AMPK: positive and negative regulation, and its role in whole-body energy homeostasis
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The AMP-activated protein kinase (AMPK) is a sensor of energy status that, when activated by metabolic stress, maintains cellular energy homeostasis by switching on catabolic pathways and switching off ATP-consuming processes. Recent results suggest that activation of AMPK by the upstream kinase LKB1 in response to nutrient lack occurs at the surface of the lysosome. AMPK is also crucial in regulation of whole body energy balance, particularly by mediating effects of hormones acting on the hypothalamus. Recent crystal structures of complete AMPK heterotrimers have illuminated its complex mechanisms of activation, involving both allosteric activation and increased net phosphorylation mediated by effects on phosphorylation and dephosphorylation. Finally, AMPK is negatively regulated by phosphorylation of the ‘ST loop’ within the catalytic subunit.

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Current Opinion in Cell Biology 2015, 33:1–7
This review comes from a themed issue on Cell regulation
Edited by Jodi Nunnari and Johan Auwerx
For a complete overview see the Issue and the Editorial
Available online 26th September 2014
http://dx.doi.org/10.1016/j.cceb.2014.09.004
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Introduction
The AMP-activated protein kinase (AMPK) is a sensor of cellular energy status, expressed in essentially all eukaryotic cells as heterotrimeric complexes containing catalytic α subunits and regulatory β and γ subunits. In mammals, AMPK is activated by increases in AMP:ATP or ADP:ATP ratios, which occur when cellular energy status has been compromised. Activation occurs in response to metabolic stresses that either interfere with ATP production or that accelerate ATP consumption, when AMPK acts to restore energy homeostasis by activating alternate catabolic processes generating ATP, while inhibiting ATP-requiring processes [1,2]. Because of the reversible reaction catalyzed by adenylate kinase (2ADP ↔ ATP + AMP), increases in AMP in stressed cells are always much larger than increases in ADP or decreases in ATP [3], so it makes sense for AMP to be the primary signal to which the system responds. This review will focus on mechanisms of positive and negative regulation of AMPK, and its role in whole body energy homeostasis; readers more interested in its downstream targets within the cell should consult other reviews [1,2].

Regulation of AMPK by nucleotides, pharmacological agents and Ca2+
As its name suggests, AMPK is allosterically activated by AMP. The physiological importance of this mechanism has been questioned [4], but studies of cells expressing only a mutant AMPK that cannot be regulated by the alternate phosphorylation mechanism suggest that the allosteric effect is a key component of the overall activation mechanism [3]. AMPK is also activated by other allosteric effectors (A-769662, 991, MT 63-78; Figure 1, top), all synthetic compounds derived from high-throughput screens targeting AMPK [5,6,7]. These preferentially activate complexes containing the β1 isoform, and bind at a site involving the α and β subunits that is distinct from those binding AMP (see below). The existence of this unique site suggests that there may be natural ligands that bind there; none have yet been identified in mammals, although salicylate (the major breakdown product of aspirin) activates AMPK in vivo by binding this site [8,9], and is a natural product of plants. Certain other activators (Figure 1, middle), such as AICAR and C13 (the latter selective for complexes containing the α1 isoform [10*,11]), are pro-drugs that are converted following cellular uptake into AMP analogs. Other pharmacological activators (e.g. metformin, berberine, resveratrol, hydrogen peroxide, Figure 1, bottom) are metabolic poisons that inhibit ATP synthesis, activating AMPK indirectly by increasing cellular AMP levels [12,13].

