

This Review is part of a thematic series on **AMP Kinase**, which includes the following articles:

AMP-Activated Protein Kinase in Metabolic Control and Insulin Signaling

Cardiac AMP-Activated Protein Kinase in Health and Disease

*Bruce Kemp, Guest Editor*

## AMP-Activated Protein Kinase in Metabolic Control and Insulin Signaling

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**Abstract**—The AMP-activated protein kinase (AMPK) system acts as a sensor of cellular energy status that is conserved in all eukaryotic cells. It is activated by increases in the cellular AMP:ATP ratio caused by metabolic stresses that either interfere with ATP production (eg, deprivation for glucose or oxygen) or that accelerate ATP consumption (eg, muscle contraction). Activation in response to increases in AMP involves phosphorylation by an upstream kinase, the tumor suppressor LKB1. In certain cells (eg, neurones, endothelial cells, and lymphocytes), AMPK can also be activated by a  $\text{Ca}^{2+}$ -dependent and AMP-independent process involving phosphorylation by an alternate upstream kinase, CaMKK $\beta$ . Once activated, AMPK switches on catabolic pathways that generate ATP, while switching off ATP-consuming processes such as biosynthesis and cell growth and proliferation. The AMPK complex contains 3 subunits, with the  $\alpha$  subunit being catalytic, the  $\beta$  subunit containing a glycogen-sensing domain, and the  $\gamma$  subunits containing 2 regulatory sites that bind the activating and inhibitory nucleotides AMP and ATP. Although it may have evolved to respond to metabolic stress at the cellular level, hormones and cytokines such as insulin, leptin, and adiponectin can interact with the system, and it now appears to play a key role in maintaining energy balance at the whole body level. The AMPK system may be partly responsible for the health benefits of exercise and is the target for the antidiabetic drug metformin. It is a key player in the development of new treatments for obesity, type 2 diabetes, and the metabolic syndrome. (*Circ Res.* 2007;100:328-341.)

**Key Words:** calcium signaling ■ diabetes ■ insulin ■ metabolism ■ signaling pathways

Genes encoding the  $\alpha$ ,  $\beta$ , and  $\gamma$  subunits of the AMP-activated protein kinase (AMPK) are highly conserved in all eukaryotic species in which genome sequences have been completed, including vertebrates and invertebrates, plants, fungi, and protozoa.<sup>1</sup> Genetic studies show that in the yeast *Saccharomyces cerevisiae*, the genes encoding these subunits are required for the response to glucose starvation.<sup>2,3</sup> In a primitive green plant, the moss *Physcomitrella patens*, genes encoding the catalytic subunits are required for growth in alternate light/dark cycles, as opposed to continuous light.<sup>4</sup> Darkness represents a period of starvation for a green plant because it is unable to produce carbohydrate by photosynthesis. The AMPK system therefore appears to have initially evolved to execute responses to carbon starvation. Because of the sophisticated endocrine systems that control glucose homeostasis in mammals (including

the effects of insulin), starvation for glucose is not a normal physiological event for mammalian cells in vivo. Nevertheless, AMPK is still ubiquitously expressed in mammalian cells, where it is involved in the response to a variety of metabolic stresses that disturb cellular energy homeostasis. More recently, it has been realized that hormones and other extracellular signals have acquired the ability to modulate the AMPK system, and it is now known to be involved in regulating energy homeostasis at the whole body, as well as the cellular, levels.

### Regulation of the AMPK Complex by 5'-AMP and Calcium

With hindsight, the first reports describing the AMPK system were 2 independent articles published in 1973 involving

Original received October 26, 2006; revision received November 30, 2006; accepted December 12, 2006.

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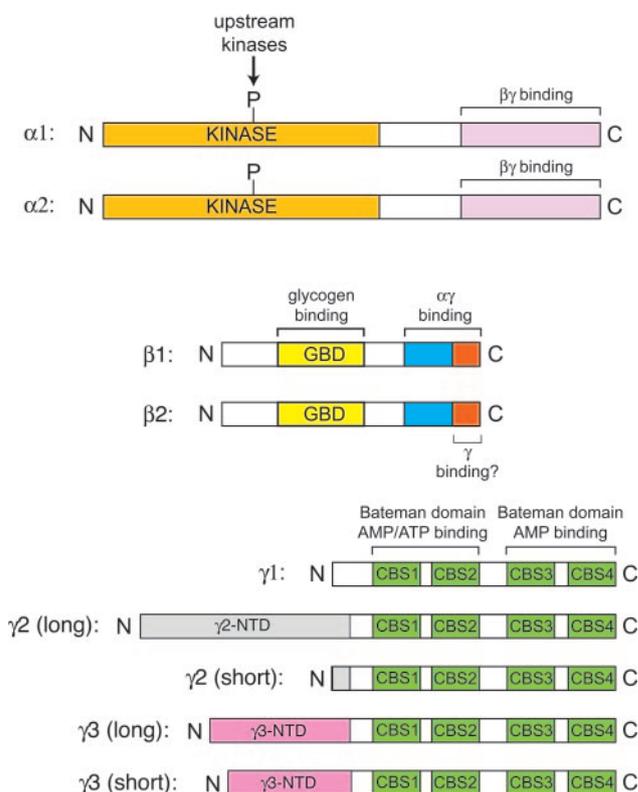
DOI: 10.1161/01.RES.0000256090.42690.05

poorly defined protein fractions that, in the presence of ATP, inactivated 2 key metabolic enzymes involved in lipid synthesis, ie, acetyl-CoA carboxylase (ACC) (involved in fatty acid synthesis)<sup>5</sup> and 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (involved in isoprenoid/cholesterol synthesis).<sup>6</sup> The protein fractions were correctly surmised to contain protein kinases, and subsequent studies revealed that both the ACC kinase and the HMG-CoA reductase kinase were stimulated by 5'-AMP.<sup>7,8</sup> However, it was not realized that both functions were performed by the same protein kinase until our laboratory provided evidence in favor of that hypothesis in 1987<sup>9</sup>; we renamed the activity AMP-activated protein kinase the following year.<sup>10</sup>

As well as allosterically activating the enzyme by up to 5-fold,<sup>9</sup> AMP also promotes its phosphorylation<sup>11</sup> at a specific threonine residue on the  $\alpha$  subunit (Thr172<sup>12</sup>) by an upstream kinase that has recently been identified as a complex between the tumor suppressor protein LKB1 and 2 accessory subunits, termed STRAD and MO25.<sup>13,14</sup> Phosphorylation of Thr172 produces at least 100-fold activation,<sup>15</sup> so that it is quantitatively much more important than the allosteric activation. The LKB1 complex is not itself activated by AMP, and the effect of the nucleotide is to make AMPK a better substrate for LKB1,<sup>13</sup> while at the same time making it a worse substrate for protein phosphatases that dephosphorylate Thr172.<sup>16</sup> The 3 effects of AMP make the system very sensitive to small increases in AMP.<sup>17</sup> All 3 effects are also antagonized by high concentrations of ATP. Because all eukaryotic cells express very active adenylate kinases, which maintains their reaction ( $2ADP \leftrightarrow AMP + ATP$ ) close to equilibrium at all times, the cellular AMP:ATP ratio varies approximately as the square of the ADP:ATP ratio,<sup>18</sup> making it a very sensitive indicator of cellular energy status.

