Mechanisms of Exercise-Induced Mitochondrial Biogenesis in Skeletal Muscle: Implications for Health and Disease

David A. Hood,[*1] *Giulia Uguccioni,*[1] *Anna Vainshtein,*[1] *and Donna D'souza*[1]

ABSTRACT

Mitochondria have paradoxical functions within cells. Essential providers of energy for cellular survival, they are also harbingers of cell death (apoptosis). Mitochondria exhibit remarkable dynamics, undergoing fission, fusion, and reticular expansion. Both nuclear and mitochondrial DNA (mtDNA) encode vital sets of proteins which, when incorporated into the inner mitochondrial membrane, provide electron transport capacity for ATP production, and when mutated lead to a broad spectrum of diseases. Acute exercise can activate a set of signaling cascades in skeletal muscle, leading to the activation of the gene expression pathway, from transcription, to post-translational modifications. Research has begun to unravel the important signals and their protein targets that trigger the onset of mitochondrial adaptations to exercise. Exercise training leads to an accumulation of nuclear- and mtDNA-encoded proteins that assemble into functional complexes devoted to mitochondrial respiration, reactive oxygen species (ROS) production, the import of proteins and metabolites, or apoptosis. This process of biogenesis has important consequences for metabolic health, the oxidative capacity of muscle, and whole body fitness. In contrast, the chronic muscle disuse that accompanies aging or muscle wasting diseases provokes a decline in mitochondrial content and function, which elicits excessive ROS formation and apoptotic signaling. Research continues to seek the molecular underpinnings of how regular exercise can be used to attenuate these decrements in organelle function, maintain skeletal muscle health, and improve quality of life. © 2011 American Physiological Society. *Compr Physiol* 1:1119-1134, 2011.

Introduction

Our understanding of the structure, function, and biogenesis of mitochondria has increased dramatically over the last two decades. In addition to its vital role in cellular energy production, this organelle is now an established participant in cell signaling in apoptosis, and its dysfunction has been found to be central to a broad range of patho-physiological conditions affecting the nervous system, heart, and/or skeletal muscle. Thus, cell biologists and clinicians alike have become interested in how mitochondrial function and dysfunction contribute to cellular homeostasis and maladaptation. In skeletal muscle, mitochondrial content can be altered in response to a change in functional demand. Indeed, the mitochondrial content of the cell corresponds closely to the energy requirements of a given tissue. One of the most dramatic phenotypic alterations in adult skeletal muscle occurs in mitochondria in response to exercise or chronic contractile activity (CCA). Contractile activity-induced mitochondrial adaptations in muscle are highly specific to, and dependent on the type of exercise (i.e., resistance vs. endurance), as well as its frequency, intensity, and duration. The result is an altered muscle phenotype and improved fatigue resistance (62, 64, 101), which is highly correlated with the increase in muscle mitochondrial density and enzyme activity. In contrast, conditions of muscle disuse (e.g., microgravity, denervation, immobilization, sedentary lifestyle, and aging) lead to diminished mitochondrial content and compromised energy production. These findings are very important for exercise physiologists who are interested in the cellular basis of muscle fatigue, and are of practical importance for athletes in training programs. From a health standpoint, these mitochondrial adaptations give us reasons to be optimistic about the role of regular physical activity in ameliorating the contractile and metabolic dysfunction of individuals who were previously sedentary. In particular, exercise alters processes that lead to cell death and myonuclear decay, and is involved in reversing the pathology associated with mitochondrial disease in skeletal muscle. Thus, mitochondrial biogenesis is now recognized to have implications for a broad range of metabolic and functional health issues. Fundamentally, the process of organelle biogenesis is a result of multiple molecular events. These processes primarily include (1) the activation of signaling kinases and phosphatases via calcium, reactive oxygen species (ROS), or metabolic

[*]Correspondence to dhood@yorku.ca

[1]School of Kinesiology and Health Science, York University, Toronto, Ontario, Canada

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intermediates, such as AMP, (2) the induction and/or activation of transcriptional regulatory proteins such as PGC-1α and nuclear respiratory factor (NRF) transcription factors, and the corresponding transactivation of their target genes, (3) the import of newly formed precursor proteins into mitochondria, and (4) the coordinated assembly of both mitochondrial and nuclear gene products into an expanding organelle reticulum. Understanding how this pathway of mitochondrial biogenesis is affected by altered physiological conditions, such as exercise training and aging, will provide valuable insight into the underlying mechanisms that help maintain cellular homeostasis. This review provides a summary of our understanding of the specific steps in the adaptation of skeletal muscle mitochondria to exercise, and examines how these molecular pathways are altered in response to conditions of regular exercise and muscle disuse.

Skeletal Muscle Fiber Types and Mitochondria

Skeletal muscle is broadly categorized into three fiber types according to their metabolic and contractile properties. These are (1) slow-twitch red (STR) fibers containing mainly the myosin heavy chain (MHC) type I isoform, (2) fast-twitch red (FTR) fibers containing mainly the MHC type IIa isoform, and (3) fast-twitch white (FTW) fibers, possessing mainly the MHC type IIb and/or IIx isoforms, depending on the species. In humans, the predominant nomenclature classifies these simply as Type I, IIa, or IIx fibers, which possess distinct contractile protein isoforms, as well as varying levels of enzymes involved in intermediary metabolism that characterize specific fiber phenotypes (100). Each of these muscle fiber types has varying steady-state mitochondrial contents that contribute to their endurance capacity. In humans, the type I fibers have the largest volume fraction of mitochondria, followed by type IIa and then type IIx fibers (65), although considerable overlap exists (111). There is an approximate 3- to 4-fold difference in the capacity for oxidative metabolism between type I (red) and type IIx (white) fibers. Repeated bouts of exercise in the form of training can induce increases in the mitochondrial content of all three muscle types (17, 60, 65), provided that these fibers are recruited. Notably, mitochondrial adaptations will not occur in muscle fibers that are not activated during the exercise bout, consistent with the idea that mitochondrial adaptations produced by exercise are initiated by stimuli within the contracting muscle, independent of humoral influences. This results in an improvement in fatigue resistance which is strongly correlated with the increase in mitochondrial enzyme activity (62).

Traditional endurance training protocols in humans and other mammals have been shown to produce an increase in mitochondrial content usually ranging from 30% to 100% within about 4 to 6 weeks. This results in improved endurance performance that is largely independent of the much smaller 10% to 20% training-induced changes in $\dot{V}o_2$ max (56). The extent of the increase in mitochondrial content in muscle cells depends upon the initial mitochondrial content. A muscle fiber with a low-oxidative white phenotype (type IIx fiber) will increase its mitochondrial content by a greater amount than a more oxidative Type IIa fiber. More recently, work has also emphasized the possibility that mitochondrial biogenesis can occur in response to high-intensity, interval training exercise bouts (45). Indeed, it was shown in 1982 that high-intensity interval training could produce large increases in cytochrome *c* expression in muscle, particularly in those fiber types that are not recruited during lower intensity, mild-to-moderate endurance training conditions (39). Thus, high-intensity, low-duration exercise is a viable alternative for the induction of mitochondrial biogenesis in muscle.

Mitochondrial biogenesis can also be produced using models of simulated exercise, such as CCA produced by electrical stimulation of the motor nerve. This model has the advantage of producing relatively large changes in mitochondrial biogenesis that can occur in a short time (1-4 weeks) (83). Thus, both chronic endurance exercise training and chronic stimulation are the most commonly used models to study the cellular processes involved in mitochondrial biogenesis. In addition, cell culture models of muscle contraction can provide further insight. Chronically stimulated cardiac or skeletal muscle myocytes (24, 33, 68) have been used to describe some of the gene expression responses that precede mitochondrial biogenesis. The term CCA will be employed to describe the role and effects of exercise in all of these experimental models.

