

## Review

# Coordination of metabolic plasticity in skeletal muscle

David A. Hood<sup>1,2,\*</sup>, Isabella Irrcher<sup>2</sup>, Vladimir Ljubovic<sup>1</sup> and Anna-Maria Joseph<sup>2</sup>

<sup>1</sup>*School of Kinesiology and Health Science and* <sup>2</sup>*Department of Biology, York University, Toronto, Ontario, M3J 1P3, Canada*

\*Author for correspondence (e-mail: dhood@yorku.ca)

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### Summary

Skeletal muscle is a highly malleable tissue, capable of pronounced metabolic and morphological adaptations in response to contractile activity (i.e. exercise). Each bout of contractile activity results in a coordinated alteration in the expression of a variety of nuclear DNA and mitochondrial DNA (mtDNA) gene products, leading to phenotypic adaptations. This results in an increase in muscle mitochondrial volume and changes in organelle composition, referred to as mitochondrial biogenesis. The functional consequence of this biogenesis is an improved resistance to fatigue. Signals initiated by the exercise bout involve changes in intracellular  $\text{Ca}^{2+}$  as well as alterations in energy status (i.e. ATP/ADP ratio) and the consequent activation of downstream kinases such as AMP kinase and  $\text{Ca}^{2+}$ -calmodulin-activated kinases. These kinases activate transcription factors that bind DNA to affect the transcription of genes, the most evident manifestation of which occurs during the post-exercise recovery period when energy metabolism is directed toward anabolism, rather than contractile activity. An important protein that is affected by exercise is the transcriptional coactivator PGC-1 $\alpha$ , which cooperates with multiple transcription factors to induce the expression of nuclear genes encoding mitochondrial proteins. Once translated in the cytosol, these mitochondrially destined proteins are imported into the mitochondrial outer membrane, inner membrane or matrix space *via* specific import machinery transport

components. Contractile activity affects the expression of the import machinery, as well as the kinetics of import, thus facilitating the entry of newly synthesized proteins into the expanding organelle. An important set of proteins that are imported are the mtDNA transcription factors, which influence the expression and replication of mtDNA. While mtDNA contributes only 13 proteins to the synthesis of the organelle, these proteins are vital for the proper assembly of multi-subunit complexes of the respiratory chain, when combined with nuclear-encoded protein subunits. The expansion of skeletal muscle mitochondria during organelle biogenesis involves the assembly of an interconnected network system (i.e. a mitochondrial reticulum). This expansion of membrane size is influenced by the balance between mitochondrial fusion and fission. Thus, mitochondrial biogenesis is an adaptive process that requires the coordination of multiple cellular events, including the transcription of two genomes, the synthesis of lipids and proteins and the stoichiometric assembly of multisubunit protein complexes into a functional respiratory chain. Impairments at any step can lead to defective electron transport, a subsequent failure of ATP production and an inability to maintain energy homeostasis.

Key words: mitochondrial biogenesis, transcription factors, reactive oxygen species, calcium signaling, mitochondrial protein import.

### Introduction

Skeletal muscle exhibits remarkable adaptive capabilities in response to a number of physiological and pathophysiological conditions. In particular, one of the most dramatic phenotypic alterations occurs in mitochondria in response to exercise or chronic contractile activity. This is most evident in low-oxidative, white muscle, which has an initial mitochondrial content ranging from only 1 to 3% of the total cellular volume (Hoppeler, 1986). Contractile activity-induced mitochondrial adaptations in muscle are highly specific and are dependent

upon the type of exercise (i.e. resistance *vs* endurance) as well as its frequency, intensity and duration. In addition, the recovery period following the exercise bout is a time of rapid, transient changes in gene expression, which contributes to the initial stages of the adaptation of each period of contractile activity. The physiological benefits of mitochondrial adaptations in muscle are an alteration in metabolic preference, with a greater reliance on lipid, rather than carbohydrate, metabolism. This reduces the formation of lactic acid, attenuates the loss of glycogen, reduces high-energy phosphate

utilization and reduces muscle fatigue. Mitochondrial adaptations in response to exercise are generally referred to as 'mitochondrial biogenesis'. In the context of this review, mitochondrial biogenesis will be used synonymously with the broader term 'metabolic plasticity', since mitochondrial adaptations to exercise represent the dominant adaptive response, while glycolytic adaptations are minimal. Mitochondrial biogenesis within muscle consists of two possible mutually inclusive alterations: (1) an increase in mitochondrial content per gram of tissue and/or (2) a change in mitochondrial composition, with an alteration in mitochondrial protein-to-lipid ratio. Although this phenomenon resulting from exercise has long been established (Holloszy, 1967), many of the detailed molecular mechanisms remain to be identified. This is important for our understanding of the general mechanisms of organelle assembly, as well as the pathophysiology of mitochondrially based diseases. Thus, mitochondrial biogenesis induced by chronic exercise is now recognized to have implications for a broader range of health issues than just the enhancement of endurance performance. Changes in mitochondrial plasticity produced by exercise are a result of multiple molecular events (Hood, 2001) (Fig. 1). These include signaling events to initiate biogenesis, the transcription of nuclear genes, the import of nuclear gene products into the organelle, the replication and transcription of mtDNA, mRNA translation into protein and the correct assembly of proteins into a functional stoichiometry. The present review will focus on (1) the initiation of metabolic plasticity, beginning with the signals leading to gene expression at the onset of exercise training, (2) the important transcriptional regulatory proteins PGC-1 $\alpha$  (peroxisome proliferators-activated receptor- $\gamma$  coactivator-1 $\alpha$ ) and mitochondrial transcription factor A (Tfam) and (3) current progress in understanding mitochondrial assembly in muscle.

