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Control of gene expression and mitochondrial biogenesis in the muscular adaptation to endurance exercise

Anna-Maria Joseph*, Henriette Pilegaard†, Anastassia Litvintsev‡, Lotte Leick† and David A. Hood*‡1

*Department of Biology, York University, Toronto, Ontario, Canada, M3J 1P3, †Copenhagen Muscle Research Centre, University of Copenhagen, Copenhagen, Denmark, and ‡School of Kinesiology and Health Science, York University, Toronto, Ontario, Canada, M3J 1P3

Abstract

Every time a bout of exercise is performed, a change in gene expression occurs within the contracting muscle. Over the course of many repeated bouts of exercise (i.e. training), the cumulative effects of these alterations lead to a change in muscle phenotype. One of the most prominent of these adaptations is an increase in mitochondrial content, which confers a greater resistance to muscle fatigue. This essay reviews current knowledge on the regulation of exercise-induced mitochondrial biogenesis at the molecular level. The major steps involved include, (i) transcriptional regulation of nuclear-encoded genes encoding mitochondrial proteins by the coactivator peroxisome-proliferator-activated receptor γ coactivator-1, (ii) control of mitochondrial DNA gene

1To whom correspondence should be addressed (email dhood@yorku.ca).
expression by the transcription factor Tfam, (iii) mitochondrial fission and fusion mechanisms, and (iv) import of nuclear-derived gene products into the mitochondrion via the protein import machinery. It is now known that exercise can modify the rates of several of these steps, leading to mitochondrial biogenesis. An understanding of how exercise can produce this effect could help us decide whether exercise is beneficial for patients suffering from mitochondrial disorders, as well as a variety of metabolic diseases.

**Introduction**

Performing regular exercise has many health benefits. The consequences of exercise include improved cardiovascular function as well as a shift in substrate oxidation toward that of lipid, rather than carbohydrate. Endurance capacity for daily work tasks is enhanced, mainly as a result of a greater oxygen delivery and extraction by the exercising muscle. Oxygen extraction is a result of an improved capillary-to-fibre ratio, as well as a higher mitochondrial content within muscle. Although these adaptations have long been recognized, the molecular basis for these changes remain a matter of intense study. This is important because an understanding of the cellular processes involved could (i) help in the development of therapeutic applications other than exercise, and (ii) achieve a greater understanding of the pathology of mitochondrial diseases. The increase in mitochondrial content that occurs as a result of regular exercise is referred to as mitochondrial biogenesis. This process is complex because mitochondria are composed of proteins encoded by both nuclear and mtDNA (mitochondrial DNA). In addition, mitochondrial structure differs among cell types and even within different regions of a specific cell type. Recently, several breakthroughs in our understanding of this process have occurred, with the discovery of an important overall regulator of mitochondrial biogenesis, termed PGC-1α (peroxisome-proliferator-activated receptor γ coactivator-1α), as well as the recognition that mitochondria continually undergo fission and fusion events, processes that have an impact on mitochondrial morphology and function. In the present paper we will review our current understanding of mitochondrial regulatory proteins and organelle assembly patterns, as well as the response to exercise. Recent reviews on the topics discussed in this essay are also included [1–8].

**PGC-1α**

**Transcriptional regulation by PGC-1α**

PGC-1α has developed a reputation as a protein that is vital for mitochondrial biogenesis. It was discovered in the search for PPARγ (peroxisome-proliferator-activated receptor γ) interacting proteins from mouse BAT (brown adipose tissue) cells. PGC-1α binds many nuclear receptors that results in an increase in the transcriptional activity of their target genes [1,9].
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Table 1. Examples of transcription factors and downstream target genes which are ultimately regulated by PGC-1α.