Mitochondrial Morphology in Muscle

Electron microscopy of both cardiac cells and skeletal muscle fibers has revealed that mitochondria are localized in two distinct regions: concentrated under the sarcolemma, termed subsarcolemmal (SS) mitochondria, and interspersed throughout the myofibrils, designated intermyofibrillar (IMF) mitochondria. SS mitochondria are proximal to peripherally localized myonuclei, and may serve to provide ATP for membrane or nuclear functions. These mitochondria may also be most relevant to the induction of myonuclear decay during apoptotic signaling (see below). In contrast, we expect that IMF mitochondria are particularly useful in delivering ATP for the myofibrillar ATPase, thereby powering actin and myosin interactions for muscle contraction. Although there are differences between fiber types and various species, the relative proportion of IMF mitochondria is usually at least 75% of the total mitochondrial volume. In addition, these mitochondrial subfractions differ in their adaptability to a stimulus (e.g., training). This suggests that their location within the cell makes them differentially sensitive to a common cellular signal. Under a variety of chronic muscle use and disuse conditions, it has consistently been shown that SS mitochondria adapt to a greater degree, or earlier, than IMF mitochondria. Biochemical analyses of these mitochondrial fractions

require that they can be isolated from the cell with the use of subcellular fractionation techniques. In doing so, it is recognized that (1) the purity of the fractions and (2) the yield relative to the actual cellular content are important issues to keep in mind. Nonetheless, comparisons among treatments (e.g., trained vs. non-trained) are valid, and important functional information about the organelles can be obtained in the absence of a confounding cellular environment. When subcellular fractionation techniques are used to isolate SS and IMF mitochondria, these subfractions exhibit subtle differences in functional, biochemical, and compositional properties (129). This is not surprising given that when membrane fractions are isolated from skeletal muscle, protein compositional differences can be observed even between continuous membrane systems. For example, the protein composition of the sarcolemma at the neuromuscular junction differs from that at other sarcolemmal locations. The sarcoplasmic reticulum (SR) at the junction with the T-tubule is also distinct from its protein composition at the longitudinal SR. Such a situation also appears to exist for mitochondria. These differences also exist between mitochondrial subfractions. In isolated mitochondria, oxygen consumption is usually measured in the absence of ADP (resting respiration, termed state 4) and in the presence of nonlimiting amounts of ADP (activated respiration, termed state 3). State 3 and 4 respiration rates are typically higher in IMF than in SS mitochondria. The resulting ATP concentration inside the organelle is about 2-fold greater in IMF mitochondria (129). Furthermore, inherent differences in ROS production and apoptotic signaling have been previously documented (3). The reasons for this difference in function may be related to their divergent enzyme and lipid compositions. In turn, these biochemical and physiological differences may be due to the local proximity of SS mitochondria to peripheral myonuclei and to a differential capacity of each mitochondrial subfraction for protein synthesis and import (see below).

Mitochondria in muscle exist in an interconnected network, or reticulum, the extensiveness of which depends on the overall oxidative capacity of the tissue, and between SS and IMF regions within the muscle cell (95). This reticular network is a product of dynamic cycles of fission and fusion. The use of fluorescent mitochondrially-targeted probes in myoblasts will ultimately be useful in revealing the continuous mitochondrial fusion and fission events occurring within these cells. The biological function of these dynamic processes permits the exchange of mitochondrial proteins, lipids, and mitochondrial DNA (mtDNA) within a pre-existing mitochondrial network. In addition, fusion and fission allows for the dynamic remodeling of mitochondria, and functions as a quality control mechanism, managing the removal of damaged portions of the mitochondrial reticulum without requiring the loss of an entire network (131). The fission proteins, Fis1 and dynamin-related protein 1 (Drp1), and the fusion proteins, mitofusin 1/2 (Mfn1/2) and optic atrophy factor 1 (OPA1), control these important remodeling processes. The dysregulation of these proteins leads to pathology and mitochondrial dysfunction (58). It is currently unknown whether exercise modulates mitochondrial fission/fusion dynamics. At least some aspects of mitochondrial fusion are altered with exercise, as Mfn2 mRNA levels were reported to increase in humans in the recovery phase following a single bout of exercise (26). Mfn2 promoter activation and mRNA expression depend, in part, on the actions of peroxisome proliferator-activated receptor-γ (PPAR-γ) coactivator (PGC)-1α, a coactivator which is increased with exercise (85). Whether some of the mitochondrial alterations associated with aging and physical inactivity can be attributed to the dysregulation of mitochondrial dynamics remains to be determined.

Implications of Biogenesis for Muscle Metabolism during Exercise

Mitochondrial biogenesis results in an improvement in muscle endurance performance. This is due to biochemical consequences of having a greater capacity for oxidative phosphorylation. First, an increase in mitochondrial content allows for a reduced requirement for glycolytic activation, leading to a lower lactic acid production rate and attenuated glycogen utilization. Evidence also shows that phosphocreatine (PCr) utilization is reduced. This sparing of glycogen and PCr permits a greater reserve of fuel to enhance exercise longevity, and the reduction in lactate levels imply that a lower acidosis will accompany muscle contractions, thereby preventing early fatigue. Second, the greater mitochondrial content promotes an increase in fatty acid fuel oxidation, contributing to the sparing effect of training on carbohydrate oxidation. Thus, exercise training-evoked increases in mitochondrial content imply that a greater fraction of the energy required at a given exercise intensity will be derived from mitochondrial metabolism, and a lower concentration of free ADP (ADP_f) will be required to attain the same level of oxygen consumption. Not only will this lower ADP_f concentration at a given $\dot{V}o_2$ reduce the rates of glycolysis and PCr utilization, but lower rates of AMP formation will also occur, which will reduce the allosteric activation of phosphorylase and AMP kinase. The consequence will be lower glycogen breakdown rates, as well as attenuated signal transduction that mediates downstream events, such as the translocation of glucose transporter 4 (GLUT4) to the plasma membrane. These adaptations predispose the individual toward greater lipid, and less carbohydrate oxidation during exercise, resulting in an enhanced endurance performance.

Overview of the Mechanisms of Mitochondrial Biogenesis

Morphological evidence of mitochondrial biogenesis produced by multiple exercise bouts can be visualized by electron microscopy as an expansion of the mitochondrial reticulum in both SS and IMF regions of muscle fibers. The final

phenotypic adaptation is the cumulative result of a complex series of signaling events that begins with the first bout of exercise in a training program. These signals are known to activate protein modifying enzymes such kinases, phosphatases, and deacetylases that alter protein conformation and activity, resulting in changes in the mRNA expression of nuclear-encoded mitochondrial proteins. Following mRNA translation, the resulting nuclear-encoded proteins are imported into the different mitochondrial compartments, such as the matrix or the inner or outer membrane of the organelle. Some of these proteins are transcription factors that act directly on mtDNA to increase the mRNA expression of mitochondrial gene products and mtDNA copy number. The mitochondrial genome encodes vital components which are critical for respiratory chain function. Thus, to produce an organelle that is functional in providing cellular ATP, contractile-activity-induced mitochondrial biogenesis requires (1) the integration of a multitude of contraction-induced cellular signals and (2) the cooperative and timely expression of the nuclear and mitochondrial genomes.

Contraction-Evoked Signaling Toward Mitochondrial Biogenesis

The initiation of mitochondrial biogenesis in muscle during an exercise training program begins with the putative signals brought about by acute muscle contractions (62). Within seconds of the onset of contractile activity in skeletal muscle, a number of rapid events occur that are involved in the initial signaling process leading to downstream protein and lipid synthesis. These changes include oscillations in cytoplasmic calcium (Ca^{2+}), increases in ATP turnover, oxygen consumption, and ROS production (Fig. 1). Accumulating evidence supports the link between chronic alterations in these events, and distinctive programs of gene expression that establish phenotypic diversity among skeletal myofibers, such as hypertrophy or mitochondrial biogenesis.

The intensity and duration of the contractile effort determines the magnitude of the signal or signals. This signaling can affect (1) the activation or inhibition of transcription factors, (2) the activation or inhibition of mRNA stability factors that mediate changes in mRNA degradation, (3) alterations in translational efficiency, (4) the post-translational modification of proteins, (5) changes in the kinetics of the transport of newly made proteins from the cytosol into the mitochondria (protein import), and (6) alterations in the rate of folding or assembly of proteins into multi-subunit complexes (Fig. 1).

Signaling to these cellular events occurs via the exercise-induced activation of a variety of enzymatic processes. Among those that appear to be involved in mitochondrial biogenesis include calcium/calmodulin-dependent protein kinase (CaMK), p38 mitogen-activated protein kinase (MAPK), and AMP-activated protein kinase (AMPK). The three fundamental cellular processes that occur during each acute exercise bout which activate these proteins include (1) an increase in cytosolic calcium, released from the SR, (2) ATP turnover, leading to the formation of AMP and the activation of AMP kinase, and (3) the production of ROS, derived from a number of sources within muscle, including mitochondria themselves.

