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Maintaining Ancient Organelles: Mitochondrial Biogenesis and Maturation

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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 to drive 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.

Keywords

mitochondrial biogenesis; cardiac myocyte; oxidative phosphorylation; transcription factors; mitochondria

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 about 60–90% of ATP production while catabolism of carbohydrates contributes the remaining 10–40%. To support this high demand for ATP production, the developing cardiac myocyte has developed a tremendous capacity for mitochondrial biogenesis in order to establish this specialized mitochondrial system. Indeed, approximately 40% of the cytoplasmic space

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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 pathologic 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 less than 5kDa 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 ($FADH_2$) generated by the oxidation of acetyl-CoA. The endosymbiotic hypothesis holds that mitochondria originated from the engulfment of aerobic eubacteria by a primordial anaerobic eukaryote. As such, the mitochondrion contains its own circular genome. This mitochondrial DNA (mtDNA) contains 37 genes encoding 13 essential protein subunits of the ETC as well as rRNAs and tRNAs necessary for the translation of the mitochondrial-encoded transcripts. Consistent with high mitochondrial density, it is estimated that each cardiac myocyte contains approximately 7,000 mtDNA copies per nuclear diploid genome.[1] In addition to mtDNA-encoded genes, a compendium of over 1000 nuclear-encoded mitochondrial proteins across multiple tissues in the mouse has been compiled,[2] and the list is growing. Nuclear genes encode all of the factors needed for transcription and replication of mtDNA. Interestingly, a significant number of the genes identified encode mitochondrial proteins of unknown function.[2] Finally, as discussed in later sections, mitochondria are not static organelles but are mobile and dynamic. Recent findings provide evidence of direct connections and communication between mitochondria as well.[3,4]

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 metazoan mtDNA replication has not been fully delineated despite simplistic evolutionary origin. There are two 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.[5] An alternative hypothesis to SDM has been proposed, termed the ribonucleotide incorporation throughout the lagging strand (RITOLS) model.[6]

Both the SDM and RITOLS models propose mtDNA replication occurs with a molecular apparatus distinct from that of which the nuclear genome is replicated.[7] The core components of the mitochondrial replisome include DNA polymerase γ (POLG),[8] the helicase T7 gp4-like protein with intramitochondrial nucleoid localization (TWINKLE), and mitochondrial single-stranded binding (mtSSB) protein (Figure 1). MtSSB protein facilitates POLG primer recognition and subsequently stimulates the polymerase activity of POLG.[9,10] Furthermore, mtSSB specifically stimulates the helicase activity of TWINKLE.[11] There is also agreement that there are *at least* two 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 2/3 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.[12] 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 mtSSB proteins 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.[13] 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 a number of pathologies.[14,15] For example, POLG mutations can cause a broad clinical spectrum including cardiomyopathy,[16,17] a phenotype confirmed in mouse models.[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.[21] There is a 90-fold increase in mtDNA deletions in POLG exonuclease deficient mice.[22] Interestingly, over-expressed TWINKLE has a protective role in certain instances.[23]

Mitochondrial DNA transcription

Transcription of the mitochondrial genome occurs bidirectionally from the L-strand promoter (LSP) and H-strand promoter (HSP) located on opposing mtDNA strands at O_H[24] and produces a polycistronic transcript spanning nearly the entire length of the mitochondrial genome.[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 POLMRT to the promoter region.[26,27] However, recent findings suggest a pre-initiation 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.[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).[28] Notably, the activity of TFB2M initiation is affected by ATP concentrations.[29] TFAM and TFB2M have no physical interaction during the pre-initiation phase. There is evidence that mtDNA associates with TFAM promiscuously in regions not directly upstream of promoters[30] and may be histone-like by supercoiling mtDNA[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 two 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 (TEFM), which likely acts as a molecular switch between the two possibilities.[33] In the absence of TEFM, POLMRT adds complementary nucleotides until reaching ~120bp downstream of LSP in a G-rich region called conserved sequence block II (CSBII). Here a hybrid G-quadruplex between nascent RNA and the nontemplate strand of DNA is formed that disrupts POLMRT activity.[34] The resultant oligonucleotide acts as a primer for DNA replication. Alternatively, when TEFM 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 CSBII region (Figure 2C).[33] Termination of mtDNA transcription appears to involve mitochondrial transcription termination factor 1 (MTERF1).[24,28] MTERF1 partially ceases transcription of the H-strand genes and exhibits nearly complete transcript termination activity with the L-strand transcript.[35] Resultant transcripts undergo extensive processing before polyadenylation and translation.[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 $FADH_2$ 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/$FADH_2$ contribute to generation of an electrochemical gradient across the inner membrane (IM) that provides the energy required to phosphorylate ADP to create ATP. The ETC is composed of 5 multi-subunit protein complexes commonly referred to as Complex I–V (CI-CV). Organization and assembly of the ETC is a complex yet critical process for maintaining maximal respiration capacity. Importantly, ETC proteins are encoded by both the nuclear and mitochondrial genomes. Each complex has several hydrophobic subunits that span the IM

and, thus, upon translation are prone to aggregation. Thus, the assembly of each complex is facilitated by many transient assembly factors encoded by two separate genomes.[36,37]