<table>
<thead>
<tr>
<th>Transcription factor</th>
<th>Ultimate downstream target genes</th>
<th>Function</th>
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<tbody>
<tr>
<td>PPARγ, α, β</td>
<td>UCP 1</td>
<td>Lipid metabolism</td>
</tr>
<tr>
<td>Retinoid X receptor</td>
<td>UCP 2</td>
<td>Lipid metabolism</td>
</tr>
<tr>
<td>Thyroid hormone receptor β</td>
<td>CPT 1</td>
<td>Lipid metabolism</td>
</tr>
<tr>
<td>Oestrogen receptor and oestrogen related receptor α, γ</td>
<td>MCAD</td>
<td>Lipid metabolism</td>
</tr>
<tr>
<td>NRF-1 and NRF-2</td>
<td>CD36</td>
<td>Lipid metabolism</td>
</tr>
<tr>
<td>Myocyte enhancer factor (MEF) 2</td>
<td>Cytochrome c</td>
<td>Electron transport</td>
</tr>
<tr>
<td>Forkhead Box 01 (FOXO1)</td>
<td>NADH dehydrogenase</td>
<td>Electron transport</td>
</tr>
<tr>
<td>Farnesoid X receptor</td>
<td>COX subunit IV</td>
<td>Electron transport</td>
</tr>
<tr>
<td></td>
<td>NRF-1</td>
<td>Transcriptional regulation</td>
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<tr>
<td></td>
<td>NRF-2</td>
<td>Transcriptional regulation</td>
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<tr>
<td></td>
<td>PGC 1α</td>
<td>Transcriptional regulation</td>
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<td></td>
<td>TFB1, TFB2</td>
<td>Transcriptional regulation</td>
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<td></td>
<td>Tfn</td>
<td>Transcriptional regulation</td>
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<tr>
<td></td>
<td>Glut4</td>
<td>Carbohydrate metabolism</td>
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<tr>
<td></td>
<td>PDK4</td>
<td>Carbohydrate metabolism</td>
</tr>
<tr>
<td>CD36, fatty acid translocase; CPT-1, carnitine palmitoyltransferase-1; Glut4, glucose transporter 4; MCAD, medium chain acetyl-coenzyme A dehydrogenase; PDK4, pyruvate dehydrogenase kinase 4.</td>
<td></td>
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Thus, PGC-1α is the transcriptional coactivator of a broad range of transcription factors regulating both nuclear and mitochondrial genes, with the potential to play an important coordinating role in regulating adaptive responses of the muscle cell. PGC-1α has the ability to increase the transcriptional activity of target genes by recruiting additional transcriptional regulators. These include proteins with histone acetyltransferase activity that are able to produce local alterations in chromatin structure, making the promoter region more accessible for the transcriptional machinery and thus more favourable for transcription.

Role of PGC-1α in BAT

The functional role of PGC-1α in BAT became evident from the findings that PGC-1α regulated the UCP1 (uncoupling protein 1) gene through activation of the transcription factors thyroid receptor and PPARγ, and that forced overexpression of PGC-1α in adipocytes elevated the mRNA content of the genes encoding UCP1 and many other mitochondrial proteins. Thus, WAT (white adipose tissue) with a low mitochondrial content obtained the characteristics of BAT with a higher mitochondrial concentration, when PGC-1α was induced. BAT is important in adaptive thermogenesis in which mitochondrial biogenesis and the increased expression of oxidative metabolism...
genes are important phenotypic characteristics. Thus, PGC-1α is important in regulating adaptive thermogenesis. This interpretation is even further strengthened by the lower body temperature and marked cold-sensitivity of PGC-1α knockout mice [10]. In these animals, no change in BAT UCP1 expression is evident upon exposure to cold, whereas wild-type animals demonstrate a 3-fold increase of UCP1 mRNA in this tissue [10].

**Role of PGC-1α in the heart**

PGC-1α protein levels are very high in the heart, coincident with the extremely high mitochondrial content of this tissue [2]. The importance of PGC-1α in regulating cardiac mitochondrial number and metabolism first became clear from the increased expression of nuclear- and mitochondrial-encoded genes produced by the forced overexpression of PGC-1α in cardiac myocytes [11]. In contrast, the hearts of PGC-1α-deficient mice have reduced mRNA content of a broad range of genes encoding mitochondrial proteins, lower mitochondrial enzyme activities and poor cardiac contraction, with defects in the ability to increase cardiac work in response to physiological stimuli [12]. These data illustrate the importance of PGC-1α in the metabolism and function of the heart. However, extreme overexpression of PGC-1α also led to cardiomyopathy, presumably as a result of the uncoordinated up-regulation of key metabolic proteins.

