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Nuclear Control of Respiratory Gene Expression in Mammalian Cells

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Abstract The mitochondrial respiratory apparatus is the product of both nuclear and mitochondrial genes. The protein coding capacity of mtDNA is restricted to the expression of 13 respiratory subunits and thus nuclear genes play a predominant role in the biosynthesis of the respiratory chain and in the expression of the mitochondrial genome. Transcriptional regulators that act on both nuclear and mitochondrial genes have been implicated in the bi-genomic expression of the respiratory chain. Mitochondrial transcription is directed by a small number of nucleus-encoded factors (Tfam, TFB1M, TFB2M, mTERF). The expression of these factors is coordinated with that of nuclear respiratory proteins through the action of transcriptional activators and coactivators. In particular, environmental signals induce the expression of PGC-1 family coactivators (PGC-1α, PGC-1β, and PRC), which in turn target specific transcription factors (NRF-1, NRF-2, and ERRα) in the expression of respiratory genes. This system provides a mechanism for linking respiratory chain expression to environmental conditions and for integrating it with other functions related to cellular energetics. J. Cell. Biochem. 97: 673–683, 2006. © 2005 Wiley-Liss, Inc.

Key words: mitochondria; gene expression; transcription; transcription factors; coactivators; respiratory chain

The mitochondrial respiratory chain requires the expression of gene products encoded by both nuclear and mitochondrial genomes [Taanman, 1999; Scarpulla, 2004]. Mitochondria have their own genetic system that includes, in vertebrates, a small covalently closed circular DNA genome of about 16.5 kb (mtDNA). It is well established that the protein coding capacity of this genome is restricted to the synthesis of 13 proteins that function as subunits for respiratory complexes I, III, IV, and V (Fig. 1). The genes specifying complex II are entirely nuclear. The mtDNA also encodes the 22 tRNAs and 2 ribosomal RNAs necessary for the translation of these respiratory subunits within the mitochondrial matrix. However, the mitochondrial genes are but a small fraction of the total number of genes necessary to account for the molecular architecture and biological functions of the organelle. Nearly 100 genes contribute to the electron transport chain and oxidative phosphorylation system alone with over 1,000 genes to maintain the organelle.

As illustrated in Figure 1, the limited coding capacity of mtDNA along with the bi-genomic synthesis of the respiratory chain necessitates that all regulatory factors directing the expression of nuclear and mitochondrial respiratory genes are of nuclear origin. This review will focus on key transcriptional regulators, which fall into two classes. The first class includes the nucleus-encoded factors governing mitochondrial gene expression. They include a mitochondrial RNA polymerase (POLRMT), a transcription and mtDNA maintenance factor (Tfam), transcription specificity factors (TFB1M and TFB2M), and a transcription termination factor (mTERF). The second class includes the nucleus-encoded factors governing nuclear respiratory gene expression. It should be noted that these factors serve to integrate respiratory gene expression with a wide range of cellular functions. This class can be divided into transcription factors that bind respiratory promoters, and coactivators

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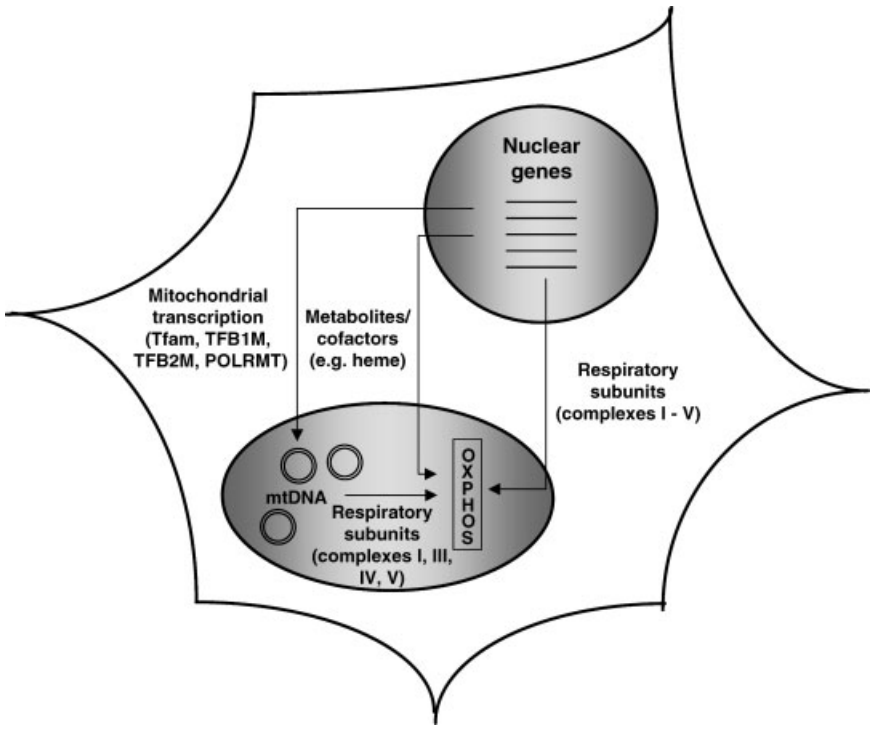

