CHAPTER FIVE

Exercise and the Regulation of Mitochondrial Turnover

David A. Hood[1], Liam D. Tryon, Anna Vainshtein, Jonathan Memme, Chris Chen, Marion Pauly, Matthew J. Crilly, Heather Carter

Muscle Health Research Centre, School of Kinesiology and Health Science, York University, Toronto, Ontario, Canada

[1]Corresponding author: e-mail address: dhood@yorku.ca

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Abstract

Exercise is a well-known stimulus for the expansion of the mitochondrial pool within skeletal muscle. Mitochondria have a remarkable ability to remodel their networks and can respond to an array of signaling stimuli following contractile activity to adapt to the metabolic demands of the tissue, synthesizing proteins to expand the mitochondrial reticulum. In addition, when they become dysfunctional, these organelles can be recycled by a specialized intracellular system. The signals regulating this mitochondrial life cycle of synthesis and degradation during exercise are still an area of great research interest. As mitochondrial turnover has valuable consequences in physical performance, in addition to metabolic health, disease, and aging, consideration of the signals which

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control this cycle is vital. This review focuses on the regulation of mitochondrial turnover in skeletal muscle and summarizes our current understanding of the impact that exercise has in modulating this process.

1. INTRODUCTION

Exercise is a powerful metabolic stress. When performed repeatedly over the course of several weeks, exercise leads to adaptations in skeletal muscle which allow it to meet the increased metabolic demand. One of the most dramatic examples of this phenotypic adaptation is the increase in mitochondrial content within muscle, which is coincident with a reduction in fatigability and an improvement in endurance performance. In contrast to the positive effects that exercise brings about to increase mitochondria, chronic muscle disuse results in the opposite change. Chronic physical inactivity leads to decrements in organelle content and function within muscle, poor performance, and an increase in apoptotic susceptibility. The metabolic derangements associated with inactivity and the loss of mitochondria include a greater storage, rather than oxidation of lipids, and a tendency for obesity and insulin resistance. Aging also brings about phenotypic changes in mitochondria within muscle, attributed to both inactivity and inherent, aging-induced changes in organelle synthesis and degradation pathways. Thus, opposing changes in the mitochondrial network within muscle cells induced by chronic exercise, aging, or disuse are now recognized to have implications for a broad range of health issues. Notably, regular exercise can counteract many of aging- and inactivity-induced detrimental effects observed on metabolism associated with defective mitochondria, and research efforts must continue to seek the molecular underpinnings of how this is brought about.

Our understanding of the structure, function, and turnover of mitochondria has increased tremendously over the last two decades, thanks to an emerging appreciation for the diversity of functions in which the organelle partakes. Long known for their vital roles in cellular energy production, mitochondria are now established participants in calcium (Ca^{2+}) handling, cellular signaling, and organelle-mediated apoptosis. In addition, mitochondrial dysfunction, brought about by either nuclear or mitochondrial DNA (mtDNA) mutations, can lead to a wide variety of pathophysiological conditions affecting a number of organ systems, notably skeletal muscle, the

heart, or the brain. Thus, both basic and clinical scientists have become keenly interested in how mitochondrial function and dysfunction contribute to cellular health and disease.

2. OVERVIEW OF MITOCHONDRIAL TURNOVER

The steady-state mitochondrial content of muscle at any time, along with the quality of the organelle pool, is a product of complex pathways of synthesis (biogenesis) and degradation (mitophagy). The synthesis of a new, higher level of functional organelles in response to exercise is the cumulative result of a series of events that begins with the very first bout of exercise in a training program. As discussed in detail below, each acute exercise bout initiates a new cascade of signaling events involving the activation of protein enzymes that modify the activity of downstream proteins such as transcription factors (TFs) that impact the expression of *nu*clear *g*enes *e*ncoding *m*itochondrial *p*roteins (NuGEMPs). The result is an increase in the level of mRNAs which encode precursor proteins destined for mitochondrial localization. Once translated in the cytoplasm, the resulting nuclear-encoded proteins interact with protein chaperones and are delivered to the mitochondria. These mitochondrial-destined proteins are subsequently imported into the different compartments, such as the matrix space or the inner or outer membrane. A subgroup of these proteins include TFs, such as mitochondrial transcription factor A (Tfam), that act directly on mtDNA to increase the mRNA expression of mitochondrial gene products and mtDNA copy number. mtDNA is critical because it encodes 13 vital proteins involved in the mitochondrial respiratory chain. Thus, mitochondrial biogenesis requires the coordinated cooperation of both the nuclear and the mitochondrial genomes to produce an organelle that is functional in providing cellular adenosine triphosphate (ATP).

In contrast to the process of biogenesis, regulation of mitochondrial content and quality is also exerted at the level of mitophagy, the selective degradation of mitochondria by the autophagosome–lysosome system. As discussed below, mitophagy is activated by similar signaling mechanisms such as biogenesis, suggesting that a coordinated regulation of these pathways exists. Rates of mitophagy are enhanced during physiological conditions of increased reactive oxygen species (ROS) production, or when the mitochondrial membrane potential decreases. When this occurs, the affected dysfunctional portion of the organelle reticulum undergoes fission to separate it from the remaining "healthy" mitochondrial network, and the

organelle fragment is targeted for degradation. Considerably less is known about the regulation of mitophagy, in comparison to biogenesis, particularly in skeletal muscle, and during exercise.

3. MITOCHONDRIAL MORPHOLOGY AND CHANGES WITH TRAINING

Skeletal muscle contains a heterogeneous pool of mitochondria, distinguished by biochemical composition, function, and subcellular location.[1] Mitochondria may be concentrated under the sarcolemma, termed the subsarcolemmal (SS) mitochondria, or localized in proximity to the contractile apparatus, regarded as the intermyofibrillar (IMF) mitochondria. However, the distinction among subpopulations is not absolute, and there is likely a degree of continuity between them,[2] as mitochondria form a dynamic reticulum,[3] the extensiveness of which is correlated to the oxidative capacity of the tissue.[4] This organelle network is continuously remodeled by fusion and fission events which add to or subtract from it, respectively. The ability of mitochondria to physically interact and alter their morphology enables quality control processes to take place,[5] serves to preserve mtDNA integrity, and influences their respiratory capacity and ROS production. Furthermore, as muscle fibers can be quite large in size, expansion of the mitochondrial reticulum favors the rapid delivery of oxygen to the mitochondrion for consumption and ATP production.

