The human heart consumes kilogram quantities of ATP daily to support persistent pump function. The vast majority of this ATP (>95%) is produced by mitochondrial oxidative phosphorylation (OXPHOS). Mitochondrial fatty acid oxidation (FAO) accounts for ≈60% to 90% of ATP production, whereas catabolism of carbohydrates contributes the remaining 10% to 40%. To support this high demand for ATP production, the developing cardiac myocyte has developed a tremendous capacity for mitochondrial biogenesis to establish this specialized mitochondrial system. Indeed, ≈40% of the cytoplasmic space within the adult cardiac myocyte is occupied by mitochondria. Importantly, the heart must continually adapt to changes in energy substrate availability, workload, and energy demands. Therefore, a complex regulatory network has evolved to dynamically match mitochondrial functional capacity with the energy demands of the heart during development and in diverse physiological contexts. Moreover, with pathological cardiac growth and remodeling, the heart also undergoes both contractile and energy metabolic reprogramming; fuel substrate preferences shift and the capacity and efficiency of mitochondrial ATP production is diminished. In this review, we describe the regulatory
pathways and machinery involved in the control of mitochondrial biogenesis in the developing and diseased heart with emphasis on the transcriptional circuitry that transduces and integrates physiological cues to the control of mitochondrial function.

Building the Mitochondrion: Genomic Assembly and Maintenance

Mitochondrial Structure and Genomic Composition

The mitochondrion is a double-membrane organelle consisting of a soluble matrix surrounded by an ion-permeable inner membrane and an outer membrane permeable to factors of <5 kDa in size. OXPHOS and ATP production is driven by a proton gradient established across the inner membrane by the electron transport chain (ETC). Electrons are donated from reducing equivalents in the form of nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH2) generated by the oxidation of acetyl-CoA. The ene.

mtDNA Replication and Transcription

Proper and repetitive mtDNA replication is essential to maintain normal mitochondrial function and to allow adaptive mitochondrial biogenic responses. The precise mechanism of mtDNA replication has not been fully delineated, despite simplistic evolutionary origin. There are 2 predominant theories of mtDNA replication. The strand-displacement model (SDM) has prevailed for decades and still persists as a viable model, albeit with modifications to the original model published in 1972. An alternative hypothesis to SDM has been proposed, termed the ribonucleotide incorporation throughout the lagging strand (RITOLS) model.

Both the SDM and the RITOLS models propose mtDNA replication occurs with a molecular apparatus distinct from that of which the nuclear genome is replicated. The core components of the mitochondrial replisome include DNA polymerase γ (POLG), the helicase T7 gp4-like protein with intramitochondrial nucleoid localization (TWINKLE), and mitochondrial single-stranded binding protein (Figure 1).

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These origins are at different loci and, thus, both models support asynchronous replication. Both models concur that mtDNA replication initiates with displacement of DNA at the O1 end of the primer. Two daughter lagging strand DNA at the 3′ end of the primer. Two daughter mtDNA molecules result from mtDNA replication.

The key point of contention between the SDM and RITOLS models of mtDNA replication regards how the single-stranded DNA resultant from the asynchronous replication is protected (Figure 1). SDM proposes mitochondrial single-stranded binding proteins that coat the H-strand and are displaced as lagging strand synthesis duplexes the single-stranded DNA. In contrast, RITOLS suggests complementary RNA produced during mtDNA transcription covers the exposed single-stranded DNA. Despite intense efforts, there is no consensus to date as to the exact mechanism of mtDNA replication.

Genetic mutations have provided key information about the function of specific components of the mtDNA replication machinery and the importance of a high-capacity
mitochondrial system for cardiac function. Mutations in replisome components including TWINKLE and POLG result in several pathologies.\textsuperscript{14,15} For example, POLG mutations can cause a broad clinical spectrum including cardiomyopathy,\textsuperscript{16,17} a phenotype confirmed in mouse models.\textsuperscript{18–20} Notably, the loss of POLG exonuclease activity in mice results in rapid buildup of mutations and deletions in the heart mitochondrion, which occurs concurrently with cardiomyopathy.\textsuperscript{21} There is a 90-fold increase in mtDNA deletions in POLG exonuclease deficient mice.\textsuperscript{22} Interestingly, overexpressed TWINKLE has a protective role in certain instances.\textsuperscript{23}

