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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-1 α , PGC-1 β and PRC) plays an important integrative role through their interactions with transcription factors (NRF-1, NRF-2, ERR α , 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.

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 FADH₂. 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

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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 percent 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]. 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-1 binds 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 expression of several E2F target genes along with Tfam and cytochrome *c* confirming the biological significance of these observations.

Physiological studies support a role for NRF-1 in integrating respiratory chain expression with diverse cellular functions. Elevated NRF-1 expression or DNA binding activity has been associated with exercise-related changes in mitochondrial biogenesis [37-39]. NRF-1 DNA binding activity, cytochrome *c* content and mitochondrial density all increase upon activation of AMP-activated protein kinase by a creatine analog that induces muscle adaptations resembling those observed during exercise [40]. Muscle-specific COX genes depend upon MEF-2 and/or E-box consensus elements for their tissue-specific expression [41]. NRF-1 regulates the expression of MEF-2A in muscle providing a potential

mechanism for indirect NRF-1 control of muscle-specific COX subunits and other muscle-specific MEF-2 target genes [42]. Interestingly, the transcriptional expression of NRF-1 is under the control of estradiol in a breast adenocarcinoma cell line mediated by the direct interaction of estrogen receptor with its recognition site in the NRF-1 promoter [43]. The induction of NRF-1 by estrogen was followed by the Tfam-regulated induction of mitochondrial transcripts and subsequent increases in oxygen consumption and mitochondrial DNA levels. The results implicate NRF-1 as a primary estrogen receptor target gene involved in the increased mitochondrial activity in estrogen-responsive cells. In addition, a link between NRF-1 and the lipopolysaccharide induced inflammatory response was demonstrated by the identification of NF- κ B-responsive elements in the NRF-1 promoter region [44]. This response is also facilitated by CREB binding to the promoter and the functional interaction between NF- κ B and CREB in controlling NRF-1-dependent mitochondrial biogenesis may help mitigate inflammatory damage. CREB is known to combine with NRF-1 in the regulated expression of cytochrome *c* in response to serum growth factors [25]. Its association with the NRF-1 promoter during the inflammatory response may be suggestive of a general CREB-dependent mechanism for the control of respiratory chain expression. Additionally, NRF-1 acts on the expression of the chemokine receptors CXCR4 and CXCR7 which bind stromal-derived factor-1, a proinflammatory α -chemokine regulating the metastatic behavior of several cancers [45,46]. These results are indicative of a link between respiratory gene expression and pro-inflammatory pathways.

A nuclear protein designated as NRF-2 was purified to homogeneity as a multisubunit transcription factor that bound essential *cis*-acting elements in the cytochrome oxidase subunit IV (COXIV) promoter [47,48]. These sites had the GGAA core motif that is recognized by the ETS-domain family of transcription factors. NRF-2 has an α subunit that binds DNA 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 [48,49]. Molecular cloning of the five subunits [49] revealed that NRF-2 is the human homologue of mouse GABP [50]. Both NRF-1 and NRF-2 have been linked directly to the expression of all ten nucleus-encoded cytochrome oxidase subunits [51-53]. Chromatin immunoprecipitations demonstrated that NRF-2 associates *in vivo* with multiple COX subunit promoters [51]. Moreover, expression of all ten nucleus-encoded COX subunits was abrogated by expression of either a dominant negative NRF-2 allele or a siRNA directed against NRF-2 α [52] pointing to an important regulatory function for NRF-2 in the control of cytochrome oxidase expression. NRF-2 sites have been found in many other nuclear genes related to respiratory chain expression (for compilations see [26,27] including genes for Tfam [29] and the TFB isoforms where both NRF-1 and NRF-2 α occupy both TFB promoters in living cells as determined by chromatin immunoprecipitation [30]. Similar control by both NRFs extend to three of the four human succinate dehydrogenase (complex II) subunit genes [54-56]. Like NRF-1, NRF-2 participates in the expression of the mitochondrial protein import machinery with functional recognition sites in TOMM70 [57,58] and TOMM20 [34] promoters. NRF-2 has also been linked to the expression of several mitochondrial translation initiation factors [59]. Involvement of both NRFs in the expression of mitochondrial transcription factors and the subunits of protein import receptor complexes is consistent with a mechanism for coordinating the expression of the respiratory chain with the biogenesis of the organelle itself. This is consistent with the induction of several members of the PGC-coactivator-NRF regulatory axis along with MyoD during mitochondrial biogenesis that occurs early in a program of skeletal muscle regeneration [60].