AMPK is also activated >100-fold by upstream kinases that phosphorylate a conserved threonine within the ‘activation loop’ of the kinase domain (Thr172 in rat α2). Interestingly, Thr172 phosphorylation is not required for full kinase activity if AMP and A-769662 are bound simultaneously [14*], although the physiological significance of this observation may remain uncertain until a natural ligand that binds at the A-769662 site is identified. The primary upstream kinases that phosphorylate Thr172 are a complex containing the tumor suppressor kinase LKB1, and the Ca2+/calmodulin-activated protein kinase, CaMKKβ [1,2]. The latter is activated when intracellular Ca2+ is increased by the action of a
hormone or drug, but the LKB1 complex has a high basal activity [15], and its ability to promote Thr172 phosphorylation is regulated instead by the binding of adenine nucleotides to its substrate, AMPK. Binding of AMP to the AMPK-γ subunit promotes Thr172 phosphorylation by causing conformational changes that promote phosphorylation of Thr172 by LKB1 and inhibit its dephosphorylation by protein phosphatases. It has been suggested that both of these effects can be triggered by ADP binding [16–18], but a recent study showed that the effect of nucleotide binding on Thr172 phosphorylation by LKB1 was specific to AMP, and that while ADP binding did protect Thr172 against dephosphorylation, this required ten-fold higher concentrations than AMP [3]. Interestingly, compounds binding at the A-769662 site also protect Thr172 against dephosphorylation in cell-free assays, although their effects in intact cells appear to be mainly through allosteric activation [6**,19,20].

Figure 1

Structures of three different classes of AMPK activating compounds. Top: direct activators that bind in the same site as A-769662 (this has not been shown directly for MT 63-78, but seems likely because, like A-769662, 991 and salicylate, it is selective for β1-containing complexes); middle: pro-drugs that are converted into AMP analogs (ZMP or C2) by intracellular enzymes; bottom: metabolic poisons which inhibit ATP synthesis (usually, but not always, by inhibiting the respiratory chain) and thus increase cellular AMP. Due to their multiple sites of action these compounds can have very varied structures.
AMPK may be activated by LKB1 at the surface of the lysosome

Recombinant LKB1 complexes phosphorylate and activate purified AMPK in reconstituted cell-free assays, with no other components apparently necessary [3]. Nevertheless, two remarkable recent papers [21**,**22*] suggest that the interaction between LKB1 and AMPK within cells may be facilitated by interactions with adapter proteins that recruit both complexes to the two-dimensional surface of the lysosome. Initial clues came from studies of axin, a scaffold protein originally identified as a negative regulator of Wnt signaling. Knocking down axin in liver and cultured cells caused defective AMPK activation, and axin was found to interact with both AMPK and LKB1, although not CaMKkβ [22*]. The same group performed a two-hybrid screen searching for other axin-interacting proteins and detected LAMTOR1 [21**], a component of the Regulator complex that promotes binding of target-of-rapamycin complex-1 (mTORC1) to the lysosomal membrane under nutrient-rich conditions [23]. They made tissue-specific knockouts of LAMTOR1, and found that AMPK activation was defective in response to starvation in liver, to exercise in muscle, and to glucose deprivation in mouse embryo fibroblasts (MEFs), although activation by the Ca2+/CaMKkβ pathway was unaffected [21**]. Finally, they provided evidence for a model in which axin acts as an adaptor that recruits LKB1 and AMPK to the Regulator complex, and hence to the lysosome, under nutrient-poor conditions (recruitment of AMPK to this complex was also enhanced by AMP binding). These results suggest that the Regulator, already known to be involved in signaling the availability of nutrients to activate mTORC1 [23], may be involved in a reciprocal manner in signaling a lack of nutrients to activate AMPK.

