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# AMPK and transcriptional regulation

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## 1. ABSTRACT

The AMP-activated protein kinase (AMPK) is an energy sensing enzyme that once activated, promotes energy production and limits energy utilisation to ensure cellular survival. In addition to targeting numerous metabolic enzymes for this purpose, it is becoming apparent that AMPK can also regulate a number of transcriptional processes. These processes ensure cell survival through the inhibition of cell cycle and growth mechanisms, and also prepare the cell for future perturbations in energy balance by increasing the capacity of the cell to produce ATP. While these adaptations might be inextricably linked through regulation of the proliferation-differentiation process, recent studies have identified a number of transcriptional regulators as AMPK substrates that give insights into the regulation of transcription by AMPK in a number of metabolically active tissues.

## 2. INTRODUCTION

The ability of an organism to respond to challenges in energy status has been a key feature in evolutionary biology. Selection for processes that ensure survival in the face of metabolic challenge has resulted in organisms that are able to sense nutrients and energy balance at a cellular level. In mammals, the AMP-activated protein kinase (AMPK) is one such molecule that when activated, limits energy utilisation and promotes energy production to ensure cellular survival. AMPK does this by inhibiting cellular growth and biosynthetic pathways such as fatty acid and sterol synthesis and activating catabolic ATP-generating processes such as glucose uptake and fatty acid oxidation. The effects of AMPK are largely mediated by direct substrate phosphorylation that ultimately controls pathway enzymatic activity and the transcriptional networks regulating these processes. This review seeks to give an overview of the diverse transcriptional processes

that AMPK regulates in a variety of tissue types. In particular, detailed discussion of the molecular mechanisms by which AMPK exerts transcriptional control will be provided.

## 3. AMPK STRUCTURE AND ACTIVATION

AMPK is a heterotrimeric enzyme consisting of catalytic α and regulatory β and γ subunits. Two α, two β and three γ subunits have been identified, making a total of 12 possible heterotrimeric combinations (1). The functional significance of these different combinations is not yet fully understood. The α subunit contains an N-terminal kinase domain and interacts with the β subunit via a domain located towards its C-terminus (1). The β subunit also interacts with the γ subunit and contains a carbohydrate binding domain that might play a role in fuel sensing (2). The γ subunit contains a number of domains involved in activation of the AMPK complex such as CBS domains that bind nucleotides and a pseudosubstrate domain (1).

Activation of AMPK occurs in response to an increase in the cellular AMP concentration, which arise during metabolic stresses such as glucose deprivation, hypoxia, ischemia and muscle contraction (1). AMP binds to the CBS regions of the γ subunit, which on its own allosterically activates AMPK. However, this also induces a conformational change where the pseudosubstrate domain can no longer interact with the α subunit kinase domain and exposes the activation T loop of this domain to upstream kinases, which activate AMPK by phosphorylation of Thr172 (3). In many cell types, the major upstream AMPK kinase (AMPKK) is the LKB1 tumor suppressor (4-6). Recent evidence also suggests that the calcium/calmodulin dependent protein kinase kinases (CaMKKs), particularly CaMKKβ, and the transforming growth factor β activated kinase 1 (TAK1) are also AMPKKs in some cell types (7-10). Additionally, AMPK is also sensitive to phosphatases, particularly PP2C (11). 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 to ATP ratio (1). Despite the fact that the AMPK system has evolved in single cell eukaryotes, it has been discovered that a number of hormones can also activate AMPK in multicellular organisms. AMPK is particularly sensitive to adipokines such as adiponectin and leptin and also signaling through α and β adrenergic receptors (12). Furthermore, AMPK is also activated by metformin, a drug that has been used for many years as a treatment for type 2 diabetes (13).

## 4. AMPK REGULATION OF CELLULAR ENERGETICS

An in depth overview of the vast cellular processes regulated by AMPK is beyond this review, however for reviews on these see (12; 14; 15). In general, AMPK exerts control over a number of energetic processes including inhibition of cellular growth and proliferation programs and biosynthetic pathways, concomitant with the activation of catabolic ATP-generating processes (1). Consistent with the LKB1 tumor suppressor being an important AMPKK in many cell types, AMPK mediates cell cycle arrest and inhibits cellular growth in response to low nutrients (16). Similarly, activation of AMPK inhibits sterol synthesis, which in addition to reducing ATP utilisation attributable to this pathway, also indirectly inhibits cellular proliferation (15). In energetically demanding tissues such as skeletal muscle and the heart, AMPK activation is associated with the augmentation of energy producing processes such as fatty acid uptake and oxidation and glucose uptake (17). AMPK activation also increases glycolysis in the heart (18). In tissues such as adipose tissue and the liver, AMPK activation suppresses the energetically costly processes of fatty acid synthesis, lipolysis and gluconeogenesis (1). Activation of AMPK in response to glucose deprivation has also been implicated in the regulation of insulin secretion from pancreatic β cells (19). Together, these actions of AMPK work in concert in multiple tissues to restore energy homeostasis at the level of the organism. The discovery that hormones such as leptin, adiponectin and adrenergic signaling can activate AMPK has further elucidated this regulatory network, where peripheral metabolism is influenced by central factors (12). As AMPK regulates such a vast number of metabolic processes in various tissues, AMPK is an attractive therapeutic target for the treatment of many diseases involving perturbed energy balance, such as obesity, type 2 diabetes and cardiovascular disease.

