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AMPK: guardian of metabolism and mitochondrial homeostasis

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Abstract

Cells constantly adapt their metabolism to meet their energy needs and respond to nutrient availability. Eukaryotes have evolved a very sophisticated system to sense low cellular ATP levels via the serine/threonine kinase AMP-activated protein kinase (AMPK) complex. Under conditions of low energy, AMPK phosphorylates specific enzymes and growth control nodes to increase ATP generation and decrease ATP consumption. In the past decade, the discovery of numerous new AMPK substrates has led to a more complete understanding of the minimal number of steps required to reprogramme cellular metabolism from anabolism to catabolism. This energy switch controls cell growth and several other cellular processes, including lipid and glucose metabolism and autophagy. Recent studies have revealed that one ancestral function of AMPK is to promote mitochondrial health, and multiple newly discovered targets of AMPK are involved in various aspects of mitochondrial homeostasis, including mitophagy This Review discusses how AMPK functions as a central mediator of the cellular response to energetic stress and mitochondrial insults and coordinates multiple features of autophagy and mitochondrial biology.

Cells constantly need to manage their energy consumption depending on the availability of nutrients and on their capacity to produce ATP. ATP is produced by catabolic processes such as the breakdown of glucose or lipids, and the majority of ATP production in cells occurs in mitochondria, the sites of oxidative phosphorylation (OXPHOS). When cells use this stored energy for all manner of cellular processes, ATP is broken down into ADP, which can be further converted to AMP. When cellular ATP levels decrease, it is essential for cells to minimize energy consumption to avoid exhausting what is left of their resources. At the same time, emergency measures have to be taken to restore the cellular energy supply, such as increasing nutrient intake, activating alternative energy-producing pathways or turning over existing macromolecules into nutrients.

Eukaryotes have evolved a very elegant system that allows them to modulate their metabolism based on nutrient availability; the key player in this system is AMP-activated protein kinase (AMPK). In single-cell eukaryotes, such as the yeast *Saccharomyces*

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cerevisiae, the AMPK homologue is responsible for activating alternative carbon source utilization pathways when glucose is absent[1,2]. In higher eukaryotes, AMPK has acquired the capacity to sense the amount of energy available in the cell by directly binding adenine nucleotides[3]. Upon changes in energy availability, and thus changes in the ATP-to-ADP or ATP-to-AMP ratio, AMPK is activated by an allosteric mechanism that stimulates its kinase activity. Once activated, AMPK redirects metabolism towards increased catabolism and decreased anabolism through the phosphorylation of key proteins in multiple pathways, including mTOR complex 1 (mTORC1)[4,5], lipid homeostasis[6–9], glycolysis[10–13] and mitochondrial homeostasis[14–17]. In addition to directly regulating key enzymes involved in these pathways, AMPK also rewires cellular metabolism in a prolonged manner by targeting transcriptional regulators[17–26]. A number of recent phospho-proteomics studies have uncovered even more AMPK substrates[27–29], and excellent recent reviews have focused on the regulation of various aspects of metabolism by AMPK[30,31].

Here, we summarize the mechanism of action of AMPK and discuss its effects on metabolism, and then focus on recent advances in research on the regulation of autophagy and mitochondrial homeostasis by AMPK. A thorough understanding of AMPK functions and its downstream targets is necessary not only to understand its role as a master regulator of metabolism but also because modulation of AMPK is an attractive avenue for therapeutic intervention in many diseases, including cancer and type 2 diabetes (BOX 1).

Box 1

Targeting AMPK for therapeutic applications

AMP-activated protein kinase (AMPK) has received a lot of attention as a potential target for treating diseases associated with metabolic perturbation. This includes diabetes, obesity and fatty liver diseases and also cancer, which is often associated with changes in metabolism.

The type 2 diabetes drug metformin had been used for decades when a study showed that its mechanism of action involved the activation of AMPK in hepatocytes[223]. Mechanistically, metformin induces energy stress through inhibition of complex I of the respiratory chain in mitochondria. This leads to a change in the ATP-to-AMP ratio and canonical AMPK activation. There has been extensive research to evaluate the contribution of AMPK to the effect of metformin on circulating glucose and lipids. AMPK phosphorylation of acetyl-CoA carboxylases (ACCs) has been proposed as a master contributor to the changes in lipid synthesis that are induced by metformin, which in turn modulates insulin sensitivity and glucose uptake in muscle. One key piece of evidence in favour of this hypothesis came from the generation of a mouse knock-in mutant lacking the AMPK site on both ACC1 and ACC2 (REF. 105). This mouse model revealed that these phosphorylation events mediate the insulin-sensitizing effect of metformin, thus establishing AMPK as a relevant target in the action of metformin. Despite controversy[224], AMPK is widely viewed as an essential component of the action of metformin at physiological concentrations[219,225]. Given the role of the AMPK–ACC pathway in regulating fatty acid synthesis, AMPK activation is also an attractive treatment option for conditions associated with increased fatty acid production, such as

nonalcoholic fatty liver disease (NAFLD)[85]. In addition to diabetes, retrospective studies have revealed that patients taking metformin had a decreased occurrence of cancer[226]. However, whether direct activation of AMPK would be sufficient to replicate the beneficial effect of metformin was not known until recent advances in the generation of potent and specific small-molecule activators of AMPK. Most notably, two recent studies have revealed that direct AMPK activation indeed improves symptoms of type 2 diabetes in multiple animal models. One study revealed that a pan-specific AMPK activator improved diabetes symptoms in several animal models, including rodents and monkeys[84]. In another study, a direct AMPK activator increased glucose uptake in muscle and reduced blood glucose in type 2 diabetes models[83]. Interestingly, inactivation of AMPK in the liver had no effect on the efficacy of the drug, whereas muscle-specific deletion of AMPK abolished the effect of the drug, establishing muscle AMPK as a key therapeutic target in type 2 diabetes[83].

AMPK activation has been suggested to have tumour-suppressive, anticancer properties in some contexts and may mediate some of the cell-autonomous benefits of mitochondrial inhibitors on tumour cell growth[227–234], although AMPK activation may also allow tumour cell survival under the metabolic stress conditions that many tumour cells may face[235–238]. Additional studies with genetic loss of AMPK and the new bioavailable small-molecule activators of AMPK will shed new light on the cancer settings in which AMPK activation or inhibition may be most desirable.

AMPK structure and activation

AMPK is a heterotrimeric complex composed of a catalytic α-subunit and two regulatory subunits, β and γ (FIG. 1). Vertebrates possess multiple isoforms of each subunit, encoded by different genes. In humans, there are two α-subunits, α1 and α2, encoded by the genes *PRKAA1* and *PRKAA2* (REF. 32), two β-subunits, β1 and β2, encoded by *PRKAB1* and *PRKAB2* (REF. 33), and three γ-subunits, γ1, γ2 and γ3, encoded by *PRKAG1*, *PRKAG2* and *PRKAG3* (REF. 34). Each AMPK complex is composed of one α-subunit, one β-subunit and one γ-subunit, and all combinations are possible, thus potentially creating 12 distinct AMPK complexes[35]. One of the remaining questions in the field is whether these different combinations really represent functionally distinct complexes with different substrate specificities or subcellular localization, as discussed below.

Subunit composition of AMPK and their functions

The α-subunit contains the kinase domain and a critical residue, Thr172, that is phosphorylated by an upstream kinase. The β-subunit contains a carbohydrate- binding module that allows AMPK to associate with glycogen[36]. The γ-subunit enables AMPK to respond to changes in the ATP-to-AMP ratio as it contains four tandem cystathionine-β-synthase (CBS) domains that bind adenine nucleotides[37]. Binding of AMP, and to a lesser extent ADP, to the γ-subunit stimulates AMPK activity[38–40] (FIG. 1).

AMP binding to the γ-subunit enhances AMPK activity through three distinct mechanisms. First, AMP has been proposed to stimulate phosphorylation of Thr172 by directly

stimulating the activity of the upstream kinase[41] or by an allosteric mechanism that would render AMPK a better substrate for the upstream kinase[42]; both of these models have been challenged by other results showing no effect of AMP on the phosphorylation of Thr172 by the upstream kinase *in vitro*[40,43–45]. Second, AMP inhibits the dephosphorylation of Thr172 by protecting it from phosphatases[46]. Last, AMP causes allosteric activation of AMPK already phosphorylated on Thr172 (REFS 39,44). Even though both ADP and AMP have been shown to bind to the CBS domains and to stimulate AMPK activity, careful evaluation of their respective binding affinity to the γ-subunit, their concentration in cells and how much their concentration varies during energy stress revealed that AMP is likely to be the major contributor to changes in AMPK activity *in vivo*[39]. It should be noted that ATP is also able to bind to the CBS domains on the γ-subunit, which allows AMPK to respond to changes in the ATP-to-AMP ratio rather than changes in total nucleotide levels and thus to act as a real sensor of energy levels[38].