In addition to the NRFs, the orphan nuclear receptors, estrogen-related receptors α , β and γ (ERRs), have emerged as important regulators of many genes involved in oxidative metabolism [61-63]. In particular, ERR α is expressed at high levels in oxidative tissues and promotes β -oxidation through its control of the medium chain acyl-coenzyme A

dehydrogenase (MCAD) gene [64-66]. $ERR\alpha$ is an important target in the control of mitochondrial biogenesis by PGC-1 α [67,68] and may provide a regulatory link between fatty acid oxidation and the respiratory chain [61]. $ERR\alpha$ engages in a direct regulatory interaction with NRF-2 (GABP) and the same computational analysis places NRF-1 downstream from $ERR\alpha$ in the regulatory cascade [68]. It is notable that genome wide surveys of $ERR\alpha$ binding suggests that $ERR\alpha$ can bind the putative GABP α promoter region and that NRF-1 sites are significantly enriched among genomic sequences showing $ERR\alpha$ occupancy [69]. More recently, the induced expression of PGC-1 β during myogenic differentiation was found to activate a number of respiratory genes by direct targeting of both NRF-1 and $ERR\alpha$ [70]. Immunoprecipitation experiments demonstrated that both NRF-1 and $ERR\alpha$ interact directly with PGC-1 β . Moreover, the deletion of NRF-1 and $ERR\alpha$ recognition sites within respiratory gene promoters or the siRNA silencing of either transcription factor attenuated the induction of respiratory gene expression or mitochondrial biogenesis by PGC-1 β . Finally, functional genomic analysis involving the genome-wide location of transcription factor occupancy has revealed that the ERRs are associated with hundreds of genes controlling all aspects of mitochondrial function [62,63]. On this basis it has been postulated that the ERRs are master regulators of mitochondrial biogenesis and function.

Additional nuclear transcription factors have been linked to the control of mitochondrial respiratory function. The cAMP response element binding protein, CREB, along with NRF-1 is involved in the growth-regulated expression of cytochrome *c* [25,71,72]. Both factors participate in the induction of cytochrome *c* in response to serum stimulation of quiescent cells [25]. CREB has been rediscovered as an activator of the cytochrome *c* promoter and is required for activation of the promoter by $ERR\alpha$ [69]. CREB sites are not generally found in the proximal promoters of nuclear respiratory genes [25] and there is no selective enrichment among genomic fragments containing ERR binding sites [69]. The initiator element binding factor, YY1, is engaged in both the positive and negative control of certain cytochrome oxidase subunit genes [73,74]. YY1 recognition sites along with those for the NRFs and $ERR\alpha$ were enriched among genes encoding OXPHOS subunits and assembly factors [75]. However, YY1 and CREB showed less specificity for respiratory genes than NRF-1 or NRF-2. Nevertheless, YY1 has been identified as a target for the nutrient sensor mTOR and YY1 silencing in myotubules results in diminished mitochondrial gene expression [76]. Finally, c-myc directs the expression of certain NRF-1 target genes [77] and myc null fibroblasts are deficient in mitochondrial content [78]. Interestingly, c-myc positively regulates mitochondrial biogenesis and the respiratory apparatus through PGC-1 β , an effect that is repressed by HIF-1 in the absence of the von Hippel-Landau tumor suppressor. This pathway may be part of a molecular switch from oxidative phosphorylation to aerobic glycolysis mediated by HIF-1 in certain cancers [79].

4. *In vivo* phenotypes associated with loss of nuclear transcription factor function

Homozygous knockout mice have been generated for many of the nuclear transcription factors associated with mitochondrial biogenesis and respiratory function. Many of these exhibit peri-implantation lethality. This is the case for NRF-1 [80], GABP α (NRF-2 α) [81] and YY1 [82]. These severe phenotypes are not surprising given the fact that the factors act on hundreds of genes affecting a range of cellular functions. In the case of NRF-1, the null blastocysts had depleted mitochondrial DNA levels and diminished mitochondrial membrane potential consistent with a respiratory chain deficiency [80]. However, they failed to progress beyond E6.5 and also failed to differentiate *in vitro*. This likely reflects the fact that NRF-1 targets include genes encoding metabolic enzymes, components of signaling pathways and gene products necessary for chromosome maintenance and nucleic acid

metabolism among others [23]. In keeping with a potential link between mitochondrial biogenesis and cell cycle progression, mouse embryo fibroblasts lacking GABP α (NRF-2 α) failed to proliferate because of an inability to replicate DNA [83]. Only a small subset of GABP target genes showed reduced expression and these did not include genes related to mitochondrial function. This was ascribed to the fact that other transcription factors that contribute to the expression of GABP targets were normally expressed and may compensate for the defect. Interestingly, the *Drosophila* homologue of NRF-2 α is required for the expression of most genes encoding mitochondrial proteins and also links mitochondrial abundance to cell growth [84]. Finally, a homozygous knockout of c-myc results in lethality between E9.5 and E10.5 [85]. This is consistent with a role for c-myc in the control of cell cycle progression and metabolic networks [86]. These *in vivo* results suggest that key factors implicated in the biogenesis of mitochondria also function in the control of the cell cycle.

