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AMPK - a nutrient and energy sensor that maintains energy homeostasis

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**Preface**

AMP-activated protein kinase (AMPK) is a crucial cellular energy sensor. Once activated by falling energy status, it promotes ATP production by increasing the activity or expression of proteins involved in catabolism, while conserving ATP by switching off biosynthetic pathways. AMPK also regulates metabolic energy balance at the whole body level. For example, it mediates the effects of agents acting on the hypothalamus that promote feeding, and entrains circadian rhythms of metabolism and feeding behavior. Finally, recent studies reveal that AMPK conserves ATP levels through the regulation of processes other than metabolism, such regulation of the cell cycle and neuronal membrane excitability.
**Introduction**

Just like a mobile electronic device, every living cell contains a “rechargeable battery” formed by pairs of interconvertible chemicals. The key chemicals within the cell are ATP and ADP, interconverted by the reaction $\text{ATP} \leftrightarrow \text{ADP} + \text{phosphate}$. This reaction is maintained by catabolism many orders of magnitude away from equilibrium, yielding a high ratio of ATP to ADP that is used to drive energy-requiring processes. In animal cells ATP is mainly generated via the mitochondrial ATP synthase, thus “charging the battery”. Almost every other function that cells perform requires energy, and most are driven by the hydrolysis of ATP back to ADP and phosphate, thus “discharging the battery”. Clearly, ATP generation needs to remain in balance with ATP consumption, and regulatory proteins that sense ATP and ADP levels would be a logical way to achieve this. However, all eukaryotic cells express high levels of adenylate kinase, and the reversible reaction it catalyzes ($2\text{ADP} \leftrightarrow \text{ATP} + \text{AMP}$) is usually maintained close to equilibrium. This means that any rise in the ADP:ATP ratio, which signifies falling energy status, causes the adenylate kinase reaction to be displaced towards ATP and AMP production. Thus, falling cellular energy is associated with increases not only in ADP, but also AMP. The relative increase in concentration is always much greater for AMP than ADP, although its absolute concentration remains lower than that of ADP unless energy stress becomes quite severe (Fig. 1). Thus, it seems logical that proteins sensing cellular energy status should monitor either the ADP:ATP or AMP:ATP ratio, or both. A small number of metabolic enzymes do directly sense the AMP:ATP ratio, including the muscle isoforms of glycogen phosphorylase [G] and phosphofructokinase [G] (which are involved in glycogen breakdown and glycolysis, and are activated by an increasing AMP:ATP ratio), and fructose-1,6-bisphosphatase (which is involved in gluconeogenesis, and is inhibited by an increasing AMP:ATP ratio). However, the principal energy sensor in most eukaryotic cells appears to be the AMP-activated protein kinase (AMPK)$^1$. In support of this, increases in AMP:ATP and ADP:ATP ratios during stresses such as muscle contraction$^2$, ischemia in cardiac muscle$^3$, or treatment of hepatocytes with metformin$^4$ are larger in cells or tissues in which AMPK, or its essential activating upstream kinase liver kinase B1 (LKB1), have been knocked out. In this Review article, we describe how AMPK monitors cellular energy status by sensing increases in the ratios of AMP:ATP and
ADP:ATP, as well as other signals. Moreover, we examine how it regulates energy balance at the cellular level, by activating catabolic pathways that generate ATP, while conserving ATP by down-regulating anabolic pathways. Finally, we discuss results showing that AMPK also regulates metabolism and energy balance at the whole body level, especially via effects on the hypothalamus, as well as recent findings suggesting that AMPK conserves energy by regulating non-metabolic processes, such as the cell cycle and neuronal membrane excitability.

**AMPK – subunits and regulation**

Genes encoding AMPK subunits are found in essentially all eukaryotes. AMPK and its orthologs appear to exist universally as heterotrimeric complexes comprising a catalytic $\alpha$ subunit and regulatory $\beta$ and $\gamma$ subunits, the domain organization of which are summarized in Fig. 2. The kinase activity of the $\alpha\beta\gamma$ complexes, in animals from nematodes to humans, is instantaneously increased by AMP by as much as 10-fold, although due to technical limitations with the assay the effect observed is usually smaller. It has been argued that this allosteric activation by AMP (which gave the kinase its name) may not be relevant under physiological conditions because of competition at the allosteric site by ADP and ATP. However, as shown in Fig. 1, an increase in the ADP:ATP ratio of only 10-fold is sufficient for the cellular concentrations of the three adenine nucleotides to become equal. Although this would represent quite a severe stress and might only occur in pathological states (such as ischemia) rather than under more physiological conditions (such as exercise), the ATP:ADP ratio would still be still many orders of magnitude away from equilibrium and could still drive energy-requiring processes.

AMPK catalytic subunits contain a conventional serine/threonine kinase domain at the N-terminus. In all species, the activity of the complex increases more than 100-fold when a conserved threonine residue in the activation loop (which is conventionally referred to as Thr172 due to its position in the original rat sequence) is phosphorylated by upstream kinases. In mammals the major upstream kinases are the LKB1:STRAD:MO25 complex (originally identified genetically as a tumor suppressor) and the Ca$^{2+}$/calmodulin-activated protein kinases kinases, especially CaMKKβ.

The LKB1 complex provides a high basal level of phosphorylation at Thr-172 that is modulated by the binding of AMP to the AMPK-$\gamma$ subunit, which promotes phosphorylation and inhibits
dephosphorylation\textsuperscript{14, 15}. Although allosteric activation is only caused by AMP, it has recently been found that the effects on phosphorylation and dephosphorylation can also be produced by ADP\textsuperscript{16, 17}. The effects of AMP and ADP on phosphorylation require \textit{N-terminal myristylation} [G] of the \(\beta\) subunit\textsuperscript{17, 18}. Since AMP and ADP bind the \(\gamma\) subunits of AMPK with similar affinity to ATP\textsuperscript{16} (which does not cause activation), and ADP is usually present in cells at higher concentrations than AMP (Fig. 1), ADP may be the key activating signal that promotes net Thr-172 phosphorylation during moderate energy stress. However, allosteric activation by AMP would further amplify activation during a more severe stress. This complex mechanism (Fig. 1) allows the system to provide a graded response of AMPK activity over a wide range of stress levels.

The alternative activating pathway, involving CaMKK\(\beta\), triggers activation of AMPK in response to increases in cell \(\text{Ca}^{2+}\) without necessarily requiring any change in AMP or ADP levels. In tumor cells that have lost the tumor suppressor LKB1 due to somatic mutations, treatments that increase AMP and ADP levels do not normally activate AMPK\textsuperscript{8}, because basal CaMKK\(\beta\) activity is too low for the effects of nucleotide binding on phosphorylation status to become evident. However, these treatments can cause AMPK activation in such cells if intracellular \(\text{Ca}^{2+}\) is also elevated\textsuperscript{19}. This emphasizes that the effects of AMP and/or ADP on Thr172 phosphorylation status are a result of their binding to AMPK, and are independent of the upstream kinases and phosphatases that phosphorylate or dephosphorylate Thr172.