There are 12 possible combinations of αβγ complexes depending on which subunits constitute the complex. *In vivo,* some cell types only contain a subset of these combinations, suggesting that some complexes have specific roles. For example, in muscle, only the α1β2γ1, α2β2γ1 and α2β2γ3 complexes are detected. Moreover, following exercise, only the α2β2γ3 complex is activated by Thr172 phosphorylation, even though this complex is not as abundant as the γ1-containing complexes[47]. This specificity for the γ3-containing complex is in contrast to the effect of the mitochondrial inhibitor PT-1, which specifically activates γ1-containing complexes in muscle[48]. Even though the consequences of specific complex activation are not known, this observation suggests that specific subunit compositions allow distinct AMPK complexes to respond to different types of stress stimuli. It has been recently shown that the different complex compositions affect activity, activation by nucleotides, sensitivity to phosphatases and allosteric regulation of phosphatase sensitivity by AMP[49].

Different subunits have also been reported to have different subcellular localization in cells; for example, the nuclear localization of α2 is increased compared with α1 in muscle during exercise[50] and following leptin treatment of myoblasts in culture[51]. Furthermore, circadian oscillation of α1 nuclear localization has been observed in the mouse liver[21], and differential localization of γ-subunits has been reported in muscle fibres[52], whereas myristoylation of β-subunits has been reported to trigger association with intercellular membranes[45] or mitochondria[53] and to result in nuclear exclusion[51].

AMPK at the lysosome

It has recently been proposed that AMPK is localized at lysosomes through an inter action with axin, a protein best characterized for its role in WNT pathway regulation[54]. In this context, axin localizes to lysosomes, where it interacts with liver kinase B1 (LKB1; also known as STK11). In response to energy stress, AMP stimulates interaction with axin, which allows Thr172 phosphorylation by LKB1 and AMPK activation. This is proposed to allow co- regulation of mTOR, a master regulator of cell growth, and AMPK under starvation conditions[55]. mTOR plays an opposing role to AMPK: it stimulates anabolic pathways under high-nutrient conditions. AMPK and mTOR are both components of ancient

conserved pathways that have evolved as a yin-yang-like antagonistic mechanism controlling catabolism and anabolism. A recent study suggests that this regulation of AMPK by axin could play an important role in the activation of AMPK following glucose starvation[56]. In this study, the authors propose that glucose starvation activates AMPK independently of the canonical AMP-dependent allosteric mechanism. Rather, binding of the glycolytic enzyme aldolase to its substrate fructose-1,6-bisphosphate (FBP) regulates AMPK–axin binding and AMPK activity[56]. Thus, under conditions of glucose starvation, FBP levels decrease, which is sensed by aldolase and stimulates the formation of the AMPK–axin complex at the lysosome[56].

Whether this same axin complex controls AMPK activation in response to distinct metabolic stresses remains to be determined, as does whether specific AMPK substrates in different subcellular locations are controlled by a lysosome-targeted pool of AMPK. However, to date, no substrate is known to be exclusively or even preferentially phosphorylated by a specific AMPK complex, so this remains an open question for future studies.

AMPK activation by upstream kinases

The AMPK complex is activated by phosphorylation on Thr172 in the activation loop of the catalytic (α) subunit by an upstream kinase (FIG. 1).

The discovery that one of the upstream kinases for AMPK is the tumour suppressor LKB1 provided a direct connection between cancer and metabolism[42,43,57]. Notably, like AMPK, LKB1 functions as a constitutive heterotrimer with the kinase-dead STE20-related kinase STRAD and the STE20 family scaffolding protein MO25 (REFS 58,59) (FIG. 1). Studies of mice in which *Lkb1* (also known as *Stk11*) has been genetically inactivated reveal that LKB1 is responsible for the majority of AMPK activation under energy stress in most mammalian tissues examined, including crucial metabolic tissues such as the liver and muscle[60–64]. Studies of cells devoid of LKB1 indicate that LKB1 mediates nearly all of the activation of AMPK in response to mitochondrial insults and low-energy conditions[65].

However, it should be noted that AMPK can also be directly phosphorylated on Thr172 in response to calcium flux by the calcium-sensitive kinase CAMKK2 (also known as CAMKKβ)[66–68], thus linking calcium signalling to the regulation of energy metabolism by AMPK[69]. Increased intracellular calcium activates CAMKK2 and leads to the activation of AMPK independently of LKB1 and nucleotide levels (FIG. 1). This has been shown to have a role particularly in response to hormones and stresses that acutely control intracellular calcium, including processes such as the regulation of food intake by hypothalamic neurons in mice[70,71], activation of antigen and thrombin receptors in immune and endothelial cells, respectively[72,73], and stimulation of mitochondrial activity by thyroid hormone[74,75]. Notably, CAMKK2 has also been shown to activate AMPK following cellular stresses, including amino acid starvation[76], hypoxia[77,78] and cell detachment from the matrix[79]. In LKB1-null cells, CAMKK2 is able to maintain some AMPK activity that is still sensitive to changes in the ATP-to-AMP ratio[80]. This CAMKK2-dependent activation of AMPK explains the observation of residual AMPK activity in LKB1-deficient tumours.

A variety of physiological conditions lead to the activation of AMPK *in vivo*, including mitochondrial inhibition, nutrient starvation and exercise (FIG. 1). It is likely that these conditions induce AMPK activation through the modulation of the ATP-to-AMP ratio. In addition, several small-molecule activators of AMPK have been developed, which bind to the interface of the α- and β-subunits and activate AMPK kinase activity independently of nucleotide levels (FIG. 1). The first of these drugs was A-769662 (REF. 81). Subsequently, other related compounds with increased potency and specificity have been developed, such as compounds 991, PF-739 and MK-8722 (REFS 82–84).

Regulation of metabolism by AMPK

During energy stress, AMPK directly phosphorylates key factors involved in multiple pathways to restore energy balance. The effect of AMPK on metabolism can be broadly divided into two categories: the inhibition of anabolism to minimize ATP consumption and the stimulation of catabolism to stimulate ATP production. We briefly review the main components of these categories below and then focus on the emerging roles of AMPK in mitochondrial processes and autophagy.

Inhibition of anabolic pathways

AMPK inhibits multiple biosynthetic pathways under conditions of energy shortage (FIG. 2). The first pathway to be identified was the inhibition of lipid and sterol synthesis by AMPK through inhibitory phosphorylation of the acetyl-CoA carboxylases ACC1 and ACC2, which catalyse the first step in *de novo* lipid synthesis, and inhibitory phosphorylation of HMG-CoA reductase (HMGCR), which catalyses the rate-limiting step in cholesterol synthesis[6,7]. This function of AMPK has major implications for the use of direct activators in the treatment of diseases associated with excess fatty acid production, such as nonalcoholic fatty liver disease (NAFLD)[85], and is consistent with the lipid-lowering effects seen upon liver-specific expression of activated AMPK[86]. AMPK also prevents the storage of glycogen by inhibitory phosphorylation of the glycogen synthases GYS1 and GYS2 (REF. 87) and inhibits hexosamine synthesis and O-GlcNAc modification of cellular proteins via phosphorylation of GFAT1 (REFS 88,89) (FIG. 2).

Under prolonged conditions of reduced energy, AMPK reprogrammes metabolism through transcriptional regulation of biosynthetic pathways. AMPK inhibits gluconeogenesis by phosphorylating CRTC2 (REF. 19) and class IIA histone deacetylases (HDACs)[24], which are co-activators of the cAMP response elementbinding protein (CREB) and forkhead box protein O (FOXO) pathways, respectively. AMPK also controls metabolism at the transcriptional level by phosphorylating sterol regulatory element-binding protein 1 (SREBP1)[23], carbohydrate-responsive elementbinding protein (ChREBP)[90] and hepatocyte nuclear factor 4α (HNF4α)[91], which are key transcriptional regulators of lipid and glucose metabolism. AMPK has been reported to phosphor late histone acetyltransferase p300 to regulate nuclear hormone receptor transcriptional activity[18].

