Transcriptional Paradigms in Mammalian Mitochondrial Biogenesis and Function

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Scarpulla RC. Transcriptional Paradigms in Mammalian Mitochondrial Biogenesis and Function. Physiol Rev 88: 611–638, 2008; doi:10.1152/physrev.00025.2007.—Mitochondria contain their own genetic system and undergo a unique mode of cytoplasmic inheritance. Each organelle has multiple copies of a covalently closed circular DNA genome (mtDNA). The entire protein coding capacity of mtDNA is devoted to the synthesis of 13 essential subunits of the inner membrane complexes of the respiratory apparatus. Thus the majority of respiratory proteins and all of the other gene products necessary for the myriad mitochondrial functions are derived from nuclear genes. Transcription of mtDNA requires a small number of nucleus-encoded proteins including a single RNA polymerase (POLRMT), auxiliary factors necessary for promoter recognition (TFB1M, TFB2M) and activation (Tfam), and a termination factor (mTERF). This relatively simple system can account for the bidirectional transcription of mtDNA from divergent promoters and key termination events controlling the rRNA/mRNA ratio. Nucleomitochondrial interactions depend on the interplay between transcription factors (NRF-1, NRF-2, PPARα, ERRα, Sp1, and others) and members of the PGC-1 family of regulated coactivators (PGC-1α, PGC-1β, and PRC). The transcription factors target genes that specify the respiratory chain, the mitochondrial transcription, translation and replication machinery, and protein import and assembly apparatus among others. These factors are in turn activated directly or indirectly by PGC-1 family coactivators whose differential expression is controlled by an array of environmental signals including temperature, energy deprivation, and availability of nutrients and growth factors. These transcriptional paradigms provide a basic framework for understanding the integration of mitochondrial biogenesis and function with signaling events that dictate cell- and tissue-specific energetic properties.
I. INTRODUCTION

A. Overview

Mitochondria are ubiquitous membrane-bound organelles that are a defining feature of the eukaryotic cell. The organelle is comprised of a soluble matrix surrounded by a double membrane, an ion impermeable inner membrane, and a permeable outer membrane. Early biochemists recognized the importance of mitochondria as the sites of aerobic oxidation of metabolic fuels. It is now well established that they contribute to many important functions including pyruvate and fatty acid oxidation, nitrogen metabolism, and heme biosynthesis among others. Most notably, the mitochondrion is the site of the electron transport chain and oxidative phosphorylation system that provides the bulk of cellular energy in the form of ATP (16, 88). Most of the chemical bond energy from the oxidation of fats and carbohydrates is converted to the reducing power of NADH and FADH$_2$ within the mitochondrial matrix. The respiratory apparatus consists of a series of electrogenic proton pumps that convert this reducing potential to an electrochemical proton gradient across the inner membrane (Fig. 1). The electrochemical potential of this gradient is converted via the ATP synthase to the high-energy phosphate bonds of ATP. In addition, the gradient can be dissipated in specialized brown adipocytes by uncoupling proteins to generate heat.

Mitochondria and their chloroplast cousins are unique among eukaryotic extranuclear organelles in that they contain their own genetic system. In vertebrates, this system is based on a mitochondrial genome consisting of a circular double-stranded DNA (mtDNA) (Fig. 2). The gene complement of mtDNA comprises but a small fraction of the number of genes necessary to account for the molecular architecture and biological functions of the organelle. Because of this limited coding capacity, mitochondria are genetically semiautonomous in that they rely heavily on the expression of nuclear genes for all of their biological functions. For example, the majority of protein subunits that comprise the five inner membrane complexes of the electron transport chain and oxidative phosphorylation system are nucleus encoded (Fig. 1). The largest of these, complex I, the NADH dehydrogenase, has 39 of its 46 subunits specified by nuclear genes, whereas the smallest, the succinate dehydrogenase (complex II), is comprised entirely of nucleus-encoded subunits (Fig. 1). Thus nuclear genes must specify most of the structural and catalytic components directly involved in energy metabolism and, in addition, control mitochondrial transcription, translation, and DNA replication (27, 46, 185, 227).

Mammalian mitochondrial biogenesis is subject to complex physiological control. Mitochondrial mass is induced in adult muscle in response to exercise (28) or chronic electrical stimulation (232), presumably as an adaptation to facilitate increased oxygen utilization. Calcium-dependent signaling has been linked to a transcriptional pathway of mitochondrial biogenesis in skeletal muscle (155, 235). The proliferation of mitochondria occurs in the brown fat of rodents during adaptive thermogenesis coinciding with the activation and induction of UCP-1, an uncoupling protein that dissipates the proton gradient to produce heat as a response to cold exposure (34, 175). During early mouse development, the mitochondrial DNA (mtDNA) content remains constant from fertilization to the blastocyst stage after which DNA replication and organelle division resumes (167). Subsequent mitochondrial proliferation increases continuously throughout development, and tissue-specific factors are thought to dictate the final adult complement of mtDNA (90). The respiratory capacity of mitochondrial membranes is en-

**TABLE 1.** Summary of protein subunits of the five respiratory chain complexes encoded by nuclear and mitochondrial genes. Depicted is a schematic of the five respiratory complexes (I–V) embedded in the lipid bilayer of the inner mitochondrial membrane. Dissociable electron carriers cytochrome c (Cyt c) and coenzyme Q (Q) are also shown. Arrows (green) show the pathway of electrons from the various electron donors. Broken arrows (blue) show the sites of proton pumping from the matrix side to the cytosolic side by complexes I, III, and IV. The red arrow shows the flow of protons through complex V from the cytosolic side to the matrix coupled to the synthesis of ATP. Indicated above each complex are the number of protein subunits encoded by nuclear (nDNA) and mitochondrial (mtDNA) genomes.

<table>
<thead>
<tr>
<th>Complex</th>
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<th>nDNA</th>
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<tr>
<td>I</td>
<td>7</td>
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<tr>
<td>II</td>
<td>1</td>
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The direction of transcription. The origin of H-strand replication (OH) is L- and H-strand promoters (LSP, HSP1, and HSP2), with arrows showing recognition (parentheses). The D-loop regulatory region contains the genes for leucine (L) and serine (S) are distinguished by their codon (red bars denoted by the single-letter amino acid code). Duplicate tRNA protein coding and rRNA genes are interspersed with 22 tRNA strands assigned as such based on their buoyant densities. Are depicted in a circular genomic map showing heavy (blue) and light (black) strands assigned as such based on their buoyant densities. Protein coding and rRNA genes are interspersed with 22 tRNA genes (red bars denoted by the single-letter amino acid code). Duplicate tRNA genes for leucine (L) and serine (S) are distinguished by their codon recognition (parentheses). The D-loop regulatory region contains the L- and H-strand promoters (LSP, HSP1, and HSP2), with arrows showing the direction of transcription. The origin of H-strand replication (OH) is within the D-loop, whereas the origin of L-strand replication (OL) is displaced by approximately two-thirds of the genome within a cluster of five tRNA genes (W, A, N, C, Y). Protein coding genes include the following: cytochrome oxidase (COX) subunits 1, 2, and 3; NADH dehydrogenase (ND) subunits 1, 2, 3, 4, 4L, 5, and 6; ATP synthase (ATPS) subunits 6 and 8; cytochrome b (Cyt b). ND6 and the 8 tRNA genes transcribed from the L-strand as template are labeled on the inside of the genomic map, whereas the remaining protein coding and RNA genes transcribed from the H-strand as template are labeled on the outside.

Enhanced postnataally as an adaptation to oxygen exposure outside the womb (217). These examples bear on a fundamental question in understanding the biology of eukaryotic cells, namely, what are the mechanisms of communication between physically separated nuclear and mitochondrial genetic systems in meeting cellular energy demands?

B. Pathophysiology

In the last 20 years, mitochondrial dysfunction has been recognized as an important contributor to an array of human pathologies. Mitochondrial defects play a direct role in certain well-defined neuromuscular diseases and are also thought to contribute indirectly to many degenerative diseases. Since the identification of the first human pathological mutation in mtDNA, many such mutations have been catalogued. Mutations in mitochondrial genes for respiratory proteins and translational RNAs, particularly tRNAs, manifest themselves in a wide range of clinical syndromes, most of which affect the neuromuscular system (55, 227). These mtDNA mutations are often maternally inherited, and in some cases, patients with certain mitochondrial myopathies exhibit excessive proliferation of abnormal mitochondria in muscle fibers, the so-called ragged red fiber (226). In addition, a subset of mitochondrial diseases exhibits a Mendelian inheritance pattern typical of nuclear gene defects (199, 205). These can affect respiratory protein subunits, assembly factors, and gene products required for mtDNA maintenance and stability.

In addition to single gene defects, dispersed lesions in mtDNA that accumulate over time may play a role in human pathologies including neurodegenerative disease (189), diabetes (131), and ageing (57). It has been widely speculated that free radical production by the mitochondrial respiratory chain contributes to the neuropathology observed in dementia and other degenerative diseases. Although there is good evidence for oxidative stress associated with neuropathology, it has been difficult to prove whether this is a cause or a consequence of neuronal death (5). The accumulation of mutations in mtDNA is also thought to contribute to human ageing. Documented changes in mtDNA, including increased prevalence of point mutations and deletions, have been observed in aged individuals (43). This along with the known age-related decrease in oxidative energy metabolism has led to the hypothesis that mtDNA mutations impair the respiratory chain leading to a decline in oxidative phosphorylation (57).

Direct experimental support for this model comes from recent studies in which a mtDNA polymerase that is defective in proofreading function was substituted for the normal polymerase in mice (213). Animals homozygous for the defective allele had a mutator phenotype with markedly increased levels of mtDNA point mutations and deletions. Although the mutations far exceed those associated with normal aging, the mutator mice had a reduced life span and a number of physiological changes related to premature aging. There has also been considerable interest in mitochondrial dysfunction as a contributing factor in the onset of type 2 diabetes (161). Insulin resistance in healthy aged individuals with no family history has been correlated with a decline in mitochondrial oxidative phosphorylation (163). The expression of a number of genes involved in oxidative metabolism is reduced in diabetic subjects as well as in those predisposed to diabetes because of family history (144, 162). Transcriptional activators and coactivators that regulate mitochondrial biogenesis have been suggested as potential contributors to this phenomenon. In addition, mitochondrial functional insuf-
ficiency has been found in the insulin-resistant offspring of patients with type 2 diabetes (164). The fact that this occurs in healthy individuals that are not diabetic suggests that an inherent defect in oxidative phosphorylation may be a contributing factor. These associations of mitochondrial dysfunction with human degenerative disease raise the basic question of how mammalian cells control mitochondrial biogenesis. It has become increasingly apparent that transcriptional mechanisms contribute to the biogenesis of mitochondria including the expression of the respiratory apparatus. Transcriptional control operates through a subset of well-defined transcription factors and transcriptional coactivators. These regulators exert their influence on the expression of both nuclear and mitochondrial genes required for the maintenance and biogenesis of the organelle.

