SYMPOSIUM REVIEW

Reactive oxygen and nitrogen species as intracellular signals in skeletal muscle

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Abstract It is well established that contracting skeletal muscles produce free radicals. Given that radicals are known to play a prominent role in the pathogenesis of several diseases, the 1980s–90s dogma was that contraction-induced radical production was detrimental to muscle because of oxidative damage to macromolecules within the fibre. In contrast to this early outlook, it is now clear that both reactive oxygen species (ROS) and reactive nitrogen species (RNS) play important roles in cell signalling pathways involved in muscle adaptation to exercise and the remodelling that occurs in skeletal muscle during periods of prolonged inactivity. This review will highlight two important redox sensitive signalling pathways that contribute to ROS and RNS-induced skeletal muscle adaptation to endurance exercise. We begin with a historical overview of radical production in skeletal muscles followed by a discussion of the intracellular sites for ROS and RNS production in muscle fibres. We will then provide a synopsis of the redox-sensitive NF-κB and PGC-1α signalling pathways that contribute to skeletal muscle adaptation in response to exercise training. We will conclude with a discussion of unanswered questions in redox signalling in skeletal muscle in the hope of promoting additional research interest in this field.

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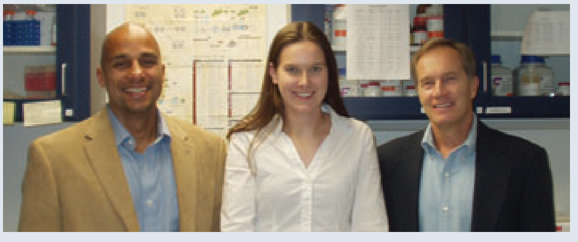
Introduction

The first report that muscular exercise increases the production of reactive oxygen species (ROS) in humans appeared in 1978 (Dillard *et al.* 1978). This initial observation did not reveal the sources of ROS production during exercise, but a subsequent study demonstrated that contracting skeletal muscles are a prominent source of ROS production (Davies *et al.* 1982). It was later observed that contracting muscles also produce nitric oxide (NO) and other reactive nitrogen species (RNS) (Balon & Nadler, 1994). Since these early observations, many studies have confirmed that muscular exercise promotes the production of both ROS and RNS in skeletal muscle fibres (Powers & Jackson, 2008).

Scott Powers (right), **Erin Talbert** (centre) and **Peter Adhihetty** (left) work in the department of Applied Physiology and Kinesiology at the University of Florida and collaborate on studies involving reactive oxygen species-linked signalling events in both skeletal and cardiac muscle. Their research backgrounds are in physiology and biochemistry. Scott Powers and Erin Talbert are currently collaborating on studies that focus on understanding the cell signalling pathways responsible for disuse muscle atrophy. Peter Adhihetty's research centres on investigating the role of mitochondrial dysfunction in both muscle and neural tissue in various diseases or disorders.



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During the 1980s–90s it was widely believed that exercise-induced ROS production was damaging to skeletal muscle fibres and limited consideration was given to the possibility that contraction-induced ROS/RNS production could play an important signalling role in muscle adaptation to exercise. However, contemporary evidence indicates that increased ROS and RNS production plays a key role in the regulation of signalling pathways that are essential for muscle adaptation in response to endurance exercise training. The discovery that ROS/RNS plays a significant role in skeletal muscle adaptation to exercise is an exciting new area of research in exercise biology and is the focus of this review. Our report begins with an overview of the sources of contraction-induced ROS and RNS production in skeletal muscles followed by a discussion of the paradox that ROS plays a significant signalling role in muscle remodelling during both exercise training and disuse-induced muscle atrophy. We will then highlight two important redox-sensitive signalling molecules in skeletal muscle, nuclear factor-κB (NF-κB) and peroxisome proliferator-activated receptor-γ coactivator-1α (PCG-1α). This will be followed by a summary of the evidence that ROS and/or RNS contribute to skeletal muscle adaptation in response to endurance exercise training. We will conclude with a discussion of the gaps in our knowledge about redox control of muscle adaptation in the hope of stimulating additional research in this exciting area of exercise biology.