In cells lacking LKB1, such as HeLa cells (tumor cells in which LKB1 is not expressed), there is still some basal phosphorylation of Thr172 and AMPK activity,<sup>13</sup> and both can be dramatically increased by addition of a  $Ca^{2+}$  ionophore. Three groups have now identified the upstream kinase responsible for phosphorylation of Thr172 under these circumstances as  $Ca^{2+}$ /calmodulin-dependent protein kinase kinase (CaMKK), especially the CaMKK $\beta$  (CaMKK2) isoform.<sup>19–21</sup> CaMKKs were originally identified as protein kinases that acted upstream of calmodulin-dependent protein kinases I and IV.<sup>22</sup> Our laboratory showed that a purified CaMKK could phosphorylate and activate AMPK in cell-free assays as long ago as 1995,<sup>11</sup> although at that time, we did not consider that this was likely to have any physiological relevance. CaMKK $\beta$  is expressed primarily in the brain but is also expressed in testis, thymus, and T cells.<sup>23</sup> Our laboratory has shown that when  $Ca^{2+}$  enters neurones in rat brain slices following  $K^+$ -induced depolarization, there is a marked phosphorylation of Thr172 and activation of AMPK that is catalyzed by a CaMKK.

Given the limited tissue distribution of the CaMKKs compared with LKB1, the  $Ca^{2+}$ -mediated pathway may be restricted to certain cell types such as neurones, although additional examples are discussed below. However, it is interesting to speculate about the function of the



**Figure 1.** Domain structure of AMPK subunit isoforms and splice variants. Regions shown in the same color are related, and their functions, where known, are indicated.

CaMKK→AMPK pathway in those cell types in which it occurs. Any treatment that causes an increase in cytoplasmic  $Ca^{2+}$  will create a subsequent demand for ATP, if only because the  $Ca^{2+}$  is immediately pumped out of the cytoplasm using ATP-driven pumps in the plasma membrane and endoplasmic reticulum. Activation of AMPK under these circumstances may represent a mechanism to anticipate the demand for ATP created by  $Ca^{2+}$  entry.

### Subunit Structure of the AMPK Complex

AMPK is a heterotrimer comprising  $\alpha$ ,  $\beta$ , and  $\gamma$  subunits. There are 2 or 3 genes encoding each subunit (Figure 1 and Table 1), giving rise to 12 possible heterotrimeric combinations, with splice variants further increasing the potential diversity. The 2 isoforms of the  $\alpha$  subunit,  $\alpha_1$ <sup>24</sup> and  $\alpha_2$ ,<sup>25</sup> contain the kinase domain in their N-terminal half, with the C-terminal regions being required to form a complex with the  $\beta$  and  $\gamma$  subunits.<sup>26</sup> They appear to have rather similar substrate specificities,<sup>27</sup> but the  $\alpha_2$  isoform is enriched in the nucleus of several cell types, including pancreatic  $\beta$  cells,<sup>28</sup> neurones,<sup>29</sup> and skeletal muscle,<sup>30</sup> whereas  $\alpha_1$  is predominantly cytoplasmic. The  $\alpha_1$  isoform is associated with the plasma membrane in carotid body type 1 cells<sup>31</sup> and airway epithelial cells,<sup>32</sup> in the latter case particularly with the apical membrane.

The  $\beta$  subunits from different eukaryotic species contain 2 conserved regions, located in the central and C-terminal regions (Figure 1).<sup>33</sup> It is now clear that the C-terminal domain is all that is required to form a functional  $\alpha\beta\gamma$

**TABLE 1. Information Regarding AMPK Subunits, Subunit Isoforms, and Domains**

Isoform	Mass (kDa)	GenBank Accession No.	Amino Acids	Domain (Approximate)	Domain Location	Domain Function	Site of Major Expression	Chromosomal Location
$\alpha_1$	63	Q13131	550	Kinase domain	1–270	Catalytic	Ubiquitous?	5p11-p14
				C-terminal domain	393–550	$\beta\gamma$ binding		
$\alpha_2$	62	P54646	552	Kinase domain	1–268	Catalytic	Muscle, liver	1p31
				C-terminal domain	397–552	$\beta\gamma$ binding		
$\beta_1$	30	Q9Y478	270	Glycogen-binding domain	72–151	Glycogen binding	Ubiquitous?	12q24.1–24.3
				C-terminal domain	179–270	$\alpha\gamma$ Binding		
$\beta_2$	30	O43741	272	Glycogen-binding domain	72–154	Glycogen binding	Muscle	1q21.1
				C-terminal domain	181–272	$\alpha\gamma$ Binding		
$\gamma_1$	38	P54619	331	N-terminal domain	1–42	?	Ubiquitous?	12q12-q14
				Bateman domain 1	43–178	AMP/ATP binding		
				Bateman domain 2	199–331	AMP/ATP binding		
$\gamma_2$	Long	Q9UGJ0	569	N-terminal domain	1–274	?	Muscle?	7q36
				Bateman domain 1	275–410	AMP/ATP binding		
				Bateman domain 2	431–556	AMP/ATP binding		
	Short	NM_024429.1	328	N-terminal domain	1–33	?	Muscle?	7q36
				Bateman domain 1	34–169	AMP/ATP binding		
				Bateman domain 2	190–315	AMP/ATP binding		
$\gamma_3$	Long	NP_059127	489	N-terminal domain	1–197	?	Skeletal muscle	2q35
				Bateman domain 1	198–333	AMP/ATP binding		
				Bateman domain 2	358–479	AMP/ATP binding		
	Short	Q9UGI9	464	N-terminal domain	1–171	?	Skeletal muscle	2q35
				Bateman domain 1	172–308	AMP/ATP binding		
				Bateman domain 2	329–454	AMP/ATP binding		

All data are for human proteins. Mass refers to the mass predicted from the amino acid sequence: some subunits migrate anomalously on SDS-PAGE, eg,  $\beta_1$  migrates more slowly than  $\gamma_1$ , even though it is smaller by sequence analysis. This may be attributable to covalent modifications;  $\beta_1$  (and possibly  $\beta_2$ ) is myristoylated at the N terminus,<sup>149</sup> whereas the  $\alpha$  and  $\beta$  subunits can be phosphorylated at multiple sites.<sup>12,149,150</sup> The 2 variants of  $\gamma_2$  and  $\gamma_3$  appear to be attributable to alternate splicing.

complex that is regulated by AMP,<sup>34</sup> whereas the central conserved region is recognized to be a glycogen-binding domain.<sup>34,35</sup> The crystal structure of the glycogen-binding domain of  $\beta_1$  was recently determined,<sup>36</sup> but the functions of this domain remain unclear, although it is present in the  $\beta$  subunits of all eukaryotic species.