**Role of PGC-1α in skeletal muscle**

A similar profound role of PGC-1α in regulating the expression of genes encoding mitochondrial proteins in skeletal muscle was convincingly shown by the changes induced by overexpressing PGC-1α both in myotubes [9] and in transgenic mice [13]. The overexpression of PGC-1α in myotubes induced mitochondrial biogenesis and increased the expression of genes involved in oxidative phosphorylation [9] (Figure 1). Interestingly, it was demonstrated that PGC-1α can coactivate the transcription factor NRF-1 (nuclear respiratory factor-1) to regulate Tfam (mitochondrial transcription factor A) levels, a nuclear-encoded protein which controls mtDNA replication and transcription (see below). This provides a mechanism through which PGC-1α can also control mitochondrially-encoded genes. In PGC-1α transgenic mice, normal 'white' muscles turned red. For example, the white vastus muscle, that normally has a low oxidative capacity, exhibited a dramatically elevated mRNA and protein content of myoglobin and COX (cytochrome c oxidase) subunits II and IV, proteins involved in oxidative metabolism. These changes occurred even when PGC-1α was expressed within the normal physiological range [13], emphasizing the physiological importance of PGC-1α. In contrast, in PGC-1α knockout mice, the lack of PGC-1α protein in skeletal muscles resulted in 30–60% reduced mRNA content of many genes encoding proteins involved in fatty acid metabolism, the tricarboxylic acid cycle and the respiratory chain [10], but surprisingly the mitochondrial volume was similar to that found...
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These data clearly support the view that PGC-1α is essential in maintaining normal metabolism in skeletal muscle. In agreement with intracellular metabolic disturbances due to changes in expression of mitochondrial proteins, skeletal muscles of PGC-1α knockout mice had an increased AMPK (AMP-activated protein kinase) activity [10]. Since this kinase is thought to be a sensor of intracellular energy charge, the increased AMPK activity is likely to be a compensatory mechanism in response to a lower intracellular ATP/ADP ratio in the PGC-1α knockout mice. PGC-1α is also suggested to contribute to the regulation of the redox status of the cell. This may be mediated by the PGC-1α-induced increase in the expression of uncoupling proteins [9], leading to reduced ROS (reactive oxygen species) production, along with a PGC-1α-regulated increased expression of scavenging enzymes [1].

Surprisingly, an influence of PGC-1α was also demonstrated on MHC (myosin heavy chain) fibre type changes, suggesting that it could act as a transcriptional coactivator that has a major influence in driving the formation of slow-twitch muscle fibres in transgenic mice. The observed change, however,

Figure 1. Exercise-induced up-regulation of PGC-1α expression and the concomitant effects of PGC-1α on the regulation of nuclear and mitochondrial encoded genes

Muscle contractions increase PGC-1α transcription, in part, through Ca²⁺-mediated signalling via calcineurin and MEF2, as well as CaMK and CREB. PGC-1α transcripts translocate to the cytosol, where mRNA is translated. PGC-1α protein translocates back to the nucleus, where it regulates the transcription of many metabolic genes and transcription factors by binding to a broad range of transcription factors (TF) and recruiting other transcriptional regulators (coactivators; CO). PGC-1α also seems to regulate its own transcription. PGC-1α activity is inhibited when repressors (R) bind to PGC-1α, and this inhibition can be released by p38-induced phosphorylation of the repressor. One of the products of PGC-1α-mediated coactivation of the NRF-1 transcription factor is Tfam. Tfam and other metabolic proteins are imported into mitochondria. Tfam enhances the transcription of mitochondrially-encoded genes and the replication of mitochondrial DNA. Repeated bouts of exercise will thus increase mitochondrial biogenesis and skeletal muscle oxidative capacity leading to improved health and improved exercise performance.
was only 10 and 20% for type I and IIa fibres respectively [13]. Although this does suggest that PGC-1α plays a role in regulating MHC expression, it should be noted that the changes in metabolic gene expression were much more pronounced, emphasizing the major role of PGC-1α in regulating the expression of genes encoding proteins in oxidative metabolism. This view is supported by the findings that PGC-1α knockout mice had similar percentages of MHC type I and IIa fibres as in wild-type animals [12].