### Early signals and gene expression responses to contractile activity

#### *Calcium, ATP turnover and reactive oxygen species*

At the onset of contractile activity, a number of rapid events occur that form part of the initial signaling process leading to downstream protein and lipid synthesis. These changes include Ca<sup>2+</sup> flux, ATP turnover and the stimulation of oxygen consumption within the contracting muscle cells. Accumulating evidence supports the link between alterations in intracellular Ca<sup>2+</sup> dynamics and distinctive programs of gene expression that establish phenotypic diversity among skeletal myofibers (Chin et al., 1998; Freyssenet et al., 1999; Freyssenet et al., 2004; Ojuka et al., 2003; Olson and Williams, 2000; Wu et al., 2000). Ca<sup>2+</sup> has been implicated as an important signal in the upregulation of several nuclear genes encoding mitochondrial proteins (Ojuka et al., 2003), in part *via* a protein kinase C (PKC)-mediated pathway (Freyssenet et al., 1999). However, although the expression of a number of genes encoding mitochondrial proteins is increased in response to elevations in intracellular Ca<sup>2+</sup>, some are not, or follow a

different time course of induction (Freyssenet et al., 2004). Thus, it is probable that elevations in Ca<sup>2+</sup> only partially mediate the contractile activity-induced changes in mitochondrial biogenesis, and that it forms part of a more complex signaling pathway, perhaps involving AMP-activated protein kinase (AMPK) (Fig. 1) (Bergeron et al., 2001; Irrcher et al., 2003; Putman et al., 2003; Winder et al., 2000; Zong et al., 2002).

The AMPK cascade is activated by cellular stresses that reduce the ATP/ADP ratio and elevate AMP as a result of myokinase activity. This can occur either by inhibiting ATP production or by accelerating ATP consumption. AMPK activity is increased in skeletal muscle with exercise (Fujii et al., 2000), as a result of 5-aminoimidazole-4-carboxamide riboside (AICAR) treatment and during chronic administration of the creatine analogue  $\beta$ -GPA (Bergeron et al., 2001).  $\beta$ -GPA treatment is associated with increased cytochrome *c* content, mitochondrial density and DNA binding activity of nuclear respiratory factor-1 (NRF-1). Thus, treatment with this agent appears to recapitulate the adaptive responses induced by endurance training or chronic contractile activity. The effect of  $\beta$ -GPA is mediated by AMPK, since enzyme inactivation ablates the  $\beta$ -GPA-induced mitochondrial biogenesis (Zong et al., 2002). Direct activation of AMPK using AICAR also mimics many of the metabolic changes evident as a result of chronic exercise training, such as augmented levels of citrate synthase activity,  $\delta$ -aminolevulinic acid synthase and uncoupling protein-3 content (Putman et al., 2003; Winder et al., 2000). Therefore, by sensing the energy status of the muscle cell, AMPK appears to be an important regulator of mitochondrial content.

However, the necessity for a multiplicity of pathways involved in metabolic plasticity is underscored by a recent study that showed that the exercise-induced activation of a number of metabolic genes was not impaired in either AMPK $\alpha$ 1 or AMPK $\alpha$ 2 knockout animals (Jorgensen et al., 2005). Thus, under those conditions, other signaling events, possibly involving Ca<sup>2+</sup> or the remaining AMPK $\alpha$  isoform, may compensate for the reduced total AMPK activity to regulate metabolic gene activation in skeletal muscle. In addition, it is likely that signals involving Ca<sup>2+</sup> and energy deficits act in concert to promote metabolic plasticity. Evidence for this is found in cells with reduced or mutated mitochondrial DNA (mtDNA), in which ATP levels are reduced, intracellular Ca<sup>2+</sup> levels are elevated (Moudy et al., 1995) and nuclear gene expression is activated (Biswas et al., 1999; Joseph et al., 2004). These data suggest the existence of a retrograde intracellular communication network between the mitochondrial and nuclear genomes. In these cells, the reduced ATP levels promote the elevation of intracellular calcium levels, resulting in the activation of calcium-responsive genes, including calcineurin and cAMP-responsive elements binding proteins (CREB) (Arnould et al., 2002; Biswas et al., 1999). The expression of several transcription factors such as NRF-1 and Tfam (Miranda et al., 1999), as well several key cytochrome *c* oxidase (COX) subunits (Biswas et al., 1999;

Marusich et al., 1997), is altered. The redundancy and complementarity in signal transduction illustrated by this energy depletion condition is an important example of the cellular compensatory mechanisms used to ensure adequate organelle synthesis for cell survival.

As part of the process of ATP production, molecular oxygen undergoes a four-electron reduction catalyzed by COX. This process accounts for 95–98% of the total oxygen consumption. The small remaining oxygen fraction can undergo a one-electron reduction with the production of reactive oxygen species (ROS) (Fig. 1). ROS production is lower in maximally activated state 3 respiration than in low-level, resting state 4 respiration, indicating that ROS production is inversely related to the rate of oxygen consumption. This is evident in mitochondria isolated from both the subsarcolemmal and intermyofibrillar regions of the myocyte (Adhietty et al., 2005). The higher ROS production in the subsarcolemmal mitochondrial subfraction may contribute to the greater potential of this mitochondrial subfraction to adapt under conditions of exercise (Hood, 2001) or disease (Ritov et al., 2005). However, it is now recognized that ROS production in muscle arises not only from mitochondria but also from other intracellular and extracellular reactions (Pattwell and Jackson, 2004). Since ROS production increases in muscle during contractile activity (Pattwell et al., 2004) and with acute exercise (Davies et al., 1982), and mitochondrial sources of ROS are reduced during active respiration, these alternative sources of ROS must increase in importance during contractile activity.