The  $\gamma$  subunits ( $\gamma_1$ ,  $\gamma_2$ , and  $\gamma_3$ ) contain variable N-terminal regions followed by 4 tandem repeats of a 60-aa sequence termed by Bateman as a CBS motif.<sup>37</sup> It is now known that these act in pairs to form 2 domains (now termed Bateman domains), each of which binds one molecule of AMP (Figure 1).<sup>38</sup> Mutations in these domains reduce both AMP binding and AMP activation, proving that these are the regulatory binding sites for the nucleotides.<sup>38</sup> The Bateman domains also antagonistically bind ATP with a lower affinity than AMP,<sup>38</sup> consistent with previous findings that high concentrations of ATP oppose activation of the AMPK complex by AMP.<sup>39</sup> The discovery that there were 2 binding domains for AMP or ATP was unexpected. The tandem domains bind AMP with a high degree of cooperativity<sup>38</sup> suggesting that the second site is inaccessible until AMP has bound at the first. This represents yet another potential mechanism to increase the sensitivity of the AMPK system to small changes in AMP.

### Regulation of AMPK by Metabolic Stress

Any metabolic stress that inhibits ATP production, or that accelerates ATP consumption, will tend to increase the cellular ADP:ATP ratio, which will be amplified by adenylate kinase into a much larger increase in the AMP:ATP ratio, with consequent activation of AMPK. 2-Deoxyglucose, which depletes ATP by being converted to the nonmetabolizable 2-deoxyglucose-6-phosphate, has sometimes been used to activate AMPK.<sup>40</sup> Not surprisingly, AMPK can also be activated by metabolic poisons that inhibit ATP production via oxidative phosphorylation, such as arsenite, oligomycin, antimycin A, azide, or dinitrophenol,<sup>41–43</sup> or by ischemia<sup>44</sup> or hypoxia.<sup>45</sup> Although the effect of ischemia is relevant to cardiovascular disease, these are all rather non-physiological stresses. However, a more physiological stress that activates AMPK in skeletal muscle is exercise or contraction,<sup>46–48</sup> and, as discussed further below, it seems likely that AMPK activation underlies many of the health benefits of regular exercise. Although there is currently no satisfactory method to measure changes in AMP during exercise in vivo, electrical stimulation of mouse hindlimb muscle in situ does cause small increases in the cellular AMP:ATP ratio.<sup>49</sup> This is

almost certainly responsible for the accompanying activation of AMPK, because no activation occurs if the same experiment is performed in mice with a muscle-specific knockout of the upstream kinase LKB1,<sup>49</sup> which is known in other cells to be required for the AMPK system to respond to changes in AMP.<sup>13</sup> Intriguingly, the changes in AMP:ATP ratio in response to electrical stimulation are significantly larger in the muscles from the knockout mice,<sup>49</sup> confirming the proposed role of AMPK in maintenance of cellular energy homeostasis.

In many tissues and organs, such as the heart, the AMPK system may be significantly activated by hypoxia only during pathological ischemic episodes, but there is evidence that, in certain specialized oxygen-sensing cells, the system is important in the response to more physiological variations in oxygen tension. These include the glomus cells in the carotid body, which sense the oxygen level in arterial blood supplying the brain, and also pulmonary artery smooth muscle cells.<sup>50</sup> In glomus cells, hypoxia causes opening of voltage-gated Ca<sup>2+</sup> channels and consequent entry of extracellular Ca<sup>2+</sup>, leading to release of neurotransmitters that cause firing of afferent neurones, triggering increased breathing. In pulmonary artery smooth muscle, hypoxia triggers release of Ca<sup>2+</sup> from intracellular stores, causing contraction of the muscle. This contrasts with the behavior of the smooth muscle in other arteries, which relax in response to hypoxia to supply more blood to the hypoxic tissue. However, this unusual behavior makes physiological sense in the pulmonary circulation, because it ensures that blood flow is diverted away from poorly oxygenated areas of the lung in favor of well-oxygenated areas. In collaboration with the groups of Evans and Peers, our laboratory has recently shown that AMPK is activated by changes in AMP:ATP that occur in response to hypoxia in pulmonary artery smooth muscle.<sup>31</sup> Unfortunately, glomus cells are too small to allow bulk measurements of AMP and ATP using the same methodology. However, AMPK-activating drugs (5-aminoimidazole-4-carboxamide riboside [AICAR] or phenformin; see below) precisely mimic all of the effects of hypoxia in both the carotid body glomus cell and in pulmonary artery smooth muscle, suggesting that hypoxia is detected by AMPK as it senses the changes in AMP:ATP ratio caused by inhibition of oxidative phosphorylation in these cells.<sup>31</sup>

As mentioned in the introduction, in lower eukaryotes such as the yeast *S. cerevisiae*, the primary role of the AMPK homolog seems to be in the response to glucose starvation. Most mammalian cells express glucose transporters and hexokinase isoforms that have a very low  $K_m$  for glucose, so that the rate of ATP synthesis from glucose only drops when blood glucose falls to pathologically low levels. However, just as there are specialized oxygen-sensing cells in the carotid body and pulmonary arteries, so too are there specialized glucose-sensing cells, including the  $\beta$  cells in pancreatic islets that release insulin and neurones in the hypothalamus of the brain that control feeding behavior. These cells express isoforms of glucose transporter (GLUT2) and hexokinase (hexokinase IV, glucokinase) with a much higher  $K_m$  for glucose, so that the rate of ATP synthesis falls in response to decreases in blood glucose within the physiological range.

Consequently, AMPK in these cells is regulated by physiological variations in blood glucose. Thus, in cell lines derived from rodent pancreatic  $\beta$  cells, AMPK is activated by low glucose and inhibited by high glucose,<sup>51,52</sup> whereas, in fasted mice, intracerebroventricular injection of glucose, or refeeding of the mice, inhibits the  $\alpha_2$  isoform of AMPK in the hypothalamus.<sup>53</sup> Moreover, the known downstream consequences of lowering external glucose in both cell types, ie, decreased insulin secretion ( $\beta$  cells) and increased feeding behavior (hypothalamus), can be mimicked by activation of AMPK at these sites, either by pharmacological or molecular biological interventions.<sup>51–55</sup> Thus, an ancient signaling pathway involved in the response to glucose starvation in single-celled eukaryotes responds to the same signal in multicellular eukaryotes, but its role has been adapted to control complex processes such as hormone release and feeding behavior.