Human CI (NADH dehydrogenase) consists of 45 different subunits,[38] seven of which are encoded by the mitochondrial genome. These subunits make up the proton pumping module (P module) of CI.[39] The 38 nuclear encoded subunits are imported into the mitochondria.[40] The oxidation of NADH and subsequent electron transfer are catalyzed by seven specific subunits; NDUFV1, NDUFV2, NDUFS1, NDUFS2, NDUFS3, NDUFS7, and NDUFS8.[41] The remaining subunits have largely unknown function but may be involved in complex stability.[42] The assembly of CI requires many assembly factors. Interestingly, a protein previously proposed to be involved in FAO, acyl-CoA dehydrogenase 9 (ACAD9), was recently found to be a necessary assembly factor for biogenesis of CI and not essential for FAO.[43] Mutations in ACAD9 have also been shown to result in cardiomyopathy.[44,45]

CII (succinate dehydrogenase, SDH) is a unique enzyme complex in that it plays a critical role in both the ETC and the tricarboxylic acid cycle (TCA cycle) by oxidizing succinate to form fumarate and simultaneously reducing FAD to form $FADH_2$. $FADH_2$ is reoxidized and the ensuing electrons travel through the ETC. Additionally, CII does not contribute protons to the gradient formed across the IM. 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).[46] Recently, several assembly factors have been established as being critical components of the assembly of CII.[47,48] All constituents of SDH as well as the assembly factors are encoded by the nuclear genome.

CIII (cytochrome bc_1 complex) and CIV (cytochrome C oxidase) comprise the remaining portion of the ETC prior to 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.[49,50] However, it should be noted that assembly of CIV appears to differ drastically between upper and lower eukaryotes and many of these assembly factors have not yet been identified in humans.[51] Finally, mutations in CIII and CIV have been shown to result in human patients with cardiomyopathy.[52–55]

The final step of OXPHOS to produce ATP is catalyzed by ATP synthase (Complex V, CV). ATP synthase has two main components named F_1 and F_0. The F_0 module is composed primarily of nine hydrophobic subunits and is embedded in the IM while the five-subunit F_1 component contains the catalytic domain responsible for phosphorylation of ADP and is located in the matrix of the mitochondrion.[56,57] Interestingly, the mitochondrial specific phospholipid cardiolipin has been shown to be a critical factor in ATP synthase oligomerization.[58] ATP synthase subunit A has been implicated in human cardiomyopathy.[59] Consistent with its role in ATP production, genetic mutations in ATP synthase subunits result in severe phenotypes, frequently presenting with cardiomyopathy.[60]

Given the complexity of the ETC, including the contribution by two 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.[61] In skeletal muscle, exercise triggers a rapid mitochondrial biogenic response.[62] Finally, changes in mitochondrial number and structure are associated with a variety of chronic diseases including heart failure, neurodegenerative diseases, and with aging.[63–66] These developmental, physiological, and pathophysiological changes in mitochondrial number and structure are driven, in part, by a complex network of nuclear transcription factors that coordinately control 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 is operative for the normal mitochondrial biogenic response during development but also serves to integrate physiologic 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.[24] NRF-1 was originally identified as a key regulator of cytochrome C expression.[67] Importantly, NRF-1 also regulates expression of several proteins acting directly on the mitochondrial genome including TFAM and TFB2M.[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 ten nuclear-encoded subunits of cytochrome oxidase.[70,71] Chromatin-immunoprecipitation followed by deep sequencing (ChIP-seq) studies have now confirmed that NRF-1 occupies sites on the promoters of genes encoding components present in all complexes of the ETC.[72] Finally, genetic deletion of NRF-1 or NRF-2 results in embryonic lethality with reduced mtDNA and ETC activity, underscoring the essential role of these factors in mitochondrial biogenesis.[73,74]

