Molecular regulation of energy balance

MOLECULAR REGULATION OF ENERGY BALANCE

An article for:

OXFORD RESEARCH ENCYCLOPAEDIA OF NEUROSCIENCE

by

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KEYWORDS: AMPK; appetite; breathing; energy expenditure; oxygen sensing

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Summary

AMP-activated protein kinase (AMPK) is a sensor of cellular energy status that monitors the levels of AMP and ADP relative to ATP. If increases in AMP:ATP and/or ADP:ATP ratios are detected (indicating a reduction in cellular energy status), AMPK is activated by the canonical mechanism involving both allosteric activation and enhanced net phosphorylation at Thr172 on the catalytic subunit. Once activated, AMPK phosphorylates dozens of downstream targets, thus switching on catabolic pathways that generate ATP and switching off anabolic pathways and other energy-consuming processes. AMPK can also be activated by non-canonical mechanisms, triggered either by glucose starvation by a mechanism independent of changes in adenine nucleotides, or by increases in intracellular Ca^{2+} in response to hormones, mediated by the alternate upstream kinase CaMKK2.

AMPK is expressed in almost all eukaryotic cells, including neurons, as heterotrimeric complexes comprising a catalytic α subunit and regulatory β and γ subunits. The α subunits contain the kinase domain and regulatory regions that interact with the other two subunits. The β subunits contain a domain that, with the small lobe of the kinase domain on the α subunit, forms the "ADaM" site that binds synthetic drugs that are potent allosteric activators of AMPK, while the γ subunits contain the binding sites for the classical regulatory nucleotides, AMP, ADP and ATP.

Although much undoubtedly remains to be discovered about the roles of AMPK in the nervous system, emerging evidence has confirmed the proposal (Evans, 2006) that, in addition to its universal functions in regulating energy balance at the cellular level, AMPK also has cell- and circuit-specific roles at the whole body level, particularly in energy homeostasis. These roles are mediated by phosphorylation of neural-specific targets such as ion channels, distinct from the targets by which AMPK regulates general cell-autonomous energy balance. Examples of these cell- and circuit-specific functions discussed in this review include roles in the hypothalamus in balancing energy intake (feeding) and energy expenditure (thermogenesis), and its role in the brainstem where it supports the hypoxic ventilatory response (breathing), increasing the supply of oxygen to the tissues during systemic hypoxia.

Introduction – sensing cellular energy

Energy-requiring processes are usually made possible in living cells by being coupled to the hydrolysis of ATP to ADP or AMP. Under ideal conditions, cells maintain a high ATP:ADP ratio (typically 10:1). Since this is many orders of magnitude away from the equilibrium position for ATP breakdown (ATP → ADP + Pi), it can act as a store of energy, analogous to a fully charged battery. Animal cells maintain a high ATP:ADP ratio primarily by two catabolic processes, i.e. glycolysis and mitochondrial oxidative metabolism, with the latter responsible for the bulk of ATP synthesis, especially in quiescent cells like neurons. This high ATP:ADP ratio is used to drive energy-requiring processes by obligate coupling of the latter to the breakdown of ATP, thus rendering the overall reactions more energetically favourable. Examples of this include the transporters that pump Ca^{2+} ions across the plasma membrane against a concentration gradient [see (1) below] and the enzymes that catalyse the modifications to glucose, amino acids and fatty acids [(2)-(4) below] that are the first steps in their incorporation into their respective macromolecular forms (glycogen, proteins, phospholipids and triglycerides):

$$Ca^{2+}_{(in)} + ATP \rightarrow Ca^{2+}_{(out)} + ADP + Pi \quad (1)$$

$$\text{glucose} + ATP \rightarrow \text{glucose-6-phosphate} + ADP \quad (2)$$

$$\text{amino acid} + \text{tRNA} + ATP \rightarrow \text{amino acyl-tRNA} + AMP + PPi \quad (3)$$

$$\text{fatty acid} + \text{CoASH} + ATP \rightarrow \text{fatty acyl-CoA} + AMP + PPi \quad (4)$$

Note that most of these reactions [e.g. (1) and (2)] generate ADP with or without phosphate (Pi), although a few [e.g. (3) and (4)] generate AMP and pyrophosphate (PPi) instead. If the rate of ATP-consuming reactions exceeds the rate at which ATP can be regenerated by catabolism, then increases in cellular ADP:ATP and/or AMP:ATP ratios will occur, these being key indicators that cellular energy status is compromised. An additional player here is the enzyme *adenylate kinase*, whose almost energy-neutral (and thus reversible) reaction ($2ADP \leftrightarrow ATP + AMP$) appears to be maintained close to equilibrium in almost all eukaryotic cells. The high ATP:ADP ratio in a fully energized cell means that the adenylate kinase reaction would normally run in a leftward direction (towards ADP), thus maintaining AMP at very low levels (if ATP:ADP is 10:1 and the adenylate kinase reaction is at equilibrium, then ATP:AMP will be ≈100:1). However, if ADP rises relative to ATP, the adenylate kinase reaction will be displaced in a rightward direction (towards ATP and

AMP). Thus, increases in cellular ADP will always be accompanied by increases in AMP, and because AMP starts at such a low level any increase will be large, much bigger than the changes in ADP or ATP. In fact, if the adenylate kinase reaction is at equilibrium, the AMP:ATP ratio will vary as the square of the ADP:ATP ratio (Hardie & Hawley, 2001). It is revealing that the small number of metabolic enzymes that directly sense the energy status of cells, including glycogen phosphorylase (Cori, Colowick, & Cori, 1938), 6-phosphofructo-1-kinase (Ramaiah, Hathaway, & Atkinson, 1964) and fructose-1,6-bisphosphatase (Taketa & Pogell, 1965), all appear to primarily sense (like AMPK) AMP and ATP rather than ADP and ATP.

AMPK – multiple subunits and regulation

AMPK occurs universally in eukaryotes as heterotrimeric complexes comprised of a catalytic α subunit and regulatory β and γ subunits (Lin & Hardie, 2017; Ross, MacKintosh, & Hardie, 2016). The α subunit contains at its N-terminal end a serine/threonine kinase domain with a small N-terminal lobe and larger C-terminal lobe, and the catalytic site located in the cleft between them, as is typical of such domains. As in many other protein kinases (Taylor & Kornev, 2011), the C-lobe contains a conserved residue within the "activation loop" that must be phosphorylated to render the kinase active (in AMPK this is a threonine, usually referred to as Thr172 for historical reasons (S. A. Hawley et al., 1996) although its exact numbering depends on the species and isoform). The principal upstream kinase that phosphorylates this site was identified in 2003 to be the tumour suppressor, LKB1 (S.A. Hawley et al., 2003; Shaw et al., 2004; Woods et al., 2003).

AMP had been identified as an allosteric activator of AMPK as early as 1980 (Yeh, Lee, & Kim, 1980). In the presence of a physiologically relevant concentration of ATP (5 mM) allosteric activation of AMPK complexes already phosphorylated on Thr172 can be as much as 10-fold, with a half-maximal effect at just over 100 μM AMP, within the physiological range (Gowans, Hawley, Ross, & Hardie, 2013; Ross, Jensen, & Hardie, 2016). AMP binding to AMPK also makes Thr172 a better substrate for the upstream kinase LKB1 (S. A. Hawley et al., 1995), and a worse substrate for protein phosphatases that dephosphorylate Thr172 (Davies, Helps, Cohen, & Hardie, 1995). There are thus three independent mechanisms by which AMP binding activates the kinase (Fig. 1), with the end result being that any small change in AMP concentration is converted into a much larger change in final output in the form of kinase activity. It has been reported that ADP mimics the

125 effects of AMP on Thr172 phosphorylation (effect #1) (Xiao et al., 2011) and dephosphorylation
126 (effect #2) (Oakhill et al., 2011), although not on allosteric activation (effect #3). However, in our
127 hands this requires concentrations of ADP around 10-fold higher than those of AMP. Although
128 ADP concentrations are indeed often higher than those of AMP, and changes in ADP might
129 therefore contribute to activation, the latter tend to be quite small and we suspect that AMP is the
130 primary activating signal (Gowans et al., 2013).
131 Using the three complementary mechanisms shown in Fig. 1 (which we now refer to as the
132 *canonical pathway* for activation), the activity of AMPK varies markedly as a function of the
133 energy status of cells. However, it has become clear that there are additional, *non-canonical*
134 mechanisms by which the kinase can be activated. In many cell types, including neurons, it can be
135 activated by Thr172 phosphorylation catalysed by the Ca^{2+}/calmodulin-dependent protein kinase,
136 CaMKK2. By this mechanism, hormones that increase intracellular [Ca^{2+}], such as thrombin
137 (Stahmann, Woods, Carling, & Heller, 2006) or vascular endothelial growth factor (Stahmann et al.,
138 2010) acting on endothelial cells, or ghrelin acting on hypothalamic neurons (Yang, Atasoy, Su, &
139 Sternson, 2011) can activate AMPK in the absence of any changes in cellular AMP:ATP ratios.
140 Another non-canonical mechanism occurs in response to glucose deprivation, which is often used as
141 a routine method to activate AMPK in cell culture. Because the original study, which was
142 conducted using insulinoma cells derived from pancreatic β cells, demonstrated that glucose
143 deprivation was associated with increases in cellular AMP:ATP/ADP:ATP ratios (Salt, Johnson,
144 Ashcroft, & Hardie, 1998), it has generally been assumed that activation by glucose deprivation is
145 always mediated by changes in AMP and/or ADP, i.e. by the canonical pathway. However, in some
146 cells (e.g. mouse embryo fibroblasts) as long as an alternate carbon source such as glutamine is
147 present, the removal of glucose from the medium is not associated with any increases in
148 AMP:ATP/ADP:ATP ratios, yet AMPK activation still occurs. Under these circumstances,
149 activation is associated with the formation of what we term a “super-complex” at the lysosomal
150 membrane involving the vacuolar proton pump (v-ATPase), the Ragulator complex containing
151 Lamtor1, and the adapter protein Axin, as well as LKB1 and AMPK (C. S. Zhang et al., 2014).
152 There is now evidence that glucose availability is sensed by the binding of the glycolytic
153 intermediate fructose-1,6-bisphosphate (FBP) to FBP aldolase, which is also associated with the
154 lysosomal v-ATPase. Lack of occupancy of aldolase by FBP appears to cause a change in

conformation of the aldolase:v-ATPase:Ragulator complex, such that the Axin:LKB1 complex is recruited from the cytoplasm. This brings LKB1 into proximity with AMPK, which also appears to translocate to the lysosomal membrane (although some may already be there), leading to Thr172 phosphorylation and activation of AMPK (C. S. Zhang et al., 2017).

AMPK – subunit isoforms and structure

In mammals, each of the three subunits of AMPK occurs as two or three isoforms (α1/α2; β1/β2; γ1/γ2/γ3) encoded by distinct genes *(PRKAA1/2; PRKAB1/2; PRKAG1-3)*. These paralogues appear to have arisen during the two rounds of whole genome duplication that occurred during early evolution of the vertebrates (Ross, MacKintosh, et al., 2016). All possible combinations of α, β and γ subunit can form complexes, so that there are up to twelve different αβγ complexes with subtle variations in their regulatory properties, expressed variably in different cell types (Ross, Jensen, et al., 2016; Ross, MacKintosh, et al., 2016).

