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 network that builds and maintains the mitochondrial genome and drives the expression of the energy transduction machinery. This finely tuned system is responsive to developmental and physiological cues, as well as changes in fuel substrate availability. Deficiency of components critical for mitochondrial energy production frequently manifests as a cardiomyopathic phenotype, underscoring the requirement to maintain high respiration rates in the heart. Although a precise causative role is not clear, there is increasing evidence that perturbations in this regulatory system occur in the hypertrophied and failing heart. This review summarizes current knowledge and highlights recent advances in our understanding of the transcriptional regulatory factors and signaling networks that serve to regulate mitochondrial biogenesis and function in the mammalian heart.

Abstract: The ultrastructure of the cardiac myocyte is remarkable for the high density of mitochondria tightly packed between sarcomeres. This structural organization is designed to provide energy in the form of ATP to fuel normal pump function of the heart. A complex system comprised of regulatory factors and energy metabolic machinery, encoded by both mitochondrial and nuclear genomes, is required for the coordinate control of cardiac mitochondrial biogenesis, maturation, and high-capacity function. This process involves the action of a transcriptional regulatory network that builds and maintains the mitochondrial genome and drives the expression of the energy transduction machinery. This finely tuned system is responsive to developmental and physiological cues, as well as changes in fuel substrate availability. Deficiency of components critical for mitochondrial energy production frequently manifests as a cardiomyopathic phenotype, underscoring the requirement to maintain high respiration rates in the heart. Although a precise causative role is not clear, there is increasing evidence that perturbations in this regulatory system occur in the hypertrophied and failing heart. This review summarizes current knowledge and highlights recent advances in our understanding of the transcriptional regulatory factors and signaling networks that serve to regulate mitochondrial biogenesis and function in the mammalian heart.

Key Words: mitochondria ■ mitochondrial turnover ■ myocytes, cardiac ■ oxidative phosphorylation ■ transcription factors
Mitochondrial Structure and Genomic Composition

Building the Mitochondrion: Genomic Assembly and Maintenance

Mitochondrial Biogenesis

Nonstandard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>AMPK</td>
<td>AMP-dependant protein kinase</td>
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<td>ERR</td>
<td>estrogen-related receptor</td>
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<td>ETC</td>
<td>electron transport chain</td>
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<td>FADH₂</td>
<td>flavin adenine dinucleotide</td>
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<td>FAO</td>
<td>fatty acid oxidation</td>
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<td>mtDNA</td>
<td>mitochondrial DNA</td>
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<td>mTOR</td>
<td>mammalian target of rapamycin</td>
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<td>NADH</td>
<td>nicotinamide adenine dinucleotide</td>
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<td>NRF</td>
<td>nuclear respiratory factors</td>
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<td>OXPHOS</td>
<td>oxidative phosphorylation</td>
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<td>PGC-1α</td>
<td>PPARγ coactivator 1α</td>
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<td>POLG</td>
<td>polymerase γ</td>
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<tr>
<td>POLMRT</td>
<td>mitochondrial RNA polymerase</td>
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<tr>
<td>PPAR</td>
<td>peroxisome-proliferator activated receptors</td>
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<tr>
<td>RITOLS</td>
<td>ribonucleotide incorporation throughout the lagging strand</td>
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<td>SDH</td>
<td>succinate dehydrogenase</td>
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<tr>
<td>SDM</td>
<td>strand-displacement model</td>
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<tr>
<td>TFAM</td>
<td>mitochondrial transcription factor A</td>
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<tr>
<td>TWINKLE</td>
<td>T7 gp4-like protein with intramitochondrial nucleoid localization</td>
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<td>YY1</td>
<td>ying yang 1</td>
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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 and an outer membrane permeable to factors of nuclear diploid genome. In addition to mtDNA-encoded proteins, there are at least 2 distinct primary origins of replication: an origin of replication (O_H) on the heavy-strand (H-strand) for leading strand synthesis and an origin of replication (O_L) on the light-strand (L-strand) for lagging strand synthesis. 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 O_H. Thereafter, POLG synthesizes the leading strand that is complementary to the L-strand. The lagging strand begins its synthesis two-thirds of the way through the mitochondrion genome at O_L after H-strand displacement. The DNA displaced at O_L folds into a stem-loop structure, which mitochondrial RNA polymerase (POLRMT) recognizes and consequently synthesizes a primer at O_L. POLG then begins synthesizing 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 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 mitochondrial, 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