Sources of ROS/RNS production in contracting skeletal muscles

The chief parent radical species produced in muscle fibres are superoxide and NO, and both species can react with other molecules to form a wide range of ROS and RNS, respectively (Halliwell & Gutteridge, 2007). An overview of the principal sites of superoxide and NO production in cells follows.

Cellular sources of superoxide. Superoxide is produced by the addition of a single electron to ground state oxygen in a variety of intracellular locations. For example, superoxide production can occur in the mitochondrion, sarcoplasmic reticulum, transverse tubules, sarcolemma, and cytosol (Fig. 1). The main sites of superoxide production in the mitochondria are complexes I and III of the electron transport chain (Barja, 1999). Further, recent findings indicate that compared to mitochondria from slow type I muscle fibres, mitochondria from fast type II muscle fibres possess unique properties that promote higher levels of ROS production (Anderson & Neufer, 2006). The mechanism(s) to explain these differences remain unknown.

Mitochondria are commonly cited as the primary source of superoxide production in contracting muscle fibres because early reports suggested that 2–5% of the total oxygen consumed by mitochondria undergoes a one electron reduction to form superoxide (Boveris & Chance, 1973; Loschen *et al.* 1974). Based upon this assertion, authors have often assumed that the increased ROS generation that occurs in contracting muscles is directly linked to the increased oxygen consumption during exercise, implying a large increase (e.g. 50- to 100-fold) in superoxide generation by skeletal muscle during aerobic contractions (e.g. see Kanter, 1995; Urso & Clarkson, 2003). Nevertheless, increasing evidence argues against this assumption as emerging evidence reveals that the rate of superoxide production by mitochondria is much less that the early estimates that 2–5% of all molecular oxygen consumed by mitochondria is converted to superoxide. For example, Brand and colleagues calculates that less than 0.15% of oxygen consumed by the mitochondria is used to form superoxide (St-Pierre *et al.* 2002). Further, abundant data indicate that mitochondria produce more ROS in state 4 (basal) respiration compared to active state 3 (maximal ADP stimulated) respiration (Di Meo & Venditti, 2001; Adhihetty *et al.* 2005; Anderson & Neufer, 2006; Kavazis *et al.* 2009). Together, these results suggest that mitochondria are not the primary source of ROS production in muscle fibres during exercise.

In addition to mitochondria, superoxide can be produced by numerous other cellular locations including NADPH oxidases located within the sarcoplasmic reticulum, transverse tubules and sarcolemma (Fig. 1) (Powers & Jackson, 2008). Unfortunately, limited information currently exists about the regulation of these systems in muscle during exercise.

Evidence also indicates that xanthine oxidase produces superoxide in the cytosol of contracting rat skeletal muscles (Gomez-Cabrera *et al.* 2005). However, compared to rats, human skeletal muscles contain lower levels of xanthine oxidase and debate continues as to whether xanthine oxidase plays an important role in superoxide production in human skeletal muscle (Linder *et al.* 1999; Gomez-Cabrera *et al.* 2003).

The dismutation of superoxide in cells produces hydrogen peroxide (H_2O_2) and this process can occur spontaneously or by action of the superoxide dismutases (SOD) (Halliwell & Gutteridge, 2007). H_2O_2 is a non-radical and a weak oxidant with a relatively long half-life, which permits its diffusion within cells and across cell membranes (Halliwell & Gutteridge, 2007). Further, H_2O_2 reacts with many different cellular molecules and can activate a variety of signalling pathways. Collectively, these properties make H_2O_2 an important ROS signalling molecule in cells (Veal *et al.* 2007).

Nitric oxide production in contracting muscles. Nitric oxide is synthesized from the amino acid L-arginine using three different isoforms of nitric oxide synthase (NOS1, NOS2 and NOS3). Further, a fourth mitochondrial nitric oxide synthase may also exist (Ghafourifar & Cadenas, 2005). Normally skeletal muscle expresses two of these isoforms (i.e. NOS1 and NOS3) (Moylan & Reid, 2007). However, NOS2 can also be expressed in skeletal muscle during inflammatory states (Moylan & Reid, 2007). Nitric oxide is known to have many signalling functions and can readily react with superoxide to form the strong oxidizing agent peroxynitrite leading to the depletion of thiol groups in cells (Moylan & Reid, 2007). This modification of cellular thiol groups could alter redox signalling and may play an important role in numerous cell signalling pathways (Jones, 2006). Peroxynitrite formation also reduces the bioavailability of both superoxide and NO, which could also influence cell signalling events (Halliwell & Gutteridge, 2007; Powers & Jackson, 2008).

