Regulation of lipid metabolism by the AMP-activated protein kinase

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The AMP-activated protein kinase (AMPK) is the central component of a protein kinase cascade which plays an important role in the regulation of many different aspects of lipid metabolism [1,2]. AMPK is activated 50- to 100-fold by phosphorylation by an upstream protein kinase, AMPK kinase (AMPKK) [3]. The key regulators of the AMPK cascade currently appear to be 5′-AMP and ATP. AMP activates the system through no less than four mechanisms [4-6]: (1) direct allosteric activation (up to 5-fold); (2) binding to AMPK, making it a better substrate for AMPKK; (3) binding to AMPK, making it a worse substrate for the inactivating phosphatase, protein phosphatase-2C; and (4) allosteric activation of AMPKK. High levels of ATP antagonize effects (1) and (3) of AMP [4,6], and also antagonize the effect of AMP on phosphorylation of AMPK by AMPKK [3], although in this case it is not yet clear whether the effect is to oppose mechanism (2) or (4), or both. Multiple isoforms of AMPK have been identified [6a].

These multiple inputs of AMP mean that, above a certain threshold concentration, a small rise in AMP will produce a large activation of the downstream kinase. The effect of AMP will also be antagonized by high concentrations of ATP. In the intact cell, ATP and AMP vary in a reciprocal manner, due to the action of adenylate kinase, which maintains the reaction 2ADP ⇌ ATP + AMP close to equilibrium (K_eq ≈ 1). In normal, fully energized cells, the ATP:ADP ratio is maintained at a high level by oxidative phosphorylation, and adenylate kinase then keeps AMP concentrations very low. However, if the cell experiences some stress that interferes with ATP production and lowers the ATP:ADP ratio, the adenylate kinase equilibrium is displaced to the right and the AMP:ATP ratio rises (approximately as the square of the ADP:ATP ratio), thus activating the AMPK system [7].

One of the original [8] and now well established targets for AMPK is acetyl-CoA carboxylase (ACC), which catalyses a key regulatory step in fatty acid synthesis. AMPK phosphorylates ACC at three sites (Ser-79, Ser-1200 and Ser-1215), and all of these sites are phosphorylated in intact cells and in vivo [9,10]. Phosphorylation at Ser-79 leads to a large decrease in V_max as well as to a decrease in sensitivity to the allosteric activator, citrate [11,12]. It has now been shown that several stresses that deplete ATP and activate AMPK lead to inactivation of ACC and marked inhibition of fatty acid synthesis in isolated rat hepatocytes. These treatments include high fructose concentrations in the medium [13], and heat shock and arsenite (a tricarboxylic acid cycle inhibitor) [14]. Similar effects are observed on incubation of cells with 5-aminoimidazole-4-carboxamide (AICA) riboside, which is taken up by cells and converted into the AMP analogue AICA riboside monophosphate, causing AMPK activation without affecting intracellular levels of AMP, ADP or ATP [15].

The product of ACC, malonyl-CoA, is a potent inhibitor of fatty acid oxidation, due to its ability to inhibit fatty acid uptake into mitochondria via inhibition of carnitine palmitoyltransferase I [16]. It is now becoming clear that inhibition of ACC by AMPK can have the dual purpose of conserving ATP by inhibiting fatty acid synthesis, and promoting ATP production by stimulating fatty acid oxidation (see for example, [17]). Skeletal and cardiac muscles express a novel, higher-molecular-mass isoform of ACC (ACC280, probably corresponding to the product of the ACC-P gene [18], otherwise known as ACC-2 [19]), but do not carry out fatty acid synthesis. Muscle tissues also express a novel isoform of carnitine palmitoyltransferase I that is much more sensitive to inhibition by malonyl-CoA [20]. In these tissues, regulation of fatty acid oxidation now appears to be one of the primary functions of AMPK. ACC purified from rat muscle (predominantly ACC280) is phosphorylated and inactivated by AMPK in vitro [21]. In rat skeletal muscle in vivo, prolonged exercise leads to activation of AMPK, inhibition of ACC, and depression of malonyl-CoA [22]. In electrically stimulated muscle, the activation of AMPK is associated with concomitant increases

Abbreviations used: AMPK, AMP-activated protein kinase; AMPKK, AMPK kinase; ACC, acetyl-CoA carboxylase; AICA, 5-aminoimidazole-4-carboxamide; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase.
in AMP and decreases in ATP [23]. Interestingly, it has recently been reported that the activation of AMPK in response to electrical stimulation is restricted to the α2 isoform [24]. In perfused rat heart, ischaemia caused by ligation of coronary arteries is associated with rapid activation of AMPK, inhibition of ACC, and decreases in malonyl-CoA. Malonyl-CoA remains depressed during subsequent reperfusion, and this may explain the very high rates of fatty acid oxidation which then occur [25,26].

The other 'classical' [27] target for AMPK is 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR), which catalyses a key regulatory step in isoprenoid and sterol synthesis. AMPK phosphorylates HMGR at Ser-871 [28], and this site is phosphorylated in intact cells under conditions where AMPK is activated [29], leading to marked inhibition of sterol synthesis [14,15]. Particularly convincing evidence that HMGR is a physiological substrate for AMPK came from the Brown and Goldstein laboratory [30]. They stably expressed recombinant HMGR in UT-2 cells, a mutant strain of CHO cells that lacks endogenous HMGR. When ATP was depleted using treatment with 2-deoxyglucose, sterol synthesis was markedly inhibited in the cells expressing wild-type HMGR, but unaffected in cells expressing a point mutant in which the AMPK site (Ser-871) was changed to alanine. Recently we have expressed the catalytic domain of HMGR in bacteria and examined the ability of its ability to inhibit enzymes of lipid bio-

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