Mitochondrial DNA Transcription
Transcription of the mitochondrial genome occurs bidirectionally from the L-strand promoter and H-strand promoter located on opposing mtDNA strands at $O_l$ and $O_h$ and produces a polycistronic transcript spanning nearly the entire length of the mitochondrial genome.\textsuperscript{25} A widely accepted model for the assembly of the mitochondrial transcription initiation complex maintains that mitochondrial transcription factor A (TFAM) interacts via its C terminus with mitochondrial transcription factor B2 (TFB2M) and subsequently recruits mitochondrial RNA polymerase (POLMRT) to the promoter region.\textsuperscript{26,27} However, recent findings suggest a preinitiation complex is formed first from POLMRT and TFAM. As shown in Figure 2A, TFAM binds mtDNA conferring promoter selectivity and subsequently recruits POLMRT. TFAM binds the N-terminus of POLMRT and establishes a polymerase interface by bending the upstream promoter DNA around POLMRT.\textsuperscript{28}

Initiation of transcription transpires as mitochondrial TFB2M transiently associates with POLMRT and binds template DNA. TFB2M facilitates promoter melting and allows complementary nucleotide binding (Figure 2B).\textsuperscript{28} Notably, the activity of TFB2M initiation is affected by ATP concentrations.\textsuperscript{29} TFAM and TFB2M have no physical interaction during the preinitiation phase. There is evidence that mtDNA associates with TFAM promiscuously in regions not directly upstream of promoters\textsuperscript{30} and may be histone-like by...
supercoiling mtDNA, which suggests a protective role in addition to its transcriptional function.

MtDNA replication and transcription use the same template DNA and begin at the same locus. Thus, these 2 processes must be coordinately regulated to prevent collisions of the replisome and transcription machinery. Whether mtDNA is replicated or transcribed depends on mitochondrial transcription elongation factor, which likely acts as a molecular switch between the 2 possibilities. In the absence of transcription elongation factor, POLMRT adds complementary nucleotides until reaching ≈120 bp downstream of L-strand promoter in a G-rich region called conserved sequence block II. Here, a hybrid G-quadruplex between nascent RNA and the nontemplate strand of DNA is formed, which disrupts POLMRT activity. The resultant oligonucleotide acts as a primer for DNA replication. Alternatively, when transcription elongation factor is present, it binds to the C terminus of POLMRT and downstream DNA, effectively interfering with the transcriptional repression caused by the G-quadruplex. This allows POLMRT to transcribe through the conserved sequence block II region (Figure 2C). Termination of mtDNA transcription seems to involve mitochondrial transcription termination factor 1. Mitochondrial transcription termination factor 1 partially ceases transcription of the H-strand genes and exhibits nearly complete transcript termination activity with the L-strand transcript. Resultant transcripts undergo extensive processing before polyadenylation and translation.

Coordinate Synthetic Actions of the Nuclear and Mitochondrial Genome During Mitochondrial Biogenesis and Assembly

The importance of coordinate actions of the nuclear and mitochondrial genome is well exemplified by the assembly of ETC subunits. Mitochondria produce ATP by oxidizing NADH and FADH₂ generated by oxidation of the energy substrates (eg, fatty acids and glucose) transferring electrons that travel through the ETC. The electrons ultimately reduce oxygen to water. Protons derived from oxidation of NADH/FADH₂ contribute to generation of an electrochemical gradient across the inner membrane that provides the energy required to phosphorylate ADP and create ATP. The ETC is composed of 5 multisubunit protein complexes commonly referred to as complex I–V. Organization and assembly of the ETC are complex yet critical processes for maintaining maximal respiration capacity. Importantly, ETC proteins are encoded by both the nuclear and the mitochondrial genomes. Each complex has several hydrophobic subunits that span the inner membrane and, thus, on translation are prone to aggregation. Thus, the assembly of each complex is facilitated by many transient assembly factors encoded by 2 separate genomes.

Human CI (NADH dehydrogenase) consists of 45 different subunits, 7 of which are encoded by the mitochondrial genome. These subunits make up the proton pumping module (P module) of complex I. The 38 nuclear encoded subunits are imported into the mitochondria. The oxidation of NADH and subsequent electron transfer are catalyzed by 7 specific subunits: NDUFV1, NDUFV2, NDUF51, NDUF52, NDUF53, NDUF57, and NDUF58. The remaining subunits have largely unknown function but may be involved in complex stability. The assembly of CI requires many assembly factors. Interestingly, a protein previously proposed to be involved in FAO, acyl-CoA dehydrogenase 9, was recently found to be a necessary assembly factor for biogenesis of CI and not essential for FAO. Mutations in acyl-CoA dehydrogenase 9 have also been shown to result in cardiomyopathy.

Complex II (succinate dehydrogenase[SDH]) is a unique enzyme complex in that it plays a critical role in both the ETC and the tricarboxylic acid cycle by oxidizing succinate to form fumarate and simultaneously reducing FAD to form FADH₂. FADH₂ is reoxidized and the ensuing electrons travel through the ETC. In addition, complex II does not contribute protons to the gradient formed across the inner membrane. Its structure is that of a heterotetramer with subunit A (SDHA) being exposed completely to the mitochondrial matrix and subunit B (SDHB) anchoring SDHA to the transmembrane subunits C (SDHC) and D (SDHD).