In mammalian cells, mitofusin-1 (Mfn1) and -2 (Mfn2), in addition to optic atrophy factor 1 (Opa1), are mitochondrial membrane proteins regulating the fusion process (Fig. 1). They are essential factors contributing to the coordinated fusion of the outer and inner membranes of the mitochondria, respectively. Fusion processes promote the generation of networks with continuous membranes and a matrix lumen which will facilitate the mobility of mitochondrial proteins, lipids, and mtDNA. Conversely, mitochondrial membrane fission is mediated by dynamin-related protein 1 (Drp1), in association with fission protein 1 (Fis1). Drp1 function requires recruitment to the mitochondria from the cytosol, where it forms an oligomeric structure triggering mitochondrial fission. These division events can be localized to specifically damaged areas of the reticulum, ensuring the selective removal of damaged regions of the network.[5]

Pioneering work by Gollnick and King provided the first suggestion that endurance training has the capability to alter mitochondrial ultrastructure.[6] Endurance training also increases the extensiveness of the mitochondrial

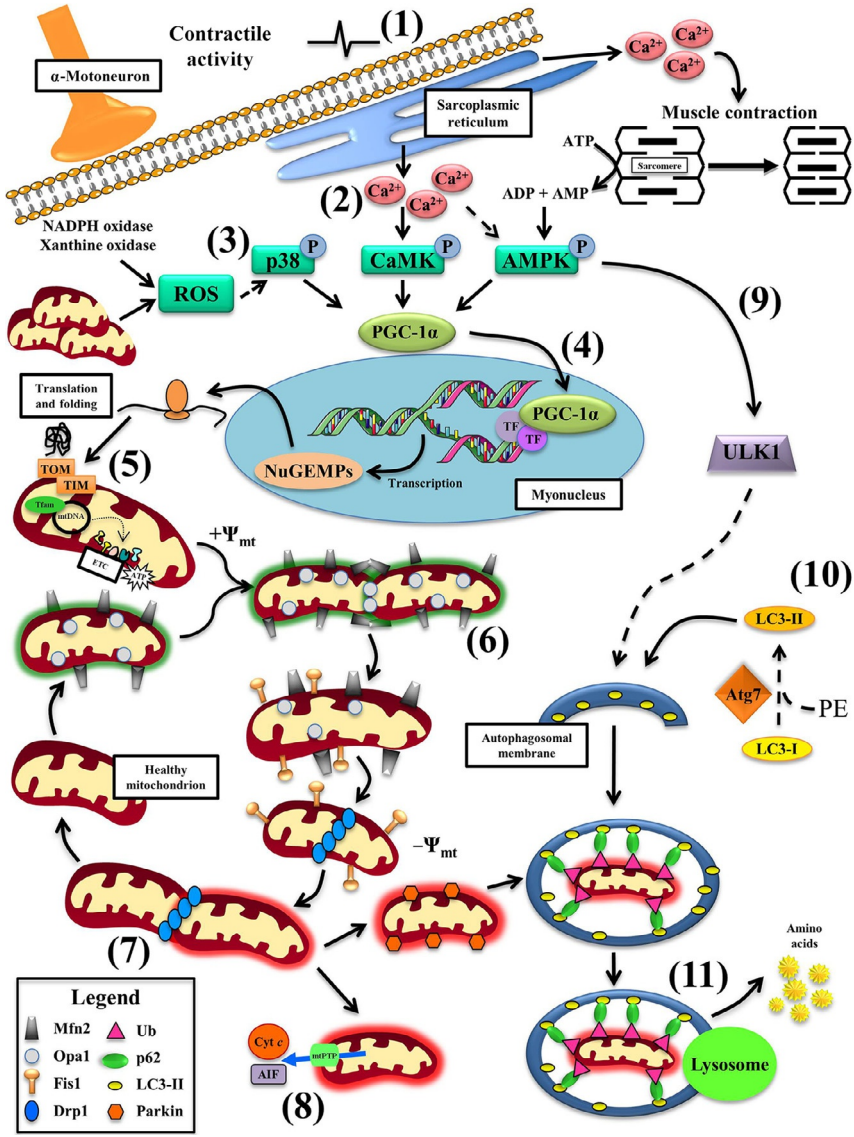

Figure 1 Effect of exercise and training on mitochondrial turnover. During muscle contraction, action potentials propagate down α-motoneurons which innervate muscle fibers (1). Electrical signals are transmitted along the sarcolemma of skeletal muscle and are coupled with the release of Ca^{2+} from the sarcoplasmic reticulum (2). Increases in intracellular Ca^{2+} levels allow for muscle contraction to occur, while also activating Ca^{2+}-sensitive signaling pathways. Contractile activity also consumes cellular ATP, causing a decrease in ATP/ADP ratio and an increase in the formation of AMP, which can activate AMP-activated protein kinase (AMPK). Reactive oxygen species (ROS)

(Continued)

reticulum.[7] Although it is well established that exercise increases mitochondrial content, the regulatory mechanisms which promote these exercise-induced adaptations have only recently been investigated. Recent work utilizing a model of chronic contractile activity showed that Opa1 and Mfn2 were significantly increased, with concomitant reductions in Drp1, providing a molecular basis for the observed changes in mitochondrial ultrastructure.[8] Additionally, the mRNA expression of mitochondrial fusion factors Mfn1 and Mfn2 also increases during the recovery period from a single

