Sensing of energy and nutrients by AMP-activated protein kinase1–4

D Grahame Hardie

ABSTRACT

AMP-activated protein kinase (AMPK) is a cellular energy sensor that exists in almost all eukaryotes. Genetic studies in lower eukaryotes suggest that the ancestral role of AMPK was in response to starvation for a carbon source and that AMPK is involved in life-span extension in response to caloric restriction. In mammals, AMPK is activated by an increasing cellular AMP:ATP ratio (which signifies a decrease in energy) caused by metabolic stresses that interfere with ATP production (eg, hypoxia) or that accelerate ATP consumption (eg, muscle contraction). Because glucose deprivation can increase the AMP:ATP ratio, AMPK can also act as a glucose sensor. AMPK activation occurs by a dual mechanism that involves allosteric activation and phosphorylation by upstream kinases. Once activated, AMPK switches on catabolic pathways that generate ATP (eg, the uptake and oxidation of glucose and fatty acids and mitochondrial biogenesis) while switching off ATP-consuming, anabolic pathways (eg, the synthesis of lipids, glucose, glycogen, and proteins). In addition to the acute effects via direct phosphorylation of metabolic enzymes, AMPK has longer-term effects by regulating transcription. These features make AMPK an ideal drug target in the treatment of metabolic disorders such as insulin resistance and type 2 diabetes. The antidiabetic drug metformin (which is derived from an herbal remedy) works in part by activating AMPK, whereas many xenobiotics or nutraceuticals, including resveratrol, quercetin, and berberine, are also AMPK activators. Most of these agents activate AMPK because they inhibit mitochondrial function. Am J Clin Nutr 2011;93(suppl):891S–6S.

INTRODUCTION

Animals derive energy from oxidation of reduced carbon compounds in food and use this to synthesize ATP from ADP. The consequent high ATP:ADP ratio (analogous to the fully charged state of a rechargeable battery) drives most of the energy-requiring reactions that occur in the cell. It is essential that ATP-producing and -consuming processes are maintained in balance, and this is achieved by the action of regulatory systems that include AMP-activated protein kinase (AMPK). In recent years, AMPK has attracted much attention because of its relevance to the epidemics of obesity and type 2 diabetes. AMPK appears to mediate the therapeutic benefits of the antidiabetic drug metformin and of xenobiotics or nutraceuticals such as resveratrol. AMPK is a target for the adipokines leptin and adiponectin and accounts for many of the health benefits of regular exercise. The purpose of this short review is to explain how the AMPK system can maintain energy balance at cellular and whole-body levels.

AMPK

Evolution

Mammalian AMPK exists as heterotrimeric complexes that comprised a catalytic \( \alpha \) subunit and regulatory \( \beta \) and \( \gamma \) subunits (1), and genes that encode these subunits are readily recognized in other eukaryotic kingdoms (ie, plants, fungi, and protists). It is instructive to consider the roles of nonmammalian orthologs of AMPK, which have been studied by genetics. In the yeast Saccharomyces cerevisiae, the AMPK ortholog is required for the response to glucose starvation. When grown in high glucose concentrations, yeast use fermentation to generate ATP and produce ethanol. It is only when the glucose concentration runs low that yeast switch to the use of oxidative metabolism or other fermentable sugars, such as sucrose, and the AMPK ortholog switches on genes required for these transitions. In the nematode worm Caenorhabditis elegans, AMPK is required for life-span extension in response to a caloric restriction or other stresses in early life (2). Finally, in the green plant Physcomitrella patens, AMPK orthologs are not required for growth in continuous light but are essential if plants are grown on a more physiologic light and dark cycle (3). Because darkness is the equivalent of starvation for a plant, these observations support the idea that the ancestral role of AMPK was in the response to starvation for a carbon source.

