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Minireview: The AMP-Activated Protein Kinase Cascade: The Key Sensor of Cellular Energy Status

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All cells must maintain a high ratio of cellular ATP:ADP to survive. Because of the adenylate kinase reaction (2ADP ↔ ATP + AMP), AMP rises whenever the ATP:ADP ratio falls, and a high cellular ratio of AMP:ATP is a signal that the energy status of the cell is compromised. The AMP-activated protein kinase (AMPK) is the downstream component of a protein kinase cascade that is switched on by a rise in the AMP:ATP ratio, via a complex mechanism that results in an exquisitely sensitive system. AMPK is switched on by cellular stresses that either interfere with ATP production (*e.g.* hypoxia, glucose deprivation, or ischemia) or by stresses that increase ATP consumption (*e.g.* muscle contraction). It is also activated by hormones that act via Gq-coupled receptors, and by leptin and adiponectin, via mechanisms that remain unclear. Once activated, the system switches on catabolic pathways that generate ATP, while switching off ATP-consuming processes that are not essential for short-term cell survival, such as the synthesis of lipids, carbohydrates, and proteins. The AMPK cascade is the probable target for the antidiabetic drug metformin, and current indications are that it is responsible for many of the beneficial effects of exercise in the treatment and prevention of type 2 diabetes and the metabolic syndrome. (*Endocrinology* 144: 5179–5183, 2003)

ALL LIVING CELLS must continuously maintain a high, nonequilibrium ratio of ATP to ADP (which are analogous to the chemicals in an electrical cell or battery) to survive. Catabolism charges up the battery by converting ADP and phosphate to ATP, whereas almost all other cellular processes tend to discharge the battery by directly or indirectly converting ATP to ADP and phosphate (or AMP and pyrophosphate). The fact that the ATP:ADP ratio in cells usually remains almost constant indicates that the mechanisms that maintain these processes in balance are very efficient. What is more surprising is that the identity of the key player in this process, the AMP-activated protein kinase (AMPK), has become apparent only in the last few years. With hindsight, its discovery can be traced back to two independent observations first reported in 1973. Gibson and co-workers (1) reported that a crude preparation of 3-hydroxy-3-methyl-CoA reductase, the key regulatory enzyme of cholesterol synthesis, became inactivated in a time-dependent manner upon incubation with MgATP, while Carlson and Kim (2) reported similar observations with acetyl-CoA carboxylase. Both groups correctly surmised that this was due to the action of a protein kinase, but it was to be another 14 yr before the current author provided evidence that these were both functions of the same protein kinase (3), which we renamed AMPK (4, 5).

Regulation of AMPK

AMPK is a heterotrimeric complex comprising a catalytic α-subunit and regulatory β- and γ-subunits (6). Each subunit exists as alternate isoforms encoded by two or three genes ($\alpha1$, $\alpha2$, $\beta1$, $\beta2$, $\gamma1$, $\gamma2$, $\gamma3$), and all twelve different combinations of isoforms appear to be able to form complexes. The predominant isoforms in most cells are $\alpha1$, $\beta1$, and $\gamma1$, but liver cells also significantly express $\alpha2$ (7), whereas skeletal and cardiac muscles also express $\alpha2$, $\beta2$, $\gamma2$, and $\gamma3$ (8–10). Although much remains to be learned, differences in function between the isoforms are already known. First, the degree of AMP dependence depends on the identity of both the α- and γ-subunits, with stimulation varying from only 50% for the $\alpha1\gamma3$ combination to more than 5-fold for the $\alpha2\gamma2$ combination (10). Second, $\alpha2$ complexes appear to be enriched in the nucleus, whereas $\alpha1$ complexes are largely cytoplasmic and appear to be largely excluded from the nucleus (11–14).

