AMPK and TOR: the Yin and Yang of cellular nutrient sensing and growth control

Asier González¹, Michael N. Hall¹, Sheng-Cai Lin² and D. Grahame Hardie³*

¹Biozentrum, University of Basel, CH4056 Basel, Switzerland
²School of Life Sciences, Xiamen University, Xiamen, 361102 Fujian, China
³Division of Cell Signalling & Immunology, School of Life Sciences, University of Dundee, Dundee, DD1 5EH, Scotland, UK

*corresponding author
ABSTRACT

The AMPK (AMP-activated protein kinase) and TOR (target-of-rapamycin) pathways are interlinked, opposing signalling pathways involved in sensing availability of nutrients and energy, and regulation of cell growth. AMPK (Yin or the “dark side”) is switched on by lack of energy or nutrients and inhibits cell growth, while TOR (Yang or the “bright side”) is switched on by nutrient availability and promotes cell growth. Genes encoding the AMPK and TOR complexes are found in almost all eukaryotes, suggesting that these pathways arose very early during eukaryotic evolution. During the development of multicellularity, an additional tier of cell-extrinsic growth control arose that is mediated by growth factors, but these often act by modulating nutrient uptake, so that AMPK and TOR remain the underlying regulators of cellular growth control. In this review we discuss the evolution, structure and regulation of the AMPK and TOR pathways, and the complex mechanisms by which they interact.
All eukaryotic cells are now thought to have arisen via a single endosymbiotic event when an archaeal host cell engulfed bacteria that were capable of oxidative metabolism, the latter eventually becoming mitochondria (Lane, 2006; Sagan, 1967). This event was followed by the transfer of most of the genes from the genome of the endosymbiont to that of the host - it has been argued that this separation of energy-generating capacity from gene expression allowed a large increase in the energy available per gene, thus permitting a major expansion in gene number in the host (Lane and Martin, 2010). This may in turn have enabled major enhancements in the complexity of eukaryotic cells compared with their prokaryotic counterparts, including the development of endomembrane systems such as lysosomes or vacuoles (de Duve, 2005), and the associated trafficking of materials between these internal compartments and the plasma membrane via membrane-bound vesicles.

New cellular functions this led to were phagocytosis and pinocytosis, used by many protists today as mechanisms of feeding, and autophagy, used by all eukaryotic cells for recycling of cellular components that are damaged or surplus to requirements, or as an emergency measure during nutrient starvation. Phagocytosis, pinocytosis and autophagy deliver proteins, lipids and carbohydrates, or even whole organelles such as mitochondria, to lysosomes or vacuoles; the latter are acidic compartments where the engulfed materials are broken down to recycle their components either for catabolism or re-use. Lysosomes or vacuoles can therefore be considered to be the “gut” or digestive systems of unicellular eukaryotes, particularly in amoeboid protists that feed by phagocytosis or pinocytosis. They would therefore have been a major source of nutrients and appear to have developed into hubs for nutrient sensing, as discussed below.

As these processes were evolving, early eukaryotes would have needed signalling pathways that could monitor the function of their new internal organelles and regulate cell growth and proliferation accordingly. For example, there would have been a need to monitor the output of ATP by mitochondria, and to up-regulate their ATP-generating capacity if or when the supply of ATP was insufficient; this is now a major function of the AMPK (AMP-activated protein kinase) signalling pathway. In addition, there would have been a requirement to monitor the supply of nutrients such as amino acids and glucose produced at the lysosome by phagocytosis, pinocytosis or autophagy, and to up-regulate cell growth when these nutrients were available; this is now a key function of the TOR (target-of-rapamycin) pathway. We propose that these two opposing pathways, which are present in almost all present-day eukaryotes, are the descendants of ancient nutrient
sensing and signalling pathways that arose very early during eukaryotic evolution. AMPK represents the Yin ("dark" or "passive") side that signals lack of nutrients or insufficient ATP and inhibits cell growth, whereas TOR represents the Yang ("bright" or "active") side that signals availability of nutrients and promotes cell growth. Just as in the Chinese philosophy of Taoism from which the Yin-Yang concept is derived, an appropriate balance between these two opposing elements ensures homeostasis and thus a healthy cell or organism.

In present-day unicellular eukaryotes, including fungi such as *Saccharomyces cerevisiae*, growth and proliferation is regulated almost entirely by nutrient availability, and the orthologs of AMPK and TOR play crucial roles in this. However, during the development of multicellular organisms, the uptake (and hence the intracellular availability) of nutrients has become modulated by an additional tier of cell-extrinsic regulation mediated by growth factors and cytokines (Palm and Thompson, 2017). It can be argued that these cell-extrinsic factors “license” or allow cells to take up nutrients, but that the AMPK and TOR pathways, which sense intracellular nutrient availability, remain the primary internal regulators of cell growth and proliferation. Interestingly, most of the mutations that cause cancer in multicellular organisms appear to affect the higher-level, cell-extrinsic regulation of cell growth. Such mutations allow cancer cells to become “rebels” that have partially reverted to their unicellular origins and that switch over to using cell-intrinsic growth control, based on nutrient availability and controlled by the AMPK and TOR pathways.

