

AMPK at the interface of nutrient sensing, metabolic flux and energy homeostasis

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The orchestration of cellular metabolism requires the integration of signals related to energy stores and nutrient availability through multiple overlapping mechanisms. AMP-activated protein kinase (AMPK) is a pivotal energy sensor that responds to reductions in adenylate charge; however, studies over the past decade have also positioned AMPK as a key integrator of nutrient-derived signals that coordinate metabolic function. This Review highlights recent advances in our understanding of how AMPK senses nutrients and regulates metabolic activity across tissues, timescales and cell types. These effects are mediated through the phosphorylation of substrates involved in metabolite trafficking, mitochondrial function, autophagy, transcription, ubiquitination, proliferation and cell survival pathways, including ferroptosis. Particular attention is given to the role of AMPK in the pathophysiology of obesity, type 2 diabetes, metabolic dysfunction-associated steatotic liver disease, cardiovascular and renal diseases, neurodegenerative disorders and cancer. Collectively, these findings reinforce AMPK as a central metabolic node that aligns cellular behaviour with energetic demand. Continued investigation into its nutrient-sensing mechanisms holds promise for identifying new strategies to restore metabolic balance in disease.

Metabolism governs how cells acquire, process and store nutrients to fuel cellular function. Control of this system requires precise monitoring of nutrient availability followed by rapid, context-dependent signalling to match the temporal metabolic demands of the cell. Acutely, cells respond directly to nutrient availability and energetic flux through allosteric regulation of core metabolic enzymes. These signals are complemented by covalent modifications such as phosphorylation that exert longer-term effects. Over extended periods of time, changes in cellular metabolism are reinforced through epigenetic modifications and transcriptional control. In the past three decades, AMPK has emerged as a critical conduit for controlling these distinct inputs

and is exquisitely sensitive to not only changes in cellular adenylate charge (energy) but also acute changes in nutrient substrates (carbohydrates, fatty acids, amino acids). These multiple nodes of AMPK regulation are critical for fine-tuning metabolic demand with nutrient availability, while dysregulation of these pathways contributes to numerous pathologies that may be pharmacologically targeted to exert beneficial effects.

In this Review, we refocus on the role of AMPK as a nutrient sensor, emphasizing new perspectives related to its sensitivity to changes in glucose and fatty acids and the isoform-specific mechanisms by which allosteric, covalent and transcriptional control of the enzyme is used

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to fine-tune activity. We then discuss new AMPK substrates that link nutrient sensing with macroautophagy, transcriptional control, protein stability and mitochondrial homeostasis. Lastly, we briefly discuss the latest discoveries and developments regarding AMPK biology as it relates to metabolic diseases, cancer and emerging areas such as neurological and infectious diseases.

AMPK subunit composition

The functional AMPK complex is made up of three different proteins: one catalytic (α) subunit and two regulatory (β and γ) subunits (Fig. 1a). Unlike invertebrates that express each AMPK subunit from a single gene¹, humans harbour seven individual genes encoding multiple isoforms of AMPK. *PRKAA1* and *PRKAA2* encode $\alpha1$ and $\alpha2$, *PRKAB1* and *PRKAB2* encode $\beta1$ and $\beta2$, and *PRKAG1*, *PRKAG2* and *PRKAG3* encode $\gamma1$, $\gamma2$ and $\gamma3$, respectively, giving rise to 12 possible heterotrimer combinations in mammalian cells². Although little evidence is currently available, splice variants of each subunit may offer even more complexity and regulatory potential. Unique AMPK complexes presumably accommodate the heterogeneous metabolic profiles and growth requirements of distinct cells and tissues. For example, it is widely recognized that the $\gamma3$ -subunit isoform is almost exclusively expressed in skeletal muscle, assembles strictly into $\alpha2\beta2$ complexes, and is found mainly in fast, glycolytic fibres^{3–5}. By contrast, $\gamma2$ expression is markedly elevated in the heart and thought to form AMPK complexes principally alongside $\alpha2$ and $\beta1$ (ref. 6). Heterotrimeric combinations of AMPK isoforms in human and rodent tissues are presented in Supplementary Table 1 and have been recently discussed in the context of how heterotrimer heterogeneity could inform regulatory mechanisms⁷. Before contextualizing the regulation of AMPK signalling in terms of nutrients, we next aim to highlight the more classical modes of upstream regulation.

Canonical AMPK activation by adenine nucleotides and upstream kinases

The primary mechanism by which AMPK is activated is through phosphorylation of a single, highly conserved threonine residue (Thr172) in its activation loop (note that for $\alpha1$ this is Thr183). While, as discussed below, other phosphorylation sites contribute to fine-tuning AMPK activity, T172 remains the dominant site controlling its covalent activation and deactivation by up to 100-fold^{8,9} (Fig. 1). Adenine nucleotide-based activation of AMPK takes place via a three-pronged mechanism involving sensing of the exchange of ATP with AMP or ADP at binding sites within cystathionine- β -synthase domains on the γ -subunit: AMP and ADP both (1) promote α -T172 phosphorylation and (2) prevent its dephosphorylation by phosphatases, and (3) only AMP further allosterically activates AMPK complexes already phosphorylated on α -T172 (refs. 10,11). Following on from the kinase domain in the α -subunit is the autoinhibitory domain (α -AID) that rotates away and disengages from the kinase domain following AMP binding¹². Part of this disinhibition involves the coordinated actions of two nucleotide-sensing 'RIM' modules embedded in the α -linker situated immediately C-terminal to the α -AID¹³. While these structural elements account for general aspects of AMPK activation, there are a multitude of isoform-specific differences regulating both canonical and non-canonical AMPK activation.

AMPK α -T172 phosphorylation is essential for full activation and is regulated by several upstream kinases. Of the most well-characterized, liver kinase B1 (LKB1) typically phosphorylates α -T172 after glucose starvation (described in greater detail below) or following changes in adenine nucleotide levels¹⁴. Calcium/calmodulin kinase kinase 2 (CaMKK2) phosphorylates α -T172 following increases in intracellular Ca^{2+} levels^{9,15}, which is particularly apparent in conditions such as genotoxic stress^{16,17}, hypoxia¹⁸ and hormone signalling in the brain^{19,20}. Facilitating this CaMKK2–AMPK interaction is the scaffolding protein IQGAP1 that directly binds to both AMPK α and CaMKK2 (ref. 21). Although initially questioned as a bonified upstream kinase^{22,23},

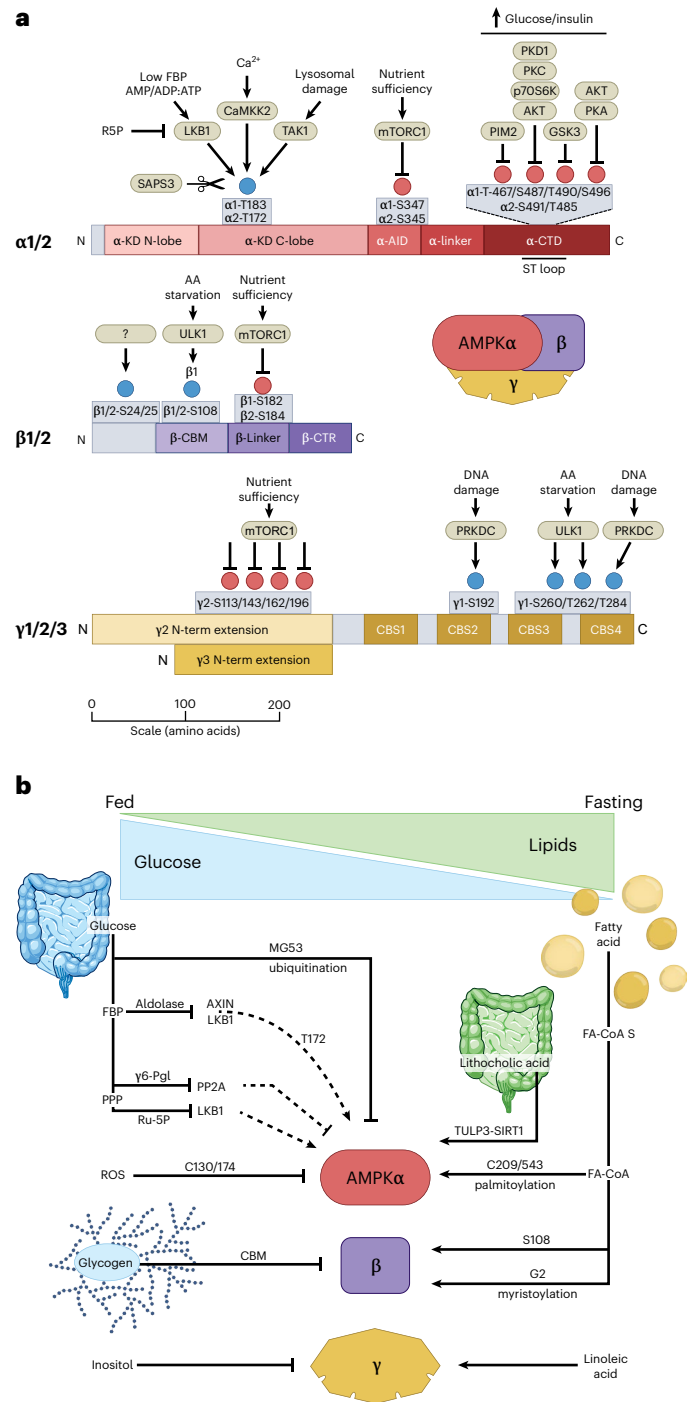


Fig. 1 | Nutrient and metabolite-sensitive regulation of AMPK complexes.

a, Nutrient regulation of AMPK subunits: domain structures of the AMPK α -, β - and γ -subunits with nutrient-sensitive phosphorylation sites. Upstream kinases that increase activity under conditions of high glucose or insulin signalling suppress AMPK activity via phosphorylation in the ST loop region. Phosphorylation sites that increase AMPK activity or positively regulate its function are depicted in blue, whereas inhibitory sites are depicted in red.

b, Metabolite control of AMPK activity during fed and fasted states occurs through changes in glucose- and lipid-related metabolites, respectively: glucose- and fatty acid-specific metabolites can directly and indirectly affect AMPK activity by influencing lipidation, phosphorylation, ubiquitination and oxidation of key residues, or through direct binding of metabolites to the α -, β - and γ -isoforms. AA, amino acid; β -CBM, β -carbohydrate-binding module; CBS, cystathionine- β -synthase; α -CTD, α -C-terminal domain; β -CTR, β -C-terminal region; KD, kinase domain; $\gamma6$ -Pgl, $\gamma6$ -phosphogluconolactone; ROS, reactive oxygen species; Ru-5P, ribulose-5-phosphate; ST loop, serine/threonine-rich loop.

recent evidence has clarified that TAK1-mediated phosphorylation of AMPK α -T172 occurs specifically under conditions of lysosomal damage, wherein TAK1 is ubiquitinated and activated by USP9X²⁴. This compartmentalized signalling cascade also explains how other TAK1-activating stimuli such as bacterial lipopolysaccharides do not increase AMPK activity²⁵. Recent studies have also found that the STE20 family of protein kinases including sterile 20-like kinase 3 (MST3) and mitogen-activated protein kinase kinase kinase 5 (MAP4K5) can phosphorylate α -T172 in human embryonic kidney cells^{26,27}; however, further validation under diverse physiological conditions and in tissues *in vivo* will be needed to further clarify their potential role. Together, the structural complexity and multifaceted mechanisms regulating AMPK activity through upstream kinase phosphorylation and adenine nucleotide abundance provide the foundation for its central role in sensing and responding to fluctuations in cellular nutrient availability detailed below.

AMPK regulation by nutrients

Throughout evolution, cells have developed intricate mechanisms to adapt to varying levels of nutrient stress and ensure survival under fluctuating energy conditions. Glucose is essential not only for glycolysis but also for generating intermediates critical for oxidative phosphorylation and other metabolic processes. While prolonged glucose deprivation leads to severe energy stress and elevated AMP/ADP to ATP ratios, even modest glucose reductions trigger metabolic adaptations aimed at maintaining energy balance. These changes are particularly relevant during natural fluctuations in nutrient availability, such as those occurring over the diurnal cycle, where decreases in glucose and insulin alongside increases in glucagon and adrenaline drive systemic metabolic responses. AMPK has been shown to regulate these interconnected endocrine pathways, with its activation linked not only to glycolytic flux under low-glucose conditions but also to direct stimulation by various lipid species. The glucose–fatty acid (Randle) cycle suggests that competition between glucose and fatty acids aligns fuel supply with metabolic demand and is influenced by dietary composition, fasting and exercise²⁸. Although its universality across cell types remains uncertain, the cycle's core principle is that metabolites such as glucose-6-phosphate (G6P), citrate, acetyl-CoA and malonyl-CoA exert feedback control over glucose uptake, pyruvate dehydrogenase activity and acetyl-CoA carboxylase (ACC) activity to regulate substrate utilization and storage. Given that AMPK is an established regulator of these pathways, it has long been considered a central regulator of carbohydrate and lipid metabolism. Recent findings, however, expand this view, highlighting AMPK's remarkable sensitivity to changes in key carbohydrate and lipid metabolites and reinforcing its role as a master nutrient sensor.