Recent studies have indicated that the chemically induced production of ROS can lead to mitochondrial reticulum elongation and branching complexity in fibroblast cells (Koopman et al., 2005a). In addition, patients with mitochondrial complex I deficiency have elevated rates of ROS production and demonstrate similar adaptive changes in mitochondrial morphology (Koopman et al., 2005b). Thus, it has become clear that ROS are important signals involved in mitochondrial adaptations and possibly in the mitochondrial responses to exercise. ROS can act through several different pathways of signal transduction, making use of signaling cascades such as protein kinases, phosphatases, phospholipases and  $\text{Ca}^{2+}$ . Downstream transcription factors that are affected by intracellular ROS include NF- $\kappa$ B, AP-1, HSF, Egr-1 and p53 (Pattwell and Jackson, 2004), resulting in their altered transcriptional activity. For example, it has recently been reported that I $\kappa$ B kinase and NF- $\kappa$ B are activated in skeletal muscle by exercise. While this pathway represents a likely ROS-activated regulator of gene expression during, and following, contractile activity (Ho et al., 2005), the precise contribution of each of these signaling cascades and their downstream targets during exercise remains an exciting avenue of investigation in metabolic plasticity.

#### *Protein kinase activation by exercise*

The alterations in cellular homeostasis brought about by contractile activity, as noted above, affect the activation of

kinases and phosphatases resulting in the posttranslational modification of proteins (Nader and Esser, 2001; Sakamoto et al., 2002). In the context of exercise, this rapid response is related to the type, intensity and duration of the contractile activity, as well as to the muscle fiber type (Bodine et al., 2001; Nader and Esser, 2001; Sakamoto et al., 2002). Multiple kinases, including AMPK, Akt and the mitogen-activated protein kinases (MAPKs) ERK1/2 and p38, are involved in the regulation of DNA transcription through the phosphorylation of nuclear transcription factors. This either enhances or inhibits the ability of transcription factors to bind DNA, affecting target gene transcription (Bergeron et al., 2001; Sakamoto et al., 2002; Sandri et al., 2004). Chronic activation of these signaling cascades brought about by muscle contractions during intermittent bouts of acute physical activity can result in either (1) nuclear and mitochondrial gene expression, leading to phenotype adaptations such as mitochondrial biogenesis (Hood, 2001) and improved endurance performance, or (2) myofiber hypertrophy and augmented force development (Glass, 2005). For example, the activation of the p38 MAPK pathway participates in contractile activity-induced PGC-1 $\alpha$  gene expression in skeletal muscle through the phosphorylation of proteins that include ATF2 (Akimoto et al., 2005). In addition, low-frequency pacing of skeletal muscle reflective of endurance activity also induced an AMPK–PGC-1 $\alpha$  signaling pathway (Atherton et al., 2005). As described below, this induction of PGC-1 $\alpha$  is an important initial mechanism involved in mitochondrial adaptations to contractile activity. In addition, a considerable amount of recent progress has been made in understanding the signaling pathways that mediate skeletal muscle hypertrophy, primarily *via* the activation of Akt-mediated events (Kubica et al., 2005; Lai et al., 2004), as well as muscle atrophy, largely *via* signaling pathways involving FOXO, Atrogin-1 and MURF-1 (Glass, 2005; Sandri et al., 2004). Given the multiplicity of signaling pathways that are active in muscle, classifying the specific cascades involved in these divergent forms of muscle plasticity is a task that requires continued investigation.

#### *Gene expression response to contractile activity and recovery*

The early cellular signals that are associated with contractile activity are known to induce the subsequent expression of mRNAs encoding transcription factors that regulate changes in skeletal muscle phenotype (Irrcher and Hood, 2004; Puntschart et al., 1998). Several studies have demonstrated that changes in the mRNA expression of transcription factors precede the contractile activity-induced regulation of mitochondrial and metabolic plasticity in skeletal muscle (Connor et al., 2001; Irrcher et al., 2003; Michel et al., 1994; Pilegaard et al., 2003; Xia et al., 1997). These include the induction of a family of immediate early genes (*c-fos*, *c-jun*), the early growth response gene-1 (*Egr-1*), specificity protein 1 (Sp1) and nuclear respiratory factor-1 (NRF-1), which respond to both acute and chronic contractile activity (Abu-Shakra et al., 1993; Connor et al., 2001; Irrcher and Hood, 2004; Michel et al., 1994; Murakami et al., 1998; Neuffer et al., 1998; Puntschart et al.,

1998; Takahashi et al., 1993). Egr-1 and Sp1 are known to be involved in regulating the transcription of cytochrome *c*, a nuclear-encoded protein of the electron transport chain (Connor et al., 2001; Freyssen et al., 2004), while NRF-1 is involved in the transcriptional activation of an even greater diversity of nuclear genes encoding mitochondrial proteins

(Kelly and Scarpulla, 2004), including the newly discovered mtDNA transcription factors TFB1M and TFB2M (Gleyzer et al., 2005). Acute contractile activity also results in a transient increase in transcriptional co-activator PGC-1 $\alpha$  promoter activity along with an increase in PGC-1 $\alpha$  mRNA (Akimoto et al., 2004) and protein (Baar et al., 2002). These increases