Mitochondrial fatty acid β-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 as well as many cellular fatty acid metabolic pathways.[24] The three PPARs (α, β, and γ) 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 (RXR). In the cardiac myocyte, PPARα and PPARβ are the most abundant with PPARγ expressed at lower levels. Although originally identified as a regulator of peroxisomal β-oxidation,[75] PPARα was subsequently shown to regulate genes involved in mitochondrial fatty acid import and oxidation.[76–78] ChIP-on-ChIP and ChIP-seq studies have confirmed PPARα occupancy in the promoters of many mitochondrial FAO genes.[79,80] PPARs are directly activated by the binding of lipid ligands to the ligand-binding domain of the receptor.[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 (ATGL) has been shown to be necessary for the generation of endogenous PPARα ligands and normal expression of PPARα target genes.[84] Lipolysis of triglyceride stores also provides most of the substrate for mitochondrial FAO, and this is augmented with increased activity of PPARα.[85] 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.[86–89] Conversely, overexpression of PPARα in the heart re-capitulates aspects of the insulin-resistant, diabetic heart with increased fatty acid uptake, storage and oxidation; a phenotype worsened by high-fat feeding.[90,91] The closely related PPARδ (also known as PPARβ) also regulates mitochondrial FAO in the heart. Loss of PPARδ results in lower cardiac FAO rates as well as cardiac hypertrophy.[92] Interestingly, mice with overexpression of PPARδ do not accumulate triglyceride and exhibit higher glucose oxidation rates.[93] Activation of the angiopoietin like 4 protein (Angptl4), an endogenous inhibitor of lipoprotein lipase, may contribute to the protection of lipotoxicity by PPARδ.[94] PPARδ also provides protection against ischemia/reperfusion injury and pressure overload.[93,95] The role of PPARγ, the least abundant PPAR, is not well understood in the heart. There is interest in cardiac PPARγ signaling due to the adverse cardiovascular risks associated with PPARγ agonists (thiazolidinediones).[96] Interestingly, both overexpression and loss of PPARγ in the mouse heart results in deleterious consequences.[97–99] However, PPARγ may also function to compensate in heart during acute periods of energy deprivation, such as occurs during sepsis.[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, ERRα, β, and γ, so named due to 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 two groups that ERRα activated expression of the mitochondrial FAO enzyme, medium-chain acyl-CoA dehydrogenase (MCAD).[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.[103,104] Specifically, genome-wide occupation studies have shown that ERRα and ERRγ regulate an

overlapping set of genes involved in mitochondrial FAO, TCA cycle, ETC, and OXPHOS.[104] ERRα can also directly regulate PPARα expression in the heart, forming a feed-forward mechanism to regulate mitochondrial FAO.[103] Generalized loss of ERRα does not result in overt deficiencies in mitochondrial biogenesis but display exaggerated cardiac dysfunction following pressure overload.[105] ERRα knockout mice also display decreased exercise capacity with higher blood lactate levels following exercise consistent with a decreased oxidative capacity.[106] 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.[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.[109,110] ERRγ directly increases type I fiber number through activation of a myosin heavy chain/miRNA circuit critical for slow muscle development.[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.[112,113] In further support of a direct role, loss of c-Myc in fibroblasts results in decreased mitochondrial mass and respiration capacity.[114] In the heart, c-Myc expression is increased following pressure overload and stimulates a mitochondrial biogenic response.[115] Activation of c-Myc also increases glucose utilization 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 following an ischemic insult.[115] These results also suggest that c-Myc may play a unique role in controlling mitochondrial function during pathologic hypertrophy when the canonical pathways (ERRs, 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 co-regulator of PPARγ in the mitochondrial-rich brown adipocyte,[116] it is now known that PGC-1α interacts with and co-activates many nuclear receptors through a specific LXXLL motif domain. The closely related PGC-1β and more distant PGC-1 related coactivator (PRC) comprise the PGC-1 family.[117–119] Interestingly, expression of PGC-1α is highly inducible in mitochondrial-rich tissues such as heart, brown adipose, and muscle by physiologic stimuli including cold exposure and exercise.[120–122] Forced expression of PGC-1α in the heart revealed a robust mitochondrial biogenic response and increased expression of nuclear-encoded mitochondrial genes.[123] The actions of PGC-1α are mediated

via its interactions with NRF-1,[122] PPARα,[124] PPARδ,[125] ERRα and ERRγ (Figure 3).[126–128] 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.[129–132] However, accelerated cardiac dysfunction is observed in PGC-1α and PGC-1β knockout mice following pressure-overload hypertrophy.[133,134]

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.[135] 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 multi-subunit complex that interacts directly with DNA-bound transcription factors as well as RNA polymerase II to facilitate formation of the pre-initiation complex.[136] This complex has been shown to interact with nuclear receptors in a ligand-dependent manner.[137] Specifically, the mediator 1 (MED1) subunit has been shown to interact directly with ERα, PPARγ and PPARα.[138–140] Somewhat surprisingly, loss of MED1 in skeletal muscle results in higher mitochondrial density particularly in white muscle groups with a high proportion of fast fibers.[141] 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 physiologic and metabolic signals with mitochondrial biogenesis