As its name suggests, AMPK is allosterically activated by AMP, but, more importantly, it is also activated by phosphorylation by one or more upstream kinases at a threonine residue within the activation loop of the α-subunit kinase domain, without which there is no detectable activity (15, 16). This phosphorylation is promoted by AMP both by stimulating phosphorylation by the upstream kinase (17) and by inhibiting dephosphorylation by protein phosphatases (18). This complex mechanism renders the cascade ultrasensitive, *i.e.* over the critical range of concentrations, a small rise in AMP produces a large increase in the final output (19). The effects of AMP are also antagonized by high concentrations of ATP, so that the system responds to rises in the AMP:ATP ratio rather than to rises in AMP alone. If the function of the AMPK system is indeed to monitor cellular energy status, a pertinent question is why it should respond to the AMP:ATP ratio rather than the ADP:ATP ratio. The likely answer is that all eukaryotic cells contain a very active adenylate kinase enzyme that maintains the reaction catalyzed (2ADP ↔ ATP + AMP) close to equilibrium at all times. This means that the

Abbreviations: AICA, 5-Aminoimidazole-4-carboxamide; AMPK, AMP-activated protein kinase; GBD, glycogen-binding domain; ZMP, 5-aminoimidazole-4-carboxamide-1-D-ribofuranosyl-5′-monophosphate.

AMP:ATP ratio varies approximately as the square of the ADP:ATP ratio (20), and the former is therefore a much more sensitive indicator of cellular energy status than the latter.

Regulation of the AMPK *in Vivo*

The AMPK system is therefore activated by any stress that causes a rise in the cellular AMP:ATP ratio, either by interfering with ATP production or by increasing ATP consumption. Stresses of the former type include heat shock and metabolic poisoning in isolated hepatocytes (21), and hypoxia and ischemia in perfused heart muscle (22, 23). These are all pathological stresses, but the kinase is also regulated by more physiological stimuli. In pancreatic β-cells, low glucose activates AMPK in the same range of concentrations over which it inhibits insulin release (13, 24). Finally, a physiological metabolic stress that activates AMPK by increasing ATP consumption is exercise in skeletal muscle, the original finding of which (25) triggered a greatly increased interest in the system. AMPK is activated by exercise or contraction in rodent (25–27) and human (28, 29) muscle, and activation is dependent on both the duration and the intensity of exercise (30, 31).

AMPK is also allosterically inhibited by physiological concentrations of phosphocreatine (32), consistent with the proposed physiological role of the kinase as a sensor of cellular energy status. Another rapidly mobilized store of energy in many tissues is glycogen. The β-subunits of AMPK contain a central conserved domain that has recently been recognized as a glycogen-binding domain (GBD) (33, 34). In both rat (35) and human (36) muscle, a high glycogen content represses AMPK activation, suggesting that the AMPK system may monitor the availability of this longer term store of energy as well as that of ATP and phosphocreatine. It is tempting to suggest that the GBD is responsible for this, although there is no direct evidence for this at present. An alternative role for the GBD, which is not necessarily mutually exclusive, is that it localizes the kinase with one of its substrates, *i.e.* glycogen synthase. Consistent with this, overexpression of AMPK in cultured cells has been found to cause the accumulation of AMPK in unusually large glycogen granules that also contain glycogen synthase (33).

Homologs of the α-, β-, and γ-subunits of AMPK are present even in the most primitive, unicellular eukaryotes such as *Giardia lamblia* (6), and it seems likely that the system primarily evolved to regulate cellular function in response to fluctuations in energy status, rather than to hormonal stimuli. Nevertheless, it has recently been found that some hormones do regulate the system. AMPK is activated by receptors coupled to phospholipase C via the G protein Gq (37), and by the adipocytokines leptin (38) and adiponectin (39). The mechanism for AMPK activation by these hormones remains unclear, and in particular, it is not known whether they act by increasing the AMP:ATP ratio or via some more novel mechanism.