### Regulation of AMPK by Hormones, Cytokines, and Other Extracellular Ligands

The AMPK system is found in all present day single-celled eukaryotes and is therefore likely to have evolved long before multicellular organisms, and the hormones and cytokines that help to integrate their functions, had developed. Nevertheless, recently it has become clear that hormones and cytokines have learnt how to exploit the cell signaling capabilities of the AMPK system. The first hints of this came with findings that AMPK complexes in CHO cells stably expressing receptors coupled via the G protein Gq, such as those for platelet-activating factor, epinephrine ( $\alpha_{1b}$ ), or bradykinin ( $B_2$ ), were activated by stimulation of the receptor with the appropriate ligand.<sup>56</sup> Because Gq couples these receptors to phospholipase C, triggering production of inositol-1,4,5-trisphosphate and Ca<sup>2+</sup> release, it seems likely that the activation of AMPK in these systems is triggered by phosphorylation by CaMKK $\beta$ , although this has not yet been demonstrated conclusively. However, in human umbilical cord vein endothelial cells Heller and colleagues<sup>57</sup> have provided evidence that thrombin (also known to act through a Gq-coupled receptor) stimulates AMPK through the Ca<sup>2+</sup>-mediated pathway. This pathway also appears to operate in other cells of the endothelial/hematopoietic lineage, such as T lymphocytes. With Cantrell and colleagues, we have recently shown that AMPK is dramatically but transiently activated when quiescent T cells are stimulated via the antigen receptor, peaking at 1 minute and returning to baseline by 60 minutes. A variety of evidence suggest that this activation is mediated by the Ca<sup>2+</sup>/CaMKK $\beta$  pathway.<sup>58</sup> Quiescent T cells have a very limited energy turnover but when stimulated, they need to generate a considerable amount of ATP for the rapid growth and proliferation that follows. We speculate that activation of AMPK allows them to rapidly switch on catabolic, ATP-generating processes to anticipate this demand. However, there is now much evidence that a high AMPK activity also represses cell growth and proliferation (see below); therefore, it may be necessary for AMPK activity to return to baseline values before T cell proliferation can commence.

Considerable excitement was generated by the discovery in the 1990s of adipokines (cytokines released by adipocytes) that control whole body energy homeostasis, especially leptin

and adiponectin. Leptin, the product of the obese gene (*ob*), appears to represent a signal that stores of fat are adequate, and exerts a feedback inhibition of food intake via effects on hypothalamic neurones that express the leptin receptor.<sup>59</sup> However, as well as inhibiting energy intake, leptin also stimulates energy expenditure by promoting uptake and oxidation of glucose<sup>60</sup> and fatty acids<sup>61</sup> in skeletal muscle. Kahn and colleagues<sup>62</sup> reported that the  $\alpha_2$  isoform of AMPK was activated in muscle by leptin injection in vivo in rats, accounting for its ability to stimulate these pathways. There appeared to be 2 effects: a rapid effect that was a direct effect on the muscle itself; and a longer-term effect that appeared to be a primary action of leptin on the hypothalamus, with a secondary effect on muscle being mediated by the sympathetic nervous system. Remarkably, the same group subsequently provided evidence<sup>53</sup> that leptin inhibited AMPK- $\alpha_2$  activity in the arcuate and paraventricular regions of the hypothalamus of fasted mice. Along with leptin, other known anorexigenic agents, including insulin and a melanocortin receptor agonist, inhibit AMPK- $\alpha_2$  in the paraventricular region, whereas agouti-related protein, an orexigenic agent, increased  $\alpha_2$  activity in the same region in fed mice. Other orexigenic agents, such as the gut hormone ghrelin<sup>55</sup> and the cannabinoids,<sup>63</sup> have also been reported to activate AMPK in the hypothalamus. Inhibition of AMPK appears to be necessary for the anorexigenic effects of leptin, because adenoviral expression of an activated form of AMPK in the hypothalamus blocked the effects of leptin.<sup>53</sup>

AMPK also appears to mediate many of the effects of adiponectin. This adipokine (also known as ACRP30<sup>64</sup>) was discovered as a protein secreted into the blood by adipocytes, although, paradoxically, its plasma concentration is reduced in obese rodents and humans.<sup>65,66</sup> AMPK has been reported to be activated by adiponectin in skeletal muscle, where it stimulates glucose uptake and fatty acid oxidation, and in liver, where it inhibits expression of gluconeogenic genes.<sup>67,68</sup> Experiments with liver-specific expression of dominant negative mutants<sup>67</sup> and knockouts<sup>69</sup> show that liver AMPK, and especially the  $\alpha_2$  isoform, is necessary for the ability of adiponectin to lower blood glucose.

### AMPK-Activating Drugs and Identification of Metabolic Pathways Regulated by AMPK

Many of the metabolic pathways regulated by AMPK were originally identified by incubating cells, or injecting whole animals, with the adenosine analog AICAR. AICAR is taken up into cells by adenosine transporters<sup>70</sup> and converted by adenosine kinase<sup>71</sup> to the phosphorylated nucleotide ZMP (5-aminoimidazole-4-carboxamide ribonucleoside), which mimics all 3 effects of AMP on the AMPK system.<sup>39</sup> Another pharmacological tool for AMPK activation became available with the discovery that the antihyperglycemic drug metformin, currently prescribed to more than 120 million people with type 2 diabetes worldwide, activates AMPK both in intact cells and in vivo.<sup>72</sup> A recent mouse gene targeting study involving a liver-specific knockout of LKB1, which prevents activation of AMPK by metformin in that tissue, suggested that activation of liver AMPK is required for the plasma glucose-lowering effects of metformin.<sup>73</sup> Thus, the major

effect of metformin seems to be inhibition of liver gluconeogenesis. Although one might expect that metformin would also activate AMPK and stimulate glucose uptake in skeletal muscle and other tissues, this may not occur in vivo because the concentrations the drug reaches in the peripheral circulation (as opposed to the portal vein, which supplies the liver direct from the gut) may not be sufficiently high to yield significant AMPK activation in those tissues. However, metformin can be used to activate AMPK in muscle and other cells in culture or ex vivo. The related biguanide drug phenformin, which is no longer used for treatment in humans because of complications resulting from lactic acidosis, is a more rapid and potent activator of AMPK in cultured cells.<sup>13</sup>

The use of AICAR and the biguanides has been extremely useful in the AMPK field, but like any pharmacological tools, results obtained using them must be interpreted with care. For example, the active derivative of AICAR, ZMP, also modulates other AMP-sensitive enzymes, such as glycogen phosphorylase<sup>74</sup> and fructose-1,6-bisphosphatase.<sup>71</sup> AICAR, itself, can compete with released adenosine for reuptake into cells, and if this allows adenosine to accumulate in the medium this can exert effects via adenosine receptors.<sup>70</sup> In the case of biguanides, they do not directly activate AMPK in cell-free assays<sup>42</sup> but instead appear to inhibit complex I of the respiratory chain,<sup>75,76</sup> leading to an increase in cellular AMP:ATP ratio.<sup>19</sup> Effects of biguanides could therefore be secondary effects of ATP depletion, rather than effects of AMPK activation per se. As an excellent example of these problems, Hue and colleagues have recently shown that effects of AICAR and metformin on the function of glucokinase in hepatocytes are still observed in cells from knockout mice lacking both AMPK catalytic subunits.<sup>77</sup> Another example is provided by findings that, although AICAR was originally reported to inhibit autophagy in isolated hepatocytes,<sup>78</sup> experiments involving expression of dominant negative mutants, or the use of compound C, now suggest that AMPK activation stimulates this process.<sup>79</sup> Compound C, an AMPK inhibitor developed by Merck<sup>72</sup> that has recently become commercially available, can be used as an AMPK inhibitor in intact cells, although its selectivity for AMPK remains uncertain.