\textit{AMPK - structure}

Although there is not yet a structure for a complete AMPK heterotrimer, the structures of various combinations of domains have been determined by X-ray crystallography. At the N-terminus of the \(\alpha\) subunit is a conventional kinase domain immediately followed by an auto-inhibitory domain (AID), so-called because constructs containing the kinase domain plus the AID are much less active than those containing the kinase domain alone\textsuperscript{20, 21}. The AID is followed by an extended “linker peptide” that connects the AID to the C-terminal domain (\(\alpha\)-CTD). A recent structure\textsuperscript{16} showed that this linker (colored red in Figs. 2 and 3) wraps around the \(\gamma\) subunit as if holding it in a tight embrace.
The β subunits contain a carbohydrate-binding module or CBM (absent in the constructs used to generate the structure in Fig. 3), which causes mammalian AMPK to associate with glycogen particles\(^\text{22, 23}\). The functional significance of this remains uncertain, although it may serve to co-localize AMPK with downstream targets located in the glycogen particle, such as glycogen synthase. The β subunit C-terminal domain (β-CTD) interacts with both the α-CTD and the γ subunit, thus forming the core of the complex. The γ subunits contain four tandem repeats, numbered CBS1 through CBS4 in Figs. 2 and 3, of a sequence motif termed a CBS repeat [G]\(^\text{24}\). These occur in a small number of other proteins (including cystathionine β-synthase (CBS)), usually as just two repeats that assemble to form a Bateman domain [G]\(^\text{25}\), with ligand-binding sites in the cleft between the repeats\(^\text{26}\). Most Bateman domains bind adenosine-containing ligands, usually ATP but in one case S-adenosyl methionine\(^\text{27}\), and mutations in them are associated with several human diseases, including a heart disease caused by mutations affecting the AMPK-γ2 subunit\(^\text{26, 27}\). In AMPK the four CBS repeats in the γ subunits form a flattened disk with one repeat in each quadrant (seen from two different sides in Fig. 3), containing four potential ligand-binding sites in the centre. These sites are numbered 1-4 according to the number of the repeat carrying a conserved aspartate residue involved in ligand binding\(^\text{6}\), and have variable occupancies in the crystal structures of partial complexes from mammals and fungi\(^\text{16, 28-30}\). In the mammalian γ1 subunit, site 2 appears to be always empty, site 4 to have a tightly-bound AMP, whereas sites 1 and 3 represent the regulatory sites that bind AMP, ADP or ATP in competition. AMP binding to site 1 appears to cause allosteric activation, while binding of AMP or ADP to site 3 appears to modulate the phosphorylation state of Thr-172\(^\text{16}\). A model\(^\text{16}\) that can explain how binding of AMP or ADP, but not ATP, protects Thr-172 against dephosphorylation has been discussed previously\(^\text{31}\).

One long-standing puzzle has been the identity of the signals that activate AMPK orthologs in fungi and plants, which are not allosterically activated by AMP\(^\text{32, 33}\). Under conditions where the \textit{S. cerevisiae} SNF1 complex is activated, such as during glucose starvation, there are very large increases in AMP:ATP and ADP:ATP ratios\(^\text{32}\). The γ subunits in fungi and plants contain four CBS repeats as in mammals, and crystal structures of partial complexes from \textit{S. pombe} have been solved with AMP, ADP and/or ATP bound at different sites\(^\text{30, 34}\). These observations support the idea that the activating signal is an adenine nucleotide in fungi as in mammals, and it was recently reported that ADP inhibits
dephosphorylation and inactivation of the $S. \textit{cerevisiae}$ complex\textsuperscript{35}, with AMP having a smaller effect\textsuperscript{36}. AMP had already been shown to inhibit dephosphorylation of a plant SnRK1 complex\textsuperscript{37}. These results suggest that ADP and/or AMP may be the elusive signals that activate the fungal and plant enzymes, but that they work only via effects on the phosphorylation state of the kinase, and not via the additional allosteric mechanism.

**Regulation of AMPK in intact cells**

In mammalian cells, AMPK is activated by many different types of metabolic stress, drugs and xenobiotics by the mechanisms described above, involving increases in cellular AMP, ADP or Ca\textsuperscript{2+}. These can now be regarded as the classical or “canonical” AMPK activation mechanisms. However, recent work suggests that other stimuli activate AMPK via mechanisms that do not involve changes in the levels of AMP, ADP and Ca\textsuperscript{2+}, which can therefore be termed “non-canonical” mechanisms. These distinct types of mechanism are addressed below.

**Activation by metabolic stresses, drugs and xenobiotics**

The canonical mechanisms of activation of mammalian AMPK, which involve increases in AMP and ADP (Fig. 1) explain why AMPK is activated by stresses that inhibit the catabolic production of ATP, such as starvation for glucose\textsuperscript{38} or oxygen\textsuperscript{39} or addition of a metabolic poison\textsuperscript{40}, as well as by stresses that increase ATP consumption such as muscle contraction\textsuperscript{41}. AMPK is also switched on by numerous drugs and xenobiotics, including anti-diabetic drugs (such as metformin, phenformin and thiazolidinediones\textsuperscript{42, 43}), plant products reputed to have health-promoting properties (resveratrol from grapes and red wine\textsuperscript{44}, epigallocatechin gallate from green tea, capsaicin from peppers\textsuperscript{45}, curcumin from turmeric\textsuperscript{46}, and even garlic\textsuperscript{47}), and plant products used in traditional Chinese medicine (berberine\textsuperscript{48} and hispidulin\textsuperscript{49}). Metformin, which is now prescribed to more than 100 million people with type 2 diabetes worldwide, was derived from the natural product galegine extracted from $Galega \textit{officinalis}$, a plant reputedly used to treat diabetes-like conditions in medieval Europe. Although metformin activates AMPK, this may not explain all of the therapeutic effects of the drug. In fact, AMPK-$\alpha$\textsubscript{1} knockout mice that also carry a liver-specific deletion of AMPK-$\alpha$\textsubscript{2} display a normal hypoglycemic response to metformin, and the acute effects of metformin on glucose production in
isolated hepatocytes are also preserved\textsuperscript{4}. The latter effect may be explained by metformin causing an increase in AMP that directly inhibits the gluconeogenic enzyme fructose-1,6-bisphosphatase. One caveat in the interpretation of these experiments is that the fall in ATP (and hence the increase in AMP) caused by metformin was larger in cells lacking AMPK than in wild type cells\textsuperscript{4}, suggesting that the inhibition of fructose-1,6-bisphosphatase by AMP may be accentuated in the absence of AMPK. Thus, although not completely ruling out a role for AMPK in metformin action, these results do indicate that other targets, such as fructose-1,6-bisphosphatase, are also important.

