Review

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Special Issue: The evolving role of mitochondria in metabolism

Transcriptional integration of mitochondrial biogenesis

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Gene regulatory factors encoded by the nuclear genome are essential for mitochondrial biogenesis and function. Some of these factors act exclusively within the mitochondria to regulate the control of mitochondrial transcription, translation, and other functions. Others govern the expression of nuclear genes required for mitochondrial metabolism and organelle biogenesis. The peroxisome proliferator-activated receptor γ coactivator-1 (PGC-1) family of transcriptional coactivators play a major role in transducing and integrating physiological signals governing metabolism, differentiation, and cell growth to the transcriptional machinery controlling mitochondrial functional capacity. Thus, the PGC-1 coactivators serve as a central component of the transcriptional regulatory circuitry that coordinately controls the energy-generating functions of mitochondria in accordance with the metabolic demands imposed by changing physiological conditions, senescence, and disease.

Regulation of mitochondrial biogenesis

A set of nucleus-encoded factors coordinately regulate mitochondrial mass and function in response to a host of energy and growth demands, including cellular metabolic stress, such as the constant production of reactive oxygen species (ROS). Dysregulation of mitochondrial function has broad implications for human disease including diabetes, heart failure, and neurodegeneration. Thus, there is a high level of interest in developing therapeutic strategies aimed at modulating the regulatory pathways that control mitochondrial function and biogenesis. The nucleus-encoded transcription factors and coactivators that govern mitochondrial function serve as one major focus. Here we review recent advances in our understanding of this regulatory circuitry and its potential role in integrating mitochondrial biogenesis with cellular stress responses.

Transcriptional circuitry controlling mitochondrial biogenesis

Factors acting upon the mitochondrial genome

The transcription and translation of the mitochondrial genome is dependent upon a host of nucleus-encoded gene products (Figure 1; Box 1). Mitochondrial DNA (mtDNA) transcription requires a single RNA polymerase (POLRMT; see Glossary) that shares sequence similarity with yeast mitochondrial and T3/T7 bacteriophage polymerases, two stimulatory transcription factors (Tfam, TFB2M), and a termination factor (MTERF1) [1,2]. Transcription takes place bidirectionally from divergent promoters, termed LSP and HSP, within the D-loop regulatory region. Because of the semi-autonomous nature of mitochondria, interest has focused on several nucleus-encoded gene products that act exclusively within the organelle to regulate the mitochondrial genetic system. The first of these was Tfam, a high mobility group (HMG)-box protein that stimulates bidirectional transcription through specific promoter recognition [3].

Loss-of-function studies have demonstrated the crucial need for the eukaryotic cell to promote mtDNA transcription and mitochondrial function early in embryonic development. Embryonic lethality in germline knockouts with severe respiratory chain defects associated with abundant atypical mitochondria has been observed upon the ablation of Tfam and other nucleus-encoded factors that function

Glossary

AMP-activated protein kinase (AMPK): an energy-sensing kinase that directly phosphorylates and enhances the activity of PGC-1.
Dimethyladenosine transferase 1, mitochondrial (Tfb1m): a mitochondrial methyltransferase that dimethylates 12S rRNA and controls the stability or assembly of the mitochondrial ribosome.
Estrogen-related receptors (ERRs): a family of orphan nuclear receptors that are involved in the regulation of virtually all aspects of mitochondrial function and biogenesis.
General control of amino acid synthesis (GCN5): an acetyltransferase that acetylates and inhibits activity of PGC-1α and β.
Nuclear respiratory factor 1 (NRF-1): a transcription factor that functions as a homodimer and activates the expression of key metabolic genes regulating cellular growth and nuclear genes required for respiration, heme biosynthesis, and mitochondrial DNA transcription and replication.
Nuclear respiratory factor 2 (NRF-2) or GA-binding protein (GABP): a multisubunit transcription factor that is involved in cytochrome oxidase expression and the nuclear control of mitochondrial function.
Peroxisome proliferator-activated receptors (PPARs): a family of ligand-activated nuclear receptors that regulate various aspects of lipid and fatty acid metabolism as well as cellular differentiation.
PGC-1 related coactivator (PRC): a third member of the PGC-1 family that also binds to transcription factors important for mitochondrial biogenesis and function.
PPARγ coactivators (PGCs): transcriptional coactivators that bind to several target transcription factors involved in cellular energy metabolism and mitochondrial function including PPARs, ERRs and NRF-1 and -2.
RNA polymerase (POLRMT): a mitochondrial DNA-directed RNA polymerase.
Transcription factor A, mitochondrial (Tfam): also termed mtTFA, is a mitochondrial transcription factor that is a key activator of mitochondrial transcription and participates in mitochondrial genome replication.
Yin-Yang 1 (YY1): a ubiquitously-expressed transcription factor that directs histone deacetylases and histone acetyltransferases to a promoter to activate or repress gene expression.