Fig. 1. Nuclear and mitochondrial gene products contributing to respiratory chain expression. Mitochondrial DNA (mtDNA) exists as closed circular genome that is present in multiple copies per organelle. It contributes directly to the respiratory apparatus (OXPHOS) by providing 13 essential subunits of the respiratory complexes I (7 subunits), III (1 subunit), IV (3 subunits), and V (2 subunits). The only other gene products encoded by mtDNA are the 22 tRNAs and 2 rRNAs required for the mitochondrial translation system. Nuclear genes contribute the majority of OXPHOS subunits as well as enzyme systems for the oxidation of respiratory substrates and the synthesis of essential cofactors such as heme. In addition, they provide all of the gene products necessary for the transcription and replication of mtDNA.

that do not bind DNA but rather dock with DNA-bound transcription factors and facilitate chromatin remodeling. Transcription factors include the nuclear respiratory factors 1 and 2 (NRF-1 and NRF-2) and estrogen-related receptor (ERRα). The discussion of coactivators will focus on the PGC-1 family of transcriptional coactivators (PGC-1α, PGC-1β, and PRC). There is abundant evidence that these coactivators are regulated physiologically both at the level of their expression and at the level of interaction with specific transcription factors. Thus, they are thought to integrate multiple transcription factors in a program of respiratory chain expression.

MITOCHONDRIAL TRANSCRIPTION FACTORS ENCODED BY NUCLEAR GENES

In contrast to the nuclear genome, mtDNA exhibits striking economy of sequence organization. The D-loop, the only substantial noncoding region, is the site of transcription initiation at divergent promoters, HSP and LSP [Shadel and Clayton, 1997; Clayton, 2000]. These promoters share an upstream enhancer that contains recognition sites for Tfam (previously mtTF-1 and mtTFA), a high mobility group (HMG) box protein that stimulates transcription through specific binding to the enhancer. Like other HMG proteins, Tfam can bend and unwind DNA, properties linked to its ability to stimulate transcription. In addition, Tfam is abundant in mitochondria and contributes to the stabilization and maintenance of the mitochondrial chromosome through its phased, sequence-independent binding to nonpromoter sites. Homozygous Tfam knockout mice exhibit embryonic lethality and depletion of mtDNA, confirming an essential role for the protein in mtDNA maintenance in mammals [Larsson et al., 1998].

In addition to Tfam, mtDNA transcription in vertebrates requires POLRMT and at least one specificity factor (mtTFB) [Bogenhagen, 1996]. POLRMT has sequence similarity to yeast mitochondrial and phage polymerases. A human mtTFB has been identified and the

protein is localized to mitochondria and stimulates transcription from an L-strand promoter in vitro [McCulloch et al., 2002]. Subsequently, two isoforms of h-mtTFB, termed TFB1M and TFB2M, were characterized [Falkenberg et al., 2002]. TFB1M is identical to the original h-mtTFB isolate and both proteins work together with Tfam and POLRMT to direct proper initiation from HSP and LSP. Like the yeast factor, both TFBs are related to rRNA methyltransferases. TFB1M can bind S-adenosylmethionine and methylate rRNA [Seidel-Rogol et al., 2003], although this activity is not required for transcriptional activation by the factor [McCulloch and Shadel, 2003].

The specific function of the two TFB isoforms remains unclear. At early times following serum stimulation of quiescent fibroblasts, mRNA for the TFB1M isoform is transiently downregulated relative to that of the TFB2M isoform suggesting that the latter is favored in the transition to proliferative growth [Gleyzer et al., 2005]. In *Drosophila* cultured cells, RNAi knockdown of the *Drosophila* B2 isoform, results in reduced mtDNA transcription and copy number [Matsushima et al., 2004]. This contrasts with RNAi knockdown of the B1 isoform, which has no effect on mtDNA transcription or replication but does result in reduced mitochondrial translation [Matsushima et al., 2005]. It is also notable that overexpression of either TFB2M or Tfam in this system increases mtDNA copy number whereas overexpression of TFB1M fails to do so. The stimulatory effect of Tfam is consistent with the observation that overexpression of human Tfam in mice increases mtDNA copy number [Ekstrand et al., 2004]. Thus, both gain of function and loss of function experiments support a role for Tfam and TFB2M in mtDNA copy number control. The inability of TFB1M to function in a similar capacity in *Drosophila* cells is surprising in light of the ability of the mammalian protein to bind the transcription activation domain of Tfam and stimulate transcription in vitro.