Recently, several assembly factors have been established as being critical components of the assembly of complex II. All constituents of SDH and the assembly factors are encoded by the nuclear genome.

Complex III (cytochrome bc₁ complex) and complex IV (cytochrome c oxidase) comprise the remaining portion of the ETC before the final OXPHOS step. Each of these complexes consists of mitochondrial and nuclear-encoded subunits. Similar to other complexes, there are multiple assembly factors that contribute to the proper assembly and structure. However, it should be noted that assembly of complex IV seems to differ drastically between upper and lower eukaryotes, and many of these assembly factors have not yet been identified in humans. Finally, mutations in complex III and complex IV have been shown to result in human patients with cardiomyopathy.

The final step of OXPHOS to produce ATP is catalyzed by ATP synthase (complex V). ATP synthase has 2 main components named Fₐ and Fᵢ. The Fₐ module is composed primarily of 9 hydrophobic subunits and is embedded in the inner membrane, whereas the 5-subunit Fᵢ component contains the catalytic domain responsible for phosphorylation of ADP and is located in the matrix of the mitochondrion. Interestingly, the mitochondrial specific phospholipid cardiolipin has been shown to be a critical factor in ATP synthase oligomerization. ATP synthase subunit A has been implicated in human cardiomyopathy. Consistent with its role in ATP production, genetic mutations in ATP synthase subunits result in severe phenotypes, frequently presenting with cardiomyopathy.

Given the complexity of the ETC, including the contribution by 2 distinct genomes, a mechanism to coordinate transcription and production of these complexes and the entire machinery of the mitochondrion has evolved. This regulatory network will be described in the next section.

Regulatory Networks Controlling Mitochondrial Biogenesis

Mitochondrial structure and number is not static, rather changes during development and in response to increased energy demands or physiological stimuli. For instance, a robust increase in the number and size of mitochondria occurs in the heart immediately before and after birth, coinciding with
increased OXPHOS and a switch to fatty acids as the major energy substrate.\textsuperscript{61} In skeletal muscle, exercise triggers a rapid mitochondrial biogenic response.\textsuperscript{62} Finally, changes in mitochondrial number and structure are associated with a variety of chronic diseases, including heart failure, neurodegenerative diseases, and with aging.\textsuperscript{53-56} These developmental, physiological, and pathophysiological changes in mitochondrial number and structure are driven, in part, by a complex network of nuclear transcription factors that coordinate control of expression of nuclear and mitochondrial genes encoding mitochondrial proteins. The components of this transcriptional regulatory network will be described in this section. The function of many of these factors has been defined in loss-of-function or gain-of-function studies in mice (Table). The importance of precision in this coordinated regulation is underscored by the complexity of the key energy transduction pathways in the mitochondria as described above for the ETC. The gene regulatory network involved in mitochondrial biogenesis not only is operative for the normal mitochondrial biogenic response during development but also serves to integrate physiological and pathophysiologic cues with fuel selection and energy production. In addition, several of these factors have been pursued as therapeutic targets for metabolic and cardiovascular diseases.

### Regulation of Mitochondrial Function and Biogenesis by Nuclear Transcription Factors

The discovery of the nuclear respiratory factors, NRF-1 and NRF-2, was the first in a series of discoveries that helped define the transcriptional regulatory network that controls many fundamental aspects of mitochondrial function and the biogenic process.\textsuperscript{24} NRF-1 was originally identified as a key regulator of cytochrome c expression.\textsuperscript{57} Importantly, NRF-1 also regulates expression of several proteins acting directly on the mitochondrial genome, including TFAM and TFB2M.\textsuperscript{68,69} These findings provided the first link between the coordinate transcriptional control of the nuclear and mitochondrial genomes. NRF-2, the human homolog of the murine GABP, is an ETS-domain containing transcription factor. Along with NRF-1, NRF-2 regulates all 10 nuclear-encoded subunits of cytochrome oxidase.\textsuperscript{57,71} Chromatin immunoprecipitation followed by deep sequencing studies have now confirmed that NRF-1 occupies sites on the promoters of genes encoding components present in all complexes of the ETC.\textsuperscript{72} Finally, genetic deletion of NRF-1 or NRF-2 results in embryonic lethality with reduced mtDNA and ETC activity, underscores the essential role of these factors in mitochondrial biogenesis.\textsuperscript{73,74}