Glucose sensing

Regulation of Thr172 phosphorylation by glucose-associated metabolic pathways. In addition to canonical adenine nucleotide sensing, AMPK activity can be acutely induced in response to low carbohydrate availability through several distinct but overlapping mechanisms (Fig. 1). Once taken across the cellular membrane, glucose is activated to G6P and further metabolized to fructose-1,6-bisphosphate (FBP), which serves as a key control point in glycolytic flux²⁹. Fasting conditions or glucose restriction can deplete FBP, forming an aldolase A-mediated ternary complex comprising aldolase A, TRPV and vacuolar H⁺-ATPase. This recruits an AXIN–LKB1 complex to the lysosome^{30,31} and facilitates LKB1-mediated phosphorylation of lysosomal AMPK at α -T172 (ref. 32). Importantly, this process has been shown to occur independent of nucleotide exchange on cystathionine- β -synthase domains³⁰ but does depend on the severity of energy stress³³. In mice, blocking FBP binding to aldolase with the small molecule aldometanib, thereby mimicking the low-glucose condition, activates AMPK and improves insulin sensitivity, liver steatosis and longevity³⁴.

Recent work has also highlighted how glycolytic metabolites and by-products can directly activate or inhibit the kinase. For example, inositol, which is generated downstream of G6P, was shown to have a suppressive effect on AMPK activity³⁵. This may be due to direct binding to the AMPK γ 1-subunit where it competes with AMP³⁵. Prolonged glucose starvation can also induce oxidative stress³⁶. While there has been some debate regarding the reactive oxygen species (ROS)-dependent^{37,38} versus AMP-dependent^{39–42} activation of AMPK in response to oxidative stress, several AMPK residues such as α 2-Cys130 and α 2-Cys174 are redox sensitive⁴³, with others having been identified through screens⁴⁴. Of note, AMPK α and other serine/threonine kinases share a conserved cysteine residue near the activating T-loop site that corresponds with human α 2-Cys174 and α 1-Cys185 (refs. 45–47), signifying the conservation of redox-based regulation of kinase signalling. Mechanistically, changes in redox state have been shown to inhibit phosphorylation of α -T172 (ref. 43); however, further studies are now needed to further evaluate the mechanistic basis for this effect and whether this is also important for controlling AMPK activity *in vivo* in response to changes in glucose availability.

Reduction in glucose also decreases flux through the oxidative pentose phosphate pathway, which has been shown to modulate α -T172 phosphorylation through two independent pathways. This includes a decrease in ribulose-5-phosphate levels that relieves LKB1 inhibition and subsequently activates AMPK⁴⁸. Alternatively, lower levels of the pentose phosphate pathway by-product γ -6-phosphogluconolactone can reduce protein phosphatase 2A activity and subsequently increase AMPK α -T172 phosphorylation⁴⁹. However, subsequent studies have indicated that in the liver, the phosphatase PP6 in complex with its regulatory subunit SAPS3 is the more likely physiological AMPK phosphatase as genetic deletion of *SAPS3* activates AMPK and protects mice from diet-induced steatosis and insulin resistance⁵⁰. Several other studies have also indicated how phosphatases can affect α -T172 phosphorylation levels during glucose-replete^{49,51–55} or glucose-starved⁵⁶ conditions; however, to date, only the role of PP6/SAPS3 has been validated *in vivo*.

Regulation of other phosphorylation events. Phosphorylation of additional residues beyond α -T172 fine-tunes AMPK activity in response to changes in glucose availability. On the catalytic subunit, phosphorylation of AMPK α 1-S487 (commonly referred to as S485 in accordance with the rat AMPK α 1 sequence) and AMPK α 2-S491, which are inhibitory and are typically elevated under anabolic conditions such as high glucose and insulin engagement⁵⁷, are regulated by a host of upstream kinases^{57–69}. Mechanistic target of rapamycin complex 1 (mTORC1) is one such upstream kinase that is itself sensitive to nutrient signals and often supports anabolic signalling, in contrast to AMPK. The interplay between AMPK and mTORC1 has recently been reviewed in detail elsewhere⁷⁰. Briefly, mTORC1, which has high activity in glucose-replete conditions⁷¹, phosphorylates AMPK α 1-S347 and α 2-S345 (ref. 72), where the α 2-S345 phosphorylation site can prevent AMPK from localizing to the lysosome⁷³. Recent work has identified additional mTORC1 phosphorylation sites on AMPK at β 1-S182 and β 2-S184, as well as several sites on the N-terminal extensions of the AMPK γ 2- and γ 3-subunits⁷⁴. Of these, β 2-S184 may have a role in nuclear AMPK activity⁷⁴. The DNA-dependent protein kinase (PRKDC) also regulates organelle-specific AMPK activity through phosphorylation of γ 1-S192 and γ 1-T284, supporting lysosomal AMPK activation downstream of glucose starvation⁷⁵. Other potential glucose-sensitive phosphorylation sites include α 1-S496 by protein kinases AKT and PKA⁷⁶, α 1-T490 and α 2-T485 by GSK3 (ref. 77) and α 1-T467 by PIM2 (refs. 78,79). How these phosphorylation sites alter the structure and activity of AMPK in addition to their physiological importance remains to be determined.

Glucose-dependent protein stability. Over longer periods of time, glucose-sensitive protein stability regulators can also affect AMPK

function. Ubiquitination and subsequent degradation of the AMPK heterotrimer in response to hyperglycaemia is regulated by the E3 ubiquitin ligase MG53 (TRIM72)⁸⁰ and the cereblon-cullin-RING ubiquitin E3 ligase 4A⁸¹. Additionally, both the GID complex⁸² and Fbxo48 (ref. 83) recognize phosphorylated AMPK α -T172 under glucose starvation conditions. However, given the complexity of measuring long-lived, phospho-regulated proteins such as AMPK, more work is required to fully understand the dynamics of α -T172 recognition by E3 ubiquitin ligases as a regulator of AMPK. Meanwhile, the deubiquitinase ubiquitin-specific protease (USP) 10 is required for full activation of AMPK in response to glucose starvation⁸⁴. Sufficient glucose levels also support glycogen synthesis through the conversion of G6P into glucose-1-phosphate and UDP-glucose. Owing to the carbohydrate-binding module on the AMPK β -subunits, direct glycogen binding has been shown to be important for maintaining liver and skeletal muscle AMPK expression^{85,86}, liver AMPK activity⁸⁷, exercise capacity and fatty acid oxidation⁸⁸. However, it should be noted the physiological stoichiometry of this binding in skeletal muscle has been questioned⁸⁹.

In summary, low glucose activates AMPK through a range of temporal mechanisms such as α -T172 phosphorylation, direct and indirect sensing of glycolytic and pentose phosphate pathway intermediates, as well as longer-term changes in ubiquitination and protein turnover. The evolution of these layered pathways probably reflects the need for cells to fine-tune responses across varying durations and intensities of nutrient stress, allowing AMPK to integrate immediate metabolic shifts with sustained adaptations. However, an important caveat of these findings with respect to mammals is that under most physiological conditions, ranging from feeding to prolonged fasting, blood glucose levels are tightly regulated in a range of 4–6 mM. This differs dramatically from the context in which many of these studies were conducted in cultured cells with either high (25 mM) or very low (<1 mM) levels of glucose. Although there are certainly biological contexts and micro-environments in which blood glucose levels can change dramatically, such as diabetes or cancer, future studies investigating the importance of these glucose-sensing mechanisms in vivo and during metabolic adaptations in response to changes in nutrient availability as occurs with fasting and refeeding will be important.

Lipid sensing

AMPK activation by FA-CoA binding. In reptiles, birds and mammals, blood glucose levels are maintained during fasting and prolonged endurance exercise by glucagon and noradrenaline⁹⁰. These hormones stimulate adipose tissue lipolysis, leading to the release of glycerol and free fatty acids that are used for gluconeogenesis in the kidney and liver and to generate ATP and reducing equivalents through β -oxidation. Notably, the magnitude of change in the circulating concentration of free fatty acids is dramatic (>10-fold), climbing from a postprandial nadir of ~0.2 mM to 2–3 mM with prolonged fasting and/or endurance exercise, a difference which, as discussed above, dwarfs the very modest changes in blood glucose that occur in parallel. Several studies over the past three decades have found that AMPK may be activated by fatty acids^{25,91–94}. However, only recently was it discovered that fatty acyl-CoA (FA-CoA) esters increase AMPK activity through direct binding to the allosteric drug and metabolite (ADaM) site within AMPK β 1 (ref. 95). This effect is specific to medium-chain and long-chain FA-CoA esters but not their closely related unesterified derivatives. Consistent with observations of small-molecule AMPK activators such as A-769662 and salicylate^{96,97}, these effects are not observed with β 2-containing complexes and are blunted in AMPK β 1p.Ser108Ala knock-in mice. Importantly, long-chain FA-CoA-induced activation of AMPK is essential to increase fatty acid oxidation as the effects of an intralipid gavage are blunted in mice lacking AMPK-inhibitory phosphorylation sites on ACC⁹⁵ or in mice with a p.Ser108Ala mutation in AMPK β 1 (ref. 98).

AMPK activation by lipids and links to lipolysis and lipophagy. As a major source of circulating fatty acids, adipose tissue lipolysis is induced by fasting, exercise and cold. Elevated lipolysis is associated with AMPK activation in adipose tissue, which was thought to involve reductions in ATP to AMP ratio related to the energetic cost of FA-CoA synthetases⁹⁹. However, recent studies using a FA-CoA FRET-based sensor and chemical inhibitors to CGI-58 (ABDH5) and ATGL have now shown that AMPK is activated by lipolysis independently of PKA due to FA-CoA-induced increases in AMPK β 1 Ser108 phosphorylation¹⁰⁰. Beyond FA-CoAs, fasting can also increase levels of linoleic acid, which was recently shown to bind to the AMPK γ 1-subunit and promote nucleotide-independent activation and synergy with AMPK⁹⁴. Although the exact binding site will have to be verified empirically, this presents a multipronged fatty acid regulatory system ensuring that AMPK is activated under fasting conditions. Caloric restriction also increases circulating concentrations of the bile acid lithocholic acid, which binds intracellularly to TUB-like protein 3 (TULP3) and causes activation of sirtuins, which deacetylate and inhibit vacuolar H⁺-ATPase, causing activation of AMPK^{101,102}. This activation of AMPK is associated with further increases in lipolysis, effects that have been linked to AMPK-mediated phosphorylation of several key proteins including lipases, HSL¹⁰³ and ATGL¹⁰⁴, and lipid droplet-associated proteins CHKA2 (ref. 105), PLIN2 (ref. 106) and PLIN3 (ref. 107). Recent studies have also shown AMPK phosphorylation of oxysterol-binding protein (OSBP)-related protein 8 (ORP8) promotes lipophagy¹⁰⁸. Together, these findings highlight a sophisticated feedback network in which AMPK not only responds to products of lipolysis but also amplifies lipid metabolism through multiple converging mechanisms, reinforcing its role as a key regulator of nutrient balance during fasting and metabolic stress.

Lipidation-dependent regulation of AMPK localization and activity. AMPK subcellular localization and activity are also regulated by fatty acids through the attachment of lipid moieties to residues on the β - and α -subunits, a process known as lipidation. One well-characterized form of lipidation is myristoylation, the covalent attachment of a 14-carbon saturated fatty acid (myristic acid) to an N-terminal glycine residue. This irreversible modification typically occurs co-translationally and facilitates membrane association and protein localization. AMPK is co-translationally myristoylated on both β -subunits at N-terminal glycine residues (G2) by NMT1 following cleavage of its N-terminal methionine^{109–111}. Myristoylation is necessary for basal association of AMPK with the lysosome^{30,31} and mitochondria¹¹² but not the Golgi¹¹³. While occupied on the membrane, this conformation may generate a 'preactivated' state, where α -T172 in the kinase domain is more accessible to upstream kinases. Furthermore, AMPK that cannot be myristoylated due to mutation of the N-terminal glycine to alanine exhibits elevated basal activity. However, non-myristoylated AMPK is unresponsive to AMP binding, suggesting that the myristoyl group has an inhibitory effect on canonical AMPK activation^{52,111}. Indeed, a myristoyl-switch mechanism has been proposed to modulate AMPK activity, wherein the myristoyl group binds within an intramolecular binding pocket on the AMPK heterotrimer until binding by AMP or ADP displaces the myristoyl group¹¹¹. Outside myristoylation, palmitoylation (attachment of a 16-carbon fatty acid chain) of both α -subunits at Cys209 and Cys543 by DHHC17 at least partially depends on myristoylation of the β -subunits, and can also influence the association of AMPK with membranes¹¹⁴. These lipidation events, in combination with 'fine-tuning' via site-specific phosphorylation that affects subcellular localization (α 2-S345 (ref. 73), β -S24/25 and S182 (refs. 74,110) and γ 1-S192/T284 (ref. 75)), ensure AMPK is directed to the appropriate organelle in a timely and signalling-specific manner.

Future considerations for the study of AMPK and nutrient sensing

While the number of upstream metabolic regulators continues to grow, future work is needed to tease out the context and importance

BOX 1**Emerging subcellular localization of AMPK**

While AMPK localization at the lysosome, mitochondria and cytoplasm is well characterized, its association with other organelles such as the nucleus, Golgi and peroxisomes remains less understood. AMPK regulates transcription via nucleo-cytoplasmic shuttling, despite lacking a canonical nuclear localization signal; both α 1- and α 2-subunits contain a nuclear export signal that supports nuclear localization^{17,379–381}. Nuclear AMPK can be activated either via CaMKK2 in response to nuclear Ca^{2+} (refs. 16,17) or by cytosolic activation followed by nuclear import³⁸². UHRF1 recruits PP2A to dephosphorylate nuclear AMPK, influencing both nuclear and cytosolic activity⁵³. During apoptosis, caspase-3 cleaves the AMPK α 1 nuclear export signal, retaining active AMPK in the nucleus³⁸³, while β -subunit phosphorylation events (Ser24, Ser25 or Ser182) can also affect nuclear localization¹¹⁰.