Finally, the mitochondrial transcription termination factor, mTERF, is involved in controlling the ratio of mRNA to rRNA by binding a transcription termination site at the end of the 16 rRNA gene. This termination event is associated with a specific initiation site for H-strand transcription suggesting that there is communication between termination and transcription. Recent work has shown that mTERF can bind sites both upstream and downstream from the rRNA transcriptional unit. This is compatible with a model whereby mTERF binding affects both initiation and termination [Martinez-Azorin, 2005].

TRANSCRIPTIONAL REGULATORS GOVERNING NUCLEAR RESPIRATORY GENE EXPRESSION

NRF-1

The isolation and characterization of cytochrome *c* and cytochrome oxidase genes in the early 1980s began a search for transcription factors that are common to the expression of nuclear respiratory genes. NRF-1 (nuclear respiratory factor 1), the first novel mammalian factor associated with genes of this class, functions as a positive regulator of transcription [Scarpulla, 2002; Kelly and Scarpulla, 2004]. It binds as a homodimer to a palindromic recognition site within the cytochrome *c* promoter and utilizes a carboxy-terminal transcriptional activation domain comprised of clustered hydrophobic amino acid residues. Phosphorylation of NRF-1 at multiple serine residues within a concise amino-terminal domain enhances both its DNA-binding [Gugneja and Scarpulla, 1997] and trans-activation functions [Herzig et al., 2000]. Glycosylation of chicken NRF-1 has been associated with transcriptional repression suggesting that other post-translational modifications may be important [Gomez-Cuadrado et al., 1995]. In mammals, NRF-1 exists as a single isoform and is related, through its DNA-binding domain, to developmental regulatory proteins in sea urchins and *Drosophila*. In addition, chicken, Zebra fish, and mouse homologs of NRF-1 have been characterized [Scarpulla, 2002].

NRF-1 acts on the majority of genes required for mitochondrial respiratory function [Scarpulla, 2002; Kelly and Scarpulla, 2004]. As summarized in Figure 2, many of these encode subunits of the five respiratory complexes while others are involved in the expression, assembly, and function of the respiratory chain. Most notably, NRF-1 acts on human genes encoding constituents of the mtDNA transcription machinery including Tfam, TFB1M, TFB2M, and POLRMT. Recent chromatin immunoprecipitation (ChIP) assays revealed that the promoters for these genes are occupied by NRF-1 in vivo [Cam et al., 2004].

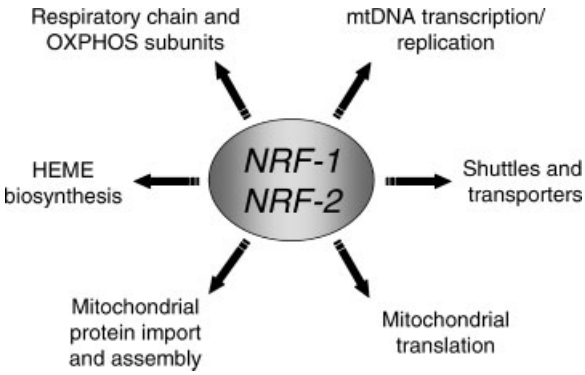

Fig. 2. Classes of NRF-1 and NRF-2 target genes implicated in mitochondrial biogenesis and function.

In addition, NRF-1 has been associated with the expression of mitochondrial and cytosolic enzymes of the heme biosynthetic pathway and components of the protein import and assembly apparatus. These associations are consistent with the hypothesis that NRF-1 plays an integrative role in nucleo–mitochondrial interactions.

Targeted disruptions of the *NRF-1* gene or its relatives have been characterized in Sea Urchins, *Drosophila*, Zebra Fish, and Mice [Scarpulla, 2002]. In general, homozygous null alleles result in an embryonic or larval lethal phenotype and in the case of *Drosophila* and Zebra Fish, a severe neurological defect. In mice, the homozygous NRF-1 knockout dies between embryonic days 3.5 and 6.5, and the null blastocysts fail to grow in culture despite having a normal morphology [Huo and Scarpulla, 2001]. They are also deficient in maintaining a mitochondrial membrane potential and have severely reduced mtDNA levels. There is no apparent defect in mtDNA amplification during oogenesis indicating that the mtDNA depletion occurs between fertilization and the blastocyst stage. Thus, the depletion of mtDNA results from the loss of an NRF-1-dependent pathway of mtDNA maintenance. However, the loss of mtDNA does not explain the early embryonic lethality of the homozygous NRF-1 nulls. Embryos from a Tfam knockout are also depleted of mtDNA but survive between embryonic days 8.5 and 10.5 [Larsson et al., 1998]. It is likely that the loss of NRF-1 affects the expression of NRF-1 target genes that are required for cell growth and development.