II. THE MITOCHONDRIAL GENETIC SYSTEM:

mtDNA STRUCTURE AND INHERITANCE

It is long established from both genetic and biochemical studies that the mitochondrial genetic apparatus has features that are distinct from that of the nucleocytosolic system. Early work pointed to a protein synthetic machinery in isolated mitochondria that could synthesize a small number of proteins utilizing mitochondrial ribosomes, rRNA, and tRNA (45). A major advance was the discovery of mitochondrial DNA. Genetic studies in yeast led to the observation that certain respiratory mutations displayed a cytoplasmic inheritance pattern that resulted from the random segregation of mtDNA molecules during mitosis. This provided genetic evidence that mitochondria had their own DNA before its existence was demonstrated physically. This conclusion was supported by physical evidence for the existence of mitochondrial DNA in yeast and in other organisms (148). Since these early discoveries, much has been done to define the structure and gene organization of mtDNA. The first complete mtDNA sequence was obtained from humans and sequences of mitochondrial genomes from many organisms have now been catalogued (227). A striking result from this work is that a similar complement of genes is conserved in mtDNAs from all multicellular organisms.

In contrast to the nuclear genome, where repetitive sequence families, introns, and vast intergenic regions account for all but a few percent of the total DNA, the mtDNA of mammals and other vertebrates exhibits striking economy of sequence organization. The vertebrate mitochondrial genome exists as a closed circular molecule of ~16.5 kb whose entire protein coding capacity is devoted to the synthesis of 13 proteins that function as essential subunits for respiratory complexes I, III, IV, and V (Figs. 1 and 2). The genes specifying complex II are entirely nuclear. The mtDNA also encodes the 2 tRNAs and 2 ribosomal RNAs necessary for the translation of these respiratory subunits within the mitochondrial matrix. Mitochondrial genes lack introns and are arranged end on end with little or no intergenic regions. Some respiratory protein genes overlap, and the adenine nucleotides of UAA termination codons are not encoded in the mtDNA but rather are supplied by polyadenylation following RNA processing (152). Protein coding and rRNA genes are interspersed with tRNA genes that are thought to demarcate the cleavage sites of RNA processing. The only substantial noncoding region is the D-loop, named after the triple-stranded structure or displacement loop that is formed by association of the nascent heavy (H)-strand in this region (Fig. 2). The D-loop contains the origin of heavy (H)-strand DNA replication and is also the site of bidirectional transcription from opposing heavy (H) and light (L) strand promoters (45). The designation of these strands is assigned based on their relative migration upon gradient centrifugation. Interestingly, the structural economy found in vertebrate mtDNA is not found in plants and fungi where the mitochondrial genomes are much larger and contain intergenic regions, introns, and multiple promoters and transcriptional units (48, 169).

The fact that mtDNA is a compartmentalized extrachromosomal element contributes to a mode of inheritance that differs from that of nuclear genes. Somatic mammalian cells generally have $10^3-10^4$ copies of mtDNA with ~2–10 genomes per organelle (179). These genomes replicate in a relaxed fashion that is independent of the cell cycle that is defined by nuclear DNA replication (46, 25). Some mtDNA molecules undergo multiple rounds of replication while others do not replicate. This, along with random sampling during cell division, allows the segregation of sequence variants during mitosis (198).

In mammals, mtDNA is maternally inherited (for references, see Refs. 101, 198). In general, paternal mtDNA is lost during the first few embryonic cell divisions and does not contribute mtDNA to the offspring, although there are reports of the presence of the paternal lineage in somatic tissues (192). In one case, recombination between maternal and paternal genomes has been documented (109). In addition, because mtDNA is a multicopy genome, an individual may harbor more than a single sequence, a condition referred to as heteroplasmy. A detrimental sequence variant may be tolerated in low copy because the defective gene product(s) it encodes do not reach the threshold for disrupting cellular function. However, sequence variants are known to segregate rapidly from heteroplasmy to homoplasmy in passing from one generation to the next (12). This can result in offspring in which the detrimental variant predominates, leading to a defective mitochondrial phenotype. The molecular basis for this rapid meiotic segregation has been ascribed to a bottleneck or sampling error in the female germ line. A massive amplification of mtDNA occurs during oogenesis from
~10^3 copies in the primary oocyte to ~10^5 copies in the mature oocyte (198). Replication of mtDNA is halted in the mature oocyte, and the existing population of mtDNA molecules is partitioned to the daughter cells during early cell divisions until the copy number is diluted to approximately that found in somatic cells. Embryonic replication does not resume until the blastocyst stage of development (167).

III. TRANSCRIPTION OF mtDNA

A. Mitochondrial Transcriptional Units

In vertebrates, the D-loop regulatory region contains bidirectional promoters for transcribing H- and L-strands as well as the H-strand replication origin (O_H) (Fig. 2). The H- and L-strand transcriptional units differ from most nuclear genes in that they are polygenic, specifying multiple RNAs (rRNA, tRNA, or mRNA). L-strand transcription is initiated at a single promoter (LSP), and RNA synthesis can traverse the entire mtDNA template producing a transcript that is processed to 1 mRNA and 8 of the 22 tRNAs (Fig. 3A). In addition, the preponderance of evidence indicates that transcription from LSP is coupled to H-strand replication (25). Mapping studies support the conclusion that transcripts truncated in the vicinity of CSB II, one of three evolutionarily conserved sequence blocks (CSB I, II, and III), serve as primers for the initiation of H-strand replication (Fig. 3A). Notably, RNA with its 5'-end mapped precisely to the LSP initiation site is covalently linked to newly synthesized H-strand DNA (38). In addition, the nascent H-strand DNA 5'-ends closely match the 3'-ends of truncated transcripts initiated at P_h (37). It is widely accepted that primer RNAs are generated by cleavage of L-strand transcripts by mitochondrial RNA processing (MRP) endonuclease at specific sites that match the in vivo priming sites (Fig. 3A). This ribonucleoprotein endonuclease also participates in the processing of 5.8S rRNA precursors and contains a nucleus-encoded RNA that is essential for catalysis (MRP RNA) (158, 197). A recent alternative model, derived from in vitro experiments using purified components, suggests that termination events associated with CSB II can also

**FIG. 3.** Schematic representation of mtDNA transcription within the D-loop regulatory region. A: transcription initiation complexes are assembled at bidirectional promoters within the D-loop. They are comprised of mitochondrial RNA polymerase (POLRMT), Tфam, a stimulatory factor that unwinds DNA, and one of the two TFB isoforms (TFB1M or TFB2M) that function as dissociable specificity factors that contact both the polymerase and Tфam. The TFBs are related to rRNA dimethyltransferases and thus may also function in RNA modification or processing. Initiation occurring at LSP can traverse the entire template or be cleaved by MRP endonuclease (RNAse MRP) at discrete RNA → DNA transition sites in the vicinity of the conserved sequence blocks (CSB I, II, III; gray shaded bars) demarcating the origin of heavy strand replication (O_H). The RNAse MRP cleavage sites correspond to the heterogeneous 5'-ends of the newly synthesized H-strand during mtDNA replication. Transcripts initiating at HSP2 can also traverse the entire template, whereas those initiating at HSP1 terminate at a transcription terminator (TERM, yellow shaded bar) localized to the tRNAL(UUR) gene to produce the 12S and 16S rRNAs. B: the transcription termination factor mTERF binds simultaneously both HSP1 and TERM resulting in the looping out of the intervening 12S and 16S rDNA. This is thought to promote efficient recycling of the transcription complexes resulting in a high rate of rDNA transcription. The mechanism likely contributes to maintaining a high ratio of rRNA to mRNA required for operation of the mitochondrial translation machinery.
account for the primer RNA 3’-ends that mark the major transition sites between RNA and DNA synthesis (165). In contrast to the LSP, H-strand transcription is initiated from two closely spaced promoters, HSP1 and HSP2 (Figs. 2 and 3A). Transcription initiating at HSP2 results in long polygenic transcripts that are processed to give 14 tRNAs, 12 mRNAs, and the 2 rRNAs. HSP1 on the other hand appears specialized for the production of ribosomal RNAs (Fig. 3A). As discussed below, termination events coupled to initiation at HSP1 provide a mechanism for controlling the relative abundance of rRNA to mRNA.

Transcription of mtDNA is completely dependent on nucleus-encoded gene products. In yeast, transcription is directed by a single subunit RNA polymerase (RPO41p), which shares sequence similarities with the T7 and T3 bacteriophage polymerases (104, 134). Although RPO41p can initiate transcription nonselectively from synthetic DNA templates, the specificity factor Rtf1p (discussed below) is required for promoter recognition and selective initiation (104). Interestingly, RPO41p, through its interactions with mitochondrial promoters and nucleoside triphosphates, may act as a sensor of ATP availability. Mitochondrial transcription abundance is correlated with respiration-coupled ATP synthesis through a requirement for a high ATP concentration for transcription initiation. This represents a potentially novel and elegant mechanism for linking energy production to the transcription of respiratory subunits (4).

Although purification of the human polymerase to homogeneity has been elusive, a human cDNA that encodes a protein with sequence similarity to yeast mitochondrial and T3/T7 bacteriophage polymerases was identified in database screenings (211). As expected for a bona fide mitochondrial polymerase, the 140-kDa protein product, designated POLRMT, is localized to mitochondria via an NH2-terminal targeting presequence. The utilization of single subunit bacteriophage-related polymerases seems nearly universal for mitochondrial transcription. A potential link between mitochondrial transcription and translation in mammalian cells is implicated by the finding of a direct interaction between POLRMT and mitochondrial ribosomal protein L12 (MRPL12) (231). MRPL12 stimulates transcription from both LSP and HSP promoters in an in vitro transcription system. Moreover, it exists in a complex with POLRMT in HeLa cell extracts, and its in vivo overexpression can enhance the steady-state levels of mtDNA-encoded transcripts. It is not yet clear whether this occurs through effects on transcription or RNA stability. Finally, an alternative splice variant of POLRMT that is missing the NH2-terminal 262 amino acids including the mitochondrial targeting presequence has been identified (108). Although it is currently unclear whether this protein functions as an independent nuclear polymerase, it has been localized to the nucleus and linked to the expression of a number of nuclear genes. Its precise role in nuclear transcription and its potential functional implications for mitochondrial biogenesis await further characterization.