Mouse knockouts of other nuclear factors required for mitochondrial function have surprisingly mild phenotypes. PPAR α null mice have diminished expression of mitochondrial fatty acid oxidation enzymes [87] while ERR α knockouts have reduced lipogenesis and exhibit cold intolerance [88], but both are viable and fertile with no gross phenotypic abnormalities. This might be explained by the fact that, unlike YY1 and the NRFs, both of these factors have multiple isoforms that may compensate for the loss of a single family member. For example, ERR γ and ERR α bind the same recognition sites within essentially the same collection of target genes [62] although the ERR γ knockouts exhibit early postnatal lethality associated with a defect in oxidative phosphorylation in the heart [89]. It is important to note that many target genes for ERR and the other nuclear factors have been identified by functional genomic analysis and there is a general paucity of information regarding the direct effects of a given factor on protein expression, complex assembly or metabolic flux through a specific pathway. This may be especially significant for the respiratory complexes which are thought to be in excess and thus must suffer substantial reductions in expression before the threshold for a respiratory defect is crossed [90]. In addition to cold intolerance [91], the ERR α null mice display a number of cardiac deficiencies when stressed by pressure overload but apparently lack any robust defect in organelle biogenesis that might be expected from the loss of a master regulator [92].

5. The PGC-1 coactivator family

The PGC-1coactivator family is a small family of nuclear transcriptional cofactors consisting of PGC-1 α , PGC-1 β and PRC. PGC-1 α , the founding member of the family, was discovered based on its induction during adaptive thermogenesis in brown fat and its interaction with PPAR γ , an important regulator of adipocyte differentiation [93]. PGC-1 α lacks histone-modifying activities but it interacts through a potent amino terminal activation domain with coactivators such as SRC-1 and CBP/p300 which have their own intrinsic ability to remodel chromatin [94,95]. The association of PGC-1 α with an increasing number of coactivator complexes is consistent with the many hormonal and nutrient signaling pathways associated with PGC-1 α . In addition to PPAR γ , PGC-1 α binds a large complement of transcription factors and nuclear hormone receptors. Among those directly associated with mitochondrial respiratory function are NRF-1, ERR α , YY1, PPAR α and MEF2C. A homologue of PGC-1 α was designated as PGC-1 β or PERC [96,97]. Both PGC-1 α and β are enriched in tissues that contain abundant mitochondria such as heart, skeletal muscle and brown fat but PGC-1 β is not induced in brown fat in response to cold exposure [96,97]. PGC-1 α and β share sequence similarities along their entire lengths consistent with a relatively recent gene duplication [96]. PRC is more divergent from the other two family members with sequence conservations restricted to discreet domains [98]. These shared domains include the amino terminal activation domain and RNA recognition

motif shared by all three family members and a central proline rich region and carboxy terminal R/S domain shared only by PGC-1 α and PRC. The sequence conservation and spatial arrangement of these domains is consistent with related function.

NRF-1 was an early transcription factor target for PGC-1 α in the regulation of mitochondrial biogenesis (Fig. 2). PGC-1 α can *trans*-activate NRF-1 target genes and a dominant negative allele of NRF-1 blocks the effects of PGC-1 α on mitochondrial biogenesis [99]. PGC-1 β resembles PGC-1 α in its functional interaction with NRF-1 and in its ability to direct the expression of nuclear respiratory genes and mitochondrial mass when expressed from viral vectors [100,101]. PGC-1 α also targets estrogen-related receptor α (ERR α) in conjunction with GABP α (NRF-2 α) in regulating respiratory genes including cytochrome *c* and β -ATP synthase [67,68]. A role for PGC-1 α as a regulator of mitochondrial biogenesis is consistent with gain of function experiments in cultured cells [99] and in transgenic mice [102]. Ectopic PGC-1 α expression in cultured cells increases COXIV and cytochrome *c* protein levels as well as the steady-state level of mtDNA [99]. Cardiac-specific over expression in transgenic mice results in massive increases in mitochondrial content in cardiac myocytes leading to edema and dilated cardiomyopathy [102].

Despite its sequence divergence from the other PGCs, PRC is similar to PGC-1 α and β in its binding to NRF-1, CREB and ERR α and in its ability to *trans*-activate nuclear genes required for mitochondrial respiratory function (Fig. 3A) including cytochrome *c*, 5-aminolevulinic synthase, Tfam and the TFB1M and TFB2M isoforms [30,72,98,103]. For each of these promoters, maximal activation by PRC requires functional NRF-1 recognition sites. Despite these similarities, PRC mRNA shows little induction in brown fat under conditions where PGC-1 α is markedly elevated [98]. However, PRC levels are higher in proliferating cells compared to those that are growth arrested because of serum withdrawal or contact inhibition [72,98]. PRC mRNA is rapidly induced upon serum stimulation of quiescent fibroblasts in the absence of *de novo* protein synthesis. This, along with its relatively short mRNA half-life, is characteristic of the class of immediate early genes implicated in the proliferative response to growth factor stimulation (Fig. 3A). These genes encode chemokines, growth factors, proto-oncogenes, serine-threonine kinases, nucleic acid enzymes and transcription factors, among others [104,105]. Recently, PRC has been shown to direct mitochondrial biogenesis through its interaction with ERR α in cultured thyroid cells [106]. PRC was a potent coactivator of ERR α -mediated gene expression and PRC enhanced the effect of ERR α on both mitochondrial mass and cytochrome oxidase activity. In addition, PRC silencing by siRNA had a negative effect on both mitochondrial mass and oxygen consumption in highly oxidative cells. Thus, PRC has the characteristics of a growth regulatory factor that can affect both respiratory chain expression and the biogenesis of mitochondria.