**Intracellular signalling regulating PGC-1α expression**

A likely role of calcium signalling in regulating PGC-1α expression became evident from the observed changes in transgenic mice expressing a constitutively active form of CaMK (calcium/calmodulin-dependent protein kinase) IV. In addition to an up-regulation of PGC-1α mRNA, the mRNA content of subunits of the NADH dehydrogenase (encoded by mtDNA), as well as nuclear gene products carnitine palmitoyl transferase I, cytochrome c and myoglobin were increased in the transgenic mice. Thus, CaMK overexpression appeared to induce a coordination of the nuclear and the mitochondrial genomes through PGC-1α [14]. In accordance with this, increasing cytosolic calcium in myotubes by caffeine incubation increased PGC-1α protein content. This induction was abolished by simultaneous treatment with dantrolene, an inhibitor of calcium release from the sarcoplasmic reticulum. In addition to the CaMK pathway, calcineurin signalling also appears to be involved in regulating PGC-1α expression in muscle cells. Co-transfection studies in C2C12 cells revealed that calcineurin seems to affect PGC-1α transcription through an effect on MEF2 (myocyte enhancer factor 2) at the PGC-1α promoter, whereas CAMKIV regulates the PGC-1α gene through phosphorylation of CREB (cAMP response element-binding protein), a binding protein that directly interacts with CRE (cAMP response element) and activates PGC-1α transcription. A cAMP signalling pathway is also likely to be involved in regulating PGC-1α expression. In hepatocytes, glucagon binding to the cell surface leads to increased cAMP, protein kinase A activation and the concomitant phosphorylation and activation of CREB. Similarly, insulin signalling through protein kinase B and FOXO1 (forkhead family transcriptional regulator) acting on insulin response sequences on the PGC-1α promoter has been demonstrated in hepatocytes [15]. In addition, AMPK has been suggested to be involved in regulating PGC-1α expression in skeletal muscle. This is because treatment of muscle cells with the AMPK activator AICAR (5-aminoimidazole-4-carboxamide-1β-D-ribofuranoside) increases PGC-1α protein content. It should be noted, however, that the exercise-induced increases in PGC-1α transcription and mRNA content are not affected in mouse skeletal muscle by knocking out either the AMPKa1 or AMPKa2 isoforms [16]. Finally, PGC-1α seems to be able to activate its own transcription by coactivating MEF2 on the PGC-1α promoter, thereby exerting an autoregulatory influence on PGC-1α.
expression. This mechanism may help to ensure the stable transcription of PGC-1α during adaptive cellular responses [17].

In addition to regulation of PGC-1α via expression changes, the activity of PGC-1α is also controlled via changes in phosphorylation state. The p38 MAPK (mitogen-activated protein kinase)-induced phosphorylation of PGC-1α increases the stability of the protein, thus maintaining its activity for a longer period of time. In addition, changes in phosphorylation state also affect the activity of repressors that interfere with the interactions between PGC-1α and transcription factors [1,17,18]. It is suggested that the phosphorylation of a repressor by p38 MAPK promotes dissociation of the repressor from PGC-1α, which then becomes accessible for the transcription factors to allow transcriptional activation [18] (Figure 1).

Effect of exercise on PGC-1α gene expression
Regular physical activity is associated with a broad range of cellular adaptations in skeletal muscle including increased capillarization and mitochondrial enzyme content, leading to the improved oxidative capacity of skeletal muscles. The current understanding is that exercise-induced gene expression responses to each single acute exercise bout contributes to the ultimate cellular adaptations observed after exercise training. The final phenotypic adaptations stem from the cumulative effects of such transient gene expression responses. For this to be possible, only genes with a sufficiently prolonged expression response to acute exercise will have the potential to accumulate mRNA/protein when exercise is performed regularly. Genes encoding proteins such as citrate synthase, 3-hydroxy-acyl-dehydrogenase and cytochrome c seem to be among those possessing such responses [19]. The products of genes with more rapid turnover responses like UCP3, hexokinase II and PGC-1α [19] are less likely to accumulate when the activity is performed regularly. However, the responses of these genes are no doubt critical for the overall adaptation to physical activity. This may be due to their roles in re-establishing intracellular energy homoeostasis, or as transcriptional regulators of other metabolic genes important in this adaptive process. Acute exercise-induced increases in PGC-1α transcription, mRNA content and/or protein content have been demonstrated in rat [20] and human [19] skeletal muscle. Typical transcriptional and mRNA responses of PGC-1α in skeletal muscle occur within the initial 6 h of recovery from exercise. Increased PGC-1α protein expression was reported 18 h after the end of exercise in rats. This increased protein content was also observed after 5–7 days of repeated 3 h/day contractile activity bouts [2]. In addition, the acute transcriptional and mRNA responses of PGC-1α were enhanced by four weeks of exercise training in humans, although all other investigated genes had lower or similar responses to acute exercise after training [19] suggesting that the exercise-induced PGC-1α gene response is potentiated by regular exercise.

