AMPK-mediated regulation of transcription in skeletal muscle

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ABSTRACT

Skeletal muscle phenotype plays a critical role in human performance and health, and skeletal muscle oxidative capacity is a key determinant of exercise tolerance. More recently, defective muscle oxidative metabolism has been implicated in a number of conditions associated with the metabolic syndrome, cardiovascular disease and muscle-wasting disorders. AMPK (AMP-activated protein kinase) is a critical regulator of cellular and organismal energy balance. AMPK has also emerged as a key regulator of skeletal muscle oxidative function, including metabolic enzyme expression, mitochondrial biogenesis and angiogenesis. AMPK mediates these processes primarily through alterations in gene expression. The present review examines the role of AMPK in skeletal muscle transcription and provides an overview of the known transcriptional substrates mediating the effects of AMPK on skeletal muscle phenotype.

INTRODUCTION

Skeletal muscle phenotype plays a critical role in human health, mainly due to its large mass and its high intrinsic capacity for oxidative metabolism. These factors are key determinants of human performance. Impaired skeletal muscle oxidative metabolism is associated with the development of a number of chronic diseases. Patients with Type 2 diabetes have impaired skeletal muscle oxidative phosphorylation secondary to a reduction in the expression of a number of metabolic and mitochondrial genes [1,2]. This could play a role in the pathogenesis of Type 2 diabetes, as first-order relatives of patients who are yet to show any clinical disease markers also have reduced skeletal muscle metabolic gene expression [3]. An association between impaired skeletal muscle oxidative capacity and cardiovascular risk factors has also emerged in rats artificially selected for low aerobic capacity [4]. In addition, defective mitochondrial metabolism is thought to contribute to the development of muscle-wasting disorders, such as that seen in aging [5], COPD (chronic obstructive pulmonary disease) [6] and some muscular dystrophies [7]. Taken together, these findings highlight the importance of oxidative metabolism and skeletal muscle phenotype in human health and performance.

The oxidative capacity of skeletal muscle is largely determined by the expression of enzymes involved in oxidative metabolism. These include genes that regulate

Key words: AMP-activated protein kinase (AMPK), angiogenesis, oxidative capacity, skeletal muscle, transcription.

Abbreviations: ACC, acetyl-CoA carboxylase; AICAR, 5-amino-4-imidazolecarboxamide riboside; AIS, auto-inhibitory sequence; AMPK, AMP-activated protein kinase; AMPKK, AMPK kinase; CaMK, Ca2+/calmodulin-dependent protein kinase; CaMKK, CaMK kinase; CBD, carbohydrate-binding domain; Cyt C, cytochrome c; Cox, Cyt C oxidase; CPT-1, carnitine palmitoyltransferase-1; CRE, cAMP-response-element; CREB, CRE-binding protein; CRTC2, CREB-regulated transcriptional co-activator 2; CS, citrate synthase; FAT, fatty acid translocase; FoxO, forkhead box O; GLUT4, glucose transporter isofrom 4; GEF, GLUT4-enhancer factor; HAT, histone acetyltransferase; HDAC, histone deacetylase; IL-6, interleukin-6; MEF2, myocyte enhancer factor 2; NRF, nuclear respiratory factor; PPAR, peroxisome-proliferator-activated receptor; PGC-1, PPARγ co-activator-1; RNAPII, RNA polymerase II; Sirt1, sirtuin 1; TBC1D, TBC (Tre-2/Bub2/Cdc16) domain family; TIC, transcriptional initiation complex.

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angiogenesis and substrate delivery to muscle, substrate transporters that take up substrate into muscle and the intrinsic enzymes of metabolic pathways that ultimately oxidize substrate within muscle. Just as the expression of these genes is down-regulated in a number of pathological states, a number of physiological situations increase the expression of these genes, including muscle contraction, or exercise, and caloric restriction [8,9]. These situations of low energy balance ultimately modulate gene transcription to increase the capacity of skeletal muscle to produce ATP and alter the phenotype of skeletal muscle to one that is more oxidative. As defective skeletal muscle oxidative metabolism is implicated in a number of chronic diseases, much research has been dedicated to identify molecular mediators that increase oxidative gene expression. In recent years, AMPK (AMP-activated protein kinase) has emerged as a critical regulator of skeletal muscle oxidative function, partly through its ability to regulate skeletal muscle transcription and phenotype. The present review examines the structure and activation of AMPK, and analyses the transcriptional responses to AMPK signalling in skeletal muscle. Finally, we provide an overview of the known AMPK substrates that mediate this transcriptional response.