It has been suggested that metformin activates AMPK in L6 cells by inhibiting AMP deaminase (the enzyme that breaks down AMP) thus causing AMP to accumulate\textsuperscript{50}. While this is an interesting proposal, the concentration of metformin used to inhibit AMP deaminase (10 mM) was very high. The same study also reported that the effect of metformin to activate AMPK was not reduced by siRNA knockdown of adenylate kinase. However, only the “cytosolic” AK1 isoform was knocked down, and not the mitochondrial isoform, AK2, which might be the more relevant isoform when studying effects of a mitochondrial inhibitor. Also, we now know that increased phosphorylation of AMPK can be triggered by an increase in ADP alone, obviating any requirement for adenylate kinase to generate AMP.

Intriguingly, some of the AMPK activators described above, including resveratrol and metformin, extend healthy lifespan in \textit{C. elegans}, and genetic studies show that the AMPK ortholog is required for these effects, as well as for the life-extending effects of dietary restriction\textsuperscript{51, 52}. In both \textit{C. elegans} and mammalian cells, AMPK up-regulates genes involved in oxidative metabolism and oxidative stress resistance by regulating transcription factors of the DAF-16/FOXO family\textsuperscript{53, 54}. This might contribute to its effects on healthy lifespan.

An important question is how these drugs and xenobiotics all manage to activate AMPK, despite the fact that their structures are so varied. Most modulate AMPK in intact cells but not in cell-free assays, suggesting that they activate AMPK indirectly. Using a cell line that expresses an AMP- and ADP-insensitive AMPK mutant, it has been shown that many of them, including metformin and resveratrol, activate AMPK indirectly by increasing cellular AMP and ADP, usually by inhibiting mitochondrial ATP synthesis\textsuperscript{55}. Many of these natural products appear to be defensive compounds produced by plants
to deter infection by pathogens, or grazing by insect or mammalian herbivores. Consistent with this, resveratrol is produced in grapes in response to fungal infection\textsuperscript{56}, while \textit{Galega officinalis} is classed as a noxious weed in the USA because it is poisonous to herbivores. Since the mitochondrial respiratory chain and ATP synthase contain several large multiprotein complexes, they might have many potential binding sites for hydrophobic xenobiotics that might inhibit their function. Production of mitochondrial poisons would be a useful general strategy for plants to deter pathogens or herbivores, with the side effect that plants would provide a rich source of AMPK activators. One could argue that, by inhibiting mitochondrial ATP synthesis, these xenobiotics are acting in animals as mimetics of dietary restriction and/or exercise, both of which can decrease cellular energy status and have favorable effects on healthy lifespan.

**Activation by oxidative stress and genotoxic treatments**

There are increasing indications that some types of cellular stress activate AMPK by “non-canonical” mechanisms that may not involve increases in AMP, ADP or Ca\textsuperscript{2+} levels. In cultured cells, AMPK is activated by reactive oxygen species (ROS) such as H\textsubscript{2}O\textsubscript{2}. At high ROS concentrations, AMPK activation may be secondary to the inhibition of mitochondrial ATP synthesis, with consequent rises in AMP and ADP levels\textsuperscript{55}. However, it has been suggested that there is also a more direct mechanism involving oxidation or glutathionylation \textsuperscript{[G]} of two conserved cysteine residues in the AMPK-\(\alpha\) subunit\textsuperscript{57}. It has also been suggested that H\textsubscript{2}O\textsubscript{2} can activate AMPK via a third mechanism involving a cytoplasmic form of the PI-3 kinase-like kinase, Ataxia-Telangiectasia Mutated (ATM)\textsuperscript{58}, the product of the gene mutated in human \textit{ataxia-telangiectasia} \textsuperscript{[G]}. Nuclear ATM is activated by double strand breaks in DNA and is part of a key DNA damage-sensing pathway. However, it has recently become clear that there is also a cytoplasmic pool of ATM that has a role in the response to oxidative stress\textsuperscript{59}. AMPK activation by low concentrations of H\textsubscript{2}O\textsubscript{2} is reduced in fibroblasts from ataxia-telangiectasia patients or mouse embryo fibroblasts lacking ATM. ATM-dependent activation of AMPK by oxidative stress seems to require LKB1, since it is attenuated in cells lacking this upstream kinase\textsuperscript{58}. Interestingly, ATM can phosphorylate LKB1, although it is not clear that this has any effect on LKB1 activity\textsuperscript{60}. 
Another class of factors that activate AMPK are genotoxic, DNA-damaging treatments such as etoposide\textsuperscript{61}, doxorubicin\textsuperscript{62} and ionizing radiation\textsuperscript{63}, which activate AMPK initially in the nucleus\textsuperscript{63}. There is evidence that these effects also require ATM but, surprisingly, they do not require LKB1 because the pathway is functional in LKB1-null cells\textsuperscript{61,63}. The detailed mechanism for this effect remains unclear. Finally, a recent genome-wide association study identified a strong association between single nucleotide polymorphisms that mapped to the \textit{Atm} gene on chromosome 11 and enhanced hypoglycemic response to metformin treatment in humans\textsuperscript{64}. Although the molecular explanation for this association remains unclear, it provides another tantalizing link between metformin, ATM and AMPK.

To summarize this section, AMPK is activated in intact cells both by “canonical” pathways involving increases in AMP, ADP or Ca\textsuperscript{2+}, and by “non-canonical” pathways such as those triggered by ROS and DNA damaging agents. The latter may involve the \textit{PI}-3-like kinase ATM, although the detailed mechanisms involved remain unclear.

\textbf{Regulation of cellular energy metabolism}

AMPK and its orthologs phosphorylate downstream targets at serine/threonine residues within a characteristic sequence motif, which has hydrophobic residues at the -5 and +4 positions and basic residues at -4 and -3, or both\textsuperscript{65,66}. Another basic residue at -6 is an additional positive determinant, while the best substrates (such as acetyl-CoA carboxylase-1/-\(\alpha\)) have additional hydrophobic residues forming an amphipathic helix N-terminal to the -5 position\textsuperscript{67}. The principal effects of AMPK activation on cell metabolism are summarized in Fig. 4. Consistent with a role in maintaining energy homeostasis, when AMPK is activated by energetic stress it switches on catabolic pathways that generate ATP, while switching off biosynthetic pathways that consume ATP.