6. Mitochondrial phenotypes associated with loss of PGC-1 family coactivator function

Over expression of PGC-1 α or PGC-1 β in cultured cells and in mouse tissues results in substantial increases in mitochondrial content [95,99,102,107]. In contrast, mice with germ line targeted disruptions of either PGC-1 α [108,109] or PGC-1 β [110,111] were viable and fertile with essentially normal energy expenditure, mitochondrial abundance and morphology. However, under physiological stress the PGC-1 knockouts exhibit more severe phenotypes. The PGC-1 α null mouse was unable to defend body temperature upon cold exposure and had reduced cardiac ATP production and work output in response to physiological stimuli [108,109,112]. A skeletal muscle-specific PGC-1 α knockout mouse exhibited multiple muscle defects including reduced exercise tolerance and abnormalities in

the maintenance of normal muscle fiber composition [113]. PGC-1 β knockout mice were viable and although the animals were not defective in energy intake or expenditure they also exhibited mitochondrial dysfunction under stress conditions [110]. Mitochondrial volume density was only marginally affected in brown fat and mRNAs encoding several nucleus-encoded respiratory proteins were diminished. COX4 and SDHB protein levels were also down and the animals exhibited defects in thermoregulation. Mitochondrial content was reduced in soleus muscle and heart along with modest reductions in respiratory gene expression. However, the mitochondria retained normal internal structure and the respiratory function of isolated mitochondria was indistinguishable between the wild type and knockout animals. Decreased respiratory mRNA expression was confirmed in an independent experiment although this was not linked to any global systemic changes in mitochondrial biogenesis or morphology [111]. These results demonstrate that either coactivator alone is not required for normal development or for the global biogenesis or maintenance of mitochondria.

The potential for functional complementation in the individual PGC-1 knockouts was addressed by the construction and analysis of PGC-1 α/β double knockout mice [114]. The viability of these mice at birth indicated that the absence of both coactivators was compatible with a full course of prenatal development. However, the majority died of cardiac failure within 24 hours with none surviving beyond 14 days. Unlike the individual knockouts, the mitochondrial volume density in the brown fat of the PGC-1 α/β null mice was reduced significantly along with diminished cristae density coinciding with reduced expression of several respiratory genes. Moreover, cardiac failure was accompanied by heart mitochondrial defects marked by a reduction in mitochondrial number and size as well as the presence of internal vacuoles with reduced cristae. These differences were ascribed to a defect in mitochondrial maturation which normally occurs during the late fetal and early postnatal stages [115,116]. The double knockout also exhibited lower expression of fatty acid oxidation and oxidative phosphorylation genes including a number of ERR α and ERR γ target genes suggesting that impairment of the PGC-1-ERR pathway contributes to the mitochondrial maturation defect [114]. The results argue that the two coactivators provide complementary functions in supporting postnatal cardiac differentiation.

Although PRC knockout mice have not been generated, stable lentiviral PRC shRNA transductants of U2OS cells having either complete or partial PRC silencing have been analyzed [117]. Complete PRC silencing led to marked inhibition of respiratory growth on galactose, diminished expression of both nuclear and mitochondrial respiratory chain subunits, lower levels of respiratory complexes I and IV and reduced production of mitochondrial ATP. Moreover, this transductant had abundant, morphologically defective mitochondria, a phenotype similar to those observed in the tissue-specific disruption of *Tfam*, *Tfb1m*, *Mterf3* and *Mterf4* whose products are localized to mitochondria and are required by the mitochondrial genetic system [13,16-18]. In addition, a gene array revealed many mitochondria-related genes whose expression was altered significantly upon PRC silencing [117]. These included respiratory chain subunits, mitochondrial protein import and assembly factors, and mitochondrial ribosomal proteins and tRNA synthetases, among others. Notably, stable expression of a PRC subfragment that binds both NRF-1 and CREB inhibits respiratory growth on galactose [72] suggesting that the interaction between PRC and its target transcription factors contributes to mitochondrial respiratory function. In addition, transient PRC silencing by siRNA also led to diminished respiratory chain expression and was most pronounced for complexes I, III and IV [118], providing independent confirmation that PRC acts as a positive regulator of nuclear genes required for expression of the mitochondrial respiratory complexes. A second, independent lentiviral transductant with partial PRC silencing to a level of about 15 percent of control cells had a milder galactose growth defect, but had no significant deficit in respiratory chain expression

and ATP production [117]. Apparently, relatively low PRC levels are sufficient to maintain a nearly normal mitochondrial phenotype in this system. These results suggest that PRC expression may account for the absence of a more severe mitochondrial defect in the PGC-1 knockout mice by maintaining mitochondrial biogenesis and respiratory function.