Structure and regulation

In mammals, the \( \alpha \) subunit occurs as 2 isoforms (\( \alpha 1/\alpha 2 \)), the \( \beta \) subunit 2 (\( \beta 1/\beta 2 \)) and the \( \gamma \) subunit 3 (\( \gamma 1/\gamma 3 \)), that give rise to \( \leq 12 \) heterotrimeric combinations. The \( \alpha \) subunit has a kinase domain at the \( N \)-terminus that is only active after phosphorylation in the activation loop at Thr-172 (4). Phosphorylation of Thr-172, which is now widely used as a biomarker for AMPK activation, can be analyzed by using phosphospecific antibodies. After a long search, the major upstream kinase phosphorylating

1 From the College of Life Sciences, University of Dundee, Dundee, United Kingdom.
3 Supported by a Programme Grant from the Wellcome Trust and by EXGENESIS, an Integrated Project of the European Commission (LSHMC-CT-2004-005272).
4 Address correspondence to DG Hardie, College of Life Sciences, University of Dundee, Dow Street, Dundee, DD1 5EH, United Kingdom. E-mail: d.g. hardie@dundee.ac.uk.

Thr-172 was shown to be liver kinase B1 (LKB1) (5, 6), which was previously identified as a tumor suppressor. Germline loss-of-function mutations in LKB1 cause an inherited susceptibility to cancer (Peutz-Jeghers syndrome), whereas somatic mutations are frequent in some cancers, especially lung and cervical cancers. Although LKB1 also acts upstream of a small family of AMPK-related kinases, AMPK is its only target known to inhibit cell growth and proliferation, and thus, its tumor-suppressor effects may be mediated by AMPK. The subunit contains a glycogen-binding domain that causes the complex to bind to glycogen particles (7, 8). The physiologic role of this is not well understood, although we have shown that AMPK can sense the structural state of glycogen (9), and our hypothesis is that it monitors the status of glycogen stores and ensures that they are rapidly replenished once depleted. Thus, AMPK may sense immediate energy availability and medium-term reserves in the form of glycogen. The subunit contains 2 sites that bind the regulatory nucleotides AMP and ATP in an antagonistic manner (10, 11). The binding of AMP, which is a signal of low cellular energy status, has the following 2 effects: 1) the promotion of the phosphorylation of the subunit at Thr-172, which causes a >100-fold activation and 2) the allosteric activation of the phosphorylated kinase by >10-fold, which yields a >1000-fold activation overall. Our current model is that LKB1 has a high basal activity and continuously phosphorylates Thr-172, but in the absence of AMP, it is immediately dephosphorylated. However, the binding of AMP causes a conformational change that inhibits the dephosphorylation of AMPK (12, 13). Soon after the discovery that LKB1 acted upstream of AMPK, another upstream kinase was found, the calmodulin-dependent kinase (CaMKK) (14–16). This pathway activates AMPK in response to a rise in cytosol Ca2+. Because increases in cytosolic Ca2+ often trigger ATP-requiring processes such as contraction or secretion, we view this as a mechanism to anticipate a demand for ATP before it actually occurred.

**DOWNSTREAM TARGETS OF AMPK**

Once activated by ATP depletion, AMPK switches on catabolic pathways that generate ATP while switching off anabolic pathways and other ATP-consuming processes, which restores the energy balance. First, I will discuss the acute activation of catabolism. AMPK activates glucose uptake via glucose transporters GLUT1 and GLUT4; in the latter case, this involves phosphorylation of a wide variety of transcription factors and coregulators (Figure 1). AMPK phosphorylates the carbohydrate response element binding protein, which suppresses the transcription of liver pyruvate kinase (26). Expressions of other hepatic transcription factors are down-regulated by AMPK at either the mRNA level [ie, sterol response element binding protein-1c (SREBP-1c) (27)] or protein concentration [ie, hepatocyte nuclear factor-4z (HNF-4z) (28)]. These effects switch off the expression of lipogenic genes such as liver pyruvate kinase, fatty acid synthase, and ACC1. Other genes that are down-regulated by AMPK in the liver encode phosphoenolpyruvate carboxykinase and glucose-6-phosphatase, which are enzymes of gluconeogenesis, in part by the phosphorylation of the coactivator CREB regulated transcription coactivator-2 (CRTC2) (29). AMPK also phosphorylates the coactivator p300, affecting its interaction with nuclear receptors such as peroxisome proliferator-activated receptor- (PPAR-γ) (30).