Cell growth and protein translation are major consumers of ATP in cells. Accordingly, AMPK regulates these processes through the modulation of the master regulator of growth mTOR. AMPK activation leads to the inhibition of mTORC1 by two independent

mechanisms: the activation of the negative mTORC1 regulator TSC2 (also known as tuberin)[4] and the inhibition of the mTORC1 subunit RAPTOR[5]. Under nutrient-rich conditions, AMPK is inactive and mTOR is active, whereas under energy-shortage conditions, increased AMPK activity leads to a decrease in mTOR activity. Lower mTOR activity leads to decreased cell growth and to decreased protein synthesis. AMPK also directly regulates protein synthesis by phosphorylating eEF2K, a negative regulator of protein elongation[92]. eEF2K is also a downstream target of the mTOR pathway[93], further reinforcing the many connections and crosstalk between these two pathways controlling cell growth and metabolism and illustrating the ancient yin-yang relationship between these two signalling hubs. In summary, AMPK-dependent inhibition of anabolic glucose, lipid and protein synthesis pathways limits ATP consumption under energy stress (FIG. 2). It should be noted that AMPK has also been reported to regulate multiple additional growth-related pathways, including Hedgehog signalling[94], the Hippo pathway[95–97], the JAK–signal transducer and activator of transcription (STAT) pathway[98] and the p53 tumour suppressor pathway[99,100], although many details remain to be fully defined. This Review focuses on metabolic targets of AMPK.

Stimulation of catabolic pathways

In order to replenish ATP stores, AMPK also actively stimulates the breakdown of macromolecules to produce energy. The stimulated pathways include increased glucose utilization, mobilization of lipid stores and turnover of macromolecules by autophagy (FIG. 2). AMPK stimulates glucose utilization by phosphorylating targets involved in the trafficking of glucose transporters to increase glucose uptake into cells. Phosphorylation of TXNIP[13] and TBC1D1 (REF. 101) increases the plasma membrane localization of GLUT1 and GLUT4, respectively. Phosphorylation of phospholipase D1 (PLD1) by AMPK has also been shown to indirectly increase glucose uptake[102]. AMPK also increases flux through the glycolytic pathway by phosphorylating PFKFB3 (6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase 3), which affects the activity of PFK1, a rate-limiting enzyme in glycolysis[11] (FIG. 2). In addition to stimulating glucose utilization, AMPK also pushes cells towards using their lipid stores. This is achieved by stimulating lipases such as adipose triglyceride lipase (ATGL; also known as PNPLA2) to release fatty acids from triglyceride stores[9]. The free fatty acids are then imported into mitochondria for β-oxidation. Import of fatty acids into mitochondria relies on a transport system that requires the acyl transferase CPT1. Interestingly, CPT1 activity seems to be modulated by AMPK activity. Indeed, malonyl-CoA, generated by ACC1 and ACC2, is a potent inhibitor of CPT1 (REFS 103,104). Therefore, by phosphorylating and inhibiting ACC, AMPK decreases the pool of malonyl-CoA, which results in decreased lipid synthesis and increased fatty acid import into mitochondria for β-oxidation. Studies using mouse models with knock-in mutations of Ser79Ala in ACC1 and Ser221Ala in ACC2 revealed that these sites are indeed important for the regulation of lipid homeostasis and the therapeutic effects of metformin downstream of AMPK[105] (BOX 1). In addition, both ACC1 and ACC2 contributed to the regulation of lipid homeostasis, showing redundancy in function between ACC1 and ACC2.

Transcriptional effects of AMPK also participte in redirecting metabolism towards increased catabolism. AMPK transcriptionally upregulates genes involved in mitochondrial biogenesis,

autophagy and lysosomal degradation (FIG. 2). These processes, as well as the direct connections between AMPK and mitochondria, are discussed in detail in the following sections.

AMPK and mitochondria

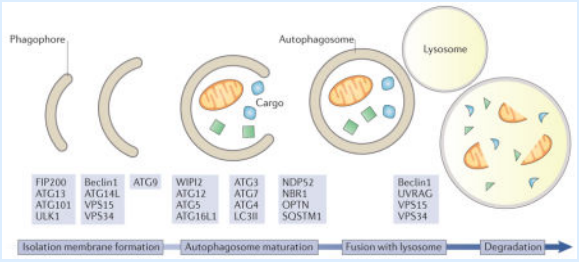
A growing body of evidence points to a specific regulation of various aspects of mitochondrial biology and homeostasis by AMPK (FIG. 3). These aspects include control of mitochondrial number through stimulation of mitochondrial biogenesis, regulation of the shape of the mitochondrial network in cells, and mitochondrial quality control through regulation of autophagy and mitophagy (BOX 2).

Box 2

Autophagy

Autophagy is a process by which cells digest their own components using a specialized machinery of adaptors and effectors. It begins with the generation of the autophagosome and recognition of the cargo, followed by the maturation of the autophagosome and fusion with lysosomes (see the figure). The term itself means ‘self-eating’ and was first coined by Belgian scientist and Nobel Prize laureate Christian de Duve. Autophagy serves two main functions: it enables the degradation of cellular structures that are too large for other surveillance pathways, such as the ubiquitin–proteasome system, and it allows cells to survive starvation by recycling building blocks such as amino acids to sustain essential cell functions. Autophagy can be either a bulk recycling of cytosolic components or a targeted removal of macromolecules and even organelles. In particular, removal of mitochondria by autophagy, a process called mitophagy, has been shown to require the canonical autophagy machinery as well as specific markers at the surface of damaged mitochondria that signal their removal. Genes essential for autophagy (ATGs) have been discovered by screening for genes that are required for autophagosome formation in the yeast *Saccharomyces cerevisiae* during nitrogen starvation [239,240]. Yoshinori Ohsumi was awarded the Nobel Prize in physiology or medicine in 2016 for the discovery of the ATG genes. Since the 1990s, the molecular events controlling autophagy execution have been characterized, and the role of ATG genes in controlling various steps of the autophagy pathway has been demonstrated[241]. The first ATG gene to be cloned, *ATG1*, encodes a protein kinase required for the initiation of autophagy. Its mammalian homologue, ULK1, plays a similar role. ULK1 forms a complex with ATG13, ATG101 and FIP200. Upon autophagy initiation, ULK1 phosphorylates and activates the class III PI3K complex I, composed of VPS34, VPS15, ATG14L and Beclin1. This complex generates phosphatidylinositol-3-phosphate (PtdIns3P) at the nascent autophagosomal membrane to help shape the phagophore membrane that also contains ATG9, the only transmembrane ATG protein. PtdIns3P recruits ATG16L1 and ATG5-ATG12 to the autophagosome through interaction with the PtdIns3P-binding protein WIPI2. The ATG16L1-ATG5-ATG12 complex then catalyses the covalent attachment of a phosphatidylethanolamine moiety to the carboxy-terminal glycine of LC3, which was previously activated by the action of ATG4, ATG7 and ATG3 (LC3I), generating LC3II[156]. LC3II is used as a marker of autophagy to visualize

autophagosomes and to quantify autophagy in cells[242] but it also allows binding of autophagy receptors such as SQSTM1, NDP52, NBR1 and OPTN[243]. Subsequently, autophagosomes mature and fuse with lysosomes, a process mediated by the class III PI3K complex II composed of VPS34, VPS15, Beclin1 and UVRAG[156], to induce degradation of the cargo by lysosomal enzymes.



AMPK promotes mitochondrial biogenesis

Mitochondrial biogenesis occurs in response to increased energy expenditure to produce more ATP. Mitochondrial biogenesis occurs via the growth and division of pre-existing mitochondria. New material is added to the existing mitochondrial network in order to increase mitochondrial mass. Mitochondrial biogenesis requires the upregulation of mitochondrial proteins and an increase in lipid production and transfer to sustain the expansion of the surface area of the inner and outer mitochondrial membranes. The mitochondrial genome encodes some mitochondrial proteins, but the vast majority of mitochondrial proteins are encoded by the nuclear genome. Thus, the signal to stimulate mitochondrial biogenesis needs to be relayed to the nucleus in order for the transcription factors to stimulate the expression of genes encoding mitochondrial proteins, as is the case for retrograde signalling[106].