Crystal structures of αβγ complexes are available for at least three combinations, i.e. α2β1γ1 (Xiao et al., 2013), α1β2γ1 (Li et al., 2015) and α1β1γ1 (Calabrese et al., 2014)), and a schematic representation of these structures is shown in Fig. 2. They were generated using constructs that were in active conformations with Thr172 phosphorylated and allosteric activators bound, and our understanding of regulatory mechanisms is therefore somewhat hampered by the lack of structures in inactive states. Nevertheless, plausible models for allosteric activation (mechanism #3) and protection against Thr172 dephosphorylation (mechanism #2) by AMP have been suggested. The kinase domain at the N-terminus of the α subunit (α-KD) is immediately followed by a small bundle of three α-helices termed the *auto-inhibitory domain (α-AID)*. This domain received its name because bacterially-expressed constructs containing the α-KD and α-AID were 10-fold less active than constructs containing the α-KD only, even when phosphorylated on Thr172 (Crute, Seefeld, Gamble, Kemp, & Witters, 1998; Goransson et al., 2007; Pang et al., 2007). In the structure of a construct containing just the α-KD and the α-AID (albeit from the fission yeast orthologue of AMPK), the α-AID binds to the "rear" of the α-KD (i.e. opposite side to the active site) interacting with both the N- and C-lobes and clamping the α-KD in an inactive conformation (Chen et al., 2009). However, in the three structures of mammalian heterotrimers, which are all in

183 active conformations, the α-AID appears to have rotated away from the N-lobe such that it now
184 interacts with the C-lobe and the γ subunit instead (Fig. 2).
185 Immediately following the α-AID in the α subunit sequence is the *α-linker*, a region of
186 polypeptide in extended conformation (depicted schematically as a chain in Fig. 2), which connects
187 the α-AID to the globular C-terminal domain (α-CTD). In the three heterotrimer structures, this α-
188 linker loops down over one surface of the nucleotide-binding γ subunit, suggesting that it forms the
189 key interface between the catalytic subunit and the regulatory γ subunit, discussed further below.
190 The β subunits (β1 and β2) contain two conserved domains, a central *carbohydrate-binding*
191 *module (β-CBM)* and a *C-terminal domain (β-CTD),* with the remaining regions (the N-terminal
192 region and the *β-linker* between the CBM and CTD) being either absent or unresolved in the crystal
193 structures. The β-CBM is known to cause a proportion of AMPK to bind to glycogen particles in
194 intact cells (Hudson et al., 2003; Polekhina et al., 2003), although the physiological function of that
195 binding remains uncertain. The β-CBM also forms part of the binding site (the *ADaM site*) for
196 potent allosteric activators of AMPK that are discussed in the next section. The β-CTD lies at the
197 "core" of the αβγ complex and appears to play a key structural role in stabilizing it, forming
198 multiple interactions with both the α-CTD and the γ subunit.
199 The γ subunits (γ1, γ2, γ3) contain the binding sites for the regulatory adenine nucleotides AMP,
200 ADP and ATP (Cheung, Salt, Davies, Hardie, & Carling, 2000; Xiao et al., 2007; Xiao et al., 2011).
201 They contain four tandem repeats (designated *CBS1* through *CBS4*) of a sequence motif termed a
202 *CBS repeat*. These motifs occur in a number of other human proteins, including the enzyme
203 *cystathione-β-synthase* after which they are named (Bateman, 1997). They invariably occur as
204 tandem repeats, although the AMPK-γ subunits are unusual in having four rather than just two
205 repeats. In general, CBS repeats appear to be involved in binding, in the cleft between each repeat
206 pair, regulatory ligands containing adenosine such as ATP (Scott et al., 2004). In the AMPK-γ
207 subunits the four repeats assemble in a pseudosymmetrical manner to form a flattened disk with one
208 repeat in each quadrant, and four potential ligand-binding sites in the centre (Fig. 2). These sites are
209 now numbered by convention (Kemp, Oakhill, & Scott, 2007) according to which repeat binds the
210 adenosine portion of the adenine nucleotide (binding of the phosphate groups can involve residues
211 from more than one repeat). Of the four potential sites only three, i.e. CBS1, CBS3 and CBS4,
212 appear to be utilized for nucleotide binding. The CBS2 site may be unused partly because it lacks a

conserved asparagine that in the other repeats binds to the ribose ring of the bound nucleotide (Xiao et al., 2007), and partly because the entrance to it appears to be blocked by the *β-loop* extending from the β-CTD (Fig. 2). The CBS1 and CBS4 sites are accessible to nucleotides entering from one side of the γ subunit (the far side in Fig. 2) and the CBS3 site from the other (the near side in Fig. 2). Of the three sites, CBS1 and CBS4 appear to constitutively bind ATP and AMP respectively; it may therefore only be at CBS3 that the regulatory nucleotides actually exchange with each other (Gu et al., 2017). It has been suggested that the function of binding of ATP at CBS1 and AMP at CBS4 may be to alter the conformation of the adjacent CBS3 site such that it has a higher affinity for AMP than ADP or ATP, thus allowing AMPK to achieve the difficult task of distinguishing small changes in AMP in the presence of much higher concentrations of ADP and/or ATP (Gu et al., 2017).

Looking at their overall shape, mammalian αβγ heterotrimers consist of two largely distinct globular regions, i.e. the *catalytic module* comprising the α-KD plus the β-CBM, and the *nucleotide-binding module* comprising the α- and β-CTDs plus the γ subunit. Interestingly, in the structures of the active heterotrimers, phospho-Thr172 lies in a deep cleft between these two modules (sandwiched between the C-lobe and the β-CTD, and not visible in Fig. 2), where its accessibility to protein phosphatases may be restricted. The "hinges" that connect the two modules are the α-linker and the unresolved *β-linker* connecting the β-CBM and β-CTD. Although the available crystal structures are all in the active conformation, there is evidence from measurements of small angle X-ray scattering (Riek et al., 2008) and singlet oxygen-mediated luminescence energy transfer (Li et al., 2015) that binding of ATP rather than AMP at site 3 causes these two modules to move apart into a less compact conformation. This is envisaged to occur because the α-linker, which is bound to the surface of site 3 when AMP is bound (Chen et al., 2013; Xiao et al., 2011; Xin, Wang, Zhao, Wang, & Wu, 2013), dissociates from the γ subunit when ATP replaces it. This movement is thought not only to allow the α-AID to move back into its inhibitory position behind the α-KD, but also to expose phospho-Thr172 for more rapid dephosphorylation by protein phosphatases. This model therefore accounts for two of the three effects of AMP binding to AMPK, although how AMP binding promotes phosphorylation of Thr172 by LKB1 is not explained.

Activation of AMPK by compounds that bind in the ADaM site

Because of its effects on metabolism, it was proposed many years ago that activators of AMPK might be useful in treatment of metabolic disorders such as Type 2 diabetes (Winder & Hardie, 1999). The first pharmacological activator of AMPK to be developed was the nucleoside 5-aminoimidazole-4-carboxamide riboside (AICA riboside), which is taken up into cells and converted to the equivalent ribotide, ZMP. ZMP is an AMP analogue that mimics all three effects of AMP on the AMPK system (Corton, Gillespie, Hawley, & Hardie, 1995), and AICA riboside was indeed subsequently shown to have favorable metabolic effects in rodent models of obesity and Type 2 diabetes [e.g. (Song et al., 2000)]. However, AICA riboside has poor oral availability, and ZMP is much less potent as an AMPK activator than AMP itself (Corton et al., 1995). Moreover, ZMP has known "off-target", AMPK-independent effects, including mimicking the regulatory effects of AMP on other AMP-sensitive enzymes such as muscle glycogen phosphorylase (Longnus, Wambolt, Parsons, Brownsey, & Allard, 2003) and liver fructose-1,6-bisphosphatase (Vincent, Marangos, Gruber, & Van den Berghe, 1991). Also, AICA riboside inhibits adenosine re-uptake into cells by nucleoside transporters, which can lead to accumulation of extracellular adenosine and consequent effects mediated by adenosine receptors (Gadalla et al., 2004).

Despite these shortcomings, initial results using AICA riboside may have encouraged pharmaceutical companies to initiate high-throughput screens that searched for novel activators of AMPK. These resulted in several compounds including A-769662 (Cool et al., 2006), MT 63-78 (Zadra et al., 2014), PF-739 and PF-249 (Cokorinos et al., 2017), PF-06409577 (Cameron et al., 2016) and MK-8722 (Myers et al., 2017). Crystal structures of some of these bound to AMPK heterotrimers (Calabrese et al., 2014; Xiao et al., 2013) showed that they bind in a cleft located between the β-CBM and the N-lobe of the α-KD (the *ADaM* site, Fig. 2). Interestingly, almost all of the activators known to bind this site are synthetic molecules, so it currently represents an "orphan receptor" and a key question is whether there are any naturally-occurring ligands that bind there. Most researchers in the field suspect that a natural ligand (most likely a metabolite) must exist, hence the name *Allosteric Drug and Metabolite* (ADaM) site (Langendorf & Kemp, 2015). However, the only naturally-occurring ligand currently known to bind there is salicylate (Calabrese et al., 2014; S. A. Hawley et al., 2012), a natural product of plants that, particularly in the form of extracts of the bark of willow (genus *Salix,* hence the name salicylate), has been used as a medicine

by humans since ancient times. It was of course the starting point for the development of perhaps the most widely used drug of the modern era, i.e. acetyl salicylic acid (ASA or aspirin) (Jeffreys, 2004). The main therapeutic targets of aspirin are thought to be the cyclo-oxygenases involved in biosynthesis of prostaglandins and other prostanoids (Ferreira, Moncada, & Vane, 1971), which are irreversibly inhibited by chemical transfer of its acetyl group to their active sites (Roth, Stanford, & Majerus, 1975). However, aspirin is broken down within minutes of entering the circulation to salicylate, which is then much more stable. Interestingly, salicylate and aspirin appear to be equipotent as anti-inflammatory agents, even though salicylate has very low potency as a cyclo-oxygenase inhibitor (Higgs, Salmon, Henderson, & Vane, 1987). This raises the possibility that some of the therapeutic effects of aspirin might be mediated by AMPK activation rather than cyclo-oxygenase inhibition (Steinberg, Dandapani, & Hardie, 2013).

Roles of AMPK in the central nervous system

Phosphorylation by AMPK of neural-specific targets

It is becoming evident that AMPK phosphorylates, and thus modulates, a variety of targets that fall outside of its originally proposed role in maintenance of metabolic homeostasis (Hardie, Schaffer, & Brunet, 2016). For example, AMPK has been shown not only to phosphorylate and thus *inactivate* the pore-forming α subunits of multiple Ca^{2+}-activated potassium channels ($K_{Ca}1.1$ and $K_{Ca}3.1$) (Klein et al., 2009; Ross et al., 2011), the voltage-gated potassium channel Kv1.5 (Andersen et al., 2015; Mia et al., 2012; Moral-Sanz et al., 2016) and the ATP-inhibited K_{ATP} channel (Kir6.2) (Chang et al., 2009), but also to phosphorylate and *activate* the α subunit of the voltage-gated potassium channel Kv2.1 (Naoko Ikematsu et al., 2011). AMPK therefore has the potential to either increase or decrease cell excitability, in a manner determined by the cell-specific expression of AMPK subunits and of specific members of ion channel superfamilies. Evidence is also now emerging that AMPK may directly phosphorylate and regulate: (i) enzymes involved in the biosynthesis of specific transmitters (Lipton et al., 2001; Murphy, Fakira, Song, Beuve, & Routh, 2009; J. Zhang et al., 2018); (ii) receptors for neurotransmitters (Ahmadi & Roy, 2016); and (3) pumps and transporters (Schneider et al., 2015).