Mitochondrial fatty acid \( \beta \)-oxidation accounts for the majority of ATP production in the normal, healthy heart. The peroxisome-proliferator activated receptors (PPARs) are now recognized as important regulators of mitochondrial FAO and many cellular fatty acid metabolic pathways.\textsuperscript{24} The 3 PPARs (\( \alpha \), \( \beta \), and \( \gamma \)) are members of the large nuclear receptor superfamily of transcription factors. PPARs bind to their cognate DNA elements as heterodimers with another nuclear receptor, the retinoid X receptor. In the cardiac myocyte, PPAR\( \alpha \) and PPAR\( \beta \) are the most abundant with PPAR\( \gamma \) expressed at lower levels. Although originally identified as a regulator of peroxisomal \( \beta \)-oxidation,\textsuperscript{75} PPAR\( \alpha \) was subsequently shown to regulate genes involved in mitochondrial fatty acid import and oxidation.\textsuperscript{76-78} Chromatin immunoprecipitation-on-chromatin immunoprecipitation and chromatin immunoprecipitation followed by deep sequencing studies have confirmed PPAR\( \alpha \) occupancy in the promoters of many mitochondrial FAO genes.\textsuperscript{79,80} PPARs are directly activated by the binding of lipid ligands to the ligand-binding domain of the receptor.\textsuperscript{81-83} In this way, PPARs are sensors to connect cellular substrate delivery and availability with mitochondrial FAO and ATP production. Interestingly, recent evidence suggests that intracellular triglyceride stores are a source of PPAR-activating ligands in the cardiac myocyte. In the heart, adipose triglyceride lipase has been shown to be necessary for the generation of endogenous PPAR\( \alpha \) ligands and normal expression of PPAR\( \alpha \) target genes.\textsuperscript{84} Lipolysis of triglyceride stores also provides most of the substrate for mitochondrial FAO, and this is augmented with increased activity of PPAR\( \alpha \).\textsuperscript{85} These results provide a mechanism, whereby PPAR\( \alpha \) senses substrate availability to regulate mitochondrial FAO in the heart.

The importance of PPARs for mitochondrial fuel metabolism and cardiac function has been defined by gain- and loss-of-function studies in mice. Mice lacking PPAR\( \alpha \) have decreased mitochondrial FAO enzyme expression and rates and develop cardiomyopathy in the context of metabolic stress.\textsuperscript{86-89} Conversely, overexpression of PPAR\( \alpha \) in the heart recapitulates aspects of the insulin-resistant, diabetic heart with increased fatty acid uptake, storage and oxidation, a phenotype worsened by high-fat feeding.\textsuperscript{90,91} The closely related PPAR\( \delta \) (also known as PPAR\( \beta \)) also regulates mitochondrial FAO in the heart. Loss of PPAR\( \delta \) results in lower cardiac FAO rates and cardiac hypertrophy.\textsuperscript{92} Interestingly, mice with overexpression of PPAR\( \delta \) do not accumulate triglyceride and exhibit higher glucose oxidation rates.\textsuperscript{93} Activation of the angiopoietin-like 4 protein (Angptl4), an endogenous inhibitor of lipoprotein lipase, may contribute to the protection of lipotoxicity by PPAR\( \delta \).\textsuperscript{94} PPAR\( \delta \) also provides protection against ischemia/reperfusion injury and pressure overload.\textsuperscript{93,95} The role of PPAR\( \gamma \), the least abundant PPAR, is not well understood in the heart. There is interest in cardiac PPAR\( \gamma \) signaling caused by the adverse cardiovascular risks associated with PPAR\( \gamma \) agonists (thiazolidinediones).\textsuperscript{96} Interestingly, both overexpression and loss of PPAR\( \gamma \) in the mouse heart result in deleterious consequences.\textsuperscript{97-99} However, PPAR\( \gamma \) may also function to compensate in heart during acute periods of energy deprivation, such as occurs during sepsis.\textsuperscript{100} These data suggest that a delicate balance of all PPAR members is needed for proper regulation of cardiac mitochondrial fuel metabolism.

The estrogen-related receptor (ERR) is another critical nuclear regulator of genes involved in mitochondrial function and biogenesis. There are 3 members of this nuclear receptor subfamily; ERR\( \alpha \), ERR\( \beta \), and ERR\( \gamma \), so named because of structural similarity to estrogen receptors (although they are not activated by estrogens). The first link to the control of mitochondrial biogenesis was made when it was shown by 2 groups that ERR\( \alpha \) activated expression of the mitochondrial FAO enzyme, medium-chain acyl-CoA dehydrogenase.\textsuperscript{101,102} In addition to the regulation of FAO, ERRs have now been shown to regulate the expression of genes involved in virtually all known mitochondrial pathways and functions.\textsuperscript{103,104} Specifically, genome-wide occupation studies have shown
that ERRα and ERRγ regulate an overlapping set of genes involved in mitochondrial FAO, tricarboxylic acid cycle, ETC, and OXPHOS. ERRα also directly regulate PPARα expression in the heart, forming a feed-forward mechanism to regulate mitochondrial FAO.