ROS as signalling molecule in skeletal muscle remodelling: the ROS paradox

Many studies have concluded that inactivity-induced ROS production in skeletal muscle contributes to disuse muscle atrophy (Powers *et al.* 2005; Powers *et al.* 2007). Paradoxically, growing evidence also suggests that intracelluar ROS production is a required signal for the normal remodelling that occurs in skeletal muscle in response to repeated bouts of endurance exercise (Hamilton *et al.* 2003; Gomez-Cabrera *et al.* 2008; Ristow *et al.* 2009). Therefore, how does the ROS production that occurs in muscle fibres during exercise avoid resulting in muscle atrophy? Unfortunately, a definitive answer to this question is not currently available. Nonetheless, our knowledge about the biological implications of exercise-induced ROS has expanded rapidly in recent years and has provided some clues to this apparent mystery. Based upon current evidence, it appears that at least two potential explanations for this apparent ROS paradox exist. First, while continuous and high rates of free radical production can damage cellular components, depress protein synthesis, and activate proteases, an acute bout of muscular exercise that results in acute production of low-to-moderate levels of oxidants does not generate this response. On the contrary, an acute and small increase in ROS production during a bout of muscular exercise appears to play an important role in the regulation of cell signalling pathways that promote gene expression leading to an increased oxidative phenotype of skeletal muscle (Droge, 2002; Jackson, 2008).

A second potential factor that may contribute to the 'ROS paradox' is that the site(s) of ROS production in muscle may differ between contracting fibres and fibres exposed to prolonged periods of inactivity. For example, a recent study indicates that prolonged muscle inactivity results in a large increase in mitochondrial ROS production (Kavazis *et al.* 2009). In contrast, it seems unlikely that mitochondria are the primary source of ROS production in contracting muscle fibres (Powers & Jackson, 2008). Therefore, it is feasible that the different sites of muscle ROS production in these two conditions may also influence redox-sensitive signalling and contribute to the ROS paradox in skeletal muscle.