Figure 1—Cont'd production from the mitochondrial electron transport chain (ETC) and other intracellular sources are also enhanced during muscle activity, likely leading to the phosphorylation of kinases such as p38 MAPK (3). These signal transduction pathways target transcription factors (TFs), as well as the transcriptional coactivator peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α), which stimulates mitochondrial biogenesis (4), among other beneficial adaptations to skeletal muscle. PGC-1α is especially critical in augmenting the transcription of NuGEMPs, which need to be translated and imported into the mitochondrion. Additionally, other nuclear-encoded factors such as mitochondrial transcription factor A (Tfam) must be imported into the mitochondrion, where they can interact with mitochondrial DNA (mtDNA) to assist the expression of mtDNA-encoded subunits of the ETC (5). This coordinated expression of both nuclear and mitochondrial genomes is key to the expansion of the mitochondrial pool. Chronic muscle activity also increases the ratio of fusion-to-fission proteins, promoting the fusion of mitochondria to form a reticular network (6). Essential mammalian skeletal muscle fusion proteins involved in these processes include Mfn2 and the inner mitochondrial membrane protein Opa1, which are necessary for fusion of the outer and inner mitochondrial membranes, respectively. Under conditions of cellular stress, mitochondrial fission can occur, allowing for the isolation of dysfunctional components of the organelle (7). Mitochondrial fission is executed by the formation of a Drp1 oligomer and can occur due to a reduction in mitochondrial membrane potential (Ψ_{mt}) to a portion of the organelle. Mitochondrial fission precedes apoptosis, and exercise training can reduce the susceptibility of both skeletal and cardiac muscle to this process, likely by reducing release of proapoptotic factors, such as cytochrome *c* and apoptosis-inducing factor (AIF) from the mitochondrion (8). Mitochondrial fission is also coupled with mitophagy, the specific degradation, and recycling of dysfunctional mitochondria through the autophagosome–lysosomal system (9). This process is thought to occur through AMPK/ULK1 signaling, in cooperation with the activation of other mitochondrial-specific kinases and ubiquitin ligases, such as Parkin. The activation of this pathway is also crucial for the formation of the autophagosomal membrane, which occurs through the Atg7-mediated lipidation of LC3-I with phosphatidylethanolamine (PE), to form LC3-II (10). Specific interactions between ubiquitinated proteins (Ub) on the mitochondrion and autophagosomal membrane are facilitated by p62, ensuring the precise engulfment of the malfunctioning component of the mitochondrial network. The autophagosome subsequently fuses with a lysosome, and its cargo is degraded and recycled within the cell (11).

bout of aerobic exercise.[9] Thus, the development of a reticular network likely begins after a single bout of exercise. Mice that were subjected to an acute exercise bout exhibited an increase in membrane interactions between skeletal muscle mitochondria in both the SS and IMF subpopulations, prior to any increase in the expression of mitochondrial fusion proteins.[10] Taken together, these data suggest that signaling to instigate mitochondrial remodeling appears to be one of the earliest events which occur in response to acute exercise. Subsequently, chronic muscle use shifts the expression of fission and fusion machinery to favor enhanced fusion, setting the stage for an intracellular environment more capable of matching the increased metabolic demands of skeletal muscle during training.

4. EXERCISE-INDUCED SIGNALING: A ROLE FOR AMPK

Mitochondrial biogenesis which occurs in response to an exercise training program originates from a variety of intracellular signaling events generated by muscular contraction (Fig. 1). Among these signals is the increase in ATP turnover within muscle cells during exercise, which consequently increases the ADP/ATP ratio of cellular ADP to ATP,[11] and simultaneously the formation of AMP. The elevated levels of AMP allosterically activate the heterotrimeric metabolic energy sensor, AMP-activated protein kinase (AMPK). Activation of this enzyme has been mechanistically linked to an enhanced expression of factors favoring oxidative metabolism, including peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), uncoupling protein 3 (UCP3), cytochrome *c*, succinate dehydrogenase, and citrate synthase.[12–15] These effects appear to be mediated by AMPK regulation of PGC-1α at both the gene and protein levels. Genetically or pharmacologically enhancing AMPK activation increases both the protein and mRNA levels of PGC-1α,[16] as well as augments the binding of TFs to regions in the PGC-1α promoter.[15] Furthermore, AMPK activation increases the phosphorylation of PGC-1α protein, which appears vital to the induction of PGC-1α-regulated genes, as well as the self-regulatory activity of PGC-1α on its own promoter.[13]

Recent studies have also noted that activity of specific subunits of the AMPK heterotrimer is also required for the basal expression of mitochondrial markers. For example, knockout (KO) of AMPK β-subunit(s) results in reduced mitochondrial content and muscle function, as well as impaired endurance performance,[17] emphasizing the necessity of this protein for basal and exercise metabolism.

5. EXERCISE-INDUCED SIGNALING: A ROLE FOR CA^{2+}

An increase in the concentration of cytosolic Ca^{2+} in skeletal muscle occurs during contractile activity (Fig. 1). This elevation of cytosolic Ca^{2+} also occurs at a concentration capable of augmenting oxidative phosphorylation in skeletal muscle mitochondria, as well as activating a number of calcium-regulated enzymes. Increased levels of cytosolic Ca^{2+} have been linked with signaling to mitochondrial biogenesis by *in vitro* experiments utilizing pharmacological agents which mimic the effects of physiological Ca^{2+} release. These studies have implicated cytosolic Ca^{2+} in stimulating the expression of a number of mitochondrial genes,[18,19] effects which appear to be mediated, in part, through enhanced signaling by calcium/calmodulin-dependent protein kinase (CaMK), protein kinase C (PKC), AMPK, and mitogen-activated protein kinases (MAPKs).[18–20]

Contractile activity-induced signaling by these Ca^{2+}-regulated kinases appears to converge on the PGC-1α promoter,[20,21] stimulating an increase in PGC-1α mRNA and protein levels.[20] While the role of PGC-1α as a downstream effector of Ca^{2+} in the context of mitochondrial biogenesis has been described, recent work has also highlighted the idea that PGC-1α may also function upstream of Ca^{2+} signaling in skeletal muscle, as it controls the expression of variety of proteins which modulate Ca^{2+} handling.[22] This provides a link for earlier findings, which have described contractile activity as a potent stimulus for inducing alterations in the expression of Ca^{2+} handling proteins.[23]

6. EXERCISE-INDUCED SIGNALING: A ROLE FOR P38 MAPK

Contractile activity also increases the phosphorylation and activation of p38 MAPK in skeletal muscle. p38 activation is sensitive to changes in ROS, inflammatory cytokines, and insulin which all may be modulated with exercise (Fig. 1). Interestingly, thyroid hormone (T_3) treatment, a well-established model for mitochondrial biogenesis, has also been documented to enhance p38 phosphorylation in skeletal muscle.[24] This may suggest a common mechanism of p38 activation which is important for muscle mitochondrial biogenesis, since both exercise and T_3 are known to result in organelle synthesis.