In the discussion of the roles of AMPK in the nervous system that follows, we will restrict our coverage to situations either where the downstream targets for AMPK responsible for the effects are

300 well understood and the critical phosphorylation sites identified, or where the anatomical locations
301 or specific cell types where AMPK plays its role are well-defined. There has been much interest in
302 the role of AMPK in pathological situations in the nervous system, such as in ischaemic stroke and
303 neurodegenerative diseases. However, some of the studies conducted in these areas are difficult to
304 interpret because they were based on use of either the AMPK activator AICA riboside, which has
305 many off-target effects as discussed above, or the kinase inhibitor compound C (dorsomorphin),
306 which has even poorer selectivity for AMPK (Bain et al., 2007). In the limited number of studies
307 where these disorders were studied more specifically using AMPK-α1 or -α2 knockout mice, they
308 were often performed with global rather than cell-type-specific knockouts, once again making the
309 results difficult to interpret. Readers interested in the role of AMPK in ischaemic stroke or
310 neurodegenerative diseases should consult other specialist reviews in those areas, bearing in mind
311 these caveats (Domise & Vingtdeux, 2016; Manwani & McCullough, 2013).

312 ***Regulation of Kv2.1 by AMPK***

313 Of the several K^+ channels that are direct targets for AMPK, Kv2.1 has been particularly well
314 studied at the molecular and cellular levels. Kv2.1 is phosphorylated by AMPK, both in cell-free
315 assays and in intact cells, at two sites within the C-terminal cytoplasmic tail (Ser440 and Ser537)
316 (N. Ikematsu et al., 2011). In HEK-293 cells that were engineered to stably express Kv2.1, AMPK
317 activation using A-769662 caused hyperpolarizing shifts in the current–voltage relationship for
318 channel activation and inactivation, which were almost abolished by single (S440A) and completely
319 abolished by double (S440A/S537A) phosphorylation-resistant mutations. In cells expressing wild
320 type Kv2.1, channel activation was also observed upon the intracellular administration of active
321 AMPK, but not an inactive control, from a patch pipette in whole-cell mode. The AMPK used was
322 a bacterially expressed α2β2γ1 complex that had been activated by thiophosphorylation of Thr172
323 (note that thiophosphorylated, unlike phosphorylated, serine/threonine residues are extremely
324 resistant to dephosphorylation), while the inactive control had a mutation in the kinase domain that
325 abolished kinase activity (N. Ikematsu et al., 2011).

326 Kv2.1 is a voltage-gated, delayed rectifier K^+ channel. Because of its relatively slow opening and
327 closing in response to depolarization, is not thought to be involved in repolarizing neurons after
328 single action potentials, but instead to contribute to adjustments in the firing frequency of action
329 potentials by raising the threshold membrane potential that must be reached before activation of the

330 voltage-gated Na^+ channels that drive action potentials. Accordingly, treatment of primary rat
331 hippocampal neurons in culture with A-769662 caused hyperpolarizing shifts in gating qualitatively
332 similar to those observed in HEK-293 cells expressing Kv2.1. This effect appeared to be mediated
333 by Kv2.1, because it was abolished by allowing an anti-Kv2.1 antibody to diffuse in from the patch
334 pipette. Moreover, administration of active, thiophosphorylated $\alpha2\beta2\gamma1$ complexes via the patch
335 pipette reduced the firing of action potentials in the neurons as predicted, whereas inactive control
336 complexes had no effect (Fig. 3) (N. Ikematsu et al., 2011). Therefore, AMPK not only regulates
337 metabolic homeostasis of neurons, but also neuronal activity.
338 The firing of action potentials, together with downstream postsynaptic events, are estimated to
339 account for up to 80% of all energy turnover in the grey matter of rodent brain (Attwell & Laughlin,
340 2001). Since Kv2.1 is widely expressed, particularly in pyramidal neurons in the hippocampus and
341 cortex (Misonou, Mohapatra, & Trimmer, 2005), its activation by AMPK could be a cell-
342 autonomous mechanism to conserve energy by reducing membrane excitability and firing of action
343 potentials in response to energy stress. It is interesting to speculate that this mechanism might also
344 contribute during sleep, during which central energy reserves are replenished. However, it is now
345 clear that AMPK also has cell- and circuit-specific functions in the nervous system involved in key
346 physiological processes operating at the whole body level, such as balancing energy intake (i.e.
347 feeding) with energy expenditure, and adjustment of oxygen supply from the lungs (i.e. breathing)
348 according to demand. These specific outputs of AMPK, which are discussed next, may be achieved
349 via cell-specific expression of hormone receptors and AMPK subunit isoforms, and of unique sets
350 of AMPK targets, such as specific ion channels. This can give rise to differential sensitivities to
351 stresses such as glucose or oxygen deprivation, or to hormones and neurotransmitters that activate
352 AMPK via the CaMKK2 pathway, according to location.

353 ***The role of hypothalamic AMPK in regulating appetite and feeding behavior***
354 Among the best established roles of AMPK in the nervous system is in the regulation of appetite
355 and food intake. Feeding is known to be promoted by stimulation of neurons located in the arcuate
356 nucleus of the hypothalamus that express agouti-related protein (AGRP) and neuropeptide Y
357 (NPY), and to be inhibited by neurons in the same anatomical location that express pro-
358 opiomelanocortin (POMC) and cocaine-and-amphetamine-regulated transcript (CART).
359 NPY/AgRP neurons increase food intake and decrease energy expenditure by antagonizing POMC

action on melanocortin receptors in neurons of the paraventricular nucleus. The first indication for a role for AMPK came from findings that it was activated in the hypothalamus of rats by treatment *in vivo* with the orexigenic hormone ghrelin, and inhibited by treatment with the anorexigenic hormone leptin (Andersson et al., 2004); other orexigenic mediators, such as cannabinoids, were subsequently found to activate AMPK (Kola et al., 2005). Moreover, injection of the AMPK activator AICA riboside into the hypothalamus led to increases in food intake (Andersson et al., 2004). Although AICA riboside is now known to have many "off-target", AMPK-independent effects (see above), the conclusion that its effects on feeding were AMPK-mediated was strengthened by findings that ectopic expression in mouse hypothalamus of inactive mutants of AMPK-α1 and -α2 (which lack kinase activity, but exert a dominant negative effect by competing with endogenous α subunits for binding to β and γ subunits) repressed food intake and body weight gain. Conversely, expression of an activated γ1 mutant (which behaves as if AMP is bound even when it is not) had the opposite effects. Moreover, leptin was found to decrease the activity of AMPK complexes containing α2, although not α1, in the hypothalamus (Minokoshi et al., 2004). The phenotypes of knocking out AMPK-α2 in AGRP/NPY and POMC/CART neurons were also initially consistent with a role for AMPK in appetite control, with the former being lean while the latter were obese. However, in both cases the effects were rather modest and age-dependent, and there were no detectable changes in food intake in the AGRP/NPY knockouts, while the POMC/CART knockouts still responded normally to leptin treatment *in vivo* in terms of food intake and body weight, and to leptin and insulin (another anorexigenic hormone) in electrophysiological studies (Claret et al., 2007). Interestingly, a proportion of both AGRP/NPY and POMC/CART neurons respond to glucose deprivation by hyperpolarization and a consequent reduction in spike frequency, although in neither case was this evident when AMPK-α2 was knocked out in these neurons. Thus, although AMPK does not appear to be required for the response to leptin and insulin in these specific (AGRP/NPY or POMC/CART) neurons, it does seem to be required for glucose-sensing (Claret et al., 2007). The latter is intriguing given the recent evidence that AMPK can sense glucose via a non-canonical mechanism (C. S. Zhang et al., 2017).

The apparent lack of effect of knocking out AMPK in AGRP/NPY or POMC/CART neurons on food intake (Claret et al., 2007) may be because AMPK is required not in these neurons themselves, but in other neurons acting immediately upstream or downstream. In one very interesting study,

AGRP/NPY neurons were identified by their fluorescence in brain slices derived from transgenic mice expressing a fluorescent protein fused to NPY, and the activity of presynaptic neurons was assessed by measuring *miniature excitatory postsynaptic currents* (mEPCs) in these cells, in the presence of tetrodotoxin to suppress firing of action potentials (Yang et al., 2011). Interestingly, treatment of brain slices from fed mice with the orexigenic hormone ghrelin increased mEPCs in the AGRP/NPY neurons to the level seen in fasted mice. Studies with pharmacological agents suggested that this was mediated by stimulation of the ghrelin receptor Ghsr1, which is coupled to intracellular release of Ca^{2+} via the G protein G_q/G_{11}. This would initiate a Ca^{2+}-dependent activation of AMPK in the presynaptic neurons via the CaMKK2 pathway. Satisfyingly, there was already evidence that CaMKK2 is involved in the response to orexigenic signals, based on studies using the CaMKK2 inhibitor STO-609 and global mouse knockouts of CaMKK2 (Anderson et al., 2008). Although this fascinating study relied heavily on different pharmacological agents, some of which have off-target effects, it does provide a plausible model to explain the role of AMPK in feeding and appetite control in specific neurons of the hypothalamus.

Intriguingly, recent work (Okamoto et al., 2018) shows that expression of constitutively active AMPK in the paraventricular nucleus of the hypothalamus led, in food choice experiments conducted on mice, to a preference for carbohydrate over fat. This effect appeared to be mediated through activation by AMPK of carnitine palmitoyltransferase-1c within a subset of corticotrophin-releasing hormone-positive neurons in the rostral region of the paraventricular nucleus, promoting Ca^{2+}-dependent activation of these neurons. As we will see, regulation of physiological processes by AMPK through such cell- and system-specific outputs is common to other regions of the brain.

The role of hypothalamic AMPK in regulating energy expenditure/thermogenesis

In another region of the hypothalamus, the ventromedial nucleus (VMH), AMPK appears to be involved in the regulation of peripheral energy expenditure rather than energy intake, by regulating the firing of sympathetic nerves that stimulate fatty acid oxidation and hence heat production (thermogenesis) in brown adipose tissue. Thus, the female sex hormone estradiol (Martinez de Morentin et al., 2014) and the thyroid hormone T3 (Lopez et al., 2010) both reduced Thr172 phosphorylation in the VMH, with the latter also decreasing expression of AMPK-α1. This was associated with an increase in activity of sympathetic nerves, increased expression of markers of thermogenesis in brown adipose tissue, and weight loss. Moreover, injection of adenoviruses

expressing an activated mutant of AMPK reduced the weight loss associated with either hormone treatment (Lopez et al., 2010; Martinez de Morentin et al., 2014).

Interestingly, while AMPK deficiency in sympathetic (catecholaminergic) neurons might increase thermogenesis and weight loss (and block the hypoxic ventilatory response, see below), it does not appear to markedly impact systemic arterial blood pressure regulation during normoxia or hypoxia, which is determined in great part by increased sympathetic outflow (MacMillan & Evans, 2018). The only way to reconcile this is if pathways leading to sympathetic control of thermogenesis differ from those involved in control of blood pressure, another example of cell- and system-specific outputs.

The role of AMPK in regulating breathing and oxygen supply

Recent studies have confirmed earlier suggestions (Evans, 2006) that AMPK is critical for the regulation of central respiratory networks that accelerate breathing during hypoxia, and thus increase the supply of oxygen to cells throughout the body, thus promoting ATP generation (Mahmoud et al., 2015). The hypoxic ventilatory response (HVR), i.e. increased breathing in response to hypoxia, is driven by afferent inputs from oxygen-sensitive chemoreceptors to the central respiratory pattern generators of the brainstem, that are distributed bilaterally in the ventrolateral medulla and generate ventilatory rhythm (Evans, Mahmoud, Moral-Sanz, & Hartmann, 2016; Smith, Ellenberger, Ballanyi, Richter, & Feldman, 1991). The HVR supports, for example, increased oxygen supply during ascent to altitude and maintenance of ventilatory function as we sleep.