A number of primate and rodent genes, whose functions are not linked directly to mitochondrial biogenesis, were identified in an initial search for NRF-1 binding sites in mammalian promoters [Virbasius et al., 1993a]. Recent chromatin immunoprecipitations (ChIP) coupled with microarrays (ChIP-on-chip) identified 691 human promoters, of the approximately 13,000 surveyed, that are bound by NRF-1 in vivo [Cam et al., 2004]. A majority of these genes are involved in mitochondrial biogenesis and metabolism, including many that had not been previously identified. Unexpectedly, a significant subset of the NRF-1 target genes are also bound by the growth regulatory transcription factor, E2F, suggesting that NRF-1 participates in the regulation of a subset of E2F-responsive genes. This subset is enriched in genes required for DNA replication, mitosis, and cytokinesis. Although NRF-1 binds its target promoters under conditions of transcriptional repression, an NRF-1 siRNA-reduced expression of several E2F target genes along with Tfam and cytochrome *c*, confirming that it functions as a transcriptional activator. NRF-1 exists in the dephosphorylated state in serum-starved cells and is phosphorylated upon serum addition [Gugneja and Scarpulla, 1997]. Since phosphorylation enhances NRF-1 transcriptional activity [Herzig et al., 2000], it is possible that phosphorylation controls the functional state of the DNA-bound factor. This along with derepression by release of E2F factors from a subset of NRF-1 target genes may help promote cell proliferation.

A central role for NRF-1 in respiratory chain expression is supported by a number of recent reports that associate elevations in NRF-1 mRNA or DNA-binding activity with enhanced mitochondrial function. Exercise training leads to an adaptive response in skeletal muscle that includes the upregulation of both NRF-1 and its coactivator PGC-1α (see below) [Hoppeler and Flück, 2003; Baar, 2004]. A similar response also occurs in cultured myotubules in response to increased calcium, which mimics exercise-induced mitochondrial biogenesis. Treatment with a creatine analog, β-guanidinopropionic acid (GPA), induces muscle adaptations in rat similar to those observed during exercise and leads to the activation of AMP-activated protein kinase (AMPK). This coincides with increased NRF-1 DNA-binding activity, cytochrome *c* content and mitochondrial density. The effects of GPA were also tested in transgenic mice expressing a dominant negative allele of AMPK.

GPA led to the activation of muscle AMPK and increased mitochondrial biogenesis in wild-type mice. This occurs in association with elevated PGC-1α and calcium/calmodulin-dependent protein kinase IV, both of which have been implicated in the regulation of mitochondrial biogenesis. In contrast, mice expressing dominant negative AMPK exhibit no increase in mitochondrial content upon GPA treatment [reviewed in Kahn et al., 2005]. These results are consistent with the proposed role of AMPK as a global regulator of energy metabolism. AMPK activation during exercise is thought to inhibit protein synthesis and cell growth pathways that consume ATP while activating carbohydrate and fatty acid oxidation to restore ATP levels.

NRF-2(GABP)

A second nuclear factor designated as NRF-2 was identified as an activator of cytochrome oxidase subunit IV (COXIV) expression [Scarpulla, 2002; Kelly and Scarpulla, 2004]. NRF-2 recognition sites in the rodent COXIV promoter have the core GGAA motifs that are common to the ETS-domain family of transcription factors. Human NRF-2 was purified to homogeneity as a five-subunit protein, which included a DNA-binding α subunit and four others (β_1, β_2, γ_1, and γ_2,) that complex with α but alone do not bind DNA. As in COXIV, NRF-2 activates transcription through an array of directly repeated ETS-domain-binding sites in COXVb suggesting that NRF-2 may regulate the expression of multiple respiratory genes [Virbasius et al., 1993b].

Molecular cloning of the five NRF-2 subunits revealed that NRF-2 is the human homolog of mouse GABP [LaMarco and McKnight, 1989]. The two additional human subunits, β_1 and γ_1, are minor splice variants of GABP subunits β_1 and β_2. The GABPβ_1 subunit, corresponding to NRF-2β_1 and β_2, has a dimerization domain that facilitates cooperative binding of a heterotetrameric complex to tandem binding sites [Scarpulla, 2002]. In solution, GABP exists as a αβ heterodimer but is induced to form the heterotetramer $\alpha_2\beta_2$ by DNA containing two or more binding sites [Chinenov et al., 2000]. The crystal structure of the heterotetramer bound to DNA has been determined [Batchelor et al., 1998]. All of the non-DNA-binding subunits contain a transcriptional activation domain. This domain resembles that found in NRF-1 and has been localized to a region upstream from the homodimerization domain [Gugneja et al., 1995].