The functional role of the exercise-induced increase in PGC-1α gene expression lies in the ability of PGC-1α to coactivate a broad range of
transcription factors, and thus a diverse set of target genes, leading to coordinated adaptive cellular responses to physical activity. In addition, the identification of specific phosphorylation sites in PGC-1α makes it possible that PGC-1α exerts its coordinating role through phosphorylation-mediated regulation of PGC-1α activity, without requiring an obligatory increase in PGC-1α protein levels. Whether such mechanisms are operative during exercise remains to be seen.

Potential role of PGC-1α in insulin resistance
The impact of PGC-1α on regulating the expression of metabolic genes has led to the search for potential roles of PGC-1α dysregulation in metabolically related diseases like T2D (type 2 diabetes). Insulin resistance and T2D were found to be associated with a lower mRNA expression of many NRF-1-regulated genes encoding proteins in oxidative metabolism. These changes coincided with reduced PGC-1α mRNA content in muscle obtained from individuals with T2D, as well as those who were non-diabetic but had a family history of diabetes [21]. PGC-1α is known to both regulate the expression of NRF-1 and to be a coactivator of NRF-1-mediated transcription. Therefore these findings suggest that lowered PGC-1α expression may be a factor leading to insulin resistance and T2D. Additionally, genetic variations in the PGC-1α gene seem to be implicated in the development of T2D. Thus, a frequent single nucleotide polymorphism (G482S) in the PGC-1α gene is associated with an increased risk of T2D in Danish Caucasians [22], and specific PGC-1α promoter polymorphisms seem to be associated with early onset T2D in a Korean population [23]. We speculate that such polymorphisms could influence the expression of PGC-1α or its rate of degradation, leading to reduced mRNA content in skeletal muscles of T2D patients, in addition to possible direct effects of a missense mutation on PGC-1α function. We propose that regular exercise may be beneficial in preventing the development of T2D because the exercise-induced increase in PGC-1α protein in skeletal muscle can lead to an increased oxidative capacity of skeletal muscle, including improved lipid oxidation.

mtDNA transcription factors
Mitochondria possess their own circular genome of about 16.5 kb termed mtDNA. mtDNA contains two ribosomal RNAs, 22 tRNAs and 13 mRNAs that encode proteins that function as subunits for respiratory complexes I, III and IV [3]. However, this represents only a small portion of the total number of genes that are necessary for the proper function of mitochondria. Indeed, the proteins that regulate the replication and transcription of mtDNA are nuclear-encoded, and need to be imported into the organelle (see below). One of the first identified, and most important of these regulatory proteins, is Tфam (Figure 2). Tфam plays a key role in maintaining the mitochondrial
Figure 2. Mitochondrial DNA transcription and replication can be induced by physical activity through numerous signal transduction cascades

Exercise signals lead to the transcription of Tfam, Tfb1 and Tfb2 into mRNAs in the nucleus, which are then synthesized in the cytosol into precursor proteins and imported into mitochondria. Mature Tfam, Tfb1 and Tfb2 proteins activate mtDNA transcription and replication within the organelle. mtDNA gene products are then assembled into the electron transport chain.

copy number and transcriptional activity since the action of Tfam along with the mitochondrial RNA polymerase is necessary for the proper initiation of transcription of mammalian mtDNA from both heavy- and light-strand promoters [3]. The importance of Tfam is evident from the phenotype exhibited by Tfam knockout mice. Homozygous Tfam knockout leads to embryonic lethality. Embryos have delayed neural development, the absence of cardiac structures and either lack or have low levels of mtDNA. mtDNA copy number and respiratory chain complex activities are reduced in the heart of heterozygous Tfam knockout mice. Disruption of Tfam in cardiomyocytes results in dilated cardiomyopathy with atrioventricular conduction blocks, whereas inactivation of Tfam in β-cells results in the inability of the β-cell to release insulin in response to a glucose challenge. Massive neuronal degeneration has also been shown to result from a defect in the Tfam gene within cortical neurons. These studies demonstrate the critical role of Tfam in the maintenance of mtDNA and mitochondrial biogenesis in a variety of tissues.