\textbf{Regulation of glucose uptake by the glucose transporter GLUT4}

Catabolic events mediated by AMPK include enhanced glucose uptake during muscle contraction, when the major metabolic fate of the glucose is catabolism to generate ATP. Muscle glucose uptake is also promoted by the hormone insulin, although this mainly occurs in resting muscle when the major metabolic fate of the glucose is glycogen synthesis, an anabolic process. In both cases, enhanced
glucose uptake is mediated through translocation of the glucose transporter GLUT4 from intracellular storage vesicles to the plasma membrane. Fusion of these vesicles with the plasma membrane requires members of the Rab family of G proteins to be in their GTP-bound state. Under basal conditions Rabs are held in an inactive GDP-bound state by Rab-GAPs such as AS160 (also known as TBC1D4) and TBC1D1, which are associated with the GLUT4 storage vesicles. The insulin-activated kinase Akt phosphorylates AS160 in muscle and adipocytes, triggering its association with 14-3-3 proteins and its consequent dissociation from the vesicles. Similarly, AMPK phosphorylates TBC1D1 in contracting muscle, with similar effects. In either case, dissociation of these Rab-GAPs triggers the conversion of Rabs to their active, GTP forms and the consequent fusion of the vesicles, carrying their GLUT4 cargo, with the plasma membrane. Consistent with this model, mice with a knock-in mutation (T649A) at a key Akt phosphorylation site in AS160 show impaired glucose disposal in vivo and impaired insulin-stimulated glucose uptake with isolated muscle. It would be very interesting to perform similar experiments with the AMPK site on TBC1D1, to confirm the mechanism by which AMPK regulates glucose uptake in contracting muscle.

Whether AMPK, working via phosphorylation of TBC1D1 and perhaps other targets, entirely accounts for the acute effects of contraction on muscle glucose transport has been a matter of some controversy. In mouse knockouts of AMPK-α2 (the major catalytic subunit isoform in muscle), the stimulatory effects of the AMPK activator 5-aminoimidazole-4-carboxamide riboside (AICAR) on glucose uptake were lost, but the effects of contraction were normal. In AMPK-α1 knockout mice, both responses were normal. By contrast, in mice with muscles lacking the upstream kinase LKB1 (in which activation of both AMPK-α1 and AMPK-α2 complexes by contraction was abolished), the effects of both AICAR and contraction on glucose uptake were lost. One explanation for these discrepancies is that AMPK-α1 may be able to compensate for AMPK-α2 when the latter is absent. Recent results with muscle-specific AMPK-β1−/− AMPK-β2−/− double knockout mice, which lack any detectable AMPK activity, have supported the idea that AMPK activation is crucial during muscle contraction. The running speed and endurance of these mice was dramatically reduced, and they exhibited blunted muscle glucose uptake in response to treadmill exercise, and markedly impaired contraction-stimulated glucose uptake in isolated muscles. These results are consistent with the idea
that AMPK represents the primary signalling pathway responsible for contraction-induced glucose uptake, although TBC1D1 may not be the only downstream target that mediates this effect.

**Regulation of other catabolic pathways**

AMPK also promotes glucose uptake into cells expressing only GLUT1 (which includes most cells other than those in muscle, liver and adipose tissue), via a mechanism that involves activation of GLUT1 that is already located at the plasma membrane76. Moreover, it promotes fatty acid uptake into cardiac myocytes via translocation of vesicles containing the fatty acid transporter CD36 to the plasma membrane77. The direct targets for AMPK that mediate these effects remain unknown. Once they have entered the cell, AMPK can promote the catabolism of glucose via glycolysis, and of fatty acids by enhancing their uptake into mitochondria and their consequent breakdown by the β-oxidation pathway. Activation of glycolysis occurs via phosphorylation of 6-phosphofructo-2-kinase (PFK2), which catalyzes the generation of fructose-2,6-bisphosphate, a key allosteric activator of the glycolytic enzyme 6-phosphofructo-1-kinase. However, this only occurs in cells expressing the PFKFB2 isoform of PFK2, such as cardiac myocytes39, or the PFKFB3 isoform, such as monocytes and macrophages78. Uptake of fatty acids into mitochondria, which appears to be the rate-limiting step in β-oxidation, is promoted by AMPK via phosphorylation and inactivation of the ACC2/ACCβ isoform of acetyl-CoA carboxylase. This results in a drop in concentration of the acetyl-CoA carboxylase product, malonyl-CoA, an inhibitor of fatty acid entry into mitochondria mediated by the carnitine-palmitoyl-CoA transferase-1 (CPT1) system79.

**Regulation of mitochondrial biogenesis and mitophagy**

Another critical process activated by AMPK is mitochondrial biogenesis (Fig. 4), which in the longer term generates increased capacity for the oxidative catabolism of both glucose and fatty acids. Repeated daily dosing of rats with AICAR results in increased expression of mitochondrial genes in muscle80 as well as increased exercise endurance81 (the latter finding prompted the World Anti-Doping Agency to ban the use of AICAR in competitive sports). The AMPK-β1−/−AMPK-β2−/− double knockout mice mentioned above, which lack any AMPK activity in muscle, also had greatly reduced muscle mitochondrial content75. The “master regulator” of mitochondrial biogenesis is PGC-1α, a co-
activator that enhances the activity of several transcription factors acting on nuclear-encoded mitochondrial genes. AMPK directly phosphorylates PGC-1α, which has been proposed to cause activation of its own transcription via a positive feedback loop. An alternative mechanism by which AMPK has been proposed to activate PGC-1α is through promotion of its deacetylation, by increasing the concentration of NAD, the co-substrate for the deacetylase SIRT1.

As well as increasing mitochondrial biogenesis, AMPK is involved in the turnover of mitochondria via the special form of autophagy termed mitophagy (Fig. 4). ULK1 and ULK2, the mammalian orthologs of the yeast Atg1 kinase that initiates the autophagy cascade, form stable complexes with AMPK, and AMPK phosphorylates and activates ULK1, thus triggering autophagy. In cells in which endogenous ULK1 was replaced by a kinase-inactive mutant, or by a mutant in which the AMPK phosphorylation sites were substituted by alanine, mitochondria with aberrant morphology and reduced membrane potential accumulated following nutrient starvation. This supports the idea that phosphorylation of ULK1 by AMPK is required for the clearance of dysfunctional mitochondria.

Mitochondria are the main site of production of reactive oxygen species in the cell, and are particularly susceptible to oxidative damage. By recycling components of damaged mitochondria, mitophagy may be as important in maintaining a healthy cellular ATP-generating capacity as is the production of new mitochondria.

**Regulation of anabolic pathways**

Consistent with its role in cellular energy homeostasis, AMPK also conserves ATP by switching off almost all anabolic pathways, including the biosynthesis of lipids, carbohydrates, proteins and ribosomal RNA. It achieves this in part by phosphorylating and/or regulating enzymes or regulatory proteins directly involved in these pathways, including acetyl-CoA carboxylase (ACC1/ACCα isoform, involved in fatty acid synthesis), glycerol phosphate acyl-transferase (triglyceride/phospholipid synthesis), HMG-CoA reductase (cholesterol synthesis), glycogen synthase (glycogen synthesis), TSC2 and RAPTOR (regulators of the target-of-rapamycin (TOR) kinase, which promotes protein synthesis) and TIF-IA/RRN3 (a transcription factor for RNA polymerase I, responsible for ribosomal RNA synthesis). In many cases, AMPK also down-regulates expression of the proteins involved in these pathways, including ACC1/ACCα and other lipogenic
enzymes (probably via phosphorylation of the lipogenic transcription factor SREBP-1C\textsuperscript{94}) and the gluconeogenic enzymes phosphoenolpyruvate carboxykinase and glucose-6-phosphatase (by phosphorylation of the transcriptional co-activator CRTC2, formerly known as TORC2\textsuperscript{95}, and/or by phosphorylation and nuclear exclusion of the Class IIa HDAC family, which deacetylate and activate FOXO family transcription factors\textsuperscript{96}). With the exception of gluconeogenesis, most of these anabolic pathways are required for cell growth, and in many cases expression of the enzymes involved is up-regulated in tumors. By down-regulating these pathways, AMPK not only conserves ATP, but also exerts a cytostatic, anti-tumor effect, consistent with the hypothesis that it exerts most, if not all, of the tumor suppressor effects of its upstream kinase, LKB1.