Calcium signaling

Ca^{2+} is released from the SR as a result of motor neuron-induced depolarization, whereupon it mediates actin and myosin interaction and muscle contraction. Ca^{2+} also acts as a second messenger to couple the initial electrical events to subsequent alterations in gene expression. Early experiments with muscle cells in culture illustrate that chronic elevations in cytosolic Ca^{2+} results in increases of mitochondrial enzyme activity, as well as increases in binding of nuclear respiratory factors-1 (NRF-1) and -2 (NRF-2) to DNA. Levels of mitochondrial transcription factor A (Tfam) and PPARγ (PGC-1α) protein content are also augmented in the presence of elevated Ca^{2+} (44, 96). Further studies indicated that Ca^{2+} regulates mitochondrial biogenesis by the activation of CaMK, primarily CaMKII. Inhibition of CaMKs *in vitro* completely blocked Ca^{2+}-mediated mitochondrial biogenesis. These data imply that Ca^{2+} released from the SR during each action potential could serve as an important second messenger leading to mitochondrial biogenesis. However, the amplitude, duration, and temporal pattern of the Ca^{2+} signals necessary to provoke such changes must be established under more physiological conditions.

p38 MAP kinase activation

MAP kinases, such as p38, can be activated by a wide variety of stress stimuli, which include cytokines, growth factors, and environmental stressors such as exercise (30). Once activated, MAPKs can regulate multiple cellular processes via phosphorylation of specific serine and threonine residues on target protein substrates. In particular, p38 has been implicated in regulating exercise-induced changes in gene expression via the phosphorylation of target substrates, including transcription factors such as ATF-2, MEF2, and p53, which are known to regulate the expression of mitochondrial proteins (139). For instance, p38 has been shown to phosphorylate the transcriptional coactivator, PGC-1α, and in doing so, increases the ability of PGC-1α to coactivate transcription factors NRF-1 and -2 which regulate the expression of nuclear genes-encoding mitochondrial proteins (NUGEMPs) (109). Using muscle cells and an *in vivo* transgenic model, Akimoto et al. (8) demonstrated that the activation of the p38 MAPK pathway by exercise stimulates PGC-1α promoter activity, and that this could be blocked by inhibition of the kinase. Further, the increase in PGC-1α was enhanced by augmented expression of the downstream transcription factor ATF-2, which was inhibited with overexpression of

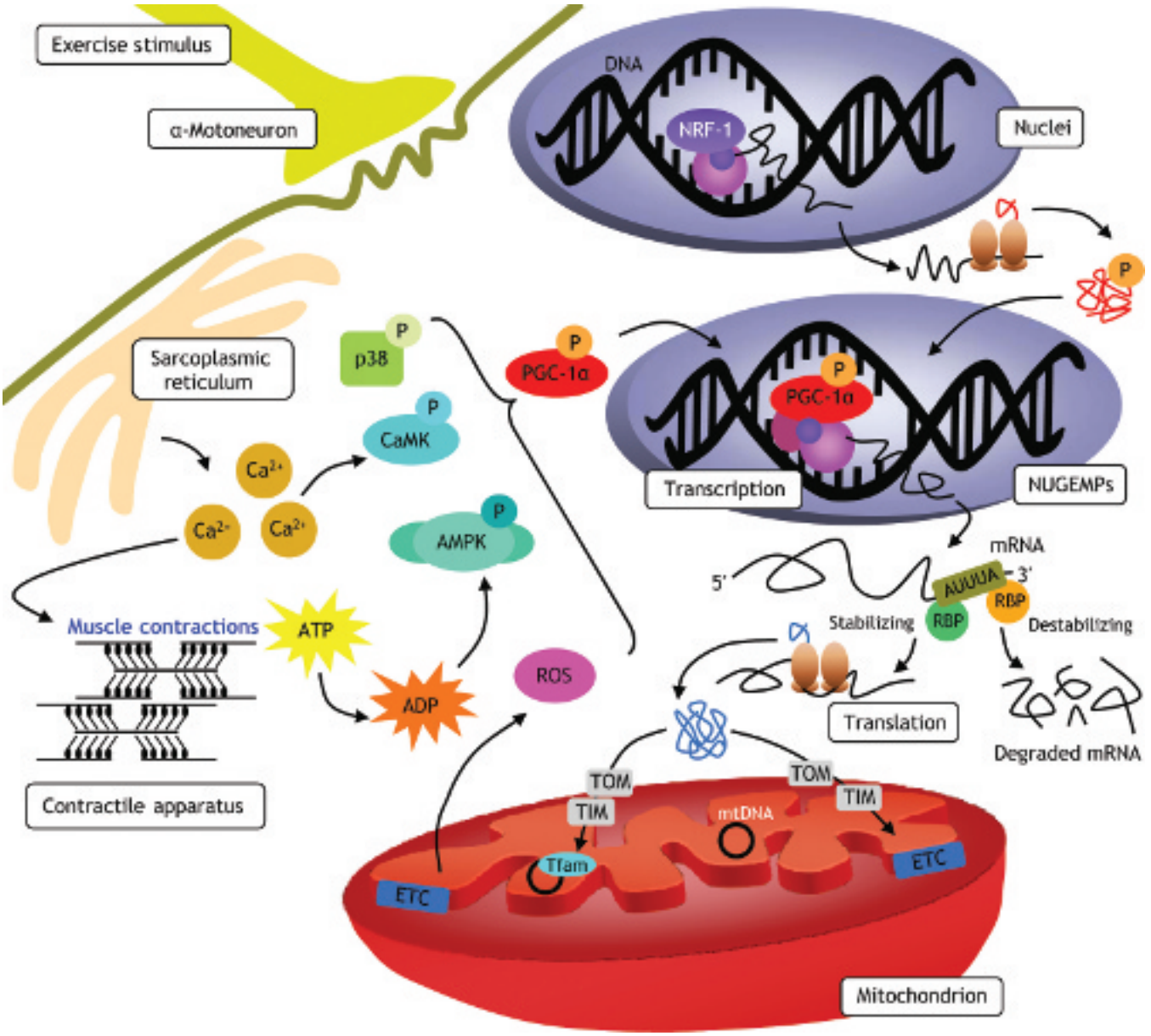

Figure 1 PGC-1α-mediated mitochondrial biogenesis. Action potentials originating at the α-motoneuron propagate electrical activity along the sarcolemma of skeletal muscle. This electrical current is coupled to the release of Ca^{2+} from the sarcoplasmic reticulum. Increases in intracellular Ca^{2+} levels (1) activate Ca^{2+}-sensitive signaling pathways, including CaMK and (2) allow for the initiation of muscle contraction. Continuous muscle contractions promote the formation of reactive oxygen species (ROS) and alter energy levels resulting in the activation of AMPK. Contractile activity also results in the phosphorylation of additional kinases such as p38 MAPK. These signal transduction pathways target transcription factors and the coactivator, PGC-1α. Activated transcription factors, such as NRF-1, regulate the expression of nuclear genes-encoding mitochondrial proteins (NUGEMPs), as well as the transcription and mRNA expression of PGC-1α. Once translated, PGC-1α binds to transcription factors (including NRF-1) to also regulate NUGEMPs. Following transcription, the nascent mRNA is translocated to the cytosol for further modification. Specific sequences (i.e., AU-rich elements) located in the 3′ untranslated region (UTR) serve as sites for RNA-binding proteins (RBPs) and microRNA (miRNA) interaction, both of which can target the transcript for degradation. Stable mRNA products avoid the decay process, and are translated into functional proteins. Newly synthesized mitochondrially-destined proteins are imported into the organelle via the translocase of the outer and inner membrane (TOM and TIM, respectively) complexes. Imported proteins may be transcription factors for mtDNA, such as Tfam. The induction of mtDNA transcription via Tfam increases the expression of proteins encoded by the mitochondrial genome that will join as subunits of complexes in the electron transport chain (ETC). PGC-1α also enhances the transcription of additional mitochondrially-destined proteins that become incorporated into the ETC once imported into the organelle. The coordinated expression of both the nuclear and mitochondrial genomes via PGC-1α contributes to the expansion of the organelle network, and enhances the process of contraction-induced mitochondrial biogenesis.

the dominant-negative version of the protein. In addition, the overexpression of constitutively active MKK3/6, the upstream kinase of p38 in skeletal muscle, also increased PGC-1α protein levels along with downstream mitochondrial targets, such as COXIV (8). Recent evidence indicates that the specific activation of p38γ, and not alternative p38 isoforms, is essential for exercise-induced increases in PGC-1α (103). Thus, these findings suggest that the exercise-mediated activation of the p38 MAPK pathway promotes the expression of PGC-1α and downstream mitochondrial adaptations.