Generalized loss of ERRα does not result in overt deficiencies in mitochondrial biogenesis but display exaggerated cardiac dysfunction after pressure overload. ERRα knockout mice also display decreased exercise capacity with higher blood lactate levels after exercise consistent with a decreased oxidative capacity. ERRγ plays an indispensible role in the postnatal transition to oxidative metabolism in the heart, a period of intense mitochondrial biogenesis, as ERRγ null mice die shortly after birth exhibiting cardiomyopathy. Consistent with these findings, ERRα and ERRγ are expressed in tissues with high mitochondrial density and oxidative capacity including brown adipose and type I and IIa skeletal muscle fibers.

Furthermore, overexpression of ERRγ in skeletal muscle promotes increased oxidative metabolism gene expression and an increase in the proportion of slow, type I fibers. ERRγ directly increases type I fiber number through activation of a myosin heavy chain/miRNA circuit critical for slow muscle development. These results underscore the importance of ERR signaling in the coordinate control of genes involved in mitochondrial function, biogenesis, and maintenance.

Significant evidence indicates that under certain circumstances, the proto-oncogene c-Myc regulates mitochondrial biogenesis. This is particularly true in rapidly dividing cells requiring high levels of ATP and metabolic intermediates to support anabolic processes. c-Myc directly activates many genes with a direct role in mitochondrial biogenesis including POLG, NRF-1, and TFAM. In further support of a direct role, loss of c-Myc in fibroblasts results in decreased mitochondrial mass and respiration capacity. In the heart,
c-Myc expression is increased after pressure overload and stimulates a mitochondrial biogenic response. Activation of c-Myc also increases glucose use and decreases FAO. In this way, c-Myc may trigger an adaptive response to increase reliance on glucose oxidation during periods of growth or ischemia. Indeed, overexpression of c-Myc in the heart results in improved recovery after an ischemic insult. These results also suggest that c-Myc may play a unique role in controlling mitochondrial function during pathological hypertrophy when the canonical pathways (ERRs and PPARs) are deactivated (see below), and the heart has increased reliance on glucose as a fuel.

Integration of Mitochondrial Biogenic Regulatory Factors
How is the activity of the various transcription factors involved in the control of nuclear and mitochondrial gene expression orchestrated for the biogenic response? An answer to this question came with the discovery of PPARγ coactivator-1α (PGC-1α). Originally identified as a coregulator of PPARγ in the mitochondrial-rich brown adipocyte, it is now known that PGC-1α interacts with and coactivates many nuclear receptors through a specific LXXLL motif domain. The closely related PGC-1β and more distant PGC-1-related coactivator comprise the PGC-1 family. Interestingly, expression of PGC-1α is highly inducible in mitochondrial-rich tissues such as heart, brown adipose, and muscle by physiological stimuli including cold exposure and exercise. Forced expression of PGC-1α in the heart revealed a robust mitochondrial biogenic response and increased expression of nuclear-encoded mitochondrial genes. The actions of PGC-1α are mediated via its interactions with NRF-1, PPARα, PPARδ, ERRα, and ERRγ (Figure 3). In the heart, PGC-1α and PGC-1β serve overlapping and partially redundant roles as loss of either protein does not result in an overt defect in mitochondrial biogenesis or energy production. However, accelerated cardiac dysfunction is observed in PGC-1α and PGC-1β knockout mice after pressure-overload hypertrophy.

In addition to the critical role of PGC-1α, other transcriptional coregulators also contribute to the upstream regulation of mitochondrial biogenesis. The recently identified PGC-1 and ERR regulator in muscle 1 (Perm1) is required for PGC-1-induced mitochondrial biogenesis and regulates the expression of certain ERR/PGC-1 target genes. Perm1 itself is activated by ERR/PGC-1 providing a positive, feed-forward mechanism to promote mitochondrial biogenesis. Its expression is highest in skeletal muscle, heart, and brown adipose tissue although its role in the heart has not yet been elucidated. The mediator complex is a large multisubunit complex that interacts directly with DNA-bound transcription factors and RNA polymerase II to facilitate formation of the preinitiation complex. This complex has been shown to interact with nuclear receptors in a ligand-dependent manner. Specifically, the mediator 1 subunit has been shown to interact directly with endoplasmic reticulum α, PPARγ, and PPARα. Somewhat surprisingly, loss of mediator 1 in skeletal muscle results in higher mitochondrial density particularly in white muscle groups with a high proportion of fast fibers. This was also associated with higher expression of slow, type I fiber contractile genes. Similar to the actions of ERRγ, these results provide a connection between the control of mitochondrial biogenesis, oxidative capacity, and the structural/contractile program of muscle.