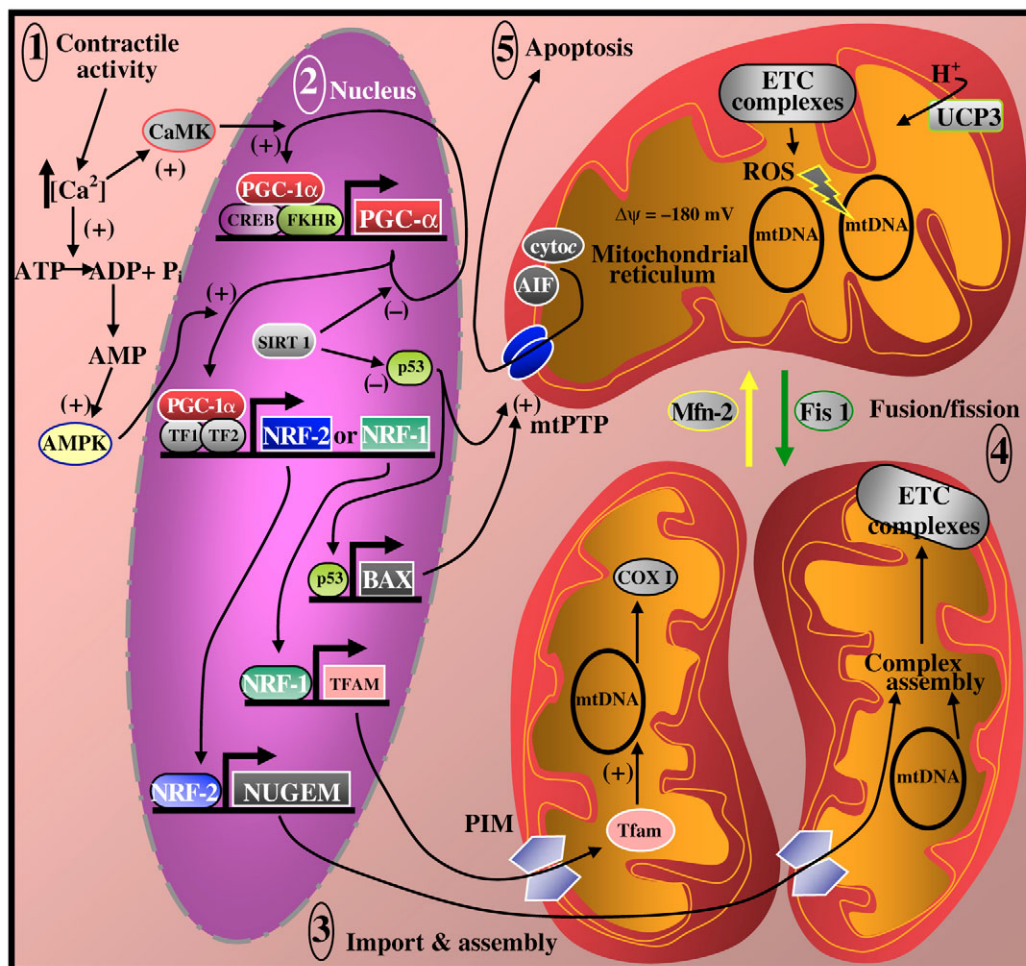


Fig. 1. Mitochondrial biogenesis is a component of contractile activity-induced muscle plasticity. Skeletal muscle contractile activity (1) is associated with elevations in intracellular  $\text{Ca}^{2+}$  and the subsequent activation of  $\text{Ca}^{2+}$ -sensitive signaling molecules such as calcium/calmodulin-dependent protein kinase (CaMK). In addition, ATP turnover resulting in the rise of AMP leads to the activation of AMP-activated protein kinase (AMPK). These two signaling kinases translocate to the myonuclei (2) and positively influence gene transcription through their interactions with the transcriptional co-activator peroxisome proliferator-activated receptor- $\gamma$  coactivator-1 $\alpha$  (PGC-1 $\alpha$ ). PGC-1 $\alpha$  autoregulates its gene expression, along with the expression of nuclear respiratory factor-1 (NRF-1) and NRF-2. NRF-1 and NRF-2 are transcription factors for numerous nuclear genes encoding mitochondrial proteins (NUGEMPS). NRF-1 also induces the expression of mitochondrial transcription factor A (Tfam), which, along with other nuclear-encoded mitochondrial proteins (NEMPS), is imported (3) into mitochondria by the protein import machinery (PIM). Tfam regulates the expression of the 13 mitochondrial DNA (mtDNA) gene products, including proteins such as cytochrome *c* oxidase subunit I (COX I). NEMPS and mtDNA-encoded proteins are assembled to form multi-subunit enzyme complexes required for oxygen consumption and ATP synthesis. This coordination between nuclear and mitochondrial genomes is necessary for organelle biogenesis. The mitochondrial phenotype is also altered through fusion and fission events (4). Mitofusion-2 (Mfn-2) influences the fusion of discrete populations of mitochondria into a larger mitochondrial reticulum, whereas Fis 1 is an important protein involved in organelle fission. Mitochondrial membrane potential ( $\Delta\psi$ ) is associated with the production of reactive oxygen species (ROS) from within the ETC, both of which can be reduced by the activity of uncoupling protein-3 (UCP3). Elevated ROS levels can trigger the opening of the mitochondrial permeability transition pore (mtPTP) and the release of the pro-apoptotic factors cytochrome *c* and apoptosis-inducing factor (AIF). Liberation of these proteins is the primary step in the mitochondrially mediated apoptotic program (5). Execution of this program is also facilitated by the actions of p53 and its downstream transcriptional target BAX. The anti-aging protein SIRT1 can inhibit the p53 pathway leading to apoptosis and it is known to negatively regulate hepatic PGC-1 $\alpha$  activity. The actions of SIRT1 in muscle remain unresolved.

appear to be dependent on the MEF2 and cAMP response element (CRE) binding sites within the PGC-1 $\alpha$  promoter, since mutation of these elements abolished contractile activity-induced transcriptional activation of the PGC-1 $\alpha$  promoter (Akimoto et al., 2004).