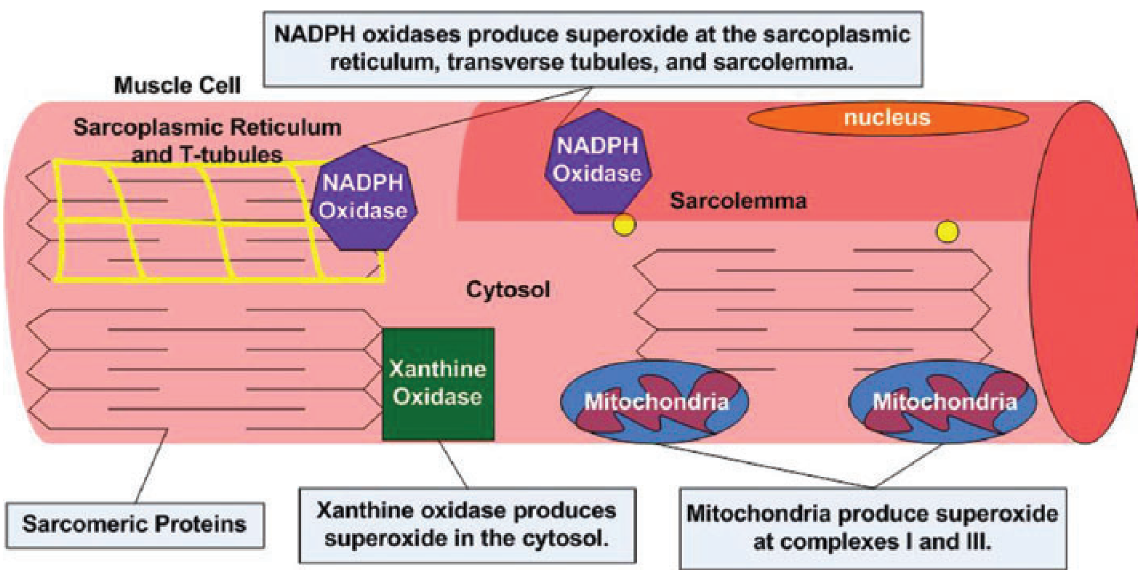

Figure 1. Illustration of the potential cellular sites for the production of superoxide in muscle fibres
Note that primary sites for cellular superoxide production include mitochondria, NADPH oxidases (located within the sarcoplasmic reticulum, transverse tubules and the sarcolemma), and xanthine oxidase. See text for more details.

Regardless of the explanation for the ROS paradox, the remainder of this report will provide a summary of two key redox sensitive signalling pathways in skeletal muscle and will highlight the evidence that ROS/RNS is required for normal adaptation of skeletal muscle to endurance exercise training.

Redox sensitive signalling pathways in skeletal muscle

Skeletal muscle is a malleable tissue that can undergo significant phenotypic changes in response to repeated bouts of exercise. Indeed, as few as five consecutive days of endurance exercise results in significant improvements in both the oxidative and antioxidant capacity of skeletal muscle fibres (Vincent *et al.* 1999, 2000). During the past decade, much has been learned about the exercise-induced cell signalling pathways that mediate these changes. Interestingly, many of these pathways appear to be initiated, or at least potentiated, by ROS and RNS signals. Several of these redox-sensitive pathways result in changes in transcription factor activity, either increasing or decreasing the transcription of target genes. The next segment will highlight the regulation of two important redox-sensitive transcription factors that are involved in muscle adaptation in response to endurance exercise training. Specifically, we will discuss the role that ROS/RNS play in the activation of exercise-induced signalling via nuclear factor-κB (NF-κB) and PGC-1α in skeletal muscle fibres.

Redox control of NF-κB activation

Again, it is well known that repeated bouts of endurance exercise result in adaptive changes in skeletal muscle fibres resulting in an oxidative phenotype with improved antioxidant capacity (Hammeren *et al.* 1992; Powers *et al.* 1992; Criswell *et al.* 1993; Powers *et al.* 1994). Understanding the cell signalling pathways responsible for exercise-induced muscle adaptation remains an important topic for research in skeletal muscle biology. In this regard, robust evidence reveals that redox-sensitive pathways use ROS or RNS to transfer signals from the cytoplasm to the nucleus to promote gene expression (Droge, 2002; Ji *et al.* 2006; Upham & Trosko, 2009). An important signalling link between contraction-induced ROS production and skeletal muscle remodelling involves the redox regulation of the NF-κB family of transcriptional activators. NF-κB transcription factors are evolutionary conserved signalling molecules that control the expression of numerous genes involved in a large number of cell processes such as inflammation, cell growth, stress responses, and apoptosis (Kramer & Goodyear, 2007). For example, several antioxidant enzymes including copper-zinc superoxide dismutase, manganese superoxide dismutase, and γ-glumatylcysteine synthetase contain NF-κB binding sites in the 5′-flanking region of their promoter (Allen & Tresini, 2000). Therefore, these genes are potential targets for ROS-mediated signalling via activation of NF-κB. A brief summary of NF-κB regulation and evidence that active NF-κB plays an important role in exercise-induced muscle adaptation follows.

NF-κB comprises a family of five transcription factors (p65, Rel B, c-Rel, p52 and p50). To act as a transcriptional factor, two of these proteins must dimerize; this dimerization facilitates nuclear translocation and the subsequent binding of NF-κB to the kB consensus sequence of the target genes (Bakkar & Guttridge, 2010). Active NF-κB transcription factors can promote a wide range of cellular outcomes depending upon the cell type (Jackman & Kandarian, 2004). All five of the NF-κB family members are expressed in skeletal muscle but evidence indicates that the p50–p65 heterodimer is responsible for the majority of NF-κB activity in muscle (Jackman & Kandarian, 2004).

