-array data sets (from which it is assumed they are unaffected) in which deficiencies originate from either mitochondrial or nuclear mutations and appear to be exclusively associated with mtDNA depletion. In fact, Cízková et al. report a decreased expression of complex IV and V genes in fibroblasts of isolated complex V deficient patients harboring nuclear mutations [41]. In Chevallet et al., differential levels of decreased respiratory complex subunits, translation apparatus (particularly mitochondria ribosomal proteins), and ion and protein import systems, such as membrane proteins, were found in 143B ρ° cells when compared to wild-type cells [43]. The decreased levels of subunits of respiratory complexes were not significant or uniform (CII and CV subunits remained unchanged), indicating that some stable sub-complexes can survive in ρ° mitochondria. It was suggested that this is because some sub-complexes have other unknown functions or because they are important for mitochondrial stability, or else because of unregulated coordinated nuclear transcription.

Similarities in the differential gene expression of mtDNA depleted (ρ°) lymphoblasts [40] and cybrids [42] have been reported. Increased expression of the genes involved in mitochondrial energy metabolism, including TCA cycle and ETC, in addition to transcription regulation occurred in these cell lines. Dissimilarities were observed in the induction of anti-apoptotic factors in cybrids, while several pro-apoptotic factors were increased in lymphoblasts. This again demonstrates the cell-specific regulation of gene expression, and indicates

that, in the case of apoptosis, the energy pathway predominance of the cell type can direct apoptosis induction [45].

Comprehensive expression profiles of nuclear encoded OXPHOS deficiencies of the OXPHOS system are limited, including only a comparison of expression under defined energy source changes in isolated complex I deficient fibroblasts [37] and, recently, in nuclear encoded complex V deficiency [41]. In both these cases as well, similarities and marked variations of expression profiles were detected, even in patients that harbored the same mutation. Furthermore, no correlation could be made with the levels of enzyme deficiency. Significant increased expression of the ROS scavenging and metal regulating family of proteins (metallothioneins) and decreased expression of pro-apoptotic protein (BNIP3) and pyruvate dehydrogenase de-activation protein (PDK1) occurred in complex I deficient cells when culture conditions were changed from glucose to galactose, in order to challenge oxidative energy production [37]. In selected patients, and notably in the patient with the most severe deficiency, significantly decreased expression of mtDNA transcripts occurred. However, increased expression of mtDNA transcripts was detected in nuclear encoded complex V deficient fibroblasts [41]. This was accompanied by increased expression of fatty acid catabolism, complex I structural genes, and apoptosis, while decreased expression of nuclear complex IV and V structural genes, cell growth, differentiation, and transduction were reported. In the same report, and in contrast to the reports referring to mitochondrial DNA mutations and deletions mentioned previously, mtDNA mutations of complex V resulted in decreased expression of genes of the TCA cycle, cell cycle regulation, mitochondrial transcription, and apoptosis.

It is thus evident from studies of differential expression in mitochondrial disorders that there is great variation in the expression of both nuclear and mitochondrial genes. For OXPHOS genes in particular, the variation in expression also occurs under steady state levels over a more than two-fold range between various tissues and cells and of different sources [44]. This is an important observation, as the varying levels of steady state expression are similar to what is often regarded as 'differential expression' when pathology is investigated. In the limited published data of a highly varied group of patient cell lines and enzyme deficiencies, induced expression of genes involved in energy metabolism occurs in most of the cases. However, the diversity of expression of these genes and apparent lack of correlation with the type and level of OXPHOS enzyme deficiency strongly underscores the significant influence of genetic make-up in cellular response.

4. Regulation of nuclear OXPHOS gene expression

Nuclear gene expression of OXPHOS and other genes involved in mitochondrial function and protection is controlled by retrograde (mitochondria-to-nucleus) signalling mechanisms. These signalling pathways are modulated in part by metabolites controlled by the mitochondrion, including Ca^{2+}, ROS, and ATP. The interplay between these metabolites in the mitochondrion and their control of the mitochondrial permeability transition pore has previously been reported on [29; 30; 44; 47]. Much less is known, however, about the downstream signalling mechanisms of these retrograde effectors in eukaryotes. Calcium-mediated signalling can involve one or more of several pathways, including activation of calcineurin (an activator of NFAT and NFκB), Ca^{2+}-dependent PKC, JNK/MAPK, and CaMK IV (and CREB) pathways [46,48]. An extensive number of enzymes and other proteins involved in cell signalling are targets of ROS or are sensitive to redox state changes. These include phospholipases A2, -C and -D; tyrosine phosphatases; guanylyl cyclase; ion and calcium channels; AP-1 and NFκB transcription factors; several protein kinases; HIF-1α; and the JNK/MAPK pathways that activate, amongst other, nuclear factor-erythroid 2 p45 subunit-related factors 1 and 2 (Nrf1 and -2), which have similar but distinct functions in the expression of antioxidant defense and xenobiotic-metabolizing genes containing one or more antioxidant responsive elements (ARE) [9,15,23,49]. Evidence also indicates that increased oxidative stress is involved in the expression of the nuclear respiratory factor-1 (NRF-1, unrelated to Nrf), which is a key transcription factor in the expression of several genes involved in mitochondrial function [50,51]. NRF-1 activation and increased cytochrome *c* expression have also been associated with the activation of the nuclear-localized, AMP-activated protein kinase (AMPK) as a result of, among others, decreased ATP/AMP ratio [52].