B. Tfam

The region of the D-loop that separates the LSP from the two HSPs contains conserved nucleotide sequence motifs that define the core promoter and mediate bidirectional transcription (212). In addition, the promoters share an upstream enhancer that binds Tfam (previously mtTF-1 and mtTFA), the first well-characterized transcription factor from vertebrate mitochondria (Fig. 3A). Tfam was first identified as a high mobility group (HMG)-box protein that stimulates transcription through specific binding to recognition sites upstream from both LSP and HSP (69). Structurally, it consists of two tandemly arranged HMG motifs and a COOH-terminal tail. Tfam resembles other HMG proteins, in that it can bend and unwind DNA, properties potentially linked to its ability to stimulate transcription upon binding DNA (159, 70). Tetrameric binding of Tfam to its recognition site is thought to promote bidirectional transcription by facilitating symmetrical interactions with other transcriptional components (7). In addition to specific promoter recognition, Tfam binds nonspecifically to apparently random sites on mtDNA (70, 71). This property, along with its abundance in mitochondria, suggests that it plays a role in the stabilization and maintenance of the mitochondrial chromosome. ABF2p, a related HMG-box factor from yeast, resembles Tfam and is required for both mtDNA maintenance and respiratory competence (54). Expression of human Tfam in ABF2p-deficient yeast cells can rescue both phenotypes (160). Thus, although highly divergent in primary structure, the human and yeast proteins are functionally interchangeable in supporting mitochondrial respiratory function in yeast. However, despite this functional complementation, ABF2p lacks an activation domain and does not stimulate transcription from yeast mitochondrial promoters in vitro (237, 50). The transcriptional activation function of Tfam resides in a COOH-terminal activation domain that is required both for transcriptional competence and for specific binding to promoter recognition sites (50).

As discussed below, a Tfam knockout mouse displays embryonic lethality and a depletion of mtDNA confirming an essential role for the protein in mtDNA maintenance in mammals (113). Interestingly, Tfam levels correlate well with increased mtDNA in ragged-red muscle fibers and decreased mtDNA levels in mtDNA-depleted cells (168). This is consistent with the observation that transgenic mice overexpressing human Tfam display increased mtDNA copy number (59). The mtDNA abundance measured in somatic tissues and in embryos is proportional to the amount of Tfam expressed in each.
suggesting that Tfam can function as a limiting determinant of mtDNA copy number. Because human Tfam is a poor activator of mouse LSP and HSP, overexpression of the human protein results in an increase in mtDNA without affecting respiratory capacity, suggesting that copy number control by Tfam is independent of its transcriptional function. In fact, both endogenous Tfam and a mutated derivative that lacks the COOH-terminal activation domain are equally competent in maintaining mtDNA copy number in cultured cells (103). These observations are reminiscent of Abf2p, which does not direct transcription but is required for mtDNA maintenance (54). Abf2p is an abundantly expressed component of mitochondrial nucleoids, protein-DNA complexes thought to be a basic segregating unit of mtDNA (105). Tfam is also expressed at levels high enough to coat mtDNA and has been recovered in vertebrate nucleoids in association with other proteins required for mtDNA genomic integrity and expression (26, 102, 230). Thus both its in vivo and in vitro properties implicate Tfam as an ideal target for regulatory pathways that control both mtDNA maintenance and transcriptional expression.

C. mtTFB

In yeast, RPO41p associates with a 43-kDa specificity factor MTF1p also known as sc-mtTFB (196). The primary structure of sc-mtTFB bears some resemblance to prokaryotic sigma factors (99), but a crystal structure reveals significant homology to rRNA methyltransferase (191). Although individually neither RPO41p nor MTF1p can interact with yeast mitochondrial promoters, together they engage in specific promoter recognition (104). Evidence for a vertebrate factor that functions in analogous fashion to the yeast sc-mtTFB came from biochemical studies in Xenopus laevis (24, 24). The partially purified factor bound DNA nonspecifically and stimulated transcription in vitro by purified mitochondrial RNA polymerase. Although purification and molecular cloning of the vertebrate factor has been elusive, this impasse has been resolved by the identification of a human mtTFB cDNA (138). The encoded protein is localized to mitochondria and stimulates transcription from an L-strand promoter in vitro, properties consistent with it being a functional homolog of sc-mtTFB. The human protein exhibits non-sequence-specific DNA binding and requires Tfam to stimulate transcription from mitochondrial templates in vitro. Two isoforms of h-mtTFB, termed TFB1M and TFB2M, have been identified independently (65). TFB1M is identical to the original mtTFB and has about 1/10 the transcriptional activity of TFB2M. As depicted in Figure 3A, both proteins work together with Tfam and mitochondrial RNA polymerase to direct proper initiation from H- and L-strand promoters in an in vitro system comprised of purified recombinant proteins (65). TFB1M has been detected in a complex with Tfam and POLRMT in transcriptionally competent mitochondrial extracts. Moreover, both TFB isoforms make direct contact with the COOH-terminal activation domain of Tfam (139). These observations support the notion that mtDNA transcription in vertebrate systems is directed by three essential components (Fig. 3A). Interestingly, like the yeast factor, both TFBs are related to rRNA methyltransferases. TFB1M can bind S-adenosylmethionine and methylate rRNA at a stem-loop structure that is conserved between bacterial and mitochondrial rRNAs (195). However, this activity is not required for transcriptional activation by the factor (139). It has yet to be determined whether the proteins are bifunctional or whether they evolved a single function from an ancestral methyltransferase.

Recent experiments suggest that the two TFB isoforms are not functionally identical. 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 (73). In Drosophila cultured cells, RNAi knockdown of the Drosophila B2 isoform results in reduced mtDNA transcription and copy number (136). 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 (135). 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 (59). Thus both gain- 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.

D. Transcriptional Termination and Mitochondrial Gene Expression

Specific termination events play an important role in governing the steady-state levels of mitochondrial transcripts. The high rate of rRNA synthesis relative to mRNA has been ascribed to more frequent initiation of H-strand transcription at HSP1. As shown in Figure 3A, HSP1 directs transcription from a site within the tRNA\textsuperscript{the} gene. Most transcripts initiated at HSP1 terminate downstream from the 16S rRNA gene at a strong bidirectional terminator localized within the adjacent tRNA\textsuperscript{len} gene (44). The 28-nucleotide terminator binds mTERF, a 34-kDa pro-
tein that specifies site-specific transcription termination in fractionated mitochondrial lysates (49). Although the purified recombinant protein displays the expected binding specificity, it is not sufficient for termination, suggesting that an additional component(s) may be required (66). Interestingly, in addition to binding the termination site, mTERF stimulates transcription from HSP1, suggesting that transcription initiation and termination are coupled (13, 111). Recent experiments demonstrate that this is indeed the case. Multiple lines of in vitro and in vivo evidence establish that mTERF binds simultaneously both the HSP1 initiation site and the terminator resulting in the looping out of intervening rDNA (Fig. 3B). These interactions are proposed to enhance the reinitiation rate at HSP1 resulting in a higher rate of rRNA synthesis. This mechanism likely accounts for the stimulatory effects of mTERF on transcription (133). Additional complexity to mTERF function is suggested by the observation of several mTERF-related genes in vertebrates (129). The protein products all have putative mitochondrial targeting signals and in one case mitochondrial localization has been confirmed (40). Interestingly, this isoform, designated as mTERFL, differs from mTERF in that it is down-regulated upon serum induction of serum-starved cells. This is reminiscent of the differential expression of TFB1M and TFB2M in response to serum stimulation (73). It is possible that functionally distinct transcription complexes arise from the differential expression of alternative isoforms of these transcription initiation and termination factors.

IV. NUCLEAR CONTROL OF MITOCHONDRIAL FUNCTION

A. Nuclear Transcription Factors Governing Respiratory Gene Expression

1. Cytochrome c and the identification of NRF-1

Isolation of cytochrome c genes in yeast and subsequently in mammalian cells opened the way to the molecular analysis of the control of nuclear genes governing mitochondrial respiratory function. In yeast, transcriptional regulation of the two cytochrome c isoforms is mediated through upstream enhancer elements within their promoters (77). These enhancers bind specific transcriptional activators and repressors that direct dramatic changes in gene expression in response to oxygen and carbon source availability (169, 242). Although cytochromes c are highly conserved at the functional level between yeast and mammals (186), the mammalian cytochrome c promoter contains recognition sites for transcription factors that bear no obvious relationship to those identified in yeast (62). In particular, systematic analysis of the cytochrome c control region revealed a palindromic recognition site for a transcription factor designated as nuclear respiratory factor 1 (NRF-1) (63) (Fig. 4). Specific NRF-1 binding sites are present in the promoters of several nuclear genes required for mitochondrial respiratory function (39, 64). The protein binds its recognition site as a homodimer through a unique DNA binding domain and functions as a positive regulator of transcription (78, 222). This is consistent with the presence of a COOH-terminal transcriptional activation domain (Fig. 4) comprised of glutamine-containing clusters of hydrophobic amino acid residues (79). NRF-1 exists as a phosphoprotein in proliferating mammalian cells and serine phosphorylation within a concise NH2-terminal domain (Fig. 4) enhances both its DNA binding (78) and trans-activation functions (92). Although other mammalian NRF-1 isoforms have not been found, NRF-1 is regulated, through its DNA binding domain, to developmental regulatory proteins in sea urchins (32) and Drosophila (53). In addition, chicken (74), zebrafish (20), and mouse (187) homologs of NRF-1 have been characterized.