A number of other genes related to respiratory chain expression are also targets for NRF-2 regulation [Scarpulla, 2002; Kelly and Scarpulla, 2004]. These include genes for Tfam, the mitochondrial transcription specificity factors, TFB1M and TFB2M, and three of the four human succinate dehydrogenase (complex II) subunit genes. NRF-1 sites are often present in NRF-2-dependent promoters but this is not a general rule. Several COX promoters and the rodent Tfam and TFB1M promoters do not have obvious NRF-1 consensus sites. This contrasts with the human Tfam and TFB promoters, which rely upon functional NRF-1 and NRF-2 recognition sites for their activities. It is possible that NRF-1 may bind sequences that deviate significantly from the known consensus.

An essential in vivo role for GABPα (NRF-2α) was revealed by a targeted disruption in mice [Ristevski et al., 2004]. As previously observed for NRF-1 knockout mice, the GABPα homozygous null mice exhibit a peri-implantation lethal phenotype. The heterozygous nulls have wild-type levels of the protein and appear normal. Although the status of mtDNA or mitochondrial function was not investigated, the early defect in the mutant embryos most likely results from a combination of mitochondrial and non-mitochondrial deficiencies. It is notable that homozygous nulls of other Ets family transcription factors also exhibit early embryonic lethality suggesting that members of this family of transcription factors are unable to compensate for one another during embryonic development.

ERRα

The estrogen-related receptor ERRα has been linked to the regulation of oxidative metabolism. ERRα, one of a family of orphan nuclear receptors including ERRβ and ERRγ, resembles the estrogen receptor but does not bind estrogen or other ligands. ERRα levels are high in oxidative tissues such as kidney, heart, and brown fat, and it acts as a regulator of β-oxidation via its control of the medium chain acyl-coenzyme A dehydrogenase (MCAD) promoter [Huss and Kelly, 2004]. The α and γ isoforms increase postnatally in the heart along with enzymes promoting mitochondrial fatty acid uptake and oxidation [Huss et al., 2002]. ERRα knockout mice have reduced fat mass and

are resistant to diet-induced obesity suggesting defects in lipid metabolism [Luo et al., 2003]. As discussed below, recent studies have also implicated ERRα in PGC-1α-induced mitochondrial biogenesis [Mootha et al., 2004; Schreiber et al., 2004].

NUCLEAR COACTIVATORS IN RESPIRATORY CHAIN EXPRESSION

The regulation of respiratory chain expression by a small number of transcription factors offers only a partial explanation for the genetic control of oxidative metabolism. For example, PPARα does not appear to control respiratory chain genes but does regulate genes involved in fatty acid oxidation. In brown adipose tissue, cold exposure triggers a cascade of events leading to the biogenesis of mitochondria and the induction and activation of UCP-1, both of which are essential to the thermogenic response [Lowell and Spiegelman, 2000; Ricquier and Bouillaud, 2000]. The brown fat-specific enhancer of the UCP-1 promoter requires several ubiquitous transcription factors including thyroid and retinoic acid receptors and PPARγ. PPARγ has a regulatory role in adipose differentiation but has not been associated with respiratory gene expression. Thus, a central issue is how the cell integrates many ubiquitous transcription factors into a program of mitochondrial biogenesis. A partial answer lies in the discovery of the PGC-1α family of transcriptional coactivators.

PGC-1α

PGC-1α is the founding member of a family of transcriptional coactivators that appears to play a major integrative function in orchestrating a program of mitochondrial biogenesis (Fig. 3). PGC-1α was identified in a differentiated brown fat cell line through its interaction with PPARγ. In addition to PPARγ, it binds several nuclear hormone receptors and can trans-activate the PPARγ- and thyroid receptor β-dependent expression of the UCP-1 promoter. The dramatic induction of PGC-1α mRNA in brown fat upon cold exposure supports its involvement in thermogenic regulation [Lin et al., 2005; Puigserver, 2005].

Perhaps the most striking property of PGC-1α is that its ectopic overexpression in cultured myoblasts induces mitochondrial biogenesis [Wu et al., 1999]. In addition to inducing