A variety of experimental and physiological conditions result in increased mitochondrial mass, one of the best-studied being exercise. Early observations in the 1950s correlated muscle activity in birds with muscle fibre mitochondrial content and indicated that active muscles contained more mitochondria than less-active muscles[107]. It is now well established that exercise and muscle activity induce a mitochondrial biogenesis programme aimed at increasing the oxidative capacity of muscles[108]. Interestingly, exercise is also a potent activator of AMPK, thus raising the possibility that AMPK could participate in this process. This is further supported by the observations that chronic AMPK activation leads to increased mitochondrial biogenesis[109] and that the AMPK-activating drug AICAR acts as an exercise mimetic[110]. Furthermore, over expression of a constitutively active AMPK γ3 subunit in mice induced mitochondrial biogenesis[111]. Loss-of-function experiments also revealed a role for AMPK in the maintenance of mitochondrial biogenesis. Mice expressing a dominant-negative mutant of AMPK failed to induce mitochondrial biogenesis under energy stress[16]. Mice lacking the AMPK β1/β2 sub units[112] or the upstream kinase LKB1 (REFS 113,114) show decreased mitochondrial content in muscle, and mice with muscle-specific LKB1 knockout fail to increase mitochondrial biogenesis after exercise[113]. Muscle-specific knockout of AMPK α-subunits also induces defects in mitochondrial biogenesis and

function[115]. In addition, AMPK has also been shown to control mitochondrial content in other tissues, including adipocytes[116], macro phages[117] and hepatocytes[118]. Collectively, these studies establish AMPK as a central regulator of mitochondrial biogenesis.

Several downstream effectors of AMPK contribute to the regulation of mitochondrial biogenesis, with no single substrate yet known to mediate the bulk of the effects. Most genes involved in mitochondrial metabolism seem to be under the control of the peroxisome proliferator-activated receptor-γ co-activator 1 (PGC1) family[119,120] through interaction with oestrogen-related receptors (ERRs) and to a lesser extent the peroxisome proliferator-activated receptor (PPAR) family of transcription factors[121] (FIG. 3). Overexpression of PGC1α in muscle is sufficient to convert type IIb (glycolytic) fibres into mitochondria-rich type II and type I fibres, establishing PGC1α as a master regulator of mitochondrial bio genesis[122]. PGC1α is regulated by several post-translational mechanisms, such as acetylation[123], methylation[124] and phosphorylation by various kinases, including AKT[125] and p38 MAPK[126]. Interestingly, it has been reported that the transcriptional upregulation of several genes involved in oxidative metabolism upon AMPK activation required PGC1α[17] and that overexpression of the constitutively active AMPK γ3 subunit increased PGC1α expression[111]. Two sites on PGC1α have been reported to be phosphorylated by AMPK *in vitro*: Thr177 and Ser538 (REF. 17). Additional studies are needed to define whether they are AMPK target sites *in vivo*. AMPK may also regulate PGC1α by several indirect mechanisms involving AMPK-dependent modulation of p38 MAPK[127], HDAC5 (REFS 24,128) or SIRT1 (REF. 129) (reviewed in REF. 130), so it seems likely that through a combination of mechanisms, AMPK communicates with PGC1α (FIG. 3). In addition, AMPK promotes the activation of transcription factor EB (TFEB; discussed below), and TFEB has been shown to directly bind to and activate the promoter of the gene encoding PGC1α, *PPARGC1A*, in some contexts[131]. In addition to PGC1α, AMPK has been shown to increase the expression of the mRNAs encoding PGC1β and ERRα in KRAS-driven colon cancer[132], and TFEB and TFE3 have also been connected to PGC1β expression in some contexts[133].

The effect of AMPK on mitochondrial biogenesis is not only important for understanding the effects of exercise on muscle mitochondria but it also has therapeutic implications in multiple pathological states, including Duchenne muscular dystrophy[134] and mitochondrial myopathy[135]. AMPK activation also increases exercise capacity in obese mice[136], improves muscle regeneration following injury[137] and protects muscle from age-related myopathies through the activation of the autophagy pathway, as discussed below[138].

Regulation of mitochondrial dynamics by AMPK

Mitochondria are highly mobile organelles that constantly undergo fusion and division events to generate a dynamic and interconnected network[139]. Different mitochondrial morphologies have been proposed to modulate OXPHOS, mitophagy and apoptosis. In particular, elongated mitochondria have been hypothesized to sustain ATP production during stress[140], protect mitochondria from mitophagy[141,142] and distribute biomaterials including lipids[143] during nutrient starvation. By contrast, in response to other cellular insults, including mitochondrial respiratory chain inhibitors, mitochondria undergo fragmentation,

which is thought to facilitate mitophagy of mitochondrial fragments lacking proper membrane potential[144], as well as to allow efficient and timely apoptosis[145].

It has long been known that environmental conditions, including mitochondrial depolarization and inhibition of mitochondrial ATP synthesis, trigger mitochondrial fragmentation by increasing mitochondrial fission rates and/or decreasing mitochondrial fusion rates[146]. However, the mechanism by which mitochondrial inhibitors affect the shape of the mitochondrial network was not clearly established, especially for mitochondrial inhibitors that do not affect the mitochondrial membrane potential[147]. Strikingly, stimuli that induce mitochondrial fragmentation, such as inhibitors of the respiratory chain, are also potent activators of AMPK. AMPK was recently shown to be required for the fragmentation of mitochondria induced by rotenone and antimycin A, which are inhibitors of complex I and complex III, respectively, of the mitochondrial respiratory chain. Surprisingly, AMPK activation through direct small-molecule activators, in the absence of mitochondrial damage, is sufficient to induce mitochondrial fission[15]. This finding established AMPK as a crucial and direct regulator of mitochondrial dynamics. Proteomics and bioinformatics screens to identify new substrates of AMPK identified two phosphorylation sites, Ser155 and Ser173, on a core component of the mitochondrial fission pathway, mitochondrial fission factor (MFF)[15,28]. MFF is the primary receptor for the dynamin-like protein DRP1 on the mitochondrial outer membrane, which mediates constriction of mitochondria during fission[148–150]. Phosphorylation of MFF on Ser155 and Ser172 by AMPK is therefore one potential mechanism that could explain the requirement of AMPK for mitochondrial fragmentation after ETC inhibition and the sufficiency of AMPK activators alone to promote mitochondrial fragmentation. Indeed, activation of AMPK results in increased localization of DRP1 at the mitochondria, an effect that is dependent on the presence of the AMPK phosphorylation sites in MFF[15]. Thus, AMPK acutely regulates the shape of the mitochondrial network during energy stress by phosphorylating MFF and controlling the localization of DRP1 (REF. 151) (FIG. 3).

Other mitochondrial targets of AMPK

As mentioned above, ACC1 and ACC2 are phosphorylated by AMPK. ACC1 is cytosolic, whereas ACC2 has an additional amino-terminal hydrophobic sequence that specifically targets it to the mitochondrial outer membrane and therefore represents the first identified mitochondrial substrate of AMPK[152]. Upon activation of AMPK, phosphorylation of Ser79 in ACC1 and Ser221 in ACC2 results in the inactivation of ACC activity. This is thought to not only inhibit the first step in fatty acid synthesis but also stimulate lipid β-oxidation in mitochondria. Specific inhibition of ACC2 at mitochondria is therefore predicted to relieve the inhibition of CPT1 by malonyl-CoA and to result in increased lipid β-oxidation. Studies have revealed that ACC1 and ACC2 both participate in the regulation of lipid homeostasis[105]. However, the presence of ACC2 at mitochondria suggests that, under certain conditions, localized regulation of ACC function at mitochondria by AMPK could play a role in metabolic adaptation to energy stress. For example, muscles from ACC2 Ser221Ala knock-in mice have decreased basal fatty acid oxidation compared with wild-type muscle and are resistant to the fatty acid oxidation-promoting effect of AICAR[153]. Interestingly,

upon exercise, these differences were lost, suggesting that exercise induces changes in fatty acid oxidation that are independent of the AMPK–ACC–malonyl-CoA axis[154].

The intricate relationship between AMPK and mitochondria is probably underestimated. As a master regulator of energy homeostasis, AMPK is very likely to target more aspects and functions of mitochondria. For example, AMPK has recently been shown to affect the movement of mitochondria at the leading edge of the cell during cell migration[155], a process probably involving a yet-to-be-identified substrate of AMPK involved in the process of mitochondrial transport. AKAP1, a mitochondria-localized protein known to act as a PKA-anchoring factor, has recently been shown to be phosphorylated by AMPK, providing a potential mechanism for localized PKA activity regulation by AMPK at mitochondria[27].