NRF-1 has now been linked to the expression of many genes required for mitochondrial respiratory function including the vast majority of nuclear genes that encode subunits of the five respiratory complexes (for recent compilations, see Refs. 106, 183, 184). In addition, considerable evidence supports a potential integrative function for NRF-1 in coordinating respiratory subunit expression with that of the mitochondrial transcriptional

![Diagram](https://via.placeholder.com/150)

**Fig. 4.** Summary of nuclear respiratory factor (NRF)-1 functional domains and DNA recognition site. NRF-1 has a central DNA binding domain (stippled box) flanked by a nuclear localization signal (horizontally hatched box) and a bipartite transcriptional activation domain (vertically hatched boxes). The protein is phosphorylated in exponentially growing cells at multiple serine residues within a concise NH2-terminal domain (cross-hatched box). NRF-1 binds a GC-rich palindrome (shown below the diagram) as a homodimer and makes guanine nucleotide contacts (filled circles) over a single turn of the DNA helix. Numbers below the NRF-1 consensus binding site represent the percentage of the time the indicated nucleotide is present at that position in 20 functional NRF-1 sites. Y, pyrimidine nucleotide; R, purine nucleotide.
machinery. As illustrated in Figure 5, NRF-1 binds and activates the promoters of Tfam (224) and both TFB isoform genes (73) whose products (as discussed above) are major regulators of mitochondrial transcription. NRF-1 has also been linked to the expression of 5-aminolevulinate synthase (30) and uroporphyrinogen III synthase (2), key enzymes of the heme biosynthetic pathway. The former is the rate-limiting enzyme in the biosynthesis of heme, a cofactor essential to the function of electron carriers encoded by both genomes (Fig. 5). Moreover, NRF-1 acts on genes whose functions are not restricted to the bigenomic expression of the respiratory apparatus (Fig. 5). The outer membrane multisubunit receptor complex designated as TOMM is required for the import of the thousands of proteins that contribute to diverse mitochondrial functions (214). TOMM20 is a key receptor subunit involved in the initial interaction with precursor proteins targeted to mitochondria. NRF-1 activates TOMM20 gene transcription through a specific promoter recognition site and is bound to the TOMM20 promoter region in vivo (23). Similarly, NRF-1 is implicated in the expression of mouse COX17, a putative cytochrome oxidase assembly factor (206). NRF-1 control over key components of the protein import and assembly machinery is suggestive of a broader role for the factor in orchestrating events in mitochondrial biogenesis beyond the transcriptional expression of the respiratory chain.

This hypothesis is reinforced by reports that associate elevations in NRF-1 mRNA or DNA binding activity with generalized effects on mitochondrial biogenesis. Both NRF-1 and its coactivator PGC-1α (see below) are upregulated during the adaptive response of skeletal muscle to exercise training (15, 147). A similar effect which mimics exercise-induced mitochondrial biogenesis occurs in cultured myotubules in response to increased calcium (154). Treatment of rats with a creatine analog, which induces muscle adaptations resembling those observed during exercise, leads to the activation of AMP-activated protein kinase. This coincides with increased NRF-1 DNA binding activity, cytochrome c content, and mitochondrial density (21). Both NRF-1 and Tfam mRNAs increase in cells depleted of mtDNA, presumably as a response to increased oxidative stress (141). NRF-1 and NRF-2 (see below) along with Tfam are also upregulated
in response to lipopolysaccharide-induced oxidative damage to mitochondria, presumably to enhance mtDNA levels and OXPHOS activity (203). Exogenous oxidants restore NRF-1 and normal cell growth to ρ0 hepatoma cells where reduced oxidant levels are associated with loss of NRF-1 and growth delay. Interestingly, NRF-1 occupancy of the Tfam promoter increases under pro-oxidant conditions, and the control of Tfam expression by NRF-1 is blocked by inhibition of NRF-1 phosphorylation by Akt (166). This suggests that redox regulation of NRF-1 target genes such as Tfam is modulated by redox-regulated phosphorylation events. Finally, both NRF-1 (DNA binding activity and mRNA) and Tfam (protein and mRNA) are upregulated in the skeletal muscle of aged human subjects (120). This may be a compensatory response to age-related reductions in energy metabolism. These associations are consistent with a general integrative role for NRF-1 in nucleomitochondrial interactions.

It is important to note that NRF-1 targets are not restricted to genes involved in mitochondrial function. An initial search for NRF-1 binding sites in mammalian promoters revealed a number of primate and rodent genes whose functions are not linked directly to mitochondrial biogenesis (222). Among these are genes encoding metabolic enzymes, components of signaling pathways, and gene products necessary for chromosome maintenance and nucleic acid metabolism among others. Moreover, NRF-1 is among seven identified transcription factors whose recognition sites are most frequently found in the proximal promoters of ubiquitously expressed genes (72). Recently, chromatin immunoprecipitations (ChIP) coupled with microarray assay (ChIP-on-chip) were used to identify human promoters that are bound by NRF-1 in vivo (33). Survey of ~13,000 human promoters by ChIP-on-chip analysis (174) identified 691 genes whose promoters are occupied by NRF-1 in living cells (33). As expected, a majority of these genes are involved in mitochondrial biogenesis and metabolism, including many that had not been previously identified. These include a collection of mitochondrial ribosomal protein and tRNA synthetase genes. Notably, a significant subset of the NRF-1 target genes was 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 was enriched in genes required for DNA replication, mitosis, and cytokinesis. Although NRF-1 was bound to 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 (78). Since phosphorylation enhances NRF-1 transcriptional activity (92), 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. Notably, NRF-1 has also been implicated in the transcriptional repression of E2F1 (58) and the activation of E2F6 (107). These results are consistent with a role for NRF-1 in regulating cell cycle progression and may explain the early embryonic lethality of NRF-1 knockout embryos (96).

2. NRF-2 (GABP) an activator of cytochrome oxidase expression

A second nuclear factor designated as NRF-2 was identified based on its specific binding to essential cis-acting elements in the cytochrome oxidase subunit IV (COXIV) promoter (181, 182). NRF-2 binding sites contained of the GGAA core motif that is characteristic of the ETS-domain family of transcription factors. Direct repeats of this sequence motif are interspersed with multiple transcription initiation sites within the mouse COXIV promoter (223, 36). Human NRF-2 was purified to homogeneity from HeLa cell nuclear extracts and is comprised of a heterotetrameric complex to tandem binding sites (210). In solution, GABP exists as a heterodimer but is induced to form the heterotetramer αβγδ by DNA containing two or more binding sites (41). The crystal structure of the heterotetramer bound to DNA has been determined (19). All of the non-DNA-binding subunits contain a dimerization domain that facilitates cooperative binding of a heterotetrameric complex to tandem binding sites (210). In solution, GABP exists as a heterodimer but is induced to form the heterotetramer αβγδ by DNA containing two or more binding sites (41). The crystal structure of the heterotetramer bound to DNA has been determined (19). All of the non-DNA-binding subunits contain a transcriptional activation domain (Fig. 6). This domain resembles that found in NRF-1 and has been localized to a region upstream from the homodimerization domain (79).

As observed for COXIV, COXIVb transcription is also activated by NRF-2 through directly repeated recognition sites within the proximal promoter, suggesting that NRF-2 is a general activator of cytochrome oxidase subunit gene expression (225). Several lines of evidence point to a direct role for NRF-2 in the expression of all 10 nucleus-encoded cytochrome oxidase subunits. NRF-2 has been associated in vivo with multiple COX subunit promoters by chromatin immunoprecipitation (157). Moreover, expression of a dominant negative NRF-2 allele reduced COX expression, and an siRNA directed against NRF-2α reduced expression of all 10 nucleus-encoded COX subunits (156). These findings are consistent with an important regulatory function for NRF-2 in cytochrome oxidase expression.
A strong correlation has been observed between NRF-2α (GABPα) and cytochrome oxidase expression in the visual cortex (149). NRF-2α and cytochrome oxidase were associated with metabolic state and neuronal activity in mature neurons, suggesting that NRF-2α is important in maintaining neuronal function. Moreover, the correlation was extended to NRF-2α mRNA, indicating that NRF-2α may be regulated at the transcriptional level by neuronal activity (82). Both NRF-2α and β subunits are enriched in the nucleus in response to neuronal stimulation (241), and both subunits are specifically translocated to the nucleus in response to neuronal depolarization (238). In fact, the neuronal factor heregulin directs the threonine phosphorylation of GABPα, stimulating both its transcriptional activity and its mobilization to the nucleus where it activates transcription of acetylcholine receptor promoters, which rely on both NRF-1 and NRF-2 for their activities (Fig. 7). Both NRF-1 and NRF-2α occupy both TFB1M and -2M promoters in vivo as determined by chromatin immunoprecipitation (73). Thus, like NRF-1, NRF-2 participates in coordinating the expression of essential respiratory chain proteins with key components of the mitochondrial transcription machinery. Such a mechanism may serve to ensure the coordinate bigenomic expression of respiratory subunits.

3. 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 (97). The α- and γ-isoforms increase postnatally in the heart along with enzymes promoting mitochondrial fatty acid uptake and oxidation (98). ERRα knockout mice have reduced fat mass and are resistant to diet-induced obesity, suggesting defects in lipid metabolism (132). Recent studies have also implicated ERRα in PGC-1α-induced mitochondrial biogenesis (143, 190). A computational approach coupled with PGC-1α-induced RNA expression profiles identified both ERRα and GABPα in a double positive-feedback loop with PGC-1α in directing expression of a subset of mitochondria-related genes (143). An inhibitor of ERRα attenuated PGC-1α-mediated...
target gene expression and total cellular respiration. In this scheme, NRF-1 was downstream of the ERR/H9251-directed changes in expression. It should be noted that PGC-1/H9251 was abundantly overexpressed from an adenovirus vector in many of the experiments utilized to validate this model. This generally results in massive changes in mitochondrial biogenesis that are not observed in genetic knockouts of PGC-1/H9251 or ERR/H9251 (discussed below). Nevertheless, ERR/H9251 does mediate the effects of virally expressed PGC-1/H9251 on mitochondria-related genes such as Tfam and ATP synthase/subunit (190). It is also of interest to note that PGC-1/H9251 functions through NRF-2 (GABP) in the control of a program of neuromuscular gene expression in skeletal muscle (85). Thus these factors likely coordinate broad adaptive changes beyond their initially observed functions in respiratory chain expression.

4. PPARs and fatty acid oxidation

A number of mitochondrial oxidative pathways feed into the respiratory apparatus. Important among these is the pathway for fatty acid oxidation, which consists of a series of enzymes within the mitochondrial matrix that oxidize fatty acids to acetyl CoA. To date, the transcriptional expression of the genes encoding this pathway has not been linked to the transcription factors that govern the expression of the respiratory chain. Rather, PPARα and -δ, members of the nuclear hormone receptor superfamily, have been implicated as transcriptional regulators of fatty acid oxidation enzymes in tissues with high rates of fat oxidation including heart, kidney, and skeletal muscle (17, 81, 97). Conversely, the PPARs have not been associated with the expression of the respiratory apparatus. This suggests that higher order integration of diverse transcription factors may be necessary to coordinate the large number of genes required for mitochondrial biogenesis and function. It is notable that ERRα regulates the expression, during adipocyte differentiation, of MCAD, a key enzyme in fatty acid oxidation (219). Thus ERRα, through its control of both respiratory and fatty acid oxidation genes, may participate in coordinating the expression of the two pathways in certain physiological contexts.