All AMPK isoforms associate with the Golgi, independent of β -subunit *N*-myristoylation¹¹³. After activation, AMPK phosphorylates GBF1 at Thr1337, triggering Golgi fragmentation and impairing cargo trafficking^{113,384}. Golgi disassembly also occurs under glucose starvation via TBC1D23-mediated activation of Golgi-bound AMPK, paralleling lysosomal activation via AXIN–LKB1 (ref. 385). Conversely, mitotic AMPK inactivation increases Golgi reassembly and mildly affects the cell cycle³⁸⁴.

Peroxisomes, partly derived from mitochondria, share dynamic regulation, including MFF-dependent morphological remodelling^{386,387}. In *Caenorhabditis elegans*, AMPK influences longevity by modulating mitochondrial and peroxisomal dynamics³⁸⁸. AMPK also inhibits pexophagy via a NIX-dependent mechanism¹²⁵, suggesting the existence of a peroxisome-specific AMPK pool whose role remains to be fully explored. Taken together, these emerging data suggest AMPK functions at the nucleus, Golgi and peroxisomes but what drives its localization, and why it might matter, remain to be determined.

of glucose and fatty acid-mediated AMPK activation. For example, are there fasting-induced energy or metabolite thresholds that dictate tissue-specific and cell-specific AMPK signalling? Does subcellular localization affect substrate selectivity? How do simultaneous changes in glucose and fatty acids interact to activate AMPK and is there synergy given their distinct mechanisms of action? To answer these and other important questions, the use of real-time activity measurements in combination with point mutant mouse models will be necessary to uncover both the physiological and potentially pathophysiological implications of metabolite-mediated AMPK regulation.

New substrates and refined models of cellular regulation by AMPK

AMPK phosphorylation of proteins is determined by intracellular localization and a well-defined recognition motif surrounding the serine or threonine phosphorylation site. While the intracellular targeting of proteins is only beginning to be understood (Box 1), substrate selectivity has been described in detail¹¹⁵ and, briefly, involves basic residues at positions –4 and/or –3, alongside hydrophobic residues at positions –5 and +4 relative to serine or threonine residues on the target protein. Despite the number of validated AMPK targets reaching just over 100 in 2022 (ref. 115), new substrates are being uncovered rapidly, with an average of 25 substrates being identified every 3 years since 2010 (Supplementary Table 2). While novel targets across diverse cellular

processes are still emerging, most recently characterized substrates over the past 5 years have been linked to the regulation of macroautophagy, transcription, ubiquitination and mitochondrial homeostasis. In the following sections, we highlight a subset of these newly identified phosphorylation events and explore their potential roles in coordinating metabolic responses to nutrient availability.

Macroautophagy

Macroautophagy (hereafter autophagy) is a conserved, ATP-dependent process that enables cells to degrade and recycle intracellular components via lysosomal pathways. Given its high energetic cost, autophagy must be tightly regulated in response to nutrient status and cellular energy availability. AMPK, alongside mTORC1, has a central role in relaying these nutrient cues to the autophagic machinery. During amino acid withdrawal, autophagy is induced primarily through the inactivation of mTORC1 and subsequent activation of the autophagy-initiating kinase, ULK1. By contrast, glucose deprivation defines a distinct regulatory paradigm in which AMPK and mTORC1 signalling intertwine to acutely reduce autophagic flux. However, AMPK signalling in the long term promotes optimal function of autophagic machinery should the nutrient stress continue, thereby balancing the energetic cost of autophagy with the type and duration of nutrient stress (Fig. 2).

Acute glucose deprivation inhibits autophagic flux^{116–120}. Recent work has clarified how in glucose-limited states, activated AMPK does not promote autophagy but instead inhibits autophagosomal maturation^{119–122}. AMPK phosphorylates ULK1 at Ser556 and Thr660, which in the presence of mTORC1-dependent phosphorylation at Ser758, stabilizes the ULK1–AMPK interaction and functionally suppresses ULK1 activity^{122,123}. The suppression of ULK1 activity is mediated by the recruitment of the scaffolding protein 14-3-3, which requires AMPK phosphorylation of Ser694 on ULK1 (refs. 124,125). Furthermore, AMPK directly phosphorylates ATG13, a member of the ULK1 complex, to oppose autophagy induction¹²⁶. Despite inhibiting ULK1 complex members, AMPK also phosphorylates ATG9A at Ser761, a site shared with ULK1, which has been shown to promote 14-3-3 binding and localization of ATG9A to autophagosomes¹²⁷. AMPK also dissociates from a basal complex containing WIPI4, ATG2 and FKBP51 after activation, with potential implications on ATG2 positioning for lipid transfer into growing autophagosomes^{128,129}. AMPK may also impair vesicle tethering required for phagophore expansion during glucose stress through a yet-unresolved mechanism¹¹⁹. The summation of these events results in stalled membrane maturation, where the cell avoids engaging in a highly energy-consuming degradative programme during acute glucose starvation.

Although AMPK acutely restrains autophagic flux under glucose starvation, mounting evidence shows that it also has a critical role in preparing and sustaining autophagy during prolonged energy stress. AMPK directly phosphorylates several components of the class III PI3K complex I, including VPS34 and Beclin-1, where the latter event promotes PI3P production, a key lipid signal required for autophagosomal nucleation and expansion^{130,131}. It also modifies auxiliary factors such as RACK1 (ref. 132), PAQR3 (ref. 133) and RASAL2 (ref. 131), which enhances VPS34 activity or stabilizes the complex under stress. In parallel, AMPK protects autophagy-related protein complexes from caspase-mediated degradation, increasing the protein stability of key players such as ULK1 and Beclin-1 during extended nutrient deprivation¹²². These effects are complemented by AMPK-dependent activation of transcriptional programmes, including phosphorylation and increased translocation of TFEB to the nucleus, which enhances lysosomal biogenesis and the expression of autophagy-related genes¹³⁴. Together, these processes enable AMPK to maintain autophagic machinery should the nutrient stress persist.

Together, these findings illustrate that AMPK does not function as a simple on–off switch for autophagy but instead acts as a context-dependent and time-dependent regulator that aligns

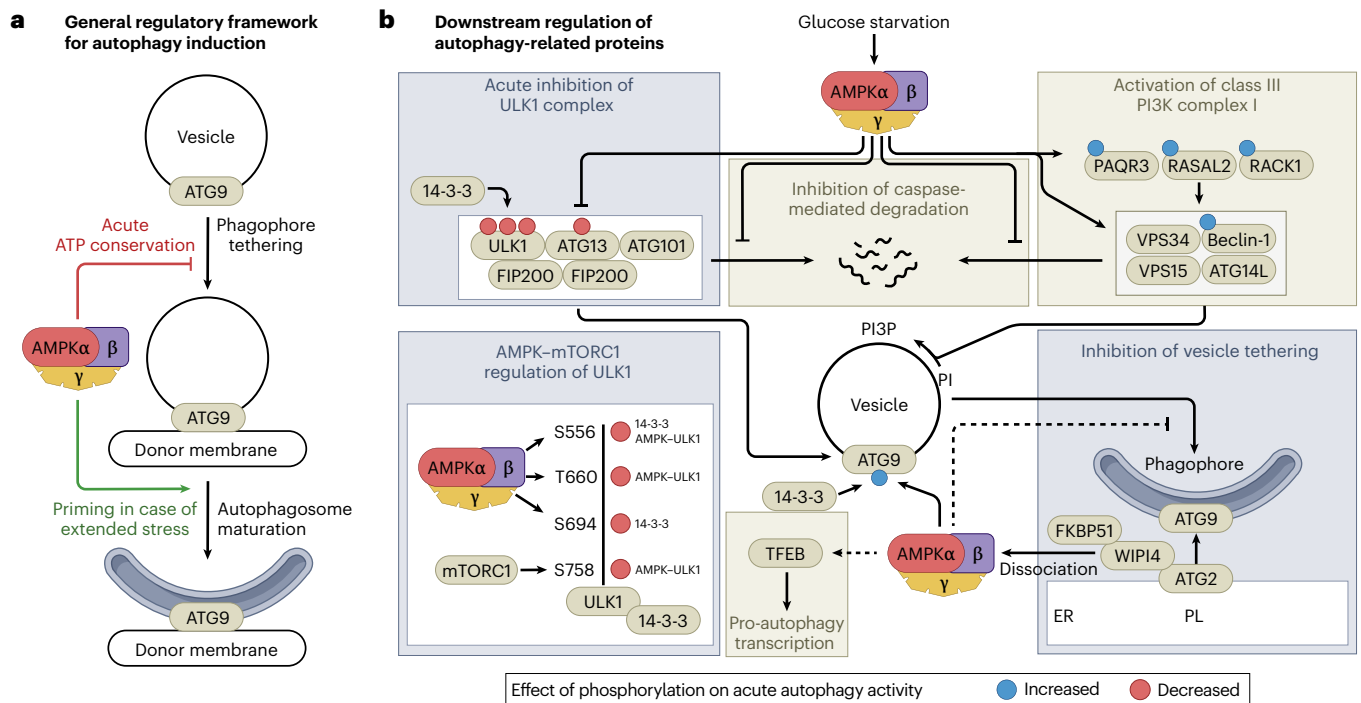


Fig. 2 Distinct acute and chronic actions of AMPK on autophagy during glucose starvation. **a**, Biphasic responses of AMPK on ATG9 vesicle dynamics during acute glucose starvation: during the initial response to glucose starvation, AMPK can either inhibit or activate autophagy depending on immediate energetic needs. When ATP levels fall sharply, AMPK inhibits ATG9 vesicle tethering to conserve energy. With continued starvation, AMPK increases ATG9 cycling and donor membrane delivery, promoting autophagosome maturation. **b**, Chronic AMPK activation supports autophagosome formation: sustained AMPK activation increases autophagy by stabilizing ULK1 and ATG13, enhancing

class III PI3KC1 activity and PI3P production, and enabling ULK1 reactivation during prolonged nutrient stress. AMPK- and ULK1-dependent phosphorylation of ATG9 promotes 14-3-3 binding, positioning ATG9 for later steps in autophagosome maturation. AMPK also increases TFEB-dependent transcription of autophagy genes and dissociates from the WIPI4–FKBP51–ATG2 complex to reduce vesicle tethering. Together, these actions strengthen autophagy when chronic nutrient stress requires ongoing recycling of cellular components. ER, endoplasmic reticulum; PL, phospholipids.

autophagic activity with cellular energetic needs. During acute glucose limitation, AMPK restrains autophagosome maturation to prevent the cell from committing ATP to a degradative programme it cannot yet support. With prolonged nutrient stress, AMPK shifts towards preserving, stabilizing and re-engaging the autophagy machinery, ensuring that autophagy can proceed efficiently once it becomes essential for sustained survival. This biphasic control highlights how AMPK integrates substrate specificity, spatial compartmentalization and the nature of the nutrient deficit to fine-tune autophagy and maintain metabolic homeostasis. Understanding how AMPK coordinates these competing signals with mTORC1 across different nutrient contexts and in different cell/tissue types will be key to resolving how autophagy is regulated in both health and disease, including in selective programmes such as mitophagy¹²⁵, xenophagy¹³⁵ and lipophagy¹⁰⁶.

Transcription

Gene transcription is a highly regulated process that depends on a variety of factors including transcription factor localization and activity, as well as epigenetic histone marks¹³⁶. AMPK phosphorylates several transcription factors and transcriptional coactivators in either the nucleus or the cytoplasm to influence metabolism in response to nutrient changes. For example, low levels of glucose can stimulate AMPK-mediated phosphorylation and activation of the FOXO transcription factors FOXO1 (refs. 137,138) and FOXO3 (refs. 139,140) while inhibiting the transcriptional coactivator p300 (ref. 141). Given the link between AMPK and glucose levels, it is becoming clear that AMPK may directly control transcription of glycolytic enzymes. Recently, AMPK phosphorylation of YBX2 at Thr115 was shown to increase glycolytic gene transcription and translation, although this is probably relevant

for only select tissues¹⁴². However, phosphorylation events on Snail1 at Ser11 (ref. 143) and RUNX1 at Ser94 (ref. 144) could also have roles in regulating glycolytic enzyme levels^{145,146}.