Gene expression data from several of the studies presented in Table 1 support the possibility that regulation of OXPHOS genes may be co-regulated. Recently, Van Waveren and Moraes have shown that not only OXPHOS genes are co-expressed, but also that subunits within OXPHOS complexes are co-expressed [53]. This co-expression is statistically associated with a selection of *cis*-acting elements in human OXPHOS gene promotors, which include well-known elements found in OXPHOS genes or related genes; NRF-1; NRF-2; and the less specific elements ERRA, SP1, MEF-2, YY1, and CREB [53–56]. Van Waveren and Moraes suggest that these factors can act independently or synergistically, in order to allow co-regulation, and with other factors can lead to diversity in expression [53]. An example of this diversity and tissue-specific expression has been described for complex IV (COX) expression in muscle [57]. In mammals, COX may contain ubiquitous liver (L) and heart/muscle-specific (H) isoforms for subunits COX6a and COX7a [54]. The promotor regions of these genes lack NRF sites but contain conserved myocyte enhancer factor 2 (MEF2) elements. Expression of MEF2A is induced by NRF-1 over expression and, with PGC1α (a co-activator that also binds PPARα), results in the induction of muscle-specific gene expression of COX [57,58].

5. Mitochondrial DNA transcription and replication, regulation, damage, and repair

The mechanism involved in the transcription and replication of mtDNA is well documented [59–61] and, as is evident from the report summary in Table 1, clearly involved in expression of OXPHOS disorders. In normal tissue, mitochondrial DNA copy number varies relative to oxidative capacity and energy needs [56]. These variations may be markedly affected in OXPHOS deficiencies, and reports show either a decreased [33–35] or increased [35] mtDNA/nDNA ratio, assuming that no mutations in replication/transcription genes exist in these patients. It has been proposed that mitochondrial gene expression is regulated not only by nuclear encoded transcriptional or post-transcriptional mechanisms, but also by the mtDNA copy number of the cell itself [62,63]. The mechanism for this is unclear, although several of the regulatory proteins involved in mtDNA transcription/replication (TFAM, POLRMT, mtSSB) are co-ordinately expressed with changes in mtDNA copy number [64,69,66]. Miranda et al., however, demonstrate induced expression of NRF-1 and TFAM mRNA in ρ° HeLa cells that correlated with ROS levels [50]. Increased expression of these factors is also found in OXPHOS deficient human cells, in aged skeletal muscle [56], and in HeLa cells treated with menadione that leads to increased superoxide formation [50]. Another key regulator of mtDNA maintenance and copy number is Twinkle helicase [67], although very little is know about its regulation. Notably, the regulation of mitochondrial DNA polymerase, DNApolγ, appears not to be affected by mtDNA levels or its maintenance and is expressed at levels sufficient to support variation among tissues [65,66].

Knowledge of the regulation of nucleus-to-mitochondria (anterograde) signalling that controls these events is still largely lacking. Mitochondrial transcription involves binding of mitochondrial RNA polymerase on three possible promotor sites, in association with TFAM and one of two transcription factor B paralogs, TFB1M and TFB2M [60]. These transcription factors have distinct roles in vivo, and