It is now known that exercise increases the expression and function of Tfam in muscle. Gordon et al. [24] demonstrated that electrically stimulated-induced contractile activity of the rat tibialis anterior muscle leads to an increase in Tfam mRNA level after four days (Figure 2). Subsequent increases
in Tfam import into mitochondria occurred by day five, leading to an accumulation of mitochondrial Tfam protein, an increase in Tfam–mtDNA binding and mtDNA transcript levels encoding COX subunit III, and a higher COX enzyme activity by day seven. A similar increase in Tfam expression has been found following endurance training in humans. Thus, the increase in Tfam expression during the progression of exercise training contributes substantially to mitochondrial biogenesis in skeletal muscle.

Although it was believed for some time that the proper initiation of transcription from heavy- and light-strand promoters was exclusively dependent upon mitochondrial RNA polymerase and Tfam [3], TFB1m and TFB2m, two human isoforms of mitochondrial specificity factor have recently been identified and shown to play an important role in transcription initiation [25]. TFB1m and TFB2m are localized to mitochondria in order to bind mtDNA and stimulate transcription from the L-strand promoter in vitro. However, TFB1m has about 10% of the transcriptional activity of TFB2m. It now appears that RNA polymerase, Tfam and the TFB isoforms are essential for proper mtDNA transcription, with Tfam playing a major role in this process since it regulates the activity of the TFB–RNA polymerase complex and transcriptional activity both in vivo and in vitro. Interestingly, TFB1m and TFB2m are transcriptionally regulated by NRF-1 and NRF-2, in a similar manner to Tfam (Figure 2). Since multiple nuclear genes encoding mitochondrial proteins also have recognition sequences for NRF-1 and -2, this indicates that common transcriptional regulators (NRF-1 and -2) link the expression of respiratory chain proteins to the mitochondrial transcriptional machinery [3]. This is likely to be important for coordinating the gene expression response to exercise, leading to an up-regulation of both nuclear and mitochondrial gene products and subsequent organelle biogenesis. More studies are necessary to reveal the expression and function of TFB1 and TFB2 in skeletal muscle during exercise.

Mitochondrial fusion and fission

Mitochondria are very dynamic structures. They have the ability to constantly fuse and divide and mitochondrial structure within the cell reflects a balance between mitochondrial fusion and division (i.e. fission). If fusion predominates, mitochondria become more interconnected and networked [26]. In contrast, excessive fission leads to mitochondrial network breakdown, the loss of mtDNA, an increase in ROS production and respiratory defects [4]. Despite the fact that the exact mechanisms responsible for mitochondrial fission and fusion events have not been identified, significant progress has been made in recognizing genes that play a critical role in these processes.

Several proteins within the outer and inner mitochondrial membrane are involved in the mitochondrial fusion process, including Mfn1 and Mfn2 (mitofusin 1 and 2) and OPA (a dynamin-related GTPase) [5]. The Mfn1 and Mfn2 isoforms are very similar in that they show a high degree of homology and
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Both are GTPases located on the outer mitochondrial membrane with the N-terminal GTPase and their coiled-coil protruding into the cytosol [5]. Despite their similarity, Mfn1 can only promote functional elongation of mitochondria in the presence of OPA1, whereas Mfn2 does not require additional fusion proteins. Of interest is that OPA1 mutations have been shown to induce optic atrophy through mitochondrial impairment. In the absence of Mfn2, the degree of fusion events is low, leading to a discontinuous mitochondrial network. In contrast, overexpression of Mfn2 protein leads to the generation of a mitochondrial reticulum-like network. There is also evidence that Mfn2 has an effect on mitochondrial metabolism. Repression of Mfn2 expression in muscle myotubes reduced glucose oxidation as well as the mitochondrial membrane potential. The impairment of lipid and carbohydrate metabolism evident in obese Zucker rats may be related to the fact that Mfn2 is significantly reduced in the muscle of this animal model [27].