AMP kinase activation

Exercise increases ATP turnover within muscle cells, resulting in a decrease in the ATP-to-ADP_f ratio, the stimulation of metabolism, and an increase in AMP formation. The rise in muscle AMP levels leads to the activation of the metabolic sensor, AMPK. The change in energy status can be achieved experimentally via the uncoupling of the mitochondrial respiratory chain, mtDNA depletion, iron deficiency, genetic disruption of the creatine phosphokinase system, or prolonged use of the pharmacological agent β-guanadinopropionic acid (β-GPA). All systems are well recognized to result in increased phosphorylation of AMPK. As first illustrated by Winder and colleagues (136), the chronic activation of AMPK using the adenosine analogue 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) also increases mitochondrial protein levels, including citrate synthase, cytochrome *c*, and the heme synthesizing enzyme δ-aminolevulinic acid synthase (ALAS). This drug has come into common usage as an exercise "mimetic" because its effects closely mimic those produced by models of chronic exercise (93), although such terminology is misleading because exercise, unlike AICAR, impacts nearly every organ system in the body. These downstream changes in mitochondrial proteins are mediated at least in part by an increase in the expression of the coactivator PGC-1α, to be discussed below.

Reactive oxygen species production

Reactive oxygen species are primarily produced by mitochondria during oxidative phosphorylation. As electrons are transferred along the ETC to form water, approximately 1% to 3% of the electrons are inappropriately donated to oxygen resulting in the production of ROS (62). Mitochondria respiring at a low level of state 4 respiration actually produce the highest amount of ROS per unit of oxygen consumed. During the accelerated respiration evident during exercise (i.e. state 3 respiration), ROS production is reduced on a per mitochondrion basis. However, ROS production from nonmitochondrial sources is increased during exercise (89, 106). The cellular consequences of ROS production depend on the magnitude and duration of the ROS produced. Low levels of ROS production can evoke signaling events that appear to have useful purposes in modifying cellular phenotype (112). However, if ROS are not regulated appropriately they can have deleterious effects on cellular function because they (1) promote the oxidation and degradation of proteins and other macromolecules, (2) lead to the accumulation of somatic mutations and oxidative damage to mtDNA, and (3) activate apoptosis. To combat excessive ROS production, muscle cells possess cytosolic and mitochondrial anti-oxidant enzymes. Therefore, ROS concentrations in muscle are determined by the balance between ROS production and their removal by anti-oxidant proteins and other ROS scavengers.

As noted above, ROS appear to act as important contributors to the signaling that leads to muscle phenotype adaptations to exercise. For example, ROS have been shown to activate both nuclear factor-kappa B (NF-kB) and activator protein-1 (AP-1), which are transcription factors involved in the expression of PGC-1α, NRF-1, manganese superoxide dismutase (MnSOD), copper-zinc superoxide dismutase (CuZnSOD), and catalase (106). Further, the treatment of muscle cells with exogenous ROS activates the promoter of PGC-1α. This leads to increases in PGC-1α mRNA expression via both AMP kinase-dependent and -independent pathways (70), and an increase in NRF-1 (63). Other studies have indicated that the chemically-induced production of ROS can lead to mitochondrial reticulum elongation and branching complexity in fibroblast cells (76). Patients with mitochondrial complex I deficiency who exhibit elevated rates of ROS production demonstrate similar adaptive changes in mitochondrial morphology (77). However, several studies have also demonstrated that an increase in ROS formation results in mitochondrial fragmentation, yielding punctate mitochondria (42, 140). While the specific effects of ROS on mitochondrial morphology remain controversial, it is apparent that ROS do have an influence on mitochondrial dynamics, and that further work is needed to decipher these effects. Thus, it has become clear that ROS are important signals involved in mitochondrial adaptations, and that they likely represent an additional stimulus involved in the mitochondrial response to exercise.

Recent work from our laboratory has illustrated that the activation of specific kinases during contractile activity is a function of contraction-induced increases in aerobic energy cost, represented by $\dot{V}o_2$ (84). During contractions, kinases were differentially sensitive to contraction-induced increments in skeletal muscle $\dot{V}o_2$, which likely influences the activation of downstream proteins during exercise of different intensities, leading to a diversity of phenotypic adaptations (84). Whether this differential kinase activation is related to the variations in ROS produced during exercise remains to be determined.

In summary, the adaptive mitochondrial responses of skeletal muscle to contractile activity appear to be closely related to the intensity and duration of cytosolic Ca^{2+}, ROS, and ATP turnover signals on kinases. It should be noted that these signals likely also converge on other protein modifying enzymes, such as phosphatases and deacetylases, however, these are poorly studied at this time in the context of exercise.

Transcription Factor Activation during Exercise

The intracellular signaling cascades evoked by muscle contractions ultimately converge upon the myonuclei to initiate transcription. Transcription is mediated by many proteins which bind to specific response elements to activate or repress gene expression. A number of these proteins have been associated with mitochondrial biogenesis, including NRF-1 and NRF-2, early growth-response gene-1 (Egr-1, c-fos, c-myc, specificity protein-1 (Sp1), cAMP response element (CRE)-binding protein (CREB), and upstream stimulatory factor-1 (USF-1). The regulatory region of each nuclear gene encoding a mitochondrial protein is heterogeneous, indicating that a universal regulatory protein, or a combination of proteins, does not likely exist. It also renders more difficult the task of fully characterizing the wide array of proteins that regulate transcriptional activity by activation or repression. The diversity evident in the regulatory network suggests that a high level of synchronization between various transcription factors is required to undertake the transcriptional process leading to a functional organelle.

Contractile activity is a powerful inducer of many transcription factors. Those associated with mitochondrial biogenesis, such as immediate early genes (Sp1, Egr-1, *c*-fos, and *c*-Jun) as well as NRF-1, have all been observed to respond rapidly to both acute and CCA at the transcript level (69, 90, 92, 138). Egr-1 and Sp1 are known regulators of cytochrome *c*, and both the Sp1 and NRF-1 binding sites appear to be relatively common features on the promoter region of many genes encoding mitochondrial proteins (40, 116). Most of the research thus far has been directed at NRF-1. NRF-1 null blastocytes (67) are unable to maintain a mitochondrial membrane potential ($\Delta\Psi_m$), and also exhibited deficient levels of mtDNA, likely in part because NRF-1 regulates the expression of proteins that control mtDNA expression, such as Tfam, mitochondrial transcription factor B-1M (TFB1M) and -2M (TFB2M) (46). Similarly, NRF-2 has also been shown to be a key protein in the regulation of multiple subunits of cytochrome *c* oxidase (COX) (115). siRNA-directed inhibition of NRF-2 expression results in decreases in the expression of all 10 COX subunits (97). It is known that the binding of both NRF-1 and NRF-2 to DNA increases during the recovery phase following a single exercise bout (15). Acute stimulation has also been shown to increase the promoter activity of PGC-1α, as well as PGC-1α mRNA levels (9). These increases likely involve the activation of multiple transcription factors, including USF-1 and CREB. Irrcher et al. (71) have shown that the overexpression of USF-1 led to significant increases in both PGC-1α transcriptional activation and mRNA expression in muscle cells. On the other hand, Akimoto et al. (9) observed an attenuation of PGC-1α promoter activity by exercise with mutations in the CRE. The activation of these contractile activity-induced transcription factors all support their roles promoting a change in muscle mitochondrial phenotype as a result of exercise.

Regulation of the PGC-1 Family of Coactivators

The PGC-1 family of coactivators are a very well studied group of nuclear regulatory proteins that are involved in multiple cellular processes including adaptive thermogenesis, glucose metabolism and oxidative capacity across a wide range of tissues (54). PGC-1α was the first identified member of the PGC-1 family, and was originally cloned from a differentiated brown adipose cDNA library on the basis of its interaction with the nuclear hormone receptor, PPARγ (110). PGC-1β and PGC-1α-related coactivator (PRC) also comprise this family and both share a similar involvement in the regulation of mitochondrial content and function, relative to PGC-1α. Specifically in muscle, strong evidence suggests that among the three PGC-1 members, PGC-1α most likely is the dominant regulator of mitochondrial function and respiration, and from this it is not surprising that the coactivator is often referred to as the master regulator of mitochondrial biogenesis.

Function

PGC-1α is a transcriptional coactivator, meaning that it modifies transcription via its ability to coactivate numerous transcription factors. PGC-1α itself does not contain enzymatic activities. Instead, it is able to augment the activity of nuclear receptors through its activation domain on the N-terminus of the protein that binds additional cofactors that contain histone acetyltransferase (HATs) activity. The binding of these proteins alters histone configuration, and thus allows for greater accessibility of the transcriptional machinery to DNA for transcription (54, 108, 133). The C-terminus of PGC-1α is also important because it binds proteins such as the thyroid hormone receptor associated complex (TRAP)/vitamin D receptor interacting protein (DRIP) mediator complex, that aid in the displacement of repressor proteins which would keep DNA in a coiled state, unfavorable for the initiation of transcription (52). Thus, PGC-1α acts as a platform to integrate many proteins involved in the process of gene transcription, and allow for the activating abilities of the coactivator.