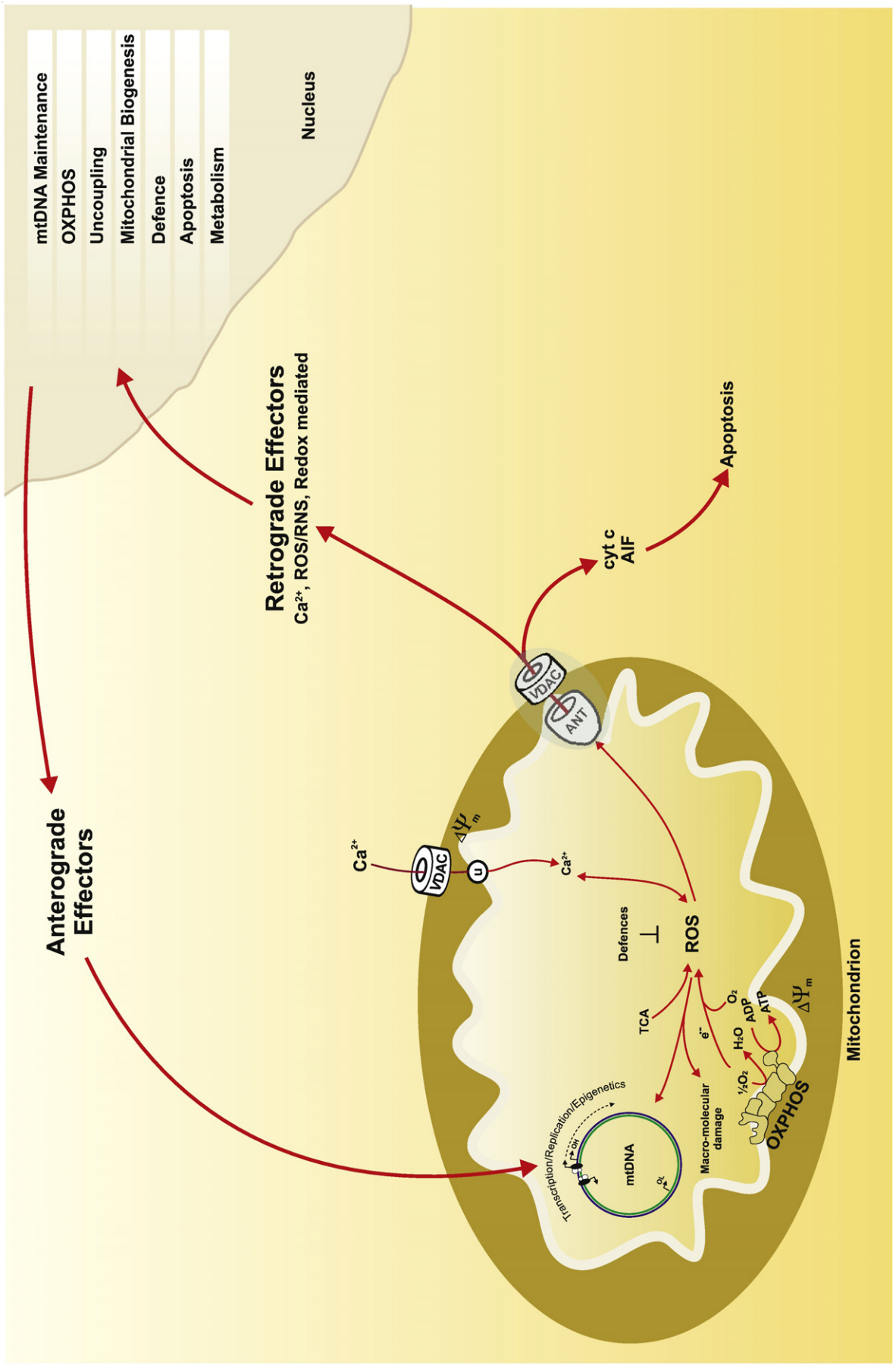

Fig. 1. Summarized display of mitochondria–nucleus interactions that control the expression of OXPHOS and related genes. Changes in homeostasis of ROS, Ca^{2+}, and ATP/ADP control the mitochondrial permeability transition pore (including ANT/VDAC). Such changes can lead to the induction of apoptosis through the intrinsic apoptosis pathway either directly or through secondary signalling pathways (retrograde effectors; see text for details), expression of nuclear genes involved in OXPHOS, and other mitochondria-related functions. Replication and transcription of mtDNA are mainly controlled by the nuclear encoded elements but are also affected indirectly by retrograde signalling and directly by epigenetic factors.

it is proposed that TFB2M is primarily involved in transcription and transcription-primed replication, while TFB1M over expression does not affect these processes but still increases mitochondrial biogenesis [68]. Furthermore, over expression of TFB2M induces TFB1M expression, which suggests that there is a retrograde signalling pathway that co-ordinately expresses these transcription factors [68]. It is interesting to note that the expression of these controlling factors of mtDNA transcription also contains the NRF-recognition sites that are *trans*-activated by the PGC1 family co-activators, PGC1α and PRC [58,69]. These controlling elements are thus shared between the expression of both nuclear- and mitochondrial-encoded OXPHOS genes.