AMPK is intimately coupled to mitochondrial metabolism through cellular AMP/ATP and ADP/ATP ratios, and mitochondria of oxygen-sensing cells appear to be uniquely sensitive to changes in oxygen supply because they express isoforms of cytochrome C oxidase whose kinetics respond to changes in PO_2 within the physiological range (Mills & Jobsis, 1970, 1972). This is due to the constitutive expression (Aras et al., 2013; Huttemann et al., 2012; Zhou, Chien, Kaleem, & Matsunami, 2016) of two nuclear-encoded, atypical subunits of the mitochondrial electron transport chain, NDUFA4L2 (Tello et al., 2011) and COX4I2 (Fukuda et al., 2007; Huttemann, Kadenbach, & Grossman, 2001). This contrasts with other cell types, where expression of NDUFA4L2 and COX4I2 expression are generally low, although they increase during prolonged hypoxia (Fukuda et al., 2007; Huttemann et al., 2001). Constitutive expression of NDUFA4L2 and COX4I2 in oxygen-

sensing cells confers, in part, their capacity to monitor changes in oxygen supply (Sommer et al., 2017), because allosteric modulation of cytochrome C oxidase is mediated in a subtype-specific manner, with COX4I1 but not COX4I2 conferring inhibition by ATP (Horvat, Beyer, & Arnold, 2006; Huttemann et al., 2001). When COX4I2 is expressed, the rate of oxygen consumption (and hence mitochondrial ATP synthesis) will therefore not increase as ATP levels fall during hypoxia (Aras et al., 2013; Fukuda et al., 2007; Horvat et al., 2006; Kocha et al., 2015). The consequent fall in ATP will in turn cause increases in the AMP:ATP ratio via the adenylate kinase reaction, thus activating AMPK.

Consistent with a role for AMPK in regulating the response of oxygen-sensing cells that adjust breathing patterns during hypoxia, the HVR is markedly attenuated by conditional deletion of both AMPK-α1 and -α2 using Cre recombinase expression from the tyrosine hydroxylase promoter (in fact, exposure of these mice to hypoxia triggers hypo-ventilation and apnoea (cessation of breathing), rather than hyper-ventilation as in the wild type) (Mahmoud et al., 2015). Note that catecholaminergic cells expressing tyrosine hydroxylase span the entire respiratory network, including the type I cells of carotid bodies as well as the brainstem.

The primary peripheral arterial chemoreceptors of mammals are the carotid bodies, of which the type I cells represent the archetypal oxygen-sensing cells. The general consensus has been that it is the afferent input responses of carotid bodies that deliver the entire ventilatory response to falls in arterial PO_2 (Prabhakar, 2000). Challenging this, however, AMPK deletion attenuated the HVR during mild and severe hypoxia without affecting these afferent input responses. This is consistent with findings that two compounds that activate AMPK via different mechanisms, i.e. AICA riboside and A-769662 (see above), do not precisely mimic the effects of hypoxia or induce pronounced activation of carotid body type I cells (Kim, Kang, Martin, Kim, & Carroll, 2014). Thus, peripheral chemosensors are not the sole arbiters of the HVR. This has also been suggested by investigations on the evolution of ventilatory control systems. Intriguingly, oxygen-sensing (and a component of the HVR) occurs at the level of the caudal brainstem in amphibians, in which both the location and influence of the primary peripheral chemosensors changes during metamorphosis from gill-breathing tadpole to lung-assisted, air-breathing adult (Porteus, Hedrick, Hicks, Wang, & Milsom, 2011). It has been proposed (Evans et al., 2016) that evolution periodically led to the reconfiguration of peripheral chemoreceptor inputs (Porteus et al., 2011) about a common, ancestral

sensor of hypoxia within the caudal brainstem, which effects signal integration and thus acts as the gatekeeper of respiratory adjustments during hypoxia. In short, the HVR may be determined by the coordinated action of the carotid body and a hypoxia-responsive circuit within the brainstem (Evans et al., 2016; Gourine & Funk, 2017; Mahmoud et al., 2015; Teppema & Dahan, 2010).

Until recently, little emphasis has been placed on the role of hypoxia-sensing by the brainstem, perhaps because the HVR is so effectively abolished by resection of the carotid sinus nerve in humans (Wade, Larson, Hickey, Ehrenfeld, & Severinghaus, 1970). However, brainstem hypoxia induces an HVR even when it receives normoxic carotid body afferent inputs (Curran et al., 2000), and directly activates subsets of catecholaminergic neurons within the nucleus tractus solitarius (NTS) and rostral ventrolateral medulla that may support partial recovery of the HVR, in a variety of animal models (Teppema & Dahan, 2010). Consistent with the effect of AMPK deletion on the HVR, dysfunction of these neurons precipitates the hypoventilation and apnoea associated with Rett syndrome, which is exacerbated during hypoxia (Roux & Villard, 2010). Moreover, it is evident that COX4I2 may, as in carotid body type I cells, be constitutively expressed by certain CNS neurons (Horvat et al., 2006), rendering mitochondrial oxidative phosphorylation sensitive to falls in local PO_2. AMPK activation could thus be triggered in a specialised subset of brainstem neurons during hypoxia to support the delivery of increased respiratory drive, required to protect against hypoventilation and apnoea. Supporting this, examination of brainstem function in AMPK-α1/α2 knockout mice by functional magnetic resonance imaging (fMRI) identified reduced activation of discrete dorsal and ventral nuclei of the caudal brainstem, despite the fact that carotid body afferent input responses were retained (Mahmoud et al., 2015). The location of the dorsal nucleus aligns with areas of the NTS that are activated during hypoxia, and represents the primary site of receipt of carotid body afferent inputs (Teppema & Dahan, 2010), well placed for signal integration. Here AMPK may attenuate activation during hypoxia of C2 (adrenergic) neurons and/or A2 (noradrenergic) neurons proximal to the midline and the area postrema (Mahmoud et al., 2015). Notably, A2 neurons provide afferent inputs to determine, together with the carotid body, activation by hypoxia of A1/C1 neurons within the ventrolateral medulla (Guyenet, 2014), the position of which aligns well with the ventral active region identified by fMRI analysis (Mahmoud et al., 2015). Through these projections of the NTS, AMPK could thus support the HVR (Evans et al., 2016) by either indirect or direct modulation of the respiratory central pattern generators (Smith,

Abdala, Borgmann, Rybak, & Paton, 2013; Smith et al., 1991) and by coordinating functional hyperaemia (Bucher et al., 2014). Because carotid body afferent discharge remains exquisitely sensitive to falls in PO_2, and ventilatory responses to hypercapnia remained unaffected even during severe hypoxia, it is unlikely that AMPK deficiency compromises the capacity during hypoxia either for activation of the peripheral carotid body type I cells or brainstem catecholaminergic neurons that govern the ventilatory response to hypercapnia, for exocytosis or for effective delivery of increased respiratory drive (Evans et al., 2016). Therefore, the mechanism by which AMPK supports the HVR is most likely neurogenic and highly localised, since AMPK deficiency in smooth muscles does not affect the HVR or systemic arterial blood pressure regulation during hypoxia, while the latter (but not the former) remains unaltered following AMPK deletion in catecholaminergic neurons (MacMillan & Evans, 2018). This represents a further example of cell- and system-specific actions of AMPK.

Accepting the possibility of a role in signal integration at the NTS, the working hypothesis (Fig. 4) is that AMPK activation by the canonical, AMP/ADP-dependent pathway may provide the capacity for sensing local hypoxic stress, i.e. decreased ATP supply, in a manner that could be effectively coupled to increased ATP usage consequent to afferent inputs from peripheral chemoreceptors to the NTS, thus increasing overall ATP demand in the receiving, activated neurons (Evans et al., 2016). Intriguingly. it is AMPK-α1 but not AMPK-α2 that supports the HVR (Mahmoud et al., 2016). Relevant to this, in high-altitude populations living in the Andes the gene encoding AMPK-α1 *(PRKAA1)* appears to have been influenced by natural selection through single nucleotide polymorphisms (Bigham et al., 2014).

Conclusions and perspectives

AMPK is almost universally expressed in cells of both unicellular and multicellular eukaryotes (Lin & Hardie, 2017). In all cells, it appears to serve the cell-autonomous role of adjusting function to ensure that the supply and demand for ATP are matched, even during fluctuations in cellular energy status and/or the availability of nutrients such as glucose. However, as multicellular organisms evolved, the roles of AMPK appear to have diversified so that it also came to serve both cell- and system-specific roles in whole body physiology; examples of this (summarized in Fig. 5) include its roles in the hypothalamus in adjusting whole body energy balance by regulating energy intake

(appetite/feeding) and expenditure, and its role in regulating ventilation of the lung during hypoxia. Mammalian AMPK is able to serve both cell-autonomous and systemic roles, in part because it exists as heterotrimers formed by up to twelve combinations of α, β and γ subunit isoforms, thus generating much potential diversity in subcellular location and function. The multiple subunit isoforms that allow this diversity appear to have arisen during the two rounds of whole genome duplication that occurred during the early development of vertebrates, with signaling proteins like AMPK being highly enriched among those genes where multiple subunit isoforms have been retained (Ross, MacKintosh, et al., 2016). During evolution, the AMPK system has also been able to serve new cell- and system-specific roles by acquiring the ability to phosphorylate and regulate cell-specific proteins, such as the ion channels, receptors and transporters that are found in neural tissue.

Acknowledgements

Recent studies in the DGH laboratory have been supported by the Wellcome Trust (204766/Z/16/Z) and Cancer Research UK (C37030/A15101). Recent studies in the AME laboratory have been supported by a British Heart Foundation Programme Grant (29885) and a Wellcome Trust Programme Grant (081195).

References

Ahmadi, M., & Roy, R. (2016). AMPK acts as a molecular trigger to coordinate glutamatergic signals and adaptive behaviours during acute starvation. *Elife, 5*. doi:10.7554/eLife.16349

Andersen, M. N., Skibsbye, L., Tang, C., Petersen, F., MacAulay, N., Rasmussen, H. B., & Jespersen, T. (2015). PKC and AMPK regulation of Kv1.5 potassium channels. *Channels (Austin), 9*(3), 121-128. doi:10.1080/19336950.2015.1036205

Anderson, K. A., Ribar, T. J., Lin, F., Noeldner, P. K., Green, M. F., Muehlbauer, M. J., . . . Means, A. R. (2008). Hypothalamic CaMKK2 contributes to the regulation of energy balance. *Cell Metab., 7*(5), 377-388. doi:10.1016/j.cmet.2008.02.011

Andersson, U., Filipsson, K., Abbott, C. R., Woods, A., Smith, K., Bloom, S. R., . . . Small, C. J. (2004). AMP-activated protein kinase plays a role in the control of food intake. *J. Biol. Chem., 279*(13), 12005-12008.