**Regulation of whole body energy metabolism**

In mammals, AMPK can also influence metabolism and energy balance at the whole body level, particularly through its actions in the hypothalamus of the brain.

**AMPK and control of appetite**

The primary appetite control centre is the arcuate nucleus [G] of the hypothalamus, in which increased electrical activity in neuropeptide Y and agouti-related protein-expressing neurons (NPY/AgRP neurons) [G] induces feeding, while increased activity in neurons expressing pro-opiomelanocortin (POMC neurons) [G] inhibits feeding\textsuperscript{97}. Kinase assays in dissected hypothalamic regions from rodents show that hormones that inhibit feeding, such as the adipokine leptin, inhibit the α2 isoform of AMPK\textsuperscript{98}, whereas those that promote it, such as the hormone ghrelin [G] from the stomach, the adipokine adiponectin, or cannabinoids, activate AMPK (the specific isoform was not determined)\textsuperscript{98-101}. Direct injection into the hypothalamus of pharmacological activators of AMPK, or DNAs encoding activated mutants, also promotes feeding\textsuperscript{98, 99}.

Although leptin inhibits AMPK-α2 in the hypothalamus\textsuperscript{98}, mice in which AMPK-α2 was specifically knocked out in NPY/AgRP neurons or POMC neurons displayed only minor changes in food intake, and still exhibited decreased food intake in response to leptin\textsuperscript{102}. A possible explanation for this anomaly was provided by a recent report suggesting that it is in presynaptic neurons [G] upstream of NPY/AgRP neurons, rather than in the NPY/AgRP or POMC neurons themselves, that
regulation of AMPK is critical\textsuperscript{103}. The frequency of \textit{miniature excitatory postsynaptic currents} \textsuperscript{G} in the NPY/AgRP neurons was used as a measure of neurotransmitter release from presynaptic neurons acting upstream. Using various pharmacological agents, evidence was obtained for a model in which ghrelin, which is released from the stomach during fasting, activates AMPK in presynaptic neurons via GHSR1 receptors (Fig. 5a). These receptors activate heterotrimeric G proteins containing \( G_q/G_{11} \), which trigger intracellular \( \text{Ca}^{2+} \) release and therefore lead to the activation of AMPK by the CaMKK\( \beta \) pathway\textsuperscript{104}. Consistent with this, CaMKK\( \beta \)-deficient mice show reduced expression of NPY and AgRP, but not POMC, in the hypothalamus, and fail to increase their food intake in response to ghrelin\textsuperscript{105}. Although the critical downstream target(s) for AMPK in the presynaptic neurons remain unclear, its activation appears to initiate a positive feedback loop in which \( \text{Ca}^{2+} \) release via \textit{ryanodine receptors} \textsuperscript{G} causes sustained activation of AMPK and release of \( \text{Ca}^{2+} \), and consequent neurotransmitter release onto the NPY/AgRP neurons. This in turn promotes sustained feeding, which due to the positive feedback loop continues even after ghrelin stimulation has ceased. According to this model, feeding only stops when leptin released by adipocytes stimulates release from neighbouring POMC neurons of opioids, which act via \( \mu \)-opioid receptors in the presynaptic neurons upstream of the NPY/AgRP neurons to inhibit AMPK (the mechanism for this latter effect remains unknown) (Fig. 5b).

An interesting parallel exists between this proposed neural circuit and the “reset-set” or “flip-flop” memory storage circuits used in electronic devices. These circuits are switched on by signals coming in via the “set” input, and remain switched on even in the absence of further stimulation until a signal is received on the “reset” input. By analogy, hunger signals such as ghrelin represent the “set” inputs that switch on AgRP neurons and trigger feeding, and feeding would continue until the satiety signal, leptin, resets the circuit by acting on the POMC neuron. This model is consistent with observed diurnal patterns of plasma ghrelin and leptin in humans eating normal meals\textsuperscript{106}. If excess food is available, it may have survival value to continue eating until the leptin signal coming from adipocytes indicates that fat stores have been replenished, rather than stopping eating as soon as the hunger signal from the stomach (ghrelin) had ceased.

**Role of AMPK in glucose sensing and modulation of the sympathetic nervous system**

AMPK in the neighboring \textit{ventromedial hypothalamus} also appears to have important roles in
controlling whole body energy balance. There is evidence that it is involved in sensing of low blood glucose, promoting release of counter-regulatory hormones (epinephrine and glucagon) that stimulate glucose production by the liver\[^{107}\]. Thyroid hormones also inhibit AMPK in this region\[^{108}\], increasing the activity of sympathetic nerves, which increase energy expenditure by promoting fat oxidation in muscle or brown adipose tissue\[^{108}\]. Interestingly, in whole body mouse knock-outs of AMPK-\(\alpha2\) there is elevated production of catecholamines\[^{109}\], consistent with the idea that inhibition of AMPK can promote activity of the sympathetic nervous system.

**AMPK and circadian rhythms**

In most mammals, whole body metabolism exhibits circadian rhythms, via which the timing of feeding and metabolism are synchronized with the daily cycle of light and darkness. The suprachiasmatic nucleus [G] of the hypothalamus is the site of the “master clock” that generates these rhythms. However, the proteins involved in establishing these rhythms are also expressed at peripheral sites such as the liver, forming “slave clocks” that can oscillate independently under certain circumstances\[^{110}\]. These proteins include the transcription factors Bmal1 and Clock, which form a heterodimer that activates the expression of many genes, two of which are the *Per (period)* and *Cry (cryptochrome)* genes. Once synthesized, Per and Cry proteins bind to each other, become phosphorylated and then enter the nucleus, where they inhibit the Clock:Bmal1 complex. This delayed negative feedback loop results in the rhythmic expression not only of Per and Cry themselves, but also of numerous other genes driven by Bmal1/Clock. AMPK has been found to phosphorylate Cry1, reducing its association with Per2 and instead increasing its binding to FBXL3, a ubiquitin ligase that promotes Cry1 ubiquitylation and degradation\[^{111}\]. This would have the effect of extending the period of the endogenous rhythm. In mouse embryo fibroblasts synchronized by serum starvation, treatment with low glucose or the AMPK activator AICAR reduced the amplitude, and increased the period, of the circadian rhythm of a luciferase reporter gene driven by the Bmal1/Clock promoter; these effects were lost in cells lacking LKB1 or AMPK. It has been known for many years that the phosphorylation of AMPK targets such as acetyl-CoA carboxylase follows a circadian rhythm in rodent liver linked to the times of feeding\[^{88}\]. The more recent results\[^{111}\] suggest that AMPK may have a crucial role in determining these circadian rhythms.
**AMPK functions beyond metabolism**

Although AMPK is best known for its effects on metabolism, it has recently become clear that it mediates other effects not directly related to metabolism. Two of these are regulation of the cell cycle and modulation of membrane excitability.