In addition, AMPK activates the transcription of catabolic genes (Figure 1). AMPK up-regulates the GLUT4 gene via effects that require the myocyte enhancer factor-2 site on its promoter (31); one potential mechanism is the phosphorylation of involved in fatty acid synthesis, 3-hydroxy-3-methylglutaryl-CoA reductase, which is involved in sterol synthesis (22), and glycogen synthase (23). An activator of anabolism that is switched off by AMPK is target-of-rapamycin complex-1 (TORC1), which stimulates protein synthesis in response to anabolic signals such as amino acids and insulin. This involves 2 effects as follows: 1) the phosphorylation of tuberous sclerosis complex protein-2 (TSC2), which converts the small G protein Rheb (which is a TORC1 activator) to its inactive GDP form (24) and 2) the phosphorylation of Raptor, which is a regulatory subunit of TORC1 (25).

As well as these rapid effects that are due to the direct phosphorylation of metabolic enzymes, AMPK switches on the expression of catabolic enzymes. This involves the phosphorylation of a wide variety of transcription factors and coregulators (Figure 1). AMPK phosphorylates the carbohydrate response element binding protein, which suppresses the transcription of liver pyruvate kinase (26). Expressions of other hepatic transcription factors are down-regulated by AMPK at either the mRNA level [ie, sterol response element binding protein-1c (SREBP-1c) (27)] or protein concentration [ie, hepatocyte nuclear factor-4z (HNF-4z) (28)]. These effects switch off the expression of lipogenic genes such as liver pyruvate kinase, fatty acid synthase, and ACC1. Other genes that are down-regulated by AMPK in the liver encode phosphoenolpyruvate carboxykinase and glucose-6-phosphatase, which are enzymes of gluconeogenesis, in part by the phosphorylation of the coactivator CREB regulated transcription coactivator-2 (CRTC2) (29). AMPK also phosphorylates the coactivator p300, affecting its interaction with nuclear receptors such as peroxisome proliferator-activated receptor- (PPAR-γ) (30).

In addition, AMPK activates the transcription of catabolic genes (Figure 1). AMPK up-regulates the GLUT4 gene via effects that require the myocyte enhancer factor-2 site on its promoter (31); one potential mechanism is the phosphorylation of
AMPK activation in muscle also increases mitochondrial biogenesis by up-regulating the expression of PPAR-γ coactivator-1α (33), which activates both mitochondrial DNA replication and nuclear-encoded mitochondrial genes. AMPK was reported to directly phosphorylate the PPAR-γ coactivator-1α (34), but another mechanism may be the deacetylation by SIRT1 (silent information regulator two number 1), which is activated downstream of the SIRT1 substrate NAD⁺ (35).

REGULATION BY METABOLIC STRESSES

The first treatments shown to activate AMPK were pathologic stresses such as heat stress or mitochondrial inhibitors (36), ischemia (37) or hypoxia (18, 19). AMPK is also activated by glucose deprivation (38), although in most cells, this only occurs at very low glucose concentrations. However, in specialized glucose-sensing cells that express GLUT2 and glucokinase (eg, pancreatic β cells and cells in the hypothalamus), AMPK is regulated by physiologic fluctuations in glucose. Although ATP-sensitive K⁺ channels may be the primary ATP sensors in these cells, AMPK appears to play a role in glucose sensing at both sites (39–41).