7. Nutrient sensing and metabolic stress

It is well established that the PGC-1 coactivators are regulated at the level of mRNA expression through signaling pathways modulating growth, differentiation and metabolism [119,120]. These include pathways that converge on the CREB-dependent induction of PGC-1 α in response to cold exposure, nutrient deprivation and exercise [121-123]. Metabolic signaling through PGC-1 α also occurs through post-translational modifications (Fig. 2). For example, nutrient sensing through the NAD⁺-dependent deacetylase, SirT1, results in the deacetylation of multiple lysine residues in PGC-1 α thereby promoting mitochondrial fatty acid oxidation in response to low glucose [124]. Moreover, energy depletion in muscle triggers the activation of AMP-activated protein kinase (AMPK) which phosphorylates PGC-1 α on specific serine and threonine residues [125]. The subsequent enhancement of mitochondrial gene expression is consistent with the notion that AMPK can mediate at least some of its effects through PGC-1 α [126]. The SIRT and AMPK pathways can also cooperate in promoting calcium dependent mitochondrial biogenesis in myocytes [127,128].

Recent findings support a role for PGC-1 α in linking nutrient deprivation to mitochondrial oxidant production through its control over SirT3 expression (Fig. 2). SirT3 is a member of the sirtuin family of deacetylases that is localized to mitochondria where it modifies key enzymatic activities in optimizing metabolic function [129]. In addition, SirT3 can exert a negative effect on oxidant stress by precipitating a series of reactions beginning with the activation of isocitrate dehydrogenase 2 and culminating in the detoxification of reactive oxygen species by glutathione peroxidase [130]. It can also deacetylate manganese superoxide dismutase in the mitochondrial matrix leading to increased activity and enhanced oxidant scavenging [131,132]. SirT3 can deacetylate specific respiratory complex subunits leading to diminished complex I, II and III activity in cells devoid of the *Sirt3* gene [133-135]. Complex III is thought to be a major site of cytosolic ROS production [136] potentially linking SirT3 to the control of cytosolic ROS levels. Thus, multiple SirT3-dependent mechanisms are implicated in mediating antioxidant effects associated with increased longevity [137].

PGC-1 α coactivates the expression of SirT3 through ERR α which binds the SirT3 proximal promoter [138,139]. Occupancy of the Sirt3 promoter region by both PGC-1 α and ERR α occurs *in vivo* and silencing of ERR α had a negative effect on the induction of Sirt3 by PGC-1 α . Importantly, SirT3 was required for the induction of several components of the ROS detoxifying machinery by PGC-1 α . Since PGC-1 α is activated by SirT1 deacetylation in response to nutrient deprivation [140], PGC-1 α can potentially serve as a link between nutrient sensing pathways and the oxidant scavenging mechanisms facilitated by SirT3 (Fig. 2). This relationship may have important implications for understanding the positive effects on longevity associated with the restriction of caloric intake [137].

A recent study has implicated PRC as a sensor of metabolic stress in cultured U2OS cells [141]. Several metabolic insults including treatment with the respiratory chain uncoupler, CCCP, expression of a dominant negative allele of NRF-1 or glucose deprivation all resulted in the marked induction of PRC protein levels (Fig. 3A). A microarray screen comparing PRC-expressing and non-expressing cells identified at least 45 PRC-responsive genes, the majority of which are involved in inflammation, cell stress and proliferation. A major

category of PRC-dependent inflammatory/stress genes included pro-inflammatory molecules (e.g. IL-1 α , IL-8, cyclooxygenase 2) as well as members of the proline-rich protein family (SPRR2D and SPRR2F). The latter are associated with the response to DNA damage and exit from the cell cycle [142,143]. Several of these PRC-dependent stress genes have been previously linked to the inflammatory microenvironment in a number of human cancers [144,145] and may also be associated with cellular senescence [146,147]. This is consistent with the observations that c-myc is induced along with PRC by chemical uncoupling [141] and that PRC is markedly elevated in several human tumor specimens (Fig. 3B). PRC protein levels are substantially increased in breast, lung and liver tumors compared to modest expression in the surrounding normal tissue (Fig. 3B). It is unclear why PRC from breast tumor migrated at a higher molecular mass. Tfam was expressed at much reduced levels in colon, breast and lung tumor specimens compared to the surrounding tissue, possibly reflecting the decrease in mitochondrial mass associated with the glycolytic nature of many tumors (Fig. 3B). These preliminary findings, along with the observation that PRC is elevated in human thyroid oncocyoma [148], suggests that PRC may contribute to the chronic inflammation associated with certain cancers.