## 5. AMPK AND TRANSCRIPTION

Regulation of these processes by AMPK involves phosphorylation of protein substrates that either directly control pathway enzymatic activities or the transcriptional programs that encodes these processes. A number of microarray analyses have established that AMPK regulates a large subset of genes involved in energy metabolism, cell signaling, cell growth and proliferation, immunity, transcription and apoptosis (20; 21). Although it is beyond this review to further discuss all the gene networks regulated by AMPK, examples of a number of AMPK responsive genes are presented in Table 1. While very few studies have established bona fide AMPK transcriptional targets, where specific phosphorylation sites and/or interacting domains have been identified, this review will be restricted to discussing major transcriptional control mechanisms where AMPK interactions with specific transcriptional regulators are established or proposed.

### 5.1. AMPK regulation of epigenetic processes

Research into transcriptional control mechanisms over the past decade has been dominated by discoveries showing that epigenetic processes are crucial for regulated gene expression. As the signaling mechanisms that control these epigenetic processes are discovered, it is not surprising to find that a number of AMPK substrates can alter the epigenetic landscape.

**Table 1.** Genes regulated by AMPK

| Tissue | Gene | Effect | General function | Reference |
|---|---|---|---|---|
| Liver | Foxo1 | ↓ | Decreased gluconeogenesis | 66 |
| | PEPCK | ↓ | | 64, 71 |
| | G-6-Pase | ↓ | | 64 |
| | pyruvate kinase | ↓ | | 74, 75 |
| | FA synthase | ↓ | Decreased fatty acid synthesis | 75 |
| | Spot 14 | ↓ | | 75 |
| Skeletal muscle | GLUT4 | ↑ | Increased oxidative capacity | 45, 46 |
| | Hexokinase II | ↑ | | 45, 46 |
| | Citrate synthase | ↑ | | 46 |
| | HAD | ↑ | | 45, 46 |
| | CoxI | ↑ | | 46 |
| | Cytochrome c | ↑ | | 45, 46 |
| | CPT1 | ↑ | | 45 |
| | UCP2 and 3 | ↑ | | 45 |
| | PGC-1 | ↑ | | 20, 21 |
| | PPARα | ↑ | | 33 |
| | Foxo1 | ↑ | | 47 |
| | PDK-4 | ↑ | | 47 |
| Hypothalamus | NPY | ↑ | Increased food intake/maintenance of plasma glucose levels | 82 |
| | AgRP | ↑ | | 83 |
| | POMC | ↑ | | 84 |
| T-cells | IL-2 | ↓ | Immunosuppression | 85 |
| | T-cell receptor | ↓ | | 20 |
| Vasculature | VEGF | ↑ | Increased vascularisation | 86 |
| | nNOS | ↑ | | 21 |
| Various | $p21^{wafl/cipl}$ | ↑ | Decreased cellular proliferation and growth/cell cycle inhibition | 39, 40 |
| | $p27^{kipl}$ | ↑ | | 87 |
| | CDK4 | ↓ | | 20 |
| | IGFBP1 + 2 | ↓ | | 20, 21 |
| | HIF | ↑ | Adaptation to hypoxia | 88 |

**Abbreviations:** AgRP: agouti-related peptide, CDK: cyclin dependent kinase, CPT: carnitine parmatoyl transferase, Cox: cyctochrome oxidase, FA: fatty acid, Foxo: forkhead box O, G-6-Pase: glucose 6 phosphatase, GLUT4: glucose transporter isoform 4, HAD: hydroxyacyl CoA, HIF: hypoxia-inducible factor, IGFBP: insulin-like growth factor binding protein, IL: interleukin, nNOS: neuronal nitric oxide synthase, NPY: neuropeptide Y, PDK: pyruvate dehydrogenase kinase, PEPCK: phosphoenolpyruvate carboxykinase, PGC: PPAR γ coactivator, POMC: propiomelanocortin, PPAR: peroxisome proliferator receptor, UCP: uncoupling protein, VEGF: vascular endothelial growth factor.