AMPK STRUCTURE AND ACTIVATION

AMPK is a highly conserved enzyme that has homologues in all eukaryotic organisms. Mammalian AMPK is a heterotrimer comprised of catalytic α- and regulatory β- and γ-subunits. There are two α-, two β- and three γ-subunits, making a total of 12 possible heterotrimeric combinations [10]. In addition, there are a number of alternative transcripts for the α1-, α2- and γ3-subunits [11]. In skeletal muscle, three heterotrimeric complexes predominate: α1/β2/γ1, α2/β2/γ1 and α2/β2/γ3 [12]. The physiological significance of these different subunit combinations, or their alternate transcripts, is not well understood. The α-subunit contains an N-terminal kinase domain, which is immediately followed by an AIS (auto-inhibitory sequence), before a C-terminal SID (subunit-interacting domain) that interacts with the β-subunit [11]. The β-subunit interacts with both the α- and γ-subunits via its C-terminal SBS (subunit-binding sequence) [11] and also contains a CBD (carbohydrate-binding domain) that might play a role in fuel sensing [13]. The γ-subunit contains a number of repeating domains involved in activation of the AMPK complex, such as Bateman domains that bind nucleotides and a pseudosubstrate domain that interacts with the AIS within the α-subunit [10].

Activation of AMPK occurs in response to increased cellular AMP concentrations, which arise during cellular metabolic stress. AMP binds to the CBS (cystathionine β-synthase) regions of the γ-subunit, which allosterically activates the AMPK complex. However, this also induces a conformational change where the pseudosubstrate domain can no longer interact with the α-subunit kinase domain. This exposes the activation T-loop of this domain to upstream kinases, which activate AMPK by phosphorylation of Thr172 [14]. In many cell types, the major upstream AMPKK (AMPK kinase) is the LKB1 tumour suppressor [15–17]. Recent evidence also suggests that CaMKs [CaMK (Ca2+/calmodulin-dependent protein kinase) kinases], particularly CaMKKβ, and TAK1 (transforming growth factor β-activated kinase 1) are also AMPKKs in some cell types [18–21]. Additionally, AMPK is also sensitive to protein phosphatases, particularly PP2C [22]. The conformational change induced by AMP binding to AMPK also reduces the affinity of AMPK as a substrate for inactivating phosphatases. As all three effects of AMP on AMPK activation are antagonized by high ATP concentrations, AMPK is extremely sensitive to alterations in the AMP/ATP ratio [10].

ACTIVATORS OF AMPK AND THE AMPK SIGNALLING RESPONSE IN SKELETAL MUSCLE

Physiological AMPK activators

In skeletal muscle, the most potent activator of AMPK is exercise, or muscle contraction. Aerobic-based exercise of intensities above approx. 60 % is sufficient to increase the activity of AMPK complexes containing the α2 catalytic subunit [23]. The activities of complexes containing the α1 catalytic subunit are unchanged by this type of exercise [23]. In addition, resistance exercise activates AMPK α2 complexes [24]. The α2/β2/γ3 heterotrimer has subsequently been identified as the AMPK complex that is primarily activated by exercise in skeletal muscle [25]. Although the AMPK system has evolved in single cell eukaryotes, multicellular organisms have evolved such that AMPK can be activated by a number of hormones to provide an inter-tissue control of energy balance. Skeletal muscle AMPK can be activated by the adipokines adiponectin [26] and leptin [27], and by IL–6 (interleukin-6) secreted by skeletal muscle in an autocrine manner [28]. Furthermore, AMPK is also activated by signalling through α- and β-adrenergic receptors in some cell systems [29]; however, adrenergic signalling does not appear to activate AMPK in skeletal muscle [30]. In some cases, hormonal control of AMPK is independent of cellular AMP concentrations and the mechanism(s) by which this occurs are currently unclear [10].