These considerations highlight the increasing importance of confirming that the effects of pharmacological agents are indeed mediated by AMPK using molecular biological methods, such as the expression of constitutively active or dominant negative AMPK mutants,<sup>80,81</sup> small interference RNAs targeted at AMPK or, best of all, AMPK or LKB1 knockouts.<sup>77</sup> Another important approach is to identify the direct target for AMPK that mediates the effect on the pathway or process, identify the sites phosphorylated on that target, and confirm that phosphorylation of those sites correlates with AMPK activation. The use of Western blotting with phospho-specific antibodies is the usual method to achieve this, and phospho-specific antibodies against Thr172 on the AMPK- $\alpha$  subunits are often now used as a surrogate for measuring AMPK activity. An even more convincing approach is to show that the effect is lost in knock-in mice or cells expressing the target protein mutated at the AMPK sites. This

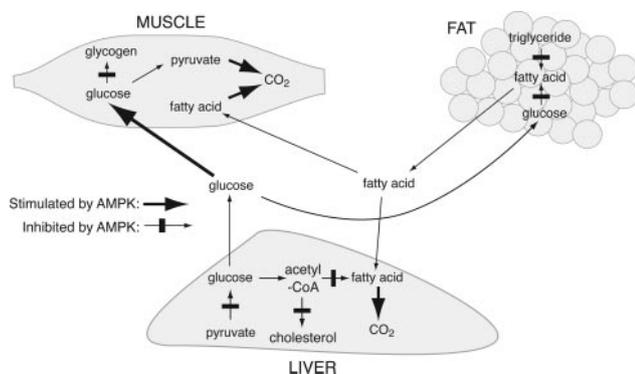
**TABLE 2. Metabolic Pathways Regulated by AMPK**

Metabolic Pathway	Effect	Immediate Target	Immediate Effects	Tissue	Reference
Glucose uptake	↑	AS160?	↑ GLUT4 translocation	Muscle	144, 151, 152
Glucose uptake	↑	?	↑ GLUT4 expression	Muscle	128, 153
Glucose uptake	↑	?	↑ GLUT1 activity	Many cells	154
Glycolysis	↑	Cardiac 6-phosphofructo-2-kinase	↑ Activity, ↑ fructose-2,6-bisphosphate	Cardiac myocytes	45, 155
Glycolysis	↑	Inducible 6-phosphofructo-2-kinase	↑ Activity, ↑ fructose-2,6-bisphosphate	Monocytes, macrophages	45, 155
Fatty acid oxidation	↑	ACC-2	↓ Activity, ↓ malonyl-CoA, ↑ CPT1 activity	Liver, muscle, others	144, 156
Fatty acid synthesis	↓	ACC-1	↓ Activity	All cells?	41, 157
Fatty acid synthesis	↓	*SREBP-1c?, HNF-4α?	↓ Expression ACC1, fatty acid synthase	Liver	72, 158–160
Cholesterol synthesis	↓	HMG-CoA reductase	↓ Activity	Liver	40, 161
Glycogen synthesis	↓	Muscle glycogen synthase	↓ Activity	Muscle	162, 163
Gluconeogenesis	↓	TORC2, others?	↓ Expression of PEPCK, glucose-6-phosphatase	Liver	108, 129
Protein synthesis	↓	Elongation factor-2 kinase*	↓ Translation elongation	All cells?	164
Protein synthesis	↓	TSC2	↓ TOR, ↓ S6K1, ↓ translation initiation	All cells?	85
Autophagy	↑	TSC2?	↓ TOR	All cells?	79
Glucose uptake	↓	GLUT4?	↓ Insulin stimulation	Adipocytes	165
Lipolysis	↓	Hormone-sensitive lipase (HSL)	↓ Adrenergic stimulation of lipolysis	Adipocytes	39, 130, 131

This is not a comprehensive summary of all literature in the field but a list of effects for which, in our opinion, there is reasonable evidence of a role for AMPK. AS160 indicates Akt substrate of 160 kDa; CPT1, carnitine palmitoyl-CoA transferase-1; PEPCK, phosphoenolpyruvate carboxykinase; S6K1, ribosomal protein S6 kinase-1. \*Although a change in function of these proteins are among the first known events downstream of AMPK activation, it has not been conclusively shown that they are direct targets of AMPK.

method was first demonstrated in a landmark study of HMG-CoA reductase phosphorylation published in 1993.<sup>40</sup>

Although there are very few pathways thought to be regulated by AMPK for which all of these criteria have been met, those with reasonably convincing evidence for an effect of AMPK are listed in Table 2. In general, AMPK switches on catabolic pathways that generate ATP (eg, glucose uptake, glycolysis) while switching off ATP-consuming anabolic pathways (fatty acid, cholesterol, glycogen, and protein synthesis). Figure 2 summarizes the major effects of AMPK activation on glucose and lipid metabolism in liver, muscle, and adipose tissue. As well as its acute and long-term effects on metabolism, for which it is best known, it is becoming



**Figure 2.** Major effects of AMPK activation on glucose and lipid metabolism in liver, muscle, and adipose tissue. Pathways stimulated by AMPK are shown with thick arrows, those inhibited by thin arrows with thick bars across them. Effects of AMPK on pyruvate oxidation are mediated by upregulation of mitochondrial biogenesis, whereas effects on fatty acid oxidation are mediated by both phosphorylation of ACC2 and activation of fatty acid entry into mitochondria, as well as upregulation of mitochondrial biogenesis.

increasingly clear that AMPK is involved in regulating other cellular processes. For example, if cells are running short of ATP, it does not make sense for them to grow and proliferate. AMPK inhibits cell growth and proliferation by inhibiting both the activity and expression of biosynthetic enzymes involved in lipid, carbohydrate, and protein synthesis, and also by switching off the target-of-rapamycin (TOR)→S6 kinase I pathway,<sup>82–84</sup> which appears to be crucial in the regulation of cell growth and cell size. AMPK appears to achieve this by phosphorylation of tuberous sclerosis complex-2 (TSC2) (tuberin), an upstream regulator of TOR.<sup>85</sup> AMPK activation also halts progress through the cell cycle at the G<sub>1</sub>→S phase transition, which is associated with accumulation of the tumor suppressor p53 and of the cyclin-dependent kinase inhibitors p21 and p27, which act downstream of p53.<sup>86–89</sup> Under these conditions, p53 becomes phosphorylated on Ser15,<sup>86,88</sup> although it is not clear that this is a direct phosphorylation. Another mechanism potentially contributing to cell cycle arrest is that AMPK activation reduces the cytoplasmic:nuclear ratio of the RNA-binding protein HuR, reducing its ability to stabilize mRNAs encoding critical cell cycle regulators, including cyclins.<sup>90</sup> AMPK activation also has complex effects on apoptosis. In some situations, it prevents the process,<sup>91–94</sup> whereas in others it induces it.<sup>95–100</sup> Successful apoptosis requires ATP, and an explanation for these conflicting findings may be that AMPK activation sometimes allows cells to recover from an energy crisis that would otherwise have triggered cell death. However, if the cells go past the “point of no return,” AMPK may become proapoptotic.