5. Other nuclear factors

Additional nuclear factors have been implicated in the expression of respiratory genes. The cytochrome c promoter contains cis-elements that recognize transcription factors of the ATF/CREB family (63, 75). These elements bind CREB both in vitro and in vivo, and phosphorylation of CREB and NRF-1 is linked to the serum induction of cytochrome c in quiescent fibroblasts (92, 220). Although CREB sites are common to cAMP- and growth-activated genes (137), these elements are not a general feature of nuclear respiratory promoters. Cytochrome c appears to be limiting for mitochondrial respiration early in the program of serum-induced entry to the cell cycle (92). CREB activation of cytochrome c expression likely contributes to its relatively rapid induction in response to serum-stimulated growth. This may be a mechanism for ramping up cellular respiration in preparation for cell growth and division.

Maximal cytochrome c promoter activity also depends on synergy between functional recognition sites for the general transcription factor Sp1 (63). Sp1 is also involved in the activation and/or repression of cytochrome c1 (125) and adenosine nucleotide translocase 2 genes (124), both of which lack NRF sites (240). Muscle-specific COX subunit genes, in contrast to their ubiquitously expressed isoforms (193), depend on MEF-2 and/or E-box consensus elements for their tissue-specific expression (228). The initiator element transcription factor YY1 has been implicated in both positive and negative control of cytochrome oxidase subunit gene expression (18, 194). Interestingly, YY1 knockout mice resemble the NRF-1 and NRF-2α knockouts in that they exhibit peri-implantation lethality (56).

![Fig. 7. Arrangement of NRF-1 and NRF-2 recognition sites in human promoters specifying key components of the mitochondrial transcription and replication machinery. Shown are linear representations of the 5’-flanking regions proximal to the putative transcription initiation sites (arrows) of human genes encoding Tfam, TFB1M, TFB2M, and POL2. Each promoter region bears a strong resemblance to a number of respiratory subunit promoters in that they contain a single NRF-1 site (blue bars) and two or more tandem NRF-2 sites (red bars). In addition, each promoter has at least one Sp1 recognition site (gray bars).](http://physrev.physiology.org/)
More recently, c-Myc has been linked to respiratory gene expression and mitochondrial biogenesis. C-Myc can induce cytochrome c and other NRF-1 target genes by binding noncanonical Myc/MAX binding sites contained within certain NRF-1 sites and can sensitize cells to apoptosis by dysregulating NRF-1 target genes (146). A dominant negative NRF-1 allele can block this c-Myc-induced apoptosis without affecting c-Myc-induced cell proliferation. In addition, Myc null fibroblasts were found deficient in mitochondrial content, and c-Myc expression in these cells partially restored mitochondrial mass (121). This along with the observation that many mitochondria-related genes including Tfam are c-Myc targets led to the conclusion that c-Myc can regulate mitochondrial biogenesis. One caveat to this conclusion is that c-Myc is estimated to have an enormous number of genome binding sites that even exceeds the number of Myc molecules in proliferating cells. Thus it is thought that only a minority of genes bound by Myc/MAX are actually regulated by c-Myc (1). In addition, because of the extremely large number of Myc target genes, it is possible that the observed effects on mitochondrial biogenesis result from indirect effects of Myc on cell growth and metabolism.

6. Dual function factors

There are several reports suggesting that certain nuclear transcription factors or their derivatives have a dual function in directing mitochondrial gene expression. Binding sites for ligand-dependent trans-activators have been observed in mtDNA (51, 234). In the best-studied example, a 43-kDa protein, closely related to the c-Erb Aα1 thyroid hormone receptor, has been localized to the mitochondrial matrix and observed to bind to the mitochondrial D-loop in vitro (234). This protein appears to be an NH₂-terminally truncated variant of the nuclear α1 thyroid hormone receptor. The overexpressed variant gives a particular cytosolic immunofluorescence and stimulates mitochondrial respiration as measured by rhodamine staining and cytochrome oxidase activity. The functional significance of this finding is supported by the observation of a direct effect of thyroid hormone on mitochondrial RNA synthesis (61). Similarly, the rat liver glucocorticoid receptor (GR) was found to translocate to the mitochondria upon treatment of animals with dexamethasone (52). Six glucocorticoid response elements (GREs) were found in mtDNA and were shown to bind GR in vitro (51). Two of the six elements are present in the D-loop, but the other four are within the COXI and COXIII genes, far removed from the in vivo sites of mtDNA transcription initiation.

IκB-α and NFκB have also been localized to mitochondria (29, 47). In one case, they were found in the intermembrane space associated with the adenine nucleotide translocator and were released upon induction of apoptosis (29). In the other, they were localized to the matrix where they were thought to act as negative regulators of mitochondrial mRNA expression (47). These seemingly contradictory results illustrate the difficulty in assigning function based on subcellular localization. It is important to note that the transcriptional activity and specificity of these shared nuclear factors using mtRNA polymerase and a defined mtDNA template has not been demonstrated. Thus it remains inconclusive as to whether these nuclear factors are true regulators of mitochondrial transcription.

B. Nuclear Coactivators in Mitochondrial Biogenesis

Most of the evidence supports a model whereby a relatively small number of nuclear transcription factors serve to coordinate the expression of nuclear and mitochondrial respiratory proteins. Of these, the NRFs, Sp1, and ERRs have been consistently associated with the majority of genes required for respiratory chain expression. These factors exert direct control over transcription of the nucleus-encoded respiratory subunits and act indirectly on the mitochondrial subunits via their regulation of mitochondrial transcription factors. In addition, other mitochondrial oxidative pathways are controlled by unrelated factors as exemplified by PPARα and the fatty acid oxidation pathway (81). This raises the question of how diverse transcription factors are integrated into a program of mitochondrial biogenesis. The discovery of the PGC-1α family of transcriptional coactivators has provided a mechanistic framework for understanding how nuclear regulatory pathways are coupled to the biogenesis of mitochondria.

1. PGC-1α

PGC-1α, the founding member of a family of transcriptional coactivators, was identified in a differentiated brown fat cell line on the basis of its interaction with PPARγ, an important regulator of adipocyte differentiation (171). Although it apparently lacks histone-modifying activities itself, it interacts, through a potent NH2-terminal transcriptional activation domain, with cofactors containing intrinsic chromatin remodeling activities (Fig. 8). In addition to PPARγ, PGC-1α binds several nuclear hormone receptors and trans-activates the PPARγ and thyroid receptor β-dependent expression of the UCP-1 promoter. Nuclear hormone receptor coactivator signature motifs (LXXLL) adjacent to the activation domain are essential for coactivation through certain nuclear receptors, including PPARα and thyroid receptor β (Fig. 8). Coupling of transcription and RNA processing by PGC-1α occurs through COOH-terminal arginine/serine rich (R/S) and RNA recognition motifs reminiscent of those found in RNA splicing factors (142). The dramatic induction of
PGC-1α mRNA in brown fat upon cold exposure supports its involvement in thermogenic regulation (126, 170). An important part of the thermogenic program is the induction of mitochondrial biogenesis, and PGC-1α is a potent inducer of this process. Ectopic overexpression of the coactivator in cultured myoblasts and other cells induces respiratory subunit mRNAs and increases COXIV and cytochrome c protein levels as well as the steady-state level of mtDNA (236).

As illustrated in Figure 9, NRF-1 has been identified as an important target for the induction of mitochondrial biogenesis by PGC-1α. The coactivator binds 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 (236). PGC-1α induction of both NRF-1 and NRF-2 transcripts may also contribute to the biogenic program. As depicted in Figure 9, PGC-1α may link neural regulatory events to the mitochondrial transcriptional machinery through its transcriptional activation of TFαm, TFB1M, and TFB2M expression. As with respiratory subunit genes, the coactivator targets the NRF-1 and NRF-2 recognition sites within Tfam and TFB promoters leading to increased mRNA expression (73). ERRα has also been associated with PGC-1α-induced mitochondrial biogenesis (143, 190). ERRα and GABPa (NRF-2α) recognition sites are conserved in the promoters of a number of oxidative phosphorylation genes, including cytochrome c and β-ATP synthase, and PGC-1α can drive expression through these sites (143, 190) (Fig. 9).

Interestingly, computer modeling indicates that PGC-1α induction of ERRα and GABPa (NRF-2α) is upstream from NRF-1 in the program of respiratory gene expression. The PGC-1α induction of mitochondrial biogenesis has been confirmed in transgenic mice. PGC-1α expression is elevated in the postnatal mouse heart, and cardiovascular overexpression of PGC-1α leads to massive increases in mitochondrial content in cardiac myocytes (116). This overexpression is associated with edema and dilated cardiomyopathy and is likely unrelated to normal PGC-1α function in the heart.

In addition to its effects on the respiratory chain and mitochondrial transcription, PGC-1α promotes mitochondrial oxidative functions by inducing the expression of genes of the mitochondrial fatty acid oxidation and heme biosynthetic pathways (Fig. 9). As we have seen, PPARα directs the transcriptional expression of fatty acid oxidation enzymes and is enriched in brown fat and other tissues with high oxidative energy demands. Ligand-dependent binding of PPARα through the LXXLL motif of PGC-1α is associated with the trans-activation of PPARα-dependent promoters (97, 218). In addition, overexpression of PGC-1α induces MCAD gene expression through its direct interaction with ERRα (98). PGC-1α can also 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 (86). Thus, as summarized in Figure 9, PGC-1α has the potential to integrate the activities of a diverse collection of transcription factors that have been implicated in the expression and function of the mitochondrial oxidative machinery.

Physiological expression of PGC-1α at the transcriptional level can be modulated through cAMP-dependent signaling. In brown adipocytes, sympathetic production of norepinephrine acts predominantly through β3-adrenergic receptors (34). These receptors are coupled to adenylyl cyclase via the Gs subtype of G proteins. The thermogenic signaling cascade results in enhanced cyclase activity and the increased production of cAMP. As shown in Figure 9, elevated cAMP levels lead to activation of CREB through its phosphorylation by protein kinase A (PKA). CREB or related CREB family members, such as activating transcription factor 2 (ATF-2), mediate both direct and indirect effects on the thermogenic program including the induction of PGC-1α and UCP1 (34, 35). The PGC-1α promoter has a potent cAMP response element (CRE) that serves as a target for CREB-mediated transcriptional activation (93). In addition to its role in the thermogenic program, the coactivator is also markedly induced in mouse liver in response to fasting where it activates the genes encoding gluconeogenic enzymes (239). The induction of gluconeogenesis by PGC-1α in fasting liver also involves the activation of CREB by serine phosphorylation in response to the catecholamine and glucagon-mediated elevation of cAMP. CREB induces the
The cAMP-dependent pathway is one of several that directly induce the transcriptional expression of PGC-1α, which in turn serves as a coactivator of gluconeogenic gene expression (93).