Pharmacological and small-compound activators of AMPK

AMPK can also be activated through pharmacological mechanisms. It has been discovered that AMPK is
activated by metformin, the most widely prescribed drug for the treatment of Type 2 diabetes [31]. Although many of the actions of metformin in patients with diabetes have been ascribed to its effect on hepatic glucose production, metformin increases skeletal muscle AMPK activity in vivo [32]. The mechanism of action of metformin is probably due to its inhibition of complex I of the respiratory chain, which ultimately increases the AMP/ATP ratio [10]. Another agent used to activate AMPK is AICAR (5-aminoo-4-imidazolecarboxamide riboside). This compound is rapidly taken up by cells and phosphorylated to ZMP, an AMP analogue that mimics its effects on AMPK [33]. AICAR has been an invaluable tool in experimental settings to acutely activate AMPK; however, infusion of AICAR into diabetic patients has found that, although AICAR has metabolic effects on the liver, it does not appear to activate skeletal muscle AMPK [34]. A screen of more specific AMPK activators has been performed and a compound termed A-769662 was synthesized using data gathered during the screen [35]. A-769662 potently activates AMPK in cell lines and in the liver in vivo; however it has limited effects in skeletal muscle [35]. It has subsequently been found that A-769662 activates AMPK complexes containing the β1-subunit, but not the β2-subunit, through interactions with the β1-subunit CBD [36]. As most skeletal muscle complexes contain the β2-subunit [12], this may explain the limited effects of systemic administration of A-769662 on AMPK activity in skeletal muscle. However, activation of AMPK with A-769662 has been observed in isolated extensor digitorum longus muscles [37]. Furthermore, Trebakh et al. [37a] have shown that AMPK trimer complexes are expressed in mouse soleus and extensor digitorum longus muscle from two strains of mice. In addition, they have shown that A-769662 activates AMPKβ1-containing complexes in intact skeletal muscle and that glucose uptake was induced independent of this activation of AMPK.

A number of natural compounds have been found to activate AMPK. These include the short-chain fatty acid α-lipoic acid, polyphenols, such as those found in green tea and red wine, and finally berberine, a plant extract used in traditional Chinese medicine [10].

### Skeletal muscle processes regulated by AMPK

Once activated, AMPK restores cellular energy balance by activating catabolic ATP-producing pathways and inhibiting anabolic ATP-consuming pathways. In this context, AMPK affects many different cellular processes in a tissue-dependent manner. In skeletal muscle, AMPK acutely increases ATP production by increasing lipid mobilization. It does this by enhancing fatty acid uptake into skeletal muscle [38] and increasing fatty acid transport into the mitochondria for β-oxidation [39]. The mechanism by which AMPK increases muscle fatty acid uptake has yet to be determined, but is likely to involve regulation of localization and/or activity of the FAT (fatty acid translocase)/CD36 lipid transporter [2]. In contrast, the mechanism by which AMPK increases fatty acid delivery into the mitochondria has been well characterized and occurs through phosphorylation and inactivation of ACC (acyetyl-CoA carboxylase) [39]. Phosphorylation of ACC reduces malonyl-CoA concentration, which relieves the allosteric inhibition of CPT-1 (carnitine palmitoyltransferase-1), the transporter of fatty acids into the mitochondria [39]. AMPK also enhances muscle ATP production by increasing skeletal muscle glucose uptake. It does this by increasing GLUT4 (glucose transporter isoform 4) content at the sarcolemma [40]. The exact mechanisms responsible are not completely understood, but are thought to involve AMPK phosphorylation of the Rab GTPase-activating proteins TBC1D1 [TBC (Tre-2/Bub2/Cdc16) domain family 1] and TBC1D4, which control the mobilization of GLUT4 storage vesicles [41]. Evidence also suggests that AMPK is able to increase rates of glycolysis by phosphorylating the glycolytic enzyme phosphofructokinase 2 [42]; however, this has yet to be confirmed in skeletal muscle. In addition, AMPK also increases substrate delivery to muscle by regulating vascular tone. This occurs through phosphorylation of eNOS (endothelial NO synthase), which increases NO production and tissue blood flow [43].

### AMPK and skeletal muscle transcription

Although AMPK promotes skeletal muscle ATP production acutely by enhancing substrate delivery to muscle and lipid and carbohydrate metabolism, AMPK also alters the metabolic phenotype of muscle to enhance ATP production capacity. While by no means comprehensive, Figure 1 provides a pathway schematic of the genes thought to be regulated by AMPK that influence skeletal muscle phenotype. Many of these findings have been gathered from studies employing the use of AICAR or genetic gain- or loss-of-function models to modulate AMPK activity to establish the role of this kinase in skeletal muscle gene expression.

### Metabolic genes regulated by AMPK

As can be seen in Figure 1, similar to its acute effects on metabolic processes, AMPK activation increases the expression of numerous genes involved in substrate supply, carbohydrate and lipid transport, and metabolism. AMPK regulates genes involved in angiogenesis including VEGF (vascular endothelial growth factor) [44], which is essential for muscle enhanced muscle capillarization, a key feature of oxidative skeletal muscle.
AMPK regulates the expression of genes involved in angiogenesis, substrate transport across the sarcolemma and key enzymes of metabolic pathways involved in substrate oxidation. The net result of this gene expression response is an increased capacity to produce ATP. β-HAD, β-hydroxyacyl-CoA dehydrogenase; HKII, hexokinase II; PDK4, pyruvate dehydrogenase kinase 4; ROS, reactive oxygen species; SOD2, superoxide dismutase 2; TCA, tricarboxylic acid; VEGF, vascular endothelial growth factor.