**Yin: the structure and regulation of AMPK/SNF1 complexes**

**Subunit structure and evolution**

AMPK appears to occur universally as heterotrimeric complexes comprising catalytic α subunits and regulatory β and γ subunits (Ross et al., 2016b). Genes encoding all three subunits are readily found within the genomes of almost all eukaryotes (Table 1 and Fig. 1). However, the orthologs in budding yeast (*S. cerevisiae*) and plants are not allosterically activated by AMP and were discovered independently of mammalian AMPK by genetic approaches (Alderson et al., 1991; Celenza and Carlson, 1986). They are therefore not usually referred to as AMPK but instead in yeast as Snf1 complexes (*SNF1* being the gene encoding the catalytic subunit), and in plants as Snf1-related kinase-1 (SnRK1) complexes.
Interestingly, the only eukaryotes known to lack AMPK subunit orthologs are parasites that spend all or most of their life cycle living inside other eukaryotic cells, including *Encephalitozoon cuniculi* and *Plasmodium falciparum*, the latter being the causative agent of human malaria (Fig. 1). These parasitic eukaryotes appear to have undergone stringent selection for small genome size, with *E. cuniculi* having one of the smallest known genome of any eukaryote, encoding only 29 conventional and 3 atypical protein kinases (compared with >500 in humans) (Miranda-Saavedra et al., 2007). Ancestors of these organisms most likely did have AMPK genes, but the modern-day descendants may have been able to dispense with them because the host cell would provide AMPK that regulates cellular energy balance on their behalf. Consistent with this, species closely related to *P. falciparum* that cause malaria in birds (*P. gallinaceum* and *P. relictum*) do still have conventional AMPK genes (Bohme et al., 2018). Interestingly, TOR genes are missing in *E. cuniculi* and *P. falciparum* (Fig. 1) but are also absent in *P. gallinaceum* and *P. relictum*.

Mammals, including humans, have two genes encoding isoforms of AMPK-α (α1 and α2), two encoding AMPK-β (β1 and β2) and three encoding AMPK-γ (γ1, γ2 and γ3) (Table 1). These multiple isoforms appear to have arisen during the two rounds of whole genome duplication that occurred during the early evolution of vertebrates (Ross et al., 2016b). All twelve combinations of these subunit isoforms are able to form heterotrimeric complexes, although it is not certain that all combinations exist in vivo. Structures for several almost complete human AMPK heterotrimers, i.e., α2β1γ1 (Xiao et al., 2013), α1β1γ1 (Calabrese et al., 2014), α1β2γ1 (Li et al., 2015) and α2β2γ1 (Ngoei et al., 2018), have been obtained via X-ray crystallography. The complexes were all crystallized in active conformations and their structures are very similar; a schematic representation of a generalized AMPK heterotrimer based on these structures is shown in Fig. 2.

**Structure of AMPK and canonical adenine nucleotide (energy)-sensing mechanism**

Although the main theme of this review is nutrient sensing, we will first discuss the classical or “canonical” mechanism by which AMPK responds to the changing energy status of cells. The catalytic α subunits of AMPK contain, at their N-termini, conventional serine/threonine kinase domains with a small N-lobe and larger C-lobe, and the catalytic site in the cleft between them. As with many other members of the ePK (eukaryotic protein kinase) family, AMPK complexes are only significantly active when phosphorylated at a critical residue within the *activation loop*, a
stretch of ∼20 amino acids in the C-lobe between the highly conserved DFG and APE motifs. In AMPK the critical phosphorylation site is a threonine, usually referred to as Thr172 after its position in the rat α2 sequence where originally mapped (Hawley et al., 1996). Thr172 is not phosphorylated by AMPK itself but by upstream kinases, principally by LKB1 (liver kinase B1) (Hawley et al., 2003; Shaw et al., 2004; Woods et al., 2003), the active form of which is a heterotrimeric complex also containing STRAD-α or −β, and the scaffold protein MO25-α or −β (Zeqiraj et al., 2009). LKB1 was originally identified as the product of the tumour suppressor gene STK11, which is mutated in Peutz-Jeghers Syndrome (an inherited susceptibility to cancer) as well as in some sporadic (i.e., non-inherited) cancers, especially lung adenocarcinomas (Alessi et al., 2006; Ji et al., 2007; Sanchez-Cespedes et al., 2002). Although LKB1 was subsequently shown to phosphorylate and activate twelve other kinases with kinase domains related to AMPK (the AMPK-related kinase family (Jaleel et al., 2005; Lizcano et al., 2004)), AMPK was the first downstream target for LKB1 to be identified, and this introduced an intriguing connection between AMPK and cancer. Indeed, it is now clear that AMPK can also act as a tumor suppressor, at least in certain animal models of cancer (Vara-Ciruelos et al., 2019).

A summary of the canonical and non-canonical mechanisms that activate AMPK, and selected downstream targets involved in its promotion of catabolic processes, inhibition of anabolic processes and effects on DNA replication, are shown in Fig. 3. In the canonical mechanism that is enshrined in its name, AMPK is activated by binding of 5′-AMP, with activation occurring not by one but three mechanisms: (1) allosteric activation of AMPK already phosphorylated on Thr172 (Carling et al., 1987; Ferrer et al., 1985; Yeh et al., 1980); (2) enhanced Thr172 phosphorylation by the LKB1 complex (Hawley et al., 1995); and (3) protection against Thr172 dephosphorylation by protein phosphatases (Davies et al., 1995). All three effects are due to binding of AMP to AMPK, not to the upstream kinase or phosphatase, and this tripartite mechanism ensures that the system responds to small increases in AMP in a very sensitive manner. Although there is general agreement that only AMP binding causes effect #1 above, ADP binding similarly triggers effects #2 and #3 (Oakhill et al., 2011; Xiao et al., 2011). However, most AMPK complexes (apart from those containing the γ2 isoform) are about 10-fold more sensitive to AMP than ADP, suggesting that increases in AMP are the primary activating signal, although increases in ADP may contribute...
(Ross et al., 2016a). All of the activating effects of AMP and ADP are antagonized by binding of ATP, so that the AMPK system effectively monitors cellular AMP:ATP and ADP:ATP ratios.

Where are the regulatory binding sites where these adenine nucleotides are sensed? The γ subunits contain four tandem repeats of a sequence termed a CBS (cystathionine β-synthase) motif (Bateman, 1997). These occur, usually as just two tandem repeats, in about 75 proteins in humans, and are also found in archaea and bacteria. Single pairs of tandem CBS repeats associate into pseudodimers (termed Bateman modules), potentially creating two pseudo-symmetrical ligand-binding sites in the intervening cleft, although in many cases only one is utilized. These sites usually bind ligands containing adenosine or (less often) guanosine (Anashkin et al., 2017; Scott et al., 2004). The two Bateman modules in each AMPK-γ subunit associate head-to-head to form a flattened disk with four potential binding sites for adenine nucleotides in the center (Fig. 2).