**Regulation of the cell cycle**

Both DNA replication (in S phase) and mitosis (in M phase) are energy-requiring processes, and it would make little sense for cells that are deficient in energy to execute them. Consistent with this, activation of AMPK in cycling cells causes a G1 arrest prior to S phase\(^{112}\). This is associated with phosphorylation of p53 at Ser-15 (although it is not clear that this is a direct target for AMPK) and up-regulation of expression of the G1 cyclin-dependent kinase inhibitor p21\(^{\text{WAF1}}\), a product of a p53-activated gene\(^{112,113}\). AMPK has also been reported to phosphorylate the C-terminal residue of another cyclin-dependent kinase inhibitor, p27\(^{\text{KIP1}}\), causing its stabilization\(^{114}\). These effects may explain, at least in part, the ability of AMPK to cause cell cycle arrest.

Surprisingly, other studies suggest that AMPK activity is required for completion of mitosis. An elegant chemical genetic screen, involving expression of AMPK-α2 with a mutation in the catalytic site that allowed it to utilize a chemically modified ATP, identified several novel targets for AMPK, all of which conformed with the established AMPK recognition motif\(^{65,66}\). Many of these novel targets had roles in mitosis and cytokinesis, including components of the anaphase promoting complex (APC1 and CDC27), three of the regulatory subunits (PPP1R12A/B/C) that target protein phosphatase-1 to dephosphorylate myosin regulatory light chain (MRLC) at Ser19, and the protein kinase PAK2, which phosphorylates MRLC at Ser19\(^{115}\). The phosphorylation of both AMPK (Thr172) and PPP1R12C at the AMPK site (Ser452) were elevated in mitotic cells, and stable expression of a S452A mutant PPP1R12C led to an increase in the proportion of multinucleated cells, indicating a defect in mitosis or cytokinesis. Relevant to this are findings that AMPK phosphorylated at Thr172 is specifically localized at several places within the mitotic apparatus in mitotic cells\(^{116}\). Intriguingly, Drosophila embryos carrying AMPK-null mutations also display a high frequency of multinucleate or polyploid cells, and this could be rescued by expressing MRLC with phosphomimetic mutations at the sites equivalent to Thr18 and Ser19 in human MRLC\(^{117}\).
It is not immediately apparent why a kinase activated by energy stress should be required for passage through mitosis. Perhaps mitosis is accelerated by AMPK in cells undergoing stress in order that an orderly cell cycle arrest may occur in the ensuing G1 phase. However, it may be that this is an ancillary function of AMPK unrelated to its role as an energy sensor.

**Regulation of membrane excitability**
Remarkably, ATP turnover in the grey matter of brain is comparable with that in leg muscle during marathon running, explaining why an organ contributing only 2% of body weight can account for >20% of resting metabolism\textsuperscript{118}. It has been estimated that the firing of action potentials accounts for 25–50% of this energy consumption, with synaptic transmission (which is triggered by action potentials) contributing most of the remainder\textsuperscript{118,119}. A mechanism that down-regulated the firing of neuronal action potentials would therefore conserve a considerable amount of energy. Recent studies with HEK-293 cells stably expressing the potassium channel Kv2.1 showed that AMPK activation was found to activate these potassium channels by causing a shift in voltage gating to more negative membrane potentials. Identical effects were observed when activated AMPK (thiophosphorylated at Thr172 to make it resistant to phosphatases), but not an inactive mutant, was introduced into the cells via the patch pipette\textsuperscript{120}. AMPK phosphorylates purified Kv2.1 at two sites in its cytoplasmic C-terminal tail, and the effect of AMPK activation on voltage gating was lost in cells in which one of these (Ser440) was substituted with a non-phosphorylatable alanine\textsuperscript{120}. Kv2.1 accounts for a large proportion of the delayed rectifier K\textsuperscript{+} channels [G] in central neurons, and their activation has been proposed to reduce the firing of action potentials down the axon\textsuperscript{121}. Interestingly, introduction of active thiophosphorylated AMPK, but not an inactive mutant, into cultured rat hippocampal neurons via the patch pipette caused a progressive decrease in the frequency of action potentials induced by a current pulse\textsuperscript{120}. These results support the idea that AMPK activation may exert a neuroprotective role by limiting the rate of firing of action potentials, thus conserving energy when the energy status of neuronal tissue is compromised.

**Conclusions and outstanding questions**
There are now around one thousand papers on AMPK and its orthologs published every year, and in
this review we have only been able to cover a small number that we found particularly interesting. The classical pathways by which AMPK is activated by increases in AMP:ATP or ADP:ATP ratios, or increases in Ca\(^{2+}\), are now becoming well understood, although our understanding of the protein phosphatases that dephosphorylate Thr-172 remains incomplete. The “non-canonical” mechanisms by which oxidative stress and genotoxic agents activate AMPK are another area that needs further investigation. Although AMPK is perhaps best known for its effects to regulate metabolism at the cellular level, in mammals it also regulates metabolism and helps to maintain energy balance at the whole body level. It does this by mediating effects of hormones and other agents acting on neurons in different hypothalamic regions, which regulate intake of food (and hence energy) and energy expenditure. AMPK also regulates diurnal rhythms of feeding and metabolism. By switching off biosynthetic pathways required for cell growth, AMPK activation would exert a cytostatic effect, helping to explain why its upstream activator, LKB1, is a tumor suppressor. Commensurate with its role in preserving cellular energy homeostasis, AMPK also down-regulates ATP-requiring processes outside of metabolism, including progress through the cell cycle (another potential tumor suppressor effect) and firing of action potentials in neurons. Although it might be said that the AMPK field is approaching maturity, it seems certain that many exciting findings about the pathway remain to be discovered, and these insights might lead to novel drugs and other means of exploiting this knowledge.


Most complete structure for an AMPK heterotrimer to date, which also suggests a model for the mechanism by which binding of AMP or ADP inhibit dephosphorylation of Thr-172.


37. Sugden, C., Crawford, R.M., Halford, N.G. & Hardie, D.G. Regulation of spinach SNF1-related (SnRK1) kinases by protein kinases and phosphatases is associated with phosphorylation of the T loop and is regulated by 5'-AMP. Plant J. **19**, 433-439 (1999).