Muscle. AMPK controls the capacity of skeletal muscle carbohydrate metabolism by regulating the expression of GLUT4, which increases the maximal rate of glucose transport into skeletal muscle [45]. In addition, AMPK also regulates the expression of HKII (hexokinase II), which phosphorylates glucose and initiates glycolysis [46]. Similarly, evidence from cardiomyocytes suggests that AMPK regulates the capacity for lipid oxidation by controlling FAT/CD36 expression, which is the primary facilitative transporter of fatty acids across the sarcolemma [47]. Although this has yet to be confirmed in skeletal muscle, AMPK does regulate CPT-1 expression in this tissue, which enhances fatty acid transport into the mitochondria [48]. Commensurate with regulating lipid supply to the mitochondria, AMPK also controls the expression of a number of mitochondrial enzymes involved in substrate oxidation. The expression of β-HAD (β-hydroxyacyl-CoA dehydrogenase), a key enzyme catalysing the β-oxidation of lipids, is increased upon AMPK activation [48], as too is the expression of PDK4 (pyruvate dehydrogenase kinase 4), which inactivates the PDH (pyruvate dehydrogenase) complex that controls substrate flux into the tricarboxylic acid cycle [49]. AMPK activation also increases the expression of CS (citrate synthase) [50], a key enzyme of the tricarboxylic acid cycle, and enzymes of the respiratory chain such as Cox [Cyt C (cytochrome c) oxidase] subunits I [50] and IV [51], which enhance substrate oxidative capacity. Consistent with this increase in oxidative capacity, AMPK activation also increases the expression of genes involved in oxidant defence, including SOD2 (superoxide dismutase 2) and catalase [51].

**Genes involved in phenotypic adaptation regulated by AMPK**

In addition, AMPK modulates the expression of a number of transcriptional regulators involved in controlling the expression of metabolic and mitochondrial genes (Figure 1). These include PPARγ (peroxisome-proliferator-activated receptor γ), PGC-1α (PPARγ co-activator 1α), NRF-1/NRF-2 (nuclear respiratory factors-1 and -2), Tfam (mitochondrial transcription factor A) [51] and FoxO1 (forkhead box O1) [49]. PGC-1α serves as a transcriptional co-activator for
a number of DNA-binding transcription factors, in addition to the PPAR family, which control metabolic gene expression [52]. PGC-1α overexpression in skeletal muscle is associated with a shift to a more oxidative phenotype and resistance to fatigue [53]. PGC-1α partly exerts its action through co-activation of NRF-1/NRF-2, which are required for the co-ordinated expression of a number of nuclear- and mitochondrial-encoded enzymes found within the mitochondrial respiratory chain [52]. Complimentary to the actions of NRF-1/NRF-2, Tfam is a mitochondrial transcription factor that is required for mitochondrial genome replication [54]. As well as regulating metabolic, mitochondrial and vascular genes directly, the co-ordinated increase in expression of these transcriptional regulators in response to AMPK activation provides further regulatory control of skeletal muscle phenotype.

Control of skeletal muscle phenotype by AMPK

The net transcriptional response to AMPK activation in skeletal muscle and shift towards a more oxidative phenotype is seen in an AMPK gain-of-function rodent model, where a R225Q mutation in the γ3-subunit results in enhanced autonomous AMPK activity [55]. This missense mutation was originally identified in Hampshire pigs with skeletal muscle glycogen storage disease. Although the γ3-subunit is not highly expressed in type 1 muscle fibres [55], mice with the R225Q gain-of-function mutation have enhanced mitochondrial and metabolic enzyme expression and total mitochondrial content in type 2 fibres [51]. Highlighting the importance of skeletal muscle AMPK-mediated transcription in health and disease, R225Q mutant mice are protected against high-fat-diet-induced skeletal muscle lipid accumulation and insulin resistance [55]. These skeletal muscle phenotypic and transcriptional adaptations are replicated in mice treated with AICAR [56]. Four weeks of daily AICAR also increased exercise tolerance in otherwise inactive mice [56]. Nonetheless, the role of AMPK in muscle fibre type transitions appears to be equivocal. The γ3 R225Q mutation [51] and daily AICAR administration for 4 weeks [57], both of which chronically activate AMPK, have no effect on muscle fibre type. However, one study has found that mice harbouring an activating γ1 R70Q mutation have a greater proportion of slow oxidative fibres in the basal state [58]. This same study also found that fibre type shifts induced by exercise training were attenuated in AMPKα2-dominant-negative mice [58]. Despite these equivocal findings on muscle fibre type, these studies clearly highlight the importance of skeletal muscle AMPK signalling in exercise performance and health and disease.