However, only three are utilized, i.e. CBS3, which is accessible from one face of the γ subunit, and CBS1 and CBS4, accessible from the other. The critical site appears to be CBS3; the α-linker, a flexible region of the α subunit that connects the α-AID (α-auto-inhibitory domain) and α-CTD (α-C-terminal domain), wraps around the face of the γ subunit containing CBS3, contacting its bound AMP (Fig. 2). This interaction is not thought to occur when ATP is bound at CBS3 instead of AMP, and the consequent release of the α-linker from the γ subunit is proposed to allow the α-AID to rotate back into its inhibitory position behind the kinase domain (Chen et al., 2009; Chen et al., 2013; Li et al., 2015; Xiao et al., 2011; Xin et al., 2013); this model thus explains allosteric activation by AMP as well as its antagonism by ATP. At the same time, the resulting conformational changes may alter the accessibility of Thr172 for phosphorylation and/or dephosphorylation, although those aspects of the mechanism are less well understood. The functions of the CBS1 and CBS4 sites are less clear, although they are close to the CBS3 site in the centre of the CBS repeats, where the three sites interact. One proposal is that CBS1 binds ATP permanently, while CBS4 binds AMP permanently, and that these constitutive binding events alter the conformation of the CBS3 site such that it has a higher affinity for AMP than ADP or ATP (Gu et al., 2017b). This helps to explain how AMPK achieves the difficult task of sensing changes in AMP in the 30-300 µM range despite the presence of mM concentrations of ATP (Gowans et al., 2013). An additional explanation is that only Mg²⁺-free ATP competes with AMP at the CBS3 site.
(Pelosse et al., 2019), although 90% of intracellular ATP is thought to be present as the Mg.ATP$^2$ complex.

Although the sequences of the α, β, and γ subunits are well conserved, the regulation by adenine nucleotides of AMPK orthologs from eukaryotes other than mammals is much less well studied. As mentioned earlier, neither Snf1 complexes from S. cerevisiae (Wilson et al., 1996) nor SnRK1 complexes from plants (Mackintosh et al., 1992) appear to be allosterically activated by AMP, although the dephosphorylation of the threonine residues equivalent to Thr172 were reported to be inhibited by ADP in S. cerevisiae (Mayer et al., 2011) and by AMP in plants (Sugden et al., 1999a).

Allosteric activation by AMP has been reported, although not well studied, using the complexes from D. melanogaster (Pan and Hardie, 2002), C. elegans (Apfeld et al., 2004) and S. pombe (Forte et al., 2019). It seems possible that allosteric activation, which is physiologically significant in intact cells (Gowans et al., 2013), was a later evolutionary refinement that increased the overall sensitivity of the system to small changes in AMP.

**Non-canonical activation of AMPK by ligands binding at the ADaM site**

The heterotrimeric AMPK complex contains other ligand-binding sites whose physiological function remains less clear. One is the glycogen-binding site on the β-CBM (β-carbohydrate-binding module), which is present in the β subunits of all eukaryotes and in mammalian cells causes a proportion of AMPK to bind to glycogen (Hudson et al., 2003; Polekhina et al., 2003; Polekhina et al., 2005). Intriguingly, as well as a conventional CBM on the β subunit, many higher plant SnRK1 complexes also contain a second CBM fused at the N-terminus of the γ subunit, forming a so-called βγ subunit (Lumbreras et al., 2001; Zhao, 2019). Although it has been proposed that the single CBM of mammalian AMPKs may allow them to sense the structural state of glycogen (McBride et al., 2009), more work is required to confirm that hypothesis. Another ligand-binding site lies in a cleft (termed the ADaM site) between the other face of the CBM (i.e., opposite to the glycogen-binding site) and the N-lobe of the kinase domain on the α subunit (Fig. 2). Several ligands that bind in this site cause a dramatic allosteric activation of AMPK with, usually, a more modest effect to promote net Thr172 phosphorylation (Goransson et al., 2007; Sanders et al., 2007; Scott et al., 2014; Yan et al., 2019). However, a curious feature is that, with the exception of salicylate (a natural product of plants, but not animals) (Hawley et al., 2012), all of the compounds
currently known to bind there are synthetic molecules that emerged from high-throughput screens searching for allosteric activators of AMPK [e.g., (Cokorinos et al., 2017; Cool et al., 2006; Myers et al., 2017)]. This binding site is therefore a type of “orphan receptor”, and many researchers in the field suspect that there is an unidentified metabolite occurring in animal cells that binds to it, hence the acronym ADaM (Allosteric Drug and Metabolite) site (Langendorf and Kemp, 2015).

Non-canonical activation of AMPK by Ca\textsuperscript{2+} and by DNA damage

Thr172 can also be phosphorylated by alternate upstream kinases, including the Ca\textsuperscript{2+}/calmodulin-dependent kinase, CaMKK2 (Hawley et al., 2005; Hurley et al., 2005; Woods et al., 2005) and TAK1 (Transforming growth factor-β-Activated Kinase-1) (Momcilovic et al., 2006). The physiological importance of TAK1 as a means of AMPK activation is not well established, although there is one report that it is involved in AMPK activation in response to TRAIL (Tumor necrosis factor-Related Apoptosis-Inducing Ligand) (Herrero-Martin et al., 2009). By contrast, there is good evidence that AMPK can be activated by the CaMKK2 pathway in response to hormones or growth factors that trigger release of Ca\textsuperscript{2+} from the endoplasmic reticulum (Fig. 3). This includes hormones acting at G protein-coupled receptors linked via G\textsubscript{q}/G\textsubscript{11} to release of the Ca\textsuperscript{2+}-mobilizing messenger IP\textsubscript{3} (inositol-3,4,5-trisphosphate), such as thrombin acting at protease-activated receptor-1 in endothelial cells (Stahmann et al., 2006), acetylcholine acting at M3 muscarinic receptors in various cell types (Jadeja et al., 2019; Merlin et al., 2010; Thornton et al., 2008; Xue et al., 2016) and ghrelin acting at GHSR1 receptors in neurons of the hypothalamus (Yang et al., 2011). AMPK is also activated via a Ca\textsuperscript{2+}/CaMKK2-dependent mechanism by the growth factor VEGF (vascular endothelial growth factor) acting at the tyrosine kinase-linked VEGF receptor in endothelial cells, which triggers release of IP\textsubscript{3} via activation of PLC\textsubscript{γ} (phospholipase C-γ) (Reihill et al., 2007; Stahmann et al., 2010).