Since PGC-1α was first discovered, much focus has been dedicated to its involvement in the regulation of mitochondrial biogenesis (7, 81, 82, 137). This effect is mediated in part by the ability of PGC-1α to coactivate multiple transcription factors involved specifically in the regulation of NUGEMPs [(Fig. 1; (85)]. In particular, coactivation of NRF-1, NRF-2, and ERRα by PGC-1α results in the transactivation of genes involved in the respiratory chain, and transcription factors of mtDNA, such as Tfam (117). More specifically, it is through the binding and regulation of NRF-1 that PGC-1α is able to stimulate mitochondrial proliferation (137). PGC-1β and PRC resemble PGC-1α in that they are also able to bind to and coactivate NRF-1 leading to increased gene expression of mitochondrial proteins (117). Over-expression of PGC-1α in skeletal muscle cells augments nuclear-encoded (e.g., COX

subunit IV) and mitochondrially (e.g., COX subunit II) encoded electron transport chain mRNA levels, and amplifies mtDNA copy number. The ability of PGC-1α, as well as -β and PRC, to integrate the transcriptional activity of these and several other nuclear transcription factors is what provides the coactivators with the capacity to coordinate the large number of genes required for mitochondrial biogenesis Thus, PGC-1α acts as a central regulator to orchestrate the expression of a variety of genes encoded by the nucleus, as well as mtDNA, leading to mitochondrial biogenesis.

Regulation of expression

PGC-1α is expressed in a tissue-specific manner, with the greatest levels of the coactivator in tissues with a high oxidative capacity, such as heart and skeletal muscle (68, 110). PGC-1α mRNA and protein levels are also induced in a variety of tissues in response to multiple stimuli involved in the regulation of mitochondrial biogenesis, including chronic exercise or thyroid hormone treatment (68, 102, 134). In skeletal muscle, the effects of exercise on PGC-1α mRNA and protein levels have been observed following a single bout, or with chronic exercise (14, 68, 102). Interestingly, the regulation of PGC-1α expression in skeletal muscle tends to coincide with signaling pathways typically activated by endurance exercise. The changes in PGC-1α mRNA expression in response to exercise and other stimuli can be largely accounted for by the activation of pathways that target transcription factors and their regulatory sequences within the promoter of PGC-1α. Thus far, the best-characterized signaling mechanisms that have been shown to transcriptionally regulate PGC-1α expression in skeletal muscle include, Ca^{2+}-mediated pathways, activation of kinases, as well as ROS-induced signaling. In particular, elevations in Ca^{2+} stimulate CaMK-dependent binding of CREB to the CRE in the PGC-1α promoter (139). Further, activation of ATF via Ca^{2+} in a p38 MAPK-dependent manner also stimulates binding of CREB to the promoter region of PGC-1α. The activation of signaling kinases, such as AMPK and p38 (as described previously), also results in the induction of PGC-1α and mitochondrial biogenesis in skeletal muscle (8, 68, 71). Treatment of muscle cells with AICAR, a pharmacological activator of AMPK, increases mRNA levels of PGC-1α. This was a result of AMPK-mediated binding of USF-1 to a GATA/Ebox consensus sequence in the PGC-1α promoter (71). ROS also provoked an increase in PGC-1α transcription, in part by H_2O_2-induced binding of USF-1 to the PGC-1α promoter in an AMPK-dependent manner (70). Although these are the major pathways that appear to regulate the transcriptional activity of PGC-1α, the coactivator is also involved in an autoregulatory loop controlling its own level of activity. Some transcription factors that PGC-1α coactivates, such as myocyte-enhancer factor (MEF)-2 and myogenic-determining factor (MyoD), also bind to, and regulate, the PGC-1α promoter (13, 53). Thus, there are multiple, likely overlapping and redundant, signaling pathways that mediate the transcription of this important metabolic coactivator. Greater insight into the mechanisms that regulate PGC-1α is essential to further our understanding on the regulatory aspects of mitochondrial biogenesis in skeletal muscle.

Role of mRNA Stability in Muscle

A myriad of processes regulate gene expression, ranging from the transcription of DNA to the synthesis of protein. While many studies have emphasized the importance of gene activation, few have examined the post-transcriptional events that also influence gene expression. Following transcription, newly synthesized mRNA transcripts are subject to modifications that alter their rate of turnover (Fig. 1). The ability of an mRNA molecule to resist degradation can provide a longer lasting substrate for the process of protein synthesis, resulting in greater protein levels within the cell. The stability of mRNA is usually mediated by protein factors or microRNAs (miRNAs) that interact at the 3′untranslated region (3′UTR) of the transcript to either stabilize or destabilize the mRNA. These are expressed in a tissue-specific fashion, leading to wide variations in the stability of mRNA across tissues. For example, nuclear transcripts encoding mitochondrial proteins appear to be least stable in liver, and most stable in skeletal muscle. The mRNA encoding ALAs, the heme metabolism enzyme, has a very short half-life ($t_{1/2}$) of 22 minutes in liver but has a $t_{1/2}$ of approximately 14 h in skeletal muscle (34). Thus, mRNA stabilization may have the greatest consequence for mitochondrial biogenesis in liver, since modest increases in stability can have a marked effect on mRNA levels in this tissue.

mRNA stability is mediated by a number of cellular factors that interact with target mRNA. For instance, RNA-binding proteins (RBPs) bind to sequences located within the 3′UTR of mRNA to alter rates of mRNA decay (Fig. 1). RBPs have the potential to stabilize or destabilize transcripts, thus influencing overall stability (114). Opposing proteins are able to act independently or synchronously to regulate the mRNAs of labile genes (36). Human-antigen R (HuR) is one of the most commonly studied RBPs, and has been shown to stabilize mRNAs encoding genes involved in myogenesis (43, 118, 119, 132), as well as the anti-apoptotic protein Bcl2 (1). HuR can also induce both the stabilization and translation of p53 mRNA (130), which is regarded as a reputable inducer of apoptosis, cell-cycle arrest, and mitochondrial biogenesis. Though HuR is primarily localized in the nucleus, its movement to the cytoplasm has been associated with mRNA stabilization and translation induction (38). Conversely, ARE/poly-(U)-binding/degradation factor 1 (AUF1) binding destabilizes mRNA, including mRNA encoding the sarcoendoplasmic reticulum calcium ATPase2a (21). Interestingly, HuR and AUF1 can bind competitively to a single ARE (43, 48), suggesting that the fate of multiple mRNA species depends upon a balance in the expression and/or activity of opposing RBPs. Other factors controlling gene expression at the post-transcriptional level

include small noncoding miRNAs or miRs that have the capacity to silence target mRNA through either the repression of translation, or the promotion of transcript degradation (51). The latter is induced by deadenylation of the mRNA transcript, signaling the RNA molecule for decay (41). The cellular factors discussed likely represent only a fraction of stability mediators involved, making additional investigation necessary.

The role of mRNA stability in mediating changes in skeletal muscle gene expression has not been extensively studied. However, mRNA stability comparisons have been made between slow-twitch and fast-twitch muscle. In the presence of extracts made from these muscle types, the half-life of utrophin A mRNA was 2- to 3-fold longer upon incubation with slow, as compared to fast muscle extracts (29). Analysis of the utrophin 3′UTR revealed one conserved ARE, which upon deletion, ablated the destabilizing effect evident in fast-twitch muscle. Thus, it is evident that muscle fiber types distinguish themselves phenotypically using divergent rates of mRNA degradation.

The mRNA stability adaptations in response to CCA have been noted in two studies. In the first, CCA of rat muscle led to rapid increases in the steady-state level of cytochrome *c* mRNA, prior to any changes in transcription rate (44). Thus, the increase in steady-state levels was due to early-onset increases in cytochrome *c* mRNA stability. In the second study it was revealed that the mRNA stability response was transcript specific. CCA led to decreases in the stability of mRNAs encoding important regulatory proteins of mitochondrial biogenesis, including PGC-1α and Tfam, but had no effect on *c*-myc and ribosomal S12 degradation (79). Thus, during conditions of enhanced organelle biogenesis, muscle cells appear to adapt to increase the capacity for mRNA turnover, with the potential of improving the kinetics of adaptation to subsequent exercise stimuli. In contrast, those transcripts which encode structural/functional elements of the electron transport chain (e.g., cytochrome *c*) are more stabilized in response to chronic exercise.