The heart must continually adapt to changes in workload, substrate availability, oxygen availability, and a myriad of other physiologic 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 (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 (CREB) directly on the PGC-1α promoter to activate its expression.[142,143] Intracellular calcium signaling pathways, e.g. calmodulin-dependent kinase (CaMK) and calcineurin, also work to increase PGC-1 levels.[144,145]

PGC-1α activity is also responsive to the energy status of the cell. This occurs at least in part through activation by AMP-dependent protein kinase (AMPK).[146] AMPK is a key cellular energy sensor activated by increasing AMP and reduced ATP levels indicative of energy depletion.[147] 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 (SIRT1) activity.[148] SIRT1 is an NAD^+-dependent deacetylase linked to the response to caloric restriction and lifespan.[149] SIRT1 directly deacetylates and activates PGC-1α, providing a connection between AMPK, SIRT1,

and mitochondrial biogenesis.[150] 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.[148,151,152] While SIRT1 deacetylates PGC-1, the acetyltransferase GCN5 carries out the reverse reaction. GCN5 directly acetylates both PGC-1α and PGC-1β.[153,154] Similar to deacetylation of PGC-1, acetylation is also regulated by metabolic inputs.[155]

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 TCA cycle and ETC/OXPHOS gene expression.[156] 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.[157] YY1 binding sites are also found in many nuclear-encoded mitochondrial genes providing further evidence of the importance of this pathway.[156,158,159] Finally, loss of YY1 results in mitochondrial defects in skeletal muscle, lower oxidative capacity, and exercise intolerance.[157] 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.[160] Restoration of Pim-3 is sufficient to restore PGC-1α expression and normal cellular growth rates. In the heart, loss of all three Pim isoforms results in a remarkably similar phenotype. Triple Pim kinase KO 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.[161] 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 following birth and in the early postnatal period.[61,162,163] 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.[164] 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.[123] Based on this perinatal expression pattern, the PGC-1 coactivators were identified as prime candidates for driving the mitochondrial biogenic surge in heart following 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 due to a complete lack of a mitochondrial biogenic response.[165] The defect in mitochondrial biogenesis in the PGC-1α/β-deficient hearts is quite 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 KO mice.[107,108] 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 PGC-1β gene with a muscle creatine kinase promoter-driven Cre recombinase (MCK-Cre) that has activity after birth during the early postnatal period. Specifically, when the cardiac PGC-1β gene is targeted in heart on a generalized PGC-1α-deficient background, animals develop a progressive, lethal, postnatal cardiomyopathy.[166] 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 as well as elongated mitochondria.[166] In addition, a distinctive mitochondrial “donut” abnormality was seen in the myocytes.[166] This latter mitochondrial abnormality has been described in Charcot-Marie-Tooth disease due to a genetic defect in mitofusin 2 (MFN2).[167] These observations suggested that the mitochondrial maturation defect was due, at least in part, to altered mitochondrial dynamics. Consistent with this conclusion, levels of a number of key proteins involved in mitochondrial fusion, including mitofusin 1 (MFN1), MFN2, and OPA1[166] were reduced in the PGC-1α/β-deficient hearts during the postnatal period prior to the development of fulminant heart failure. MFN1 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-deficient 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 sarcomeres or as a quality control mechanism.[168]

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 quite 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.[3,169] 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 approximately 16 days.[169] 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 MFN1.[166] Moreover, adult PGC-1α/β-deficient mice do not exhibit an overt abnormality in cardiac function.[166] However, in other studies, combined loss of MFN1 and MFN2 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.[169–172] 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, TCA cycle, and ETC/OXPHOS.[166] 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.[173,174] 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,[166] PGC-1α null mice develop ventricular dilation and reduced contractile function in response to chronic pressure overload.[133] In addition, ERRα loss-of-function mice develop severe heart failure in the context of pressure overload and are sensitive to ischemic insult.[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, a number of studies have now shown reduced expression of PGC-1α, PPAR, ERR, and many of its downstream targets in the failing rodent and human heart.[175–182] Changes in fuel substrate selection is one consequence of deactivation of the PGC-1 circuit. For instance, it is now well-established that rates of FAO are decreased in pathologic cardiac hypertrophy.[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, very 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.[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 *en route* to heart failure. Accordingly, transcriptional events and alterations in PGC-1 signaling likely occur later in the pathogenic process.[183] Notably, the role of altered mitochondrial biogenesis and dynamics in this pathologic process remains to be fully defined.