Another non-canonical AMPK activation mechanism occurs in response to DNA damage and/or replicative stress (Fig. 3), which can be induced by etoposide, hydroxyurea, aphidicolin or ionizing radiation (Fu et al., 2008; Li et al., 2019b; Sanli et al., 2010). Interestingly, the effects of etoposide, hydroxyurea or aphidicolin require CaMKK2 but not LKB1, correlate with increases in nuclear Ca\textsuperscript{2+}, only activate AMPK in the nucleus and (at least for etoposide) only activate the α1 isoform (Li et al., 2019b; Vara-Ciruelos et al., 2018). Studies with AMPK knockout cells reveal that they
are hypersensitive to cell death induced by DNA damage or replicative stress (Vara-Ciruelos et al., 2018), and this correlates with increased resection of replication forks as well as other chromosomal abnormalities (Li et al., 2019b). The defects in the knockout cells have been attributed, at least in part, to lack of phosphorylation by AMPK of the 5′-3′ exonuclease EXO1, which normally causes its association with 14-3-3 proteins, thus restraining its ability to resect replication forks (Li et al., 2019b). Since many of these genotoxic treatments are used in cancer therapy, it seems likely that they would be more efficacious if administered together with an AMPK inhibitor, thus preventing the protective effects of AMPK against cell death induced by DNA damage or replicative stress.

**Non-canonical activation of AMPK by glucose starvation**

Recent studies in mammalian cells have revealed, perhaps surprisingly, that activation of AMPK in response to glucose starvation can occur via a non-canonical, AMP-independent mechanism. The first clues came from administration of siRNAs targeting AXIN1 into the tail vein of mice, using adenoviral vectors that direct expression to the liver. After overnight starvation, animals receiving siRNA showed diminished AMPK activation and increased fat storage in liver. This led to the discovery that AXIN1, which was initially identified as a central scaffold protein for Wnt signalling (Zeng et al., 1997), binds constitutively to LKB1 and acts as an adapter for LKB1 to associate with and phosphorylate AMPK; this initial characterization of the role of AXIN1 was based on an in vitro reconstitution experiment where high levels of AMP were required for the interaction to occur (Zhang et al., 2013), which can now be classified as a cytosolic, AXIN/AMP-dependent mechanism (Zong et al., 2019). A subsequent yeast two-hybrid screen searching for novel AXIN1-interacting proteins (Zhang et al., 2014) identified p18/LAMTOR1, a protein anchored to the lysosomal membrane by N-terminal myristoyl and palmitoyl modifications (Nada et al., 2009).

p18/LAMTOR1 is a key component of the Ragulator complex, which (as will be discussed later) plays a central role in the activation of mTORC1 via interaction with the vacuolar ATPase (v-ATPase) (Bar-Peled et al., 2012; Sancak et al., 2010; Zoncu et al., 2011). In LAMTOR1-null cells or cells with knockdown of the v0c subunit of the v-ATPase, AMPK activation induced by glucose starvation was no longer observed. In addition, AXIN1, in complex with LKB1, was found to translocate to the lysosomal surface, forming a supramolecular complex with the Ragulator and v-ATPase, which was not observed in LAMTOR1-null cells or cells with knockdown of the v-
ATPase v0c subunit (Zhang et al., 2014). By this mechanism, LKB1 is brought to the vicinity of a pool of AMPK that appears to permanently reside on the lysosomal membrane due to N-terminal myristoylation of the β subunit. This overall mechanism is now referred to as the lysosomal AMPK activation pathway (Fig. 3).

It should be noted that AXIN has two isoforms, AXIN1 and AXIN2, which are functionally redundant both in Wnt signaling (Chia and Costantini, 2005), and in the lysosomal AMPK activation pathway (Zong et al., 2019). While AXIN1 is ubiquitously expressed, AXIN2 is mainly expressed in neuronal cells and some actively proliferating cells. For example, AXIN2 is not expressed in differentiated hepatocytes (Zong et al., 2019), although it was detected in a small population of self-renewing cells adjacent to the central vein in the liver lobule (Wang et al., 2015a). Similarly, while mouse embryo fibroblasts (MEFs) only express AXIN1, AXIN2 is also expressed in HEK293T cells, so that if AXIN1 expression is knocked out in HEK293T cells, the lysosomal AMPK activation pathway remains intact (Zong et al., 2019). In addition, in some cell types that rely on glycolysis for ATP production, glucose starvation may also activate AMPK by the canonical AMP-dependent pathway, rendering the lysosomal activation pathway redundant. For example, in HEK293 cells (unlike in MEFs) there are rapid increases in cellular AMP:ATP and ADP:ATP ratios after glucose removal even when an alternative carbon source such as glutamine is provided (Zhang et al., 2017). In these cells, the canonical AMP-dependent pathway for AMPK activation operates independently of the lysosomal AMP-independent pathway in response to glucose starvation (Zong et al., 2019). Thus, studies of the lysosomal pathway in some cell types or tissues need to take into account the possibility not only of expression of AXIN1 or AXIN2, but also of changing AMP levels.