The cAMP-dependent pathway is one of several that directly induce the transcriptional expression of PGC-1α in various tissues (126). PGC-1α along with Tfam and NRF-1 are induced via cGMP-dependent signaling resulting from elevated levels of nitric oxide (NO) (150) (Fig. 9). The NO induction of PGC-1α is correlated with increased mitochondrial biogenesis in several cell lines. The induced mitochondrial mass is accompanied by increased oxidative phosphorylation-coupled respiration consistent with an increase in functional mitochondria (151). These results were obtained by pharmacological increases in either NO or cGMP, raising the question of whether physiological fluctuations in endogenous NO can regulate mitochondrial content (114). Interestingly, tissue mitochondria in mice with a homozygous disruption of the gene encoding endothelial NO synthase (eNOS<sup>−/−</sup>) were somewhat smaller and less densely packed than in wild-type mice (150, 151). These changes were accompanied by reductions in energy expenditure and mRNA levels for PGC-1α, Tfam, and NRF-1, arguing that basal mitochondrial content is affected by the loss of eNOS-generated NO. Establishment of an NO-dependent pathway of mitochondrial biogenesis awaits confirmation of these findings.

A number of reports link the expression of PGC-1α to exercise-induced mitochondrial biogenesis in skeletal muscle (3, 15, 76, 154, 178, 207–209, 233). In each case, PGC-1α mRNA and/or protein increase as an adaptive response to endurance exercise of varying intensity and duration. These findings are satisfying in the context of the long-standing relationship between endurance exercise and enhanced mitochondrial respiratory chain expression and function (95). Both NRF-1 and NRF-2 DNA binding activities were elevated along with the expression of several NRF target genes in rat skeletal muscle following an exercise regimen (15). Similar changes in the PGC-1α-NRF pathway were mimicked in cultured myotubules in response to increased calcium levels, suggesting that PGC-1α signaling contributes to adaptive changes in mitochondrial biogenesis in skeletal muscle cells (154). Exercise and other neuromuscular activity may also lead to the activation of the p38 mitogen-activated protein kinase (MAPK) pathway resulting in PGC-1α induction through ATF2 and MEF2 (3). There is evidence that depletion of ATP during exercise leads to elevated AMP/ATP ratios.
which enhances AMP-activated protein kinase (AMPK) activity (Fig. 9). In addition, increased calcium activates Ca2+/calmodulin-dependent protein kinase (CaMK), and this along with AMPK results in the induction of PGC-1α and activation of NRFs during energy deprivation (153, 243). In fact, as seen in other instances of PGC-1α induction, CREB phosphorylation, in this case by CaMK, directs CREB-activated PGC-1α transcription (Fig. 9). This pathway is also driven by calcineurin A through myocyte enhancer factor 2 (MEF2), which is a potent trans-activator of PGC-1α transcription in muscle (87). This same factor has been linked to the expression of muscle-specific COX subunits (228). Although it is not clear whether the NRFs are direct targets of these kinases, the associated increase in NRF-1 DNA binding activity coincides with elevated expression of cytochrome c and 5-aminolevulinate synthase and enhanced mitochondrial density (21). It should be noted that PGC-1α mRNA is induced to similar levels in wild-type and in both AMPK α1 and α2 knockout mice, arguing that these isoforms of AMPK are not essential to exercise-induced gene expression by PGC-1α (100). This study was based on patterns of mRNA expression alone, and thus a definitive conclusion awaits a systematic analysis of mitochondrial biogenesis in these mice.

Finally, a recent study shows that NRF-1 and NRF-2 occupancy of cytochrome c and cytochrome oxidase subunit IV promoters, respectively, occurs prior to the exercise-induced increase PGC-1α protein levels (233). Exercise also leads to the activation of ATF-2 through p38 mitogen-activated protein kinase (p38 MAPK). This transcription factor, a member of the CREB/ATF family, binds the PGC-1α promoter and induces its transcription, perhaps as a secondary phase of the adaptive response. It is unclear whether direct phosphorylation of PGC-1α by p38 MAPK is required in this model.

2. PGC-1β

Two mammalian relatives of PGC-1α have been characterized. PGC-1β, although somewhat larger than PGC-1α, shares sequence similarity with PGC-1α along its entire length (110, 127). Like PGC-1α, PGC-1β has an NH2-terminal activation domain, LXXLL coactivator signatures, and a COOH-terminal RNA recognition motif. Although PGC-1β lacks an R/S domain, it is not clear whether it differs from PGC-1α in its ability to couple transcription and RNA slicing. Steady-state tissue expression of PGC-1β mRNA parallels that of PGC-1α with the highest levels in brown fat, heart, and skeletal muscle, tissues high in mitochondrial content and oxidative energy production. 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 (140). 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. PGC-1β also exhibits a marked preference for promoting the ligand-dependent activity of ERα while having a minimal effect on ERβ (110). These examples illustrate that differences in transcription factor interactions mediate differences in functional specificity between members of the PGC-1 family.

Despite their differential utilization of nuclear hormone receptors, PGC-1β binds NRF-1 and is a potent coactivator of NRF-1 target genes leading to increased mitochondrial gene expression (127). Forced expression of PGC-1β in cultured muscle cells results in increased mitochondrial biogenesis and oxygen consumption, although PGC-1α has been associated with higher proton leak rates than PGC-1β (202). Thus, although PGC-1α and -β are functionally distinct, they both utilize NRF-1 and other transcription factors to induce mitochondrial biogenesis. This has been confirmed in transgenic mice in which PGC-1β is abundantly overexpressed in skeletal muscle (10). The coactivator promotes massive increases in mitochondrial content that are accompanied by marked elevations in the expression of respiratory mRNAs and proteins derived from the expression of both nuclear and mitochondrial genes. This mitochondrial biogenesis is associated with the induction of type IIX oxidative muscle fibers and increased capacity for aerobic exercise. These changes are presumed to result from activation of a specific program of gene expression by PGC-1β through transcription factor targets such as MEF2, ERRα, and PPARα among others. Notably, the induction of type IIX fibers was absent in the soleus muscle despite high-level expression of PGC-1β in this tissue, suggesting that abundant expression of the coactivator alone is not sufficient. It will be important to confirm the regulation of muscle fiber type by PGC-1β in loss of function experiments.

3. PRC

A database search for sequence similarities to PGC-1α identified the partial sequence of a large cDNA with a COOH-terminal RS domain and RNA recognition motif (6). Molecular cloning of the full-length cDNA revealed additional sequence similarities with PGC-1α including an acidic NH2-terminal region, an LXXLL signature for nuclear receptor coactivators, and a central proline-rich region (Fig. 10). Although significant sequence similarity with PGC-1α is confined to these distinct regions, their spatial conservation is highly suggestive of related function. Thus the protein encoded by this cDNA was designated as PGC-1α-related coactivator (PRC) (6). PRC resembles both PGC-1α and -β in that it binds NRF-1 both in vitro and in vivo and can utilize NRF-1 for the trans-activation of NRF-1 target genes (6). PRC has a
potent NH2-terminal activation domain that is highly similar to that of PGC-1/H9251 and is required for NRF-1-dependent trans-activation. These properties along with its nuclear localization and in vivo interaction with NRF-1 attest to a transcriptional function for PRC. As with PGC-1/H9251, binding of NRF-1 occurs through the NRF-1 DNA binding domain. In addition to cytochrome c and 5-ALAS promoters, PRC can trans-activate the human TFB1M and TFB2M promoters in a manner indistinguishable from PGC-1/H9251 (73). The NRF binding sites within the proximal promoters of these genes serve as targets for trans-activation by both coactivators.

Despite these similarities, 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 (6). In addition, PRC expression is not particularly enriched in highly oxidative tissues with abundant mitochondria. However, as illustrated in Figure 11, PRC is rapidly induced upon serum treatment of quiescent fibroblasts and is expressed more abundantly in proliferating cells compared with growth-arrested cells. Moreover, serum induction of PRC is accompanied by a pattern of gene expression that is qualitatively similar to that observed in response to elevated PGC-1α (73). This includes increased expression of Tfam, TFB1M and -2M mRNAs, as well as those for both nuclear and mitochondrial respiratory subunits. It is of interest in this context that PRC, NRF-1, and Tfam are markedly upregulated in thyroid oncocytes in conjunction with increases in cytochrome oxidase activity and mtDNA content (180). These thyroid tumors are characterized by dense mitochondrial accumulation and are apparently devoid of PGC-1α. Both PRC and PGC-1α mRNAs are rapidly induced in human skeletal muscle in response to endurance exercise (178). PRC mRNA expression is high initially and persists for ~4 h but then modulates to a basal level at 24 h after exercise. Thus PRC may direct early adaptive changes in gene expression in response to exercise.

Serum induction of PRC has the characteristics of immediate early genes, those genes whose expression is induced by serum growth factors in the absence of de novo protein synthesis (91). Their induction is among the very earliest events in the genetic program of cell growth.

Despite these similarities, 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 (6). In addition, PRC expression is not particularly enriched in highly oxidative tissues with abundant mitochondria. However, as illustrated in Figure 11, PRC is rapidly induced upon serum treatment of quiescent fibroblasts and is expressed more abundantly in proliferating cells compared with growth-arrested cells. Moreover, serum induction of PRC is accompanied by a pattern of gene expression that is qualitatively similar to that observed in response to elevated PGC-1α (73). This includes increased expression of Tfam, TFB1M and -2M mRNAs, as well as those for both nuclear and mitochondrial respiratory subunits. It is of interest in this context that PRC, NRF-1, and Tfam are markedly upregulated in thyroid oncocytes in conjunction with increases in cytochrome oxidase activity and mtDNA content (180). These thyroid tumors are characterized by dense mitochondrial accumulation and are apparently devoid of PGC-1α. Both PRC and PGC-1α mRNAs are rapidly induced in human skeletal muscle in response to endurance exercise (178). PRC mRNA expression is high initially and persists for ~4 h but then modulates to a basal level at 24 h after exercise. Thus PRC may direct early adaptive changes in gene expression in response to exercise.

Serum induction of PRC has the characteristics of immediate early genes, those genes whose expression is induced by serum growth factors in the absence of de novo protein synthesis (91). Their induction is among the very earliest events in the genetic program of cell growth.
Like other genes in this class, PRC mRNA is increased rapidly by serum induction of quiescent fibroblasts in the presence of the protein synthesis inhibitor cycloheximide (220). The mRNA for PRC and other immediate early genes is superinduced and also markedly stabilized by the drug, probably because of a requirement for protein synthesis for mRNA turnover. Cytochrome c is also serum induced in part through the NRF-1 and CREB sites within its promoter (92). NRF-1 and CREB have been implicated as regulators of cell growth. As discussed above, NRF-1 occupies the promoters of many genes required for DNA replication, cytokinesis, and mitosis including a number of those targeted by the E2F family of growth regulators (33). CREB is well known as a target of mitogenic signaling pathways (137). Interestingly, PRC binds CREB through the same binding sites used for NRF-1, and both factors exist in a complex with PRC in cell extracts (220). In addition, NRF-1 and CREB both occupy the cytochrome c promoter in vivo, and PRC occupancy of the cytochrome c promoter increases upon serum induction of quiescent fibroblasts. This is consistent with a model whereby PRC targets these transcription factors in response to mitogenic signals. This is supported by the observation that expression of the PRC NRF-1/CREB binding domain in trans leads to dominant negative inhibition of cell growth on galactose as a carbon source (220). Growth on galactose requires mitochondrial respiration, suggesting that PRC may link the expression of genes required for both cell growth and mitochondrial respiration.