Conclusions

In summary, 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 is 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Nonstandard Abbreviations and Acronyms

mtDNA	mitochondrial DNA
ETC	electron transport chain
OXPHOS	oxidative phosphorylation
TCA cycle	tricarboxylic acid cycle
FAO	fatty acid oxidation
SDM	strand-displacement model
RITOLS	ribonucleotide incorporation throughout the lagging strand
ChIP-seq	chromatin immunoprecipitation followed by deep sequencing

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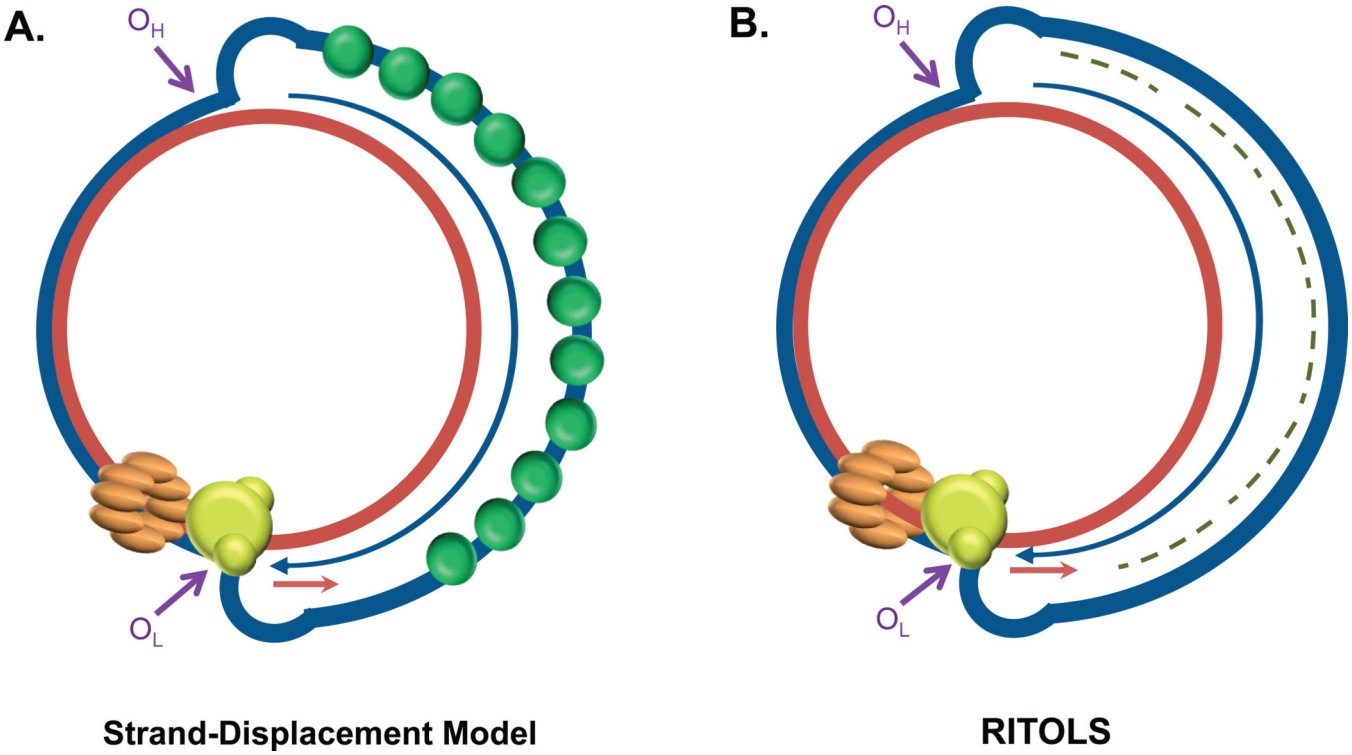

Figure 1. The two predominant models of mtDNA replication are shown here

Both models concur the replisome consists of at least a helicase, TWINKLE (orange) and POLG (yellow). Leading strand synthesis begins at O_H and lagging strand synthesis at O_L (red arrow). A) Strand-displacement model (SDM) proposes SSB proteins (green spheres) bind the displaced H-strand during leading strand replication. B) Alternatively, the ribonucleotide incorporation throughout the lagging strand (RITOLS) model proposes portions of transcribed mtDNA bind the H-strand (green dotted line).