Aras, S., Pak, O., Sommer, N., Finley, R., Jr., Huttemann, M., Weissmann, N., & Grossman, L. I. (2013). Oxygen-dependent expression of cytochrome c oxidase subunit 4-2 gene expression is mediated by transcription factors RBPJ, CXXC5 and CHCHD2. *Nucleic Acids Res, 41*(4), 2255-

2266. doi:10.1093/nar/gks1454
Attwell, D., & Laughlin, S. B. (2001). An energy budget for signaling in the grey matter of the brain. *J. Cereb. Blood Flow Metab., 21*(10), 1133-1145. doi:10.1097/00004647-200110000-00001
Bain, J., Plater, L., Elliott, M., Shpiro, N., Hastie, C. J., McLauchlan, H., . . . Cohen, P. (2007). The selectivity of protein kinase inhibitors: a further update. *Biochem J, 408*(3), 297-315.
Bateman, A. (1997). The structure of a domain common to archaebacteria and the homocystinuria disease protein. *Trends Biochem Sci, 22*(1), 12-13.
Bigham, A. W., Julian, C. G., Wilson, M. J., Vargas, E., Browne, V. A., Shriver, M. D., & Moore, L. G. (2014). Maternal PRKAA1 and EDNRA genotypes are associated with birth weight, and PRKAA1 with uterine artery diameter and metabolic homeostasis at high altitude. *Physiol Genomics, 46*(18), 687-697. doi:10.1152/physiolgenomics.00063.2014
Bucher, E. S., Fox, M. E., Kim, L., Kirkpatrick, D. C., Rodeberg, N. T., Belle, A. M., & Wightman, R. M. (2014). Medullary norepinephrine neurons modulate local oxygen concentrations in the bed nucleus of the stria terminalis. *J Cereb Blood Flow Metab, 34*(7), 1128-1137. doi:10.1038/jcbfm.2014.60
Calabrese, M. F., Rajamohan, F., Harris, M. S., Caspers, N. L., Magyar, R., Withka, J. M., . . . Kurumbail, R. G. (2014). Structural basis for AMPK activation: natural and synthetic ligands regulate kinase activity from opposite poles by different molecular mechanisms. *Structure, 22*(8), 1161-1172. doi:10.1016/j.str.2014.06.009
Cameron, K. O., Kung, D. W., Kalgutkar, A. S., Kurumbail, R. G., Miller, R., Salatto, C. T., . . . Xiao, J. (2016). Discovery and preclinical characterization of 6-chloro-5-[4-(1-hydroxycyclobutyl)phenyl]-1H-indole-3-carboxylic Acid (PF-06409577), a direct activator of adenosine monophosphate-activated protein kinase (AMPK), for the potential treatment of diabetic nephropathy. *J. Med. Chem., 59*(17), 8068-8081. doi:10.1021/acs.jmedchem.6b00866
Chang, T. J., Chen, W. P., Yang, C., Lu, P. H., Liang, Y. C., Su, M. J., . . . Chuang, L. M. (2009). Serine-385 phosphorylation of inwardly rectifying K+ channel subunit (Kir6.2) by AMP-dependent protein kinase plays a key role in rosiglitazone-induced closure of the K(ATP) channel and insulin secretion in rats. *Diabetologia, 52*(6), 1112-1121. doi:10.1007/s00125-009-1337-4
Chen, L., Jiao, Z. H., Zheng, L. S., Zhang, Y. Y., Xie, S. T., Wang, Z. X., & Wu, J. W. (2009). Structural insight into the autoinhibition mechanism of AMP-activated protein kinase. *Nature, 459*(7250), 1146-1149. doi:nature08075 [pii] 10.1038/nature08075
Chen, L., Xin, F. J., Wang, J., Hu, J., Zhang, Y. Y., Wan, S., . . . Wu, J. W. (2013). Conserved regulatory elements in AMPK. *Nature, 498*(7453), E8-E10. doi:10.1038/nature12189
Cheung, P. C. F., Salt, I. P., Davies, S. P., Hardie, D. G., & Carling, D. (2000). Characterization of AMP-activated protein kinase g subunit isoforms and their role in AMP binding. *Biochem. J., 346*, 659-669.
Claret, M., Smith, M. A., Batterham, R. L., Selman, C., Choudhury, A. I., Fryer, L. G., . . . Withers, D. J. (2007). AMPK is essential for energy homeostasis regulation and glucose sensing by

611 POMC and AgRP neurons. *J. Clin. Invest., 117*(8), 2325-2336.
612 Cokorinos, E. C., Delmore, J., Reyes, A. R., Albuquerque, B., Kjobsted, R., Jorgensen, N. O., . . .
613 Miller, R. A. (2017). Activation of skeletal muscle AMPK promotes glucose disposal and
614 glucose lowering in non-human primates and mice. *Cell Metab., 25*(5), 1147-1159 e1110.
615 doi:10.1016/j.cmet.2017.04.010
616 Cool, B., Zinker, B., Chiou, W., Kifle, L., Cao, N., Perham, M., . . . Frevert, E. (2006).
617 Identification and characterization of a small molecule AMPK activator that treats key
618 components of type 2 diabetes and the metabolic syndrome. *Cell Metab., 3*(6), 403-416.
619 Cori, G. T., Colowick, S. P., & Cori, C. F. (1938). The action of nucleotides in the disruptive
620 phosphorylation of glycogen. *J. Biol. Chem., 123*, 381-389.
621 Corton, J. M., Gillespie, J. G., Hawley, S. A., & Hardie, D. G. (1995). 5-Aminoimidazole-4-
622 carboxamide ribonucleoside: a specific method for activating AMP-activated protein kinase in
623 intact cells? *Eur. J. Biochem., 229*, 558-565.
624 Crute, B. E., Seefeld, K., Gamble, J., Kemp, B. E., & Witters, L. A. (1998). Functional domains of
625 the alpha1 catalytic subunit of the AMP-activated protein kinase. *J. Biol. Chem., 273*(52),
626 35347-35354.
627 Curran, A. K., Rodman, J. R., Eastwood, P. R., Henderson, K. S., Dempsey, J. A., & Smith, C. A.
628 (2000). Ventilatory responses to specific CNS hypoxia in sleeping dogs. *J Appl Physiol (1985),*
629 *88*(5), 1840-1852.
630 Davies, S. P., Helps, N. R., Cohen, P. T. W., & Hardie, D. G. (1995). 5'-AMP inhibits
631 dephosphorylation, as well as promoting phosphorylation, of the AMP-activated protein kinase.
632 Studies using bacterially expressed human protein phosphatase-2Ca and native bovine protein
633 phosphatase-$2A_C$. *FEBS Lett., 377*, 421-425.
634 Domise, M., & Vingtdeux, V. (2016). AMPK in neurodegenerative diseases. *Experientia, 107*, 153-
635 177. doi:10.1007/978-3-319-43589-3_7
636 Evans, A. M. (2006). AMP-activated protein kinase and the regulation of Ca2+ signalling in O2-
637 sensing cells. *J Physiol, 574*(Pt 1), 113-123.
638 Evans, A. M., Mahmoud, A. D., Moral-Sanz, J., & Hartmann, S. (2016). The emerging role of
639 AMPK in the regulation of breathing and oxygen supply. *Biochem J, 473*(17), 2561-2572.
640 doi:10.1042/BCJ20160002
641 Ferreira, S. H., Moncada, S., & Vane, J. R. (1971). Indomethacin and aspirin abolish prostaglandin
642 release from the spleen. *Nat. New Biol., 231*(25), 237-239.
643 Fukuda, R., Zhang, H., Kim, J. W., Shimoda, L., Dang, C. V., & Semenza, G. L. (2007). HIF-1
644 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells.
645 *Cell, 129*(1), 111-122. doi:10.1016/j.cell.2007.01.047
646 Gadalla, A. E., Pearson, T., Currie, A. J., Dale, N., Hawley, S. A., Sheehan, M., . . . Frenguelli, B.
647 G. (2004). AICA riboside both activates AMP-activated protein kinase and competes with
648 adenosine for the nucleoside transporter in the CA1 region of the rat hippocampus. *J.*
649 *Neurochem., 88*(5), 1272-1282.
650 Goransson, O., McBride, A., Hawley, S. A., Ross, F. A., Shpiro, N., Foretz, M., . . . Sakamoto, K.

(2007). Mechanism of action of A-769662, a valuable tool for activation of AMP-activated protein kinase. *J. Biol. Chem., 282*(45), 32549-32560.

Gourine, A. V., & Funk, G. D. (2017). On the existence of a central respiratory oxygen sensor. *J Appl Physiol (1985)*, jap 00194 02017. doi:10.1152/japplphysiol.00194.2017

Gowans, G. J., Hawley, S. A., Ross, F. A., & Hardie, D. G. (2013). AMP is a true physiological regulator of AMP-activated protein kinase by both allosteric activation and enhancing net phosphorylation. *Cell Metab., 18*(4), 556-566. doi:10.1016/j.cmet.2013.08.019

Gu, X., Yan, Y., Novick, S. J., Kovich, A., Goswami, D., Ke, J., . . . Melcher, K. (2017). Deconvoluting AMP-dependent kinase (AMPK) adenine nucleotide binding and sensing. *J. Biol. Chem.* doi:10.1074/jbc.M117.793018

Guyenet, P. G. (2014). Regulation of breathing and autonomic outflows by chemoreceptors. *Compr Physiol, 4*(4), 1511-1562. doi:10.1002/cphy.c140004

Hardie, D. G., & Hawley, S. A. (2001). AMP-activated protein kinase: the energy charge hypothesis revisited. *BioEssays, 23*, 1112-1119.

Hardie, D. G., Schaffer, B. E., & Brunet, A. (2016). AMPK: an energy-sensing pathway with multiple inputs and outputs. *Trends Cell Biol., 26*(3), 190-201. doi:10.1016/j.tcb.2015.10.013

Hawley, S. A., Boudeau, J., Reid, J. L., Mustard, K. J., Udd, L., Makela, T. P., . . . Hardie, D. G. (2003). Complexes between the LKB1 tumor suppressor, STRADa/b and MO25a/b are upstream kinases in the AMP-activated protein kinase cascade. *J. Biol., 2*(4), 28. doi:10.1186/1475-4924-2-28

Hawley, S. A., Davison, M., Woods, A., Davies, S. P., Beri, R. K., Carling, D., & Hardie, D. G. (1996). Characterization of the AMP-activated protein kinase kinase from rat liver and identification of threonine 172 as the major site at which it phosphorylates AMP-activated protein kinase. *J. Biol. Chem., 271*(44), 27879-27887.

Hawley, S. A., Fullerton, M. D., Ross, F. A., Schertzer, J. D., Chevtzoff, C., Walker, K. J., . . . Hardie, D. G. (2012). The ancient drug salicylate directly activates AMP-activated protein kinase. *Science, 336*(6083), 918-922. doi:10.1126/science.1215327

Hawley, S. A., Selbert, M. A., Goldstein, E. G., Edelman, A. M., Carling, D., & Hardie, D. G. (1995). 5'-AMP activates the AMP-activated protein kinase cascade, and Ca2+/calmodulin activates the calmodulin-dependent protein kinase I cascade, via three independent mechanisms. *J. Biol. Chem., 270*(45), 27186-27191.

Higgs, G. A., Salmon, J. A., Henderson, B., & Vane, J. R. (1987). Pharmacokinetics of aspirin and salicylate in relation to inhibition of arachidonate cyclooxygenase and antiinflammatory activity. *Proc. Natl. Acad. Sci. USA, 84*(5), 1417-1420.

Horvat, S., Beyer, C., & Arnold, S. (2006). Effect of hypoxia on the transcription pattern of subunit isoforms and the kinetics of cytochrome c oxidase in cortical astrocytes and cerebellar neurons. *J Neurochem, 99*(3), 937-951. doi:10.1111/j.1471-4159.2006.04134.x

Hudson, E. R., Pan, D. A., James, J., Lucocq, J. M., Hawley, S. A., Green, K. A., . . . Hardie, D. G. (2003). A novel domain in AMP-activated protein kinase causes glycogen storage bodies similar to those seen in hereditary cardiac arrhythmias. *Curr. Biol., 13*(10), 861-866.

Huttemann, M., Kadenbach, B., & Grossman, L. I. (2001). Mammalian subunit IV isoforms of cytochrome c oxidase. *Gene, 267*(1), 111-123.

Huttemann, M., Lee, I., Gao, X., Pecina, P., Pecinova, A., Liu, J., . . . Grossman, L. I. (2012). Cytochrome c oxidase subunit 4 isoform 2-knockout mice show reduced enzyme activity, airway hyporeactivity, and lung pathology. *FASEB J, 26*(9), 3916-3930. doi:10.1096/fj.11-203273

Ikematsu, N., Dallas, M. L., Ross, F. A., Lewis, R. W., Rafferty, J. N., David, J. A., . . . Evans, A. M. (2011). Phosphorylation of the voltage-gated potassium channel Kv2.1 by AMP-activated protein kinase regulates membrane excitability. *Proceedings of the National Academy of Sciences, 108*(44), 18132-18137. doi:10.1073/pnas.1106201108

Ikematsu, N., Dallas, M. L., Ross, F. A., Lewis, R. W., Rafferty, J. N., David, J. A., . . . Evans, A. M. (2011). Phosphorylation of the voltage-gated potassium channel Kv2.1 by AMP-activated protein kinase regulates membrane excitability. *Proc. Natl. Acad. Sci. USA, 108*(44), 18132-18137. doi:1106201108 [pii] 10.1073/pnas.1106201108

Jeffreys, D. (2004). *Aspirin: the remarkable story of a wonder drug*. London: Bloomsbury Publishing.