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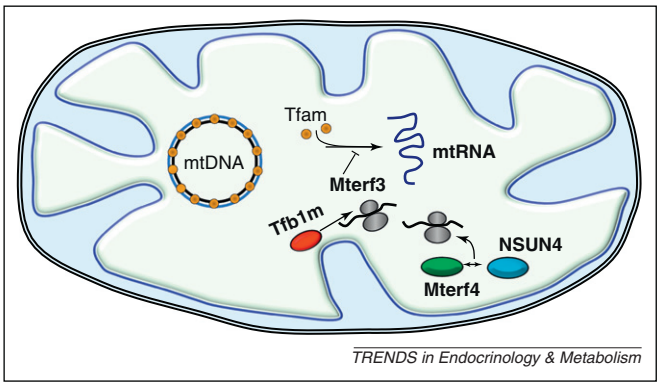

Figure 1. Nucleus-encoded factors acting within mitochondria. Targeted gene disruptions in mice (knockouts) have helped to define the functions of several nuclear genes encoding products that function within mitochondria (as described in the text). These include Tfam (orange spheres), that binds to the mtDNA at multiple sites and functions in both mtDNA maintenance and transcription initiation; Mterf3, that functions as a negative regulator of mtDNA transcription; and Tfb1m (red ellipse) and Mterf4 (green ellipse), that participate in mitochondrial ribosome assembly. Tfb1m is a dimethyltransferase 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 (blue ellipse), participates in the assembly of the large ribosomal subunit.

within the mitochondria. A germline Tfam knockout mouse exhibited embryonic lethality at embryonic day (E)10.5 associated with a severe oxidative phosphorylation defect and a marked reduction in mtDNA content, demonstrating a requirement for Tfam in mtDNA maintenance *in vivo* [4]. Embryonic lethality also occurs with germline knockouts of *Mterf3*, encoding a negative regulator of mitochondrial transcription initiation [5], *Tfb1m*, encoding a dimethyltransferase [6], and, most recently, *Mterf4*, encoding a regulator of ribosome biogenesis and translation through its actions with the rRNA methyltransferase NSUN4 [7]. Loss of these functions is often accompanied by defects in respiratory complexes I, III, IV and V which rely upon mitochondrion-encoded subunits. Interestingly, mice with a homozygous germline knockout of *Ant1*, encoding an adenine nucleotide translocator, also exhibit ragged red muscle fibers with abundant abnormal mitochondria and their hearts display cardiac hypertrophy with massive mitochondrial proliferation [8]. The mitochondrial proliferation probably represents a compensatory response.