PRC may have a dual role in cell growth control. Under normal conditions it is transiently expressed upon initiation of the proliferative cycle [72]. However, under conditions of metabolic stress, PRC departs from its usual transient expression pattern and becomes constitutively elevated [141]. In this capacity it may promote an inflammatory/stress response that triggers adaptive changes in cell structure, metabolism and growth (Fig. 3A). Interestingly, the induction of PRC by respiratory chain uncoupler and the associated inflammatory/stress response is highly sensitive to antioxidant [141], suggesting that this pathway is optimized in a pro-oxidant environment. This contrasts with the PGC-1 α /SirT3 pathway which, along with ERR α , links nutrient deprivation to oxidant scavenging through induction of the ROS detoxifying machinery [138,139]. Thus, these seemingly opposing pathways, directed by different members of the PGC-1 coactivator family, may provide a balance between oxidant detoxification linked to longevity and the chronic inflammation associated with cellular senescence and disease. In this scenario, PGC-1 α promotes an antioxidant environment through SirT3 in support of the increased mitochondrial fatty acid oxidation precipitated by nutrient limitation [124]. This may exert positive effects in promoting growth and longevity (Fig. 2). However, under conditions of severe stress, possibly resulting from genetic mutation, environmental toxins or oncogene activation, PRC may contribute to the induction of an inflammatory/stress response that accompanies cellular senescence (Fig. 3A). It is tempting to suggest that balance between the PGC-1 α /SirT3 and PRC pathways is reflected in the diminished levels of SirT3 in human cancer cells [135,149] as opposed to the elevated PRC protein levels (Fig. 3B).

8. Summary and perspective

Nucleus-encoded regulatory proteins are essential to the control of mitochondrial biogenesis and oxidative function. Those factors that act exclusively within the mitochondrion (Tfam, Tfb1m, Mterf3, Mterf4) are absolutely required to maintain the morphology, biogenesis and function of the organelle and their loss in mouse knockouts has severe consequences for embryonic development and tissue function. Knockouts of several nuclear transcription factors acting on nuclear respiratory genes (NRF-1, NRF-2, YY1) display early embryonic lethality whereas others (ERR α , PPAR α) yield relatively mild phenotypes. The latter may appear non-essential because they exist in families of multiple isoforms that may compensate for the loss of a single member. This is illustrated in the PGC-1 coactivator family where the individual PGC-1 α or β knockout mice are viable with a mild mitochondrial phenotype compared to the severe defect in postnatal cardiac mitochondrial maturation observed in the double PGC-1 α / β knockout. In addition to its contributions to

the energy generating functions of mitochondria, PGC-1 α may help maintain a pro-oxidant environment in response to nutrient limitation by inducing the SirT3-dependent detoxification of reactive oxygen species. Thus, this coactivator may contribute to the effects of nutrient limitation on longevity by promoting efficient metabolic function coupled to oxidant detoxification. A third member of the PGC-1 family, PRC, appears to function as an immediate-early gene during cell cycle entry but can also orchestrate an anti-oxidant-sensitive inflammatory response to severe metabolic stress. The nutrient sensing pathways directed by these members of the PGC-1 coactivator family may help balance the cellular responses to nutrient availability and may serve as essential links between the energy generating functions of mitochondria and the cellular REDOX environment associated with longevity, senescence and disease.

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Highlights

Nucleus-encoded factors control the expression mitochondrial respiratory function
Nucleus-encoded factors acting within mitochondria are essential for embryogenesis
Ablation of factors acting on nuclear respiratory genes results in a range of phenotypes
Nuclear coactivators governing respiratory gene expression respond to metabolic stress

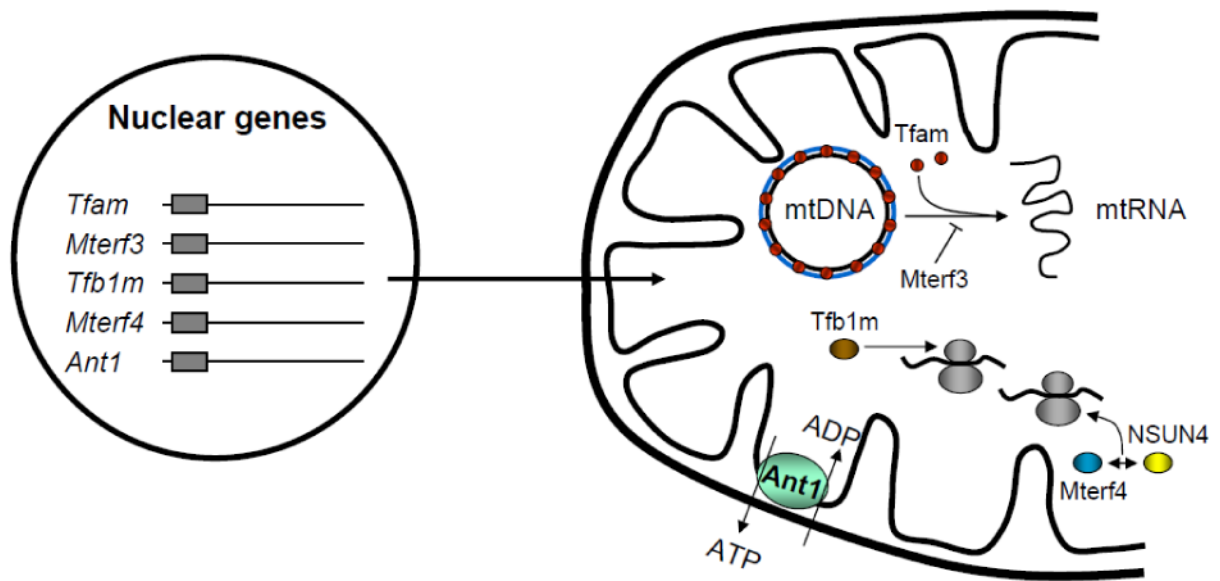


Fig. 1.