In the study of skeletal muscle mitochondrial biogenesis, mRNA stability is becoming recognized as an important regulatory control point in the gene expression pathway. While modifications to skeletal muscle mRNA stability are apparent with chronic exercise, little information is available regarding the protein participants governing such change. Future work will aim to tease out the mechanisms involved, as well as ascertain if opposing results are evident in conditions of muscle disuse.

Mitochondrial Protein Import

Eukaryotic cells are distinctive in their ability to house both nuclear and mtDNA. Although the mitochondrial proteome comprises about 1500 proteins, only 13 are encoded by mtDNA. Accordingly, an elaborate system is required to transport nuclear-encoded proteins from the cytosol into the mitochondrion. The protein import machinery (PIM) is responsible for taking up these proteins and assembling them into functional complexes within the organelle. It is composed of two primary assembly complexes, the translocase of the outer membrane (TOM complex) and the translocase of the inner membrane (TIM complex; Fig. 1). Despite the extensive characterization of this system in lower eukaryotic cells (23), the components of this pathway, and the mechanisms regulating this process, remain poorly understood in mammalian cells.

Protein import machinery

Many mitochondrial-related proteins are fabricated as precursor proteins, containing a cleavable, positively charged N-terminal pre-sequence that dictates the route of import and final destination (16). Precursor proteins are translocated to the mitochondria and maintained in an import competent state via interaction with cytosolic chaperone proteins, including heat shock proteins (e.g., Hsp70), and mitochondrial import stimulation factor (MSF). Import-ready precursors are subsequently directed to TOM complex receptors, including Tom20 and Tom70. The precursor protein continues its path through the import machinery upon delivery to the TOM complex. This complex consists of a ~400 kDa general import pore (GIP) that is primarily comprised of Tom22, the β-Barrel protein Tom40, and three smaller proteins, Tom7 (61), Tom6 (10, 25, 74), and Tom5 (37). Tom5 is believed to transfer proteins from Tom22 to Tom40, while Tom6 and Tom7 are involved in the import of the Tom40 precursor protein (23). Single-particle imaging of the TOM complex has revealed that each import pore contains a dimer of Tom40, and each ~400 kDa complex consists of ~6 molecules of Tom40. Therefore each TOM complex contains up to 3 import channels (18). In addition to the TOM complex, the outer membrane also contains a separate group of multi-subunit proteins that form the sorting and assembly (SAM) complex. The SAM complex is responsible for the insertion and sorting of outer membrane proteins (i.e., Tom40 and voltage-dependent anion channel (VDAC)) that rely on these protein complexes to mediate their insertion (78).

The arrival of the precursor protein to the TIM complex signals the formation of a TOM-TIM super complex (28). Such cooperation facilitates efficient transport of mitochondrial-destined proteins. Translocation of precursor proteins through the TIM complex is an energy-dependent process, requiring both ATP and a membrane potential ($\Delta\Psi_m$). Similar to the TOM complex, the TIM machinery is made up of a group of proteins that assist in targeting the precursor proteins to the intermembrane space, inner membrane, and matrix. Though several TIM complexes are present in the inner membrane, the majority of mitochondrial proteins are imported through the Tim23 complex. The basic components of this inner membrane complex include: Tim23, Tim50, Tim17, and Tim21 (22, 23). Tim17 and Tim23 span the inner membrane, and have domains associated with both the

matrix and intermembrane space. In a manner analogous to the TOM complex, Tim17 and Tim23 bind the precursor protein and form a pore through which the precursor can travel. The TIM22 complex forms the second complex of the TIM pathway, transporting inner membrane proteins responsible for the exchange of metabolites involved in the inner membrane carrier pathway. Note that these inner membrane proteins possess internal targeting signals that encourage their integration into the inner membrane. Examples include the ADP/ATP (AAC) metabolite carrier family, as well as Tim17 and Tim23 (121). In addition to the TIM23 and TIM22 complexes, the TIM pathway also consists of small TIM proteins that are found within the intermembrane space. These proteins are 8 to 12 kDa in size and are typically characterized by two short motifs, each possessing a pair of cysteine residues that are separated by three other amino acids (twin C×3C motifs). Mutations in the twin C×3C motifs prevent the assembly of the small TIM complexes, as well as the import of Tim23 (59, 113).

Import studies in skeletal muscle mitochondria

Initial studies on the protein import pathway began in the late 1970s, using mitochondria isolated from yeast Saccharamyces cerevisiae and the fungus Neurospora crassa (23). This work has since been extended to mammalian tissues, including liver, heart, and skeletal muscle. In muscle, it is now known that isolated IMF mitochondria have a 2- to 3-fold higher rate of import as compared to the SS subfraction. Experimental inhibition of mitochondrial respiration through inhibition of the electron transport chain dramatically reduced rates of import (129). These results suggest that divergent rates of import are attributed to the distinctive biochemical properties innate to each mitochondrial subfraction, and potentially link rates of import to rates of ATP production and mitochondrial respiration.

A number of studies have shown that exercise in the form of CCA results in an increase in the transport rate of precursor proteins into the matrix of skeletal muscle mitochondria. Part of the reason for this contractile activity-induced adaptation is due to altered expression of PIM components, including Tom20 (47, 98, 127). Contractile activity was also shown to increase expression of the cytosolic chaperones Hsp70 and MSF, as well as the intramitochondrial chaperones Hsp60, mtHsp70, and Cpn10 (94, 98). CCA likely facilitates an increase in the transcriptional activation of these import-assisting genes, thus altering their expression. This is supported by evidence that outlines consensus binding sites for transcription factors NRF-1 and NRF-2 in the promoter region of several import genes, including Tom20 (20, 57). Interestingly, cardiolipin also rapidly increases with chronic muscle use. This occurs at a rate which is faster than the increase in mitochondrial protein accumulation (128), suggesting that the assembly of the mitochondrial reticulum in response to exercise occurs first through the synthesis of membrane phospholipids, followed by the insertion of membrane proteins into the membrane.

Exercise in the form of contractile activity induces increases in the rate of import into the matrix for proteins such as malate dehydrogenase (MDH) and Tfam. This effect is thought to be mediated, in part, by Tom20 (50). The acceleration of Tfam import into the mitochondrial matrix is of particular importance due to its role as an essential mtDNA transcription factor (Fig. 1). Tfam has been shown to promote the transcription and replication of the mitochondrial genome, leading to increased mtDNA copy number (135), and a greater transcription of COX subunits (47). While CCA appears to consistently accelerate the import of matrix-destined proteins, the same trend is not observed with outer membrane proteins. For instance, contractile activity had no effect on the import of Bcl-2 import into the outer membrane (127), but was able to increase Tom40 import (72). This suggests that the import of proteins is protein-specific, and is perhaps related to protein function within the organelle.

Disuse and aging

Muscle disuse results in an impaired pattern of PIM expression that subsequently reduces import of matrix proteins (120). This reduction in protein import is closely correlated to the changes in mitochondrial respiration observed, suggesting that endogenous ATP production is vital for the import mechanism. In addition, the reduction may be a result of increases in ROS production by the mitochondria as a result of chronic muscle disuse, since exogenous ROS have a marked inhibitory effect on the rate of import into the organelle. It is also noteworthy that this adaptation is clearly distinct from the changes observed in aging muscle, despite similar changes in mitochondrial content. Surprisingly, mitochondria from aging muscle display an increase in mitochondrial protein import, despite augmented rates of ROS production (66). An accelerated assembly of the outer membrane multi-subunit TOM complex was also observed in mitochondria isolated from muscle of old animals (72). Similarly, partial or complete depletion of mtDNA in cultured cells induced accelerated protein import (73, 86) despite exhibiting impaired maintenance of $\Delta\Psi_m$ and reduced ATP synthesis (35, 73). These results implicate the involvement of a compensatory response of the protein import apparatus that attempts to sustain protein translocation and mitochondrial assembly under conditions of reduced mitochondrial content or energetic deficiency.

Mitochondrial protein import is an important constituent of the biogenesis process, permitting the integration of many nuclear-encoded proteins required for proper organelle development. The rate of import is altered in response to changing energy demands, and is likely a result of modified PIM gene expression, as well as the rate of ATP production. The physiological value of this adaptation is that the capacity for protein import is increased, producing mitochondria that are more sensitive to small changes in precursor protein concentration in the cytoplasm.