AMPK also has a role in cell senescence. Increased cellular AMP:ATP and AMPK activity appear to contribute to the senescence observed in human fibroblasts after a high num-

**TABLE 3. Protein Targets Directly Phosphorylated by AMPK**

Protein Target	Site	Effect on Protein Function	Pathway	Tissue	Effect on Pathway	Reference
<b>Lipid metabolism</b>						
ACC-1	S80	↓ Activity	Fatty acid synthesis	All cells?	↓ Fatty acid synthesis	166
ACC-2	S221	↓ Activity	Fatty acid oxidation	Muscle, liver	↑ Fatty acid oxidation	156
HMG-CoA reductase	S872	↓ Activity	Isoprenoid synthesis	Liver	↓ Cholesterol synthesis	161
Hormone-sensitive lipase	S554	↓ Activity	Lipolysis	Adipose tissue	↓ Lipolysis	131, 133
<b>Carbohydrate metabolism</b>						
Glycogen synthase	S8	↓ Activity	Glycogen synthesis	Muscle	↓ Glycogen synthesis	162, 163
<b>6-Phosphofructo-2-kinase</b>						
Cardiac isoform	S466	↑ Activity	Regulation of glycolysis	Heart	↑ Glycolysis	45
Inducible isoform	S461	↑ Activity	Regulation of glycolysis	Monocytes, macrophages	↑ Glycolysis	155
<b>Cell signaling</b>						
Endothelial NO synthase	S1177	↑ Activity	NO production	Endothelial cells	↑ NO, increased blood flow?	113
TSC2 (tuberin)	S1387	↑ Rheb-GAP	Regulation of TOR	All cells?	↓ Cell growth, protein synthesis	85
Insulin receptor substrate-1	S794	↑ PI3K binding	Insulin signaling	All cells?	↑ Insulin signaling?	167
AS160	?	↓ Rab-GAP?	GLUT4 trafficking	Muscle	↑ Glucose uptake	123
<b>Transcription</b>						
p300	S89	↓ Interaction	Gene expression	All cells?	↓ Transcription by nuclear receptors	168
HNF4- $\alpha$	S313	↓ DNA binding; ↑ degradation	Gene expression	Liver, others	↓ Transcription	107
ChREBP	S568	↓ DNA binding	Gene expression	Liver	↓ Transcription, L-pyruvate kinase gene	106
TORC2	S171	↑ Cytoplasmic localization	Gene expression	Liver	↓ Transcription, gluconeogenic genes	108
<b>Ion transport/ion balance</b>						
CFTR	?	↓ Channel opening	Ion transport, fluid secretion	Airway, gut, epithelium	↓ Ion transport	32

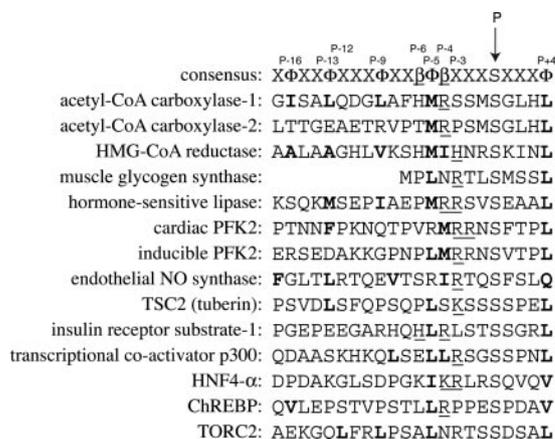
PI3K indicates phosphatidylinositol 3-kinase; CFTR, cystic fibrosis transmembrane conductance regulator.

ber of passages in culture.<sup>101</sup> Intriguingly, this is consistent with studies of life span in the nematode worm, *Caenorhabditis elegans*. Senescence in the worms is associated with increases in cellular AMP:ATP, and environmental stresses such as starvation or heat shock that increase cellular AMP:ATP, or mutations that inactivate genes involved in insulin signaling such as decay accelerating factor-2 (encoding the ortholog of the insulin/insulin-like growth factor-1 receptor), extend life span only in worms expressing AAK-2, which is 1 of the 2 catalytic subunits of AMPK in *C elegans*.<sup>102</sup> This raises the intriguing possibility that AMPK may also be implicated in the increase in life span induced by caloric restriction in mammals.

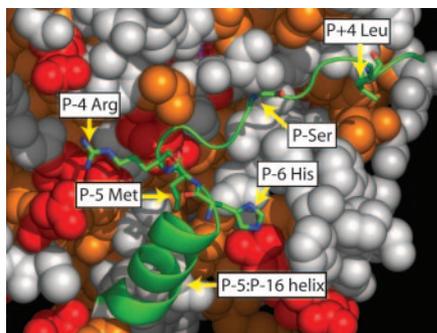
### Direct Downstream Targets for AMPK and Target Recognition

The number of direct physiological targets identified for AMPK is now approaching 20 (Table 3). However, given the critical importance of maintaining energy balance at both the cellular and whole body levels, it seems likely that this may eventually run into hundreds. How does AMPK recognize its downstream targets? Figure 3 shows an alignment of 14 sequences containing sites phosphorylated by AMPK in cell-free assays, all of which are also believed to be targets in vivo. In every case, it is a serine residue that is phosphorylated, although threonine can be phosphorylated by AMPK in synthetic peptide substrates.<sup>103</sup> In most cases, there are bulky hydrophobic residues (shown in bold) at positions P-5 and P+4 (ie, 5 residues N terminal and 4 residues C terminal to the phosphorylated serine), and at least 1 basic residue (underlined) at either P-4 or P-3. The importance of this motif ( $\Phi$ -[ $\beta$ ,X]-X-X-S/T-X-X-X- $\Phi$ , where  $\Phi$  is hydrophobic and  $\beta$

is basic) was initially confirmed using variant synthetic peptides substrates.<sup>103,104</sup> Understanding of substrate recognition was further extended by modeling the binding of the sequence around Ser79 on rat ACC1 (still probably the most efficient substrate) to a 3D model of the  $\alpha_1$  kinase domain, based on crystal structures of related protein kinases (Figure 4). This model was thoroughly tested by making complementary mutations of the substrate and the kinase.<sup>15</sup> In the model,



**Figure 3.** Alignment of the consensus recognition motif for AMPK and sequences around sites believed to be phosphorylated by AMPK on physiological substrates. Sequences are from the human, although in many cases the sites were identified with enzymes from other species. The serine residues phosphorylated are shown by the arrow labeled "P" at the top, and "P-x or P+x" refers to residues x residues N or C terminal to the phosphorylated serine. In the consensus recognition motif,  $\Phi$  refers to a hydrophobic residue (M, L, I, F, or V) and  $\beta$  to a basic residue (R, K, or H). Hydrophobic and basic residues believed to be important in recognition of the target sequences are in bold or underlined respectively.