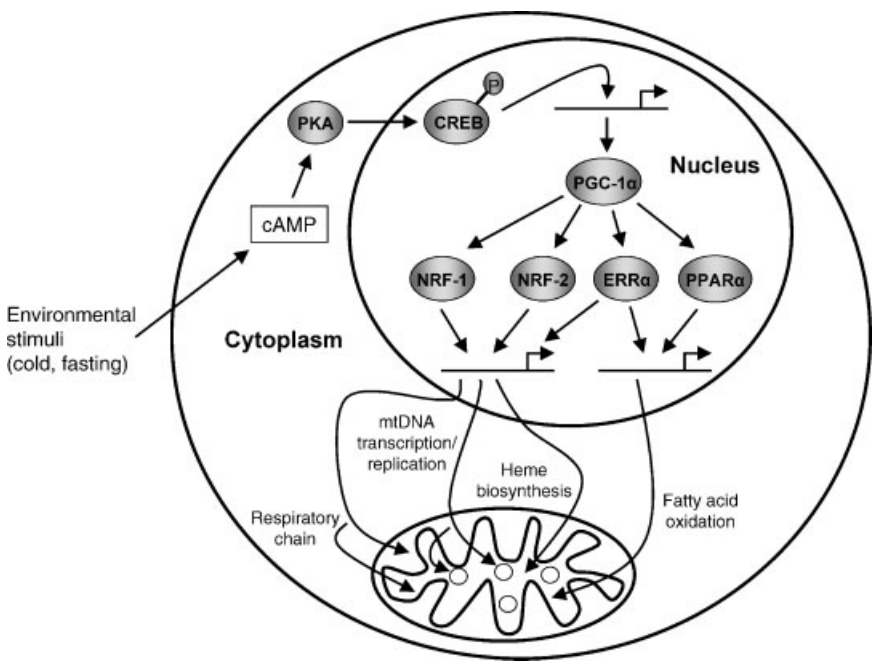

Fig. 3. PGC-1α as an intermediary between environmental stimuli and transcriptional responses. Signaling pathways that respond to environmental stimuli by increasing cAMP levels lead to the activation of CREB through phosphorylation by protein kinase A (PKA). CREB is a potent inducer of PGC-1α, which in turn targets multiple transcription factors (NRF-1, NRF-2, and ERRα) required for the expression of nuclear respiratory genes. PGC-1α interacts with ERRα and PPARα in activating the fatty acid oxidation pathway. PGC-1α and ERRα may serve to integrate both pathways in promoting a tissue-specific bioenergetic phenotype.

respiratory subunit mRNAs, PGC-1α increases COXIV and cytochrome *c* protein levels as well as the steady-state level of mtDNA (Fig. 3). These changes coincide with increased oxygen uptake in differentiated myotubules and a visible increase in mitochondrial number. PGC-1α interacts with NRF-1 and can trans-activate NRF-1 target genes involved in mitochondrial respiration. Moreover, a dominant negative allele of NRF-1 blocks the effects of PGC-1α on mitochondrial biogenesis providing in vivo evidence for a NRF-1-dependent pathway [Wu et al., 1999]. In addition to its effects on respiratory chain expression, PGC-1α can induce the expression of genes of the mitochondrial fatty acid oxidation and heme biosynthetic pathways. PPARα is an activator of fatty acid oxidation enzymes and is enriched in brown fat and other tissues with high oxidative energy demands such as heart and liver. Ligand-dependent binding of PPARα through the LXXLL motif of PGC-1α is associated with the trans-activation of PPARα-dependent promoters [Huss and Kelly, 2004]. Similarly, PGC-1α can utilize both NRF-1 and FOXO1, a forkhead box family member, to induce transcription of δ-aminolevulinate synthase, the first and rate-limiting enzyme of the heme biosynthetic pathway [Handschin et al., 2005].

PGC-1α expression is elevated in the postnatal mouse heart and in response to fasting, conditions that increase mitochondrial energy production [Lin et al., 2005; Puigserver, 2005]. As observed for other cell types, overproduction of PGC-1α in cardiac myocytes induces respiratory subunits, oxidative enzymes, oxygen uptake, and mitochondrial biogenesis. In transgenic mice, expression of PGC-1α from a cardiac-specific promoter leads to massive proliferation of enlarged mitochondria in the heart [Lehman et al., 2000]. This is associated with edema and dilated cardiomyopathy. Surprisingly, mice with a targeted disruption of the *PGC-1α* gene are viable and show no changes in mitochondrial abundance or morphology in liver or brown fat [Lin et al., 2004]. However, they do show reductions in oxygen consumption in isolated hepatocytes and in the expression of several mRNAs linked to mitochondrial function. Interestingly, the only tissues to display morphological abnormalities were brown adipose tissue and the striatum of the brain. Defects in the striatum have been associated with movement disorders and the PGC-1α null mice were markedly hyperactive. This hyperactivity correlated with a loss of axons in the striatum as well as with reductions in nucleus-encoded mRNAs for respiratory and brain-specific genes. These findings are intriguing in light of the longstanding link between mitochondrial function and neurodegenerative disease.

Recently, estrogen-related receptor α (ERRα) has been associated with PGC-1α-induced mitochondrial biogenesis [Mootha et al., 2004; Schreiber et al., 2004]. PGC-1α interacts with ERRα and induces its expression leading to enhanced MCAD gene expression particularly during brown adipocyte differentiation [Huss and Kelly, 2004]. DNA sequence motifs required for transcriptional activation by PGC-1α have strong similarities with ERRα and GABPα (NRF-2α) recognition sites [Mootha et al., 2004]. These motifs are conserved in the promoters of some oxidative phosphorylation genes and PGC-1α can drive the expression of promoters containing the sites. ERRα-binding sites are present in both cytochrome *c* and β-ATP synthase promoters and these sites contribute to trans-activation by PGC-1α but have no effect on these promoters in the absence of PGC-1α [Schreiber et al., 2004]. Interestingly, computer modeling indicates that PGC-1α induction of ERRα and GABPα (NRF-2α) are upstream from NRF-1 in the program of respiratory gene expression.