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. Mterf3 serves as a negative regulator of this process. Tfb1m (brown ellipse) and Mterf4 (blue ellipse) 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 (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.

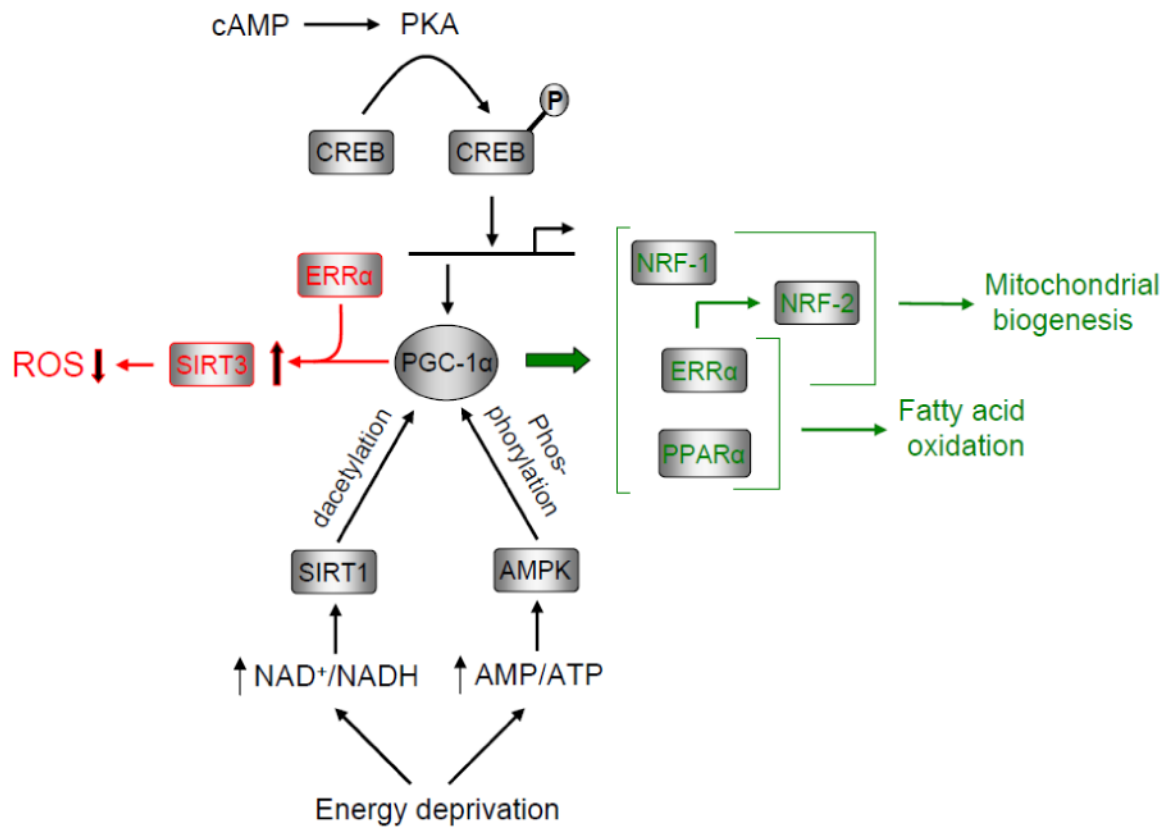
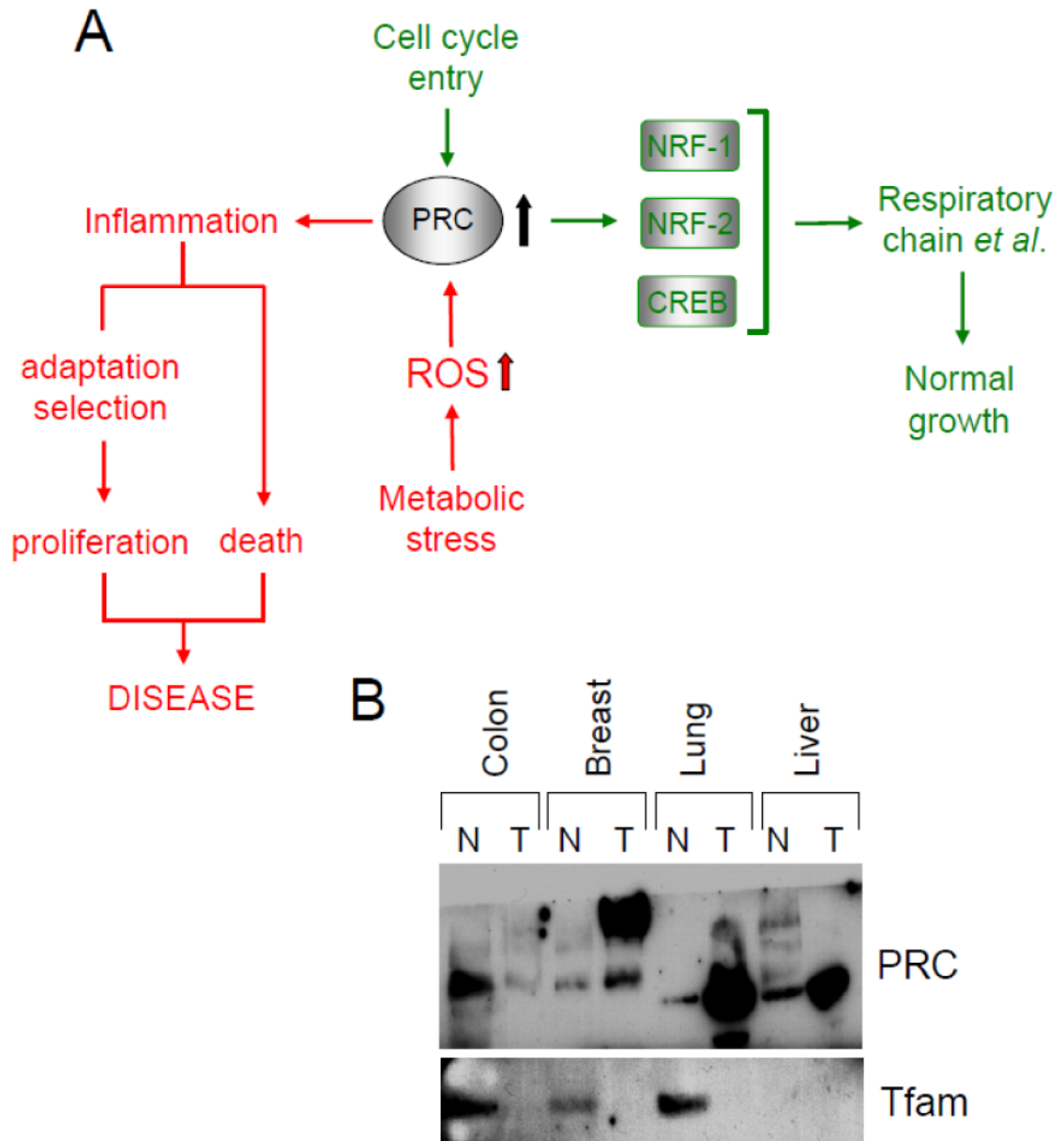