#### 5.1.1. Histone 3 (H3)

In eukaryotes, double stranded DNA wraps around a core of four histones (comprising of histone 2A, 2B, 3 and 4) at 146 bp intervals that together forms the basis of the nucleosome, a structural subunit of chromatin and chromosomes (22). Gene regulation is highly dependent on chromatin modifications that result in reorganisation of distinct nucleosomes. Methylated histones create a highly compact structure containing DNA that is inaccessible to initiators of transcription, resulting in transcriptional inactivity (22). However, destabilization of the interaction between histones and DNA exposes DNA to transcriptional regulators. This is largely mediated by altered post translational modification of the histone octamer that forms the nucleosome core. Histone demethylation and phosphorylation promotes further chromatin modifications including histone acetylation, and provides the molecular basis for the chromatin remodeling required for initiation of gene transcription (23). The AMPK yeast homologue, sucrose non-fermenting protein 1 (Snf1) is a histone 3 (H3) kinase (24). In response to low glucose conditions, Snf1 increases INO1 gene expression by phosphorylating ser10 on H3, which promotes acetylation of lys14 by the histone acetyltransferase (HAT) Gcn5 (24). The abundance of H3 throughout the genome makes it tempting to speculate that Snf1, and perhaps AMPK, could increase transcription rates for multiple gene subsets via H3 phosphorylation. However, a recent study

suggests promoter specific functions for H3 phosphorylation (25). While ser10 phosphorylation by Snf1 also occurs at the Gal1 promoter, the recruitment of Gcn5 to this region occurs independently of ser10 phosphorylation (25). This suggests that histone phosphorylation has generalized functions on a number of different gene promoters, but additional promoter specific requirements are needed to initiate transcription. Likewise, this suggests that other transcriptional regulators in addition to Snf1/AMPK are required for a specific transcriptional response.

**5.1.2. p300/CREB binding protein (CBP)**

In mammals, the family of HATs includes p300 and its paralogue the CREB binding protein (CBP). These proteins differ only in a small C-terminal region unique to CBP and functionally overlap to regulate many common processes (26). p300/CBP are ubiquitously expressed throughout development and function through recruitment to specific nucleosomes via interactions with DNA binding transcription factors. This occurs through a number of distinct interacting domains found throughout the entire p300/CBP sequence and places p300/CBP in proximity to acetylate histones and modify chromatin structure to favor transcriptional activation (26). These transcriptional coactivators are known to associate with at least 40 different transcription factors (27). Consequently, p300/CBP regulate a vast number of cellular processes, and paradoxically, these enzymes appear to be able to regulate completely opposing cellular processes. The best example of this is the ability of p300/CBP to act as both a tumor suppressor and also as a coactivator of many oncogenes (27). In addition to its role in regulating cell cycle, p300/CBP also regulates the differentiation of specialized cell lineages and have been implicated in myogenesis (28) and hemopoiesis (29). Phosphorylation modulates p300/CBP activity and while p300/CBP HAT activity is upregulated by a number of signaling pathways (26), phosphorylation of ser89 attenuates p300/CBP activity. This site was originally identified as being protein kinase C (PKC) dependent (30), however it has also been described as an AMPK phosphorylation site (31). Specifically, AMPK phosphorylation of p300/CBP at ser89 reduced its interaction with nuclear receptors such as peroxisome proliferator activated receptor γ (PPARγ), retinoic acid receptors and thyroid receptor (31). However, the interaction of p300/CBP with other transcription factors such as p53, GATA and E1a were not affected. This could be explained by the nuclear receptor interacting domain on p300/CBP being located at the extreme N-terminus of the protein, and phosphorylation of ser89 would presumably alter the structure of this domain. This suggests that modulation of p300/CBP activity by AMPK is specific to regulation of the nuclear receptor superfamily of transcription factors. Given that the enhanced transcriptional activity of many nuclear receptors, such as the PPARs, is associated with enhanced expression of lipid oxidative genes and an enhanced capacity for ATP production (32), the relationship between AMPK and p300/CBP seems counterintuitive, particularly as AMPK activation appears to increase PPARα expression (33). Nonetheless, it provides another example of AMPK regulating the epigenetic machinery, yet providing relative specificity in the physiological response.

**5.1.3. RNA polymerase I (Pol I)**

It has been reported that AMPK complexes, specifically those containing the $\gamma_3$ subunit, are localized to the nucleolus, the site of rRNA synthesis within the nucleus (34). Together with ribosomal proteins, rRNA forms the structural basis of the ribosome and plays a key role in mRNA translation. Activation of AMPK reduced the activity of RNA polymerase I (Pol I; (34), which transcribes rRNA. While no direct link between AMPK and Pol I was confirmed, this could suggest that AMPK seeks to reduce the rate of translation in response to perturbations in energy balance, by reducing the rRNA transcriptional rate.

In recent times, examination of gene expression regulation has revealed the importance of epigenetics in this process. Insights into the epigenetic mechanisms thought to be regulated by AMPK reveal that the field is only just beginning to understand how AMPK might control large related gene subsets (Figure 1). It seems likely that further AMPK targets that regulate the epigenetic landscape will be identified in the future.