Akimoto *et al.*[25] first demonstrated that an acute exercise bout was capable of increasing p38 phosphorylation, which was later followed by an

increase in PGC-1α mRNA content. The increase in PGC-1α transcript levels was likely accomplished through increased gene transcription. Indeed, PGC-1α promoter activity was increased with overexpression of p38. Furthermore, both promoter activity and PGC-1α protein activity are attenuated during contractile activity if p38 is inhibited.[20] The observed increase in PGC-1α promoter activity may be achieved through the ability of p38 to phosphorylate the TFs, myocyte enhancer factor-2 (MEF2), and activating transcription factor-2 (ATF2), which both bind to promoter elements within the upstream regulatory region of the PGC-1α gene.[26] Therefore, these data suggest that p38 is important for the increase in PGC-1α gene transcription in skeletal muscle with exercise.

p38 has also been shown to directly phosphorylate PGC-1α protein on multiple residues.[27] This posttranslational modification of PGC-1α increases the stability of the protein and alleviates normal coactivator repression, resulting in enhanced PGC-1α activity when compared to its dephosphorylated form. Collectively, these observations place p38 as an important signaling kinase for exercise-induced mitochondrial biogenesis through the activation of PGC-1α at multiple molecular levels.

7. EXERCISE-INDUCED SIGNALING: ACTIVATION OF PGC-1α

Contractile activity-induced signaling from the aforementioned cellular changes acts on a variety of TFs linked with mitochondrial biogenesis, such as nuclear respiratory factor-1/2 (NRF-1/2), early growth response gene-1 (Egr-1), specificity protein-1 (Sp1), c-fos, c-myc, cAMP response element binding protein (CREB), and upstream stimulating factor (USF1). The expression of these factors is upregulated and they translocate to myonuclei to stimulate transcription of mitochondrial genes.[28–31]

These signals are linked to the transcription of genes associated with mitochondrial content and oxidative phosphorylation by a member of a family of transcriptional coactivators, PGC-1α (Fig. 1). In skeletal muscle, PGC-1α is regarded as the most significant regulator of mitochondrial biogenesis and function.[32,33] As it is a transcriptional coactivator, it lacks the capacity to bind to nuclear DNA directly. Instead, it enhances the activity of TFs, such as NRF-1/2 and others,[32,34] by the recruitment of histone-modifying factors and direct interactions with the transcription initiation machinery.[35] The activation of these TFs is coupled with the transcriptional control of other genes involved in mitochondrial function and biogenesis. These include subunits of

protein complexes in the electron transport chain (ETC) and factors involved in their assembly, mtDNA transcription and replication machinery, and mitochondrial protein import.[36] PGC-1α also coactivates NRF-1 on the Tfam promoter,[32] a required factor for the expression of the mtDNA, emphasizing its role in the coordinated expression of mitochondrial genes from both nuclear and mitochondrial genomes. It is not surprising then that the expression of PGC-1α is tissue dependent, with the highest levels of this protein existing in the most oxidative tissues, namely cardiac and skeletal muscle.[37] Furthermore, PGC-1α expression can be induced in muscle by contractile activity both *in vitro* and *in vivo*,[31,37] an effect which is preceded by PGC-1α translocation to the nucleus.[30] Taken together, it is clear that PGC-1α sits at the crux of the control of mitochondrial content and function.

Skeletal muscle-specific overexpression of this protein is sufficient to enhance basal mitochondrial content and endurance performance.[38,39] Similarly, whole-body PGC-1α KO mice display compromised basal mitochondrial function and content,[40] while skeletal muscle-specific ablation of PGC-1α is associated with impaired endurance performance, likely owing to abnormalities in mitochondrial structure and function, in addition to a reduced expression of metabolic genes,[41] highlighting the constitutive role of this coactivator in the maintenance of the size and quality of the mitochondrial pool. However, the absolute necessity of PGC-1α in exercise-induced adaptations is still up for debate. Changes in mitochondrial gene expression to an exercise training program are unaffected in a whole-body PGC-1α KO,[42] suggesting that this protein may not be crucial for exercise-induced adaptations to skeletal muscle, and that other factors may be at play in causing these alterations. Furthermore, knockdown of PGC-1α in myotubes revealed that some mitochondrial proteins require PGC-1α for contractile activity-induced organelle synthesis, while others do not.[43] In stark contrast to these findings, skeletal muscle-specific KO of PGC-1α attenuated the expression of mitochondrial proteins, but not a shift in fiber-type, following an endurance training program.[44] These data perhaps imply a specific role for PGC-1α in the adaptations of skeletal muscle to exercise. While the role for PGC-1α in skeletal muscle in an unstressed state is quite clear cut, the function of this coactivator in a state of energetic imbalance is still disputed.

8. AGING AND MUSCLE MITOCHONDRIA

Mitochondrial content and function in skeletal muscle decline with age. Recent reviews by Nair and colleagues[45,46] have summarized the factors

which contribute to changes in mitochondrial function in aging muscle. A decline in organelle content is supported by many studies which report reduced Krebs' cycle and ETC enzyme activities, protein markers, and mtDNA content. Electron micrograph evidence of diminished IMF mitochondrial size and a reduced thickness of the SS mitochondrial layer[8] also exist. The fragmented mitochondria evident in aging skeletal muscle are likely a result of altered ratios in the expression of fusion and fission regulatory proteins which govern mitochondrial morphology. Reports from muscle of aged rodents and humans have observed that the balance of these regulatory factors is skewed toward favoring greater rates of fission, compared to fusion, within aged muscle. Mitochondrial respiration and maximal ATP production rates are also impaired with aging, possibly a consequence of reduced mitochondrial protein synthesis,[47] or increased uncoupling of oxygen consumption to ATP synthesis.[48] It is important to recognize that many of these decrements remain even when physical activity levels between young and old subjects are carefully matched,[47,49] suggesting true age-related deficits in mitochondrial function. However, this conclusion remains controversial.[45,50] The level of physical activity of the individual is certainly one of the most important determinants of organelle function in aging muscle.

As noted above, PGC-1α is an important regulator of mitochondrial content in a variety of tissues including muscle. PGC-1α mRNA and protein content are reduced in both slow- and fast-twitch muscles with age,[51] suggesting that reductions in mitochondrial function or content could be attributable to the loss of this coactivator. When PGC-1α was overexpressed in muscle of both young (6 months) and aged (22 months) mice,[39] PGC-1α prevented atrophy and retained mitochondrial content and function. Leick *et al.*[52] also showed that the absence of PGC-1α was necessary to extend the benefits of exercise training into older age. Markers of mitochondrial content were decreased with age as expected, and this was prevented with endurance exercise training. However, training was incapable of preventing the mitochondrial decline in animals that lacked PGC-1α, indicating that PGC-1α is necessary to stimulate the beneficial effects of exercise on mitochondrial content during the aging process. Thus, PGC-1α is important for mitochondrial biogenesis during the aging process.