Kemp, B. E., Oakhill, J. S., & Scott, J. W. (2007). AMPK structure and regulation from three angles. *Structure, 15*(10), 1161-1163.

Kim, D., Kang, D., Martin, E. A., Kim, I., & Carroll, J. L. (2014). Effects of modulators of AMP-activated protein kinase on TASK-1/3 and intracellular Ca(2+) concentration in rat carotid body glomus cells. *Respir Physiol Neurobiol, 195*, 19-26. doi:10.1016/j.resp.2014.01.020

Klein, H., Garneau, L., Trinh, N. T., Prive, A., Dionne, F., Goupil, E., . . . Sauve, R. (2009). Inhibition of the KCa3.1 channels by AMP-activated protein kinase in human airway epithelial cells. *Am J Physiol Cell Physiol, 296*(2), C285-295. doi:10.1152/ajpcell.00418.2008

Kocha, K. M., Reilly, K., Porplycia, D. S., McDonald, J., Snider, T., & Moyes, C. D. (2015). Evolution of the oxygen sensitivity of cytochrome c oxidase subunit 4. *Am J Physiol Regul Integr Comp Physiol, 308*(4), R305-320. doi:10.1152/ajpregu.00281.2014

Kola, B., Hubina, E., Tucci, S. A., Kirkham, T. C., Garcia, E. A., Mitchell, S. E., . . . Korbonits, M. (2005). Cannabinoids and ghrelin have both central and peripheral metabolic and cardiac effects via AMP-activated Protein Kinase. *J. Biol. Chem., 280*(26), 25196-25201.

Langendorf, C. G., & Kemp, B. E. (2015). Choreography of AMPK activation. *Cell Res., 25*(1), 5-6. doi:10.1038/cr.2014.163

Li, X., Wang, L., Zhou, X. E., Ke, J., de Waal, P. W., Gu, X., . . . Melcher, K. (2015). Structural basis of AMPK regulation by adenine nucleotides and glycogen. *Cell Res., 25*(1), 50-66. doi:10.1038/cr.2014.150

Lin, S. C., & Hardie, D. G. (2017). AMPK: sensing glucose as well as cellular energy status. *Cell Metab., 27*, 299-313. doi:10.1016/j.cmet.2017.10.009

Lipton, A. J., Johnson, M. A., Macdonald, T., Lieberman, M. W., Gozal, D., & Gaston, B. (2001). S-nitrosothiols signal the ventilatory response to hypoxia. *Nature, 413*(6852), 171-174. doi:10.1038/35093117

Longnus, S. L., Wambolt, R. B., Parsons, H. L., Brownsey, R. W., & Allard, M. F. (2003). 5-

731 Aminoimidazole-4-carboxamide 1-beta -D-ribofuranoside (AICAR) stimulates myocardial
732 glycogenolysis by allosteric mechanisms. *Am. J. Physiol., 284*(4), R936-R944.
733 Lopez, M., Varela, L., Vazquez, M. J., Rodriguez-Cuenca, S., Gonzalez, C. R., Velagapudi, V. R., .
734 . . Vidal-Puig, A. (2010). Hypothalamic AMPK and fatty acid metabolism mediate thyroid
735 regulation of energy balance. *Nat. Med., 16*(9), 1001-1008. doi:nm.2207 [pii] 10.1038/nm.2207
736 MacMillan, S., & Evans, A. M. (2018). AMPK-alpha1 or AMPK-alpha2 Deletion in Smooth
737 Muscles Does Not Affect the Hypoxic Ventilatory Response or Systemic Arterial Blood Pressure
738 Regulation During Hypoxia. *Front Physiol, 9*, 655. doi:10.3389/fphys.2018.00655
739 Mahmoud, A. D., Lewis, S., Juricic, L., Udoh, U. A., Hartmann, S., Jansen, M. A., . . . Evans, A.
740 M. (2015). AMPK Deficiency Blocks the Hypoxic Ventilatory Response and Thus Precipitates
741 Hypoventilation and Apnea. *Am J Respir Crit Care Med*. doi:10.1164/rccm.201508-1667OC
742 Mahmoud, A. D., Lewis, S., Juricic, L., Udoh, U. A., Hartmann, S., Jansen, M. A., . . . Evans, A.
743 M. (2016). AMP-activated Protein Kinase Deficiency Blocks the Hypoxic Ventilatory Response
744 and Thus Precipitates Hypoventilation and Apnea. *Am J Respir Crit Care Med, 193*(9), 1032-
745 1043. doi:10.1164/rccm.201508-1667OC
746 Manwani, B., & McCullough, L. D. (2013). Function of the master energy regulator adenosine
747 monophosphate-activated protein kinase in stroke. *Journal of neuroscience research*.
748 doi:10.1002/jnr.23207
749 Martinez de Morentin, P. B., Gonzalez-Garcia, I., Martins, L., Lage, R., Fernandez-Mallo, D.,
750 Martinez-Sanchez, N., . . . Lopez, M. (2014). Estradiol regulates brown adipose tissue
751 thermogenesis via hypothalamic AMPK. *Cell Metab., 20*(1), 41-53.
752 doi:10.1016/j.cmet.2014.03.031
753 Mia, S., Munoz, C., Pakladok, T., Siraskar, G., Voelkl, J., Alesutan, I., & Lang, F. (2012).
754 Downregulation of Kv1.5 K channels by the AMP-activated protein kinase. *Cell Physiol*
755 *Biochem, 30*(4), 1039-1050. doi:10.1159/000341480
756 Mills, E., & Jobsis, F. F. (1970). Simultaneous measurement of cytochrome a3 reduction and
757 chemoreceptor afferent activity in the carotid body. *Nature, 225*(5238), 1147-1149.
758 Mills, E., & Jobsis, F. F. (1972). Mitochondrial respiratory chain of carotid body and
759 chemoreceptor response to changes in oxygen tension. *J Neurophysiol, 35*(4), 405-428.
760 Minokoshi, Y., Alquier, T., Furukawa, N., Kim, Y. B., Lee, A., Xue, B., . . . Kahn, B. B. (2004).
761 AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the
762 hypothalamus. *Nature, 428*(6982), 569-574.
763 Misonou, H., Mohapatra, D. P., & Trimmer, J. S. (2005). Kv2.1: a voltage-gated k+ channel critical
764 to dynamic control of neuronal excitability. *Neurotoxicology, 26*(5), 743-752.
765 Moral-Sanz, J., Mahmoud, A. D., Ross, F. A., Eldstrom, J., Fedida, D., Hardie, D. G., & Evans, A.
766 M. (2016). AMP-activated protein kinase inhibits Kv 1.5 channel currents of pulmonary arterial
767 myocytes in response to hypoxia and inhibition of mitochondrial oxidative phosphorylation. *J*
768 *Physiol, 594*(17), 4901-4915. doi:10.1113/JP272032
769 Murphy, B. A., Fakira, K. A., Song, Z., Beuve, A., & Routh, V. H. (2009). AMP-activated protein
770 kinase and nitric oxide regulate the glucose sensitivity of ventromedial hypothalamic glucose-

771 inhibited neurons. *Am J Physiol Cell Physiol, 297*(3), C750-758. doi:10.1152/ajpcell.00127.2009
772 Myers, R. W., Guan, H. P., Ehrhart, J., Petrov, A., Prahalada, S., Tozzo, E., . . . Sebhat, I. K. (2017).
773 Systemic pan-AMPK activator MK-8722 improves glucose homeostasis but induces cardiac
774 hypertrophy. *Science, 357*(6350), 507-511. doi:10.1126/science.aah5582
775 Oakhill, J. S., Steel, R., Chen, Z. P., Scott, J. W., Ling, N., Tam, S., & Kemp, B. E. (2011). AMPK
776 is a direct adenylate charge-regulated protein kinase. *Science, 332*(6036), 1433-1435.
777 doi:10.1126/science.1200094
778 Okamoto, S., Sato, T., Tateyama, M., Kageyama, H., Maejima, Y., Nakata, M., . . . Minokoshi, Y.
779 (2018). Activation of AMPK-regulated CRH neurons in the PVH is sufficient and necessary to
780 induce dietary preference for carbohydrate over fat. *Cell Rep., 22*(3), 706-721.
781 doi:10.1016/j.celrep.2017.11.102
782 Pang, T., Xiong, B., Li, J. Y., Qiu, B. Y., Jin, G. Z., Shen, J. K., & Li, J. (2007). Conserved alpha-
783 helix acts as autoinhibitory sequence in AMP-activated protein kinase alpha subunits. *J. Biol.*
784 *Chem., 282*(1), 495-506.
785 Polekhina, G., Gupta, A., Michell, B. J., van Denderen, B., Murthy, S., Feil, S. C., . . . Stapleton, D.
786 (2003). AMPK beta subunit targets metabolic stress sensing to glycogen. *Curr. Biol., 13*(10),
787 867-871.
788 Porteus, C., Hedrick, M. S., Hicks, J. W., Wang, T., & Milsom, W. K. (2011). Time domains of the
789 hypoxic ventilatory response in ectothermic vertebrates. *J Comp Physiol B, 181*(3), 311-333.
790 doi:10.1007/s00360-011-0554-6
791 Prabhakar, N. R. (2000). Oxygen sensing by the carotid body chemoreceptors. *Journal of Applied*
792 *Physiology, 88*(6), 2287-2295.
793 Ramaiah, A., Hathaway, J. A., & Atkinson, D. E. (1964). Adenylate as a metabolic regulator.
794 Effect on yeast phosphofructokinase kinetics. *J. Biol. Chem., 239*, 3619-3622.
795 Riek, U., Scholz, R., Konarev, P., Rufer, A., Suter, M., Nazabal, A., . . . Wallimann, T. (2008).
796 Structural properties of AMP-activated protein kinase. Dimerization, molecular shape, and
797 changes upon ligand binding. *J. Biol. Chem., 283*(16), 18331-18343.
798 Ross, F. A., Jensen, T. E., & Hardie, D. G. (2016). Differential regulation by AMP and ADP of
799 AMPK complexes containing different gamma subunit isoforms. *Biochem. J., 473*(2), 189-199.
800 doi:10.1042/BJ20150910
801 Ross, F. A., MacKintosh, C., & Hardie, D. G. (2016). AMP-activated protein kinase: a cellular
802 energy sensor that comes in 12 flavours. *FEBS J., 283*(16), 2987-3001. doi:10.1111/febs.13698
803 Ross, F. A., Rafferty, J. N., Dallas, M. L., Ogunbayo, O., Ikematsu, N., McClafferty, H., . . . Evans,
804 A. M. (2011). Selective Expression in Carotid Body Type I Cells of a Single Splice Variant of
805 the Large Conductance Calcium- and Voltage-activated Potassium Channel Confers Regulation
806 by AMP-activated Protein Kinase. *J Biol Chem, 286*(14), 11929-11936. doi:M110.189779 [pii]
807 10.1074/jbc.M110.189779
808 Roth, G. J., Stanford, N., & Majerus, P. W. (1975). Acetylation of prostaglandin synthase by
809 aspirin. *Proc. Natl. Acad. Sci. U S A, 72*(8), 3073-3076.
810 Roux, J. C., & Villard, L. (2010). Biogenic amines in Rett syndrome: the usual suspects. *Behav*

Genet, 40(1), 59-75. doi:10.1007/s10519-009-9303-y

Salt, I. P., Johnson, G., Ashcroft, S. J. H., & Hardie, D. G. (1998). AMP-activated protein kinase is activated by low glucose in cell lines derived from pancreatic b cells, and may regulate insulin release. *Biochem. J., 335*(3), 533-539.