A physiologic stress that activates AMPK by accelerating ATP consumption is muscle contraction (42). Contraction induced by intense electrical stimulation has been associated with increases in the cellular AMP:ATP ratio (43), and this is likely to be the trigger for AMPK activation, although a milder stimulation may activate AMPK via the Ca²⁺-CaMKK pathway (44). AMPK is not required for the activation of glycogen breakdown and glycolysis; the key enzymes in these pathways (phosphorylase and phosphofructokinase) are directly activated by increases in the AMP:ATP ratio. Most evidence has suggested that AMPK is not a key player in resistance exercise (eg, weight-lifting or sprinting when phosphocreatine and glycogen are the main energy sources), but becomes important during endurance exercise, when the use of oxidative metabolism and blood-borne fuels increases. It appears that AMPK activation is responsible for many, if not all, acute responses to prolonged contraction, as well as chronic adaptations to repeated exercise. Studies that used mouse models, including muscle-specific knockouts of the upstream kinase LKB1 (43), and mice that express a dominant negative AMPK mutant (45), suggested that AMPK is a major, although probably not the only, mediator of contraction-stimulated glucose uptake. Experiments with AMPK-α1 or -α2 knockouts did not support a crucial role for either the subunit alone in contraction-stimulated glucose uptake (46), but these results may have been affected by redundancy between isoforms, and double knockouts have not been studied. An interesting effect in α1 knockout mice is the increased muscle hypertrophy in response to a mechanical overload (47). This can be explained by AMPK inhibition of the mammalian target-of-rapamycin (mTOR) complex, which is activated during resistance exercise and is thought to be important for the accompanying hypertrophy (48). AMPK activation and the consequent inhibition of mTOR during endurance exercise may explain why muscles of endurance athletes are not as hypertrophied as those of sprint- or strength-trained athletes.

REGULATION OF AMPK BY CYTOKINES

Although AMPK evolved in unicellular eukaryotes and regulates energy balance in a cell-autonomous manner, cytokines that evolved in metazoans to regulate energy balance at the whole-body level interact with the system (49) (Figure 2). Thus leptin, which is released from adipocytes as a signal that fat stores are adequate, stimulates AMPK in skeletal muscle, which explains how it activates fat oxidation and energy expenditure. By contrast, leptin has been reported to inhibit AMPK in the hypothalamus, which potentially explains how it suppresses food intake. Although some leptin functions in the hypothalamus appeared to be normal in the absence of AMPK (50), other treatments that increase food intake, such as ghrelin, cannabinoids and hypoglycemia, also activated hypothalamic AMPK (41, 51, 52). Adiponectin, which is also released from adipocytes but has a plasma concentration that is paradoxically increased in lean individuals, lowers plasma glucose by activating AMPK in the liver (53).

FIGURE 2. Cytokines, drugs, and xenobiotics that activate AMPK-activated protein kinase (AMPK). As discussed in the text, most of the drugs and xenobiotics [with the exception of 5-aminooimidazole-4-carboxamide ribonucleoside (AICAR)] activate AMPK by inhibiting mitochondrial function.

EFFECTS OF AMPK ACTIVATION ON GLUCOSE HOMEOSTASIS

As research on AMPK has progressed, it became increasingly clear that AMPK activators might be useful as drugs to treat insulin resistance or type 2 diabetes. Thus, AMPK acutely increases muscle glucose uptake via a mechanism that remains functional in insulin-resistant individuals and also increases GLUT4 expression (31) so that insulin would promote greater muscle glucose uptake even with no change in insulin sensitivity. AMPK also promotes glucose metabolism by increasing mitochondrial biogenesis, which is relevant because people at risk of developing type 2 diabetes appear to have a relative deficit in mitochondrial function (54). Another mechanism by which AMPK could increase insulin sensitivity is by promoting fat oxidation and reducing triglyceride storage; an excess amount of muscle triglyceride is associated with insulin resistance (54). Finally, an important source of the high glucose concentrations in type 2 patients with diabetes is elevated hepatic glucose production, which AMPK inhibits by down-regulating gluconeogenic genes.