It is well-known that methylation of DNA plays an important role in epigenetic events: DNA-methyltransferases (DNA-MTase) catalyse the addition of a methyl group to a cytosine ring in CpG dinucleotides leading to 5-methylcytosine, which is generally associated with reduced gene expression. This occurs either by blocking binding of transcription factors, binding transcription repressors, or changing chromatin structure [70]. For the mitochondrion evidence of DNA methylation is lacking. However, TFB1M and TFB2M are homologues to rRNA methyltransferases and the role of TFB1M in ribosome biogenesis is proposed [60,64,68,71]. Although methylation and other epigenetic events play an important role in nDNA transcription regulation and maintenance, information on the occurrence and role of these factors in mtDNA expression and maintenance needs further study.

Damage to mtDNA is often highlighted as a factor affecting expression of mtDNA genes and thus contributing to secondary consequences of OXPHOS deficiencies. Considering its structure, maintenance, and close localization to sources of oxidative damage, this may well be a significant factor in disease expression. Owing to its close proximity to the site of mitochondrial ROS production and comparatively less efficient mtDNA damage repair, mtDNA is more sensitive to oxidative damage than nDNA [72]. It has also been demonstrated that oxidative damage is more likely to occur in the controlling D-loop region [56,73]. The compounding factors for oxidation of mtDNA are the close proximity of metal ions that act as catalysts and ROS damage to OXPHOS complexes that result in secondary ROS [72]. The lack of protective histones around the mtDNA is also suggested to contribute to the sensitivity of the mtDNA to oxidative damage, although the presence of regulating proteins, such as TFAM, on mtDNA may have a protective effect against ROS damage [74,75]. It has now been established that mtDNA damage is primarily repaired through the ATP-dependent base excision repair pathway [76–80].

The factors regulating mtDNA replication and transcription are thus highly diverse and tightly controlled by mitochondria–nucleus signalling. In addition, more immediate factors such as ROS, metabolic regulation and defense mechanisms that determine levels of oxidative stress and possibly epigenetic factors, control expression and maintenance. These factors are frequently evaluated separately. Considering the diverse expression profiles that were reported for mtDNA in OXPHOS deficiencies, as well as the regulation of the factors controlling its expression, the interplay of all these factors need to be evaluated to obtain a better understanding of how the mitochondrion responds to OXPHOS deficiencies.

6. Conclusion

The complex and almost unpredictable nature of disease phenotypes associated with OXPHOS deficiency has been a considerable impediment in the characterization and treatment of OXPHOS deficiencies. The effect of OXPHOS deficiencies on mitochondrial and nuclear DNA expression and regulation has been investigated in several studies in an attempt to clarify the adaptive responses in OXPHOS disease phenotypes. These include genes involved in mtDNA maintenance, uncoupling, biogenesis, defense mechanisms, apoptosis, and metabolic regulation. A striking observation from these investigations is the great variation that exists in the differential expression of both the nuclear and mitochondrial DNA, which prompted this review of expression profiles and the mechanisms involved.

As summarized in Fig. 1, the immediate (such as ROS and ATP) and secondary (such as Ca^{2+}) consequences of OXPHOS deficiencies are key mediators in retrograde signalling events that induce expression of nuclear OXPHOS and other genes involved in various cellular processes. Many of the signalling elements and promotor binding sites that control expression of these nuclear genes have now been identified and demonstrate that, in the case of OXPHOS and mtDNA maintenance genes, coordinate (ROS and Ca^{2+} sensitive) expression occurs. The origin and role of ROS is diverse, as its function in the oxidation of macromolecules, including mtDNA and RNA, also contributes to the way mtDNA transcription and replication occur. An aspect that needs to be investigated further is the occurrence and possible role of epigenetic events in the mitochondrion, which may, similar to nuclear DNA, have a marked effect on the expression and maintenance of mtDNA.

Although current data of the expression of genes involved in energy metabolism in OXPHOS deficiencies exhibit diverse profiles and (often) inconsistencies, it is clear that the expression of these genes contributes to the disease expression of OXPHOS deficiencies. In order to obtain an improved understanding of the intricate consequences and adaptive responses in OXPHOS disorders, the expression of nuclear and mitochondrial genes needs to be evaluated holistically, in combination with the signalling processes and metabolites involved and in well-defined disease models. Such an investigation poses a significant challenge, but recent developments in systems biology technologies may soon overcome any difficulties posed.

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