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\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure1}
\caption{Two predominant models of mtDNA replication are shown here. Both models concur the replisome consists of at least a helicase, T7 gp4-like protein with intramitochondrial nucleoid localization (TWINKLE; orange) and polymerase \( \gamma \) (POLG; yellow). Leading strand synthesis begins at \( O_{L} \) and lagging strand synthesis at \( O_{H} \) (red arrow). \textbf{A,} Strand-displacement model proposes single-stranded binding proteins (green spheres) bind the displaced H-strand during leading strand replication. \textbf{B,} Alternatively, the ribonucleotide incorporation throughout the lagging strand (RITOLS) model proposes portions of transcribed mitochondrial DNA bind the H-strand (green dotted line).}
\end{figure}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure2}
\caption{Mitochondrial RNA polymerase (POLMRT) plays a critical role in mitochondrial transcription and replication. \textbf{A,} The transcription preinitiation begins with mitochondrial transcription factor A (TFAM) binding and recruiting POLMRT. TFAM enables POLMRT interaction with upstream promoter by bending the DNA around POLMRT. \textbf{B,} Transcription initiation occurs when mitochondrial transcription factor B2 (TFB2M) binds POLMRT and facilitates promoter melting forming the characteristic D-loop region. POLMRT synthesizes an RNA primer (green dotted line) until reaching conserved sequence block II (CSBII) where the transcription/replication switch occurs. \textbf{C,} In the presence of TEFM, the G-quadruplex that stalls POLMRT is disrupted allowing POLMRT to continue adding nucleotides and completing transcription (top). In the absence of TEFM, POLMRT dissociates from mtDNA (DITOLS) and transcription is terminated at CSBII and the oligonucleotide strand is used as a primer for DNA replication (bottom). Replication then proceeds after the recruitment of the replisome apparatus.}
\end{figure}
superoiling mtDNA,\textsuperscript{31,32} 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.\textsuperscript{33} In the absence of transcription elongation factor, POLMRT adds complementary nucleotides until reaching \(\approx 120\) 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.\textsuperscript{34}

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).\textsuperscript{33} Termination of mtDNA transcription seems to involve mitochondrial transcription termination factor 1.\textsuperscript{24,28} 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.\textsuperscript{35} Resultant transcripts undergo extensive processing before polyadenylation and translation.\textsuperscript{25}

### 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 FADH\textsubscript{2} generated by oxidation of the energy substrates (e.g., fatty acids and glucose) transferring electrons that travel through the ETC. The electrons ultimately reduce oxygen to produce water. Protons derived from oxidation of NADH/ FADH\textsubscript{2} 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.\textsuperscript{36,37}

Human CI (NADH dehydrogenase) consists of 45 different subunits,\textsuperscript{38} 7 of which are encoded by the mitochondrial genome. These subunits make up the proton pumping module (P module) of complex I.\textsuperscript{39} The 38 nuclear encoded subunits are imported into the mitochondria.\textsuperscript{40} The oxidation of NADH and subsequent electron transfer are catalyzed by 7 specific subunits: NDUFV1, NDUFV2, NDUF51, NDUF52, NDUF53, NDUF57, and NDUF58.\textsuperscript{41} The remaining subunits have largely unknown function but may be involved in complex stability.\textsuperscript{42} 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.\textsuperscript{43} Mutations in acyl-CoA dehydrogenase 9 have also been shown to result in cardiomyopathy.\textsuperscript{44,45}

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 FADH\textsubscript{2}. FADH\textsubscript{2} 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).\textsuperscript{46} Recently, several assembly factors have been established as being critical components of the assembly of complex II.\textsuperscript{47,48} All constituents of SDH and the assembly factors are encoded by the nuclear genome.

Complex III (cytochrome bc\textsubscript{1} 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.\textsuperscript{49,50} 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.\textsuperscript{51} Finally, mutations in complex III and complex IV have been shown to result in human patients with cardiomyopathy.\textsuperscript{52–55}

The final step of OXPHOS to produce ATP is catalyzed by ATP synthase (complex V). ATP synthase has 2 main components named F\textsubscript{0} and F\textsubscript{1}. The F\textsubscript{0} module is composed primarily of 9 hydrophobic subunits and is embedded in the inner membrane, whereas the 5-subunit F\textsubscript{1} component contains the catalytic domain responsible for phosphorylation of ADP and is located in the matrix of the mitochondrion.\textsuperscript{56,57} Interestingly, the mitochondrial specific phospholipid cardiolipin has been shown to be a critical factor in ATP synthase oligomerization.\textsuperscript{58} ATP synthase subunit A has been implicated in human cardiomyopathy.\textsuperscript{59} Consistent with its role in ATP production, genetic mutations in ATP synthase subunits result in severe phenotypes, frequently presenting with cardiomyopathy.\textsuperscript{50}