**Figure 4.** View of a model of the polypeptide around Ser79 on acetyl-CoA carboxylase-1 (ACC1) phosphorylated by AMPK, bound to the  $\alpha_1$  kinase domain of AMPK.<sup>38</sup> The model was visualized and drawn using PyMOL version 0.97, with the kinase domain represented as spheres and hydrophobic residues in orange, acidic residues in red. The ACC1 peptide is shown in “cartoon” view with the side chains of selected amino acids in “stick” representation, with nitrogen atoms in blue, carbon in green and oxygen in red. Hydrogen atoms are omitted throughout. “P-x or P+x” refers to residues x residues N or C terminal to the phosphorylated serine.

the protein substrate binds in a long groove that runs from bottom left to top right in Figure 4, with the serine to be phosphorylated (P-Ser) toward the right-hand end. The  $\gamma$  phosphate of ATP (which is buried by the “glycine-rich loop” at top left in Figure 4) is well positioned to transfer its  $\gamma$  phosphate to this serine. The side chains of the critical hydrophobic residues at P-5 (Met) and P+4 (Leu) bind in hydrophobic pockets within this substrate-binding groove, whereas the critical P-4 basic residue (Arg) binds to an acidic patch (shown in red in Figure 4, on the left-hand side of the binding groove). In addition to the basic residue at P-4 or P-3, many physiological targets also have a basic residue at P-6 (Figure 3), and mutagenesis of this residue (His in ACC1) showed that it is an additional positive determinant. In our model, this P-6 basic side chain binds to another acidic patch located on the opposite side of the substrate-binding groove from the patch that binds the P-4 side chain (Figure 4).

As well as the crucial hydrophobic residue (Met) at P-5, ACC1 has additional hydrophobic side chains on the N-terminal side occurring every third or fourth residue, ie, at P-9, P-13, and P-16. This pattern of hydrophobic residues is also discernible in a few of the other substrates, including HMG-CoA reductase, endothelial NO synthase, TSC2, and TORC2 (transducer of regulated CREB-2) (Figure 3). In the case of HMG-CoA reductase, a crystal structure of the human protein has been determined<sup>105</sup> and the residues from P-12 to P-5 do indeed form an  $\alpha$  helix, with the hydrophobic residues pointing outward into the solvent. According to our model, these residues form an amphipathic  $\alpha$  helix (labeled “P-5:P-16 helix” in Figure 4), with the hydrophobic residues that line 1 face fitting into the substrate-binding groove. Although this  $\alpha$  helix almost certainly increases the affinity of binding between AMPK and the substrate, it is clearly not essential, because in muscle glycogen synthase, the leucine at the P-5 position is only the second amino acid in the whole protein, following cleavage of the N-terminal methionine (Figure 3). Finally, inspection of Figure 3 shows that 2 of the 13 substrates (cardiac PFK2 and endothelial NO synthase

[eNOS]) have the basic–hydrophobic–basic motif that usually occurs at the P-6, P-5, and P-4 or P-3 positions displaced by 1 residue, ie, at P-5, P-4, and P-3. It may be significant that in both these cases the 2 basic residues at P-5 and P-3 are arginine. The side chain of arginine can probably position its positively charged nitrogen atoms with more flexibility than either lysine or histidine. It is conceivable that this, coupled with the large size of the 2 acidic patches on the kinase domain that bind the basic side chains, provides sufficient flexibility to allow a different spacing of the basic residues from the phosphoacceptor. This flexibility means that predicting phosphorylation sites for AMPK from raw sequence data are not straightforward.

Many of the direct targets for AMPK identified in Table 3 and Figure 3 are transcription factors (hepatocyte nuclear factor-4 [HNF4- $\alpha$ ], ChREBP) or transcriptional coactivators (p300, TORC2), and it is now clear that mammalian AMPK has many effects on transcription, as has always been known to be the case for the yeast homolog. In most of the cases listed in Table 3, phosphorylation by AMPK inhibits transcription, either by inhibiting DNA binding (ChREBP,<sup>106</sup> HNF4- $\alpha$ <sup>107</sup>), triggering degradation (HNF4- $\alpha$ <sup>107</sup>), or promoting binding of 14-3-3 proteins, thus causing cytoplasmic sequestration (TORC2<sup>108</sup>). However, AMPK activation can also increase gene expression, particularly in skeletal muscle. In a microarray study in mice, transgenic expression of a dominant negative AMPK mutant in muscle reduced AMPK activity and caused increased expression of 234 genes and decreased expression of 130 genes.<sup>109</sup> AMPK activation has also been shown to downregulate expression of the transcription factor SREBP-1c (sterol response element binding protein-1c) in liver,<sup>72</sup> and upregulate expression of the transcription factor peroxisome proliferator-activated receptor- $\alpha$  (PPAR- $\alpha$ ) and the coactivator PPAR- $\gamma$  coactivator-1 $\alpha$  (PGC-1 $\alpha$ )<sup>110</sup> in muscle, although in these cases, this does not appear to be attributable to direct phosphorylation.

### Role of AMPK in the Vasculature

The role of AMPK in the heart is discussed in another article in this issue, so will not be discussed in detail here. In endothelial cells, AMPK activation has the same effects on metabolism as it does in other cells. In human umbilical vein endothelial cells, AMPK can be activated using AICAR or glucose deprivation, and when activated it, stimulates acetyl-CoA carboxylase phosphorylation and fatty acid oxidation.<sup>111,112</sup> One AMPK target that may be particularly important in the circulatory system is the endothelial isoform of NO synthase (eNOS). Phosphorylation of Ser1177 on eNOS increases the  $V_{max}$  of the enzyme and its sensitivity to  $Ca^{2+}$  and calmodulin.<sup>113</sup> Because the classical effect of NO is to relax the smooth muscle lining blood vessels, it is conceivable that this is a mechanism to increase blood flow to hypoxic tissues. Consistent with this, Salt and colleagues<sup>114</sup> have shown that activation of AMPK in human aorta endothelial cells causes increased phosphorylation of eNOS at Ser1177 and increased NO production. AMPK is also activated by hypoxia in human umbilical vein endothelial cells (HUVECs), and adenoviral expression of a dominant negative AMPK mutant in those cells blocked increased eNOS

phosphorylation at Ser1177 in response to hypoxia.<sup>115</sup> However, AMPK activation does not seem to account for eNOS phosphorylation in HUVECs treated with thrombin.<sup>57</sup>

AMPK may also be important in the process of angiogenesis. Mice with an embryonic knockout of the upstream kinase LKB1 die in mid-gestation with vascular abnormalities and abnormal expression of vascular endothelial cell growth factor.<sup>116</sup> In addition, adenoviral expression of a dominant negative AMPK mutant in HUVECs inhibited their migration toward vascular endothelial cell growth factor and inhibited angiogenesis using *in vitro* and *in vivo* assays.<sup>115</sup>

### Interactions Between the AMPK and Insulin/Insulin-Like Growth Factor-1 Signaling Pathways

The insulin/insulin-like growth factor-1 signaling pathway is activated when nutrients are available, whereas the AMPK pathway is activated when cells are starved for a carbon source. One would therefore expect these 2 pathways to oppose each other, and this is often the case. In mammals, insulin promotes lipid, protein, and glycogen synthesis, whereas AMPK inhibits these biosynthetic pathways. The effect of insulin on protein synthesis is mediated in part by activation of the TOR pathway via phosphorylation of TSC2, whereas activation of AMPK causes phosphorylation of different sites on TSC2 and inhibits TOR.<sup>85,117</sup> There also appears to be direct crosstalk between the 2 pathways. In some tissues, such as cardiac muscle, insulin antagonizes activation of AMPK,<sup>118,119</sup> and this appears to involve activation of the protein kinase Akt (also termed protein kinase B [PKB]).<sup>120</sup> It has recently been proposed that this is attributable to phosphorylation by Akt of the AMPK  $\alpha$  subunits on Ser485 or Ser491 (equivalent residues on the  $\alpha_1$  or  $\alpha_2$  isoforms), which antagonizes AMPK activation via phosphorylation at Thr172 by LKB1.<sup>121</sup> Whether this mechanism operates in other tissues remains unclear.