Mitochondrial DNA and Exercise

Mitochondria are unique in their ability to house multiple copies of a small circular DNA molecule (mtDNA) comprising 16,569 nucleotides. While mtDNA is tiny in comparison to the 3 billion nucleotides found in the nuclear genome, it contributes 13 mRNA, 22 tRNA, and 2 rRNA molecules that are essential for mitochondrial biogenesis and function. The 13 mRNA molecules all encode protein components of the electron transport chain. The import of nuclear-encoded mtDNA maintenance proteins is necessary to transcribe and replicate this genome. These proteins include Tfam, mtRNA polymerase, DNA polymerase (POL-γ), single-stranded-binding protein (mtSSB), RNA processing enzymes, and TFBM1 and TFBM2 (87, 91). Tfam has been studied most extensively because of its importance in both mtDNA transcription and replication. Tfam participates in the transcription of mtDNA by binding to, and subsequently fostering a change in shape of the DNA molecule that permits mitochondrial RNA polymerase interaction (Fig. 1). Tfam does not act alone in this process, and is instead reliant on the assistance of either TFBM1 or TFBM2 (31, 99). Transcription yields a single RNA molecule, which is appropriately spliced into different mRNA, tRNA and rRNA products, contributing to the expression of proteins incorporated within the electron transport chain (31). Unlike transcription, Tfam regulation of mtDNA replication is indirect, and occurs independently of nuclear gene replication. Regulation of replication is an essential function of Tfam, as its levels correlate well with mtDNA abundance (80, 105). Loss of Tfam, either by experimental disruption of the gene, or the development of mitochondrial myopathies leads to partial or total depletion of mtDNA.

mtDNA content is proportional to oxidative capacity in a variety of tissues. During the induction of mitochondrial biogenesis using CCA, mtDNA copy number and mRNA transcripts are elevated (135). In humans, endurance training in healthy aged men and women also induced an improvement in mitochondrial ETC activity in conjunction with a ~50% increase in mtDNA copy number (88). This is likely to be due, in part, to an exercise-induced increase in the expression of Tfam. Skeletal muscle exposed to CCA for 7 days resulted in elevated Tfam mRNA levels on day 4 (47), followed by an accelerated rate of protein import into the mitochondrial matrix, increasing mitochondrial Tfam content. Subsequently, this resulted in greater protein-DNA binding on day 7, producing a parallel increase in mitochondrial transcripts and function, as measured by COX activity (47) A similar response in human muscle was observed following a 4-week endurance training regime (19). Tfam mRNA levels preceded concomitant changes to mitochondrial content and function, suggesting that endurance exercise induces mitochondrial biogenesis, in part, through the regulation of Tfam transcriptional activity. An investigation of the Tfam promoter and expression indicates that the transcription of Tfam is regulated by PGC-1α, through the co-activation of NRF-1 (137). Given the importance of Tfam in organelle biogenesis, further investigation into its regulation is required.

Mitochondrially-Mediated Apoptosis in Muscle

Muscle is unique in its ability to adapt to environmental demands. It has been well documented that under conditions of chronic muscle disuse, such as prolonged bed rest, limb immobilization (i.e., casting), denervation, and microgravity (e.g., space flight), skeletal muscle atrophies and loses strength and function. This type of muscle wasting has been observed with aging as well. Conversely, endurance training results in muscular adaptations that are beneficial in promoting a healthier muscle phenotype. With exercise training, skeletal muscle becomes more oxidative and is better protected from oxidative stress. An elevation in the generation of ROS has been documented, with disuse as well as with progressive aging, in skeletal muscle (27, 55). ROS production from mitochondria is also accelerated in muscle subject to chronic disuse. When the production of ROS exceeds cellular antioxidant capability, resulting in oxidative stress, apoptosis is triggered (126). Apoptosis is a tightly regulated form of programmed cell death, the aberrant regulation of which may lead to pathological conditions including tumorigenesis, autoimmune, and neurodegenerative diseases (5, 6). Apoptosis has also been implicated in disuse-related muscular atrophy as well as sarcopenia (104). Thus, the increases in ROS observed with aging may predispose the myofiber to mitochondrially-mediated apoptosis. To date, although these apoptotic signaling cascades have been studied extensively, some of the functions and interactions of the major participants in the apoptotic machinery remain poorly characterized. ROS-induced apoptotic events have been linked to the phosphorylation of *c*-Jun-*N*-terminal kinase (JNK) and the recruitment to the mitochondria of the structurally-related Bcl-2 family of proteins (Fig. 2). These proteins function collectively to regulate organelle membrane integrity (5). Bcl-2-associated X protein (Bax) and Bak are pro-apoptotic members of the Bcl-2 family which exist as monomers in the cytosol. Upon the initiation of an apoptotic stimulus, Bax undergoes a conformational change which allows it to homo- or hetero-oligomerize with Bak, and subsequently translocate and insert into the mitochondrial membrane (3). This step is believed to be critical, but not exclusive, for mitochondrial permeabilization and the formation of the mitochondrial permeability transition pore (mtPTP). In addition, the actin binding protein cofilin-2 was recently found to translocate into the mitochondria and help facilitate membrane permeabilization (75). The pathway by which this takes place, however, remains unknown. The opening of the mtPTP is rapidly followed by an efflux of apoptogenic factors such as cytochrome *c*, apoptosis-inducing factor (AIF), endonuclease G (Endo G), and Smac/DIABLO (Smac D). These proteins can either directly or indirectly cause DNA fragmentation, resulting in the decay of the myonucleus, and