**5.2. AMPK transcriptional regulation of cellular growth and differentiation**

A key feature of the AMPK signaling pathway is not only to promote energy production, but also to limit energy utilisation to ensure cellular survival. Consistent with the fact that the LKB1 tumor suppressor is a major AMPKK, activation of AMPK is associated with reduced cellular growth and proliferation (16). Previous studies have elucidated a role for AMPK in this process through modulation of the mammalian target of rapamycin (mTOR) activity, a key regulator of cell growth and proliferation, via phosphorylation and activation of the Tuberous Sclerosis Complex 2 (TSC2), a negative regulator of mTOR (16; 35). The raptor division of the mTOR pathway (TORC1) regulates cell size and proliferation through mRNA translation, ribosome biogenesis and autophagy (36). While ribosome biogenesis by TORC1 involves a transcriptional component and control of transcriptional regulators such as Forkhead-like-transcription factor (FHL1; (37) and upstream binding factor (UBF; (38), the role of AMPK in regulating these transcriptional pathways downstream of TORC1 are unknown. However, recent studies have identified a number of AMPK substrates that are directly involved in transcriptional control of cell growth and proliferation.

**5.2.1. p53**

In response to nutrient deprivation, cells respond by not only by promoting maintenance of energy production, but also through suppression of cell cycle progression and in serve cases of metabolic stress, initiation of apoptosis. A number of lines of evidence suggest that AMPK is involved in this process. For example, glucose starvation induces a G1 cell cycle arrest concomitant with a reduction in the number of cells in S phase and is

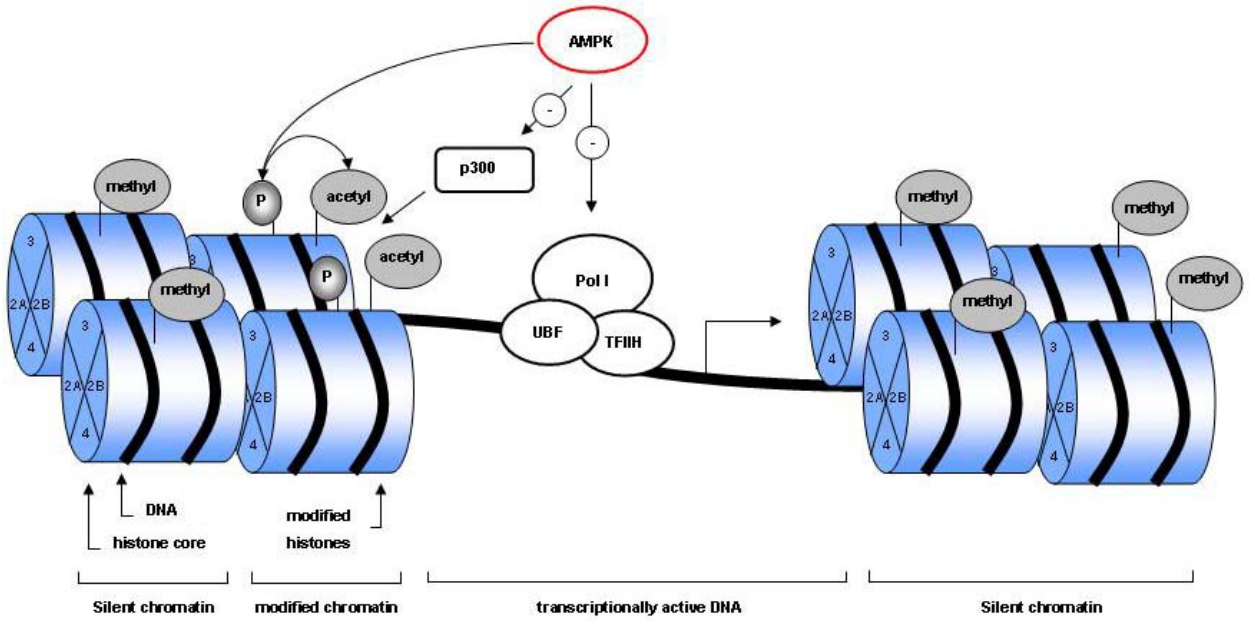


**Figure 1.** AMPK regulates epigenetics. AMPK directly phosphorylates ser10 on histone 3 (H3), which promotes acetylation of H3 and chromatin remodeling. Phosphorylation of the HAT p300/CBP on ser89 reduces its transcriptional activity towards nuclear receptors. Finally, AMPK has been linked to inhibition of polymerase I activity and transcription of rRNA.

associated with activation of AMPK (39). Furthermore, a number of studies have shown that AICAR treatment induces a similar cell cycle arrest to glucose starvation in a number of cell types (39; 40). Recent evidence suggests that AMPK regulates the p53 transcription factor to achieve this. Known as a tumor suppressor, p53 is activated by cellular stress signals such as DNA damage, ultraviolet light and oncogenes (41). Under normal conditions, p53 is rapidly degraded by ubiquitin mediated proteolysis, however other posttranslational modifications such as phosphorylation and acetylation stabilize p53, attenuating its subsequent degradation and enhancing its DNA binding activity towards regulatory promoter regions of genes involved in cell cycle inhibition, apoptosis and genetic stability (41). It should be noted that the cellular response to p53 activation is cell type and context dependent, and initiation of apoptosis does not occur in all cell types. Treatment with AICAR (40) or expression of constitutively active (CA) AMPK (39) results in phosphorylation of p53 at ser15 (ser18 in mice) and enhanced expression of $p21^{waf1/cip1}$, a gene that encodes an inhibitor of the cyclin dependent kinases (CDKs), which are key regulators of the cell cycle. Inhibition of the CDKs arrest cells in both the G1 to S and the G2 to mitosis transitions. Jones *et al.* (39) established that AMPK ser15 phosphorylation of p53 provides a metabolic checkpoint through enhanced p21 expression that ensures cellular sustainability, as genetic deletion of p53 in the face of metabolic stress permits unsustainable cellular proliferation.