The cause of the decline in mitochondrial content and function in skeletal muscle with age remain controversial. Research has shown that the protein import pathway, a route employed for the incorporation of new proteins into the organelle reticulum, is unaffected with age.[53] mtDNA

deletions and point mutations occur with increasing incidence with age, but appear to occur in later life, after the onset of the decline in mitochondrial function.[54] In contrast, the decline in PGC-1α and a reduction in its transcriptional activity may be one of the most compelling reasons for the decrease in organelle content with age.

Exercise is an important therapeutic intervention to ameliorate this decline and restore organelle function with age. It is well known that exercise is a potent inducer of mitochondrial biogenesis in younger individuals, and many studies report that aging muscle adapts to exercise as well. However, is aging muscle equally adaptive to an exercise regimen as muscle from younger individuals? In assessing this question, it is important to provide equivalent relative workloads to both young and older subjects. In addition, many studies have investigated mitochondrial adaptations in older humans; however, they have not consistently employed the use of a younger group for comparison. This makes it difficult to conclude on the degree of mitochondrial adaptation in young compared to older individuals. Nonetheless, many studies using both cross-sectional and longitudinal designs indicate that mitochondrial concentration can increase in both older men and women with exposure to exercise. Studies which compare active older adults to sedentary counterparts show that the active older groups have preserved mitochondrial content and function, higher PGC-1α expression, and a greater capacity to defend against oxidative stress.

Rodent models of exercise have provided considerable insight into the molecular regulation of mitochondrial biogenesis in aging muscle and afford the possibility of strict control over the absolute training workload. Several studies have utilized the chronic contractile activity model[55] to study the effects of "exercise" on mitochondrial adaptation in aging muscle. Walters *et al.*[56] found that chronic contractile activity-invoked exercise of the flexor digitorum longus in young and aged rodents increased citrate synthase activity; however, the rate of increase in the aged animals was attenuated at the onset of the exercise protocol. Yet, by 90 days, young and aged animals had equivalent levels of this mitochondrial enzyme marker. Work from our laboratory using a short-term chronic contractile activity protocol (7 days) of the rat tibialis anterior muscle indicated reduced mitochondrial biogenesis in old muscle as compared to young muscle.[53] This blunted response in mitochondrial proliferation was attributable to reduced elevations of PGC-1α and Tfam, in addition to lack of alterations in protein import machinery components in aged muscle. These data illustrate the potential corrective nature of chronic exercise in ameliorating organelle dysfunction, but also

suggest that the kinetics of mitochondrial adaptations in old muscle are delayed in response to an exercise regimen. This may be a result of the fact that aged muscle is less capable of activating upstream kinases, such as AMP kinase, p38 MAPK, or CaMK in response to exercise.[57] Interestingly, this attenuated signaling, leading to a reduced mRNA response, has also been repeatedly demonstrated in response to resistance exercise protocols in old, compared to young subjects.[58,59] Thus, the reduced activation of important kinases regulating mitochondrial biogenesis may be partly responsible for the delayed and diminished adaptation of mitochondria to exercise in senescent muscle.

In summary, these data suggest that aged muscle is capable of increasing mitochondrial content in response to exercise, but that the rate of onset at which this increase takes place may be reduced. However, it is clear that exercise is an important therapeutic intervention to repair dysfunctional mitochondria and improve aerobic energy provision in aging muscle.

9. ALTERNATIVE EXERCISE PROGRAMS: HIGH-INTENSITY INTERVAL TRAINING

Endurance training has been known for many years to stimulate mitochondrial biogenesis, an effect which contributes to an increased aerobic endurance capacity in trained subjects.[11,60] However, endurance training requires a notable time commitment, in terms of both the frequency and duration of exercise sessions, in order to elicit positive results. In order to circumvent these challenges, alternative training programs to induce similar outcomes have been explored.

In addition to exercise duration and frequency, exercise intensity is a central determinant of the cellular and biochemical alterations which occur in response to a training program. As different muscle fiber types are recruited in a hierarchical manner determined by the intensity of exercise employed, it would appear that an intensity of exercise sufficient enough to recruit the greatest number of fiber types might be the most useful in facilitating whole muscle adaptations. Dudley *et al.* provided one of the first pieces of evidence, suggesting that exercise intensity is directly related to the magnitude with which skeletal muscle mitochondrial enzyme content is augmented.[61] This work also put forward the idea that a variety of high-intensity interval training (HIIT) programs may be capable of yielding increases in skeletal muscle oxidative capacity similar to that of a continuous endurance training program. This has since been corroborated by evidence

in human skeletal muscle, as both endurance and interval training approaches provide similar increases in the expression of oxidative genes[62] and mitochondrial content markers.[63,64] The increase in mitochondrial proteins is likely due to the increase in the phosphorylation of p38, AMPK, and p53, which can be evoked by a single bout of HIIT,[65,66] and can mediate the translocation of PGC-1α protein to the nucleus. This translocation has been associated with increases in the mRNA and protein levels of a variety of mitochondrial markers, such as citrate synthase and cytochrome *c* oxidase subunit IV during the recovery phase.[67–69] These changes appear to be a product of the accruing effects of repeated mitochondrial mRNA "bursts" which follow each individual training bout.[70]

In addition to promoting mitochondrial biogenesis, it has been suggested that interval training programs can foster the remodeling of the mitochondrial network. An increase in the protein expression of the mitochondrial fusion protein Mfn1 as well as the fission proteins Fis1 and Drp1 as a result of a HIIT program has been documented.[70] This may have implications for mitochondrial function, as skeletal muscle mitochondria isolated from individuals who have undergone a HIIT program display an increase in the maximal ADP-stimulated respiration rate and respiratory control ratio.[71]

In sum, when contrasting these two approaches to increasing skeletal muscle mitochondrial content at this point in time, the HIIT routine appears to afford a notable reduction in the time invested in exercise, perhaps providing an attractive substitute for continuous training regimens. Whether this exercise regime is suitable for all populations remains under debate.