AMPK regulates autophagy and mitophagy

Autophagy is a process by which cellular components such as proteins, macromolecules, organelles and pathogens are recycled by a specialized cellular machinery that mediates the engulfment of the cargo in membranous structures called autophagosomes, their fusion with lysosomes, and subsequent degradation of the cargo in autophagolysosomes (BOX 2 and reviewed in REF. 156). The autophagy machinery involves several multi-protein complexes that control every step, and AMPK regulates various aspects of this machinery.

Autophagy mainly serves two distinct functions: the turnover of old or damaged molecules and the replenishment of nutrient stores during times of starvation. These two functions are similar in their execution but differ in their mechanism of initiation. While the turnover of molecules is thought to be a continuous process in cells, the induction of autophagy during starvation involves the nutrient-sensing pathway mediated by mTORC1. Upon nutrient starvation, mTORC1 is inactivated, which relieves the inhibitory phosphorylation on ULK1, a kinase essential for autophagy induction[157,158]. mTORC1 also phosphorylates and inhibits other components of the autophagy pathway, such as ATG14L (also known as Barkor)[159] and the ULK1-interacting protein ATG13 (REF. 160). These phosphorylation events participate in the inhibition of autophagy under nutrient-rich conditions. Upon activation, ULK1 phosphorylates several proteins involved in the execution of autophagy, including its binding partners FIP200, ATG101 (REF. 161) and ATG13 (REF. 162), the downstream effectors ATG9 (REF. 163), ATG14L[159], VPS34 (also known as PI3K type 3), Beclin1 and AMBRA1 (REFS 161,164), and the trafficking protein Sec16A[165] (BOX 2, FIG. 4 and reviewed in REF. 166).

Regulation of autophagy by AMPK

AMPK has been shown to regulate autophagy both in yeast[167] and in mammalian cells[168,169]. Molecular characterization of this regulation has revealed that AMPK controls autophagy at different steps. First, AMPK directly phosphorylates the mTOR upstream regulator TSC2 on Thr1227 and Ser1345 (REF. 4) and the mTORC1 subunit RAPTOR on Ser722 and Ser792 (REF. 5). Both of these phosphorylation events participate in reducing mTOR activity under conditions of energy stress. As mentioned above, reduced mTOR activity relieves the inhibitory phosphorylation on ULK1 to activate autophagy.

A direct link between AMPK and the core autophagy pathway was established when it was shown that AMPK directly phosphorylates ULK1 on at least four residues: Ser467, Ser555, Thr574 and Ser637 (REFS 14,170) (FIG. 4). Using ULK1-knockout cell lines and reconstitution with non-phosphorylatable mutants, phosphorylation of ULK1 by AMPK was shown to be important for autophagy and cell survival during starvation and metabolic stress. Strikingly, cells expressing ULK1 mutants that cannot be phosphorylated by AMPK also appear to accumulate defective mitochondria, suggesting that the AMPK–ULK1 axis is important not only for general autophagy but also specifically for the selective removal of damaged mitochondria[14]. Subsequent studies confirmed an essential role for ULK1 in mitophagy under various conditions[75,162,171–177]. Moreover, efficient removal of damaged mitochondria following AMPK activation has been shown to be important in various contexts, such as in brown adipose tissue[116] and during fasting and ageing in muscles[138], as well as in macrophages[178] and hepatocytes[179]. In addition to phosphorylating ULK1, AMPK has been reported to phosphorylate residues on other core components of the autophagy pathway, such as Ser761 on ATG9 (REF. 180), Thr133 and Ser135 on VPS34 (REF. 181), Ser91 and Ser94 on Beclin1 (REFS 181,182), Thr50 on the VPS34-associated protein RACK1 (REF. 183) and Thr32 on PAQR3, an ATG14L–VPS34 scaffolding protein[184]. Some of these phosphorylation events represent suboptimal AMPK sites and have not been independently validated, so many of the molecular details of AMPK and ULK1 control of autophagy remain to be elucidated (FIG. 4).

Another pathway controlling mitophagy is the PINK1–Parkin-mediated removal of depolarized mitochondria[185]. This pathway involves the stabilization of PINK1 at mitochondria upon loss of the mitochondrial membrane potential[186]. PINK1 in turn recruits and phosphorylates Parkin, an E3 ubiquitin ligase that marks mitochondria for degradation by the autophagy machinery[177]. In addition, PINK1 also phosphorylates ubiquitin on mitochondria, a process that amplifies the signal by stimulating Parkin activity[187,188]. This pathway requires complete mitochondrial depolarization to be activated and is therefore different from the basal surveillance of mitochondria carried out by the AMPK–ULK1 axis. However, it remains to be established whether AMPK and ULK1 play a role in the regulation of mitophagy by the PINK1–Parkin pathway. It should be noted that inducers of PINK1–Parkin-mediated mitophagy, such as the proton ionophore CCCP, are strong inducers of AMPK through the inhibition of ATP synthesis in mitochondria. Therefore, it is conceivable that some targets downstream of AMPK and/or ULK1 could be involved in the PINK1–Parkin pathway.

Mitochondrial localization of AMPK

The presence of at least two well-characterized substrates of AMPK at mitochondria, MFF and ACC2, raises the possibility that a pool of AMPK exists at or close to mitochondria. However, it must be noted that MFF and ACC2 are localized in the mitochondrial outer membrane and are therefore exposed to the cytosol, where they could be phosphorylated by freely floating cytosolic AMPK. Given the central role of mitochondria in energy production, one could hypothesize that a mitochondrial pool of AMPK would be able to sense and respond more rapidly and efficiently to changes in energy status. This hypothesis is supported by some experimental evidence suggesting that a mitochondrial pool of AMPK

indeed exists. One such piece of evidence is the observation that myristoylation of the β-subunit of AMPK causes AMPK to localize to mitochondria, where it could regulate the specific removal of damaged mitochondria by mitophagy[53]. Interestingly, phosphorylation of ULK1 on Ser555 by AMPK has been shown to regulate recruitment of ULK1 to mitochondria during mitophagy, suggesting that localized signalling at mitochondria regulates the removal of damaged mitochondria[189].

Small-peptide probes for AMPK activity also suggest the existence of a mitochondrial pool of AMPK that can specifically regulate ATP levels independently of cytosolic AMPK[190]. However, biochemical fractionations are hard to interpret because of the inherent nature of organelles; they tend to associate in cells and are therefore almost impossible to purify without contamination from other organelles or membrane structure. Imaging techniques allow for a better resolution and identification of subcellular organelles but rely on the specificity of the antibodies used and need to be appropriately controlled. Careful evaluation of the subcellular localization of the seven endogenous AMPK subunits is required to establish the existence and regulation of a potential mitochondrial pool of AMPK.

AMPK acts as a signal integration platform to control mitochondrial health

AMPK regulates at least three essential aspects of mitochondrial homeostasis: biogenesis, fission and mitophagy. During amino acid starvation, autophagy is activated by inhibition of mTORC1. Under these conditions, mitochondria elongate via a process called mitochondrial hyperfusion[140] to evade degradation by mitophagy and maximize OXPHOS[141,142]. This process is beneficial only if the mitochondria are functional and the cell contains the appropriate substrates to support OXPHOS. However, efficient removal of damaged mitochondria and segregation of dysfunctional parts of the mitochondrial network have been reported to rely on mitochondrial fission[144,191,192]. Therefore, in response to mitochondrial damage, as well as under other low ATP conditions where AMPK is activated, phosphorylation of MFF and ULK1 could facilitate the segregation of damaged mitochondria and their removal by mitophagy, while activation of mitochondrial biogenesis would ensure that mitochondrial mass is restored by the generation of new, healthier mitochondria. The response to starvation therefore depends on whether cells lack amino acids and growth signals, which leads to mTORC1 inactivation but does not activate AMPK, or whether cells lack both nutrients and energy, leading to activation of AMPK, mitochondrial fragmentation and subsequent mitophagy (FIG. 3). It remains to be established whether the strength and/or duration of the signal governs the AMPK response, from a rescue effort to a dismantling and rebuilding mission.