If correct, this model would represent a ‘paradigm shift’ in the field. Although independent corroboration is currently lacking, there are some related findings consistent with it. Firstly, there is evidence that AMPK interacts with the folliculin complex. The latter contains folliculin and folliculin-interacting proteins (FNIP1 or FNIP2), is associated with the lysosome [24], and is also involved in regulation of mTORC1 [23]. AMPK has been reported to interact with FNIP1 [25] and to phosphorylate folliculin [26], and was recently found to be activated in folliculin-deficient MEFs, suggesting that folliculin is a negative regulator of AMPK as well as a positive regulator of mTORC1 [27]. Secondly, both the LKB1 and AMPK complexes have lipid modifications (C-terminal farnesylation of LKB1 and N-terminal myristoylation of AMPK-β subunits). Point mutations that prevent these modifications reduced association of both complexes with intracellular membranes, and mice carrying the LKB1 mutation displayed reduced AMPK activation by contraction or AICAR in skeletal muscle [17,28*]. Although the identities of the intracellular membranes that bind the LKB1 and AMPK complexes were not determined, it is tempting to speculate that these lipid modifications help the LKB1 and AMPK complexes to co-localize with axin on the surface of the lysosome.

AMPK regulates whole-body as well as cellular energy balance

AMPK orthologs are clearly present in unicellular eukaryotes, suggesting that the system originally evolved to regulate energy homeostasis in a cell-autonomous manner. However, in multicellular organisms hormones that regulate whole-body energy balance, particularly through effects on the hypothalamus, appear to have adapted to interact with AMPK (Figure 2). For example, the hormones ghrelin released from the stomach during fasting, and adiponectin released from adipocytes of lean individuals, activate AMPK in the arcuate nucleus of the hypothalamus and promote food intake [29,30]. Conversely, leptin, released from adipocytes of obese individuals, inhibits AMPK in the arcuate nucleus and inhibits food intake [reviewed in [31]].

Figure 2

Regulation of whole body energy balance via effects of gut hormones, adipokines and thyroid hormones on AMPK in the arcuate nucleus and ventromedial regions of the hypothalamus. See main text for details.
As well as these effects of AMPK in the arcuate nucleus to modulate appetite and hence energy intake, inhibition of AMPK in the ventromedial hypothalamus (VMH) appears to promote whole body energy expenditure by triggering activation of the sympathetic nervous system (SNS), which stimulates fat oxidation in muscle and white adipose tissue, and thermogenesis in brown adipose tissue (BAT). Thus, administration of the thyroid hormone T3 to the VMH inhibited AMPK, and this was required for the associated increase in SNS activity and expression of thermogenic markers in BAT [32]. More recently, it was found that injection into rat VMH of liraglutide, an agonist for the glucagon-like peptide-1 (GLP-1) receptor, decreased phosphorylation of Thr172 and the AMPK target ACC, increased SNS activity and expression of thermogenic markers in BAT, and promoted weight loss without affecting food intake [33]. GLP-1 is an incretin hormone released from the gut following feeding and, as well as these effects on the VMH, it decreases appetite via effects on the arcuate nucleus.

The mechanisms by which these hormones regulate AMPK are known in some but not all cases. There is evidence that ghrelin, binding to Ghsr1 receptors in the arcuate nucleus, activates AMPK by the Ca2+-CaMKKβ pathway; this occurs in presynaptic neurons that activate neurons expressing the orexigenic peptides neuropeptide Y (NPY) and Agouti-related peptide (AgRP), thus promoting feeding [34]. It was further proposed that leptin triggers release (from neurons expressing the anorexigenic peptide pro-opiomelanocortin) of an opioid that inhibits AMPK in the same presynaptic neurons, thus terminating the feeding impulse [34]. A potential mechanism for this comes from findings that μ-opioid receptor agonists activate the PI 3-kinase (PI3K–Akt–mTORC1–S6K1 pathway [35]; it has been proposed that S6K1 phosphorylates AMPK-α2 at a putative inhibitory site, Ser491 [36] (however, this is discussed further below). Finally, in endothelial cells liraglutide has been shown to activate AMPK via release of intracellular Ca2+ and activation of CaMKKβ [37], but how it inhibits AMPK in the VMH is unclear.