Metabolic plasticity and adaptations to contractile activity are augmented by an intervening recovery period between bouts of activity (Michel et al., 1994; Neuffer et al., 1998; Pilegaard et al., 2003; Puntchart et al., 1998; Takahashi et al., 1993). This applies to transcription factor expression (Irrcher and Hood, 2004), as well as the expression of several genes that encode various regulatory and metabolic proteins, including PGC-1 $\alpha$ . The activation of most of these genes takes place during the initial 1–4 h of recovery, returning to basal levels within 24 h (Pilegaard et al., 2000). This activation appears to be markedly attenuated if glycogen repletion post-exercise is maximized by the consumption of a high carbohydrate diet. By contrast, the response is increased if glycogen repletion is inhibited by the consumption of a low carbohydrate diet (Pilegaard et al., 2005). Thus, the enhanced gene expression response post-exercise is likely related to ATP availability, involving a shift in ATP utilization away from contractile activity toward the energy requirements of transcription, translation and post-translational modification reactions, as well as the availability of carbohydrate and lipid sources of energy.

#### Mitochondrial transcription factor A (Tfam)

Mitochondria contain multiple circular 16 kb genomes (mtDNA) (Fig. 1) that encode 13 protein, 22 tRNA and 2 rRNA genes, representing only a small fraction of the total number of proteins involved in the production and synthesis of mitochondria. The remaining mitochondrial proteins are encoded by the nuclear genome and must be imported into the organelle *via* the protein import machinery (Fig. 1). Thus, mitochondrial biogenesis requires the co-expression of both the nuclear and mitochondrial genomes to ensure the proper assembly and expansion of the mitochondrial reticulum. mtDNA replication and transcription are regulated independently of the nuclear genome but are dependent on the expression and import of a number of nuclear-derived transcription and regulatory factors. One of the most important proteins involved in these processes is Tfam. Tfam is imported into mitochondria *via* Tom20 (Grey et al., 2000) and it has a presequence that is cleaved during the import process. Within mitochondria, Tfam is responsible for binding to the regulatory D-loop region of mtDNA and is involved in mtDNA transcription and replication (Larsson et al., 1998). Recent work has indicated that Tfam is enriched in a nucleoid complex with mtDNA within the organelle (Legros et al., 2004), possibly serving to protect mtDNA from excessive ROS-induced damage or helping maintain mtDNA stability. Indeed, Tfam levels are closely associated with mtDNA levels in patients with mtDNA depletion (Larsson et al., 1994). The reduced levels of Tfam under these conditions may be a result of impaired

translation or import into the organelle (Larsson et al., 1994). Tfam is essential for embryonic development, and heterozygous knockout animals have reduced mtDNA copy number, transcription and respiratory chain dysfunction (Larsson et al., 1998; Li et al., 2000). Interestingly, Tfam levels have been reported to increase in human muscle (Lezza et al., 2001) and rat tissues (Dinardo et al., 2003) during aging, in a possible effort to maintain mtDNA stability. Exercise training also stimulates the expression of Tfam in humans (Bengtsson et al., 2001), as does chronic contractile activity produced by electrical stimulation of skeletal muscle in rats (Gordon et al., 2001). The increase in Tfam protein expression is preceded by a time-dependent increase in Tfam mRNA expression, import into the organelle and mtDNA binding (Gordon et al., 2001). Although a complete picture of the transcriptional regulation of the Tfam gene is lacking, it appears that NRF-1 is important in conferring transcriptional activation (Choi et al., 2004). The increase in the level of this transcription factor as an early event at the onset of contractile activity (Irrcher et al., 2003; Murakami et al., 1998) is likely important in mediating the subsequent involvement of Tfam in exercise-induced mitochondrial biogenesis. Recent work indicates that NRF-1 is also involved in the transcriptional regulation of accessory mtDNA transcription factors TFB1M and TFB2M (Gleyzer et al., 2005). The expression and function of these during exercise-induced metabolic plasticity remains to be determined.

#### Peroxisome proliferator-activated receptor- $\gamma$ coactivator-1 $\alpha$ (PGC-1 $\alpha$ )

The discovery of PGC-1 $\alpha$  represents a major advancement in the elucidation of the molecular mechanisms driving mitochondrial biogenesis. PGC-1 $\alpha$  was initially cloned from a brown fat cell library and was found to play a key role in linking the actions of nuclear hormone receptors to the transcriptional control of adaptive thermogenesis in brown fat, of which the biogenesis of mitochondria is a key process (Puigserver et al., 1998). Given the role of skeletal muscle in adaptive thermogenesis, PGC-1 $\alpha$  was found to be equally important in controlling mitochondrial content in this tissue (Wu et al., 1999). In fact, both gain- and loss-of-function studies in cell culture and *in vivo* point to PGC-1 $\alpha$  as an important regulator of energy metabolism and mitochondrial biogenesis in tissues relying mainly on oxidative metabolism for ATP production (i.e. brain, heart, skeletal muscle, brown fat and liver) (Leone et al., 2005; Lin et al., 2004; Puigserver et al., 1998; Wu et al., 1999).

In heart and skeletal muscle, the ectopic expression of PGC-1 $\alpha$  induces mitochondrial biogenesis and increases cellular respiration (Lehman et al., 2000; St-Pierre et al., 2003; Wu et al., 1999). The molecular mechanisms involved, at least in skeletal muscle, include the upregulation of Tfam transcription *via* co-activation of NRF-1 and NRF-2 (Wu et al., 1999). Thus, by this mechanism, PGC-1 $\alpha$  is able to coordinate nuclear and mitochondrial gene expression, a key requirement in the control of organelle biogenesis.