Although the results of Zhang et al (2014) demonstrated that glucose starvation activated AMPK via the lysosomal pathway in mammals, it remained unclear how the presence or absence of glucose was sensed. Pursuing this, it became apparent that aldolase, the glycolytic enzyme that converts FBP (fructose-1,6-bisphosphate) into triose phosphates, which can also be associated with the v-ATPase complex, is a direct (physical) sensor for FBP. When aldolase is unoccupied by FBP (whose levels rapidly decrease upon glucose deprivation) the v-ATPase complex undergoes conformational changes that inhibit its activity as a proton pump (as suggested by increased pH levels in the lysosomal lumen (Zhang et al., 2017)) and also allow the AXIN1:LKB1 complex to
interact with the v-ATPase and Ragulator. Multiple lines of evidence support the idea that aldolase
is the direct sensor. Firstly, knockdown of all isoforms of aldolase caused constitutive activation of
AMPK, even in high glucose. Secondly, in cells expressing the D34S mutant of aldolase, which has
a greatly reduced $k_{cat}$ despite an almost unchanged $K_m$ for FBP (Morris and Tolan, 1993) (meaning
that FBP will accumulate in the active site of aldolase even in low glucose), AMPK was not
activated by glucose starvation (Zhang et al., 2017). Importantly, this mechanism for AMPK
regulation by glucose can occur in the absence of any changes in adenine nucleotide ratios. For
example, in MEFs transferred from medium with high glucose (25 mM) to medium containing
glucose concentrations below 5 mM, or in livers of mice starved overnight (when blood glucose
dropped from 9 to 3 mM), AMPK was activated without any associated changes in cellular
AMP:ATP or ADP:ATP ratios. Interestingly, however, if glutamine (the other major carbon source
in the medium) was removed from the medium as well as glucose, there was an additional, delayed
(but ultimately larger) activation of AMPK that did correlate with increases in AMP:ATP and
ADP:ATP ratios (Zhang et al., 2017). These results indicate that the non-canonical glucose-sensing
mechanism for AMPK activation can act in parallel with the canonical AMP-dependent mechanism.
In line with the concept that glucose availability can be sensed independently of cellular energy
status, neither pyruvate nor glutamine, which both feed into the TCA cycle for ATP production,
prevent lack of glucose from activating AMPK. Indeed, it is now clear that the AXIN/lysosome-
dependent and AMP–dependent mechanisms can co-exist, with their contributions to overall
AMPK activation depending on the magnitude of any increases in AMP, as well as the subcellular
location (Zong et al., 2019).

Another recent study has uncovered the mechanism that signals the presence or absence of FBP
in the active site of aldolase to the formation of the AXIN-LKB1-AMPK complex on the lysosomal
membrane. It was demonstrated that TRPV (transient receptor potential V) channels located on the
ER (endoplasmic reticulum) membrane are required for AMPK activation in response to low
glucose. The current model is that aldolase that is unoccupied by FBP interacts with TRPV at
lysosome:ER contact sites, inhibiting its $Ca^{2+}$-releasing activity. Once the $Ca^{2+}$ concentrations at the
ER-lysosome contact sites falls below a certain level, TRPV gains affinity for the v-ATPase, re-
configuring its association with aldolase and causing the formation of the AXIN-based complex to
activate AMPK (Li et al., 2019a). It should be pointed out that the concentration of the TRPV-
released \( \text{Ca}^{2+} \) (\(<1 \mu\text{M}\)) is well below that required for activation of CaMKK2, which is not involved in the lysosomal AMPK activation mechanism. It has been proposed that the pool of \( \text{Ca}^{2+} \) at the ER-lysosome contact sites acts as a kind of buffer or damper, smoothing the output and thus preventing fluctuations in AMPK caused by rapid oscillations of FBP binding in the active site of aldolase (Li et al., 2019a).

Glucose starvation also causes rapid activation of the Snf1 complex in *S. cerevisiae* (Wilson et al., 1996; Woods et al., 1994a) and, intriguingly, complexes containing Sip1 (one of three \( \beta \) subunit orthologs in yeast) translocate to the vacuolar membrane upon glucose removal (Vincent et al., 2001). However, the detailed mechanism appears to be different from that in mammalian cells because no clear AXIN orthologs are found in yeast. Once activated, the Snf1 complex phosphorylates the transcriptional repressor Mig1 (Smith et al., 1999; Treitel et al., 1998), triggering both its inactivation (Papamichos-Chronakis et al., 2004) and nuclear export (DeVit and Johnston, 1999). Mig1 binds to and inhibits the promoters of many glucose-repressed genes, including the *SUC2* gene encoding a secreted invertase that is required to metabolize alternate carbon sources such as sucrose or raffinose (Hedbacker and Carlson, 2008). As in mammalian cells, the Snf1 complex also phosphorylates and inactivates acetyl-CoA carboxylase, potentially inhibiting fatty acid biosynthesis under glucose-limiting conditions (Mitchelhill et al., 1994; Woods et al., 1994b).

Although the effects of starvation for a carbon source are less well studied in plants, knockout or silencing of the genes encoding the AMPK-\( \alpha \) orthologs in the moss *Physcomitrella patens* (Thelander et al., 2004) and the higher plant *Arabidopsis thaliana* (Baena-Gonzalez et al., 2007) causes failure to respond appropriately to periods of darkness, the equivalent of starvation in plants. In cells of *A. thaliana* the AMPK-\( \alpha \) ortholog KIN10 is responsible for triggering extensive reprogramming of transcription affecting thousands of genes, some of which are required for adaptive responses such as starch breakdown during starvation (Baena-Gonzalez et al., 2007; Baena-Gonzalez and Sheen, 2008). SnRK1 complexes also phosphorylate and inactivate both sucrose phosphate synthase and HMG- (3-hydroxy-3-methylglutaryl-) CoA reductase, potentially inhibiting the anabolic pathways of sucrose and sterol synthesis (Nukarinen et al., 2016; Sugden et al., 1999b).
Since activation by starvation for key carbon sources (especially glucose) appears to be a common feature of the AMPK orthologs from mammals, plants and budding yeast, yet they differ in their regulation by adenine nucleotides, it is tempting to speculate that sensing of glucose rather than energy may have been the ancestral role of the kinase. However, it remains unclear exactly how carbon starvation causes activation of the orthologs in plants and yeast.