V. LESSONS FROM MOUSE GENE KNOCKOUTS

The complexity of the pathways of mitochondrial biogenesis is illustrated by the phenotypes of knockout mice. As summarized in Table 1, these studies reveal that both essential and nonessential genes define the mitochondrial phenotype. The essential genes are generally single copy and encode both nuclear and mitochondrial transcription factors. These genes are not only required for the maintenance of mitochondrial function but also for proper embryogenesis and organismal viability. They include Tfam, the NRFs, and other factors that are fundamental to the expression of nuclear and mitochondrial genes. The nuclear transcription factors implicated in mitochondrial biogenesis are not exclusive to this process but rather integrate the expression of mitochondria with that of other fundamental cellular functions. The nonessential genes are members of small gene families and function as important transcriptional modulators of mitochondrial expression and function. These include the transcription factors ERRα and PPARα as well as the PGC-1 family of coactivators that target key transcription factors in specifying tissue-specific energetic properties, a subset of which is mitochondrial content. Individually they are not required for viability or for maintaining basal functional levels of mitochondria (Table 1). However, through their regulated expression and transcriptional specificities, these regulatory factors play an important role in relaying extracellular signals to the transcription machinery.

A. Transcription Factor Knockouts

A targeted disruption of Tfam confirmed its in vivo function in mitochondrial transcription and replication (Table 1). A homozygous Tfam knockout results in postimplantation lethality between embryonic days E8.5 and E10.5 (113). The E8.5 embryos are severely depleted of mtDNA and are deficient in both cytochrome oxidase...

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**Table 1. Mouse knockouts of nucleus-encoded transcriptional regulators implicated in mitochondrial biogenesis and function**

<table>
<thead>
<tr>
<th>Gene Knockout</th>
<th>Viability</th>
<th>Mitochondrial Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tfam (germ line)</td>
<td>Embryonic lethal between days E8.5 and E11.5</td>
<td>mtDNA depletion, severe respiratory chain deficiency</td>
</tr>
<tr>
<td>PolgA</td>
<td>Embryonic lethal between days E7.5 and E8.5</td>
<td>mtDNA depletion, cytochrome oxidase deficiency</td>
</tr>
<tr>
<td>NRF-1</td>
<td>Embryonic lethal between days E3.5 and E6.5</td>
<td>mtDNA depletion, loss of mitochondrial membrane potential, blastocyst growth defect</td>
</tr>
<tr>
<td>NRF-2 (GABP)</td>
<td>Preimplantation lethal</td>
<td>Not determined</td>
</tr>
<tr>
<td>YY1</td>
<td>Embryonic lethal between days E3.5 and E8.5</td>
<td>Not determined</td>
</tr>
<tr>
<td>PPARα</td>
<td>Viable and fertile</td>
<td>Reduced constitutive expression of mitochondrial fatty acid oxidation enzymes, defective fatty acid oxidation in response to fasting</td>
</tr>
<tr>
<td>ERRα</td>
<td>Viable and fertile</td>
<td>Normal food consumption and energy expenditure, reduced lipogenesis, cold intolerance</td>
</tr>
<tr>
<td>PGC-1α (Lin et al.)</td>
<td>Viable and fertile, increased postnatal mortality</td>
<td>Normal mitochondrial morphology, reduced O2 consumption in hepatocytes, fasting hypoglycemia, cold intolerance, defective cardiac ATP production</td>
</tr>
<tr>
<td>PGC-1α (Leone et al.)</td>
<td>Viable and fertile, normal mortality</td>
<td>Increased body mass, modest reduction in skeletal muscle mitochondria, normal glucose homeostasis</td>
</tr>
<tr>
<td>PGC-1β</td>
<td>Viable and fertile</td>
<td>Normal energy intake and expenditure, reduction in skeletal muscle and heart mitochondria</td>
</tr>
</tbody>
</table>
and succinate dehydrogenase activities. In addition, tissue-specific Tfam knockout animals show a strong correlation between Tfam and mtDNA abundance (122, 201). These studies establish an essential role for Tfam in the maintenance of mtDNA copy number in vivo. Interestingly, a homozygous knockout of mitochondrial DNA polymerase γ also results in developmental arrest at E7.5–E8.5, severe depletion of mtDNA, and loss of cytochrome oxidase activity (83). These similarities in mitochondrial phenotype between Tfam and DNA polymerase γ knockouts (Table 1) illustrate that intact mtDNA transcription and replication machinery is required for mtDNA maintenance and for development beyond late gastrulation and early organogenesis. It is also notable that overexpressing Twinkle, a mtDNA helicase, in transgenic mice leads to increased mtDNA copy number (215). In humans, dominant mutations in Twinkle result in the accumulation of multiple mtDNA deletions in several somatic tissues. These results reinforce the conclusion that mtDNA transcription and replication factors are essential to the expression of the mitochondrial respiratory chain in mammalian tissues.

Mouse gene knockouts of several of the nuclear transcription factors implicated in mitochondrial biogenesis have also been analyzed (Table 1). A homozygous NRF-1 mouse knockout results in early embryonic lethality between embryonic days E3.5 and E6.5 (96). Although they are morphologically normal, the NRF-1 null blastocysts degenerate rapidly in culture and have severely reduced mtDNA levels accompanied by a deficiency in maintaining a mitochondrial membrane potential. The mtDNA depletion in the blastocyst does not result from a defect in mtDNA amplification during oogenesis, arguing that the mtDNA depletion occurs between fertilization and the blastocyst stage. The phenotype is consistent with the loss of an NRF-1-dependent pathway of mtDNA maintenance. However, depletion of mtDNA alone does not explain the peri-implantation lethality of the homozygous NRF-1 nulls because Tfam knockout embryos are also depleted of mtDNA but survive to between embryonic days E8.5 and E10.5 (113). Thus the earlier lethality of NRF-1 nulls may result from the decreased expression of NRF-1 target genes that are required for other cell growth and developmental functions. Among these may be the subset of genes identified as targets of both NRF-1 and the E2F family by ChIP on chip assay (33). It is of interest in this context that loss-of-function mutations in NRF-1 have a dramatic negative effect on both Drosophila and zebrafish development. Partial loss-of-function mutations in EWG, a NRF-1 relative in Drosophila, result in aberrant intersegmental axonal projection pathways in the embryo (53). Total loss-of-function mutations in EWG are embryonic lethal. The NRF-1 gene in zebrafish is expressed in the eye and central nervous system of developing embryos. Its disruption gives a larval-lethal phenotype accompanied by defects in the development of the central nervous system (20).

As observed for NRF-1, GABPa (NRF-2α) homozygous null mice also exhibit a peri-implantation lethal phenotype (176). Although no determination of the state of mtDNA or mitochondrial function was made in the mutant embryos, the early lethality may result from a combination of mitochondrial and nonmitochondrial defects. Homozygous knockouts of other Ets family transcription factors also exhibit early embryonic lethality, suggesting that members of the family are unable to compensate for one another during embryonic development. It is surprising in light of the NRF knockouts that mice homozygous null for ERα are viable and fertile and display no defects in food intake or energy expenditure (Table 1), although they do have reduced fat mass and are resistant to high-fat diet-induced obesity (132). The ERα knockouts show a modest reduction in cytochrome c mRNA, but no changes in the global expression of other respiratory subunit genes have been noted. The absence of a profound mitochondrial deficiency in these mice may be explained by the fact that ERα is one of a small family of related factors whose members may compensate for the loss of a single isoform. In contrast, Tfam and NRF-1 are the products of unique genes. Recent work demonstrates that the ERα knockout mice are deficient in adaptive thermogenesis because of decreased mitochondrial mass in brown adipose tissue (221). Although PGC-1α and UCP-1 were induced normally by cold exposure in the ERα knockouts, the decrease in mitochondria was accompanied by reductions in respiratory gene expression, mtDNA copy number, and the oxidative capacity of the tissue. The remaining mitochondria were morphologically and functionally normal. The results argue that ERα has a specific function in differentiation-induced mitochondrial biogenesis in brown adipose tissue without affecting basal levels of mitochondrial maintenance. A similar theme is echoed in mice with a targeted disruption of PPARα (Table 1). The PPARα knockouts are also viable and fertile with no obvious phenotypic abnormalities (115). However, they do have a markedly blunted response to peroxisome proliferators, demonstrating a crucial role for PPARα in the pleiotropic response to these agents. In addition, the mice display lower constitutive expression of several mitochondrial fatty acid metabolizing enzymes (8) and, in contrast to wild-type mice, the PPARα knockouts were resistant to the induction of these mitochondrial enzymes in both liver and heart in response to fasting (119). Thus, although not required for normal development, viability, or mitochondrial maintenance, PPARα plays a crucial role in orchestrating regulatory responses necessary for optimal mitochondrial oxidative functions.
B. Coactivator Knockouts

As we have seen, gain-of-function experiments have established that overexpression of either PGC-1α or PGC-1β can orchestrate massive increases in mitochondrial biogenesis both in cultured cells and in mouse tissues (10, 116, 236). These changes have been ascribed to their ability to induce the expression of key transcription factors (NRF-1, NRF-2, ERRα) and to interact specifically with these factors in mediating the activation of genes that promote the biogenesis of mitochondria. Thus it is surprising that mice with a targeted disruption of PGC-1α are viable and show no changes in mitochondrial abundance or morphology in liver or brown fat (128). They do show reduced oxygen consumption in isolated hepatocytes and reduced expression of several mRNAs linked to mitochondrial function. The only tissues to display obvious 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. Subsequent experiments with the PGC-1α null mice demonstrated that PGC-1α is not required for mitochondrial biogenesis (9). However, the mice are deficient in the expression of genes necessary for mitochondrial function and in oxidative enzyme activities in heart and skeletal muscle. These changes coincide with reduced cardiac ATP production and a defect in work output in response to physiological stimuli. Recently, a skeletal muscle-specific PGC-1α knockout mouse was characterized (84). These mice clearly show multiple muscle defects including reduced exercise tolerance and abnormalities in the maintenance of normal muscle fiber composition. The results illustrate that the true tissue-specific functions of PGC family coactivators may be masked by pleiotropic phenotypes in mice harboring a generalized gene disruption in all of their tissues.