*Mechanism of PGC-1 $\alpha$  action and regulation of gene expression*

PGC-1 $\alpha$  is part of an important family of transcriptional co-activators that includes PGC-1 $\beta$  and PGC-related co-activator [PRC; see Lin et al. (Lin et al., 2005), for review]. These interact with a broad spectrum of nuclear hormone receptors and transcription factors to activate physiological processes intrinsically linked with energy metabolism in tissues possessing a high mitochondrial content. Both PGC-1 $\beta$  and PRC share a high degree of homology with PGC-1 $\alpha$ , in particular within domains necessary for protein–protein interactions. They also share some redundancy with respect to the manner in which they function, although the extent of this has yet to be defined in the context of the coordination of metabolic plasticity in skeletal muscle.

The basis for the PGC-1 $\alpha$ -mediated regulation of mitochondrial gene expression and biogenesis lies in its ability to transcriptionally activate a variety of transcription factors, as noted above, and also in its association with a growing list of proteins that are involved in regulating PGC-1 $\alpha$  activity. These include proteins that alter PGC-1 $\alpha$  transcriptional activity *via* post-translational modifications or protein–protein interactions. For example, PGC-1 $\alpha$  activity in skeletal muscle is positively regulated by the activation of the p38 MAPK pathway. Knutti et al. first described that the biological activity of PGC-1 $\alpha$  was tightly coupled to the binding of a putative repressor protein (Knutti et al., 2001). Upon phosphorylation by p38 MAPK, the inhibitory effect of the repressor protein was relieved, permitting PGC-1 $\alpha$  to recruit, interact with and co-activate proteins to induce target gene expression (Fan et al., 2004; Knutti et al., 2001). Puigserver et al. subsequently described the p38 MAPK-mediated phosphorylation of PGC-1 $\alpha$  on ser/thr residues within its inhibitory domain (Puigserver et al., 2001). This action was shown to stabilize and increase the transcriptional potency of PGC-1 $\alpha$  protein and to stimulate mitochondrial respiration. The subsequent identification of the repressor protein first characterized by Knutti et al. as p160<sup>MBP</sup> (Knutti et al., 2001) permitted the elucidation of distinct dual actions of p38 MAPK in stimulating mitochondrial biogenesis *via* PGC-1 $\alpha$ : (1) the direct effect on increasing PGC-1 $\alpha$  stability *via* ser/thr phosphorylation and (2) the augmentation of PGC-1 $\alpha$  transcriptional activity *via* the disruption of the PGC-1 $\alpha$ /p160<sup>MBP</sup> interaction. It appears that p160<sup>MBP</sup> is functional in skeletal muscle, since overexpression of this protein in PGC-1 $\alpha$  expressing C<sub>2</sub>C<sub>12</sub> muscle cells suppressed mitochondrial oxygen consumption and gene expression (Fan et al., 2004). Thus, the phosphorylation and activation of p38 MAPK are likely vital events at the onset of contractile activity-induced mitochondrial biogenesis.

It is also becoming clear that, in addition to post-translational phosphorylation, the acetylation status of PGC-1 $\alpha$  represents an important mechanism that could regulate its transcriptional activity, and thus affect mitochondrial biogenesis. For example, PGC-1 $\alpha$  has been shown to associate with histone acetyltransferases such as CBP/p300 and SRC-1 to activate the transcription of target genes (Puigserver et al.,

1998) and with the NAD-dependent deacetylase SIRT1. In liver, the SIRT1-mediated deacetylation of PGC-1 $\alpha$  stimulates the up-regulation of gluconeogenic genes and liver glucose output without affecting mitochondrial gene expression (Rodgers et al., 2005) whereas in neural cells, deacetylation of PGC-1 $\alpha$  causes a reduction in cellular oxygen consumption (Nemoto et al., 2005). Interestingly, these results suggest that the cellular context plays an important role in determining the ultimate effect of SIRT1-mediated PGC-1 $\alpha$  deacetylation. It is currently unknown whether PGC-1 $\alpha$  and SIRT1 physically interact to modulate mitochondrial function in skeletal muscle.

Despite indications that PGC-1 $\alpha$  is a key player in mitochondrial biogenesis, two recent independent studies using loss-of-function strategies to investigate the necessity of PGC-1 $\alpha$  in inducing mitochondrial biogenesis have determined that PGC-1 $\alpha$  is not an absolute requirement for the induction of mitochondrial biogenesis (Arany et al., 2005; Leone et al., 2005). However, these studies used different loss-of-function strategies to eliminate PGC-1 $\alpha$  expression, resulting in discrepant phenotypes and metabolic profiles. Thus, precise conclusions on the necessity of PGC-1 $\alpha$  for mitochondrial biogenesis, and the possible compensatory roles of other transcriptional co-activators, require further study, particularly in the context of exercise.