**Downstream targets of AMPK**

AMPK phosphorylates downstream targets containing well-defined recognition motifs, and at least 60 have now been well validated – a full discussion of these is beyond the scope of this article and readers are referred to a previous review (Hardie et al., 2016). In general, AMPK phosphorylates and activates proteins involved in catabolic pathways, thus enhancing ATP synthesis, while phosphorylating and inactivating proteins involved in anabolic (biosynthetic) pathways, thus inhibiting cell growth while conserving ATP. AMPK also causes a cell cycle arrest in G1 phase (Fogarty et al., 2016; Imamura et al., 2001), although in that case the direct downstream targets responsible for the effect are not clear. In this section, we will mention only a few key targets that are important for the effects of AMPK on catabolic and anabolic pathways.

Starting with effects on catabolism, in many cell types AMPK activation increases glucose uptake via effects on the trafficking of the glucose transporters, GLUT1 (Barnes et al., 2002) or GLUT4 (Kurth-Kraczek et al., 1999). This is achieved in part via phosphorylation and consequent degradation of TXNIP, an α-arrestin family member that normally promotes reuptake of GLUT1 and GLUT4 from the plasma membrane by endocytosis (O'Donnell and Schmidt, 2019; Wu et al., 2013). In the case of GLUT4, AMPK also phosphorylates TBC1D1, a GTPase activating protein (GAP) for members of the Rab family, causing dissociation of TBC1D1 from intracellular GLUT4-storage vesicles (GSVs) with consequent conversion of Rabs to their GTP-bound forms, thus promoting trafficking of GSVs to the plasma membrane (Pehmoller et al., 2009). AMPK can also phosphorylate and activate 6-phosphofructo-2-kinase, the enzyme that generates fructose-2,6-bisphosphate, a potent allosteric activator of the key glycolytic enzyme 6-phosphofructo-1-kinase. However, this effect is cell type-dependent because only the PFKFB2 (Marsin et al., 2000) or PFKFB3 (Marsin et al., 2002) isoforms of 6-phosphofructo-2-kinase, which are not expressed ubiquitously, are direct targets for AMPK. AMPK also acutely promotes fatty acid oxidation by
phosphorylating and inactivating the mitochondrial isoform of ACC2 (acetyl-CoA carboxylase-2), thus reducing the local pool of malonyl-CoA, an inhibitor of uptake of fatty acids into mitochondria via the transport system involving carnitine palmitoyl-CoA transferase-1 (Winder and Hardie, 1996).

In the longer term, AMPK activation tends to promote the oxidative metabolism typical of quiescent cells, rather than the rapid glucose uptake and glycolysis typical of cells undergoing rapid proliferation, including tumor cells. Firstly, it promotes mitochondrial biogenesis (Zong et al., 2002) as well as expression of oxidative enzymes (Winder et al., 2000), perhaps by direct phosphorylation (Jager et al., 2007) or deacetylation (Canto et al., 2009) of the transcriptional co-activator, PGC-1α. Secondly, AMPK maintains the cellular content of functional, healthy mitochondria by promoting both mitophagy, via phosphorylation of the autophagy kinase ULK1 (Unc-51-like kinase 1) (Egan et al., 2011b), and mitochondrial fission, perhaps via phosphorylation of proteins involved in mitochondrial fission such as MFF (mitochondrial fission factor) or MTFR1L (mitochondrial fission regulator-1-like) (Ducommun et al., 2015; Schaffer et al., 2015; Toyama et al., 2016). Because mitochondria can exist in cells as elongated branching networks that can be of lengths close to that of the cell diameter, mitochondrial fission may be necessary to break these networks down into smaller segments suitable for mitophagy. Consistent with this, the phenotypes of muscle-specific double knockouts of α1/α2 (Lantier et al., 2014) or β1/β2 (O'Neill et al., 2011) in mice include exercise intolerance associated with the appearance in electron micrographs of mitochondria of abnormal size and morphology.

Along with these effects on catabolism, AMPK acutely switches off most anabolic pathways. It was discovered for its ability to phosphorylate and inactivate ACC1 (acetyl-CoA carboxylase-1) and HMG-CoA reductase, two key enzymes of fatty acid and cholesterol synthesis, respectively (Hardie et al., 1989). Indeed, phosphorylation of ACC1 at Ser80 (Ser79 in rodents), monitored using phosphospecific antibodies, remains the most widely used biomarker for AMPK activation in intact cells. Moreover, mice with knock-in Ser→Ala mutations of the AMPK sites on ACC1 and ACC2 (Fullerton et al., 2013) or HMG-CoA reductase (Loh et al., 2019) have elevated levels of triglycerides and cholesterol, respectively, demonstrating that these phosphorylation sites have regulatory significance in vivo. AMPK also switches off glycogen synthesis via phosphorylation of the GYS1 (Jorgensen et al., 2004) and GYS2 (Bultot et al., 2012) isoforms of glycogen synthase,
nucleotide synthesis via phosphorylation of the PRPS-1 and -2 isoforms of phosphoribosyl pyrophosphate synthetase (Qian et al., 2018), and ribosomal RNA synthesis via phosphorylation of TIF-1A/RRN3, a transcription factor for RNA polymerase-1 (Hoppe et al., 2009). Finally, AMPK switches off the elongation step of protein synthesis in part via phosphorylation of elongation factor-2 kinase (Johanns et al., 2017), an atypical Ca\(^{2+}\)-dependent kinase that phosphorylates elongation factor-2 and causes pausing in elongation. Other effects on protein synthesis are mediated indirectly by inactivation of mTORC1, which is discussed in more detail in a separate section below.