Integration of Upstream Physiological and Metabolic Signals With Mitochondrial Biogenesis
The heart must continually adapt to changes in workload, substrate availability, oxygen availability, and a myriad of other physiological or pathophysiologic conditions. With respect to mitochondrial energy production, multiple independent signaling pathways serve to link these physiological inputs to the transcriptional control of mitochondrial function and biogenesis. Many of these signaling pathways converge on PGC-1 including AMP-kinase (AMPK), calcium dependent signals, and cAMP. This latter response is primarily mediated by the actions of the cAMP-response element binding protein directly on the PGC-1α promoter to activate its expression. Intracellular calcium signaling pathways, eg, calmodulin-dependent kinase (CaMK) and calcineurin, also work to increase PGC-1 levels.

PGC-1α activity is also responsive to the energy status of the cell. This occurs, at least in part, through activation by AMPK. AMPK is a key cellular energy sensor activated by increasing AMP and reduced ATP levels indicative of energy depletion. Activation of PGC-1α by AMPK provides a link between the energy status of the cell and mitochondrial biogenesis. AMPK also stimulates nicotinamide adenine dinucleotide (NAD+) synthesis and sirtuin 1 activity. Sirtuin 1 is an NAD+-dependent deacetylase linked to the response to caloric restriction and lifespan. Sirtuin 1 directly deacetylates and activates PGC-1α, providing a connection between AMPK, sirtuin 1, and mitochondrial biogenesis. Therefore, this network provides a mechanism to connect the energy status of the cell, sensed by ATP/AMP levels, and the redox state (NAD+/NADH) to mitochondrial biogenesis and function. In support of this, the acetylation status of PGC-1α has been shown to be altered in states of caloric excess and exercise. Although sirtuin 1 deacetylases PGC-1, the acetyltransferase GCN5 carries out the reverse reaction. GCN5 directly acetylates both PGC-1α and PGC-1β. Similar to deacetylation of PGC-1, acetylation is also regulated by metabolic inputs.

Nutrient availability and other cellular growth signals also serve to regulate mitochondrial biogenesis and oxidative respiration under certain circumstances. For instance, in skeletal myocytes, inhibition of the mammalian target of rapamycin (mTOR) leads to decreased levels of PGC-1α and a corresponding decrease in tricarboxylic acid cycle and ETC/OXPHOS gene expression. These effects are mediated, at least in part, through the transcription factor yin yang 1 (YY1). Phosphorylation of YY1 by mTORC1 recruits PGC-1α providing a link between mTOR signaling, YY1 and mitochondrial biogenesis. YY1 binding sites are also found in many nuclear-encoded mitochondrial genes providing further evidence of the importance of this pathway.
loss of YY1 results in mitochondrial defects in skeletal muscle, lower oxidative capacity, and exercise intolerance. However, regulation of PGC-1 by mTOR is complex. For example, an independent and directionally opposite connection with mTOR signaling was observed with the serine/threonine kinases Pim-1, Pim-2, and Pim-3. Inhibition or loss of the Pim kinases results in inactivation of mTORC1 and secondary activation of AMPK through reduced cellular ATP levels concomitant with a significant decrease in PGC-1α levels. Restoration of Pim-3 is sufficient to restore PGC-1α expression and normal cellular growth rates. In the heart, loss of all 3 Pim isoforms results in a remarkably similar phenotype. Triple Pim kinase knockout mice display cardiac myocyte senescence with mitochondrial defects, decreased ATP levels, activation of AMPK and markedly reduced expression of PGC-1α and PGC-1β and their target genes. Interestingly, overexpression of c-Myc, a known Pim kinase target, reversed the mitochondrial derangements observed with loss of Pim kinase. Taken together, these results demonstrate the complexity of PGC-1 signaling and its control of mitochondrial biogenesis and various states of nutrient availability and cellular growth states.

**Mitochondrial Biogenesis and Maturation in the Developing Heart**

**Role of Mitochondrial Biogenesis and Integration With Dynamics During Cardiac Development**

The constant energy demands of the mammalian heart requires the development of a specialized, high-capacity mitochondrial system. The same is true for other highly oxidative tissues, such as brown adipose and slow-twitch muscle. The major mitochondrial biogenic surge during cardiac development occurs immediately after birth and in the early postnatal period. Similar but less robust mitochondrial biogenic responses occur in all tissues during the perinatal period. The perinatal mitochondrial biogenic surge in heart is followed by a period of mitochondrial maturation including fusion, fission, and redistribution among the sarcomeres during the postnatal growth period. Similar but less robust mitochondrial biogenic responses occur in all tissues during the perinatal period. The perinatal mitochondrial biogenic surge in heart is followed by a period of mitochondrial maturation including fusion, fission, and redistribution among the sarcomeres during the postnatal growth period. Ultimately, the fully differentiated adult cardiac myocyte is characterized by a tightly packed mitochondrial network between the sarcomeres. This maturation process involves an intense period of mitochondrial fusion and fission, together with an induction in the expression of nuclear- and mitochondrial-encoded proteins to equip the
organelles with high capacity for energy substrate oxidation (primarily fatty acids and glucose) and OXPHOS.