*Regulation of PGC-1 $\alpha$  gene expression*

Since PGC-1 $\alpha$  is an important regulator of mitochondrial biogenesis, it follows that the transcription of PGC-1 $\alpha$  plays an important regulatory role in the induction and maintenance of mitochondrial content in skeletal muscle. Recently, several signaling pathways directly influencing the activity of the PGC-1 $\alpha$  promoter have been identified. The best characterized are the signaling events occurring through a proximal cAMP responsive element (CRE) *via* CREB, a CRE-binding protein. Activation of CREB *via* phosphorylation appears to be an important step in a cascade of events that results in the integration of several signaling pathways leading to the execution of PGC-1 $\alpha$ -mediated actions in various tissues, including adaptive thermogenesis in brown fat (Cao et al., 2004; Puigserver et al., 1998), induction of gluconeogenic genes in the liver (Herzig et al., 2001) and calcium signaling through calcium/calmodulin-dependent protein kinase (CaMK) and calcineurin (CnA) in skeletal muscle and heart (Handschin et al., 2003; Schaeffer et al., 2004). Other signaling mechanisms involved in regulating the transcriptional activity of PGC-1 $\alpha$  have been described, including the p38 MAPK-mediated activation of ATF2, which leads to enhanced ATF2–CRE binding and activation of the PGC-1 $\alpha$  promoter (Akimoto et al., 2005). Interestingly, some transcription factors that PGC-1 $\alpha$  co-activates also regulate PGC-1 $\alpha$  promoter activity. An example of this autoregulatory mechanism was recently described in the MEF2-mediated regulation of PGC-1 $\alpha$  in skeletal muscle. It was shown that MEF2 proteins bind to the PGC-1 $\alpha$  promoter causing transcriptional activation, which is enhanced when PGC-1 $\alpha$  is ectopically expressed (Handschin et al., 2003). The continued identification of

factors that regulate PGC-1 $\alpha$  transcription will help us understand the stimulus-specific regulation of PGC-1 $\alpha$  expression, eventually leading to the identification of potential therapeutic strategies.

#### *Response to exercise*

It is known that single bouts of exercise can elicit changes in the expression of a number of transcription factors implicated in mitochondrial biogenesis, including PGC-1 $\alpha$ . When exercise is performed successively over a period of time, progressive increases in the accumulation of PGC-1 $\alpha$  protein and the important co-activated transcription factor NRF-1, as well as Tfam, the downstream target of PGC-1 $\alpha$  and NRF-1, are observed (Baar et al., 2002; Gordon et al., 2001; Irrcher et al., 2003; Terada et al., 2002). Thus, the exercise-induced coordination of these increases establishes the likelihood that these proteins play important roles in mediating the mitochondrial adaptations to exercise. The recent finding that skeletal muscle function in PGC-1 $\alpha$ <sup>-/-</sup> mice is reduced, but not completely compromised, supports this hypothesis (Leone et al., 2005). However, the absence of a complete compromise in muscle function in PGC-1 $\alpha$ <sup>-/-</sup> animals highlights the potential of discovering novel and unexplored mechanisms of exercise-induced adaptations in muscle that are not mediated by PGC-1 $\alpha$ . Thus, a more complete characterization of the existing PGC-1 family members and/or identification of novel PGC-1-like co-activators will be important in further elucidating the exercise-mediated induction and maintenance of mitochondrial content.

#### **Mitochondrial multi-subunit complex assembly in health and disease**

The ability of the mitochondrion to function as the predominant energy-producing organelle within a cell is dependent on the electron transport chain (ETC) complexes that are embedded within the inner mitochondrial membrane. The assembly of these large hetero-oligomeric complexes is unique because it requires the coordinated incorporation of subunits from both the nuclear and mitochondrial genomes. In recent years, defects in energy metabolism, which arise from mutations in mitochondrially associated genes, have been shown to serve as the molecular basis for a plethora of human diseases (Shoubridge, 2001; Wallace, 1992). These mitochondrial diseases, which usually manifest in early childhood, primarily affect high energy-producing tissues such as the brain, muscle and heart (Wallace, 1992).

The most widely studied ETC complex is COX, the terminal electron acceptor in the ETC, which converts oxygen to water and provides the protons required for ATP synthesis (Nijtmans et al., 1998). The assembly of the 200 kDa mammalian COX holoenzyme is a highly regulated process that involves the sequential incorporation of 13 subunits and several prosthetic groups into specific intermediate subcomplexes (Nijtmans et al., 1998; Wielburski and Nelson, 1983). COX assembly is further complicated by the fact that COX subunits are of both

nuclear and mitochondrial origin and that assembly requires numerous nuclear-encoded accessory proteins including SCO1, SCO2, COX 10 and Surf-1 (Barrientos et al., 2002; Carr and Winge, 2003). Three of the subunits that form the catalytic and structural core of the enzyme (COX I–III) are transcribed and translated by mtDNA and are subsequently incorporated into the inner membrane (Capaldi, 1990). The remaining smaller subunits are coded for in the nucleus and must therefore be targeted and imported into the organelle. Currently, the exact function of these nuclear-encoded subunits in holoenzyme assembly is not known, but the transcriptional regulation of the genes encoding these subunits is being elucidated (Lenka et al., 1998). The existence of tissue-specific isoforms of these nuclear-encoded enzymes suggests that they may function to alter the catalytic activity of the COX complex during altered states of energy metabolism within different tissues (Kadenbach et al., 1990; Linder et al., 1995). Furthermore, in rat liver cells, the dissociation of numerous nuclear-derived subunits results in an increase in the enzymatic activity of the COX holoenzyme (Kadenbach et al., 1991). Based on these observations, it is generally believed that these smaller proteins may play an important role in regulating the stability and maintenance of the COX holoenzyme complex (Kadenbach et al., 1991).