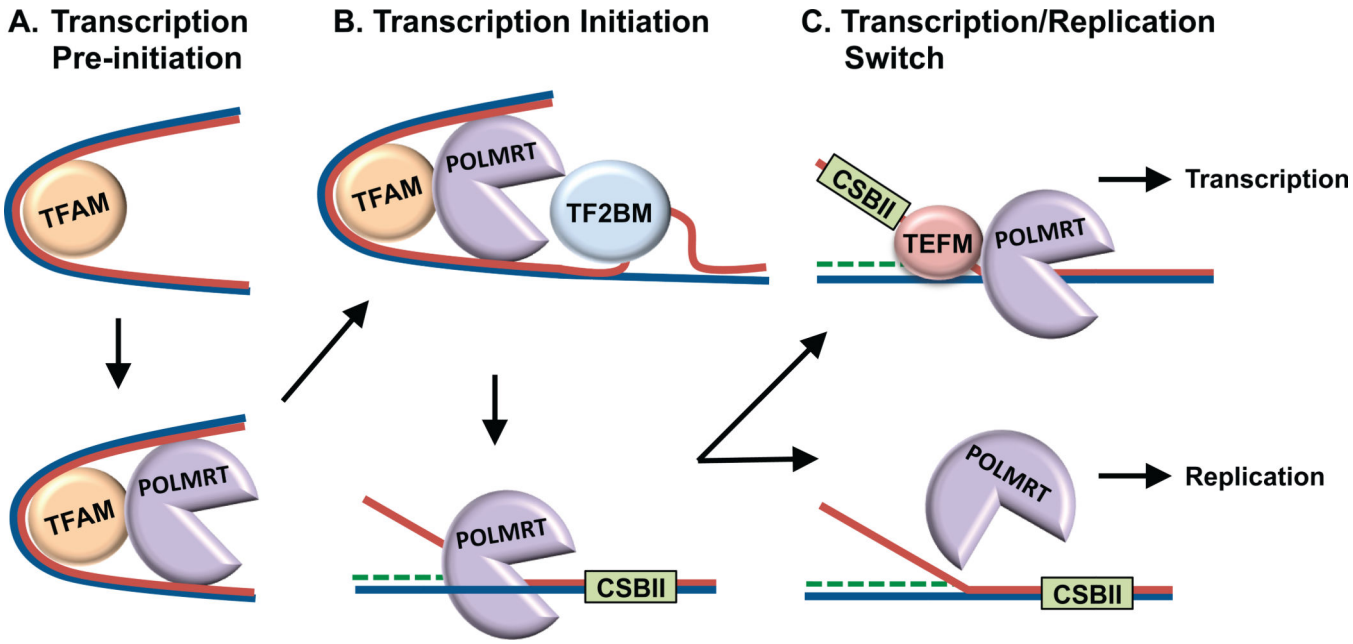

Figure 2. POLMRT Plays a Critical Role in Mitochondrial Transcription and Replication

A) The transcription pre-initiation begins with mitochondrial transcription factor A (TFAM) binding and recruiting POLMRT. TFAM enables POLMRT interaction with upstream promoter (P) by bending the DNA around POLMRT. B) Transcription initiation occurs when TF2BM binds POLMRT and facilitates promoter melting forming the characteristic D-loop region. POLMRT synthesizes an RNA primer (green dotted line) until reaching CSBII where the transcription/replication switch occurs. 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 disassociates from mtDNA, transcription is terminated at CSBII and the oligonucleotide strand is used as a primer for DNA replication (bottom). Replication then proceeds following the recruitment of the replisome apparatus. TFAM, mitochondrial transcription factor A; POLMRT, mitochondrial RNA polymerase; TFB2M, mitochondrial transcription factor B2; CSBII, conserved sequence block II; TEFM, mitochondrial transcription elongation factor.