A point of contention in the AMPK field is that a number of different loss-of-function models fail to show an overt skeletal muscle phenotype. Indeed, AMPKα1-and α2-knockout mice have no difference in basal metabolic and mitochondrial gene expression when compared with wild-type mice [45]. In addition, these knockout mice have normal gene expression responses to exercise, but in some instances have a blunted AICAR response [49]. There are issues with compensation between the AMPK catalytic isoforms in these knockout models; however, a muscle-specific AMPKα2-dominant-negative mouse model has similar gene expression results [59]. Nonetheless, genetic inactivation of AMPKα2 in skeletal muscle does impair exercise performance [60–62], and has had conflicting involvement in the metabolic response to high-fat feeding [63,64]. But in terms of gene expression, the mild phenotype of these AMPK loss-of-function models raises the possibility that other signalling pathways might compensate in the absence of AMPK signalling under conditions of skeletal muscle metabolic stress. Supporting this idea are findings showing that muscle contraction in AMPKα2-dominant-negative mice results in greater activation of nearly 20 other kinases when compared with muscle contraction in wild-type mice [65]. This apparent redundancy in AMPK signalling in skeletal muscle has recently been examined in the context of regulation of the GLUT4 gene [66]. That study showed that the CaMKII and calcineurin signalling pathways can maintain GLUT4 expression in the absence of AMPK signalling, in a fibre-type-dependent manner [66]. Nonetheless, the phenotypic adaptations seen in AMPK gain-of-function models show that activation of AMPK is a very important mediator of skeletal muscle transcription.

AMPK substrates regulating skeletal muscle transcription

Consistent with its role as a signalling kinase, AMPK phosphorylates a variety of transcriptional substrates to mediate its transcriptional responses. These include traditional DNA-binding transcription factors, but also transcriptional co-activators and co-repressors. This section of the review examines the few bona fide AMPK transcriptional substrates that have been identified and how these substrates contribute to AMPK-mediated transcription in skeletal muscle. This section also highlights that AMPK targets a number of transcriptional substrates with overlapping, yet distinct, functions to achieve its role in regulating skeletal muscle gene expression. In addition, the nexus between AMPK and other signalling mechanisms that help AMPK achieve phenotypic specificity is discussed.

p53

Well studied in cancer fields, p53 is a DNA-binding transcription factor that acts as a tumour suppressor
following activation by cellular stress signals such as DNA damage, UV light and a number of oncogenes [67]. p53 regulates a programme of genes involved in cell-cycle inhibition, apoptosis and genetic stability in a context-dependent manner [67]. In addition to these processes, p53 also regulates aerobic respiration by directly regulating the promoter regions of many metabolic and mitochondrial genes [68]. Indeed, loss-of-function p53 mutations, which are common in many cancers, result in a shift towards anaerobic metabolism as a result of reduced oxidative capacity [68]. Knockout of p53 shows a metabolic phenotype in skeletal muscle with reduced mitochondrial content, total Cox activity and PGC-1α expression [69]. Although examined in the context of cell-cycle arrest in response to glucose deprivation, it has been found that AMPK is a p53 kinase [70]. Under normal conditions, p53 is rapidly degraded by ubiquitin-mediated proteolysis; however, other post-translational modifications, such as phosphorylation and acetylation, stabilize p53, attenuating its subsequent degradation [70]. This enhances the DNA-binding activity of p53 towards its regulatory promoter regions, including those that control the expression of genes involved in aerobic metabolism [67]. Treatment with AICAR [71] or expression of constitutively active AMPK [70] results in phosphorylation of p53 at Ser15 (Ser18 in mice). In addition, phosphorylation of p53 at this residue has been observed in response to muscle contraction in association with AMPK activation, and has been proposed as one mechanism mediating phenotypic adaptations of skeletal muscle to exercise [69].

