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Nucleus-encoded regulators of mitochondrial function: Integration of respiratory chain expression, nutrient sensing and metabolic stress ☆

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ABSTRACT

Nucleus-encoded regulatory factors are major contributors to mitochondrial biogenesis and function. Several act within the organelle to regulate mitochondrial transcription and translation while others direct the expression of nuclear genes encoding the respiratory chain and other oxidative functions. Loss-of-function studies for many of these factors reveal a wide spectrum of phenotypes. These range from embryonic lethality and severe respiratory chain deficiency to relatively mild mitochondrial defects seen only under conditions of physiological stress. The PGC-1 family of regulated coactivators (PGC-1α, PGC-1β and PRC) plays an important integrative role through their interactions with transcription factors (NRF-1, NRF-2, ERRα, CREB, YY1 and others) that control respiratory gene expression. In addition, recent evidence suggests that PGC-1 coactivators may balance the cellular response to oxidant stress by promoting a pro-oxidant environment or by orchestrating an inflammatory response to severe metabolic stress. These pathways may serve as essential links between the energy generating functions of mitochondria and the cellular REDOX environment associated with longevity, senescence and disease. This article is part of a Special Issue entitled: Mitochondrial Gene Expression.

1. Introduction

Mitochondria are the sites of an array of biochemical activities but are best known for their role in biological oxidations. In particular, they house enzyme systems that convert the chemical bond energy derived mainly from the oxidation of carbohydrates and lipids to NADH and $FADH_2$. These powerful reducing agents are utilized by the electron transport chain and oxidative phosphorylation system of the mitochondrial inner membrane to generate an electrochemical proton gradient across the membrane. This is accomplished by a series of electrogenic proton pumps that couple electron flow to the extrusion of protons to the cytosolic side of the membrane. The resulting proton motive force, comprised of both a voltage potential and a pH gradient, is used by the membrane bound ATP synthase to drive the synthesis of ATP [1,2] or by uncoupling proteins to generate heat [3]. Although the mitochondrial electron carriers are highly efficient in delivering electron pairs to molecular oxygen, the terminal acceptor, occasionally molecular oxygen can become partially reduced by a single electron forming superoxide anion, a highly reactive and toxic species. This occurs predominately at complexes I and III presumably under conditions of excess reducing power and abundant oxygen. The resulting superoxide anion can be converted to hydrogen peroxide via superoxide dismutases localized to both the cytosol and the mitochondrial matrix. However, cells contain a number of powerful scavenging systems to deal with these toxic products making it unlikely that they accumulate in large amounts under normal conditions. Hydrogen peroxide is converted to water by catalase, peroxiredoxin or glutathione peroxidase accounting for the protection of cellular constituents from large transient amounts of free radicals [4].

Mitochondria and their chloroplast cousins are unique among eukaryotic organelles in having their own genetic systems. Mammalian mitochondria have a covalently closed circular genome of approximately 16 kilobases that encodes 37 genes. In contrast to the nuclear genome, where long and short interspersed repeats, introns and vast intergenic regions account for more than 95% of the total DNA, the mtDNA of mammals and other vertebrates exhibits a highly economical sequence organization. In mammals, mitochondrial genes are lacking introns and are arranged head to tail with little or no intergenic regions. The only protein coding genes direct the synthesis of 13 mRNAs for essential respiratory chain subunits while the remaining 24 genes encode the 22 tRNAs and 2 rRNAs required for translation of these mRNAs within the mitochondrial matrix. Thus, the entire mitochondrial genetic system is retained solely for the purpose of providing 13 essential protein subunits of respiratory complexes I, III, IV and V. These are expressed through the bi-directional synthesis of multigenic transcripts, followed by the processing, polyadenylation and translation of individual mRNAs [5,6]. Surprisingly, the organizational economy found in vertebrate mtDNA is not observed in plants and fungi where the mitochondrial genomes are much larger and contain intergenic regions, introns and multiple promoters and transcriptional units [7,8].

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Nevertheless, nearly the same small complement of mitochondrial genes exists over the entire evolutionary spectrum necessitating that nuclear genes control mitochondrial transcription, translation and DNA replication as well as provide the vast majority of gene products required for the biochemical functions and molecular architecture of the organelle.

2. Phenotypic similarities associated with loss of essential nucleus-encoded mitochondrial functions

Because of the semiautonomous nature of mitochondria, interest has focused on a number of nucleus-encoded gene products that act exclusively within the organelle to regulate the mitochondrial genetic system (Fig. 1). In particular, loss-of-function studies provide insights into the phenotypic effects of ablating the expression of nuclear gene products that regulate mitochondrial gene expression. The first of these was Tfam, a high mobility group (HMG)-box protein that stimulates bi-directional transcription through specific promoter recognition [9]. Tfam has been recovered in vertebrate nucleoids in association with other proteins required for mtDNA genomic integrity and expression [10–12]. A germ line Tfam knockout mouse exhibited embryonic lethality at E10.5, a severe oxidative phosphorylation defect and a marked reduction in mtDNA content, demonstrating a requirement for Tfam in mtDNA maintenance *in vivo* [13]. Interestingly, the hearts of a cardiac/skeletal muscle-specific *Tfam* knockout, in addition to having respiratory chain defect and diminished mtDNA levels, also had abundant atypical mitochondria with tubular cristae [14]. Skeletal muscle-specific *Tfam* knockout mice had several features characteristic of mitochondrial myopathy in humans including cytochrome oxidase-deficient ragged red muscle fibers that derive their appearance from the accumulation of abundant abnormal mitochondria [15].