Downstream Targets for AMPK Activation

Much of what has been learned about the downstream targets for AMPK in intact cells and *in vivo* has come from the use of the compound 5-aminoimidazole-4-carboxamide (AICA) riboside. This adenosine analog is taken up into cells and converted by adenosine kinase to the monophosphorylated nucleotide 5-aminoimidazole-4-carboxamide-1-D-ribofuranosyl-5′-monophosphate (ZMP). ZMP mimics all of the activating effects of AMP on the AMPK system, although it is much less potent than AMP itself (40). Nevertheless, in most cells, it accumulates to sufficiently high concentrations that it activates AMPK without disturbing cellular levels of AMP, ADP, or ATP (40, 41). An important caveat in the use of AICA riboside is that ZMP does affect certain other AMP-regulated enzymes (42, 43). Recently, it has been found that the antidiabetic drug metformin activates AMPK (44) by a mechanism that involves phosphorylation by the upstream kinase but with no alteration in the cellular AMP:ATP ratio (45, 46). Because AICA riboside and metformin activate AMPK by different mechanisms, if both agents produce the same physiological effect, one can have more confidence that it is mediated by AMPK activation. For a complete analysis of the effects of AMPK on a physiological process, it is also necessary to identify the actual target protein for AMPK phosphorylation, identify the sites phosphorylated, and show that these are phosphorylated in intact cells or *in vivo* in response to AMPK activation. At present, this level of analysis has been achieved only in a few cases.

In general, activation of AMPK switches on catabolic pathways that generate ATP, while switching off anabolic pathways and any other nonessential processes that consume ATP. It achieves this both by direct phosphorylation of regulatory proteins involved in the process, and by indirect effects on gene expression. A full discussion of the targets for AMPK is beyond the scope of this minireview, but a summary of those that are reasonably well established is shown in Fig. 1. The interested reader should consult earlier reviews for detailed citations (6, 20, 47), and only a few more recently established examples are discussed below.

As well as inhibiting biosynthetic pathways such as fatty acid and cholesterol synthesis, it has been found that activation of AMPK inhibits protein synthesis (48, 49). Although the direct targets for phosphorylation by AMPK responsible for the inhibition of translation remain unclear, there seem to be two mechanisms involved. First, AMPK activation causes activation of elongation factor-2 kinase and increased phosphorylation of elongation factor-2, leading to inhibition of the elongation step of translation (48, 50). Second, AMPK activation also appears to down-regulate the mammalian target of rapamycin pathway, which can activate translation both through phosphorylation and activation of p70 S6 kinase, and through increased phosphorylation of 4E-binding protein 1, which relieves inhibition of initiation of translation (49, 51, 52). Another recently discovered target for AMPK is the cystic fibrosis transmembrane regulator protein (53–55), which is involved in regulating transepithelial transport of ions and, as a consequence, secretion of fluid into the airways and the gut. The overall process of fluid secretion requires not only direct hydrolysis of ATP by the cystic fibrosis transmembrane regulator protein itself, but also ATP turnover catalyzed by the plasma membrane Na^+/K^+ ATPase. As an energetically expensive process, it makes sense for it to be switched off in response to AMPK activation. Finally, the expression of numerous genes is regulated by AMPK (*e.g.*