Yang – the structure and regulation of TOR complexes

Subunit structure and evolution

TOR is a serine/threonine protein kinase belonging to the PIKK (phosphatidylinositol kinase-related kinase) family, which also includes DNA-PK and ATM (Keith and Schreiber, 1995). TOR is conserved in all eukaryotes except (as for AMPK) in the case of a few obligate intracellular parasites such as *E. cuniculi* and *P. falciparum* (Tatebe and Shiozaki, 2017; van Dam et al., 2011) (Fig. 1), which may be able to exploit TOR signalling in the host cell. Whereas most eukaryotes contain a single *TOR* gene, a few possess more than one, for example budding yeast (*S. cerevisiae*) and fission yeast (*S. pombe*) have two (Shertz et al., 2010) (Table 1), while trypanosomes have up to four (Saldivia et al., 2013). Early eukaryotes presumably possessed a single *TOR* gene that was duplicated and/or lost multiple times during evolution (Shertz et al., 2010).

TOR was originally identified genetically in *S. cerevisiae* via mutations that render cells resistant to the growth-inhibitory properties of the antibiotic rapamycin (Heitman et al., 1991; Kunz et al., 1993). It was identified in mammalian cells shortly thereafter (Brown et al., 1994; Chiu et al., 1994; Sabatini et al., 1994; Sabers et al., 1995), and the name mTOR (mammalian TOR) was eventually adopted based on the yeast precedent. More recently, the HUGO Gene Nomenclature Committee changed the definition of the mTOR acronym to “mechanistic TOR” in order to create a common nomenclature for TOR in vertebrates (Hall, 2013). However, this has led to TOR from nematodes or even yeast sometimes being referred to as mTOR.

TOR forms two structurally and functionally distinct multiprotein complexes termed TOR complexes-1 and -2 (TORC1 and TORC2), of which only TORC1 is acutely sensitive to rapamycin
The two TOR complexes, like TOR itself, are conserved from yeast to humans, although TORC1 appears to be absent from ciliates and TORC2 from plants (Tatebe and Shiozaki, 2017; van Dam et al., 2011) (Fig. 1). In mammals, mTOR and the adaptor protein mLST8 (mammalian lethal with SEC13 protein 8) are common to both TOR complexes. RAPTOR (regulatory-associated protein of TOR) is the defining subunit of mTORC1, whereas RICTOR (rapamycin-insensitive companion of mTOR) and mSIN1 (stress-activated MAP kinase interacting protein 1) define mTORC2.

The domain organization of TOR is also conserved. The C-terminal half of TOR contains a FAT (FRAP, ATM, and TRRAP) domain followed by the FRB (FKBP-rapamycin binding) domain, the catalytic kinase domain, and a C-terminal FAT domain termed FATC. Structural biologists often refer to the FAT, FRB, kinase and FATC domains collectively as the FATKIN region (Baretić et al., 2016; Imseng et al., 2018) (Fig. 4). FATKIN regions are found in all PIKK family members, although only the FRB domain in TOR binds the FKBP-rapamycin complex. All PIKKs contain long, N-terminal extensions that serve as docking surfaces for binding partners. The N-terminal half of TOR consists of tandem arrays of HEAT (Huntingtin, Elongation Factor 3, PP2A, andTOR) and TPR (tetratricopeptide) repeats. The HEAT repeats of mTOR bind RAPTOR (Hara et al., 2002; Kim et al., 2002), which also has several characteristic regions: the RAPTOR N-terminal conserved (RNC) CASPase-like domain, a central set of seven α-helical repeats termed the armadillo (ARM) domain, and a C-terminal seven-bladed WD40 β-propeller (Hara et al., 2002; Kim et al., 2002). By contrast, mLST8 is a small protein consisting entirely of a WD40 β-propeller.

**Structure of the mTORC1 complex**

TORC1 architecture was solved by a combination of X-ray crystallography and cryo-EM (cryo-electron microscopy) on truncated mTOR-mLST8 (Yang et al., 2013), RAPTOR from the fungus Chaetomium thermophilum (Aylett et al., 2016) or the plant *A. thaliana* (Yang et al., 2017), and TOR-Lst8 from the fungus *Kluyveromyces marxianus* (Baretić et al., 2016). These studies described mTORC1 at 4.4 Å (Yang et al., 2016) and 3.0 Å resolution (Yang et al., 2017), mTORC1 in complex with FKBP-rapamycin at 5.9 Å (Aylett et al., 2016), and mTORC1 bound to its activator RHEB at 3.4 Å (Yang et al., 2017).

mTORC1 is a 1 MDa homodimer of heterotrimerers (each of the latter containing mTOR,
RAPTOR and mLST8) that adopts a rhomboidal (lozenge) shape with a large central cavity (Fig. 4). It exhibits two-fold (C2) symmetry with the axis of symmetry passing through the central cavity. The FATKIN region of each of the two copies of mTOR forms a compact unit located near the central cavity, on opposite sides of the C2 axis. The two FATKIN regions come close to each other but make little or no contact. Each kinase site is located at the bottom of a deep catalytic cleft that is partly obscured by surrounding structural elements, suggesting that the kinase activity is regulated by physically restricting access to the catalytic site (Yang et al., 2017; Yang et al., 2013). The HEAT repeats of each mTOR subunit form two distinct helical solenoids, one a low curvature bridge/M-HEAT (hereafter referred to as the “bridge”) and the second a high curvature horn/spiral/N-HEAT (hereafter referred to as the “horn”) peripherally linked to the bridge (Aylett et al., 2016; Baretić et al., 2016; Yang et al., 2017). The horn of one copy of mTOR packs against the bridge of the other to mediate dimerization and form the central cavity. The two-fold symmetry is likely conserved among TORC orthologs because: (i) there is a high degree of conservation throughout the HEAT repeat region of TOR; and (ii) TOR from K. marxianus (Baretić et al., 2016) and humans (Aylett et al., 2016) are architecturally identical. The horn and bridge, in addition to forming the dimer interface, are exposed, suggesting an additional role in binding regulatory or accessory proteins. mLST8 binds to the kinase domain of mTOR and thereby constitutes the ends of the short axis of the mTORC1 rhomboid. RAPTOR has an extended Z-like shape with the RNC domain and WD40 β-propeller located at opposite ends, connected by the ARM domain (Aylett et al., 2016; Yang et al., 2017). RAPTOR also contributes to the mTORC1 dimer interface, because the ARM domain of one RAPTOR binds the horn of one mTOR molecule and the bridge of the other, thereby linking the two copies of mTOR. The RAPTOR β-propeller domains are at the ends of the long axis of mTORC1.