The phenotype of an independently constructed PGC-1α null mouse confirmed that the coactivator is not required for normal development or for the global biogenesis or maintenance of mitochondria (118) (Table 1). However, these mice had reduced mitochondrial density in slow-twitch skeletal muscle and modestly reduced respiratory capacity in both liver and skeletal muscle. There are also a number of significant differences with the previously described knockout including the absence of a defect in gluconeogenesis and a disparity in cardiac phenotype. These have been ascribed to differences in genetic background or targeting strategies (68).

Recently, a targeted disruption of the mouse PGC-1β gene was constructed by removal of exons containing the nuclear hormone receptor coactivator signature motifs (LXXLL) and introduction of a premature stop codon (117). Mice with a homozygous disruption of the gene were viable and fertile and exhibited no gross changes in whole body substrate utilization or total energy input or output (Table 1). They did however have a higher overall metabolic rate associated with a lean phenotype compared with wild-type littermates. Closer examination revealed a number of tissue alterations in mitochondrial gene expression and respiratory function. There were clear reductions in some genes associated with mitochondrial electron transport chain function in brown and white fat, skeletal muscle, and heart. These included respiratory subunits encoded by both nuclear and mitochondrial genes. Although diminished mRNA levels were not always accompanied by similar changes in the encoded protein, mitochondrial volume was reduced in both heart and skeletal muscle. In these cases, the accompanying decreases in electron transport and oxidative phosphorylation activities resulted from reduced numbers of functionally normal mitochondria. In brown fat, mitochondrial content was normal, but the knockout mice exhibited a blunted response to norepinephrine-induced thermogenesis. These phenotypes clearly point to an important modulatory role for PGC-1β in maintaining optimal tissue energetics.

The absence of a dramatic mitochondrial biogenesis defect in either the PGC-1α or PGC-1β null mice may result from mechanisms that compensate for the individual loss of these coactivators. Since the PGC-1 family members share several functional motifs and can all trans-activate NRF target genes, the remaining family members may substitute functionally for the ablated member. This is seen in the brown fat of the PGC-1β knockout where PGC-1α levels are markedly upregulated, possibly resulting in the maintenance of normal mitochondrial content in this tissue (117). This issue was addressed by knocking down the expression of PGC-1β by shRNA in a preadipocyte cell line derived from PGC-1α knockout mice (216). Although PGC-1α appears not to be required for adipocyte differentiation, its loss is correlated with an inability to induce cAMP-dependent expression of genes necessary for thermogenesis, including UCP-1 and cytochrome c. UCP-1, however, is induced by insulin and retinoic acid in the absence of PGC-1α. Reduced expression of PGC-1β in the PGC-1α null background led to a failure to establish and maintain differentiated levels of mitochondrial density and function in mature brown adipocytes. Mitochondrial content is reduced to a level present in undifferentiated preadipocytes. Thus, although neither coactivator is required to direct the program of adipocyte differentiation, one or the other is essential to achieve a differentiated mitochondrial phenotype. This is reminiscent of the case of ERRα, suggesting that the PGC-ERRα regulatory pathway directs differentiation-specific changes in mitochondrial content in brown fat and possibly other tissues. It remains an
open question as to whether PRC can support basal levels of mitochondrial biogenesis in the absence of PGC-1α and -β. It is of interest in this context that expression of a dominant negative allele of PRC from a lentivirus vector inhibits respiratory growth in cultured cells (220). Clearly, the PGC-1 coactivators exhibit highly specialized regulatory functions but, individually, do not appear to be the sole determinants of mitochondrial content in the majority of tissues.

VI. RETROGRADE PATHWAYS

A. The RTG Pathway in Yeast

The regulated expression of the PGC-1 family of inducible coactivators provides a mechanism for modulating mitochondrial biogenesis and function in response to a variety of extracellular signals. In addition, pathways exist for the communication of the functional state of mitochondria back to the nuclear transcriptional machinery. This phenomenon of retrograde signaling is well studied at the transcriptional level in the yeast *Saccharomyces cerevisiae* (130). In yeast cells that are depleted of mitochondrial DNA (ρ0 cells), the resulting respiratory deficiency reduces the supply of glutamate, which is derived from the α-ketoglutarate of the citric acid cycle. Cells compensate by increasing production of peroxisomes, the sites of fatty acid oxidation in yeast. This generates acetyl-CoA, which fuels the citric acid cycle. The nuclear CIT2 gene encoding peroxisomal citrate synthase is markedly induced in ρ0 cells. This enzyme is part of the glyoxylate cycle, and its induction in response to a defect in mitochondrial respiration allows cells to generate citrate (200). Positive transcriptional control of peroxisomal citrate synthase and other peroxisomal proteins in ρ0 cells is mediated by basic helix-loop-helix-leucine zipper transcription factors, Rtg1p and Rtg3p (177). A third factor Rtg2p facilitates the translocation of Rtg1p and Rtg3p from the cytoplasm to the nucleus where they bind to their target genes as a heterodimer and drive transcription. Rtg2p has an ATP binding site and is thought to function as a sensor of the functional state of mitochondria in part through its interaction with the phosphoprotein Mks1p. The phosphorylated state of Mks1p dictates its interaction with Rtg2p or negative regulators creating a molecular switch for control of the RTG pathway (67).

B. Mammalian Retrograde Regulation

The Rtg pathway has not been identified in vertebrate cells, but there are a number of examples where the expression of nuclear genes is altered by deficiencies in mitochondrial function (31). Defective mitochondria that result from certain mitochondrial disease mutations proliferate in diseased muscle fibers giving rise to ragged red fibers (145, 226). Specific nuclear genes involved in ATP production also display elevated expression in cells with mtDNA mutations (89). An altered pattern of nuclear gene expression, involving proteins of the mitochondrial inner membrane, intermediate filaments, and ribosomes, occurs in human cells upon depletion of mtDNA (123). Likewise, chicken cells either depleted of mtDNA or treated with the mitochondrial protein synthesis inhibitor chloramphenicol have increased levels of mRNAs for elongation factor 1α, β-actin, v-myc, and GAPDH (229). Although the mechanistic bases for these phenomena have not been elucidated, they are thought to represent nuclear responses to deficiencies in ATP production.

Recent studies suggest that retrograde signaling in animal cells may be mediated by calcium (22). Inhibition of mitochondrial respiratory function either by depletion of mtDNA or by metabolic inhibitors results in a stress response that coincides with elevated cytosolic calcium levels. The response includes increased expression of calcium-responsive transcription factors and cytochrome oxidase subunit Vb. Calcium signaling has also been associated with a PGC-1-dependent pathway of mitochondrial biogenesis in skeletal muscle (155, 235). Constitutive expression of calcium/calmodulin-dependent protein kinase IV (CaMKIV) in the skeletal muscle of transgenic mice leads to increased mtDNA copy number and respiratory enzymes as well as elevated PGC-1α levels. Thus calcium may be an important link between the relay of extracellular signals to the nucleus and the bidirectional communication between nucleus and mitochondria. This notion is reinforced by the observation that CREB is found in its transcriptionally active, phosphorylated state in cells exhibiting mitochondrial insufficiency resulting either from the loss of mtDNA or by pathological mutation in mtDNA (11). This coincides with the activation of CaMKIV, which, as discussed, can lead to CREB phosphorylation in response to calcium. It remains to be determined whether calcium signaling through CREB and the PGC-1 family coactivators represents a physiologically meaningful pathway of retrograde regulation in vertebrate cells.

VII. CONCLUSION

Much progress has been made in uncovering the transcriptional mechanisms that govern the function and biogenesis of mitochondria. The identification and characterization of important regulatory factors has led to a framework for understanding the continuity between signaling events affecting cellular energetics and the expression of both nuclear and mitochondrial genes that dictate the mitochondrial phenotype. The preponderance of evidence suggests that nucleus-encoded transcription fac-
tors acting on both nuclear and mitochondrial genes serve to coordinate the expression of the gene products required for maintenance of the respiratory apparatus as well as other essential mitochondrial functions. A subset of these is also required for proper embryonic development and organellar viability. Because the organelle is intimately associated with a myriad of cellular functions, it is not surprising to find that the factors governing mitochondrial expression are engaged in other cellular activities as well. Thus they likely play a fundamental integrative role in coordinating mitochondrial functions with cellular events necessary for growth and development. This likely accounts for the embryonic lethality observed in gene knockouts for several of these factors. Among the nucleus-encoded factors are those that are imported into mitochondria where they direct the transcription and replication of mtDNA. Recent progress has led to the elucidation of all of the major components of the vertebrate mitochondrial transcriptional machinery including the polymerase and requisite initiation and termination factors. The stage is set for further understanding of the regulatory mechanisms by which this elegant system governs mitochondrial gene expression and mtDNA abundance. An unresolved issue is whether factors can be shared between nuclear and mitochondrial transcription systems and, if so, what roles such factors play in nuclear-mitochondrial interactions.

Superimposed on this core of transcription factors are regulators that modulate mitochondrial biogenesis in response to extra- and possibly intracellular signals. An important breakthrough has been the discovery of the PGC-1 family of regulated coactivators. Although individually these factors are not essential for embryonic development or viability in mice, they are important determinants of cell- and tissue-specific bioenergetic phenotypes. PGC-1 family members are differentially expressed or modified in response to a variety of extracellular signals including temperature, energy deprivation, and availability of nutrients and growth factors. They also exhibit differential specificities for transcription factor utilization, which in turn governs the pattern of gene expression orchestrated by each coactivator. All three family members activate gene expression through the nuclear respiratory factors (NRF-1, NRF-2) consistent with the fact that their biological responses all encompass changes in mitochondrial expression. It is clear that the PGC-1 coactivators play a role in specifying differentiation-induced mitochondrial content in tissues such as brown fat and skeletal muscle. However, individually they are unlikely to be the only determinants of basal mitochondrial content in most tissues. Perhaps there is sufficient functional redundancy among the three family members such that the loss of any one is at least partially compensated for by the others. Additional loss of function experiments combined with a better understanding of the biological functions of PRC and PGC-1β should clarify this issue. It will also be of interest to determine whether retrograde signaling is mediated by any of these coactivators.

ACKNOWLEDGMENTS

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GRANTS

Work in the author’s laboratory was supported by National Institute of General Medical Sciences Grant GM-32525–25.

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Physiol Rev • VOL 88 • APRIL 2008 • www.prv.org
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