In unstressed cells, the nuclear localization sequence of NF-κB is bound to inhibitory IkB proteins in the cytosol and these inhibitory proteins prevent the dimerization of p50 to p65. However, increased levels of ROS in the cytosol can activate IkB-α kinase (IKK) resulting in the phosphorylation of IkB proteins; this initiates ubiquitination and subsequent IkB degradation via the proteasome (Kabe *et al.* 2005). Degradation of IkB removes the inhibition and liberates NF-κB complexes so that dimerization and nuclear translocation can occur (Kabe *et al.* 2005) (Fig. 2).

Many studies have reported that an acute bout of endurance exercise results in NF-κB activation in skeletal muscle (Ji *et al.* 2006; Ji, 2007). In this regard, time course studies reveal that a decrease in cytosolic IkB and an increase in phosphorylated IkB proteins occurs immediately post-exercise (Ji *et al.* 2004). Current evidence indicates that NF-κB binding to DNA reaches a peak at $\sim$2 h following an acute bout of exercise (Ji *et al.* 2004). Similarly, exposure of C2C12 muscle cells to 1–2 mmol l^{-1} H_2O_2 results in NF-κB activation with maximal NF-κB/DNA binding occurring at 2 h following exposure to ROS (Zhou *et al.* 2001).

Although ROS can promote NF-κB activation and subsequent gene expression, the DNA binding activity of oxidized NF-κB is diminished suggesting that ROS may also inhibit NF-κB transcriptional activity (Kabe *et al.* 2005). Hence, although NF-κB was once considered to be a prototypic redox sensitive transcription factor, the observation that ROS can both promote and inhibit NF-κB transcriptional activation has led to debate regarding the redox control of NF-κB signalling (Pantano *et al.* 2006). Nonetheless, a growing body of evidence suggests that exercise-induced ROS production promotes contraction-induced NF-κB activation in skeletal muscle

(Ji *et al.* 2007; Ji, 2008). Indeed, several studies reveal that exercise-induced NF-κB activation in skeletal muscle requires ROS as an upstream signal and that NF-κB activation is essential for exercise training-induced expression of antioxidant enzymes (Hollander *et al.* 2001; Ji *et al.* 2004; Gomez-Cabrera *et al.* 2005). For example, Gomez-Cabrera and colleagues have demonstrated that antioxidant-mediated suppression of exercise-induced ROS emission in contracting skeletal muscles results in blunted NF-κB activation as evidenced by reduced nuclear binding of NF-κB (Gomez-Cabrera *et al.* 2005). Further, the decrease of contraction-induced ROS production and NF-κB activation in these experiments also prevented the exercise-induced increase in muscle levels of manganese SOD (MnSOD) mRNA and MnSOD protein.

To summarize, ROS generated by contracting muscles plays an important role in the activation of NF-κB in skeletal muscle in response to exercise. Moreover, exercise-induced activation of NF-κB appears to be a requirement for exercise-induced expression of MnSOD and perhaps many other cellular proteins that are critical to training-induced muscle adaptation. Identifying other important gene targets of NF-κB in skeletal muscle is likely to remain an important topic for future studies of exercise-induced cell signalling.

Redox control of PGC-1α

An important skeletal muscle adaptation that occurs with endurance training is the increase in mitochondrial content in the muscle fibre due to mitochondrial biogenesis. Exercise-induced increases in mitochondria in skeletal muscle are associated with a variety of health-related benefits including improvements in tissue oxidative capacity, exercise tolerance and insulin resistance (Holloszy, 1967; Holloszy & Coyle, 1984; Irrcher *et al.* 2003*a*, 2008; Hawley & Zierath, 2004). Mitochondrial biogenesis is a unique and complicated process since mitochondria are composed of gene products from both the nuclear and mitochondrial genomes. Thus, mitochondrial biogenesis requires the coordinated response of the nuclear and mitochondrial genomes to maintain the correct stoichiometric arrangement of proteins during organelle synthesis. This coordinated response is accomplished by a vast array of transcription factors and transciptional coactivators (Hood, 2001; Adhihetty *et al.* 2003).