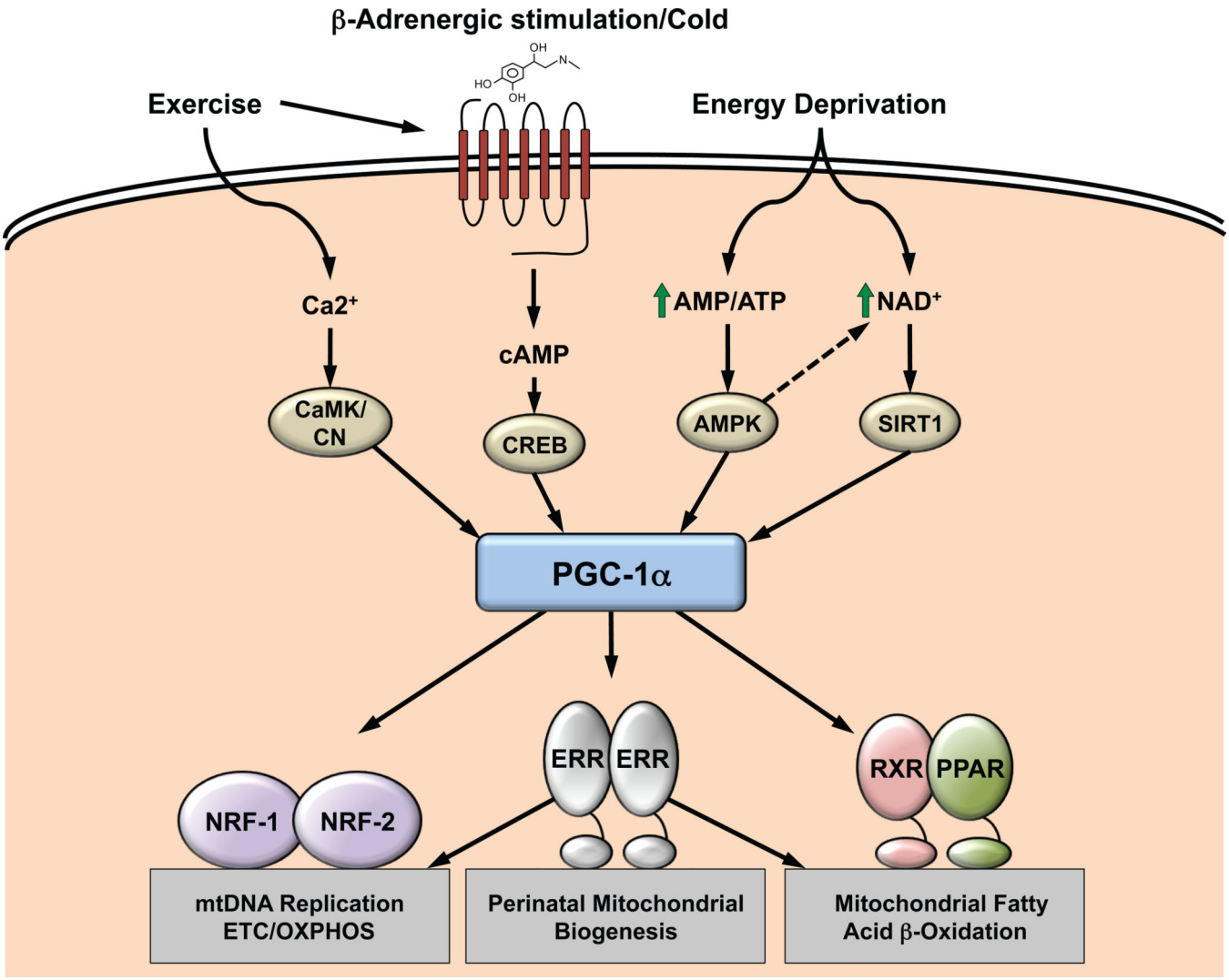

Figure 3. PGC-1α mediates physiologic control of mitochondrial biogenesis and function

The transcriptional coactivator PGC-1α interacts directly with multiple transcription factors to integrate upstream signaling events with mitochondrial biogenesis and functional capacity. The downstream transcription factors control virtually every aspect of mitochondrial function and energy production including biogenesis, dynamics, and maintenance of protein levels. The control of PGC-1α expression and activity is dynamic, responding to multiple intracellular second messengers and signaling molecules transmitting inputs from various physiologic and metabolic stimuli (top). CREB, cAMP-response element binding protein; AMPK, AMP-activated protein kinase; SIRT1, sirtuin 1; CaMK, calmodulin-dependent kinase; CN, calcineurin; NAD^+, nicotinamide adenine dinucleotide; PGC-1α, PPARγ coactivator 1; PPAR, peroxisome proliferator-activated receptor; NRF, nuclear respiratory factor; ERR, estrogen-related receptor; ETC/OXPHOS, electron transport chain/oxidative phosphorylation.