Mitochondrial fission is required during cell division when proper inheritance of mitochondria by daughter cells is of critical importance [6]. The

Figure 3. Schematic model of mitochondrial fusion and fission
GTPases are enzymes that have the ability to hydrolyse GTP. Mfn is an outer membrane mitochondrial protein. It has a cytosolic GTPase domain and two coiled-coil regions. Adjacent mitochondria start fusing by a process of oligomerization between Mfn molecules at the C-terminal coiled-coil region. Mfns coordinate fusion with the help of an OPA1 protein which is an intermembrane space GTPase. Mitochondrial fission is initiated by the cytosolic Drp1 protein that is recruited to the mitochondrial outer membrane by a presently unknown signal. It is proposed that Fis1 recruits Drp1 to the mitochondrial outer membrane, where activated Drp1 forms a ring-like complex and conveys signals to (or from) the inner membrane to coordinate mitochondrial membrane fission (modified from [23,25]).
mechanisms of mitochondrial fission are still poorly understood. Drp1 (dynamin-related protein 1) is a large GTPase protein and, along with Fis1 (mitochondrial fission protein), regulates fission in mammalian cells. The assembly of fission machinery occurs at scission points on the outer mitochondrial membrane, where Fis1 recruits the Drp1 protein (Figure 3). The mitochondrial network becomes more fragmented when Fis1 expression is increased, whereas a decrease in Fis1 expression leads to more interconnected mitochondria.

Skeletal muscles have highly interconnected mitochondria, particularly in the intermyofibrillar region. In contrast, mitochondria within the subsarcolemmal region of muscle cells appear more fragmented [7]. Endurance exercise training leads to an expansion of the mitochondrial reticulum during organelle biogenesis [8], yet little is known with regard to the specific mechanisms that control this process in muscle. A recent study has demonstrated an increase in Mfn1 and Mfn2 mRNA levels in human skeletal muscle 24 h post-exercise [28], but the regulation of the expression of these mitofusin isoforms, or the involvement of Fis1 or OPA have not yet been investigated. This remains an important area for future investigation in the study of mitochondrial structure and function in muscle.

Protein import

The biosynthesis of the mitochondrion is unique because it requires a high degree of intracellular communication between not one, but two distinct genomes. Given the limited capacity of the mitochondrion for gene transcription and translation, the nuclear genome is primarily responsible for encoding the majority of proteins essential for reticulum expansion and organelle biogenesis. In order for these polypeptides to transverse the mitochondrial membrane and be targeted to specific organelle compartments, they must first associate with, and utilize, a subset of proteins collectively referred to as the mitochondrial protein import machinery [8].

The protein import machinery is divided into two intricate sets of proteins (Figure 4). The first is the TOM (translocase of the outer membrane) complex, which in addition to several outer membrane receptor proteins, contains an approx. 400 kDa general import pore where precursor proteins traverse the outer membrane. The presence of specific targeting signals within the primary structure of a newly synthesized protein dictates not only its localization within the mitochondria, but also the specific translocation pathway that it will take. The TIM (translocases of the inner membrane) complex comprise the second protein import machinery complex. These proteins rely on the mitochondrial membrane potential ($\Delta \psi_m$) for the translocation of precursor proteins across the inner membrane to the matrix.

To date, the regulation of the protein import pathways, and more specifically the dynamics of assembly of these protein import complexes in response
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...has remained elusive. However, there is evidence to suggest that the protein import machinery has the ability to respond to energy perturbations within the cell. For example, distinct mitochondrial subpopulations within skeletal muscle display different import capacities, which are believed to contribute to some of the biochemical and functional differences observed between these mitochondria. Furthermore, in response to an exercise stimulus, it has been shown that several components...