As described above, the PGC-1 coactivators serve as inducible boosters of the transcriptional regulators involved in mitochondrial biogenesis, including ERRs, PPARs, and NRF-1. Levels of PGC-1α increase just before birth and this increased expression is partially maintained during the postnatal period.\(^1\) On the basis of this perinatal expression pattern, we identified the PGC-1 coactivators as prime candidates for driving the mitochondrial biogenic surge in heart after birth. Gene targeting strategies in mice demonstrated that PGC-1α and PGC-1β are absolutely required for cardiac perinatal mitochondrial biogenesis. Mice with germline deficiency of PGC-1α and PGC-1β die of heart failure shortly after birth because of a complete lack of a mitochondrial biogenic response.\(^1\) The defect in mitochondrial biogenesis in the PGC-1α/β−/− mice is profound. Indeed, cardiac myocyte mitochondrial morphology and density in the postnatal hearts of the mutant mice cannot be distinguished from that of the fetal period, indicating a complete lack of the biogenic response. Interestingly, single gene knockouts for either PGC-1α or PGC-1β do not exhibit this phenotype indicating significant functional redundancy among the coactivators for this mitochondrial biogenic function. Generalized or cardiac-specific ERRγ deficiency largely phenocopies the mitochondrial biogenic defect and heart failure seen with the PGC-1 knockout mice.\(^1,10\) Taken together, these results indicate that induction of the PGC-1 coactivators trigger the mitochondrial biogenic response that prepares the cardiac myocyte for postnatal energy metabolic maturation.

The PGC-1/ERR circuit has also recently been shown to play an important role in the postnatal mitochondrial maturation process in heart. This was accomplished by generating mice with targeted disruption of the Ppargc1b (PGC-1β) gene with a muscle creatine kinase promoter–driven Cre recombinase that has activity after birth during the early postnatal period. Specifically, when the Ppargc1b gene is targeted in heart on a generalized PGC-1α/β−/− background, animals develop a progressive, lethal, and postnatal cardiomyopathy.\(^1\) Interestingly, mitochondrial morphology is severely altered during the postnatal period in these PGC-1α/β−/− mice. The mice exhibit signatures indicative of altered mitochondrial fusion and fission, including small fragmented and elongated mitochondria.\(^1\) In addition, a distinctive mitochondrial donut abnormality was seen in the myocytes.\(^1\) This latter mitochondrial abnormality has been described in Charcot-Marie-Tooth disease because of a genetic defect in mitofusin 2.\(^2\) These observations suggested that the mitochondrial maturation defect was, at least in part, because of altered mitochondrial dynamics. Consistent with this conclusion, levels of many key proteins involved in mitochondrial fusion, including mitofusin 1, mitofusin 2, and OPA1\(^1\) were reduced in the PGC-1α/β−/− hearts during the postnatal period before the development of fulminant heart failure. Mitofusin 1 was dramatically reduced, and its gene was found to harbor an ERR binding site that conferred transcriptional activation by PGC-

Mitochondrial Biogenesis and Maintenance in the Normal and Diseased Adult Heart

Several lines of evidence suggest that the general activity of mitochondrial dynamics and biogenesis of the adult heart is limited in contrast to the perinatal and postnatal periods. For example, rates of mitochondrial fusion and fission have been shown to be low in adult cardiac myocytes.\(^3,10\) Although direct measurements of mitochondrial fusion and fission are not possible in vivo, measurements of mitochondrial size in cardiac-specific, inducible knockouts of mitofusin 1 and 2 estimate the cardiomyocyte fusion/fission cycle to be ≈16 days.\(^10\) Consistent with these observations, conditional targeting of the PGC-1α and β genes in the adult mouse heart does not result in significant alterations in mitochondrial ultrastructure or density, despite reduced levels of mitofusin 1.\(^1,16\) Moreover, adult PGC-1α/β−/− mice do not exhibit an overt abnormality in cardiac function.\(^16\) However, in other studies, combined loss of mitofusin 1 and mitofusin 2 in the adult heart results in a significant mitochondrial ultrastructural and cardiac functional phenotype, suggesting that mitochondrial dynamics is active in the adult heart at some level.\(^10–12\) Despite the lack of an overt cardiac and mitochondrial structural phenotype, gene expression profiling of the adult PGC-1α/β−/− mice revealed significant and widespread downregulation in the expression of genes involved in mitochondrial energy transduction pathways, including FAO, tricarboxylic acid cycle, and ETC/OXPHOS.\(^16\) Consistent with the gene expression results, state 3 respiration rates of mitochondria isolated from the PGC-1α/β−/− hearts are significantly reduced. These results suggest that the general activity of mitochondrial biogenesis and dynamics (and likely mitochondrial turnover) is relatively low in the adult heart.