Class IIa HDACs (histone deacetylases)
The class IIa HDACs are a family of transcriptional repressors that are highly expressed in striated muscle [72]. Comprising isoforms 4, 5, 7 and 9, they inhibit gene expression by deacetylating histone lysine residues within the nucleosome core [72]. This maintains a highly compact structure containing DNA that is inaccessible to initiators of transcription such as RNAPII (RNA polymerase II) and the TIC (transcriptional initiation complex). The class IIa HDACs associate with a number of DNA-binding transcription factors, such as MEF2 (myocyte enhancer factor 2), which provides specificity to the gene subsets that this family of co-repressors regulate [73]. Through these transcription factors, class IIa HDACs regulate gene networks involved in myogenic differentiation and metabolism [72]. Indeed, conserved MEF2-binding motifs are found in many metabolic genes, including PGC-1α [74]. Recently, it has been discovered that AMPK phosphorylates HDAC5 on Ser259 and Ser498 [75]. These sites are highly conserved in all class IIa HDACs and their phosphorylation dissociates the HDAC from MEF2 and provides binding sites for the 14-3-3 chaperone proteins, which then export the HDAC out of the nucleus [76]. This was also found to be the case following phosphorylation by AMPK, and was directly examined in the context of regulation of the GLUT4 gene [75]. Significantly, mutation of the key regulatory Ser259 and Ser498 residues on HDAC5 in the heart leads to sudden cardiac death due to mitochondrial abnormalities [74]. Furthermore, gene expression screening in animals containing this mutation revealed a co-ordinated suppression of genes involved in oxidative and mitochondrial metabolism, including that of PGC-1α [77]. In addition, HDAC5 has been found to control angiogenesis in endothelial cells by directly regulating the promoter regions of genes including FGF2 (fibroblast growth factor 2) [74]. Vascular defects have also been observed in HDAC7-knockout animals [78]. Taken together, these findings suggest that AMPK phosphorylation of class IIa HDACs might contribute to many of the gene expression responses to AMPK activation in skeletal muscle and its surrounding vasculature.

A recent screen for potential class II HDAC kinases has potentially revealed why a number of AMPK loss-of-function transgenic models fail to show overt skeletal muscle phenotypes [79]. From these experiments, it is evident that there is considerable redundancy in class II HDAC signalling, with multiple kinases capable of phosphorylating Ser259 and Ser498 on HDAC5 [79]. The kinases identified that are expressed in skeletal muscle include PKD (protein kinase D) and Mark2 [microtubule-associated regulated kinase 2; also known as Par1/EMK1 (ELKL motif kinase 1)], an AMPK-related kinase [79]. These results suggest that, although AMPK is a key regulator of muscle oxidative genes through the class IIa HDACs, it is not strictly essential.

PGC-1α
As discussed above, PGC-1α has been described as a master regulator of oxidative metabolism through its ability to coactivate a number of transcription factors that control metabolic and mitochondrial gene expression [52]. PGC-1α functions in a reciprocal manner to the class II HDACs in that it associates with DNA-bound transcription factors and subsequently recruits enzymes with HAT (histone acetyltransferase) activity to increase histone acetylation [52]. This unwinds DNA from its core of histone proteins, exposing promoter and gene regions to the TIC and RNAPII, resulting in transcriptional activation. As mentioned previously, the effects of PGC-1α on skeletal muscle transcription and phenotype are profound, with PGC-1α overexpression resulting in a marked increase in muscle oxidative capacity and performance, which is associated with an increase in the expression of a large group of angiogenic and oxidative genes [53]. Extreme overexpression of PGC-1α (i.e. 6-7 fold) can result in skeletal muscle insulin resistance, probably due to an increase in lipid uptake into muscle that exceeds oxidative capacity [80].
AMPK controls multiple points of the PGC-1α feed-forward loop

Regulation of PGC-1α feed-forward loop by AMPK involves multiple interactions between signalling molecules, transcriptional co-activators, transcriptional repressors and transcription factors. AMPK phosphorylates PGC-1α at Thr^{177} and Ser^{388} and also activates Sirt1 by increasing NAD^{+} concentrations. Phosphorylation of PGC-1α primes it for deacetylation by active Sirt1, which increases PGC-1α transcriptional activity, such that it co-activates the MEF2 transcription factor, thereby increasing the expression of its own gene. In addition, AMPK phosphorylates HDAC5 at Ser^{259} and Ser^{498}, relieving MEF2 transcriptional repression and also phosphorylates CREB at Ser^{133}, increasing the transcriptional activity of this transcription factor. These factors together further potentiate PGC-1α expression.

However, moderate overexpression of PGC-1α to levels equivalent of those observed following physiological skeletal muscle metabolic stress (i.e. approx. 25%), such as that seen following exercise, results in enhanced fatty acid oxidation and insulin action [81]. This highlights the importance of PGC-1α in muscle health and performance. In addition to increases in PGC-1α expression, post-translational modification of PGC-1α also modulates its activity. In this context, AMPK has been identified as a PGC-1α kinase, phosphorylating Thr^{177} and Ser^{388} [82]. In certain skeletal muscle cell culture systems, AMPK-mediated transcription of many oxidative genes is dependent on PGC-1α, and mutation of these sites to alanine residues completely ablates AICAR-mediated transcription in a gene reporter system [82].