The basic pattern of embryonic lethality at approximately E8.5 in germ line knockouts and severe respiratory chain defects associated with abundant atypical mitochondria in the heart-specific counterparts has been observed upon the ablation of other nucleus encoded factors that supply diverse functions within the mitochondria (Fig. 1). This is true for *Mterf3*, encoding a negative regulator of mitochondrial transcription initiation [16], *Tfb1m*, encoding a dimethyltransferase [17], and, most recently, *Mterf4*, encoding a regulator of ribosome biogenesis and translation [18]. Loss of these functions is often accompanied by defects in respiratory complexes I, III, IV and V which rely upon mitochondria-encoded subunits. Interestingly, mice with a homozygous germ line knockout of *Ant1*, encoding an adenine nucleotide translocator (Fig. 1), also had ragged red muscle fibers with abundant abnormal mitochondria and their hearts displayed cardiac hypertrophy with massive mitochondrial proliferation [19]. It is also notable that a germ line mouse knockout of *PolgA*, encoding a subunit of mitochondrial DNA polymerase, also resulted in mid gestation lethality [20].

These findings illustrate that ablation of nuclear genes, whose products provide diverse functions within the mitochondria to maintain electron transport and oxidative phosphorylation share similar phenotypic features in both germ line and tissue-specific knockout mice. Embryonic lethality at mid-gestation is common to most of the germ line knockouts. Although multiple genes contribute diverse regulatory functions, the muscle-specific knockouts of each resulted in an increased abundance of morphologically and functionally defective cardiac mitochondria. This suggests that there is a similar adaptive response to genetic lesions affecting non-redundant nuclear functions that act exclusively to maintain the biogenesis and function of the mitochondrial respiratory apparatus.

3. Nuclear transcription factors

Characterization of cytochrome *c* and cytochrome oxidase promoters led to the identification of nuclear respiratory factors, NRF-1 and NRF-2, as activators of nuclear genes providing multiple mitochondrial functions. NRF-1binds a palindromic recognition site in the cytochrome *c* promoter as a homodimer and functions as a positive regulator of transcription [21–24]. Serine phosphorylation of the protein in proliferating cells enhances both its DNA binding [24] and *trans*-activation functions [25]. NRF-1 has been implicated in the expression of many nuclear genes required for expression of the mitochondrial respiratory chain [26–28]. In addition to respiratory chain subunits, NRF-1 is linked to the expression of nuclear genes necessary for mitochondrial transcription [29,30], heme biosynthesis [31,32], and protein import and assembly [33–35]. In addition, NRF-1 is among seven transcription factors whose recognition sites are most frequently found in the proximal promoters of ubiquitously expressed genes [36]. Chromatin immunoprecipitations (ChIP) coupled with microarray assay (ChIP-on-chip) identified 691 genes whose promoters are occupied by NRF-1 in living cells [28]. The majority are involved in mitochondrial biogenesis and metabolism. A major subclass of the NRF-1 targets also bound the growth regulatory transcription factor E2F and this subset was enriched in genes required for DNA replication, mitosis and cytokinesis. An NRF-1 siRNA reduced

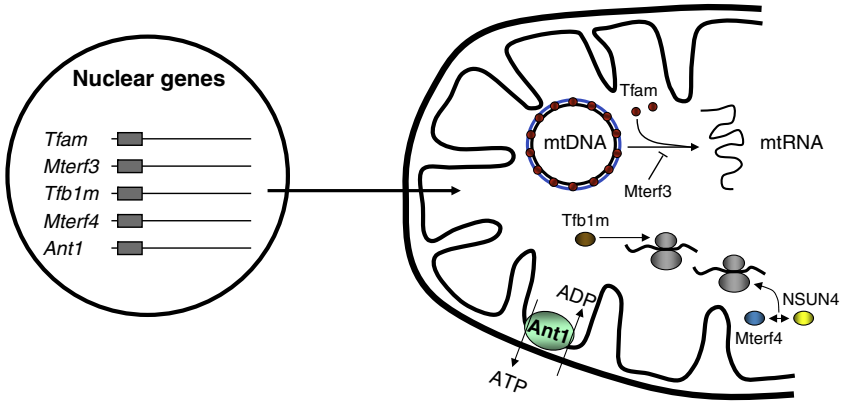

Fig. 1. Summary of the sites of action for nuclear gene products that act within the mitochondria to control mitochondrial transcription and translation. Mouse knockouts of the indicated nuclear genes share common phenotypes consisting of embryonic lethality and severe respiratory chain deficiency accompanied by abundant abnormal cardiac mitochondria. Tfam (red spheres) binds the mitochondrial chromosome at multiple sites and functions in both mtDNA maintenance and transcription initiation. Mter3 serves as a negative regulator of this process. Tfb1m (brown ellipse) and Mterf4 (blue ellipse) participate in mitochondrial ribosome assembly. Tfb1m is a dimethytransferase that catalyzes the adenine dimethylation of the small ribosomal RNA required for ribosome assembly and translation. Similarly, a complex containing Mterf4 and the rRNA methyltransferase, NSUN4 (yellow ellipse), participates in the assembly of the large ribosomal subunit. Although not a direct regulator of gene expression, mouse knockouts of the adenine nucleotide translocator, Ant1 (green ellipse), share several phenotypic features with *Tfam*, *Tfb1m*, *Mterf3* and *Mterf4* knockouts. Thus, ablations of different nuclear genes whose products function exclusively within the mitochondria have similar consequences for the biogenesis and function of the organelle.