In other cases, the insulin and AMPK signaling pathways work in the same direction, particularly in processes that regulate plasma glucose levels. In skeletal muscle, both insulin and AMPK activation stimulate glucose uptake by increased translocation of GLUT4 to the plasma membrane, although the subsequent fate of the glucose is different (glycogen synthesis in the case of insulin, which is anabolic; glycolysis/oxidation in the case of AMPK, which is catabolic). The 2 pathways appear to converge on the phosphorylation of AS160,<sup>122,123</sup> a protein with a Rab-GTPase-activating protein (Rab-GAP) domain that is involved in regulation of GLUT4 translocation. Activation of AMPK plays a major part in the well-known ability of muscle contraction to stimulate glucose uptake, because in mice overexpressing a dominant negative AMPK mutant,<sup>80</sup> and in a skeletal muscle-specific knockout of the upstream kinase LKB1,<sup>49</sup> the effects of contraction on glucose uptake are reduced. There is also evidence that AMPK activation may be involved in the ability of a single bout of acute exercise to increase the insulin sensitivity of glucose uptake.<sup>124</sup> The mechanism for this effect remains unclear, although a possibility is that it is attributable to the ability of AMPK to inhibit the TOR pathway because the latter, which is activated by

insulin, is believed to exert a feedback regulation on insulin signaling by downregulating IRS1.<sup>125–127</sup> AMPK activation may also be involved in the effects of repeated exercise (ie, training) to improve insulin-sensitive glucose uptake, because of its ability to increase expression of GLUT4<sup>128</sup> and perhaps other effects.

A second case in which insulin and AMPK act in the same direction occurs in the liver, in which both repress the expression of enzymes of gluconeogenesis, such as phosphoenolpyruvate carboxykinase and glucose-6-phosphatase.<sup>129</sup> It makes obvious sense that insulin, a hormone released in response to high blood glucose, should repress hepatic glucose production, whereas in the case of AMPK it may perhaps have evolved as among its antianabolic actions. Gene repression by AMPK is thought to occur via phosphorylation and consequent cytoplasmic sequestration of the transcriptional coactivator TORC2.<sup>108</sup> Repression of gluconeogenesis appears to be the major cause of the plasma glucose-lowering effects of the adipokine, adiponectin, and the antidiabetic drug metformin on plasma glucose levels, and in both cases, there is evidence that the mechanism involves activation of AMPK.<sup>67,73</sup>

A final case in which insulin and AMPK act in the same direction occurs in adipocytes, where both suppress activation of hormone-sensitive lipase, and hence lipolysis, by cAMP-elevating agonists.<sup>39,130,131</sup> The 2 pathways act by different mechanisms, with insulin causing phosphorylation and activation of phosphodiesterase 3B by Akt, thus lowering cAMP,<sup>132</sup> whereas AMPK phosphorylates hormone-sensitive lipase at a site (Ser565) that antagonizes activation by cAMP-dependent protein kinase.<sup>133</sup> In the case of insulin, this can be regarded as among its anticatabolic actions, but the reason why AMPK should suppress lipolysis requires more explanation. If fatty acids released by lipolysis are not removed from the cell rapidly enough, they are known to recycle into triglyceride, thus consuming ATP.<sup>134</sup> Inhibition of lipolysis by AMPK has been proposed<sup>135</sup> as a mechanism to limit this recycling, ensuring that the rate of lipolysis does not exceed the rate at which fatty acids can be removed or metabolized by other routes, such as fatty acid oxidation.

The parallel effects of insulin and AMPK on muscle glucose uptake and hepatic glucose production formed part of the basis for the idea first proposed in 1999<sup>136</sup> that activators of AMPK could be used to treat type 2 diabetes and perhaps obesity. This was initially tested successfully using AICA riboside in animal models such as genetically obese or fat-fed rodents,<sup>137–140</sup> and findings that activation of AMPK underlies the glucose-lowering effects of metformin<sup>72,73</sup> provided a “proof-of-concept” for the idea in humans. Intriguingly, in at least 3 animal models that are resistant to diet-induced obesity, ie, mice overexpressing the uncoupling proteins UCP1<sup>141</sup> or UCP3,<sup>142</sup> or mice with a knockout of stearoyl-CoA desaturase-1,<sup>143</sup> there appears to be a persistent activation of AMPK in the affected cell types.

The ability of AMPK to inhibit adipocyte lipolysis and to stimulate fatty acid oxidation in many tissues, both acutely via phosphorylation of ACC2<sup>144</sup> and chronically by upregulating PGC-1 $\alpha$  and mitochondrial biogenesis,<sup>110,145</sup> is also likely to be beneficial in insulin resistance and type 2

diabetes. There is much evidence that these conditions are partly caused by abnormal accumulation of triglyceride in muscle and liver and that this can be at least partly caused by a defect in mitochondrial function.<sup>146</sup> Interestingly, incubation of cultured cells with high levels of the saturated fatty acid palmitate affected the integrity of the endoplasmic reticulum and led to cell death, and this was ameliorated using AICAR to activate AMPK and stimulate fatty acid oxidation.<sup>147</sup> One caveat is that it has also been proposed that high rates of fatty acid oxidation can be damaging because of oxidative stress, for example, in ischemic heart following reperfusion<sup>44</sup> and in endothelial cells.<sup>148</sup> It will be important to establish whether any adverse effects of AMPK activation in certain cell types are outweighed by the overall improvements in metabolic status.

### Conclusions and Perspectives

Based on the known effects of AMPK on carbohydrate and lipid metabolism, it is already clear that the system is a major player in the development and/or treatment of obesity, diabetes, and the metabolic syndrome, which have reached epidemic proportions in modern industrialized society. AMPK is probably involved in the beneficial effects of regular exercise on these conditions and now appears to mediate many of the effects of the adipokines leptin and adiponectin, as well as the antidiabetic drug metformin. AMPK also appears to be involved in cancer because of the ability of the LKB1→AMPK pathway to act as a tumor suppressor, together with potential roles of this pathway in angiogenesis, apoptosis, and senescence of tumors. There are also now indications the AMPK system is involved in the effects of caloric restriction on life span. All of these represent fruitful areas for further investigation.

### Sources of Funding

Supported by a Programme Grant from the Wellcome Trust and the EXGENESIS Integrated project (LSHM-CT-2004 to 005272) from the European Commission.

### Disclosures

None.

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JOURNAL OF THE AMERICAN HEART ASSOCIATION



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*Circ Res.* 2007;100:328-341

doi: 10.1161/01.RES.0000256090.42690.05

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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