The exact mechanisms by which AMPK phosphorylation augments PGC-1α transcriptional activity have not yet been established; however, it has been found previously that phosphorylation of PGC-1α by p38 MAPK (mitogen-activated protein kinase) can remove the inhibitory effect of a repressor protein on PGC-1α transcriptional activity [83]. As PGC-1α can co-activate its own gene through a feed-forward loop [84], it has been proposed that phosphorylation of PGC-1α might mediate all of the AMPK-mediated transcriptional responses in skeletal muscle [82]. This has not yet been conclusively shown experimentally and it is likely that numerous transcriptional substrates are required to fully co-ordinate the AMPK transcriptional response. For example, HDAC phosphorylation, which regulates PGC-1α expression (Figure 2) [74], as well as other metabolic genes [75], might be equally important in the AMPK response. As the PGC-1α feed-forward mechanism is under multiple points of control by AMPK (Figure 2), the exact role that PGC-1α plays in AMPK-mediated transcription is difficult to dissect. However, skeletal-muscle-specific PGC-1α-knockout animals have similar gene expression responses to exercise when compared with wild-type animals [85], suggesting that the effects of AMPK on skeletal muscle gene expression are not completely dependent on PGC-1α. In addition, a previously uncharacterized PGC-1α promoter situated within the first exon has been identified [86]. It is unclear how AMPK might affect PGC-1α transcription through this alternate promoter.

CREB [CRE (cAMP-response-element)-binding protein] family of transcription factors

The CREB family of DNA-binding transcription factors include CREB, CREB1, CREM (CRE modulator), CREBL2 (CREB-like 2) and ATF1 (activating transcription factor 1) [87]. These proteins form homo- and hetero-dimers that bind to CRE sequences within the promoter regions of many metabolic and mitochondrial genes, including PGC-1α [88]. In addition to these genes, expression of a dominant-negative CREB transgene in skeletal muscle results in a dystrophic phenotype [89], strengthening further the link between oxidative metabolism and muscle function. Phosphorylation of the CREB proteins in AMPK-mediated transcription in skeletal muscle has been difficult to...
establish. AMPK also phosphorylates CRTC2 (CREB-regulated transcriptional co-activator 2) that results in nuclear exclusion of this co-activator and inhibition of CREB transcriptional activity [91]. This mechanism is critical for AMPK-mediated inhibition of hepatic gluconeogenesis, yet its role in skeletal muscle gene expression is unclear. Nonetheless, CRTC2 appears to be a regulator of skeletal muscle oxidative metabolism [92], consistent with its role as a CREB co-activator. This apparent discrepancy in AMPK signalling and its potential effect on skeletal muscle gene expression and phenotype are yet to be resolved.

**FoxO family of transcription factors**
The FoxO family includes isoforms 1, 3, 4 and 6, and these DNA-binding transcription factors regulate a cluster of skeletal muscle genes involved in glucose metabolism, tumour suppression and longevity [93]. The FoxO proteins are primarily regulated by phosphorylation, with Akt being the most well-characterized kinase [93]. Phosphorylation of FoxOs by Akt results in their nuclear export and transcriptional inactivation. FoxO transcriptional activity can also be regulated by acetylation [94]. Interestingly, skeletal-muscle-specific overexpression of FoxO1 results in muscle atrophy, consistent with its role in the regulation of a group of autogenes, including atrogin 1 and MuRF1 (muscle ring finger 1) [95]. Associated with this loss of muscle mass, these mice suffered impaired glycaemic control [95]. In contrast, however, the FoxO family members have also been observed to regulate a broad programme of metabolic genes in a manner similar to AMPK in a number of experimental systems. This led to the hypothesis that the FoxOs might be transcriptional substrates of AMPK. Indeed, AMPK has been shown to phosphorylate FoxO1, 3, 4 and 6 in vitro [93]. The sites phosphorylated on FoxO3 have been characterized further and six unique sites were identified. In contrast with Akt phosphorylation, AMPK phosphorylation did not cause nuclear exclusion of FoxO3, but instead increased its transcriptional activity [93]. The exact mechanisms involved remain unclear, but it has been hypothesized that, with five of the six phosphorylation sites being within the transactivation domain, this might make this domain more acidic, thereby increasing its transcriptional activating potential [93]. Non-biased microarray analyses also revealed that these phosphorylation sites regulate the transcription of genes involved in oxidative stress resistance and mitochondrial metabolism [93]. These findings, however, contradict studies showing that AICAR represses FoxO1-dependent transcription [96]. Although the AMPK phosphorylation sites on FoxO1 have yet to be identified and characterized, this could suggest that AMPK differentially regulates the FoxO family members to achieve transcriptional specificity.