10. EFFECT OF TRAINING ON mtDNA AND mtDNA DISEASES

Mitochondria are unique organelles as they possess their own genetic material. Human mtDNA is organized as circular and double-stranded DNA that encodes 2 ribosomal RNAs, 22 transfer RNAs, and 13 polypeptide subunits of the respiratory chain complexes found across the inner mitochondrial membrane. However, mtDNA contributes to comprise only a small fraction of the mitochondrial proteome. Thus, the proper assembly of a functional mitochondrion requires the coordinated expression of both mitochondrial and nuclear genomes. Improper transcription and/or translation of either genome can lead to organelle dysfunction. Furthermore, mtDNA is particularly susceptible to ROS-induced damage due in part to its proximity to the ETC.[72] Although the cell has measures in place allowing it to

repair mtDNA, these mechanisms are not as effective as those found in the nucleus. Thus, mtDNA mutations which arise from oxidative damage can blunt the expression of mtDNA which is vital for the proper functioning of the ETC. As a result, increases in mitochondrial dysfunction can perpetuate a vicious cycle of mtDNA damage and ROS production.

Pathological mtDNA mutations can be distributed in a heteroplasmic fashion, meaning that the proportion of mutant to wild-type mtDNA copies within a mitochondrion can vary. The level of heteroplasmy can range from 99:1 to 1:99, with the severity of symptoms depending on the extent of the pathology. Symptoms are tissue specific but mutations that directly impair mitochondrial function often affect multiple tissues such as muscle and neurons, which rely heavily on mitochondria for energy. Mitochondrial diseases that specifically affect the neuromuscular system are termed mitochondrial myopathies and include Kearns–Sayre syndrome, myoclonic epilepsy and ragged red fibers, neuropathy, ataxia, retinitis pigmentosa, and Leber's hereditary optic neuropathy.[73]

In healthy individuals, exercise-induced mitochondrial biogenesis occurs concomitant with an increase mtDNA copy number,[74] which parallels the increase in skeletal muscle oxidative capacity.[75] This effect is likely mediated, in part, by an increase in Tfam expression and import into the mitochondrion[76,77] (Fig. 1). However, with mtDNA mutations, ATP production levels are often diminished as a result of defective ETC complexes. In skeletal muscle, a decrease in ATP levels can directly impact muscle contraction and performance. Individuals with cytochrome *b* gene mtDNA mutations suffer exercise intolerance as the predominant clinical feature, with symptoms of fatigue and higher than normal levels of lactic acid.[78] Furthermore, mitochondrial myopathies with high mtDNA heteroplasmy correlate to lower whole-body oxygen consumption and lower exercise capacity. Interestingly, aerobic exercise is an intervention capable of stimulating mitochondrial biogenesis by increasing the expression of respiratory chain enzymes and oxidative capacity in mitochondrial myopathy patients; however, it does not improve the mtDNA mutation levels observed in these patients.[79,80] Resistance exercise training may also offer an alternative therapeutic approach through the activation and recruitment of muscle satellite cells to mature myofibers, thereby reducing the proportion of mutant mtDNA.[81] While considerable progress has been made, more work is needed using larger sample sizes and different training protocols to resolve our understanding of exercise training as a therapeutic option for improving the quality of life for mitochondrial myopathy patients.

11. EXERCISE AND TRAINING ON ROS PRODUCTION AND ANTIOXIDANT ENZYMES

Exercise and contractile activity have the capacity to encourage the production of moderate amounts of ROS in muscle[82,83] (Fig. 1). This occurs due to the improper donation of electrons from the ETC to oxygen during oxidative phosphorylation. The electrons which escape, termed an "electron leak," form reactive superoxide, which is then rapidly transformed into H_2O_2 by cellular antioxidant enzymes, such as the superoxide dismutases. While electron leakage from the ETC has historically been suggested to be the predominant source of cellular ROS, current experimental evidence suggests that NADPH oxidase is likely the major ROS-generating source in contracting muscle.[84] Although high levels of ROS production can be detrimental to cellular components, physiological levels are important regulators of cell signaling pathways, and consequently the regulation of gene expression.[85]

During basal state 4 respiration, mitochondrial ROS production is elevated.[86] ROS formation is reduced under the ADP-stimulated state 3 respiration, similar to what would be observed during aerobic exercise, likely due to more efficient electron transfer between ETC complexes. However, this ROS production appears to have a role in the induction of mitochondrial biogenesis following contractile activity. *In vitro* experiments have identified that exogenous ROS treatment increases PGC-1α expression in muscle cells, through both AMPK-dependent and -independent activation of the PGC-1α promoter.[87] Furthermore, the human PGC-1α promoter contains a variety of binding sites for other ROS-sensitive TFs, including Sp1, CREB, ATF2, nuclear factor-κB, p53, and MEF2,[15] supporting the hypothesis that moderate alterations in ROS during exercise may regulate the activity of the PGC-1α promoter through these or other factors. Additionally, inhibition of the cytosolic ROS source xanthine oxidase during exercise attenuates PGC-1α expression and signaling for mitochondrial biogenesis.[88] These data highlight a shared signaling pathway between these metabolic byproducts and the resulting increase in PGC-1α expression, providing a mechanism for the increase in mitochondrial mass[89] and elongation of the mitochondrial network[90] with ROS treatment.

To control cellular ROS levels, skeletal muscle contains a system of enzymatic and nonenzymatic antioxidants that are strategically located in many compartments of the cell. Enhancement of this antioxidant defense

system is one of the many adaptive responses that occur with exercise training. This response is associated with increased expression and activity of nuclear erythroid 2 p45-related factor 2 (Nrf2), a TF that serves as the central regulator of antioxidants and detoxification enzymes.[91] Although limited work analyzing the effect of exercise on the expression and regulation of Nrf2 has been done, targeting this factor and its regulatory axis is an attractive area of research.