The collective results of conditional PGC-1 and ERR loss-of-function studies in mice define developmental stage-specific roles for PGC-1 signaling in heart; PGC-1/ERR signaling is required for mitochondrial biogenesis and maturation during postnatal cardiac development (Figure 4). In contrast, PGC-1 coactivators are dispensable for maintenance of mitochondrial density and cardiac function under basal conditions in the adult, but serve to maintain high-capacity mitochondrial respiratory function by driving expression of genes involved in mitochondrial energy transduction and ATP synthetic pathways. Emerging evidence indicates that mitochondrial dysfunction contributes to the genesis of heart failure.\(^17\) Accordingly, what is the contributory role of alterations in the circuitry that regulates cardiac mitochondrial biogenesis and function in common causes of heart failure such as hypertension or ischemic insult? Whereas the adult PGC-1α/β−/− mice do not exhibit a functional cardiac phenotype under basal conditions,\(^16\) PGC-1α null mice develop ventricular dilation and reduced contractile function in response to chronic pressure.
overload.\textsuperscript{133} In addition, ERR\textalpha loss-of-function mice develop severe heart failure in the context of pressure overload and are sensitive to ischemic insult.\textsuperscript{105} These results suggest that reduced activity of the PGC-1 transcriptional regulatory circuit reduces energetic reserves and predisposes to the development of cardiac remodeling and reduced ventricular function in the context of stressors, such as chronic pressure overload or ischemic insult. Consistent with this notion, several studies have now shown reduced expression of PGC-1\textalpha, PPAR, ERR, and many of its downstream targets in the failing rodent and human heart.\textsuperscript{175–182} Changes in fuel substrate selection are one consequence of deactivation of the PGC-1 circuit. For instance, it is now well-established that rates of FAO are decreased in pathological cardiac hypertrophy.\textsuperscript{175,177} However, the contributory role of changes in fuel substrate preference is not well understood. These observations and reports of mitochondrial derangements and myocyte death in the failing heart suggest that a vicious cycle develops in which reduced activity of the PGC-1 cascade causes reduced capacity for mitochondrial fuel oxidation and ATP synthesis resulting in contractile dysfunction, calcium homeostatic abnormalities, and myocyte death as a final pathway (Figure 4). However, recent evidence suggests that alterations in the PGC-1 transcriptional circuit or transcriptional events in general may not serve as the primary event in the energy metabolic derangements of the failing heart. Specifically, combined transcriptional and metabolomic profiling of hearts from well-defined mouse models representing the spectrum from pressure overload–induced compensated cardiac hypertrophy to overt remodeling revealed the surprising finding that relatively few changes in mitochondrial energy metabolic pathways occur at the transcriptional level.\textsuperscript{183} Rather, altered metabolite levels correlated with the onset of heart failure and altered mitochondrial function, suggesting that post-transcriptional alterations in mitochondrial function drive the early metabolic abnormalities \textit{en route} to heart failure. Accordingly, transcriptional events and alterations in PGC-1 signaling likely occur later in the pathogenic process.\textsuperscript{183}

Notably, the role of altered mitochondrial biogenesis and dynamics in this pathological process remains to be fully defined.

**Conclusions**

Significant progress has been made in delineating the mechanisms involved in the control of cardiac biogenesis and maturation in the developing, normal, and diseased heart. A transcriptional regulatory network has evolved to allow the heart to match ATP-producing capacity with energy demands under diverse developmental and physiological circumstances. The transcriptional regulatory circuit described herein finely tuned to activate mitochondrial biogenesis at precise times during the perinatal stages, followed by a maturation process that involves mitochondrial dynamics. This same circuit maintains high-level expression of nuclear and mitochondrial genes involved in the various energy metabolic pathways in the adult heart. Interestingly, the specific targets and actions of this mitochondrial biogenic regulatory network are dictated by developmental stages. The biogenic and mitochondrial maintenance circuitry must be tightly orchestrated with mitochondrial quality control and turnover. An important frontier in this field relates to the delineation of specific alterations in this control network relevant to the development of myocardial diseases, such as heart failure. Delineation of adaptive and maladaptive responses could lead to new therapeutic approaches aimed at the prevention and early stage treatment of heart failure. Mitochondrial-targeted therapies for myocardial disease are likely to be most effective if aimed at the appropriate developmental process and stage of heart failure, possibly necessitating distinct therapies depending on the age of the patient and severity of disease.

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