The complexity of the pathways of mitochondrial biogenesis is illustrated by the phenotypes of knockout mice. The PGC-1α knockout displays surprisingly mild mitochondrial impairment in light of the impressive potency of the coactivator in directing mitochondrial biogenesis in both cultured cells and in transgenic mice. Likewise, mice that are homozygous null for ERRα are viable and fertile and display no defects in food intake or energy expenditure, although they do have reduced fat mass and are resistant to high-fat diet-induced obesity [Luo et al., 2003]. The ERRα knockouts show a modest 1.4-fold reduction in cytochrome *c* mRNA but no changes in the expression of other respiratory subunit genes have been noted. This contrasts sharply with homozygous knockouts of other genes that are essential to mitochondrial biogenesis. For example, both Tfam [Larsson et al., 1998] and NRF-1 [Huo and Scarpulla, 2001] knockout mice die during embryonic development and embryos are severely depleted

of mtDNA. These differences may arise from the fact that Tfam and NRF-1 are unique whereas both PGC-1α and ERRα are members of small families of related factors whose members may display compensatory interactions. The analysis of multiple gene knockouts may shed light on the complex relationships that likely exist among these factors.

PGC-1β

PGC-1β or PERC is a close relative of PGC-1α with sequence similarity between the two distributed along their entire lengths with greater sequence conservation in the amino-terminal activation domain and the carboxy-terminal RNA-binding domain [Lin et al., 2005]. Steady-state tissue levels of PGC-1β mRNA parallel that of PGC-1α with the highest levels in brown fat, heart and skeletal muscle. However, PGC-1β differs from PGC-1α in that it is not induced in brown fat upon cold exposure and it is a poor inducer of gluconeogenic gene expression in hepatocytes and liver [Meirhaeghe et al., 2003]. This most likely results from the absence of an interaction between hepatic nuclear receptor 4α (HNF4α) and forkhead transcription factor 01 (FOXO1), which mediate the expression of gluconeogenic genes. However, despite these differences, PGC-1β binds NRF-1 and is a potent coactivator of NRF-1 target genes leading to increased mitochondrial gene expression [Lin et al., 2003]. Moreover, ectopic expression of PGC-1β results in increased mitochondrial biogenesis and oxygen consumption although PGC-1α has been associated with higher proton leak rates than PGC-1β [St. Pierre et al., 2003]. These results demonstrate that although PGC-1α and β are functionally divergent, they retain the ability to potentiate the biogenesis of mitochondria through their interaction with NRF-1 and possibly other transcription factors. Since the tissue-specific expression pattern of the two coactivators is very similar, PGC-1β may compensate for the absence of PGC-1α in PGC-1α null mice in maintaining mitochondrial function.

PRC

A database search identified the partial sequence of a large cDNA with sequence similarity to PGC-1α in the carboxy-terminal domain comprising the RNA recognition motif and RS domain [Andersson and Scarpulla, 2001]. Analysis of the cloned full-length cDNA revealed additional sequence similarities with PGC-1α including an acidic amino-terminal region, an LXXLL signature for nuclear receptor coactivators and a proline-rich region. Although the overall sequence similarity with PGC-1α is weak, the spatial conservation of these features is highly suggestive of related function and the protein encoded by this cDNA was designated as PGC-1α-related coactivator (PRC) [Andersson and Scarpulla, 2001]. It is of interest that PRC, NRF-1, and Tfam are markedly upregulated in thyroid oncocytomas in conjunction with increases in cytochrome oxidase activity and mtDNA content [Savagner et al., 2003]. These thyroid tumors are characterized by dense mitochondrial accumulation and are apparently devoid of PGC-1α.

Like PGC-1α, PRC binds NRF-1 both in vitro and in vivo and can utilize NRF-1 for the transactivation of NRF-1 target genes [Andersson and Scarpulla, 2001]. The amino-terminal activation domain conserved in both PGC-1α and PRC is required for NRF-1-dependent transactivation. In addition, NRF-1 binding to both coactivators occurs through the NRF-1 DNA binding domain. Recently, PRC was observed to be indistinguishable from PGC-1α in its ability to trans-activate the human TFB1M and TFB2M promoters [Gleyzer et al., 2005]. The NRF binding sites within the proximal promoters serve as targets for trans-activation by both coactivators. Moreover, the expression of TFB1M and TFB2M is upregulated in response to serum growth factors and during L1 adipocyte differentiation where mitochondrial biogenesis is evident [Wilson-Fritch et al., 2003].