Additionally, AMPK regulates the translocation of transcription factors between the cytosol and nucleus after nutrient stress. Phosphorylation of YAP causes its cytosolic retention^{147–149}, while phosphorylation of the redox-sensitive NRF2 at Ser558 increases its nuclear localization¹⁵⁰. Additional phosphorylation of Ser374, Ser408 or Ser433 may influence target-specific transcription including *HMOX1* (refs. 151,152). AMPK phosphorylation of PHGDH following glucose deprivation does not affect its nuclear translocation but does increase malate oxidation in the nucleus¹⁵³. Activation of AMPK due to low glucose also reduces flux through de novo lipogenesis via phosphorylation of sterol response element-binding protein-1c (SREBP1c)¹⁵⁴, effects which are further reinforced through phosphorylation of insulin-induced gene-1 (Insig1) at Thr222 (ref. 155) and Krüppel-like factor 10 (KLF10) at Thr189 (ref. 156). Additionally, AMPK was recently shown to inhibit skeletal muscle calcium cycling through phosphorylation of cFeimin, which binds to the forkhead transcription factor FOXO2, leading to suppression of sarcolipin¹⁵⁷. As reductions in skeletal muscle sarcolipin have been linked to lower caloric expenditure following weight loss¹⁵⁸, a process known as adaptive thermogenesis or metabolic adaptation, these data suggest that AMPK reduces energy expenditure in muscle through transcriptional mechanisms.

Epigenetic modification of histones by acetylation and methylation can regulate accessibility of DNA to upstream transcription factors. These histone marks are increasingly recognized to be linked to the metabolic state of the cell¹⁵⁹, wherein AMPK exerts several direct effects. For example, histone acetylation depends on nuclear acetyl coenzyme

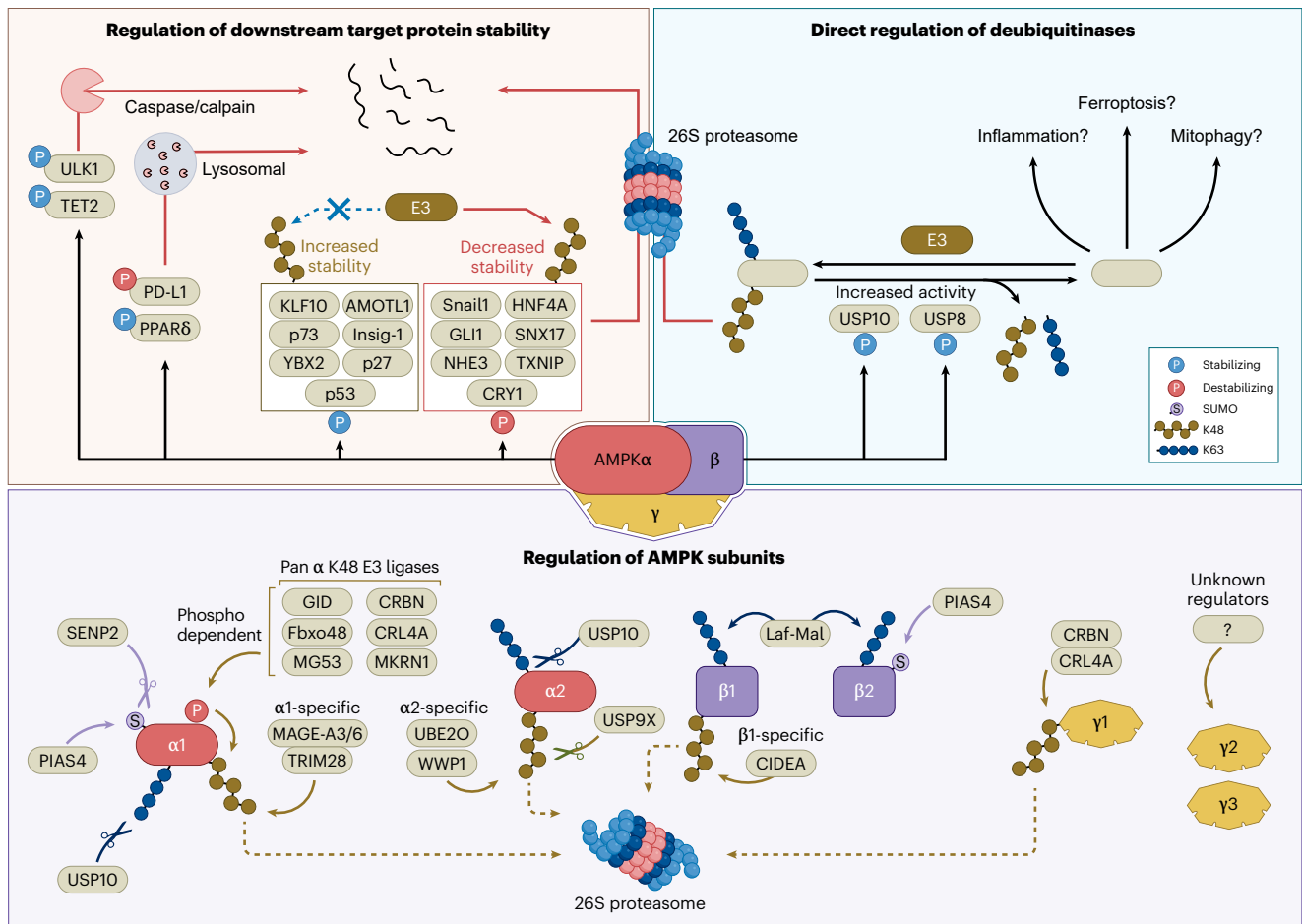


Fig. 3 | AMPK-dependent regulation of protein stability through ubiquitin- and protease-linked pathways. Stabilizing and destabilizing phosphorylation (top left): AMPK phosphorylation can either increase or decrease the stability of downstream proteins. Substrates marked in blue indicate phosphorylation events that increase protein stability, typically by reducing lysosomal, proteasomal or caspase–calpain turnover. Substrates marked in red indicate phosphorylation events that decrease stability, directing proteins involved in growth, transcriptional repression or inflammatory signalling towards enhanced degradation. Deubiquitinases and ubiquitin chain editing (top right): AMPK increases the activity of USP8 and USP10, altering the removal of K48- and

K63-linked ubiquitin chains from downstream proteins. These changes affect pathways including mitophagy, inflammatory signalling and other stress-responsive processes, helping determine substrate fate during energetic stress. Regulation of AMPK subunits (bottom): ubiquitin- and SUMO-dependent regulation of the AMPK α -, β - and γ -subunits. Subunit-specific E3 ligases add K48-linked ubiquitin to target AMPK subunits for proteasomal degradation, while K63-linked ubiquitin influences signalling or localization. SUMOylation and deSUMOylation further adjust AMPK complex abundance and isoform-specific activity during changing metabolic conditions.

A (acetyl-CoA) generated via ATP citrate lyase (ACLY) and acetate via acetyl-CoA synthetase 2 (ACSS2). AMPK increases histone acetylation by promoting nuclear acetate uptake and conversion through phosphorylation of ACSS2 at Ser659 (ref. 160). These effects are further promoted through AMPK phosphorylation of histone deacetylases including SIRT1 (ref. 161), HDAC5 (ref. 162) and EZH2 (ref. 60). AMPK also influences histone and DNA methylation by targeting and activating the histone demethylase PHF2 (ref. 163) or stabilizing the transcription factor TET2 (ref. 61), respectively. For transcription initiation, chromatin immunoprecipitation assays have shown that AMPK α 2 complexes phosphorylate TATA-box-binding protein-associated factor 1 (TAF1) at Ser1353, which impairs TAF1 interaction with RNA polymerase (Pol) II and suppresses transcription¹⁶⁴. Similar observations were reported with Pol I-mediated transcription, where AMPK activity inhibits rRNA synthesis¹⁶⁵. Lastly, following genotoxic stress, calcium-dependent nuclear AMPK activation promotes phosphorylation of Exo1 and modulation of the DNA damage response^{16,17}. These events, in combination with AMPK-mediated phosphorylation of histone H2B⁶⁴, highlight how AMPK can enhance or repress the activation of transcription factors through epigenetic modifications. However, further detailed

assessment of knock-in models will be important to fully understand how important these modifications are for transcriptional responses to changes in glucose and fatty acid availability.

Protein stability

Protein turnover is a key regulator of protein function and is subject to phosphorylation-dependent regulation following glucose starvation^{166,167}. In addition to autophagy and transcriptional control, emerging evidence indicates that AMPK broadly controls protein levels of target substrates by affecting protein stability (Fig. 3). Ubiquitin is an 8.6-kDa protein that can be attached to target lysine residues either singularly or in chains to affect protein–protein interactions or target proteins for degradation in the 26S proteasome. This occurs through an elaborate system featuring E1 ubiquitin-activating, E2 ubiquitin-conjugating and E3 ubiquitin-ligating enzymes, the latter of which number nearly 700 (ref. 168). Despite this number, the only established AMPK E3 ubiquitin ligase target is Parkin¹²⁵, which has an important role in mitophagy (discussed in greater detail below). Removal of ubiquitin chains is catalysed by USPs. Of these, AMPK phosphorylates USP10 at Ser76 to enhance its activity, resulting in a feedforward loop

considering USP10 also deubiquitinates and activates AMPK⁸⁴. Additionally, recent evidence suggests that AMPK phosphorylation of USP8 at Ser718 inhibits 14-3-3 binding and increases its stability, thereby enhancing deubiquitination of selected targets¹⁶⁹. USP8 has been shown to influence AMPK-regulated processes such as mitophagy¹⁷⁰, ferroptosis¹⁷¹ and inflammatory signalling¹⁷², necessitating further investigation of the AMPK–USP8 axis. Beyond direct modulation of ubiquitin regulators, phosphorylation of numerous downstream targets results in either stabilization^{142,149,155,156,173–176} or destabilization^{143,177–183} of target proteins, although some of these are due to modulating lysosomal¹⁸¹, calpain-mediated¹⁸⁴ or caspase-mediated degradation¹²². These data support an important role for AMPK in the regulation of proteostasis, although links between downstream targets and conditions of nutrient stress *in vivo* will require further refinement.

While AMPK exerts notable regulation of ubiquitination and degradative mechanisms of downstream substrates, it is itself regulated by ubiquitination on the $\alpha 1$ (refs. 80,81,185), $\alpha 2$ (refs. 80,81,186–188), $\beta 1$ (refs. 189,190), $\beta 2$ (ref. 190) and $\gamma 1$ (ref. 191) subunits across a range of tissues and with varying effects on AMPK protein stability and activity. Taking advantage of this, a recent study used deubiquitinase-targeting chimeras to force deubiquitination of AMPK β -subunits and increase protein levels¹⁹². This could be of relevance in combination with AMPK activators in diseases where AMPK expression and activity is suppressed. Closely related to ubiquitination is SUMOylation, which involves the attachment of small ubiquitin-related modifiers (SUMOs) onto target lysine residues and often competes with ubiquitination. Although AMPK does not regulate any core SUMO machinery, phosphorylation of mitochondrial fission factor (MFF) enhances MFF SUMOylation and leads to mitochondrial fragmentation¹⁹³. AMPK is SUMOylated itself by PIAS4 on both the $\beta 2$ -subunit¹⁹⁴ and $\alpha 1$ -subunit¹⁹⁵, the latter of which inhibits its activity, while SENP2 causes deSUMOylation of AMPK α and renders it more susceptible to ubiquitination and subsequent degradation¹⁹⁶. Further evaluation of AMPK regulation of and by ubiquitination and SUMOylation, particularly in different tissues and cell types under different nutrient starvation conditions, will be important.

In summary, an emerging number of substrates have linked AMPK nutrient signals with the control of macroautophagy, as well as epigenetic, transcriptional and ubiquitination-based regulation of protein expression. These mechanisms may be important for reinforcing the effects of AMPK over long durations to adapt to changes in chronic alterations in nutrient availability; however, in many cases the physiological significance of this regulation remains unclear. For example, while it is well documented that AMPK reduces the expression of genes important for lipogenesis through phosphorylation of SREBP1 (ref. 154), whether this is physiologically important given metabolic flux through the pathway is already inhibited due to phosphorylation of the rate-limiting metabolic enzyme (for example, ACC) remains to be established. Future studies validating these phosphorylation events using knock-in mouse models will be important for delineating which pathways are vital for changes in nutrient partitioning in response to physiological and pathological adaptations.

Updates on AMPK-mediated control of mitochondrial homeostasis

Cellular adaptations to nutrient availability are heavily dependent on the maintenance of mitochondrial function. For example, in the liver the process of converting glucose to palmitate through *de novo* lipogenesis and generating glucose from pyruvate via gluconeogenesis requires ~54 ATP equivalents. As such, the finely tuned interplay between the formation of new mitochondria (biogenesis), dynamic remodelling of existing mitochondrial networks (fission and fusion) and degradation of non-functional or damaged mitochondria (mitophagy), is essential to fuel appropriate cellular responses to nutrient availability (Fig. 4). Recent work has provided numerous examples of AMPK serving as an essential upstream regulator of mitochondrial homeostasis. Below,

we highlight advances in our understanding of AMPK regulation of mitochondrial dynamics, mitophagy and biogenesis.

Fission

Mitochondrial fission can generate distinct pools of daughter mitochondria depending on whether the scission initiation site is located at the midzone or periphery¹⁹⁷. This is important for propagating the mitochondrial network¹⁹⁸, targeting mitochondria with low membrane potential for mitophagy¹⁹⁷ or enhancing fatty acid oxidation¹⁹⁹. AMPK is localized to mitochondria³³ through *N*-myristoylation¹¹², and this localization is often enhanced with activation^{18,200,201}. Recent work has expanded the number of mitochondrial AMPK targets with many involved in regulating mitochondrial fission. After mitochondrial AMPK activation through impaired mitochondrial function³³ or glucose starvation³⁵, AMPK acutely phosphorylates mitochondrial fission regulators such as MFF^{202,203}, MTFRIL²⁰⁴, ARMC10 (ref. 205), INF2 (ref. 206), AKAP1 (ref. 207) and MCU²⁰⁸. Interestingly, in the cases of MFF²⁰², MTFRIL²⁰⁴ and ARMC10 (ref. 205), introducing alanine point mutations at AMPK phosphosites prevents AMPK-dependent fission in response to pharmacological activation. Therefore, addressing whether these sites function synergistically will require further evaluation. Most of these targets are associated with midzone fission, suggesting that AMPK-induced fission promotes network propagation, or at the very least, network remodelling¹⁹⁷. However, ARMC10 has been shown to associate with both MFF and FIS1 (ref. 205), the latter of which is enriched during peripheral fission events¹⁹⁷.