Peroxisome proliferator-activated receptor-γ (PPAR-γ) coactivator-1α (PGC-1α) has been shown to be an integral regulator of mitochondrial biogenesis. PGC-1α interacts with and coactivates a variety of transcription factors and nuclear receptors involved in the upregulation of both nuclear- and mitochondrial-encoded genes involved in organelle synthesis, which include oestrogen-related receptor-α (ERR-α; Huss *et al.* 2002), nuclear respiratory factors (NRF-1 and NRF-2; Scarpulla, 2006), thyroid hormone receptor (TR; Irrcher *et al.* 2003*b*) and myocyte enhancer factor (MEF; Lin *et al.* 2002). PGC-1α has been shown to regulate a variety of cellular processes such as adaptive thermogenesis, glucose metabolism, muscle fibre-type specialization and oxidative phosphorylation in many tissues (Puigserver *et al.* 1998; Wu *et al.* 1999; Lin *et al.* 2002). Within skeletal muscle, numerous studies have shown that PGC-1α is capable of dictating mitochondrial content and function, and potently activating mitochondrial biogenesis (Ljubicic *et al.*; Wu *et al.* 1999; Vega *et al.* 2000; Huss & Kelly, 2004). Thus, PGC-1α has emerged as a key protein that can regulate mitochondrial content in tissues and has been referred to as the 'master regulator' of mitochondrial biogenesis.

PGC-1α expression in muscle can be induced by a variety of stimuli associated with muscular exercise (Goto *et al.* 2000; Baar *et al.* 2002; Irrcher *et al.* 2003*a*,*b*; Pilegaard

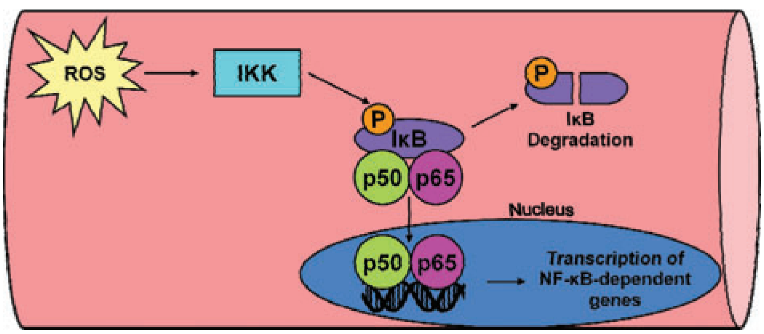

Figure 2. Steps leading to NF-κB activation and signalling in cells
In unstressed cells, the nuclear localization sequence of NF-κB is bound to inhibitory IkB proteins in the cytosol and these inhibitory proteins inhibit the dimerization of p50 to p65. During periods of oxidant stress, increased levels of ROS in the cytosol can activate IkB-α kinase (IKK), which results in phosphorylation of IkB proteins. This phosphorylation initiates ubiquitination and subsequent IkB degradation via the proteasome. Degradation of IkB removes the inhibition and releases NF-κB complexes so that dimerization and nuclear translocation can occur. See text for more details.

et al. 2003; Russell *et al.* 2003). The exercise-induced signalling mechanisms leading to PGC-1α induction have been primarily attributed to contractile activity-mediated increases in AMPK and p38 MAPK activation. While many studies have confirmed that both acute and chronic exercise can activate pathways leading to PGC-1α induction, much less is known about the specific role that ROS plays in mitochondrial biogenesis. Nonetheless, emerging studies reveal that exercise increased ROS and RNS production in muscle occurs coincident with enhanced mitochondrial biogenesis signalling markers. However, these observations are correlative and do not demonstrate cause-and-effect. Thus, two important questions emerge: (1) is PGC-1α transcriptional activity influenced by redox control? and (2) are the exercise inductions of PGC-1α expression in skeletal muscle dependent upon ROS and/or RNS production?