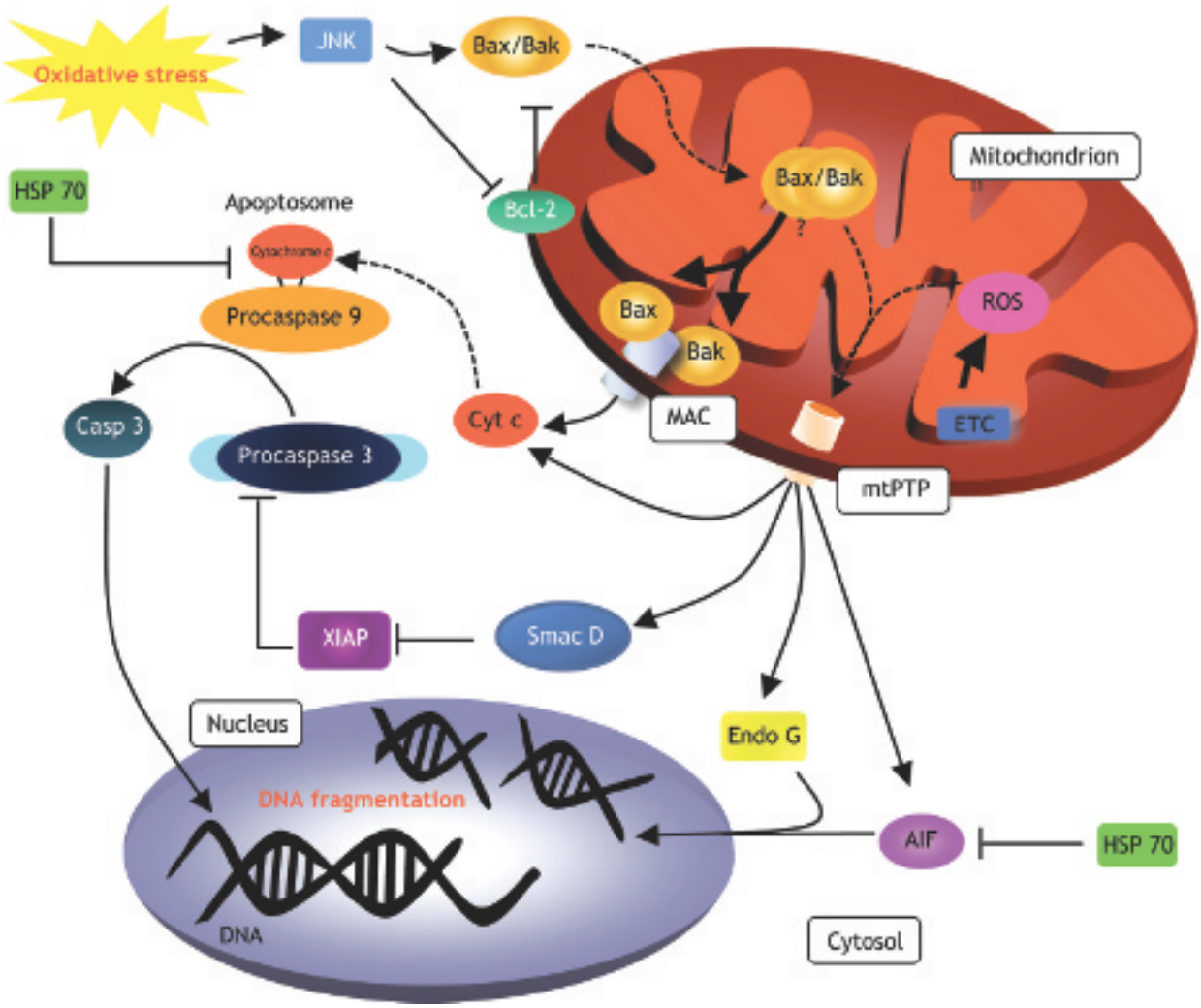

Figure 2 Mitochondrially mediated apoptotic pathway. In response to oxidative stress, kinases such as JNK activate Bax/Bak and other Bcl-2 family member proteins. Bax/Bak then translocate to the mitochondria where they oligomerize and insert into the mitochondrial outer membrane. Bax translocation induces mitochondrial permeabilization either via the permeability transition pore (mtPTP) or via formation of the MAC. The pores facilitate the rapid release of cytochrome c, apoptosis-inducing factor (AIF), EndoG and Smac/DIABLO (Smac/D). All of these proteins can either directly or indirectly cause DNA fragmentation, ultimately inducing nuclear decay. The indirect pathway is caspase-dependent and occurs upon the liberation of cytochrome c from the mitochondria. Cytochrome c then interacts with apoptotic protease-activating factor 1 (APAF-1), dATP as well as caspase 9 (casp9) to form the apoptosome. The apoptosome activates caspase 3 (casp3) which translocates into the nucleus and induces DNA fragmentation. AIF and Endo G induce cell death in a caspase-independent manner, by inserting into the nucleus and causing DNA fragmentation directly. Bcl-2 is localized to the mitochondrial outer membrane where it opposes pro-apoptotic activity by (1) preventing Bax oligomerization and (2) prohibiting the release of proapoptotic factors. Heat shock protein 70 (HSP 70) is another apoptotic inhibitor that prevents apoptosis by directly inhibiting AIF-induced chromatin condensation, or by disrupting the APAF-1 and cytochrome c association, thus halting apoptosome formation. XIAP acts to inhibit DNA fragmentation by inhibiting caspase activity. The role of exercise in mediating changes in the expression, translocation and function of these proteins remains to be fully elucidated.

ultimately in the demise of the myonuclear domain that it governs (Fig. 2).

Differential susceptibility of SS and IMF mitochondria to apoptosis

Muscle possesses several unique properties which make it an interesting tissue for the study of apoptosis. Muscle is multi-nucleated in nature, possessing both SS mitochondria and IMF mitochondria (32, 64). When compared to SS mitochondria, IMF mitochondria release 2.5- and 10-fold greater amounts of cytochrome *c* and AIF, respectively (3). In accordance with this, the maximum rate of mtPTP opening in IMF mitochondria was also found to be significantly greater than that of SS mitochondria. Despite releasing less AIF and cytochrome *c* in response to ROS, SS mitochondria possesses a 10-fold greater Bax:Bcl-2 ratio, 2.7-fold higher rate of ROS production, and an approximate 2-fold greater membrane potential (3). Given the apoptotic vulnerability of IMF mitochondria, and the fact that IMF mitochondria constitute a much larger percentage

(≈80%) of total mitochondria in muscle compared to SS mitochondria (~20%), IMF mitochondria appear to play a more prominent role in mediating apoptosis in muscle.

Chronic muscle disuse

Hindlimb suspension, microgravity, limb immobilization, and denervation are well-established animal models of muscle disuse. These conditions lead to a reduction in muscle activity and cause dramatic declines in both overall muscle mass and mitochondrial content (107). Although the exact mechanisms responsible for disuse-related muscular atrophy are currently unknown, apoptosis has been implicated in this condition. Muscle disuse resulting in atrophy, correlates closely with increased incidence of mitochondrially mediated decay (4, 120, 124), along with a pronounced reduction in mitochondrial content. Work by Allen et al. demonstrated that the typical decreases in muscle mass observed with chronic disuse occurred coincident with a reduction in the number of myonuclei (11). Apoptotic deletion of myonuclei has been found to lead to a genetic shortage that initiates remodeling processes. A reduction in fiber size follows, to maintain a constant nuclear-to-cytoplasm ratio (i.e., the myonuclear domain). Given the prominent role of mitochondria in apoptotic induction, a reduced mitochondrial content following muscle disuse may suggest reduced apoptotic susceptibility. However, it appears that the remaining reduced mitochondrial pool is more susceptible to apoptotic stimuli, coinciding with increased DNA fragmentation.

A number of studies have attempted to elucidate the initial signaling pathways involved in triggering atrophy and apoptosis during muscle disuse. Early experiments in this area showed that the expression of the inhibitor of differentiation-2 (Id2) protein, a negative regulator of the myogenic transcription factor family, was elevated following 7 or 14 days of unloading in quail skeletal muscle (12). Increased Id2 expression occurred coincident with the highest caspase activation and the greatest loss of muscle mass during the unloading phase, suggesting that Id2 may be involved in mediating the apoptosis-related atrophy of skeletal muscle associated with muscle unloading. A subsequent study in rat muscle illustrated that Id2 and p53 were both significantly elevated following hindlimb unloading (125). In contrast, denervation in rats did not result in elevated Id2 expression, but significant increases in Id1 and Id3 levels were found. This difference emphasizes that the model of muscle disuse may determine the signaling pathway leading to apoptosis in muscle. In addition, 2 weeks of microgravity led to augmented p53 protein levels, a transcription factor that increases the expression of Bax (49). Given that Bax is an important pro-apoptotic component in mitochondrially mediated cell death, Siu and Alway (122) investigated denervation-induced muscle apoptosis in Bax null mice. This study showed that DNA fragmentation, cytochrome *c* release, oxidative stress markers, and caspase-3 activity were either attenuated, or unaltered, in denervated muscle from Bax null mice. Taken together, these data indicate that multiple pathways and proteins are involved in mediating the mechanisms of apoptosis following muscle unloading.

Chronic muscle use/exercise

The extent to which apoptotic mechanisms are evoked in normal, healthy muscle following exercise is largely unknown. Endurance training and CCA induce a multitude of adaptations within skeletal muscle, providing improved endurance performance which is due, in part, to an increase in mitochondrial biogenesis (62). Although mitochondrial biogenesis is clearly an advantageous metabolic adaptation, the effect on mitochondrially mediated apoptotic signaling has only recently been investigated. CCA has been found to attenuate both cytochrome *c* and AIF release when subjected to a maximal ROS stimulus, despite the presence of higher levels of these proteins within the mitochondrial subfractions. Furthermore, ROS production was also significantly suppressed by CCA, however, this was only evident in IMF mitochondria (2).Thus it appears that contractile activity-induced anti-apoptotic adaptations occurred preferentially within the more ROS-sensitive IMF subfraction. Since IMF mitochondria comprise the largest fraction of the total mitochondrial content, these data suggest a protective effect of CCA is mediated, at least in part by reduced apoptotic susceptibility to stimuli such as ROS (2). Another study showed that apoptosis and apoptotic-related signaling was also significantly attenuated in both cardiac and skeletal muscle following 8-week endurance training. These attenuated apoptotic responses following training were likely attributed to elevations in the apoptotic suppressors x-linked inhibitor of apoptosis protein (XIAP), apoptosis repressor with caspase recruitment domain (ARC), and FLICE inhibitory protein (FLIP) (123). Thus, CCA or exercise may serve as a therapeutic intervention to combat the onset of apoptotic events evident during age-induced sarcopenia, muscle diseases, or periods of chronic muscle disuse. Further investigation is required to establish: (1) the kinetics of the signaling response and the translocation of proteins to apoptotic triggers; (2) the quantitative contributions of apoptosis to muscle atrophy; and (3) the role of exercise in attenuating myonuclear decay in muscle wasting conditions.

Significance and Health Implications

Skeletal muscle mitochondrial biogenesis and turnover are highly intricate processes, offering many opportunities for regulation. Chronic exercise has been well established as a major stimulant for organelle synthesis, and it does so through the induction of a number of concomitant changes that range from the upregulation of nuclear gene expression, to the acceleration of protein import and mRNA turnover. Enhanced mitochondrial content observed as a result of chronic muscle use serves to attenuate the possible mitochondrial dysfunction that arises during aging and conditions of muscle disuse, thereby improving work performance and the quality of life.

As such, further understanding of the molecular mechanisms involved is warranted, as it can lead to the development of alternative therapeutic interventions that will benefit those unable to perform regular physical activity.

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