REGULATION OF AMPK BY DRUGS AND XENOBIOTICS

The first drug shown to activate AMPK was the nucleoside 5-aminooimidazole-4-carboxamide ribonucleoside (AICAR) (Figure
2), which is taken up into cells and converted to an AMP mimetic, 5-aminomidazole-4-carboxamide ribonucleoside monophosphate (ZMP) (55). AICAR reversed many metabolic effects in animal models of insulin resistance such as the ob/ob mouse (56), the fa/fa rat (57, 58), or the fat-fed rat (59). In 2001 it was reported that the anti-diabetic drug metformin activated AMPK (27), and studies of mice with a liver knockout of LKB1 suggested that the activation of hepatic AMPK was essential for its anti-hyperglycemic effects (60). Metformin is currently the frontline treatment of type 2 diabetes and has been prescribed to >100 million patients worldwide. Remarkably, another major class of anti-diabetic drug, the thiazolidinediones, also activates AMPK (61), although the main therapeutic target of thiazolidinediones may be the nuclear receptor PPAR-γ. However, a major effect of PPAR-γ activation appears to be to trigger the release of adiponectin from adipocytes, which is supported by findings that low doses of thiazolidinediones failed to improve glucose tolerance in adiponectin knockout mice (62). Thus, thiazolidinediones activate AMPK by 2 mechanisms as follows: a cell-autonomous effect and an indirect effect that involves adiponectin release.

Finally, many plant-derived xenobiotics or nutraceuticals that were claimed to have health benefits in diabetes or cancer have been reported to activate AMPK (Figure 2). These include resveratrol from red wine, epigallocatechin gallate from green tea, capsaicin from peppers, berberine, which is a yellow dye of the genus Berberis that is used in traditional Chinese medicine, and quercetin (63–66).

An interesting question concerns how such a variety of xenobiotics with differing structures could all activate AMPK. Our hypothesis was that many of them inhibit mitochondrial function, which would increase the cellular AMP:ATP ratio. Although some drugs, such as thiazolidinediones, were reported to alter cellular nucleotide ratios, this was not shown for others, including metformin (61). However, there are distinct pools of adenine nucleotides within cells, and thus, increases in the AMP:ATP ratio in one pool might be masked by unaltered ratios at others. As a more sensitive test, we made use of a mutation in the γ2 subunit of AMPK (R531G) that abolishes AMP binding and activation (10) and constructed isogenic cell lines that expressed either wild-type or mutant γ2 (67). Our prediction was that compounds that inhibit mitochondrial function would activate AMPK in the wild-type cells but not in mutant cells. This was the case for the classical inhibitor of mitochondrial ATP synthase, oligomycin, as well as metformin, resveratrol, and berberine (67). By contrast, agents that act via AMP-independent mechanisms, including A23187 (which activates the CaMKK pathway) and A-769662 (which directly activates AMPK by binding to a site distinct from AMP) (68, 69), activated the wild-type cells but not the mutant cells (67). Thus, although oxidative stress activates AMPK, it does this indirectly by damaging the respiratory chain.

CONCLUSIONS

AMPK acts as a sensor of cellular energy by monitoring the cellular AMP:ATP ratio. It can also act as a sensor of glucose, which is consistent with its ancestral role in lower eukaryotes in response to glucose starvation. In cells that express GLUT2 and glucokinase, such as pancreatic β cells and cells in the hypothalamus, glucose metabolism and, hence, the AMP:ATP ratio may vary in response to physiologic fluctuations in plasma glucose concentrations. In other cells that express GLUT1 or GLUT4 and hexokinase, glucose uptake and phosphorylation are saturated at low glucose concentrations, and thus, AMPK in those cells is not sensitive to the glucose supply.

By switching from anabolism to catabolism, AMPK restores the energy balance after stresses that cause ATP depletion. This makes it an ideal target for drugs aimed at the treatment of metabolic disorders such as type 2 diabetes. Surprisingly, many existing anti-diabetic drugs, as well as other xenobiotics and nutraceuticals claimed to have health benefits, appear to act, in part, via AMPK. Many of these activate AMPK indirectly by inhibiting mitochondrial ATP synthesis. At first, it might seem surprising that so many of these compounds should be mitochondrial poisons. However, many of them, including resveratrol, are secondary plant metabolites that may have evolved as defensive compounds to deter grazing or infection by pathogens.

The funding sources had no influence on this article. The author had no conflicts of interest.

REFERENCES