Schneider, H., Schubert, K. M., Blodow, S., Kreutz, C. P., Erdogmus, S., Wiedenmann, M., . . . Pohl, U. (2015). AMPK Dilates Resistance Arteries via Activation of SERCA and BKCa Channels in Smooth Muscle. *Hypertension, 66*(1), 108-116. doi:10.1161/HYPERTENSIONAHA.115.05514

Scott, J. W., Hawley, S. A., Green, K. A., Anis, M., Stewart, G., Scullion, G. A., . . . Hardie, D. G. (2004). CBS domains form energy-sensing modules whose binding of adenosine ligands is disrupted by disease mutations. *J. Clin. Invest., 113*(2), 274-284. doi:10.1172/JCI19874

Shaw, R. J., Kosmatka, M., Bardeesy, N., Hurley, R. L., Witters, L. A., DePinho, R. A., & Cantley, L. C. (2004). The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. *Proc. Natl. Acad. Sci. USA, 101*(10), 3329-3335.

Smith, J. C., Abdala, A. P., Borgmann, A., Rybak, I. A., & Paton, J. F. (2013). Brainstem respiratory networks: building blocks and microcircuits. *Trends Neurosci, 36*(3), 152-162. doi:S0166-2236(12)00201-9 [pii]
10.1016/j.tins.2012.11.004

Smith, J. C., Ellenberger, H. H., Ballanyi, K., Richter, D. W., & Feldman, J. L. (1991). Pre-Botzinger complex: a brainstem region that may generate respiratory rhythm in mammals. *Science, 254*(5032), 726-729.

Sommer, N., Huttemann, M., Pak, O., Scheibe, S., Knoepp, F., Sinkler, C., . . . Weissmann, N. (2017). Mitochondrial Complex IV Subunit 4 Isoform 2 Is Essential for Acute Pulmonary Oxygen Sensing. *Circ Res, 121*(4), 424-438. doi:10.1161/CIRCRESAHA.116.310482

Song, X. M., Fernström, M., Galuska, D., Fiedler, M., Rincon, J., Ryder, J. W., . . . Zierath, J. R. (2000). *AICAR treatment improves glucose homeostasis in ob/ob mice.* Paper presented at the Joint Congress of the Scandinavian Physiological Society and the American Physiological Society, Uppsala University and Karolinska Institutet Stockholm, Sweden.

Stahmann, N., Woods, A., Carling, D., & Heller, R. (2006). Thrombin activates AMP-activated protein kinase in endothelial cells via a pathway involving Ca2+/calmodulin-dependent protein kinase kinase beta. *Mol. Cell. Biol., 26*(16), 5933-5945.

Stahmann, N., Woods, A., Spengler, K., Heslegrave, A., Bauer, R., Krause, S., . . . Heller, R. (2010). Activation of AMP-activated protein kinase by vascular endothelial growth factor mediates endothelial angiogenesis independently of nitric-oxide synthase. *J. Biol. Chem., 285*(14), 10638-10652. doi:M110.108688 [pii] 10.1074/jbc.M110.108688

Steinberg, G. R., Dandapani, M., & Hardie, D. G. (2013). AMPK: mediating the metabolic effects of salicylate-based drugs? *Trends Endocrinol. Metab., 24*(10), 481-487. doi:10.1016/j.tem.2013.06.002

Taketa, K., & Pogell, B. M. (1965). Allosteric inhibition of rat liver fructose 1,6-diphosphatase by adenosine 5'-monophosphate. *J. Biol. Chem., 240*, 651-662.

Taylor, S. S., & Kornev, A. P. (2011). Protein kinases: evolution of dynamic regulatory proteins. *Trends Biochem. Sci., 36*(2), 65-77. doi:S0968-0004(10)00183-0 [pii] 10.1016/j.tibs.2010.09.006

Tello, D., Balsa, E., Acosta-Iborra, B., Fuertes-Yebra, E., Elorza, A., Ordonez, A., . . . Landazuri, M. O. (2011). Induction of the mitochondrial NDUFA4L2 protein by HIF-1alpha decreases oxygen consumption by inhibiting Complex I activity. *Cell Metab, 14*(6), 768-779. doi:10.1016/j.cmet.2011.10.008

Teppema, L. J., & Dahan, A. (2010). The ventilatory response to hypoxia in mammals: mechanisms, measurement, and analysis. *Physiol Rev, 90*(2), 675-754. doi:10.1152/physrev.00012.2009

Vincent, M. F., Marangos, P. J., Gruber, H. E., & Van den Berghe, G. (1991). Inhibition by AICA riboside of gluconeogenesis in isolated rat hepatocytes. *Diabetes, 40*(10), 1259-1266.

Wade, J. G., Larson, C. P., Jr., Hickey, R. F., Ehrenfeld, W. K., & Severinghaus, J. W. (1970). Effect of carotid endarterectomy on carotid chemoreceptor and baroreceptor function in man. *N Engl J Med, 282*(15), 823-829. doi:10.1056/NEJM197004092821501

Winder, W. W., & Hardie, D. G. (1999). The AMP-activated protein kinase, a metabolic master switch: possible roles in Type 2 diabetes. *Am. J. Physiol., 277*, E1-E10.

Woods, A., Johnstone, S. R., Dickerson, K., Leiper, F. C., Fryer, L. G., Neumann, D., . . . Carling, D. (2003). LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. *Curr. Biol., 13*(22), 2004-2008.

Xiao, B., Heath, R., Saiu, P., Leiper, F. C., Leone, P., Jing, C., . . . Gamblin, S. J. (2007). Structural basis for AMP binding to mammalian AMP-activated protein kinase. *Nature, 449*(7161), 496-500.

Xiao, B., Sanders, M. J., Carmena, D., Bright, N. J., Haire, L. F., Underwood, E., . . . Gamblin, S. J. (2013). Structural basis of AMPK regulation by small molecule activators. *Nature Commun., 4*, 3017. doi:10.1038/ncomms4017

Xiao, B., Sanders, M. J., Underwood, E., Heath, R., Mayer, F. V., Carmena, D., . . . Gamblin, S. J. (2011). Structure of mammalian AMPK and its regulation by ADP. *Nature, 472*(7342), 230-233. doi:10.1038/nature09932

Xin, F. J., Wang, J., Zhao, R. Q., Wang, Z. X., & Wu, J. W. (2013). Coordinated regulation of AMPK activity by multiple elements in the alpha-subunit. *Cell Res., 23*(10), 1237-1240. doi:10.1038/cr.2013.121

Yang, Y., Atasoy, D., Su, H. H., & Sternson, S. M. (2011). Hunger states switch a flip-flop memory circuit via a synaptic AMPK-dependent positive feedback loop. *Cell, 146*(6), 992-1003. doi:S0092-8674(11)00882-8 [pii] 10.1016/j.cell.2011.07.039

Yeh, L. A., Lee, K. H., & Kim, K. H. (1980). Regulation of rat liver acetyl-CoA carboxylase. Regulation of phosphorylation and inactivation of acetyl-CoA carboxylase by the adenylate energy charge. *J. Biol. Chem., 255*(6), 2308-2314.

Zadra, G., Photopoulos, C., Tyekucheva, S., Heidari, P., Weng, Q. P., Fedele, G., . . . Loda, M. (2014). A novel direct activator of AMPK inhibits prostate cancer growth by blocking lipogenesis. *EMBO Mol. Med., 6*(4), 519-538. doi:10.1002/emmm.201302734

Zhang, C. S., Hawley, S. A., Zong, Y., Li, M., Wang, Z., Gray, A., . . . Lin, S. C. (2017). Fructose-1,6-bisphosphate and aldolase mediate glucose sensing by AMPK. *Nature, 548*(7665), 112-116. doi:10.1038/nature23275

Zhang, C. S., Jiang, B., Li, M., Zhu, M., Peng, Y., Zhang, Y. L., . . . Lin, S. C. (2014). The lysosomal v-ATPase-Ragulator complex Is a common activator for AMPK and mTORC1, acting as a switch between catabolism and anabolism. *Cell Metab., 20*(3), 526-540. doi:10.1016/j.cmet.2014.06.014

Zhang, J., Dong, J., Martin, M., He, M., Gongol, B., Marin, T. L., . . . Shyy, J. Y. (2018). AMPK Phosphorylation of ACE2 in Endothelium Mitigates Pulmonary Hypertension. *Am J Respir Crit Care Med*. doi:10.1164/rccm.201712-2570OC

Zhou, T., Chien, M. S., Kaleem, S., & Matsunami, H. (2016). Single cell transcriptome analysis of mouse carotid body glomus cells. *J Physiol*. doi:10.1113/JP271936

FIGURE LEGENDS

Figure 1: **Summary of the three mechanisms by which AMP activates AMPK in the canonical pathway.** AMP binding to AMPK has three effects: (1) promoting phosphorylation at Thr172 by LKB1 (converting AMPK to AMPK-P); (2) inhibiting dephosphorylation at Thr172 by protein phosphatases (preventing conversion of AMPK-P back to AMPK until AMP dissociates); (3) causing allosteric activation (conversion of AMPK-P to the more active **AMPK-P)**. Effects (1) and (2), but not (3), are mimicked by the binding of ADP, and all three are antagonized by binding of ATP. The numbers in parentheses underneath the three states of AMPK represent their approximate relative activities.

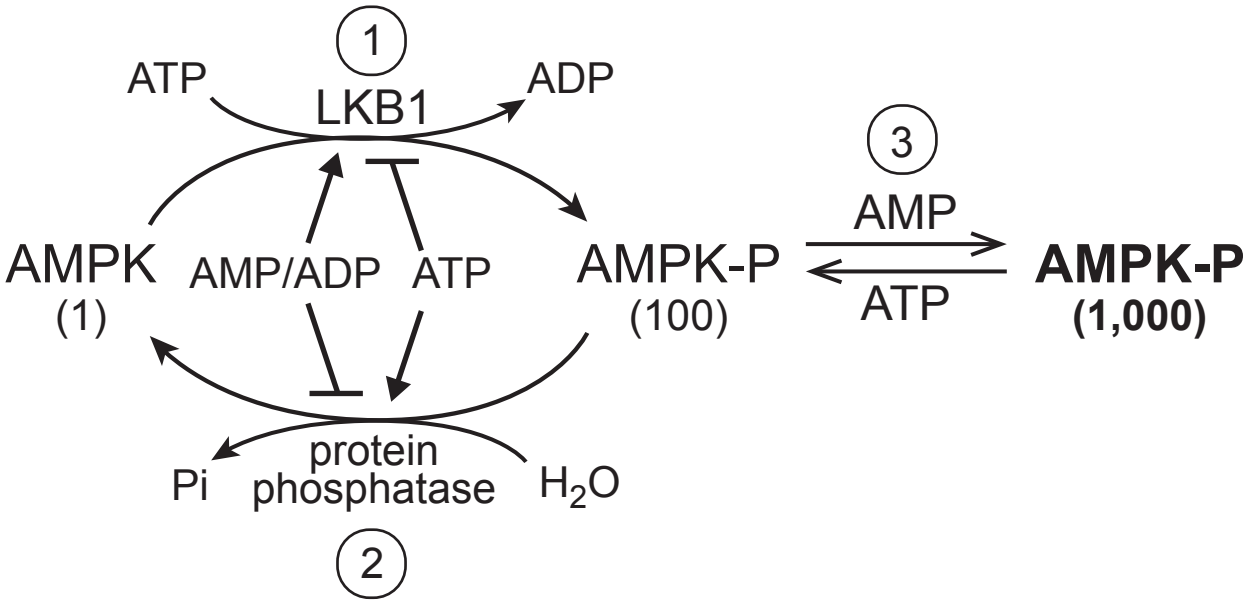
Figure 2: **Highly schematized representation of the structure of an AMPK heterotrimer in active conformation, showing approximate locations of domains mentioned in the text.** Based on (Calabrese et al., 2014; Li et al., 2015; Xiao et al., 2013). Three molecules of AMP are bound at the CBS1, CBS3 and CBS4 sites in the γ subunit. Thr172 is phosphorylated in these structures, but is not visible because it is located on the far side of the kinase domain C-lobe, close to the catalytic site. The α-linker is depicted as a chain physically linking the α-AID and the α-CTD. Binding of ATP at the CBS3 site is thought to cause dissociation of the α–linker from this site, allowing the α-AID to rotate back into its inhibitory position behind the N- and C-lobes of the kinase domain.