**OTHER TRANSCRIPTIONAL SUBSTRATES**

In addition to the well-characterized AMPK substrates outlined above, evidence exists that AMPK targets other transcriptional regulators in mediating skeletal muscle gene expression. Although comprehensive results might be lacking to class these transcriptional regulators as bona fide AMPK substrates, some of these putative targets are discussed. In addition, other signalling systems that are regulated by AMPK which affect skeletal muscle transcription, and their impact on AMPK-mediated gene expression specificity will be discussed.

**NRF-1**

Another potential AMPK target is NRF-1 which, as discussed above, is required for the co-ordinated expression of many nuclear- and mitochondrial-encoded enzymes found within the mitochondrial respiratory chain [52]. As highlighted previously, AMPK increases the expression of NRF-1 [53]. Furthermore, chronic activation of AMPK in the skeletal muscle of mice is associated with an increase in the DNA-binding activity of NRF-1 and an increase in mitochondrial density and expression of mitochondrial enzymes [97]. It is unclear whether the increase in NRF-1 expression with AMPK activation is sufficient to mediate this response or whether direct regulation of NRF-1 is required. Indeed, these effects could also be mediated through increased activation and expression of PGC-1α, a potent co-activator of NRF-1 [52]. Further research will clarify how AMPK regulates NRF-1-mediated gene expression.

**GEF (GLUT4-enhancer factor)**

As its name suggests, GEF was originally identified as a regulator of the GLUT4 gene that directly binds to a region on the GLUT4 promoter to enhance GLUT4 transcription [98]. AMPK can phosphorylate GEF in vitro, which is associated with enhanced GEF DNA-binding activity [99]. The specific phosphorylation site(s) was not identified, nor was this interaction confirmed in vivo. If GEF is indeed a bona fide AMPK substrate, it could further suggest that AMPK targets multiple regulators of a given gene or gene cluster to achieve specificity in its transcriptional response.

**Sirt1 (sirtuin 1)**

The NAD⁺-dependent class III deacetylase Sirt1 has emerged as a key regulator of PGC-1α and FoxO function and, therefore, skeletal muscle phenotype [100]. Sirt1-dependent deacetylation of PGC-1α and FoxO1 alters their transcriptional activity and enhances the transcription of skeletal muscle genes involved in energy metabolism [100]. AMPK has recently been found to activate Sirt1 by increasing NAD⁺ levels, suggesting that AMPK can regulate skeletal muscle transcription.
indirectly through the actions of Sirt1 [100]. The two points of AMPK signalling to PGC-1α, first through direct phosphorylation and second through deacetylation via Sirt1, appear to be inter-dependent in that phosphorylation of PGC-1α provides a priming motif for deacetylation [100]. This adds another degree of complexity to regulation of PGC-1α by AMPK (Figure 2) and highlights further the emerging complexity of AMPK signalling on muscle phenotype. In addition, it should be noted that Sirt1 also deacetylates p53, which exposes lysine residues for ubiquination and enhanced degradation [101]. This goes against the general role of AMPK-mediated transcription in skeletal muscle is clear, as skeletal muscle phenotype and oxidative function play a critical role in human health and performance.

CONCLUSIONS AND FUTURE DIRECTIONS

AMPK-mediated transcription in skeletal muscle plays a critical role in skeletal muscle phenotype through regulation of angiogenesis and oxidative capacity. This energy-sensing kinase regulates a large subset of genes involved in substrate delivery to muscle, substrate transport and oxidation. The co-ordinated increase in transcription of these genes in response to AMPK activation is mediated by a number of transcriptional regulators whose functions are altered in response to AMPK signalling. As more bona fide AMPK substrates with transcriptional regulatory function are identified, and interactions with other signalling pathways are discovered, the complexities of this transcriptional regulation are becoming apparent. Indeed, it is expected that numerous more AMPK transcriptional targets will be identified. Future research should also focus on the role of AMPK in regulating epigenetic mechanisms, which are emerging as a key control point of co-ordinated gene expression. A further challenge for the field will be to dissect the overlapping functions of AMPK transcriptional substrates and determine whether these are redundant mechanisms or are required to obtain exact response specificity. Nonetheless, the importance of AMPK-mediated transcription in skeletal muscle is clear, as skeletal muscle phenotype and oxidative function play a critical role in human health and performance.

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