The functional implications of AMPK-mediated fission will depend in part on the mitochondrial pools generated downstream. For example, PSCS-enriched mitochondria lack cristae and ATP synthase, are generated through consecutive rounds of mitochondrial fission and catalyse proline synthesis²⁰⁹. Alternatively, mitochondrial fission can also generate fatty acid oxidation-specialized mitochondria due to membrane curvature-induced decreases in the binding capacity of CPT1 for malonyl-CoA, thereby relieving CPT1 inhibition¹⁹⁹. Whether or not AMPK-dependent mitochondrial fission produces these or other subpools of mitochondria remains to be seen. Evidence *in vivo* supports that AMPK activation by the AMP-mimetic drug AICAR produces more spherical mitochondria, phenocopying mitochondrial differences between periportal and pericentral hepatocytes²¹⁰. Additionally, populations with obesity and ageing display impaired liver mitochondrial fission that can be reversed by relieving AMPK inhibition⁷⁶. These observations linking AMPK and mitochondrial morphology may also explain why CPT1 is not inhibited during muscle contractions in mice lacking AMPK-inhibitory phosphorylation sites on ACC2 that retain high levels of malonyl-CoA²¹¹, despite an important role for muscle AMPK in controlling fatty acid oxidation²¹². AMPK activation can also promote fatty acid oxidation by increasing Rab8A binding to the lipid droplet-associated protein PLIN5 (ref. 213), or by promoting endoplasmic reticulum–mitochondrial contact sites through an interaction with MFN2 (ref. 214), thereby providing contact sites to facilitate mitochondrial fission¹⁹⁸. Therefore, in addition to reducing local malonyl-CoA levels through inhibition of ACC²¹⁵, AMPK-mediated mitochondrial fission could render CPT1 less sensitive to malonyl-CoA and provide tighter lipid droplet and endoplasmic reticulum membrane contact sites to synergistically enhance fatty acid oxidation.

Altogether, AMPK phosphorylates several regulators that support mitochondrial fission. Whether increases in fission are essential to stimulate fatty acid oxidation in response to alterations in nutrient availability, such as increases in fatty acids or reductions in glucose, requires further investigation.

Mitophagy

Mitophagy is a selective form of autophagy that degrades mitochondria. There are two main mitophagy subtypes: ubiquitin-mediated mitophagy that targets mitochondrial fragments with low membrane

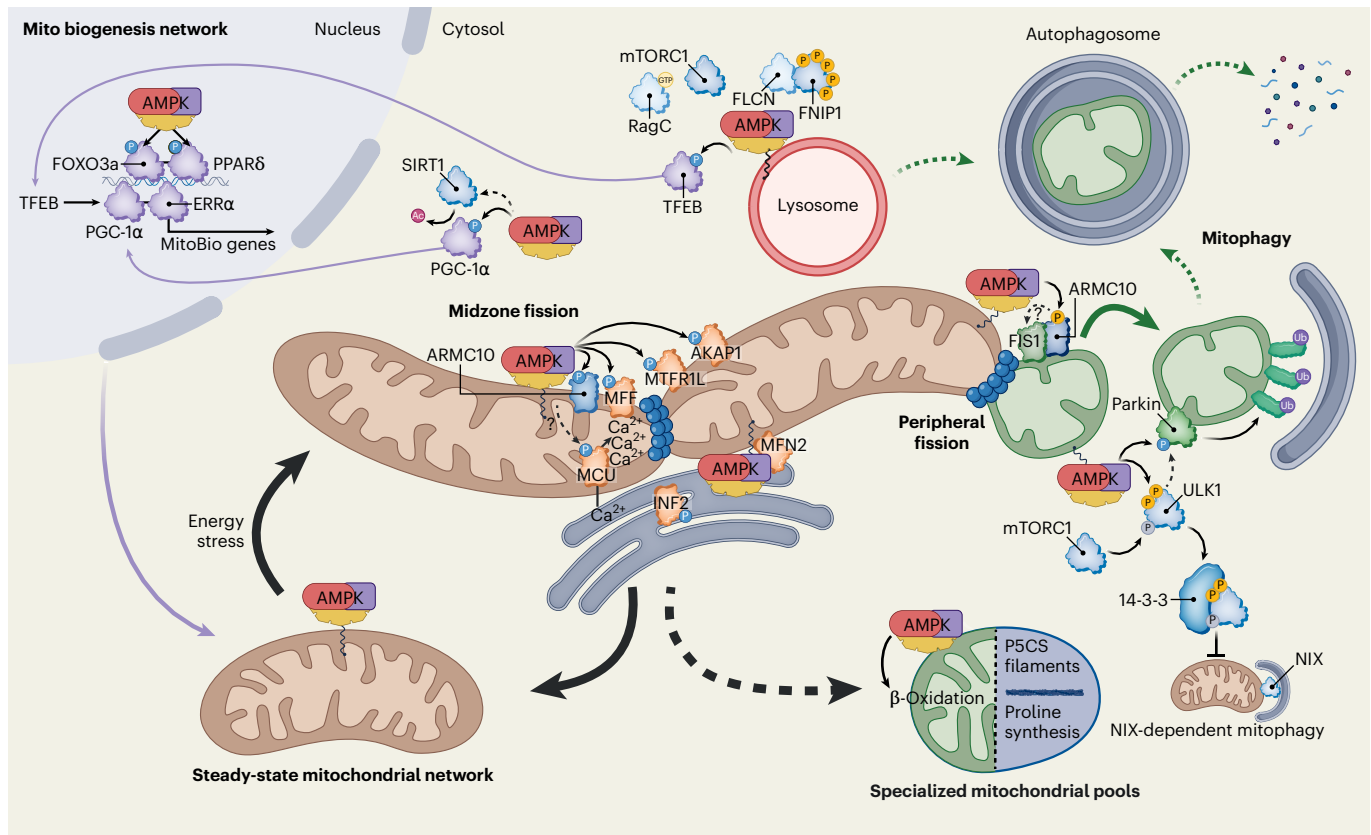


Fig. 4 | AMPK as a master regulator of mitochondrial quality control. Mitochondrial biogenesis (top left): AMPK phosphorylates SIRT1 and TFEB in the cytosol. Activated SIRT1 deacetylates PGC-1 α , promoting its nuclear translocation, while TFEB also translocates into the nucleus. These events along with AMPK phosphorylation of FOXO1 and PPAR δ and interactions with ERR α drive expression of mitochondrial biogenesis. Mitophagy (top right): AMPK supports selective removal of damaged mitochondria by promoting PINK1–Parkin-dependent mitophagy. AMPK- and ULK1-dependent phosphorylation of Parkin enhances its recruitment to impaired mitochondrial fragments. By contrast, AMPK-dependent sequestration of ULK1 by 14-3-3 decreases NIX-dependent mitophagy of functional mitochondria, helping maintain mitochondrial content during stress. Specialized mitochondrial pools (bottom right): repeated fission events generate mitochondrial subpopulations with

distinct metabolic roles, including mitochondria enriched for β -oxidation and mitochondria associated with P5CS filaments that support proline synthesis. Mitochondrial fission (bottom centre): AMPK phosphorylates MFF, ARMC10, MTRFRL, INF2, AKAP1 and MCU to increase midzone fission and establish endoplasmic reticulum–mitochondrial contact sites. AMPK interaction with MFN2 at these junctions assists mitochondrial division. ARMC10 and FIS1 in peripheral regions promote fission events that are more frequently directed towards mitophagy. Integration of stress signals (bottom left): across the mitochondrial network, AMPK responds to energetic stress, mitochondrial toxins or hypoxia by coordinating acute fission, selective mitophagy and subsequent biogenesis. These integrated pathways preserve mitochondrial quality and support metabolic flexibility under fluctuating nutrient conditions.

potential and depends on PINK1–Parkin signalling, and receptor-mediated mitophagy that degrades functional mitochondria and requires receptors such as NIX^{216,217}. While AMPK may influence mitophagy through its pro-mitochondrial fission signalling, several recent studies have additionally placed AMPK as a direct upstream regulator of mitophagy-specific proteins. One mechanism involves AMPK phosphorylation of Parkin at Ser108, which can stimulate PINK1–Parkin-dependent mitophagy¹²⁵. This can occur even in the absence of ULK1, which can also phosphorylate Parkin¹²⁵. However, AMPK-induced sequestration of ULK1 by 14-3-3 prevents NIX-dependent mitophagy of functional mitochondria¹²⁵. This presents a two-pronged approach through which AMPK supports the removal of damaged mitochondria while sparing functional mitochondria, reminiscent of its role in opposing the wholesale, energetically costly induction of macroautophagy. However, this framework is complicated by the fact that rapid phosphorylation of ULK1 at Ser556 is needed for mitophagic induction in response to the mitochondrial uncoupler CCCP²⁰⁰. Therefore, the temporal and context-dependent nature of these phosphorylation events must be reconciled in the future. Regardless, both AMPK and ULK1 accumulate in mitochondrial fractions quickly following mitochondrial uncoupling or exercise, where non-myristoylated AMPK fails to induce mitophagy^{112,218,219}. Importantly, defects in AMPK-induced

mitophagy are observed in several mouse models in which AMPK is impaired including hepatocytes⁹⁸, muscle²²⁰ and brown adipose tissue (BAT)²²¹ and is associated with metabolic dysfunction-associated steatohepatitis (MASH), sarcopenia and cold intolerance, respectively, indicating a vital role for this process in maintaining homeostasis. Taken together, AMPK activity is responsible for the induction of mitophagy, although it accomplishes this in a selective manner that spares functional mitochondria from energetically wasteful degradation.

Mitochondrial biogenesis

The replenishment of mitochondria via the transcriptional upregulation of new respiratory chain subunits is essential for maintaining an adaptive mitochondrial network that can readily respond to varying stressors. AMPK-activating conditions such as chronic exposure to free fatty acids stimulates mitochondrial biogenesis⁹⁸. Recent work has identified some of the key regulators that contribute to this phenotype including PPAR δ ¹⁷⁴, FOXO3 (refs. 139,140) and TFEB/TFE3 (ref. 222), as well as FNIP1, which regulates the localization of TFEB/TFE3 (refs. 134,223). Phosphorylation of FNIP1 on five distinct phosphorylation sites disrupts mTORC1-mediated cytosolic retention of TFEB/TFE3 (refs. 134,223), which, in addition to the well-characterized lysosomal biogenesis network downstream of TFEB/TFE3 activation, leads to