Figure 3: **Diffusion of active, thiophosphorylated AMPK (α2β2γ1) complex from a patch pipette into cultured rat hippocampal neurons caused time-dependent depression of firing of action potentials.** Immediately on insertion of the patch pipette with active (A) or inactive (B) complex, or 10 minutes later with active (C) or inactive (D) complex, action potentials were triggered by current injection and recorded. (E) shows the frequency of action potentials (mean ± SEM, n = 7) as a function of time. Redrawn from (N. Ikematsu et al., 2011).

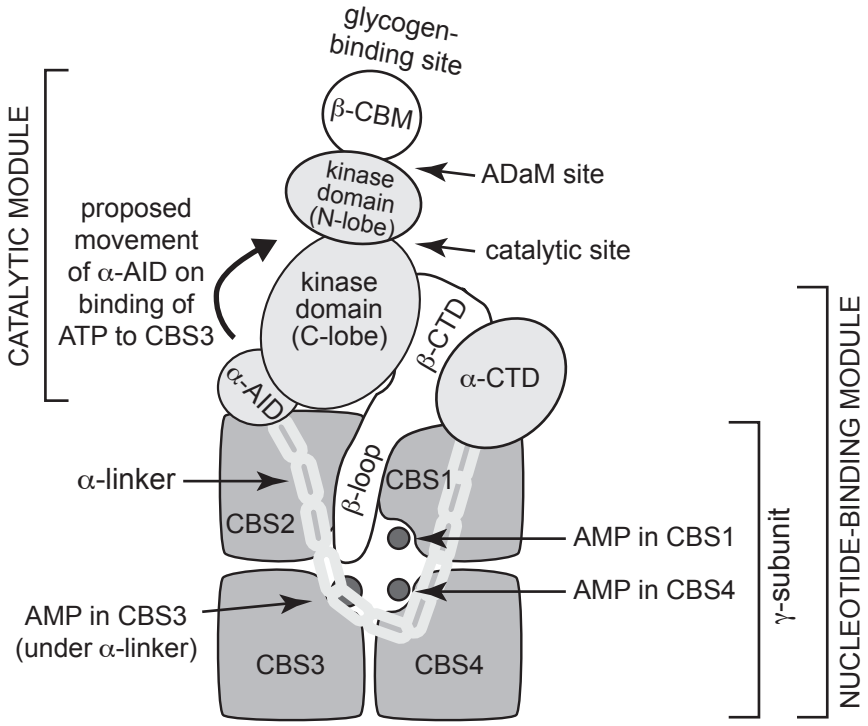
Figure 4: **Regulation of the HVR by AMPK.** Schematic describing the new hypothesis on the regulation by AMPK of the hypoxic ventilatory response, through integration of local and applied metabolic stresses. AP = area postrema; NTS = nucleus tractus solitarius. From (Mahmoud et al., 2015).

Figure 5: **AMPK in the nervous system controls whole body energy supply.** The diagram shows a tracing of a sagittal section of a whole mouse brain, indicating anatomical locations where AMPK may regulate the neural control of appetite/feeding (arcuate nucleus), energy expenditure (ventromedial hypothalamus) and breathing (NTS + respiratory central pattern generators).

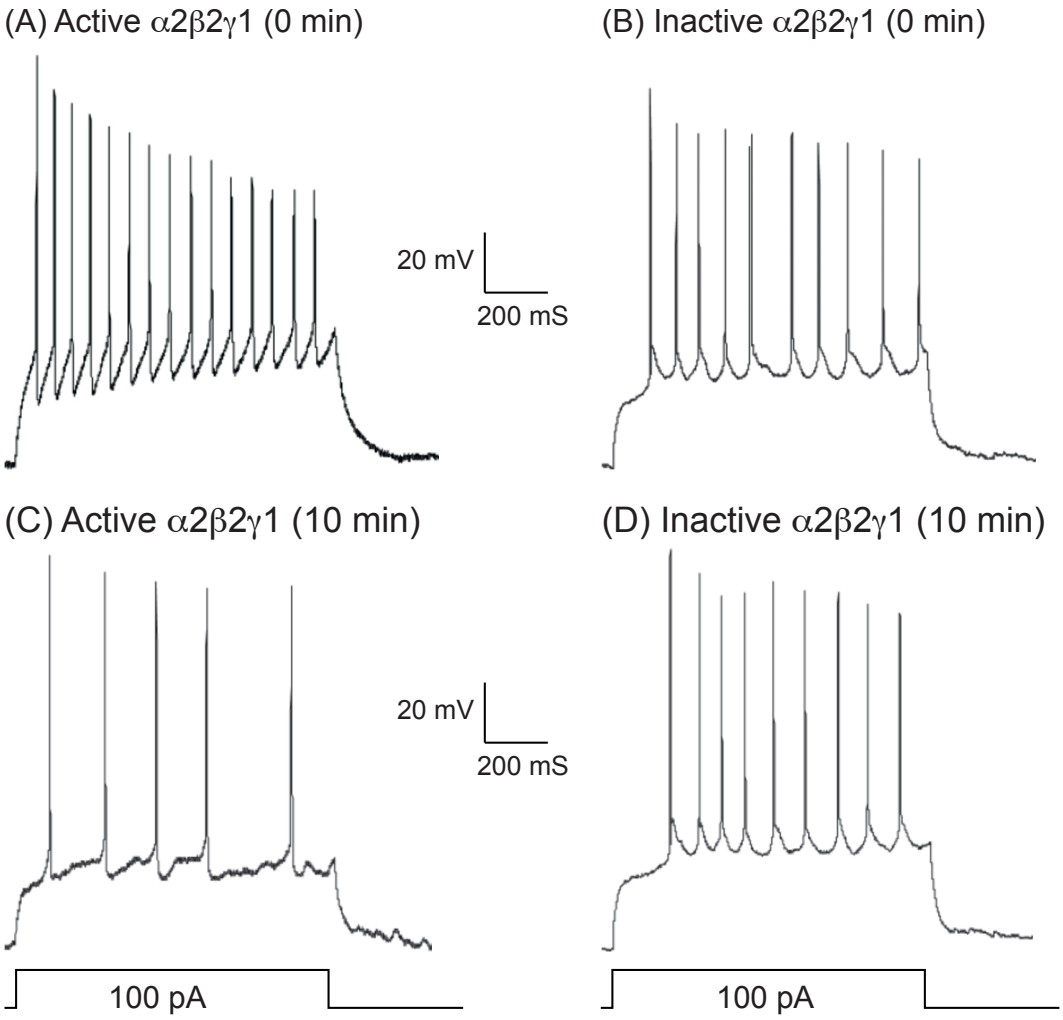
Hardie & Evans Figure 1



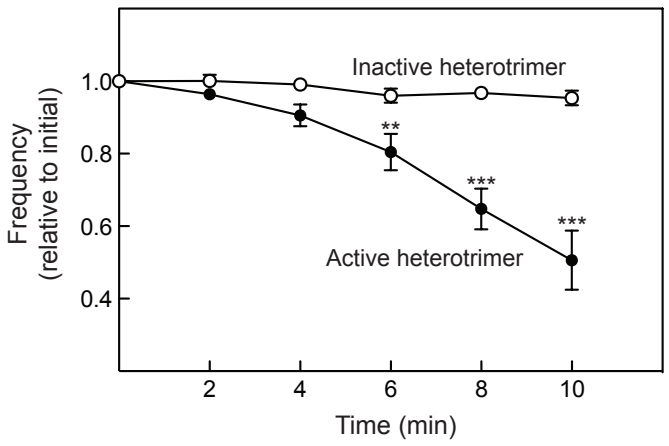
Hardie & Evans Figure 2



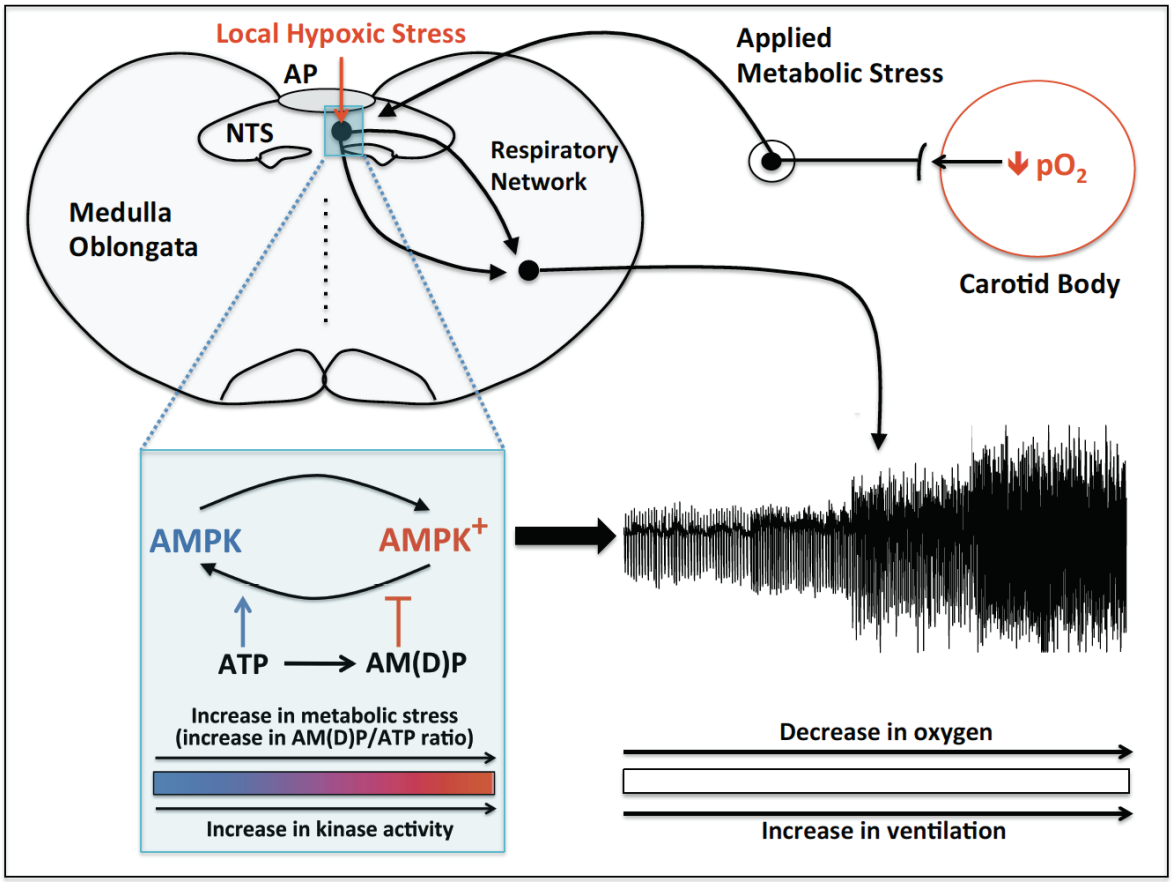
Hardie & Evans Figure 3



E) Time course of changes in action potential frequency



Hardie & Evans Figure 4



Hardie & Evans Figure 5