Box 1. Mitochondrial structure and function

Mitochondria engage in an array of biochemical activities and are the major sites of oxidative ATP production in eukaryotic cells [93]. The organelle consists of a soluble matrix bounded by a double membrane, an inner ion-impermeable membrane, and an outer membrane permeable to molecules of molecular mass up to approximately 5 kDa. The electron donors, NADH and $FADH_2$, derived from the oxidation of acetyl-CoA, are utilized by the electron transport chain (ETC) of the mitochondrial inner membrane to establish an electrochemical proton gradient across the membrane. The resulting proton motive force, comprising both a voltage potential and a pH gradient, is used by the membrane-bound ATP synthase to drive the synthesis of ATP [93], or by uncoupling proteins to generate heat [94]. Although the mitochondrial ETC efficiently delivers electron pairs to molecular oxygen, the terminal acceptor, occasionally molecular oxygen, becomes partially reduced by a single electron, forming superoxide anion, a highly reactive and toxic species.

According to the endosymbiont hypothesis, mitochondria originated from the engulfment of aerobic eubacteria by a primordial anaerobic eukaryote, an event possibly coincident with the origin of eukaryotic cells [95]. As a result, the organelle has its own genetic system with several bacteria-like features including a compact circular DNA genome (mtDNA), a simple transcription system that produces multigenic RNA transcripts, and a translational apparatus with antibiotic sensitivities similar to prokaryotic cells. Gene loss to the nucleus over evolutionary time left the mammalian organelle with only 37 genes. Only 13 mitochondrial genes encode proteins, all of which are essential subunits of the respiratory chain. The remaining genes specify tRNAs and rRNAs required for protein translation within the mitochondrial matrix [96]. Nearly the same small complement of mitochondrial genes exists over the entire evolutionary spectrum, and thus nuclear genes control mitochondrial transcription, translation, and DNA replication. They also provide the vast majority of gene products required for the biochemical functions and molecular architecture of the organelle.

Transcription factors acting upon the nuclear genome

The nuclear respiratory factors, NRF-1 and NRF-2, were the first nuclear transcription factors implicated in the expression of multiple mitochondrial functions in vertebrates. NRF-1, initially identified through its binding to the cytochrome *c* promoter, functions as a positive regulator of transcription [9]. It acts on genes encoding respiratory subunits [10] as well as Tfam [9] and both TFB isoform genes [11] whose products (as discussed) are major regulators of mitochondrial transcription and ribosome assembly [6,12,13]. Human NRF-2 was identified as a multisubunit transcriptional activator of the cytochrome oxidase subunit IV (COXIV) promoter and its mouse ortholog is GABP, an ETS-domain transcription factor [9]. The human protein has a DNA-binding α subunit and four others (β_1, β_2, γ_1 and γ_2) that complex with α to provide an activation domain and to modulate binding affinity. Evidence points to a direct role for both NRF-1 and NRF-2 in the expression of all 10 nucleus-encoded cytochrome oxidase subunits [14,15]. Both factors also participate in the expression of the mitochondrial import receptor complex and of COX17, a putative cytochrome oxidase assembly factor [9]. Control of the mitochondrial transcription and import machinery by both NRFs may be part of a mechanism coordinating the expression of the respiratory chain with the biogenesis of the organelle itself. Genetic deletion of the NRFs and other nuclear respiratory factors, such as the transcriptional repressor protein YY1, results in peri-implantation lethality [16–18]. NRF-1 null blastocysts failed to progress beyond E6.5 and had depleted mitochondrial DNA levels and diminished mitochondrial membrane potential, consistent with a respiratory chain deficiency [16]. In keeping with a potential link between mitochondrial biogenesis and cell cycle progression, mouse embryo fibroblasts lacking GABPα (NRF-2α) failed to proliferate because of an inability to replicate DNA [19].

Members of the nuclear receptor (NR) superfamily also play a key role in the transcriptional control of nuclear genes encoding mitochondrial enzymes and proteins. The peroxisome proliferator-activated receptor α (PPARα) was the first NR shown to be involved in the transcriptional control of mitochondrial metabolism. Originally discovered as a regulator of genes encoding peroxisomal fatty acid oxidation (FAO) enzymes, PPARα is now known to coordinately regulate nuclear genes encoding mitochondrial FAO enzymes (reviewed in [20]). PPARα is a member of a family of related NRs including the ubiquitously-expressed