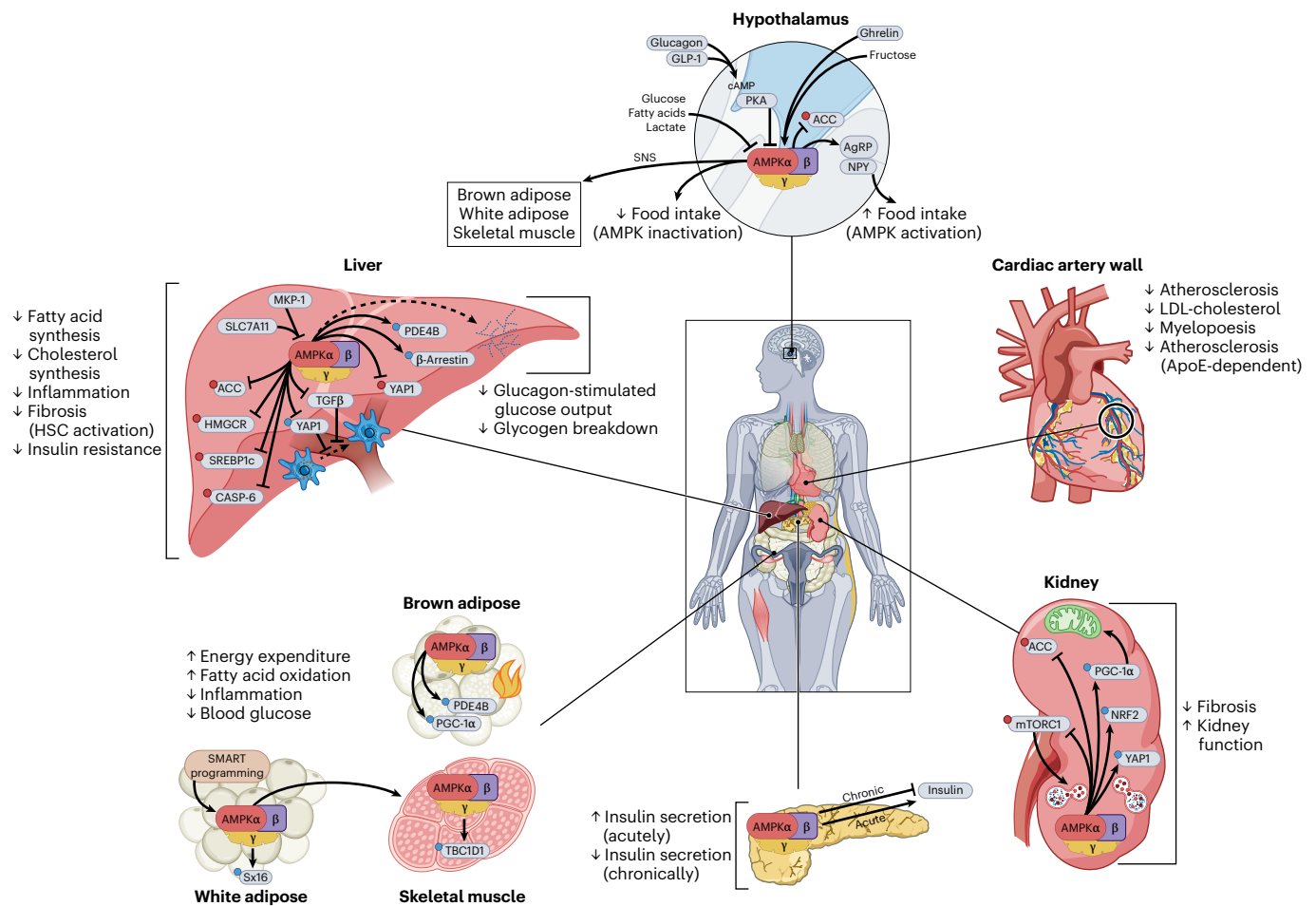


Fig. 5 | AMPK control of tissue-specific metabolism in cardiometabolic disease. Hypothalamus: nutrient and hormone signals regulate AMPK activity in hypothalamic neurons to control appetite and energy expenditure. GLP1R agonists reduce hypothalamic AMPK activity, suppressing food intake, whereas AMPK activation in this region promotes feeding and reduces thermogenesis. Cardiac and vascular system: AMPK activation in the heart increases glucose uptake, fatty acid oxidation and ATP production during increased workload or ischaemia, helping maintain cardiac function. In the vascular wall, AMPK reduces inflammation, improves endothelial function and limits smooth muscle cell proliferation. In macrophages, AMPK decreases pro-inflammatory signalling and foam cell formation, contributing to atheroprotection and reduced lesion development. Kidney: renal AMPK maintains mitochondrial function and autophagy, supports tubular cell survival and helps prevent the development

and progression of CKD under metabolic stress conditions. Pancreas: AMPK acutely enhances glucose-stimulated insulin secretion, contributing to the regulation of systemic glucose levels during nutrient challenges. White and brown adipose tissue and skeletal muscle: AMPK promotes thermogenesis and energy expenditure by increasing glucose uptake, fatty acid oxidation and mitochondrial biogenesis, collectively improving insulin sensitivity. Liver: AMPK activation decreases lipogenesis, cholesterol synthesis and gluconeogenesis while reducing inflammation and fibrosis, reducing MASLD and diabetes. Through these coordinated actions, AMPK improves systemic glucose metabolism, lipid handling and mitochondrial function and reduces cardiometabolic risk. HSC, hepatic stellate cell; LDL, low-density lipoprotein; SNS, sympathetic nervous system.

upregulation of an N-terminally truncated form of PGC-1 α . This in turn promotes activation of the transcription factor ERR α and PGC-1 α target genes¹³⁴. The importance of TFEB activation is supported by observations that mice with a transgenic muscle-specific alanine mutation in *FNIP1* (*FNIP1*^{S220A}) show impaired exercise tolerance²²³, similarly to mice lacking AMPK²²⁴. These data suggest that phosphorylation of FNIP1 and the resulting liberation of TFEB/TFE3 from the lysosome is probably a critical driver of the induction of mitochondrial biogenesis, although multiple overlapping mechanisms contribute to the maintenance of the mitochondrial pool.

In summary, mitochondrial homeostasis is orchestrated by AMPK through its integrated regulation of mitochondrial fission, mitophagy and biogenesis, thereby enabling cells to adapt metabolically by removing damaged mitochondria, preserving functional organelles and replenishing the mitochondrial pool under nutrient stress. Given this central role, studies involving chronic AMPK inhibition must carefully account for downstream alterations in mitochondrial dynamics, as

observed phenotypes may stem primarily from impaired mitochondrial function rather than from direct loss of AMPK signalling. Moreover, because mitochondrial homeostasis influences a wide range of physiological processes, its preservation may be a key determinant of therapeutic efficacy across diverse disease contexts, including metabolic disorders, cancer and rare diseases.

AMPK and metabolic diseases

Metabolic inflexibility, or the inability to switch efficiently between fuel sources during fasting and refeeding, is a hallmark of cardiometabolic diseases including obesity, type 2 diabetes, metabolic dysfunction-associated steatotic liver disease (MASLD) and chronic kidney disease (CKD). Given the role of AMPK as a central nutrient sensor that coordinates glucose and fatty acid metabolism with mitochondrial function, impaired AMPK signalling contributes to the development and progression of these disorders and is viewed as an important therapeutic target (Fig. 5 and Box 2).

BOX 2

AMPK activators and inhibitors—an update

Since the 2019 review³⁸⁹ of synthetic AMPK activators such as MK-8722, SC4 and PF-739, progress has been made towards developing clinical candidates (Supplementary Table 3). ATX-304 (formerly O304) has shown benefits in various indications, although its recently identified mitochondrial uncoupling activity raises questions about the importance of AMPK in mediating these effects^{278,279,390,391}. PXL770, an allosteric β 1-AMPK activator (and β 2 at higher doses), stabilizes α -T172 phosphorylation via ADaM site binding²⁵⁸. Clinical trials demonstrated its safety, lipogenesis suppression (phase 1)³⁰⁹ and liver steatosis reduction in people with type 2 diabetes (phase 2a)²⁵⁹. It also improved outcomes in rodent models of polycystic kidney disease³⁴⁸ and adrenoleukodystrophy³⁵⁸. BI-9774, a pan- β AMPK activator, suppresses leptin³⁹² and migration inhibitory factor³⁹³ release from adipocytes and macrophages, respectively.

By contrast, AMPK inhibition may be therapeutic in certain contexts such as appetite suppression and cancer. Notable inhibitors include compound C (dorsomorphin), SBI-0206965 and BAY-3827 (Supplementary Table 3). Compound C is a non-selective ATP-competitive inhibitor and should not be relied upon to delineate AMPK function due to extensive off-target effects^{394–396}. SBI-0206965, initially described as a ULK1 inhibitor³⁹⁷, has improved selectivity but still targets AMPK-related kinases^{394,398}. BAY-3827, the most potent inhibitor, affects p90 ribosomal S6 kinases with a distinct off-target profile³⁹⁹. Using both SBI-0206965 and BAY-3827 is the most reliable approach to study AMPK inhibition. MT47-100, an ADaM site modulator, acts as a β 2-AMPK inhibitor and β 1-AMPK activator, offering therapeutic potential in β 2-overexpressing tumours or β 1-driven tumour suppression²⁷⁵.

Obesity

The hypothalamus has a central role in regulating energy balance, controlling food intake and energy expenditure. Consistent with promoting anabolism, a truncated constitutively active AMPK mutation injected into the hypothalamus of mice increases food intake²²⁵. Mice expressing a γ 2-AMPK mutation (p.Arg299Gln), which makes AMPK constitutively active, are also hyperphagic due to increased Agouti-related peptide sensitivity to ghrelin, leading to obesity even when fed a chow diet²²⁶. Mechanistically, AMPK stimulation of appetite in response to ghrelin is dependent on AMPK phosphorylation of ACC, which lowers malonyl-CoA²²⁷, an important regulator of appetite. This pathway also seems to be important in humans as people with the equivalent AMPK mutation (γ 2-p.Arg302Gln) have increased adiposity²²⁶. Similarly, a polymorphism in fatty acid amide hydrolase in mice, which increases the endocannabinoid anandamide and is associated with obesity in humans, increases hypothalamic AMPK activity and weight gain²²⁸. Consistent with the role of AMPK in controlling appetite, genetic inhibition of AMPK activity in the hypothalamus blunts orexigenic Agouti-related peptide and neuropeptide Y expression, food intake and ghrelin-induced food intake²²⁹. Glucose²²⁵, lactate²³⁰ and free fatty acids also exert anorexigenic effects through inhibition of hypothalamic AMPK activity with recent studies indicating that the latter may exert effects through free fatty acid receptor 1 (FFAR1)/GPR40 (ref. 231). These data suggest that the activation of AMPK promotes food intake, whereas inhibition reduces food intake, and that these effects can also be translated to humans.

Glucagon-like peptide 1 receptor (GLP1R) agonists are approved for weight loss and have been shown to reduce the incidence of type 2 diabetes, cardiovascular disease (CVD), MASLD and CKD. The GLP1R is predominately expressed in the hindbrain, with projections involving proopiomelanocortin-expressing neurons in the arcuate nucleus of the hypothalamus. GLP1R agonists reduce adiposity primarily by suppressing appetite; this involves increases in cAMP/PKA, which suppress hypothalamic AMPK activity^{232–234}, effects that are blocked when AMPK activity is elevated by low glucose or intracerebroventricular injection of fructose²³⁴. These effects are cell autonomous as exendin-4 also reduces AMPK activity in a dose-dependent manner in a GT1-7 hypothalamic cell line²³². AMPK α -T172 phosphorylation is also elevated in the hypothalamus of GLP1R null mice, which are known to have increased food intake and obesity. Similarly, intracerebroventricular glucagon administration also reduces food intake, activating PKA and inhibiting AMPK²³⁵, effects that are blocked with a constitutively active AMPK adenovirus delivered to the arcuate nucleus of the hypothalamus²³⁵. Notably, AMPK activity is increased in many tissues following treatment with GLP1R agonists, although whether this reflects secondary effects of weight loss, improved insulin sensitivity and reduced inflammation, or instead involves direct activation of AMPK, remains unresolved. Addressing this will require studies in calorically matched or pair-fed controls, as well as time-course experiments to determine whether AMPK activation precedes weight loss. It will also be important to clarify whether incretin-based therapeutics such as semaglutide or tirzepatide directly modulate hypothalamic AMPK activity and, if so, whether this contributes to reduced food intake and the broad spectrum of disease benefits attributed to these agents. Resolving these questions will be essential for determining the extent to which AMPK acts as a mediator of GLP1R agonist efficacy and whether it represents a tractable target for enhancing or extending their therapeutic effects.

White adipose tissue and BAT are important for controlling energy expenditure through release of endocrine factors and control of futile cycling pathways, and AMPK seems to have an important role in both facets. Many endocrine factors regulate energy expenditure and metabolism through activation of AMPK (reviewed in ref. 90). AMPK is highly expressed in BAT and is activated by catecholamines in response to cold. Consistent with these observations, high-throughput screening of 190 protein kinases controlling the formation of BAT identified AMPK as being critical in this process²³⁶. In *db/db* mice, neuronal-targeted small extracellular vesicles that inhibit hypothalamic AMPK stimulate BAT thermogenesis and browning of white adipose tissue²³⁷. Adipocyte-specific deletion of either AMPK α 1 α 2 or the inducible deletion of AMPK β 1 β 2 isoforms leads to impaired thermogenesis and a lethal drop in core body temperature when mice are housed in the cold (4 °C)²²¹. Importantly, reductions in adipose tissue AMPK lead to increases in adiposity, insulin resistance and fatty liver disease when mice are fed a high-fat diet. Inhibition of BAT AMPK by microRNA cluster miR-130b/301b²³⁸, the pesticide chlorpyrifos²³⁹ or hyperuricaemia²⁴⁰ also promotes obesity, liver steatosis and insulin resistance. Consistent with these findings, mice expressing a constitutively active AMPK in adipose tissue have increases in energy expenditure and are protected against diet-induced obesity owing to enhanced expression of the calcium pump SERCA1, which is normally found only in muscle²⁴¹. Recent studies suggest that AMPK may promote BAT thermogenesis through phosphorylation of Y-box binding protein 2 (YBX2) at Thr115, which stabilizes PGC-1 α ¹⁴². AMPK may also promote glucose uptake in adipocytes through phosphorylation of syntaxin-16 (Sx16) at Thr7 (ref. 242). Adipocyte AMPK α 2 is also a critical regulator of circadian programmes that coordinate communication between adipose tissue and muscle to enhance exercise capacity and prevent obesity²⁴³.

These data indicate a critical role for adipose tissue AMPK in promoting energy expenditure, and although this may seem counterintuitive given the role of AMPK in reducing energy demand, maintaining

euthermia is essential for mammals, and futile cycling in adipose tissue is energetically more efficient than shivering thermogenesis.