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**Figure 4. Effect of chronic contractile activity on mitochondrial protein import**

Following an exercise stimulus, signalling pathways are activated which up-regulate the expression of several nuclear genes encoding mitochondrial proteins, including genes of the protein import machinery, as well as Tfam. Newly synthesized precursor proteins are subsequently targeted from the cytosol to the outer mitochondrial membrane (OM) via the cytosolic chaperones heat shock protein 70 (cHsp70) and mitochondrial import stimulating factor (MSF). The precursor protein associates with Tom20, Tom22 and Tom70 receptors and is transferred to the intermembrane space (IMS) via the TOM complex. Tim50 and the smaller TIM proteins direct the precursor protein either to the Tim22 channel to be inserted into the inner membrane, or to the Tim23 channel to be pulled into the matrix via the ATP-driven action of mtHsp70 and the membrane potential ($\Delta \Psi_m$). Once inside the matrix, the presequence is cleaved by MPP (mitochondrial processing peptidase) and refolded by Hsp60 and Cpn10 into a mature protein. Mitochondrial proteins such as Tfam bind to mtDNA to induce the transcription and translation of proteins required for oxidative phosphorylation processes and ATP production.
of the import machinery, including cytosolic molecular chaperones, outer membrane receptor proteins, and matrix chaperonins are increased [24,29]. These changes in the expression of the protein import apparatus are believed to contribute to the higher rate of precursor protein import that occurs following chronic contractile activity. As noted above, an increase in the import rate of Tfam observed with seven days of contractile activity may promote the transcription and replication of the mitochondrial genome, leading to increased mtDNA copy number and increased transcription of COX subunits.

The protein import machinery also adapts in response to pathological conditions of disease. In patients harbouring mtDNA mutations, a retrograde signalling pathway is activated, leading to a compensatory response within the nuclear genome. The result of this is an increased expression of specific protein import machinery components in an effort to maintain, or even increase protein import of nuclear-encoded mitochondrial proteins into the organelle [30,31]. The dynamic role of the protein import machinery has further been solidified with the finding that a mutation in DDP (the deafness dystonia protein), contributes to the human disease termed Mohr–Tranebjaerg syndrome, a rare neurodegenerative disorder characterized by hearing loss and dystonia. DDP is the human homologue of Tim8a which is located in the intermembrane space and is responsible for the proper insertion and assembly of the Tim23 protein. In addition, the recent finding that in response to an apoptotic stimulus, DDP is released from the mitochondria into the cytoplasm to promote Drp-1 mediated mitochondrial fission further implicates the importance of protein import in mitochondrial regulation [32].

Thus, given the above findings, it is now known that both changes in the expression, as well the assembly of the protein import machinery complexes into the mitochondrial membrane will affect the rate at which precursor proteins are imported into the mitochondria. Exercise stimulates import machinery expression, as well as import kinetics within the organelle. Whether this is also a result of an augmented assembly of protein import complexes remains to be determined. Theoretically, this improved capacity for import would allow for a lower requirement for rates of transcription and translation, implying a more efficient process of organelle assembly. Much work is still required to solidify the dynamic interactions of the assembly of these complexes in exercise-induced mitochondrial biogenesis.

Conclusions

Mitochondrial biogenesis is now recognized as a vital and exciting area of cell biology, the comprehension of which is relevant to an understanding of a large number of cellular pathological conditions. Exercise can play a significant role in accelerating the rate of mitochondrial biogenesis and likely serves to attenuate mitochondrial dysfunction present in a number of metabolic diseases. The study of the molecular basis of these exercise-induced effects remain
relevant to exercise physiologists, clinicians dealing with mitochondrially based diseases, as well as molecular biologists seeking an understanding of the underlying mechanisms of organelle biogenesis.

Summary

- Mitochondrial synthesis in muscle (biogenesis) is a consequence of endurance training and leads to fatigue resistance.
- Mitochondrial biogenesis is regulated at transcriptional and post-transcriptional levels of gene expression, as well as by fission and fusion processes.
- PGC-1α is an important transcriptional coactivator of nuclear genes encoding mitochondrial proteins, whilst Tfam regulates the expression of mitochondrial DNA.
- Exercise regulates the expression of PGC-1α, Tfam and protein import, but little is known about how exercise affects mitochondrial fission and fusion.
- Understanding the mechanisms by which exercise affects mitochondrial biogenesis can help us understand whether exercise is a viable treatment modality for metabolic diseases involving the organelle.

Owing to space restrictions, references to certain works have not been included in the list below. A complete set of references is available from the corresponding author.

References


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