12. EXERCISE AND THE PROTEIN IMPORT PATHWAY

The mammalian mitochondrial proteome contains over 1000 proteins.[92] Of these, only 13 are encoded by mtDNA, thereby obligating the existence of an elaborate transport system for the import of nuclear-derived proteins from the cytosol into mitochondria. Defects in the import pathway are lethal in some organisms and lead to disease in humans.[93] Since mitochondria have several compartments, specific pathways have evolved to deliver proteins to the matrix, inner membrane, outer membrane, and intermembrane space. Proteins are synthesized in the cytosol with either internal or N-terminal mitochondrial targeting sequences (MTSs) which interact with specific chaperones to unfold and direct the precursor to the translocase of the outer membrane (Tom) receptor complex (Fig. 1). Cytosolic chaperones include heat-shock proteins 70 and 90 (Hsp70 and Hsp90) and mitochondrial import-stimulating factor (MSF). Precursors are then transferred to Tom40 and its accessory proteins which form an aqueous channel through which the precursor protein passes to be sorted to the outer membrane, the inner membrane, or the translocase of the inner membrane (Tim complex). In a fashion similar to the Tom complex, inner membrane import channel proteins Tim17 and Tim23 bind the precursor protein and form a pore through which the precursor can travel. On the inner face of the inner membrane, precursor entry to the matrix is facilitated by the ratchet-like action of mitochondrial Hsp70 (mtHsp70). Once inside, the N-terminal MTS of the precursor is cleaved by a mitochondrial processing peptidase to form the mature protein, and it is then folded into its active conformation by Hsp60 and chaperonin 10 kDa (cpn10). The import process requires both energy in the form of ATP to assist in cytosolic protein unfolding, as well as an intact membrane potential to help pull the positively charged presequence into the matrix.

The protein import process adapts to conditions of muscle use and disuse and determines, in part, the mitochondrial content within the cell. In response to chronic exercise, the cellular content of a number of protein import

machinery components is increased, including the cytosolic chaperones MSF and Hsp70, the intramitochondrial proteins mtHsp70, Hsp60, and cpn10, as well as subunits of both the Tom and Tim complexes.[94,95] Coincident with these changes are parallel contractile activity-induced increases in the rate of import into the matrix and outer membrane.[76,94,95] Tom20 appears to be particularly important in mediating the increase in protein import, since experiments in muscle cells in which this outer membrane protein was artificially over- or underexpressed led to parallel concomitant changes in the rates of protein import into the matrix.[96] Included in the accelerated pathway of import into the matrix is Tfam, the TF that mediates mtDNA transcription and replication. Skeletal muscle contractile activity increases Tfam import, leading to greater Tfam–mtDNA binding, as well as augmented mtDNA gene products.[76] Recent work has shown that tumor suppressor p53 also plays a critical role in this Tfam–mtDNA complex, as the ablation of p53 completely attenuates the expression of mtDNA following acute exercise.[97]

The physiological importance of the adaptation of protein import to exercise is that the capacity for import is increased, producing mitochondria that are more sensitive to small changes in precursor protein concentration. Thus, at any production rate of cytosolic precursor proteins, a higher rate of protein import would occur, a situation that would be advantageous for mitochondrial biogenesis. This would be of particular benefit during conditions of impaired mitochondrial protein import, a situation which could arise during chronic muscle disuse or disease. Indeed, we now have experimental evidence that endurance exercise training can rescue the protein import defect produced in mitochondria by the absence of the outer membrane proteins Bax and Bak.[98]

In summary, the protein import pathway is a complex protein targeting and transfer system which is vital for organelle biogenesis, given the limited coding capacity of mtDNA. This capacity of this pathway is malleable; it decreases during muscle disuse and adapts in a positive manner to contractile activity. In this way, it is an important determinant of mitochondrial content in muscle during alterations in level of physical activity.

13. EFFECT OF EXERCISE ON MITOCHONDRIALLY MEDIATED APOPTOSIS

Apoptosis is the process of programmed cell death which plays an important role in cell function and homeostasis, but that is also capable of contributing to the pathogenesis of physiological aging and skeletal muscle disuse.[99] This process can be induced through various mechanisms, most

commonly converging on the caspase proteases, which cleave select nuclear and cytosolic targets leading to DNA fragmentation. Apoptosis can be signaled extrinsically via binding of external ligands to cell death receptors on the outer surface of the cell membrane, or by intrinsic stimuli, resulting in DNA fragmentation contributing to cell death. One of the most notable intrinsic apoptotic pathways is mediated by the mitochondrion (Fig. 1). These organelles contain many proapoptotic factors, including cytochrome *c*, apoptosis-inducing factor (AIF), and endonuclease G that can be released following the opening of the *m*itochondrial *p*ermeability *t*ransition *p*ore (mtPTP) and the *m*itochondrial *a*poptosis-inducing *c*hannel (MAC). A variety of cellular conditions, including excessive ROS production, can cause mitochondrial permeabilization and the release of these proapoptotic factors, inducing DNA fragmentation and apoptosis.[100]

Research has indicated that exercise training can reduce the susceptibility of skeletal muscle to apoptosis. Initial studies have shown that following an endurance training protocol, an increase in the expression of Bcl-2 and mitochondrial superoxide dismutase (MnSOD) occurred, along with a diminished expression of the proapoptotic factor Bax. Chronic contractile activity also revealed a reduction in the release of proapoptotic factors from isolated mitochondria of young animals[101] and reduced DNA fragmentation in older animals.[53] Furthermore, endurance training also resulted in a decreased Bax:Bcl2 ratio, as well as AIF levels in both soleus and cardiac muscle.[102]

This protective effect of endurance exercise on skeletal muscle apoptotic susceptibility may also be relevant in several animal models of human disease. Obese Zucker rats typically display elevated levels of apoptotic factors in cardiac muscle. Interestingly, exercise training can reduce the expression of these factors to levels near that of a nonobese control.[103] More recently, it has been established that hypertension is associated with enhanced skeletal muscle myopathy and apoptosis. Endurance training of the spontaneously hypertensive rat led to a reduction in fragmented nuclei and cytochrome *c* release into the cytosol, while increasing Bcl-2 and MnSOD expression.[104]

Taken together, exercise training appears to be a feasible approach to diminish mitochondrially mediated apoptosis, both basally and under stressed conditions, in turn yielding a more favorable muscle phenotype.