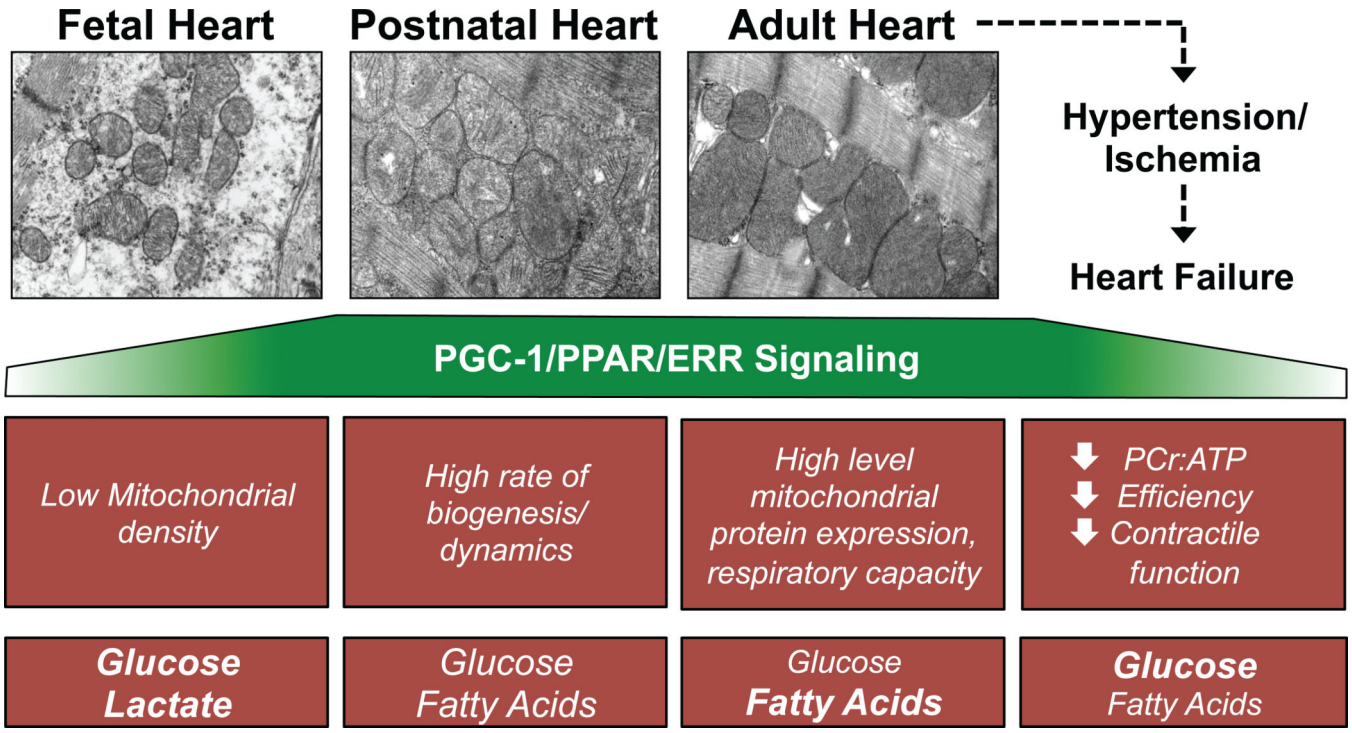

Figure 4. Dynamic changes in cardiac mitochondrial number, structure, and function during developmental stages and in the failing heart

Electron micrographs reveal a developmentally regulated increase in cardiac myocyte mitochondrial number and organization during the transition from the fetal period to the adult. The surge of mitochondrial biogenesis at birth and subsequent maturation in the postnatal heart is driven by induction of PGC-1. In the adult heart, PGC-1 maintains high level, coordinated expression of nuclear and mitochondrial genes encoding mitochondrial machinery. Mitochondrial function and energy production is compromised in the failing heart concomitant with decreased ERR/PPAR/PGC-1 signaling. PPAR, peroxisome proliferator-activated receptor; ERR, estrogen-related receptor; PGC-1, PPARγ coactivator 1; PCr, phosphocreatine.

Table

Nuclear transcription factors regulating mitochondrial biogenesis and maturation

Transcription factor	Class	Model	Phenotype	References
NRF-1	Nrf1 DNA-binding	General KO	Embryonic lethality of KO at e6.5 with reduced mtDNA and ETC activity	73
NRF-2/GABP	ETS-domain	General KO	Embryonic lethality prior to implantation	74
PPARα	Nuclear receptor	General KO	Reduced FAO rates, cardiomyopathy with metabolic stress	86–89
		Cardiac-specific Tg	Increased FAO uptake, storage and oxidation; recapitulates diabetic cardiomyopathy phenotype	90,91
PPARβ/δ	Nuclear receptor	General KO	Reduced FAO and glucose oxidation; cardiac hypertrophy	92
		Cardiac-specific Tg	Increased FAO and glucose oxidation, protected against lipotoxicity	93,95
PPARγ	Nuclear receptor	General KO	Cardiac hypertrophy, increased susceptibility to oxidative stress	98,99
		Cardiac-specific Tg	Lipid accumulation and cardiomyopathy	97
ERRα	Nuclear receptor	General KO	Cardiomyopathy following pressure overload with phosphocreatine depletion and reduced ATP synthesis rate	105,106
ERRγ	Nuclear receptor	General KO	Postnatal lethality with inability to shift to oxidative metabolism	107,108
		Skeletal muscle specific Tg	High mitochondrial density, increased type I fibers	109,110
YY1	GLI-Kruppel zinc finger	Skeletal muscle specific KO	Defects in mitochondrial structure and energy production	157
c-Myc	Basic helix-loop-helix/leucine zipper	Cardiac-specific Tg	Cardiac transgene results in mitochondrial biogenesis, increased glucose oxidation, and protection from I/R injury	115
PGC-1	Coactivator	General PGC-1α or PGC-1β KO	Decreased function following pressure overload	133,134
		Combined PGC-1α/β KO	Postnatal lethal, lack of mitochondrial biogenic response	165
		Postnatal PGC-1α/β KO	Mitochondrial defects with impaired postnatal dynamics	166
		Adult cardiac-specific PGC-1α/β KO	Normal mitochondrial density but marked downregulation of mitochondrial gene expression	166
		Cardiac-specific PGC-1α Tg	Marked mitochondrial biogenesis resulting in cardiomyopathy	123
MED1	Coactivator	Skeletal muscle specific KO	Increased mitochondrial density, fast to slow fiber type shift	141