### 5.2.2. Thyroid receptor interacting protein 6 (TRIP6)

A recent study has found that thyroid receptor interacting protein 6 (TRIP6) is an AMPK substrate (42). As its name suggests, TRIP6 was initially described as a protein that interacts with the thyroid receptor. However, TRIP6 has recently been reclassified as a member of the zyxin group of proteins, which are typically localized at focal adhesion plaques in the plasma membrane. TRIP6 can also localize to the nucleus (43) and discovery of the TRIP6 N-terminal transctivating domains led to the finding that TRIP6 could also act as a transcriptional coactivator for the NFκβ signaling pathway. Solaz-Fuster *et al.* (42) discovered a functional interaction between AMPK and TRIP6 through two-hybrid screening, and confirmed this interaction *in vivo*. It was also found that AMPK could phosphorylate TRIP6 *in vitro*. The exact site(s) were not identified, but were localized to the first 290 amino acids of TRIP6. Solaz-Fuster *et al.* (42) also showed that AMPK does not affect the interaction of TRIP6 with specific components of focal adhesion plaques, such as the lysophosphatidic acid receptor. Rather, AMPK appears to enhance the coactivating properties of TRIP6 towards NFκβ regulated reporter gene expression (42). This raises the possibility that AMPK can regulate processes such as cell proliferation, apoptosis, differentiation and oncogenesis via intersection with the NFκβ signaling pathway. Future studies are needed to discern the role that AMPK might play in regulating these diverse processes through TRIP6. Of note however, knockdown of TRIP6 results in increased features associated with malignant transformation of epithelial cells and malignant myeloid diseases, such as increased cell motility and a loss of cell to cell adhesion (44). These data might suggest that AMPK could regulate tumourogenesis through interaction with TRIP6 and provides another example of transcriptional regulation by AMPK that limits cellular proliferation and growth to ensure cellular survival.

### 5.3. AMPK transcriptional regulation of skeletal muscle metabolism

Perhaps the most significant effects that AMPK exerts on transcription are observed in skeletal muscle. In addition to the putative role AMPK plays in skeletal muscle glucose uptake and fatty acid oxidation, it is clear that AMPK regulates a number of enzymes involved in carbohydrate and lipid metabolism and the electron transport chain (20; 21; 45-47). Indeed, AMPK is required for skeletal muscle mitochondrial biogenesis in response to chronic energy deprivation (48). These adaptations presumably enhance the capacity of this tissue to meet future metabolic challenges. It has been hypothesized that AMPK induces this phenotypic change by increasing the expression of the peroxisome proliferator activated receptor γ coactivator 1α (PGC-1α), a transcriptional coactivator that regulates many metabolic genes in skeletal muscle (49). However, studies show that AMPK directly regulates a number of transcriptional regulators independently of PGC-1α in skeletal muscle to control the expression of a number of metabolic gene subsets.

#### 5.3.1. Histone deacetylase 5 (HDAC5)

Histone deacetylase 5 (HDAC5) is a transcriptional repressor that is highly expressed in striated muscle that inhibits gene expression by deacetylating histone lysine residues within the nucleosome core. This maintains a highly compact structure containing DNA that is inaccessible to initiators of transcription such as RNA polymerase 1 and the generic transcription factors. HDAC5 associates with a number of DNA binding transcription factors, such the myocyte enhancer factor 2 (MEF2) and the ankyrin repeat proteins ANKRA2 and RFXANF, which provides specificity to the gene subsets that HDAC5 regulates (50; 51). Through these transcription factors, HDAC5 regulates gene networks involved in myogenic differentiation (52). Recently, it has been discovered that AMPK phosphorylates HDAC5 on ser259 and ser498, which dissociate HDAC5 from MEF2 and provides binding sites for the 14-3-3 chaperone proteins, which then export HDAC5 out of the nucleus (McGee *et al.*, unpublished observations). It was also shown that ser259 and ser498 are required for AMPK induction of the glucose transporter isoform 4 (GLUT4) gene. Significantly, mutation of the key regulatory ser259 and ser498 residues on HDAC5 in the heart leads to cardiac sudden death due to mitochondrial abnormalities (53). Furthermore, expression screening in animals containing this mutation revealed a coordinated down regulation of genes involved in oxidative and mitochondrial metabolism, including that of PGC-1α (53). Together, these data suggest that AMPK phosphorylation of HDAC5 might account for many of the gene expression responses to AMPK activation in skeletal muscle. A recent screen for potential class II HDAC kinases has possibly revealed why a number of AMPK transgenic models fail to show striking skeletal muscle phenotypes. From these experiments, it is evident that there is considerable redundancy in class II HDAC signaling, with multiple kinases capable of phosphorylating serines 259 and 498 on HDAC5 (54). The kinases identified that are expressed in skeletal muscle include protein kinase D (PKD) and microtubule associated regulated kinase 2 (Mark2, also known as Par1/EMK1), an AMPK related kinase (54). These data suggest that although AMPK is a key regulator of this network of genes, likely through HDAC5, it is not essential.