### Structural analysis of the AMPK complex

Two key events during the last year were the publication of the first almost complete crystal structures for AMPK, of the human α2β1γ1 [6**] and α1β1γ1 [9*] complexes. Both had been phosphorylated on Thr172 and crystallized in the presence of AMP and either A-769662 or 991, so were in a fully activated state. The structures, which are broadly similar, can be divided into two distinct halves that I will term the catalytic and nucleotide-binding modules (Figure 3); these probably correspond to the twin lobes of the ‘cashew nut-like’ or L-shaped particles seen in earlier negatively-stained electron micrographs [38]. The catalytic module contains a typical eukaryotic kinase domain (KD) with small and large lobes, and a conserved domain from the β subunit known as the carbohydrate-binding module (β-CBM). The latter is known to cause binding of cellular AMPK to glycogen particles [39,40], and it interacts with the small lobe of the kinase domain via the surface opposite to its well-defined glycogen-binding site [41]. Intriguingly, A-769662 and 991 bind in the cleft between the CBM and the KD (Figure 3) [6**,9*], a binding site that is unique to AMPK.

The regulatory module contains the C-terminal domains of the α and β subunits (α-CTD/β-CTD), as well as the entire γ subunit containing the binding sites for the regulatory nucleotides AMP, ADP and ATP. These sites are formed by four tandem repeats within the γ subunit of a sequence known as a CBS motif, which assemble in a pseudosymmetric manner to give four potential ligand-binding clefts. One of these (site 2) always appears to be empty, but in the α2β1γ1 structure sites 1, 3 and 4 were occupied by AMP; the precise roles of the three sites are still being debated.

The ‘hinges’ connecting the catalytic and regulatory modules comprise the α subunit auto-inhibitory domain (α-AID) together with the ‘α-linker’ that connects the α-AID to the α-CTD, and the linker connecting the β-CBM and the β-CTD, which unfortunately was not resolved in either structure. The α-AID, a compact bundle of three α-helices, is so-called because constructs containing the KD and α-AID were found to be 10-fold less active than those containing the KD alone [19,42]. In a truncated KD:AID structure from the fission yeast ortholog, the AID contacts the small and large lobes of the KD, appearing to hold the latter in an inactive conformation [43]. By contrast, in the human α2β1γ1 structure the AID appears to have undergone a rotation such that it interacts with the large lobe and the γ subunit instead. Intriguingly, in this structure the α-linker, which has an extended conformation, wraps around one face of the γ subunit, contacting AMP bound in site 3 (Figure 3, left). A current model to explain allosteric activation, based on a combination of studies [6**,18,44,45], proposes that when ATP (rather then AMP) is bound at site 3 its extra phosphate groups prevent the binding of the α-linker, whose release from site 3 allows the AID to rotate back into its inhibitory position behind the KD. Supporting this, point mutations of conserved residues within the α-linker [44,45], or its replacement by an unrelated shorter linker [9*], yield complexed that, while fully active, are no longer allosterically activated by AMP. Furthermore, Thr172 lies in the cleft between the catalytic and nucleotide-binding modules (Figure 3), where relative movements between the two modules could affect its accessibility to protein phosphatases, potentially explaining the ability of AMP binding to protect against Thr172 dephosphorylation [18]. Such a conformational change would be consistent with previous results from X-ray scattering in solution, which indicated that the α2β2γ1
and α1β1γ1 complexes adopt more compact conformations when they bind AMP [38].