Diabetes

Elevations in hepatic glucose output are a hallmark of diabetes, contributing to hyperglycaemia and controlled by the balance between glucagon and insulin. In the fasted state, glucagon induces a cAMP–PKA signalling cascade culminating with the transcription of gluconeogenic targets. While glucagon activates AMPK in the liver²⁴⁴, an inhibitory role for AMPK in the regulation of hepatic glucose production was suggested²⁴⁵ and strengthened by the observation that metformin activated hepatic AMPK²⁴⁶. Conflicting evidence that AMPK promotes or inhibits hepatic glucose production has been presented over the years, with conclusions based on an array of genetic mouse models^{247–250}. With the advent of next-generation AMPK activators, it was recently demonstrated that AMPK activation lowered glucagon-induced gluconeogenesis in primary mouse hepatocytes as well as in vivo^{251,252}. AMPK suppresses glucagon-stimulated hepatic glucose production by phosphorylating and activating phosphodiesterase 4B, which degrades cAMP, thereby antagonizing the cAMP–PKA pathway²⁵³. AMPK also phosphorylates CRTC2 (CREB-regulated transcription coactivator 2), which prevents its nuclear translocation and upregulation of gluconeogenic genes²⁵⁴, an effect that is augmented by low glycogen levels during fasting⁸⁷. Lastly, recent studies have found that at least in the cardiac cells, AMPK phosphorylates β -arrestin at Ser330 increasing phosphodiesterase 4 expression and activity and lowering cAMP²⁵⁵, which if also observed in the liver could explain previous findings showing metformin suppressed cAMP accumulation and lowered hepatic glucose production²⁵⁶. In addition to reductions in gluconeogenesis, direct metabolic flux analyses in conscious unrestrained mice show that glycogenolysis is also reduced in liver-specific AMPK $\alpha 1\alpha 2$ knockout mice²⁵⁷. Consistent with these findings, in mice²⁵⁸ and humans²⁵⁹ activation of AMPK complexes containing the $\beta 1$ -subunit with PXL770 is associated with reductions in blood glucose. These data strongly support the model that activation of AMPK suppresses hepatic glucose production.

Skeletal muscle is the primary tissue contributing to glucose disposal in humans, yet no diabetes medications directly target this tissue. In mice and humans, AMPK $\alpha 2\beta 2\gamma 3$ -containing complexes are important for increasing glucose uptake into skeletal muscle^{260–265}. AMPK activation with exercise also enhances muscle insulin sensitivity, which is mediated through the phosphorylation of TBC1D1 (refs. 266,267). Pan-AMPK $\beta 1\beta 2$ activators PF-739 and MK-8722 both increase glucose uptake in skeletal muscle and lower blood glucose in mice and non-human primates^{252,268}. Despite these promising results, the path to clinical use has been hindered by the observation that chronic administration of MK-8722 promoted non-pathological cardiac hypertrophy. Considering AMPK overactivity has been linked to cardiac abnormalities in Wolff–Parkinson–White syndrome²⁵², a condition caused by *PRKAG2* mutations producing constitutively active $\gamma 2$ -AMPK in the heart²⁶⁹, these fears have sidelined pan-AMPK $\beta 1\beta 2$ activators. Such unintended effects signified a major bottleneck for the design of clinically effective AMPK drugs, with the goal now shifting towards discovery of pharmaceuticals that exclusively target $\alpha 2\beta 2$ - (ref. 270) or $\gamma 3$ -containing heterotrimers⁷.

In addition to enhancing glucose disposal in muscle, preserving β -cell mass and enhancing insulin secretion are important for improving glucose homeostasis in diabetes. Over the past two decades there have been many disparate findings in this research area with studies showing that activation of AMPK in pancreatic islets leads to inhibition^{271,272} or stimulation^{273–275} of insulin secretion. However, the use of more targeted small-molecule direct AMPK-activating compounds, including 991, RA089 and PF-06409577, have found that transiently activating AMPK potentiates glucose-stimulated insulin secretion from islets, whereas chronic activation inhibits secretion²⁷⁶. AMPK activation

in islets in response to exercise, the GLP1R agonist exendin-4 or the AMPK activator 991 also protects against β -cell senescence, an important factor contributing to type 2 diabetes²⁷⁷. The dual AMPK activator and mitochondrial uncoupler O304 (now known as ATX-304) also prevented hyperglycaemia in streptozotocin-treated mice by increasing insulin secretion²⁷⁸. This molecule also stimulates insulin-independent glucose uptake and utilization in skeletal muscle and heart in vivo while paradoxically reducing glycogen accumulation²⁷⁸. Critically, O304 reverts diabetic cardiomyopathy^{278,279}. Mechanistically, this may be because glucose uptake promoted by AMPK activation can be shuttled towards oxidation, due to the uncoupling activity, rather than glycogen synthesis as occurs with β -pan activators²⁷⁸. These data with O304 highlight the multimodal mechanism by which AMPK activation may potentially exert positive effects on glucose homeostasis in the absence of cardiac dysfunction.

CKD

The kidney contains numerous cell types with distinct functions and energy requirements. AMPK is highly expressed in certain kidney regions, such as the proximal tubule and the ascending limb of the loop of Henle, while other areas, such as renal tubular cells and podocytes, have lower expression levels²⁸⁰. Shear stress from urine flow activates AMPK in kidney epithelial cells, which is essential for regulating lipophagy and mitochondrial biogenesis through AMPK–mTOR and AMPK–PGC1 α pathways²⁸¹. Additionally, fluid flow-induced AMPK activation controls epithelial cell size by phosphorylating proteins such as YAP1, thereby enhancing autophagy within the cilium²⁸². Notably, deletion of AMPK α subunits in renal tubular cells disrupts salt and water balance, underscoring AMPK's importance in kidney homeostasis²⁸³. In diabetes-associated CKD, reduced AMPK activity is linked to impaired kidney function and increased renal hypertrophy in animal models^{284,285}. The activation of AMPK using small-molecule activators lowers serum creatinine and reduces proteinuria, oxidative injury and fibrosis^{286–292}, effects which may involve the phosphorylation of ACC²⁹³ and NRF2 (ref. 151). These data suggest that AMPK activators could provide valuable new treatment options for patients with CKD and other kidney-related ailments.

MASLD and MASH

MASLD is the most common cause of chronic liver disease and encompasses a spectrum of liver pathologies ranging from steatosis to MASH, which is characterized by hepatocyte death, inflammation and fibrosis. MASLD and MASH are associated with reduced hepatic AMPK activity in many studies^{294–296}, an effect that has been linked to increased oxidative stress mediated by solute carrier family 7 member 11 (SLC7A11)²⁹⁷ or mitogen-activated protein kinase phosphatase-1, which sequesters LKB1 in the nucleus²⁹⁸; however, it should be noted that some studies have failed to detect changes in LKB1 localization in response to phosphorylation²⁹⁹. Many microRNAs also inhibit AMPK activity and promote steatosis³⁰⁰ and inflammation³⁰¹. Genetic inhibition of AMPK α ³⁰², AMPK β ^{25,302} or the ADaM binding site ($\beta 1$ -p.Ser108Ala mutation)⁹⁸ promotes MASH in mice while introducing a constitutively active AMPK through modulation of α ³⁰³, $\beta 1$ (ref. 52) or $\gamma 1$ (refs. 241,304) isoforms protects mice from MASH. These beneficial effects have been attributed to phosphorylation of several substrates including ACC^{305,306}, HMGCR³⁰⁷, SREBP1 (ref. 154) and pro-caspase-6 (ref. 295). There have been over 100 distinct xenobiotics and small molecules tested in mouse models of MASLD that have been shown to have positive effects on reducing steatosis, inflammation and in some cases also fibrosis, indicating the reproducibility of early findings describing these effects. Importantly, AMPK $\beta 1$ -biased compounds including PF-06409577 (ref. 308) and PXL770 (ref. 258) also advanced into studies with non-human primates and humans, with the latter also reducing de novo lipogenesis³⁰⁹ and safely lowering liver steatosis and insulin resistance in people with type 2 diabetes²⁵⁹. PXL770 also reduced TGF β -induced activation of hepatic

stellate cells, key drivers of liver fibrosis²⁵⁸. Mechanistically, activation of AMPK may reduce fibrosis by suppressing the proliferation of hepatic stellate cells through inhibition of cholesterol and fatty acid synthesis²⁵⁸. AMPK also phosphorylates and inhibits YAP³¹⁰, a key regulator of the Hippo pathway that is upregulated in MASH and is important for activation of hepatic stellate cells³¹¹. These data indicate that activation of AMPK may exert beneficial effects in MASH by targeting steatosis, inflammation and fibrosis simultaneously.

CVD

Individuals with comorbidities such as obesity, MASLD, CKD and diabetes are at increased risk of morbidity and mortality from CVD, which encompasses a spectrum of conditions. As a regulator of lipid homeostasis and inflammatory programmes, AMPK is positioned as a potentially important therapeutic target for atherosclerosis, which stems from imbalances in lipid metabolism and maladaptive inflammatory responses that promote plaque formation and lead to acute coronary events³¹². Genetic deletion of various AMPK subunits ($\alpha 1$, $\alpha 2$, $\alpha 1/\alpha 2$, $\beta 1$) has failed to bring clarity as to the endogenous role of AMPK in atherosclerosis with disparate results potentially related to different models (ApoE or LDLR knockout) and time of analysis (early or late disease)^{313–317}. Similarly, in a mouse model where AMPK was unable to phosphorylate and inhibit HMGCR (p.Ser871Ala knock-in mice), atherosclerosis was more severe when mice were deficient for ApoE³¹⁸, but not LDLR³¹⁹. Interestingly, lysosomal accumulation of the cholesterol derivative 25-hydroxycholesterol causes inhibition of mTORC1, leading to AMPK activation and subsequent STAT6 phosphorylation at Ser564, which may be atheroprotective³²⁰. Irrespective of the role of endogenous AMPK in controlling disease development, there is strong evidence that activation of AMPK $\beta 1$ -containing heterotrimers using salicylate^{314,321} or PF-06409577 (ref. 313) inhibits cholesterol synthesis and atherosclerosis development. Importantly, PF-06409577 also lowers plasma low-density lipoprotein cholesterol in non-human primates³⁰⁸. Metabolic comorbidities including obesity, diabetes, CKD and MASH are associated with low AMPK activity and heightened cardiovascular risk (Fig. 5). Therefore, pharmacological activation of AMPK, particularly $\beta 1$ -containing complexes, shows promise in reducing cholesterol levels and atherosclerotic progression, while simultaneously exerting positive effects on glucose homeostasis (Box 2).

Cancer

A growing area of interest in the field of cancer biology is how changes in diet and nutrient partitioning affects outcomes through direct modulation of tumour proliferation or through changes in tumour immunogenicity. In contrast with metabolic disorders, in which AMPK activation generally improves disease outcomes, AMPK exhibits both tumour-suppressive and tumour-promoting effects depending on the type and stage of tumour development, effects which may involve the context of activation (Fig. 6). Potential anti-tumorigenic effects of AMPK activation have been observed alongside reduced cell proliferation in hepatocellular carcinoma, prostate cancer and non-small-cell lung cancer^{306,322–324}. Suppression of proliferation has been linked to AMPK-mediated inhibition of protein synthesis via phosphorylation of components of the mTORC1 complex^{325–327}, HIF1 α ³²³ or eEF2K³²⁸, which deprive cells of the necessary building blocks for growth and division³²⁹. Fasting or ketogenic diet conditions, which lower glucose and increase free fatty acids, also suppress translation due to AMPK phosphorylation of MAP kinase-interacting protein kinase, which phosphorylates and inhibits eIF4E⁹⁴. Lower levels of glucose can also influence anti-tumorigenic transcription through phosphorylation of FOXM1 (ref. 330), the methyltransferase EZH2 (ref. 181) or the transcription factor TET2 (ref. 184). Lastly, activation of AMPK in response to ketogenic diets can promote immunogenicity through phosphorylation of programmed death-ligand 1 (PD-L1; ref. 181). These data

suggest multiple mechanisms by which AMPK–nutrient interactions may mediate positive effects on tumour burden.

Despite these anti-tumour effects, AMPK activation can also support pathways beneficial for tumour growth in established cancers. To sustain growth, tumour cells rely on amino acids such as glutamine for protein synthesis, metabolism and redox balance³³¹. Recent work has elucidated how under low glucose conditions, AMPK phosphorylates PDZD8 to activate GLS1 (ref. 332), increasing glutaminolysis and the funneling of glutamine into the tricarboxylic acid (TCA) cycle. Additionally, asparagine synthetase converts aspartate and glutamine to asparagine and glutamate, respectively, where asparagine levels link mitochondrial respiration rates to mTORC1 activity³³³ and can boost the import of other amino acids³³⁴. Interestingly, LKB1 activity is suppressed by elevated intracellular asparagine levels^{335,336}, suggesting that the interplay between glutamine and asparagine may serve as a key regulatory node for AMPK activity. Additionally, AMPK can reside within the mitochondrial matrix by an unclear mechanism, where it phosphorylates the α -subunit of the pyruvate dehydrogenase complex²⁰¹. This increases the flow of pyruvate into the TCA cycle and worsens metastasis in breast cancer models²⁰¹.

Beyond metabolism, recent studies have highlighted how AMPK regulates multiple forms of cell death. Direct phosphorylation of RIPK1 (ref. 337) and pro-caspase-6 (ref. 295) limits apoptosis during glucose restriction and hepatic steatosis, respectively. Pyroptosis, which can be induced by mannose supplementation and is considered anti-tumorigenic³³⁸, is fuelled by gasdermin family members that perforate the cell membrane. AMPK is activated by mannose owing to direct interaction with *N*-acetyl-D-glucosamine 6-phosphate, leading to phosphorylation of GSDME and suppression of pyroptosis³³⁹. Additionally, AMPK opposes hypoxia-induced cell death by inhibiting aldolase B via phosphorylation and suppressing glycerol excretion³⁴⁰. Iron-dependent ferroptosis requires lipid peroxidation to facilitate cell death and is generally considered to be tumour suppressive³⁴¹. Through inhibition of ACC, AMPK minimizes the abundance of polyunsaturated fatty acids available for peroxidation, thereby suppressing ferroptotic cell death³⁴². However, in combination with pyrimidine synthesis inhibitors, AMPK activity induces ferroptosis through phosphorylation of UMP5³⁴³. AMPK activation can also induce cell death in several leukaemias^{144,344}, highlighting the complex and context-dependent role of AMPK in cell death regulation. This is further complicated by the phosphorylation of RRM1, which functions to increase dNTPs and promote cancer cell survival³⁴⁵. Therefore, both AMPK activators and inhibitors could be used to treat various cancers depending on their type and stage.