PGC-1α appears to be sensitive to the redox status of the cell because treatment of cultured muscle myotubes with exogenous hydrogen peroxide causes induction of PGC-1α, and the antioxidant *N*-acetylcysteine inhibits this upregulation (Irrcher *et al.* 2009). In this regard, Irrcher *et al.* (2009) predict that elevated cellular levels of ROS induce PGC-1α transcription indirectly, via AMPK activation (Fig. 3). Thus, PGC-1α appears to be part of a redox-sensitive pathway similar to the ROS-sensitive transcription factor NF-κB. In fact, the human PGC-1α promoter contains an NF-κB binding site, which suggests NF-κB may also regulate the expression of PGC-1α (Irrcher *et al.* 2008). Analysis of the human PGC-1α promoter has revealed a variety of consensus transcription binding sites to the following transcription factors: specificity protein 1 (SP1), cAMP response element binding protein (CREB), CREB related family member, activating transcription factor (ATF2), forkhead transcription factor (FKHR), p53, EBox binding proteins, GATA and MEF2 (Irrcher *et al.* 2008). Many of these transcription factors have been shown to be ROS-sensitive which indicates numerous potential possibilities for redox control of PGC-1α expression. However, these are only theoretical possibilities which have not been confirmed and further studies are necessary. Additionally, RNS, particularly NO, may also be involved in the regulation of PGC-1α (Lira *et al.* 2010). Specifically, Lira *et al.* (2010) report that NO production promotes PGC-1α expression via NO-mediated activation of AMPK (i.e. AMPK α1 isoform) (Fig. 3). Therefore, the current evidence suggests that both ROS and RNS can contribute to PGC-1α expression via a common signalling pathway (i.e. AMPK activation).

It has recently been shown that PGC-1α activity is also regulated by a variety of post-translational modifications including phosphsphorylation, acetylation, methylation and ubiquination (Jager *et al.* 2007; Rodgers *et al.* 2008; Dominy *et al.* 2010). Studies have demonstrated that p38 MAPK and AMPK are capable of phosphorylating PGC-1α at a variety of amino acid residues, which results in a more stable and active PGC-1α protein. The acetylase transferase (GCN5) acetylates PGC-1α at several lysine residues to inactivate PGC-1α while the NAD$^+$-dependent deacetylase Sirt1 removes acetyl groups leading to activation of PGC-1α (Fig. 3). These post-translational modifications have been proposed to activate and/or deactivate PGC-1α located in the nucleus or within the cytosol of the cell. Recently, Wright *et al.* (2007) reported that acute exercise causes the activation

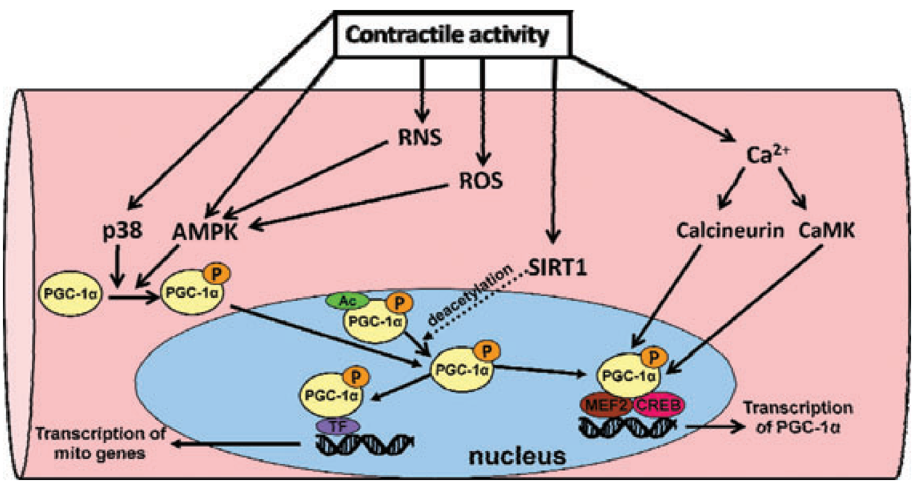

Figure 3. Schematic diagram of the key steps involved in PGC-1α expression and activation in skeletal muscle fibres
Note that the dotted arrow leading from SIRT1 and directed toward the arrow leading from PCG-1α is driving this reaction forward resulting in deacetylation of PGC-1α and increasing the pool of non-acetylated PGC-1α in the nucleus. See text for more details. Legend: Ac, acetylation; p, phosphorylation.

a cytosolic pool of PGC-1α, which then translocates to the nucleus to initiate mitochondrial gene expression prior to increases in overall PGC-1α expression. Thus, post-translational modifications appear to represent an immediate mechanism to activate PGC-1α and initiate PGC-1α-dependent gene expression. To date, it is unclear whether these post-translational modifications are under redox control but this certainly represents another potential complexity that may be involved in ROS-mediated induction of PGC-1α.