#### 5.3.2. GLUT4 enhancer factor (GEF)

As its name suggest, the GLUT4 enhancer factor (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 (55). Recently, it has been shown that AMPK can phosphorylate GEF *in vitro* (56). Phosphorylation of GEF by AMPK was associated with enhanced GEF DNA binding activity (56). The specific site(s) were not identified, nor was this interaction confirmed *in vivo*. If GEF is in fact a bona fide AMPK substrate, it could suggest that AMPK targets multiple regulators of a given gene or gene cluster to achieve specificity in its transcriptional response.

#### 5.3.3. Nuclear respiratory factor 1 (NRF-1)

Although the effect of AMPK mediated transcription in skeletal muscle is profound, very few mechanisms have been identified to explain this response. While targeting of HDAC5 and GEF by AMPK could be involved, another potential target is the nuclear respiratory factor 1 (NRF-1). NRF-1 is a transcription factor required for the expression of many nuclear and mitochondrial encoded enzymes found within the mitochondrial respiratory chain (57). In mice, chronic activation of AMPK is associated with an increase in the DNA binding activity of NRF-1 and an increase in mitochondrial density and expression of mitochondrial enzymes (58). However, these effects could also be mediated through increased activation and expression of PGC-1, a potent coactivator of NRF-1 (59). While it is clear that AMPK increases the expression of PGC-1 (20; 21), it has also been proposed that AMPK might acutely regulate PGC-1 activity through phosphorylation of PGC-1, which releases PGC-1 from its repressor protein p160myb and increases PGC-1 coactivational activity (60). Further research will clarify how AMPK regulates NRF-1 mediated gene expression.

### 5.4. AMPK and transcriptional regulation of hepatic metabolism

Circulating blood glucose levels are a balance between glucose uptake in skeletal muscle and adipose tissue and glucose production by the liver. Elevated hepatic glucose production is a major cause of the hyperglycemia observed in insulin resistant states such as type 2 diabetes (61). Glucose production in the liver is regulated by the dynamic expression of a number of enzymes involved in gluconeogenesis, such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase). Insulin suppresses hepatic glucose output and gluconeogenic gene expression through Akt-mediated phosphorylation and nuclear exclusion of Foxo1, which reduces PGC-1α expression and subsequent coactivation of gluconeogenic genes (62). Conversely, glucagon enhances gluconeogenic gene expression and hepatic glucose output through cAMP-protein kinase A (PKA) mediated phosphorylation of the CREB transcription factor (63). Activation of AMPK is associated with reduced expression

of PEPCK and G6Pase (64), suggesting that AMPK regulates the transcription of these genes. Indeed, liver specific AMPK $\alpha_2$ knockout mice are hyperglycemic and glucose intolerant, consistent with increased gluconeogenic gene expression (65). While it has been shown that AMPK activation increases the proteolytic degradation of Foxo1 in H4 cells (66), possibly providing a link between AMPK activation and reduced gluconeogenic gene expression, a number of studies have identified transcriptional regulators of the hepatic gluconeogenic program as AMPK substrates.

**5.4.1. Transducer of regulated CREB activity 2 (TORC)**

Recently, the importance of a CREB coactivator, termed the transducer of regulated CREB (TORC) activity 2 was identified as being a central regulator of the hepatic gluconeogenic transcriptional program (67). The mechanism by which TORC2 coactivates CREB mediated gene expression involves association with CREB at its basic region/leucine zipper (bZIP) DNA binding domain, which enhances CREB interaction with the $TAF_{II}130$ component of TFIID (68). Phosphorylation of ser171 sequesters TORC2 in the cytoplasm via interactions with 14-3-3 proteins (69), similar to the paradigm presented for HDAC5. This reduces the expression of CREB dependent gluconeogenic genes including PEPCK, G6Pase and PGC-1α (67). Originally, the AMPK related salt inducible kinase (SIK) was identified as a kinase that phosphorylates TORC2 on ser171 (69). However, AMPK was subsequently found to be a TORC2 kinase, it too phosphorylating ser171 (67). Furthermore, activation of AMPK was sufficient to sequester TORC2 to the cytoplasm even in the presence of cAMP agonists (67). This suggests that hepatic AMPK activation might override the induction of the gluconeogenic transcriptional program in response to fasting signals such as glucagon. The importance of AMPK and other AMPK related kinases in regulating this response was recently highlighted by a report showing that LKB1 regulates the gluconeogenic program via TORC2, and that metformin, a widely prescribed drug treatment for type 2 diabetes, requires LKB1 for its therapeutic effects in lowering hepatic glucose output (70).