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
and oxidation. Chromatin immunoprecipitation-on-chromatin immunoprecipitation and chromatin immunoprecipitation followed by deep sequencing studies have confirmed PPARα occupancy in the promoters of many mitochondrial FAO genes. PPARs are directly activated by the binding of lipid ligands to the ligand-binding domain of the receptor. In this way, PPARα 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 ligands and normal expression of PPARα target genes. Lipolysis of triglyceride stores also provides most of the substrate for mitochondrial FAO, and this is augmented with increased activity of PPARα. These results provide a mechanism, whereby PPARα 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 PPARα have decreased mitochondrial FAO enzyme expression and rates and develop cardiomyopathy in the context of metabolic stress. Conversely, overexpression of PPARα 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. The closely related PPARδ (also known as PPARβ) also regulates mitochondrial FAO in the heart. Loss of PPARδ results in lower cardiac FAO rates and cardiac hypertrophy. Interestingly, mice with overexpression of PPARδ do not accumulate triglyceride and exhibit higher glucose oxidation rates. Activation of the angiopoietin-like 4 protein (Angptl4), an endogenous inhibitor of lipoprotein lipase, may contribute to the protection of lipotoxicity by PPARδ. PPARδ also provides protection against ischemia/reperfusion injury and pressure overload. The role of PPARγ, the least abundant PPAR, is not well understood in the heart. There is interest in cardiac PPARγ signaling caused by the adverse cardiovascular risks associated with PPARγ agonists (thiazolidinediones). Interestingly, both overexpression and loss of PPARγ in the mouse heart result in deleterious consequences. However, PPARγ may also function to compensate in heart during acute periods of energy deprivation, such as occurs during sepsis. 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: ERRα, ERRβ, and ERRγ, 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 ERRα activated expression of the mitochondrial FAO enzyme, medium-chain acyl-CoA dehydrogenase. 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. 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.\textsuperscript{106} ERRα can also directly regulate PPARα expression in the heart, forming a feed-forward mechanism to regulate mitochondrial FAO.\textsuperscript{103} Generalized loss of ERRα does not result in overt deficiencies in mitochondrial biogenesis but display exaggerated cardiac dysfunction after pressure overload.\textsuperscript{105} ERRα knockout mice also display decreased exercise capacity with higher blood lactate levels after exercise consistent with a decreased oxidative capacity.\textsuperscript{106} ERRγ plays an indispensable 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.\textsuperscript{107,108} 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.\textsuperscript{109,110} ERRγ directly increases type I fiber number through activation of a myosin heavy chain/miRNA circuit critical for slow muscle development.\textsuperscript{111} 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.\textsuperscript{112,113} In further support of a direct role, loss of c-Myc in fibroblasts results in decreased mitochondrial mass and respiration capacity.\textsuperscript{114} 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 LXLL 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 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 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 pathophysiological 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 (Figure 3). PGC-1α expression is rapidly increased by β-adrenergic stimulation in response to multiple stimuli including cold exposure and exercise. 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 deacetylates 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 ying 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.

Finally,
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. 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. The defect in mitochondrial biogenesis in the PGC-1α/β-deficient hearts 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. Taken together, these results indicate that induction of the PGC-1 coactivators triggers 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 development, a generalized PGC-1α-deficient background, animals develop a progressive, lethal, and postnatal cardiomyopathy. 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. In addition, a distinctive mitochondrial donut abnormality was seen in the myocytes. This latter mitochondrial abnormality has been described in Charcot-Marie-Tooth disease because of a genetic defect in mitofusin 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 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-1α/ERRα. In addition, cardiac-specific ERRα and ERRγ null mice also exhibit a postnatal phenotype similar to that of the PGC-1α/β-/- mouse, providing additional evidence that the PGC-1/ERR transcriptional regulatory circuit is important for the increased mitochondrial fusion and fission rates during postnatal mitochondrial maturation in heart. The reason for increased mitochondrial dynamics during the postnatal period is unknown, but it is possible that it is necessary for distribution and alignment between the sarcocemes or as a quality control mechanism.

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. 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. 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. Moreover, adult PGC-1α/β-deficient mice do not exhibit an overt abnormality in cardiac function. 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. Despite the lack of an overt cardiac and mitochondrial structural phenotype, gene expression profiling of the adult PGC-1α/β-deficient 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. Consistent with the gene expression results, state 3 respiration rates of mitochondria isolated from the PGC-1α/β-deficient 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. 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-deficient mice do not exhibit a functional cardiac phenotype under basal conditions, PGC-1α null mice develop ventricular dilation and reduced contractile function in response to chronic pressure.
overload. In addition, ERRα loss-of-function mice develop severe heart failure in the context of pressure overload and are sensitive to ischemic insult. 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-1α, PPAR, ERR, and many of its downstream targets in the failing rodent and human heart. 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. 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. 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 en route to heart failure. Accordingly, transcriptional events and alterations in PGC-1 signaling likely occur later in the pathogenic process. 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.

Acknowledgments
We thank Ling Lai for assistance with Figure preparation, Teresa Leone for critical reading and Lorenzo Thomas for assistance in the preparation of this article.
Sources of Funding
This work was supported by National Institutes of Health grants R01 DK045416, R01 HL058493, and R01 HL101189 (Dr Kelly).

Disclosures
Dr Kelly serves on a Scientific Advisory Board for Pfizer. The other authors report no conflicts.

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Maintaining Ancient Organelles: Mitochondrial Biogenesis and Maturation
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Circ Res. 2015;116:1820-1834
doi: 10.1161/CIRCRESAHA.116.305420
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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