The mitochondrial assembly of multi-subunit complexes can be altered under various physiological and pathological conditions (Hood, 2001). In iron deficiency, the lack of functional heme results in decreased mitochondrial mass within the muscle, as well as mitochondria with abnormal structure (Hood et al., 1992). In addition, impaired COX function arising from mutations in mitochondrially encoded COX enzymes or assembly factor genes has been reported to be the primary cause of COX deficiency associated with ETC defects (Nijtmans et al., 1998). Using a blue-native polyacrylamide gel electrophoresis (BN-PAGE) approach (Schagger and von Jagow, 1991), the assembly of specific subunits into nascent complexes can be directly monitored, and alterations in the assembly profile that may be the underlying molecular cause of several mitochondrially associated diseases can be examined. A prime example of the importance of this assembly process has recently been demonstrated with the finding that patients harboring mutations in the Surf-1 COX assembly gene have an accumulation of unassembled COX subunits and intermediate complexes, leading to reduced COX activity (Tiranti et al., 1998; Williams et al., 2004). Furthermore, the use of BN-PAGE, in conjunction with denaturing (SDS) electrophoretic separation in the second dimension, has allowed for the characterization of the assembly pathway of other ETC complexes such as Complex I and the ATP synthase complex. These studies have subsequently led to the identification of assembly defects that impair the stability of the holoenzyme complexes (Antonicka et al., 2003; Nijtmans et al., 2001). Thus, these electrophoretic techniques appear to be very useful for the study of mitochondrial complex assembly in physiological and pathological conditions.

Despite an emergence of studies examining mitochondrial complex assembly, the molecular mechanisms regulating the biosynthesis of these complexes remain generally unresolved, particularly in mammalian cells. The series of steps in mitochondrial assembly that is best studied is that of protein import into the matrix of the organelle (Hood and Joseph, 2004). Precursor proteins translated in the cytosol are directed to the multi-subunit translocase of the outer membrane (Tom) complex by molecular chaperones such as mitochondrial import stimulating factor (MSF) or cytosolic heat-shock protein 70 (Hsp70). The N-terminal targeting sequence of the precursor protein interacts with outer membrane receptors such as Tom20 or Tom70, the protein is then subjected to ATP-dependent unfolding and is subsequently drawn into the matrix of the mitochondrion through the translocase of the inner membrane (TIM) complex. This occurs *via* the ATP-dependent action of mtHsp70 and accessory proteins. Once inside the matrix, the targeting sequence is cleaved, and the mature protein is re-folded into a functional component for Krebs cycle or for other matrix functions. Divergent import pathways are utilized for import into the outer membrane, intermembrane space or inner membrane (Koehler, 2004; Wiedemann et al., 2004).

Mitochondrial plasticity in muscle is dependent on the adaptive capacity of the protein import pathway, since approximately 1000 proteins localized within the organelle require entry *via* this route during biogenesis. In muscle, protein import rates into subsarcolemmal (SS) mitochondria are less than that for mitochondria isolated from the intermyofibrillar (IMF) region, in part because of differential amounts of intramitochondrial ATP and divergent rates of respiration. Chronic contractile activity of muscle results in an enhanced rate of import of precursor proteins into the matrix of both mitochondrial subfractions (Gordon et al., 2001; Takahashi et al., 1998). This is likely due to the greater expression of protein import machinery components, such as Tom20 and mtHsp70. Tom20 appears to be critical for the initial recognition and import of precursor proteins, since antisense-induced reductions in Tom20 expression lead to parallel declines in import rate (Grey et al., 2000).

Thus, there is conclusive evidence that the import and assembly of multi-subunit complexes are modified during altered states of mitochondrial biogenesis. These findings demonstrate another level of control through which mitochondria maintain their protein composition, as well as cellular homeostasis, in response to physiologically induced increases in mitochondrial function.

#### Future directions in metabolic plasticity in muscle

This review has focused on the currently viewed vital proteins and processes involved in regulating mitochondrial biogenesis in muscle. Much remains to be learned in areas such as the assembly of multi-subunit complexes, lipid incorporation into the organelle, mitochondrial fission and fusion mechanisms, the role of mitochondrial nucleoids and the mtDNA transcription factors TFB1M and TFB2M, as well as the control of PGC-1 $\alpha$

expression. Continued study in this area will undoubtedly reveal the existence of other important mechanisms involved in regulating mitochondrial biogenesis in health, disease and as a result of exercise. This will also have implications for our understanding of mitochondrial dysfunction following skeletal muscle disuse or as a result of disease.

#### List of abbreviations

AICAR	5-aminoimidazole-4-carboxamide riboside
AMPK	AMP-activated protein kinase
ATF2	activating transcription factor 2
$\beta$ -GPA	$\beta$ guanidinopropionic acid
BN-PAGE	blue-native polyacrylamide gel electrophoresis
CaMK	calcium/calmodulin-dependent protein kinase
CBP	CREB-binding protein
CnA	calcineurin
COX	cytochrome <i>c</i> oxidase
CRE	cAMP response element
CREB	CRE-binding protein
ERK1/2	extracellular regulated kinase 1/2
ETC	electron transport chain
FOXO	Forkhead box, o-class
Hsp70	heat shock protein 70
I $\kappa$ B	NF- $\kappa$ B inhibitor
IMF	intermyofibrillar region
MAPKs	mitogen-activated protein kinases
MEF2	myocyte enhancer factor-2
MSF	mitochondrial import stimulating factor
mtDNA	mitochondrial DNA
NEMPS	nuclear-encoded mitochondrial proteins
NF- $\kappa$ B	nuclear factor $\kappa$ B
NRF-1	nuclear respiratory factor-1
NUGEMPS	nuclear genes encoding mitochondrial proteins
PGC-1 $\alpha$	peroxisome proliferators-activated receptor- $\gamma$ coactivator-1 $\alpha$
PIM	protein import machinery
PKC	protein kinase C
PRC	PGC-related co-activator
ROS	reactive oxygen species
SCO1/2	synthesis of cytochrome <i>c</i> oxidase
SDS	sodium dodecyl sulfate
SIRT1	sirtuin 1
SRC-1	steroid receptor coactivator 1
Tfam	mitochondrial transcription factor A
TFB1/2M	mitochondrial transcription factor B1/B2
TIM	translocase of the inner membrane
Tom	translocase of the outer membrane

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