Role of AMPK in autophagy beyond mitochondria

Autophagy is also involved in the removal of intracellular pathogens, a process called xenophagy[193]. The AMPK pathway has been shown to be activated and to modulate the autophagy response during viral[194,195], bacterial[178] and prion infections[196], thus expanding the repertoire of conditions that involve the regulation of autophagic flux by AMPK. Interestingly, to sustain their replication, several pathogens either induce or repress AMPK activity depending on their specific needs, such as evasion of xenophagy, increased glucose or lipid oxidation, or nutrient availability (reviewed in REF. 197).

Transcriptional regulation of autophagy by AMPK

In addition to regulating general transcriptional regulators such as p300 (REF. 18) and class IIa HDACs[24], AMPK activation leads to selective regulation of a subset of genes involved in metabolism, autophagy and lysosomal function. This is achieved through direct and indirect mechanisms that could participate in long-term modulation of autophagy flux by AMPK. First, AMPK directly phosphorylates members of the FOXO family of transcription factors[20] that regulate genes involved in autophagy[198]. AMPK phosphorylation of FOXO3 stimulates its transcriptional activity under conditions of energy stress[20]. Interestingly, two other members of the FOX transcription factor family, FOXK1 and FOXK2, compete with FOXO3 to repress genes involved in autophagy[199]. FOXK1 and FOXK2 are phosphorylated by mTOR under conditions of high nutrient availability. Upon phosphorylation by mTOR, FOXK proteins translocate to the nucleus, where they compete with FOXO3 for binding to the same genomic regulatory sites and repress autophagy genes[199]. As a result, when AMPK is activated, phosphorylation of FOXO3 activates autophagy genes, while repression of mTOR through phosphorylation of TSC2 (REF. 4) and RAPTOR[5] induces FOXK exclusion from the nucleus to allow for maximal activation of FOXO3 targets[199] (FIG. 5). In addition to FOXK, mTOR also regulates TFEB and TFE3, which are master regulators of lysosomal function[200–203]. Recently, it was shown that AMPK activity controls lysosomal biogenesis through TFEB during embryonic stem cell differentiation into embryoid bodies[26]. Under the conditions stated above, when AMPK is active and mTOR is inhibited, TFEB dephosphorylation drives its import into the nucleus, where it turns on a network of genes called the CLEAR network[200]. Products of genes in the CLEAR network control lysosomal activity, which is required for autophagy.

In addition to control of TFEB via suppression of mTORC1, two additional mechanisms by which AMPK may activate TFEB-dependent gene expression were reported. AMPK phosphorylation of FOXO3 was shown to repress the expression of the mRNA encoding SKP2, which is part of the SCF E3 ubiquitin ligase complex that degrades the histone demethylase CARM1 (REF. 25). The net effect of AMPK activation was increased levels of CARM1, which acted as a co-activator for TFEB to promote the expression of lysosomal genes (FIG. 5). Very recently, it was reported that AMPK-dependent phosphorylation of the lipogenic enzyme ACSS2 induced its translocation to the nucleus, where it stimulated TFEB-dependent gene expression[204]. ACSS2 has been hypothesized to control the local synthesis of acetyl- CoA pools in the nucleus to control acetylation of his-tones and other transcriptional regulators[205] (FIG. 5). Like CARM1, it is curious that potentially broad effects on histone modification could yield a selective induction of a TFEB-dependent gene expression programme. This is a very interesting developing area at the intersection of AMPK and the control of gene expression under starvation conditions.

Conclusions and perspectives

AMPK has been identified in the past few years to act as a central integrator of mitochondrial homeostasis by controlling various aspects of the mitochondrial life cycle, from biogenesis and dynamics to removal by mitophagy. Given the function of mitochondria as the major source of ATP production in the cell, it makes perfect sense that AMPK, a low

ATP sensor that restores ATP homeostasis, would engage downstream effectors to ensure optimal mitochondrial function. Almost every mitochondrial insult or defect activates AMPK, including respiratory chain complexes inhibitors such as the diabetes drug metformin, the Parkinson disease- causing herbicide rotenone, proton ionophores, ATP synthase inhibitors and mitochondrial DNA depletion or mutation. AMPK therefore acts as a guardian of the mitochondria. This is an ancient and conserved role of AMPK, with connections between AMPK and mitochondria occurring in yeast[206], *Caenorhabditis elegans*[207–209] and flies[210].

Given that across all eukaryotes, AMPK is activated under conditions in which mitochondrial function and mitochondrial production of ATP are compromised, it is surprising that there are not more AMPK substrates dedicated to restoring mitochondrial health, as mitochondrial integrity is a central mediator of cellular energetics. Several questions remain and deserve careful evaluation in the coming years. For example, it remains to be established whether specific AMPK complex compositions preferentially localize at or close to mitochondria and respond to specific stresses involving mitochondria. How such mitochondrial pools of AMPK might interact with or relate to the recently described populations of AMPK and LKB1 at lysosomes[56] will be an interesting area for future study. The interplay between AMPK, ULK1 and other kinases activated by mitochondrial and energetic stress (for example, PINK1 and TBK1)[177,211–213] and how distinct phosphorylation events dictate mitophagy and fission and/or fusion versus apoptotic cell fate decisions remain poorly defined. Interestingly, recent findings implicate mitochondrial dynamics and mitochondrial health in immune cell function and stem cell pluripotency[214–218].

Finally, expansion of the repertoire of known and confirmed substrates of AMPK at mitochondria will help to identify novel aspects of mitochondrial function regulated by AMPK. Given that metformin is a potent AMPK activator, these questions are not only important in understanding fundamental biological processes but also have real-life applications for the more than 150 million people worldwide who take metformin[219]. Moreover, recent efforts to generate direct activators of AMPK for the treatment of cancer and metabolic diseases (BOX 1) also highlight the importance of understanding how AMPK affects metabolism in general and mitochondrial biology in particular. Regulation of mitochondrial health could represent one of the ways by which metformin and other AMPK activators improve the symptoms of type 2 diabetes[105,220], as well as how exercise and calorie restriction improve health and lifespan[221,222].

Note added in proof

In an elegant study[244] in skeletal muscle of mice bearing a fluorescent reporter gene *pMitoTimer* that measures oxidative stress and mitophagy *in vivo*, the authors demonstrated that dominant-negative AMPK suppresses acute-exercise-induced ULK1 phosphorylation, mitophagy and lysosomal biogenesis. Conditional deletion of *Ulk1* resulted in loss of acute-exercise-induced mitophagy but had no effect on lysosomal biogenesis, collectively suggesting a broad role for AMPK in restoring metabolic homeostasis after exercise, and a crucial role of ULK1 downstream of AMPK in mitophagy after exercise.

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Glossary

Allosteric mechanism
Modulation of protein activity by the binding of a molecule to a specific site, often associated with a change in conformation.

Axin
A protein involved in WNT pathway signalling regulation and in mTOR signalling at the lysosome.

Acetyl-CoA carboxylases
Enzymes that catalyse the first step in *de novo* lipid synthesis, the carboxylation of acetyl-CoA to malonyl-CoA.

Metformin
A widely prescribed type 2 diabetes drug. Mechanistically, metformin inhibits complex I of the respiratory chain and leads to changes in the ATP-to-AMP ratio and activation of AMP-activated protein kinase (AMPK).

Mitophagy
Specific removal of mitochondria by autophagy.

Complex I and complex III
Complexes of the respiratory chain in the mitochondrial inner membrane that couple the transfer of electrons to proton pumping. The proton gradient created by the respiratory chain is used to produce ATP, while the electrons are transferred to molecular oxygen.

Dynamin-like protein DRP1
A protein necessary for mitochondrial fission. DRP1 is recruited to mitochondria at sites of future division and mediates the constriction of mitochondria.