Mice with a muscle-specific double AMPK-β1/β2 subunit knockout display dramatically reduced running speed and endurance, blunted muscle glucose uptake in response to treadmill exercise, and markedly impaired contraction-stimulated glucose uptake in isolated muscles.


AMPK activation switches on autophagy, especially of mitochondria (mitophagy), and disruption of this pathway leads to the accumulation of dysfunctional mitochondria in cells.


Class IIa lysine deacetylases are physiological targets of AMPK, and deacetylation of FOXO family transcription factors by this mechanism contributes to inhibition of gluconeogenic gene expression by AMPK.


100. Kola, B. et al. Cannabinoids and ghrelin have both central and peripheral metabolic and cardiac effects via AMP-activated Protein Kinase. J. Biol. Chem. 280, 25196-25201 (2005).


Ghrelin activates AMPK via a Ca\(^{2+}\)-dependent mechanism in presynaptic neurons upstream of NPY/AgRP neurons in the hypothalamus, activating a positive feedback loop that causes continued neurotransmitter release and feeding, until action of leptin on POMC neurons causes release of opioids that inhibit AMPK in the presynaptic neurons.


A novel chemical genetic screen identifies many new targets for AMPK, some of which appear to be phosphorylated to allow completion of mitosis.


Acknowledgements

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Figure Legends

Figure 1: Model for the mechanism by which AMPK is activated by increases in AMP and ADP (top), as the cellular concentrations of ATP, ADP and AMP change (bottom). The model at the top represents different states of the three subunits of AMPK (see Fig. 2 for details of domains). The graph at the bottom shows changes in the predicted concentrations of ATP, ADP and AMP on going from an unstressed, fully charged cell (left) to a cell undergoing a severe energy stress (right), corresponding to a 10-fold increase in ADP:ATP ratio. The graph was generated by assuming that the adenylate kinase reaction was at equilibrium. Note that in a fully charged cell (left), AMP concentration is very low, but that its % change in concentration as ADP/ATP increases is always much greater than those of ATP or ADP. In the model at the top, the basal state (top left) has sites 1 and 3 in the γ subunit occupied by ATP (site 4 is always occupied by AMP). Replacement of ATP by ADP (or AMP) at site 3 during moderate stress (top centre) promotes phosphorylation of Thr172 (bottom centre), causing a 100-fold increase in activity (indicated by two stars). Replacement of ATP by AMP at site 1 during more severe stress causes a further 10-fold allosteric activation (indicated by a third star, bottom right). As cellular energy status returns to normal, AMP at site 1 and ADP or AMP at site 3 are progressively replaced by ATP (moving from right to left on the bottom row). This promotes the dephosphorylation of Thr-172 and a return to the basal state.

Figure 2: Domain map of typical mammalian AMPK. Colour coding of domains are similar to those in Figs. 1 and 3. AMPK complexes are heterotrimers composed of α, β and γ subunits in a 1:1:1 ratio. Key to acronyms: N-lobe, N-terminal lobe of kinase domain; C-lobe, C-terminal lobe of kinase domain; AID, auto-inhibitory domain; α-CTD, α subunit C-terminal domain; CBM, carbohydrate-binding module; β-CTD, β subunit C-terminal domain; CBS1-4, CBS repeats in γ subunit. The β-CTD forms the core of the complex, binding to the α-CTD and the N-terminus of the γ subunit prior to CBS1.
Figure 3: Two views of a crystal structure of a partial heterotrimeric complex of mammalian AMPK; the right-hand view is rotated about 180° about the y axis compared to the left-hand view. The constructs crystallized contained only the C-terminal domain of the β subunit and also lacked a flexible loop in the C-terminal domain of the α subunit. The α subunit AID was present but was not resolved in this crystal form; in the left-hand view, its approximate location would just to the right of the junction between the α subunit C-lobe (green) and the α-linker (red). The crystals contained AMP bound at sites 3 and 4. Note how in this structure, access to the Thr172 site is restricted by the close association of the α-CTD with the kinase domain (left-hand view). In addition, note how AMP bound in site 3 is not visible because it is covered by the “α-hook” structure of the linker peptide (right-hand view, linker peptide in red). Key to acronyms: N-lobe, N-terminal lobe of kinase domain; C-lobe, C-terminal lobe of kinase domain; γ-NTD, N-terminal region of γ subunit; α-CTD, α subunit C-terminal domain; β-CTD, β subunit C-terminal domain; γ-CBS1-4, CBS repeats in γ subunit.

Figure 4: Summary of effects of AMPK activation on cellular metabolism. The likely protein targets that mediate the metabolic effects of AMPK, as well as the final metabolic outcomes, are depicted. Proteins shown on the inner wheel with question marks may not be directly phosphorylated by AMPK. Catabolic pathways, including glucose uptake via GLUT4 and GLUT1, glycolysis, fatty acid uptake via CD36, fatty acid oxidation, mitochondrial biogenesis and autophagy are invariably activated by AMPK. Anabolic pathways, including fatty acid synthesis, transcription of lipogenic enzymes, triglyceride synthesis, cholesterol synthesis, transcription of gluconeogenic enzymes, glycogen synthesis, protein synthesis and rRNA synthesis, are invariably inhibited by AMPK.
**Figure 5:** AMPK-regulated control of feeding behavior by modulation of neuropeptide Y- and agouti-related protein-expressing neurons (NPY/AgRP neurons) and pro-opiomelanocortin neurons (POMC neurons), as proposed by Yang et al\textsuperscript{103}. A) in the fasted state ghrelin, a “hunger signal” derived from the stomach, activates AMPK in the presynaptic neurons acting upstream of NPY/AgRP neurons via the CaMKKβ pathway. This causes release of Ca\textsuperscript{2+} by Ca\textsuperscript{2+}-induced release from intercellular stores via ryanodine receptors, creating a feedback loop that causes continued release of neurotransmitter onto the NPY/AgRP neuron, even when ghrelin stimulation ceases. The NPY/AgRP neurons promote feeding (and inhibits the POMC neurons, which inhibit feeding). b) Feeding continues (even in the absence of ghrelin) until the POMC neurons are stimulated by the “satiety signal”, leptin. Activity of these neurons inhibits feeding and also promotes release of opioids that inhibit AMPK in the presynaptic neurons upstream of the NPY/AgRP neurons, switching them back to an inactive state.