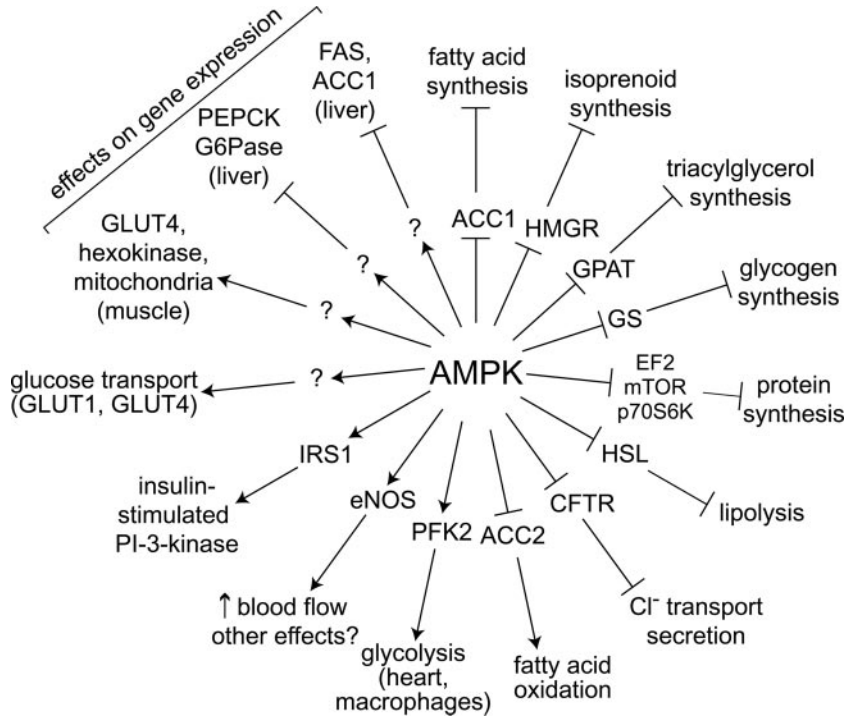

FIG. 1. Known physiological target proteins and pathways regulated by the AMPK system (modified from Ref. 6). The list is not exhaustive: other targets have been proposed, but in some cases the evidence is not yet conclusive. ACC, Acetyl-coenzyme A carboxylase [ACC1 (α) and ACC2 (β) isoforms]; HMGR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; GPAT, glycerol phosphate acyl transferase; GS, glycogen synthase; EF2, elongation factor-2; mTOR, mammalian target of rapamycin; p70S6K, p70 ribosomal protein S6 kinase; HSL, hormone-sensitive lipase; CFTR, cystic fibrosis transmembrane regulator; PFK2, 6-phosphofructo-2-kinase; eNOS, endothelial nitric oxide synthase; IRS1, insulin receptor substrate-1; PI-3-kinase, phosphatidylinositol 3-kinase; GLUT1 and -4, glucose transporter 1 and 4; PEPCK, phosphoenolpyruvate carboxykinase; G6Pase, glucose-6-phosphatase; FAS, fatty acid synthase.

Fig. 1). In a recent microarray study of mice expressing a dominant-negative mutant AMPK in muscle (where AMPK activity was undetectable), a total of 234 genes were up-regulated, and 130 were down-regulated by more than 2-fold compared with control mice (56). Intriguingly, the genes that are up-regulated by AMPK in muscle are similar to those induced by endurance exercise training, including glucose transporter GLUT4, and oxidative enzymes of mitochondria (57). Thus, activation of AMPK during exercise may help to prepare the muscle for subsequent exercise bouts by increasing the capacity of muscle to rapidly take up and oxidize glucose.

In most cases, the direct target proteins for AMPK responsible for the effects on gene expression are not known. However, AMPK phosphorylates the carbohydrate response element binding protein at Ser^{568}, which inhibits its DNA binding activity and may be involved in the regulation of expression of the liver pyruvate kinase gene (58). It also phosphorylates the transcriptional coactivator p300 at Ser^{89}, and this reduces its interaction with nuclear hormone receptors such as peroxisome proliferator-activated receptor-γ (59). Moreover, AMPK activation has been shown to reduce the expression of several important transcription factors, including sterol regulatory element binding protein-1c (44), hepatocyte nuclear factor-4α (60), CCAAT/enhancer binding protein-α, and peroxisome proliferator-activated receptor-γ (61). AMPK directly phosphorylates hepatocyte nuclear factor-4α, and this seems to have a 2-fold effect, both reducing its ability to form homodimers and bind DNA, and stimulating its degradation (62).

Relevance to Type 2 Diabetes and the Metabolic Syndrome

Via mechanisms indicated in Fig. 1, AMPK activation causes many metabolic changes that would be beneficial in subjects with type 2 diabetes and the metabolic syndrome, such as increased glucose uptake and metabolism by muscle and other tissues, decreased glucose production by the liver, and decreased synthesis and increased oxidation of fatty acids. Indeed, experiments with animal models of type 2 diabetes and the metabolic syndrome show that activation of AMPK using 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside riboside can reverse many of the metabolic defects of these animals *in vivo* (63–66). The AMPK system is the probable target of the major antidiabetic drug metformin (44–46) and may even be one target for another antidiabetic drug, rosiglitazone (45). It is responsible for the increased fat oxidation in response to the adipocyte-derived hormones, leptin (38) and adiponectin (39), thus promoting their action to prevent fat accumulation in other tissues. It is also activated by exercise, which is known to have beneficial effects in the treatment and prevention of type 2 diabetes. Given the inexorable rise in the incidence of type 2 diabetes and the metabolic syndrome in modern society, these recent findings help to explain the intense current interest in the AMPK system.

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