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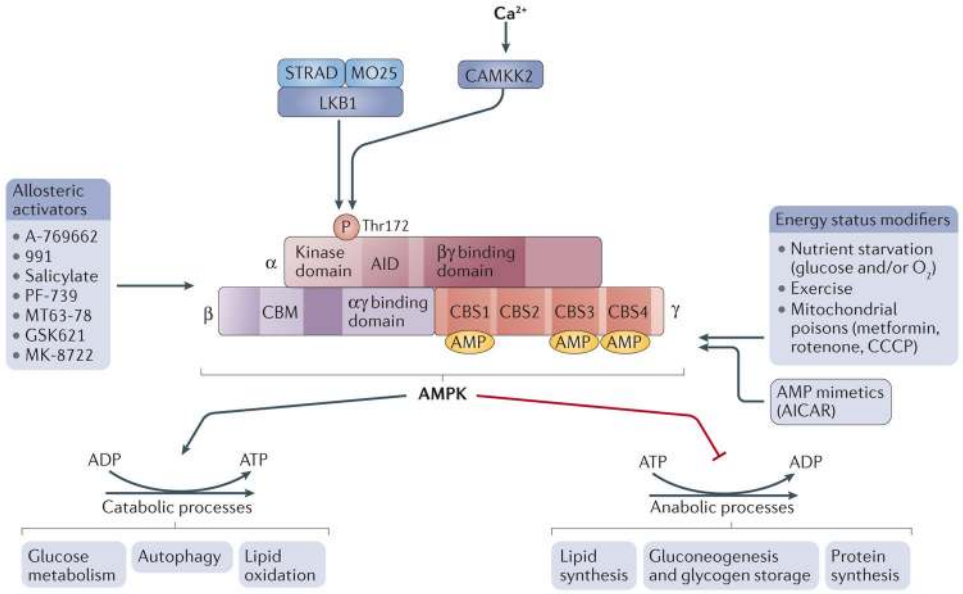

Figure 1. AMPK structure and activation

Domain structure of the AMP-activated protein kinase (AMPK) trimer, showing the α-, β- and γ-subunits with their respective domains. The upstream kinases CAMKK2 and liver kinase B1 (LKB1) are shown above the AMPK complex. LKB1 is a heterotrimer composed of LKB1, STRAD and MO25; CAMKK2 is activated by intracellular calcium. Several factors lead to AMPK activation, such as mitochondrial poisons and oxygen or glucose starvation, as well as exercise. Drugs that activate AMPK include the AMP mimetic AICAR and several small-molecule allosteric activators (listed on the left-hand side). The effect of AMPK activation is to rewire metabolism to decrease anabolic processes (that is, ATP consumption) and increase catabolism (that is, ATP production) to restore a more favourable energy balance. Downstream substrates are grouped by biological functions. ATP-producing processes are activated, while ATP-consuming processes are inhibited. AID, auto-inhibitory domain; CBM, carbohydrate-binding module; CBS, cystathionine-β-synthase.

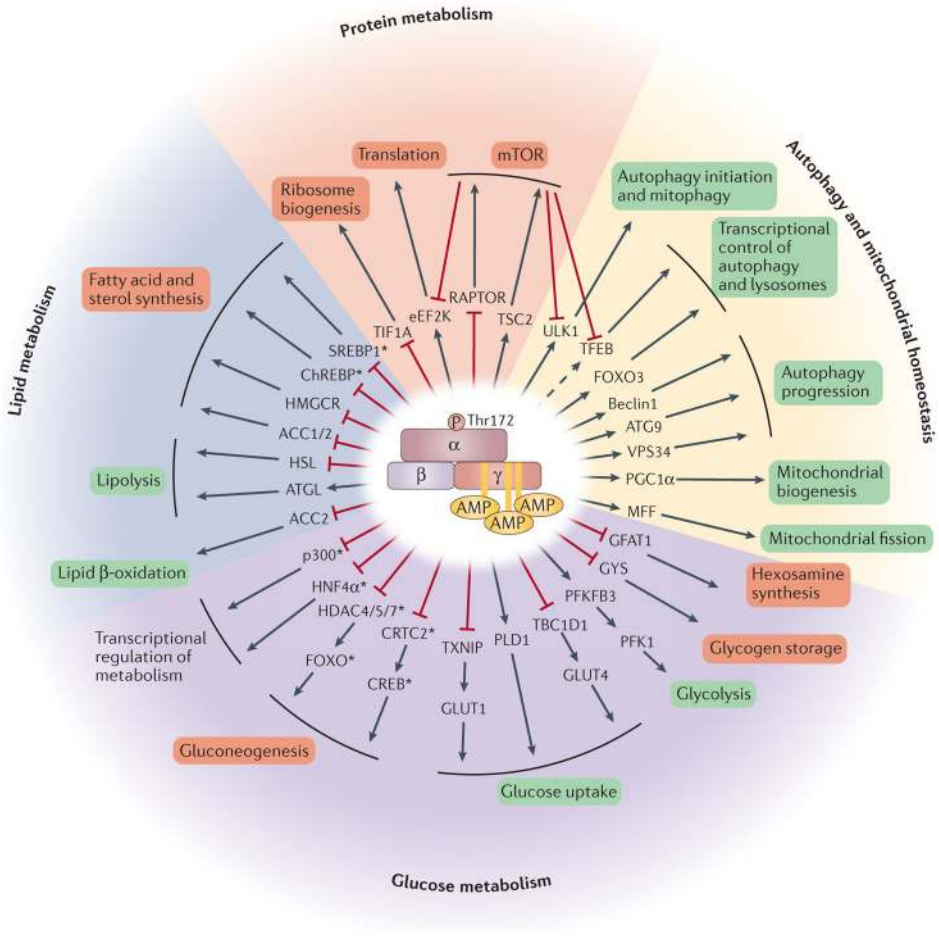

Figure 2. AMPK regulates a variety of metabolic processes

Once activated, the AMP-activated protein kinase (AMPK) complex phosphorylates key targets to rewire metabolism. The direct targets of AMPK are shown in the first concentric circle. The arrow indicates whether the phosphorylation is activating or inhibitory for the function of the target protein. The general process in which each target is involved is indicated in the next circle, and the box colour indicates whether that general process is activated (green) or inhibited (red). For certain targets, an intermediate mediator of the effect is indicated between the two circles. The pathways modulated by AMPK are grouped into four general categories — protein metabolism, lipid metabolism, glucose metabolism, and autophagy and mitochondrial homeostasis — denoting the wide range of processes that are controlled by AMPK. mTOR is modulated by AMPK while also modulating several direct or indirect targets of AMPK. This is illustrated by arrows from mTOR to its targets and serves to emphasize the complex relationship between these two signalling pathways. Transcriptional regulators are denoted by an asterisk. It is important to note that only a subset of AMPK substrates is included in the figure. ChREBP, carbohydrate-responsive element-binding protein; CREB, cAMP response element-binding protein; FOXO, forkhead box protein O; HDAC, histone deacetylase; HMGCR, HMG-CoA reductase; HNF4α, hepatocyte nuclear factor 4α; MFF, mitochondrial fission factor; PGC1α, peroxisome

proliferator-activated receptor-γ co-activator 1α; PLD1, phospholipase D1; SREBP1, sterol regulatory element-binding protein 1; TFEB, transcription factor EB.

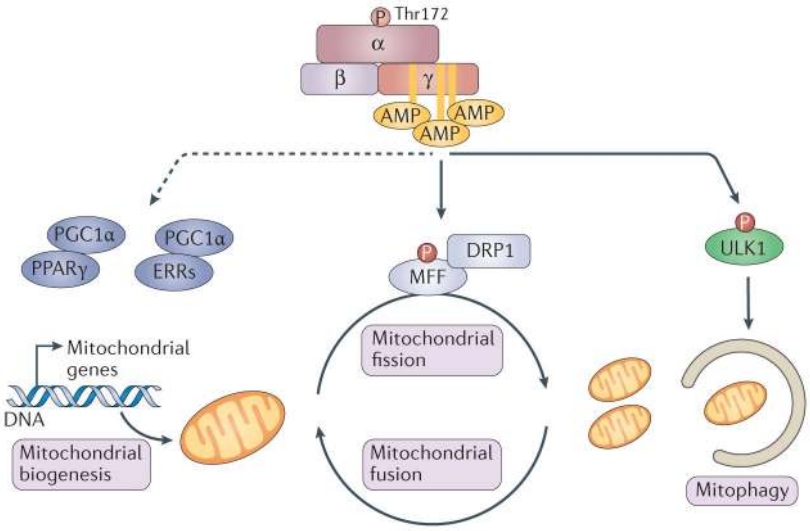

Figure 3. Regulation of mitochondrial homeostasis by AMPK

AMP-activated protein kinase (AMPK) directly phosphorylates mitochondrial fission factor (MFF) to regulate mitochondrial fission through dynamin-like protein DRP1 and activates ULK1, the upstream kinase in autophagy and mitophagy. Mitochondrial fission is required for mitophagy and allows damaged mitochondria to be degraded by mitophagy. During energy stress, AMPK also activates peroxisome proliferator-activated receptor-γ (PPARγ) co-activator 1α (PGC1α), which activates mitochondrial biogenesis genes through interaction with PPARγ or oestrogen-related receptors (ERRs). The dashed arrow indicates potentially indirect regulation.