Fig. 2. Potential integration of mitochondrial oxidative and antioxidant pathways by PGC-1 α . PGC-1 α gene expression is induced by cAMP signaling through protein kinase A (PKA) phosphorylation of the transcription factor CREB. This pathway is observed upon β -adrenergic receptor activation during thermogenesis in brown fat. PGC-1 α is also activated by posttranscriptional phosphorylation by AMPK or by deacetylation by SirT1 in response to nutrient deprivation. Induction or activation of the coactivator can enhance mitochondrial biogenesis and oxidative function through the coactivation of multiple transcription factors involved in respiratory gene expression (green pathway). PGC-1 α activation may also promote an antioxidant environment by combining with ERR α in the induction of SirT3, a mitochondrial sirtuin that can trigger ROS detoxification (red pathway).

**Fig. 3.**

A potential role for PRC in orchestrating a balance between cell cycle entry and the inflammatory response to metabolic stress. (Panel A) Under normal conditions (green pathway), PRC protein levels are transiently upregulated during entry to the cell cycle. This coincides with the normal initiation of cell growth or as a response to serum stimulation of quiescent fibroblasts. In this capacity PRC is thought to activate genes encoding the respiratory chain and other growth-related functions through interactions with its target transcription factors. Alternatively, PRC protein levels can be induced in response to severe metabolic stress (red pathway) brought about by nutrient starvation or chemical uncoupling. This is associated with the activation of an antioxidant-sensitive inflammatory/stress program possibly leading to abnormal proliferation or cell death. Genes from this program have been associated with the tumor microenvironment and with cellular senescence. (Panel B) Evidence for elevated PRC protein levels in some solid tumors. A commercial

immunoblot from DNA Technologies containing protein samples from several different tumors and the adjacent normal tissue was probed with affinity-purified rabbit anti-PRC antibody or rabbit anti-Tfam. The results show a robust PRC signal in breast, lung and liver tumors compared to modest expression in the surrounding normal tissue. Surprisingly, the PRC signal from breast tumor migrated at a higher molecular mass. The Tfam control was expressed at much reduced levels in several tumor samples compared to the surrounding tissue possibly reflecting the decreased mitochondrial mass associated with the glycolytic nature of many tumors.