Emerging therapeutic areas

ADPKD is the most common inherited kidney disease characterized by a progressive increase in cyst number and size, often leading to kidney failure. Activation of AMPK $\beta 1$ -containing heterotrimers using salsalate³⁴⁶, PF-739 (ref. 347) or PXL770 (ref. 348) alleviates proliferation of cyst-lining epithelial cells in mouse models of autosomal dominant polycystic kidney disease; with salsalate effects being additive with the current standard of care tolvaptan³⁴⁹. The effects of AMPK on disease development probably involve inhibition of cystic fibrosis transmembrane conductance regulator³⁵⁰ and mTORC1 (ref. 351) while enhancing mitophagy/autophagy and mitochondrial biogenesis³⁵². Despite these positive effects of therapeutics, surprisingly, chronic constitutive AMPK activation is linked to the development of cysts in distal convoluted tubules and cortical collecting ducts^{353,354}, suggesting further studies may be needed to evaluate long-term safety.

Neuromuscular disorders, including Duchenne muscular dystrophy, myotonic dystrophy type 1 and spinal muscular atrophy, are distinct disorders but exhibit common alterations in mitochondrial biology. Consistent with this concept, removing AMPK from muscle accelerates ageing²²⁰ and muscle disuse-induced atrophy³⁵⁵. Treatment

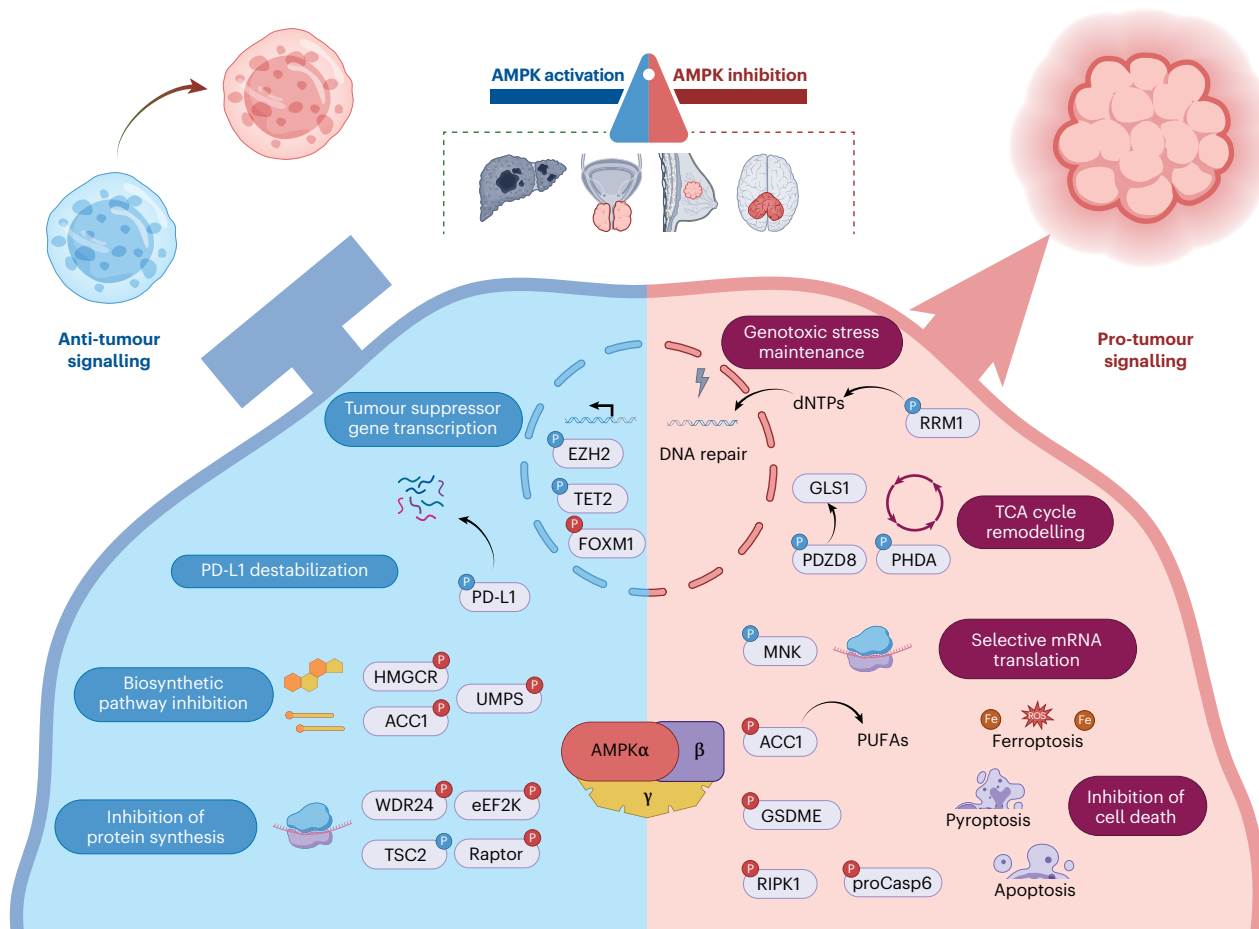


Fig. 6 | Context-dependent roles of AMPK in tumour suppression and tumour adaptation. Tumour-suppressive actions (left): AMPK limits tumour initiation and early growth by inhibiting anabolic pathways required for biosynthesis. Phosphorylation of HMGCR, ACC1 and UMPS reduces cholesterol, fatty acid and nucleotide synthesis, and phosphorylation of WDR24, RAPTOR, TSC2 and eEF2K suppresses protein translation. AMPK also destabilizes PD-L1, supporting anti-tumour immunity, and regulates tumour-suppressive transcription through EZH2, TET2 and FOXM1. These mechanisms are consistent with the anti-tumour effects seen during nutritional and metabolic interventions (caloric restriction, exercise, metformin), which activate AMPK and reduce growth-promoting signalling. Tumour-promoting actions (right): in established tumours, AMPK

can enhance survival under nutrient limitation, hypoxia or therapeutic stress. AMPK reduces ferroptotic and pyroptotic sensitivity (ACC1, GSDME, RIPK1, pro-caspase-6), supports glutaminolysis through PDZD8 and GLS1, and increases dNTP supply via RRM1 to promote DNA repair. These responses allow tumour cells to adapt to metabolic and immune stress, including immune-mediated killing, and can reduce the effectiveness of treatments that rely on energetic or oxidative stress. Thus, AMPK activation may be beneficial for cancer prevention, but may promote stress adaptation in existing tumours, an important consideration for nutritional strategies and combination therapies. PUFAs, polyunsaturated fatty acids.

with MK-8722 evokes a gene profile indicative of a disease-resistant slow oxidative muscle phenotype in the *mdx* mouse model of Duchenne muscular dystrophy³⁵⁶. It also improves muscle histology and gene expression profiles in a mouse model of myotonic dystrophy³⁵⁷. AMPK activation using PXL770 also improves the survival of a mouse model of X-linked adrenoleukodystrophy, a severe disease caused by mutations in the peroxisomal *ABCD1* transporter gene, leading to toxic accumulation of very long-chain fatty acids and eventually inflammation, mitochondrial dysfunction and axonal degeneration of the spinal cord and peripheral nerves³⁵⁸. Similarly, GSK773 treatment corrects several hallmarks of CPT2 deficiency, one of the most common inherited fatty acid oxidation defects, including promoting mitochondrial biogenesis, a shift towards oxidative fibre type, and corrects the impaired muscle differentiation in CPT2-deficient myotubes³⁵⁹.

Defects in mitochondrial quality-control pathways are also a hallmark of neuronal disorders. In growing dendrites, AMPK is activated by neuronal activity via oscillations in calcium, which increases CaMKK2 activity³⁶⁰. This increase in AMPK activity is essential for promoting mitochondrial fission as the inhibition of AMPK or fission results in multiple defects in neuronal development. Mechanistically, recent

studies have found that in addition to phosphorylating ULK1 and MFF, in neuronal cells AMPK also phosphorylates synaptojanin 2 binding protein (SYNJ2B) at Ser21 and this promotes PTEN-induced kinase 1 (PINK1) mitochondrial localization and enhances the survival of cultured cortical neuronal cells³⁶¹. Consistent with the critical role for AMPK in controlling neuronal development, genetic deletion of the AMPK α 2 or AMPK β 2 (but not α 1 or β 1) isoforms reduces recognition memory and hippocampal synaptic plasticity, effects that are associated with decreased dendritic spine density and abnormal spine morphology in the hippocampus^{362,363}. Genetic deletion of AMPK α isoforms in the medial prefrontal cortex also induces anxiety-like behaviours³⁶⁴, whereas acute psychological stress activates AMPK-dependent autophagy in the lateral habenula³⁶⁵. Despite the importance of AMPK α 2 and β 2 isoforms in maintaining neuronal function, it seems that Alzheimer's disease is associated with elevations in AMPK α 1 activity and inhibition of this isoform improves memory in an Alzheimer's disease mouse model. This effect is independent of changes in A β ₄₂ or p-tau but did reduce eEF2 hyperphosphorylation and restored protein synthesis in hippocampi³⁶⁶. These data suggest that although AMPK α 2 and β 2 isoforms are essential for neuronal development and

memory, hyperactivation, which potentially overrides typical neuronal oscillations regulated by CAMKK2, may be detrimental for neuronal development and function.

Highly conserved defence pathways promote pathogen control and host survival to various bacterial, viral and fungal pathogens through activation of AMPK. A common link between intracellular bacterial infection and activation of AMPK is lysosomal damage^{24,135,367,368}. This compromised lysosomal function leads to TAK1-mediated activation of AMPK and enhanced pathogen clearance^{24,369,370}. Various species of bacteria³⁷¹ and viruses³⁷² also activate AMPK through indirect mechanisms by disrupting mitochondrial dynamics. Additionally, viral³⁷³ and bacterial infections³⁷⁴ induce systemic hypoglycaemia in line with the infection severity, leading to AMPK activation. Although direct modification of AMPK during infection is not well established, AMPK α 1 has been identified as an itaconate-modified protein in inflammatory macrophages³⁷⁵. As AMPK activity is inhibited in mice lacking the enzyme required to produce itaconate³⁷⁶, this suggests itaconate may serve as an exogenous regulator of AMPK that links metabolic and immune signalling in a pathogen-specific manner. In a similar vein, nitric oxide is produced by innate immune cells to eliminate intracellular bacteria and AMPK is S-nitrosylated at γ 1-Cys131, which reduces its responsiveness to AMP³⁷⁷ and could influence the response to infection, although this requires empirical investigation. Downstream, activation of AMPK in bacterially and virally infected cells may limit fatty acid and cholesterol synthesis, which are vital for cell division³⁷⁸. Thus, AMPK seems to have a role in responding to infectious diseases; however, whether this can be harnessed to improve health is likely to be context dependent.

Conclusions

AMPK phosphorylation of metabolic enzymes, regulation of transcriptional programmes and modulation of mitochondrial and autophagic dynamics coordinates nutrient partitioning and enhances cellular resilience under metabolic stress. Over the past decade, many phosphorylation events beyond α -T172 have been shown to control AMPK activity, but how they alter enzyme structure to influence activity is still not understood. Furthermore, how these multiple phosphorylation sites interact with allosteric regulators to ultimately dictate AMPK activity has yet to be investigated. However, an understanding of this structure–function relationship will be vital to appreciate how AMPK integrates temporal and tissue-specific signals to optimize nutrient partitioning and whole-body energy homeostasis. To answer these questions, future studies will need to harness spatial-omics alongside live-cell imaging technologies to visualize the isoform-specific roles and subcellular dynamics of AMPK signalling. For example, might low glucose activate a select pool of AMPK proximal to glucose transporters while fatty acids activate an alternative mitochondria-specific pool? Understanding this relationship will facilitate the development of innovative, targeted therapies aimed at improving metabolic flux and mitochondrial health that may have applications across a range of diseases.

Moreover, with the development of targeted genetic strategies, it may be possible to overexpress specific isoform mutations that may render AMPK active or inactive in specific tissues or cell types. Such an approach may be especially amenable to Duchenne muscular dystrophy or potentially MASLD and CVD where genetic manipulation has been approved for clinical development, thereby potentially alleviating concerns around chronic activation in the heart. Such strategies may also be useful in cancer where cell-targeted therapies for inhibiting AMPK in the tumour cell while activating AMPK in the tumour microenvironment may maximize therapeutic responses. Ultimately, a deeper mechanistic understanding as to the complexity of AMPK regulatory signalling will be essential for unlocking its full therapeutic potential. By leveraging these advanced tools and targeted approaches, future research may enable precision modulation of AMPK activity, tailoring interventions to specific diseases and patient populations while minimizing off-target effects.

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Author contributions

All authors contributed to writing and proofreading the manuscript.

Competing interests

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Additional information

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