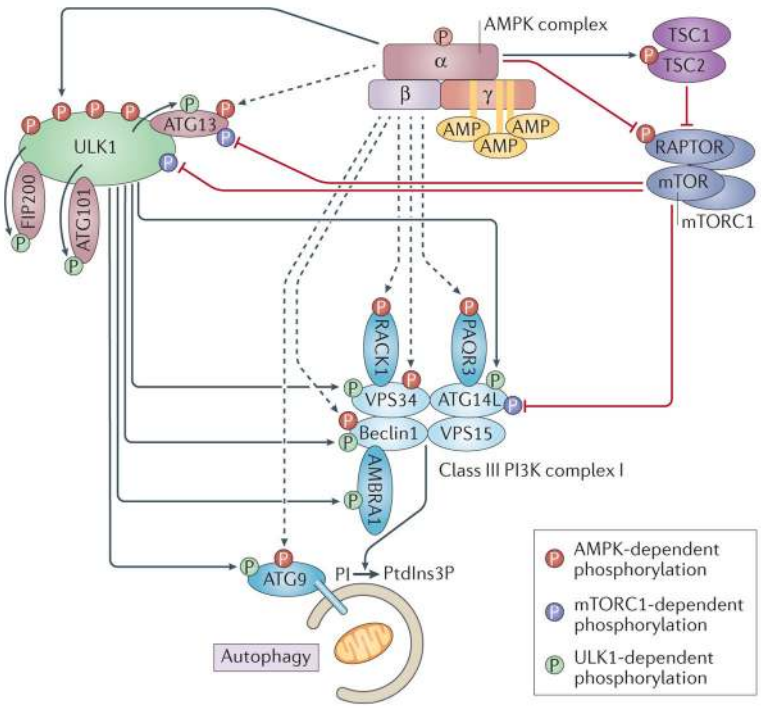

Figure 4. Details of the regulation of autophagy by mTOR, AMPK and ULK1

ULK1-mediated phosphorylation events are shown in green, mTOR-mediated phosphorylation events in purple and AMP-activated protein kinase (AMPK)-mediated phosphorylation events in red. Dashed arrows indicate reported phosphorylation events by AMPK that are insufficiently documented or that involve sites that do not conform to the AMPK motif. Upon activation, AMPK phosphorylates TSC2 and RAPTOR, which leads to the downregulation of mTOR complex 1 (mTORC1) (purple complex, composed of mTOR, RAPTOR, mLST8, DEPTOR and PRAS40 (also known as AKT1S1); not all shown) activity. AMPK phosphorylates ULK1 on at least four serines to promote its activity. ULK1 is part of a complex with ATG101, ATG13 and FIP200. All of these proteins have been shown to be targets of ULK1, while ATG13 has also been reported as a target of both AMPK and mTORC1. PI3K complex I mediates the conversion of phosphatidylinositol (PI) to phosphatidylinositol-3-phosphate (PtdIns3P) at the surface of the forming autophagosome (beige membrane shown engulfing cellular components, including a mitochondrion), a step required for proper recruitment of cargo and adaptor proteins. The PI3K complex I components (light blue) and accessory factors (dark blue) are shown with their reported phosphorylation. ATG9 is an integral membrane protein localized at the autophagosome membrane. The arrows indicate whether the phosphorylation event is activating or inhibitory for the function of the protein.

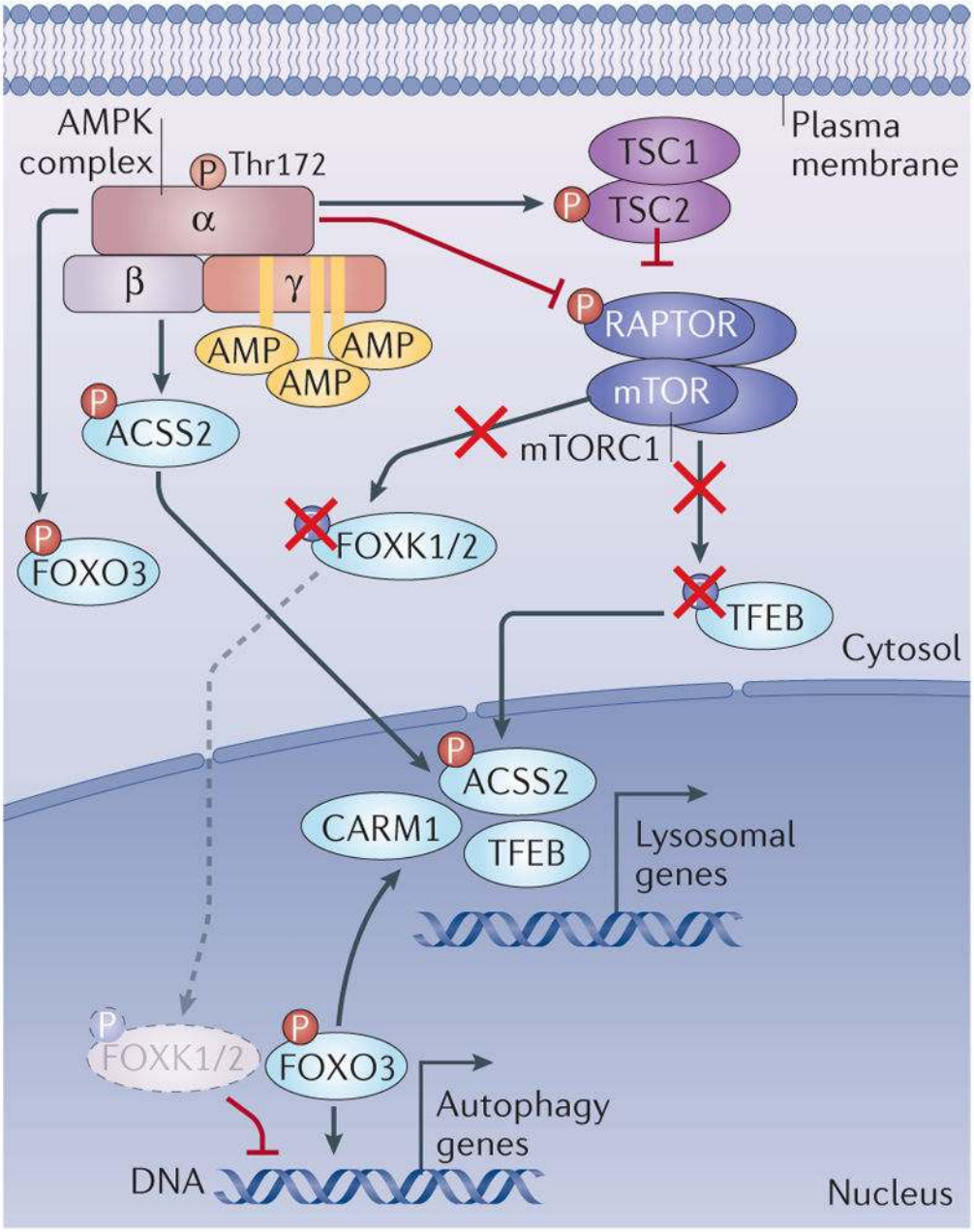

Figure 5. Modulation of the transcription of autophagy and lysosome genes by AMPK

AMP-activated protein kinase (AMPK) regulates the activity of forkhead box protein O3 (FOXO3) through direct phosphorylation. In parallel, AMPK decreases mTOR complex 1 (mTORC1) (purple complex, composed of mTOR, RAPTOR, mLST8, DEPTOR and PRAS40; not all shown) activity through phosphorylation of RAPTOR and the upstream regulator TSC2. This leads to downregulation of mTORC1 activity. This results in dephosphorylation of mTORC1 targets FOXK1 and FOXK2, as well as transcription factor EB (TFEB). As a result, dephosphorylated FOXK1 and FOXK2 can no longer act as transcriptional repressors of FOXO3 targets, allowing higher transcription of autophagy genes downstream of FOXO3 binding. However, dephosphorylation of TFEB allows its

nuclear translocation and activation of its target genes, including genes involved in lysosome biogenesis. In addition, an increased CARM1 protein level resulting from FOXO3-dependent gene activation further enhances TFEB-dependent gene expression. Acetyl-CoA carboxylase 2 (ACC2) phosphorylation by AMPK stimulates its nuclear import where it enhances TFEB target gene expression.