**5.4.2. AICAR responsive element binding protein (AREBP)**

In addition to phosphorylating TORC2 to inhibit hepatic gluconeogenesis, it appears that AMPK targets other transcriptional regulators to exert additional control over this process. Recently, the AICAR response element binding protein (AREBP) was identified as a transcriptional repressor of PEPCK expression through cloning of proteins binding to an AICAR responsive element on the PEPCK promoter (71). At its N-terminus, AREBP contains a nuclear localization signal and contains five $C_2H_2$-type zinc finger motifs at its C-terminus, which shares high homology with the zinc finger domains of repressors of nuclear receptors (71). AMPK phosphorylation of AREBP on ser470 reduces the expression of PEPCK (71). Although a DNA binding protein, overexpression of AREBP has no effect on PEPCK expression. To repress PEPCK expression, AREBP must be phosphorylated by AMPK at ser470, which reduces AREBP DNA binding activity (71). It is unclear exactly how this unique mechanism represses PEPCK expression, but it is thought that the AREBP release from DNA could then form interactions with and inactivate PEPCK transcriptional coactivators (71). Further investigations are needed to firmly elucidate this mechanism. Northern blotting revealed that AREBP is ubiquitously expressed, however the function of this transcriptional repressor in other tissues is unknown and could reveal a wider role for AMPK in the regulation of transcription.

**5.4.3. Carbohydrate response element binding protein (ChREBP)**

In response to conditions of low energy flux, an additional mechanism by which the liver spares glucose is through inhibition of hepatic glycolysis. Like hepatic gluconeogenesis, this is largely regulated by modulating the transcription of enzymes in this pathway, such as liver pyruvate kinase (L-PK; (72). The carbohydrate response element binding protein (ChREBP) transcription factor mediates this effect. In response to glucose, ChREBP, which is specifically expressed in the liver, binds to a region on the L-PK promoter and activates L-PK expression (73). However, activated AMPK is thought to phosphorylate ser568 on ChREBP, which reduces ChREBP DNA binding activity and reduced L-PK transcriptional activity (74). Activation of hepatic AMPK also reduces FA synthase expression (75), which together with reduced glycolytic flux, results in a shift toward fatty acid oxidation to meet the energetic demands of the liver.

**5.4.4. Hepatocyte nuclear factor 4α (HNF4α)**

Another mechanism by which AMPK controls the expression of glucose dependent genes in the liver is through regulation of the hepatocyte nuclear factor 4α (HNF4α). Activation of AMPK is associated with reduced HNF4α DNA binding activity, HNF4α degradation and reduced expression of HNF4α target genes such as L-PK, glucose transporter isoform 2 and aldolase B (76). This was revealed to be due to AMPK mediated phosphorylation of ser304 (ser313 in humans) on HNF4α (77). The important role that HNF4α plays in the regulation of metabolic genes in other tissues such as the pancreas, the intestine and the kidney suggest that AMPK regulation of HNF4α could be important in the regulation of metabolism in numerous tissues. In particular, this relationship could be significant in the pancreas, as a null allele in the HNF4α gene causes a form of diabetes termed maturity onset diabetes of the young (MODY), which is characterised by a defect in pancreatic insulin secretion (78).

Analysis of AMPK transcriptional targets in the liver reveals that AMPK is able to regulate a specific physiological response, such as hepatic fuel sparing, through different mechanisms that include both gluconeogenesis and glycolysis (Figure 2). It is unclear if this is designed to provide exquisite control over this response, or if these mechanisms have evolved to ensure redundancy in the signaling regulating physiologically important functions. Nonetheless, AMPK regulation of hepatic glycolysis, together with gluconeogenesis, highlights that this energy sensing molecule can regulate a number of different transcriptional processes that result in