Is contraction-induced ROS production required for PGC-1α induction in skeletal muscle?

Numerous training studies have provided *in vivo* evidence that ROS are important signalling molecules mediating many of the exercise-induced adaptations in skeletal muscle. The majority of these studies utilize dietary antioxidant supplementation to suppress exercise-induced redox signalling in skeletal muscles. For example, Hamilton *et al.* (2003) illustrated that a diet rich in antioxidants prevents training-induced increases in heat shock protein 72 (HSP72) in the heart. This study provided the first evidence that exercise-induced ROS production was critical for the training-induced adaptive responses of HSP72. These findings were confirmed and extended by Jackson *et al.* (2004) who demonstrated that training-induced increases in HSP60, HSP72 and HSP73 in human skeletal muscle were blunted by a diet high in vitamin E and β-carotene (Jackson *et al.* 2004). Thus, antioxidant treatments were initially shown to blunt the HSP72 responses to exercise, which clearly indicates the importance of ROS as signalling molecules. However, the link between exercise-induced ROS production and exercise-induced elevations in muscle PGC-1α was only recently identified and a brief summary of this work follows.

Gomez-Cabrera *et al.* (2008) reported that exercising rats supplemented with vitamin C do not exhibit the normal phenotypic changes observed in skeletal muscle following a program of endurance training. Specifically, vitamin C supplementation diminished the exercise-induced increase in maximal oxygen consumption and also prevented the training-mediated rise in muscle antioxidant enzymes and PGC-1α protein levels in rat skeletal muscle. These investigators also supplemented human subjects with vitamin C and found the results to be in agreement with their findings in rodents (Gomez-Cabrera *et al.* 2008). Recent work has further confirmed that supplementing human subjects with vitamins C and E leads to diminished training-induced improvements in insulin sensitivity, as well as the expression of antioxidant defence enzymes and PGC-1α (Ristow *et al.* 2009). Taken together, these results support the notion that contraction-induced ROS molecules are critical signalling molecules for exercise-induced adaptations *in vivo* and that PGC-1α is under redox control.

Conclusions and future directions

A growing body of literature reveals that ROS and RNS are important signalling molecules for exercise-training induced adaptations in skeletal muscles. Indeed, both human and animal studies confirm that prevention of exercise-induced redox signalling via antioxidant supplementation results in a blunted training response in skeletal muscles.

Although our understanding of redox signalling pathways in skeletal muscle has grown in recent years, many unanswered questions remain. For example, the primary sites of superoxide production in contracting muscle remain controversial and additional work is required to determine the main sites of ROS production in contracting muscle and how this varies with exercise conditions (i.e. low intensity exercise *versus* high intensity exercise). Further, the inability to perform quantitative measures of ROS production in muscle cells is a significant limitation to investigators. Indeed, significant technological advances are required to provide investigators with the necessary tools to better understand the sites of ROS production and redox compartmentalization in muscle fibres. This is a critical area for future work because improving our understanding of ROS production and redox compartmentalization in muscle fibres will likely provide important insight into specific redox signalling pathways.

Also, whilst it is established that activation of NF-κB contributes to exercise-induced signalling in skeletal muscle, the identification of unknown gene targets of NF-κB in skeletal muscle remains an important topic for future research. Moreover, details regarding the specific signalling role that NF-κB plays in promotion of exercise-induced expression of PGC-1α should receive additional experimental attention.

Finally, emerging evidence indicates that ROS are required for contraction-induced expression of PGC-1α in the active muscle fibres. However, limited information exists regarding the explicit redox controlled signalling pathways that regulate PGC-1α activation and mitochondrial biogenesis in skeletal muscle. Clearly, there is much more to be learned about the redox control of skeletal muscle adaptation to exercise.

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