**Negative regulation of AMPK by phosphorylation**

The C-terminal domains of vertebrate α subunit isoforms contain the ‘ST’ loop, a serine/threonine-rich insert of 50–60 amino acids not present in orthologs from most lower eukaryotes [46*]. In the two recent crystal structures the ST loop was either unresolved [6**] or had been replaced by a short artificial linker [9*]. The residues defining the ends of this loop are close to Thr172 (Figure 3, right), and the loop appears to contain several regulatory phosphorylation sites. For example, Ser485 (rat α1 numbering) is phosphorylated by the insulin-activated protein kinase, Akt [47*], or the cyclic AMP-dependent protein kinase, PKA [48*], inhibiting subsequent phosphorylation of Thr172 by either LKB1 or CaMKKβ [46*]. Recent studies suggest this is because the phosphorylated ST loop interacts with basic residues in the KD small lobe, thus physically blocking access to upstream kinases [46*]. AMPK-α2 contains an equivalent conserved residue (Ser491) whose phosphorylation is likely to exert the same inhibitory effect, although Ser491 is a poor substrate for Akt and appears to be modified by autophosphorylation instead [46*]. As discussed above, it has been suggested that phosphorylation of Ser491 by S6K1 explains the inhibitory effects of leptin on AMPK in hypothalamic neurons [36], although our group has been unable to obtain evidence that this site is phosphorylated by S6K1 in HEK-293 cells [46*]. The ST loop can also be phosphorylated at sites N-terminal to Ser485/491 by GSK3, and it has been proposed that this inhibits net Thr172 phosphorylation by promoting dephosphorylation [49]. Finally, PKA can also phosphorylate Ser-173 (adjacent to Thr172 within the activation loop) and this too appears to inhibit Thr172 phosphorylation [50].

**Conclusions and perspectives**

It is becoming clear that AMPK, a signaling protein that may have originally evolved to regulate energy balance in
a cell-autonomous manner, has adapted in multicellular animals to regulate whole-body energy balance. It does this primarily by mediating effects of hormones that act in the hypothalamus to regulate energy intake (ghrelin, leptin, adiponectin) and/or energy expenditure (T3, GLP-1). Given this role, pharmacological activation of AMPK is an obvious target for treatment of metabolic disorders such as obesity, diabetes and cardiovascular disease. As discussed above, several new direct allosteric activators have recently been reported, and the publication of two crystal structures for complete AMPK complexes should aid the further development of such compounds. The finding that LKB1 may activate AMPK at the surface of the lysosome was unexpected, and there are likely to be further developments in this story. Phosphorylation of the ST’ loop is now well established as a means for negative regulation of AMPK, and is likely to be important under physiological conditions.

Acknowledgements
Recent studies in the author’s laboratory have been supported by a Senior Investigator Award from the Wellcome Trust [097726], a Programme Grant from Cancer Research UK [C37038/A15101], and the pharmaceutical companies supporting the Division of Signal Transduction Therapy at the University of Dundee (AstraZeneca, Boehringer-Ingelheim, GlaxoSmithKline, Merck KGaA, Janssen Pharmaceuticals and Pfizer).

References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


6. Xiao B, Sanders MJ, Carmena D, Bright NJ, Haire LF.


- Describes the discovery of a novel, synthetic AMPK activator (MT 63-78 or Debio 0930) which most likely binds at the same site as A-769662; also describes pre-clinical studies suggesting its potential use for treatment of prostate cancer, due to its ability to inhibit fatty acid and cholesterol syntheses.


- Describes the structure of a second heterotrimeric complex of AMPK (α1β1γ1), confirming the binding site for A-769662 and providing insight into its selectivity for β1 complexes.


- Describes a novel AMPK activator, C13, a phosphonate ester that is taken up into cells and converted by cellular esterases into an AMP mimetic.


- Describes another AMPK activator, C14.


- Describes the surprising discovery that AMPK can be fully active in the absence of Thr172 phosphorylation, as long as the AMP and A-769662 binding sites are occupied.


- Provides evidence that activation of AMPK by LKB1 may occur in intact cells on the surface of the lysosome, where the two complexes are recruited by binding to the adapter protein axin, which in turn binds to the lysosomal protein LAMTOR1; also suggests that the system may detect nutrient lack independently of changes in adipine nucleotides.
AMPK — regulation and role in energy homeostasis


Establishes a likely mechanism by which phosphorylation of Ser485 by Akt within the ST loop acts to negatively regulate AMPK.