Despite these structural and functional similarities, the expression pattern of PRC differs significantly from that of PGC-1α [Andersson and Scarpulla, 2001]. PRC mRNA is not enriched in brown versus white fat and is only slightly elevated in brown fat upon cold exposure, arguing against a major role for PRC in adaptive thermogenesis. As summarized in Figure 4, PRC is rapidly induced upon serum treatment of quiescent fibroblasts and is expressed more abundantly in proliferating cells compared to growth-arrested cells. These cell cycle changes in PRC mRNA expression were observed in the absence of detectable PGC-1α and suggest that PRC is a growth-regulated coactivator. The pattern of gene expression that accompanies PRC induction is qualitatively similar to the pattern observed in response to

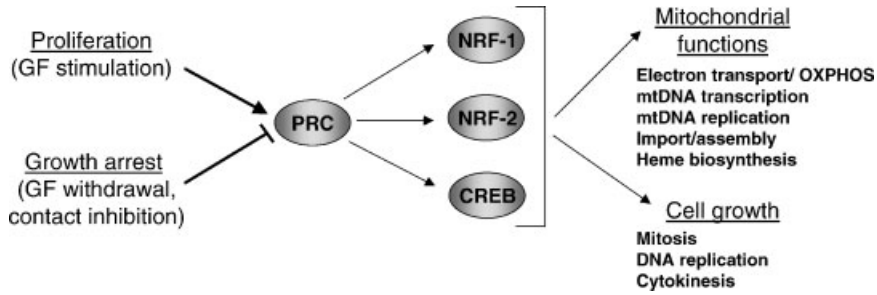

Fig. 4. Model for PRC-dependent integration of respiratory gene expression with cell growth. PRC is induced in proliferating cells and trans-activates respiratory target genes through NRF-1, NRF-2, and CREB. NRF-1 and CREB are known to also target genes involved in cell growth providing a potential mechanism for coordinating cell proliferation with respiratory gene expression. PRC is potently downregulated during growth arrest brought about by serum withdrawal or contact inhibition.

elevated PGC-1α [Gleyzer et al., 2005]. This includes increased expression of mitochondrial transcription factors and both nuclear and mitochondrial respiratory subunits. Recently, PRC was observed to bind the cAMP response element-binding protein (CREB) both in vitro and in vivo (unpublished). CREB is activated in response to serum growth factors and its association with PRC in vivo may facilitate the growth factor response. These results are consistent with a role for PRC in mitochondrial biogenesis during proliferative growth.

CONCLUSIONS

It is clear that the interplay of transcription factors and regulated coactivators contributes to the expression of both nuclear and mitochondrial respiratory genes. The PGC-1 family of coactivators mediates the effects of environmental stimuli on the transcriptional machinery through interactions with cognate transcription factors that bind respiratory gene promoters (Fig. 3). The integration of respiratory subunit genes with those contributing to the transcription and replication of mtDNA results in the bigenomic expression of the respiratory apparatus. Importantly, respiratory chain expression may be coordinated with other functions such as fatty acid oxidation, thermogenesis, gluconeogenesis, and cell growth through the action of PGC-1 family members on an array of transcription factors.

Specificity of the transcriptional program occurs both at the level of environmental signaling and at the level of transcription factor recognition by the coactivators. As shown in Figure 3, PGC-1α responds to cAMP-dependent signaling pathways through the control of the PGC-1α promoter by CREB family transcription factors. By contrast, PRC transcriptional expression is thought to involve growth factor activation through as yet unidentified factors (Fig. 4). PGC-1α, PGC-1β, and PRC exhibit specificity in the spectrum of transcription factors that they target. Some, like NRF-1, are targets for all three coactivators while others, such as nuclear hormone receptors, are differentially regulated through PGC-1α and β. For example, PGC-1α and β differ in their ability to interact with gluconeogenic factors and to induce gluconeogenesis [Lin et al., 2003]. Differences in the response to environmental signals and in transcription factor interactions likely contribute to specialization in the functions of the PGC-1 family members. However, it is unlikely that induction of a coactivator can fully account for tissue-specific differences in the genetic program, for example thermogenesis in brown fat and gluconeogenesis in liver. It is likely that additional levels of specificity are "hard wired" in particular cells and tissues. In addition, little is known about the role of post-translational modifications in modulating the effects of these transcriptional components, perhaps in a tissue-specific manner. Much emphasis has been placed on the effects of the transcription factors and coactivators discussed in this review on cellular energetics. However, factors such as the NRFs that are targets of PGC-1 coactivators act on hundreds of genes that are not involved in the expression of the respiratory chain [Cam et al., 2004]. Thus, it is likely that these activator–coactivator interactions may serve to integrate seemingly unrelated cellular functions.

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