14. AUTOPHAGY AND MITOPHAGY WITH EXERCISE

The cellular remodeling which occurs in response to contractile activity necessitates the turnover of dysfunctional organelles, as well as oxidized

and damaged proteins. Macroautophagy (hereafter referred to as autophagy) is a cellular recycling mechanism that is characterized by the encapsulation of damaged organelles and protein aggregates in doubled membrane vesicles known as autophagosomes, and their subsequent delivery to and degradation within the lysosome (Fig. 1). Enhanced lysosomal degradation with strenuous exercise was observed in skeletal muscle over three decades ago.[105] Since then, an acute bout of exercise has been demonstrated to induce autophagy in various organs and tissues including skeletal muscle of humans and rodents.[106–110]

Exercise-induced autophagy involves the phosphorylation of Bcl-2, an antiapoptotic and antiautophagic protein. This allows for the release of Beclin1 from a complex with Bcl-2, consequently allowing for the formation of autophagosomes. It has been thought that exercise may require autophagy for the breakdown of fuel sources to be utilized during an exercise bout. Mice overexpressing a mutated Bcl-2 incapable of being phosphorylated (AAA knock-in, $Bcl2^{AAA}$) exhibit normal basal autophagy, but are deficient in stimulus-induced autophagy, and have an impaired endurance capacity, suggesting that autophagy may be required for exercise performance. However, given that the inability to initiate autophagy in this experimental model is not specific to any one tissue, teasing out the requirement for autophagy specifically within skeletal muscle as a determinant of exercise performance is essential. Intriguingly, skeletal muscle-specific ablation of Atg7 ($Atg7^{-/-}$), an indispensable protein involved in autophagy, does not result in alterations in endurance performance.[111] In fact, these mice have an improved metabolic profile and are protected from obesity, findings which were attributed to the mitochondrial stress and the release of the mitokine FGF21 in $Atg7^{-/-}$ mice.[112] These contrasting findings bring into question the requirement for autophagy as an energy source for muscle contraction. However, repeated bouts of running exercise reveal a progressive drop in performance as well as diminished mitochondrial membrane potential in muscle-specific $Atg7^{-/-}$ mice, suggesting that autophagy may be critical for organelle turnover postexercise. This autophagy activation is, in part, ROS dependent, as treatment with general or mitochondria-specific ROS scavengers attenuates autophagy induction in wild-type mice.[111] Moreover, aerobic exercise enhances mitochondrial LC3II, p62, and ubiquitination following exercise, suggesting the induction of mitochondrial-specific autophagy with exercise.[110] These findings indicate that autophagy is vital for organelle turnover postexercise, thus contributing to exercise-induced adaptations.

An increase in the expression of autophagy genes and proteins has also been noted in humans following ultra-endurance exercise, which consisted of 24–28 h of treadmill running.[109] While limited data have been compiled with regard to the autophagic processes in human skeletal muscle following acute or chronic endurance training, this is certainly an avenue of research which would be attractive to explore.

Autophagy is also essential for exercise-induced benefits, including protection against high-fat diet (HFD)-induced metabolic impairments. $Bcl2^{AAA}$ mice are more susceptible to HFD-induced obesity and fail to exhibit exercise training-mediated protection against HFD-induced disturbance in glucose tolerance. These findings point to the involvement of autophagy in metabolic renovation with repeated bouts of physical activity.[107] Moreover, voluntary exercise training results in increased basal autophagy and mitophagy protein expression.[113] However, this increase is compromised in mice that are heterozygous for Atg6 ($Atg6^{-/+}$). $Atg6^{-/+}$ mice also fail to induce skeletal muscle mitochondrial biogenesis and angiogenesis in response to an exercise training program, along with an inability to improve their endurance capacity. These results point to a role for basal autophagy and/or mitophagy as a requirement for exercise training-induced skeletal muscle adaptations and improvement of physical performance.[113]

There is also evidence to suggest that regular exercise may be able to restore conditions where autophagy may be deficient, or ineffective. Indeed, long-term exercise training was also demonstrated to reactivate autophagy flux in skeletal muscle of animals treated with the lysosomal inhibitor chloroquine.[114] Furthermore, lifelong exercise, in combination with caloric restriction, has been found to improve the reduction in autophagy observed with aging, as well as to dampen the age-related increase in oxidative damage and apoptosis.[115] This suggests that exercise may serve as a potential form of therapy for myopathies characterized by impaired autophagy. However, exercise should be prescribed with caution, as in certain autophagy-deficient conditions, such as those involving a mutation in the extracellular matrix protein collagen VI, the myopathy may be exacerbated.[106]

Although the early events leading to autophagy activation with exercise have not been thoroughly examined, several possibilities exist. Muscle contraction represents a form of energetic imbalance, much like nutrient deprivation or starvation, all of which are well characterized as activators of autophagy. The increased metabolic demands of contracting muscle, along with elevated ROS production, activate the cellular stress responders such as AMPK, SIRT1, and p38, which may lead to the induction of

autophagic machinery. In particular, activation of AMPK has been definitively linked with the control of mitophagy.[116] An acute bout of exercise also leads to reduced Akt phosphorylation, which would inhibit mTOR signaling and thereby also promote autophagy.[106,107]

Another possible mechanism involved in the induction of autophagy may be the unfolded protein response (UPR), which is activated in response to sarcoplasmic reticulum stress induced by contractile activity.[117,118] Certain UPR factors have been implicated in the activation of autophagy in various cell types.[119–121] Interestingly, PGC-1α was shown to contribute to UPR induction with exercise acting through the UPR ATF6, and mice lacking ATF6 are exercise intolerant.[117]

Therefore, although several studies indicate that autophagy is activated during an acute bout of exercise, the mechanisms underlying this activation remain to be elucidated. It is also becoming increasingly evident that autophagy is involved in chronic physical activity-induced adaptations through increased organelle turnover. However, the kinetics of autophagy activation during and following an exercise bout still require further exploration.

15. CONCLUSIONS

Exercise training-induced adaptations are wide ranging and can positively impact a variety of organ systems. In particular, alterations to skeletal muscle, such as the increase in mitochondrial content and improvement in the health of the mitochondrial pool, are keys to the enhanced metabolic capacity of the organ. These changes are a product of the signals which originate from shifts in the myocellular environment as a result of exercise, including the consumption of ATP, the production of moderate levels of ROS, and the intracellular release of Ca^{2+}, among others. Concomitant with an increase in the quantity of mitochondria is the expansion of the organelle network and augmented antioxidant capacity. In addition, exercise results in an enhanced removal of dysfunctional mitochondria. Altogether, these alterations are beneficial to muscle in that they afford the organ the ability to function more efficiently and effectively, and are vital for improvements in endurance capacity and well-being, particularly during the aging process. Thus, continued attention to the understanding of the molecular basis of theses mitochondrial adaptations is essential.

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