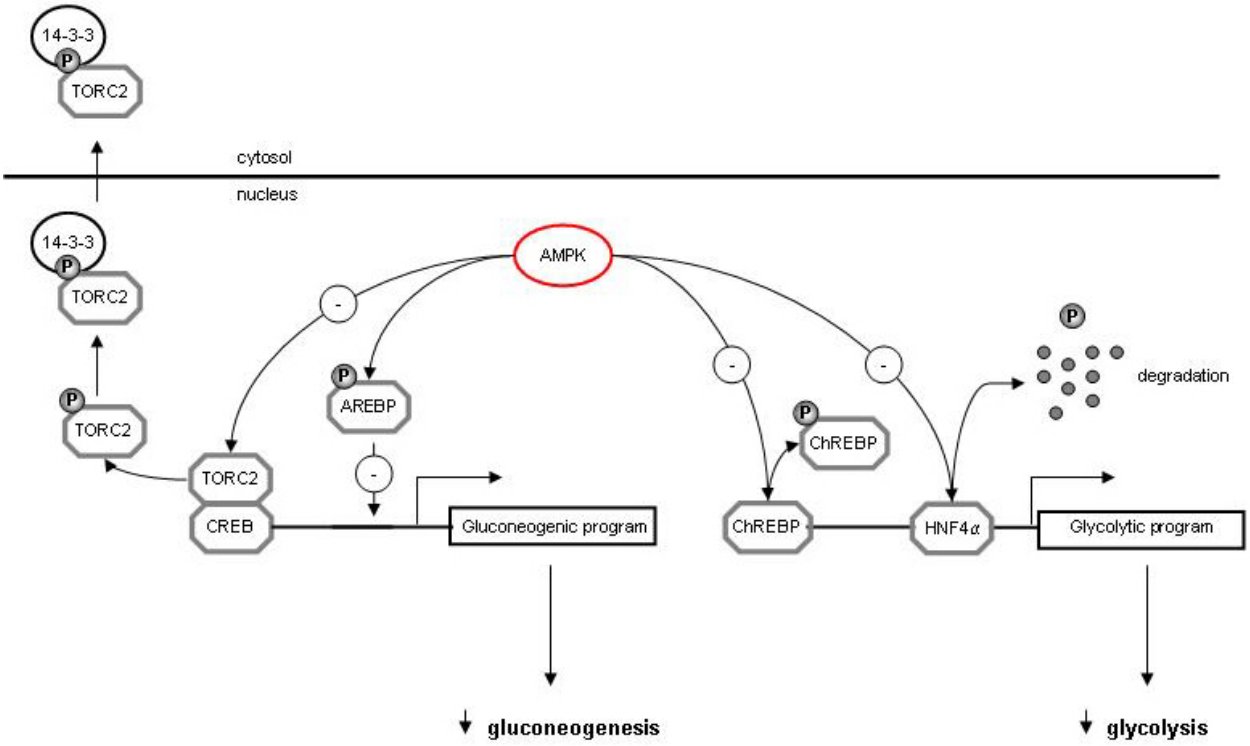


**Figure 2.** Transcriptional control of hepatic metabolism by AMPK. Phosphorylation of the transducer of regulated CREB activity (TORC2) by AMPK at ser171 dissociates TORC2 from CREB and results in TORC2 nuclear export by 14-3-3s, reducing CREB transcriptional activity. Phosphorylation of the AICAR response element binding protein (AREBP) at ser470 by AMPK similarly inhibits hepatics gluconeogenic gene expression. Glycolytic gene expression is inhibited by phosphorylation at ser568 of the carbohydrate response element binding protein (ChREBP), which reduces its DNA binding and therefore transcriptional activity. AMPK phosphorylation of HNF4α at ser313 promotes HNF4α degradation, also reducing glycolytic gene expression.

complimentary effects on tissue metabolism and physiology.

### 5.5. Other AMPK transcriptional targets

#### 5.5.1. Peroxisime proliferator activated receptor α (PPARa)

The peroxisime proliferator activated receptor α (PPARα) is a member of the nuclear receptor superfamily and is highly expressed in the liver, and to a lesser extent adipose tissue, skeletal muscle, heart and brain (79; 80). Thus, PPARα exerts its effects on multiple metabolically active tissues. Like many PPARs, PPARα is a DNA binding receptor for lipid ligands. Upon ligand binding, PPARα undergoes a conformational change that results in transcriptional corepressors being released in exchange for transcriptional coactivators (32). This activates transcription of a number of PPARα dependent genes, which promote fatty acid oxidation (79). In perhaps the most unique mechanism yet describing AMPK regulated transcription, AMPK has been found to enhance PPARα mediated transcription, not through a phosphorylation event, but through direct physical interaction with PPARα (81). Interestingly, AMPK kinase deficient mutations and mutation of a putative AMPK phosphorylation site on PPARα did not affect the ability of AMPK to coactivate PPARα (81). Furthermore, binding of AMPK to PPARα was increased by ATP and coactivation of PPARα by AMPK was inhibited by AICAR (81). Together, these results suggest that AMPK coactivates PPARα only when in its inactive confirmation. The function of this mechanism is unknown, but it could form a feed-forward mechanism designed at preserving cellular ATP. The relationship between AMPK and PPARα could be another example the central regulation of metabolism by AMPK given that a recent study has suggested that it is activation of PPARα in the brain, as opposed to the liver or adipose tissue, that is important in the regulation of whole body glucose metabolism (80).

## 6. PERSPECTIVES

The role of AMPK in the control of transcriptional events is a relatively new area of study, however it is clear that AMPK is an important regulator of a number of transcriptional programs in a variety of cell types and tissues. Although many transcriptional regulators are known AMPK substrates and have been described in this review, in many instances full characterization of the gene networks regulated by these AMPK substrates has yet to be established under the context of AMPK activation.

Additionally, it is anticipated that new interactions between AMPK and as yet unidentified regulators of transcription will be discovered in the future. Understanding these interactions will uncover the mechanisms by which AMPK regulates diverse processes such as energy metabolism, cell signaling, cell growth and proliferation, immunity, transcription and apoptosis. Identification of these mechanisms will also establish therapeutic targets for diseases such as cancer, obesity and type 2 diabetes.

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