**Glossary**

- **activation loop** a sequence segment in the C-terminal lobe of protein kinases that often plays a key role in switching the kinase on; in many cases the kinase is only active after phosphorylation of this loop
- **allosteric activation** the activation of an enzyme by non-covalent binding of a ligand (an *allosteric activator*) that binds at a site distinct from the catalytic site
- **arcuate nucleus** an anatomical region of the hypothalamus at the base of the brain that appears to have a particular role in feeding and appetite
- **ataxia-telangiectasia** an inherited human disorder of which the clinical signs include *ataxia* (uncoordinated movement) and *telangiectasia* (dilated blood vessels in the skin or mucous membranes); caused by
mutation of the *ATM* gene, encoding a protein kinase of the 
*phosphatidylinositol-3- kinase-like kinase* (PIKK) family

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Bateman domain</td>
<td>a domain formed by two tandem CBS repeats that associate together with central clefts that bind small molecules, especially adenosine derivatives</td>
</tr>
<tr>
<td>CBS repeat</td>
<td>sequence motif usually occurring as two tandem repeats that form a Bateman domain; named after cystathionine β-synthase, in which the Bateman domain binds S-adenosyl methionine</td>
</tr>
<tr>
<td>circadian rhythm</td>
<td>a biological rhythm that follows the normal 24 hour cycle; although endogenously driven and thus continuing in the absence of external cues, they are often entrained or modified by external stimuli such as light or food availability</td>
</tr>
<tr>
<td>delayed rectifier K⁺ channels</td>
<td>a group of voltage-gated K⁺ channels that open and close slowly in response to membrane depolarization; by allowing K⁺ ions to flow out of cells down their concentration gradient and thus oppose subsequent depolarization, these channels regulate the frequency of action potentials</td>
</tr>
<tr>
<td>fructose-1,6-bisphosphatase</td>
<td>enzyme that catalyzes a key regulatory step in gluconeogenesis (hydrolysis of fructose-1,6-bisphosphate to fructose-6-phosphate) in the liver and kidney</td>
</tr>
<tr>
<td>ghrelin</td>
<td>a 28 amino acid peptide that is released by cells of the stomach and represents a “hunger signal”</td>
</tr>
<tr>
<td>glutathionylation</td>
<td>the covalent attachment of glutathione to a protein, via the formation of a mixed disulfide between the cysteine moiety of glutathione and a cysteine side chain of the protein</td>
</tr>
<tr>
<td>glycogen phosphorylase</td>
<td>the primary enzyme that mobilizes stores of glucose in glycogen, catalyzing the release of glucose-1-phosphate from the non-reducing ends of glycogen by a phosphorylisis reaction</td>
</tr>
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LKB1:STRAD:MO25 complex

a heterotrimeric complex containing the tumor suppressor protein kinase, LKB1, and the accessory subunits STRAD and MO25; LKB1 was discovered as the gene mutated in a form of inherited cancer susceptibility (Peutz-Jeghers syndrome), and is also lost due to somatic mutation in many human cancers.

membrane excitability

some biological membranes, such as the plasma membranes of neurons, are excitable because they contain voltage-gated \( \text{Na}^+ \) channels that open in response to depolarization, allowing \( \text{Na}^+ \) ions to flood into the cell down their concentration gradient; this amplifies the depolarization and causes a wave of depolarization (an action potential) to travel along the membrane.

miniature excitatory postsynaptic currents

small depolarizing currents measured by patch clamping of a neuron, due to release of packets of neurotransmitter from a presynaptic neuron upstream of that neuron; they can be observed by applying tetrodotoxin to inhibit the firing of action potentials in the neuron.

mitophagy

the special form of autophagy by which mitochondria (probably in a damaged or defective state) are engulfed by autophagosomes and degraded, recycling their contents for re-use.

NPY/AgRP neuron

a neuron that expresses the neuropeptides neuropeptide Y and agouti-related protein.

N-terminal myristylation

the covalent attachment of 14 carbon saturated fatty acid (myristic acid), usually to the N-terminus of a protein following cleavage of the initiating methionine.

phosphofructokinase

enzyme that catalyzes a key regulatory step in glycolysis, the transfer of phosphate from ATP to fructose-6-phosphate to generate fructose-1,6-bisphosphate.

POMC neuron

a neuron that expresses the neuropeptide pro-opiomelanocortin.
presynaptic neuron: a neuron acting immediately upstream of the neuron under study, which releases neurotransmitters directly onto that neuron.

Rab-GAP: protein carrying a Rab-GTPase activator protein function, i.e. the ability to promote conversion of small G proteins of the Rab family from their active Rab-GTP state to their inactive Rab-GDP state.

Ryanodine receptors: Ca$^{2+}$ release channels in the sarcoplasmic/endoplasmic reticulum of muscle cells and neurons, which are activated by Ca$^{2+}$ and blocked by the plant product, ryanodine.

Ventromedial nucleus: an anatomical region of the hypothalamus at the base of the brain that appears to have a role in glucose sensing and activation of the sympathetic nervous system.
Hardie et al Figure 2

α subunits: N  KINASE DOMAIN  C
- N-lobe
- C-lobe
- AID
- Linker

β subunits: N  CBM  β-CTD  C
- glycogen binding
- α binding

γ subunits: N  CBS1 CBS2 CBS3 CBS4  C
- Bateman domain
- AMP/ATP binding

upstream kinases

P

α-CTD  β binding

γ binding
Hardie et al Figure 3

**α-kinase domain**
- N-lobe
- C-lobe

Approx. location of α-AID (not resolved)

**N-lobe**
- catalytic cleft
- Thr-172

**C-lobe**
- α-CTD
- β-CTD

**γ-CBS1**
- γ-CBS2
- γ-CBS3
- γ-CBS4

AMP bound in site 4

**γ-NTD**

amp bound in site 3

**γ-subunit**

α-hook of linker peptide covers AMP in site 3
Hardie et al Figure 5

- **AMPK**
- **rRNA synthesis** (RNA pol I)
- **glucose uptake** (GLUT4)
- **glucose uptake** (GLUT1)
- **glycolysis** (phosphofructokinase)
- **glycogen synthesis**
- **glycogen synthase**
- **gluconeogenic enzymes** (transcription)
- **cholesterol synthesis**
- **triglyceride synthesis**
- **lipogenic enzymes** (transcription)
- **fatty acid synthesis**
- **fatty acid oxidation**
- **mitochondrial biogenesis**
- **autophagy** (mitophagy)

**Pathways:**
- **Protein synthesis** (mTOR)
- **TSC2**
- **Raptor**
- **TBC1D1**
- **PFKFB3**
- **PFKFB4**
- **ACC2**
- **ACC1/α**
- **SREBP1c**
- **GPAT**
- **HMGR**
- **HDACs**
- **CRTC2**
- **SIRT1**
- **PGC-1α**
- **ULK1/2**
- **Raptor**
- **proteinsynthesis (mTOR)**
- **gluconeogenic enzymes (transcription)**
- **cholesterol synthesis**
- **triglyceride synthesis**
- **lipogenic enzymes (transcription)**
- **fatty acid synthesis**
- **fatty acid uptake (CD36)**
- **glycolysis** (phosphofructokinase)
- **fatty acid oxidation**
- **mitochondrial biogenesis**
- **autophagy** (mitophagy)
Hardie et al Figure 6

[Diagram showing the interaction between ghrelin and leptin in the bloodstream, with pre-synaptic neurones, AMPK, AGRP, and POMC.]