Importantly, RAPTOR is also required for mTORC1 substrate recruitment. The region in RAPTOR responsible for substrate binding is in a cleft between the RNC and the ARM domains, located ≈65 Å from the catalytic site (Fig. 4) (Yang et al., 2017), via which RAPTOR binds a sequence of five amino acids termed the TOS (TOR signaling) motif. The TOS motif is defined as FXΦ[E/D]Φ, where Φ is a hydrophobic residue and X any residue (Gouw et al., 2018; Nojima et al., 2003; Schalm and Blenis, 2002; Yang et al., 2017). TOS motifs are present in some TORC1 substrates, such as ribosomal protein S6 kinase (S6K; TOS motif FDIDL) and eukaryotic
translation initiation factor 4E binding protein (4EBP; TOS motif FEMDI) (Nojima et al., 2003; Schalm and Blenis, 2002; Schalm et al., 2003). However, the mTORC1 substrates ULK1 (Dunlop and Tee, 2013) and TFEB (transcription factor EB) (Roczniak-Ferguson et al., 2012; Settembre et al., 2012) interact with RAPTOR yet lack an obvious TOS motif. Furthermore, although the TOS-binding region of RAPTOR is highly conserved from yeast to mammals, TORC1 substrates in lower eukaryotes seem to lack TOS motifs, so that it is unclear how TORC1 recognizes its substrates in those organisms.

Inhibition of TOR by rapamycin depends on the formation of a complex between rapamycin and the cytoplasmic immunophilin FKBP12 (FK506-binding protein of 12 KDa) (Benjamin et al., 2011). An FKBP-rapamycin complex binds the FRB domain at the lip of the TOR catalytic cleft, forming a lid that physically prevents access of substrates to the catalytic site. FKBP-rapamycin does not induce a conformational change in mTOR, suggesting that FKBP-rapamycin indeed acts by obstructing substrate access (Aylett et al., 2016; Yang et al., 2017; Yang et al., 2013). TORC2 is not acutely inhibited by rapamycin, because the FKBP-rapamycin binding site in the TOR FRB domain in TORC2 is masked by RICTOR (Chen et al., 2018; Gaubitz et al., 2015; Karuppasamy et al., 2017; Stuttfeld et al., 2018). Cryo-EM studies have resolved S. cerevisiae (Karuppasamy et al., 2017) and human (Chen et al., 2018; Stuttfeld et al., 2018) TORC2 at intermediate resolution. The two mTOR complexes share many features, including C2 symmetry, similar binding sites for RAPTOR and RICTOR, and a deep catalytic cleft. However, full structural interpretation of mTORC2 awaits higher resolution structural data.

**Regulation of mTORC1 by lysosomal recruitment and growth factors**

TOR controls cell growth and metabolism in response to nutrients, growth factors, and (in part through AMPK) cellular energy status. Nutrients, especially amino acids, are likely to be the ancestral TORC1 activating inputs, as they are sufficient to activate TORC1 in unicellular organisms such as yeast. However, in multicellular organisms, TORC1 activation requires additional input from growth factors. Mechanistically, amino acid and growth factor inputs converge on mTORC1 as follows: (i) amino acids stimulate translocation of mTORC1 from the cytosol to the lysosome where it encounters the small G protein RHEB (RAS homologue enriched in brain), and (ii) growth factors activate lysosomal RHEB, enabling it to activate mTORC1 in turn
Amino acid availability is transmitted to TORC1 mainly via the RAGs (Ras-related family of small GTPases) (González and Hall, 2017; Nicastro et al., 2017; Wolfson and Sabatini, 2017) (Fig. 5). There are four RAGs in mammals (RAGA through RAGD) and two in *S. cerevisiae* (Gtr1 and Gtr2) that form obligate heterodimers of RAGA or RAGB with RAGC or RAGD, and Gtr1 with Gtr2. RAGs are attached to the lysosome in mammalian cells through the pentameric Ragulator complex (Bar-Peled et al., 2012; Sancak et al., 2010), while the Gtr1-Gtr2 heterodimer is attached to the vacuole in yeast through the trimeric Ego complex (Kogan et al., 2010; Levine et al., 2013; Powis et al., 2015; Zhang et al., 2012). Clearly, the lysosome or vacuole is the TORC1 signalling hub in all eukaryotic cells. Amino acid sufficiency promotes the TORC1-activating conformation of the RAG-Gtr heterodimer (RAGA/B or Gtr1 loaded with GTP, and RAGC/D or Gtr2 loaded with GDP). In mammals, the active RAG heterodimer binds RAPTOR and thereby recruits mTORC1 from the cytosol to the lysosomal surface, while in budding yeast TORC1 is constitutively bound to the vacuolar surface and the active Gtr1-Gtr2 heterodimer binds Kog1 (yeast ortholog of RAPTOR) to stimulate TORC1 via an unknown mechanism (Binda et al., 2009). From yeast two-hybrid experiments, it has been proposed that a region of Kog1 comprising amino acids 777-814 in the central ARM domain, interacts with Gtr1 (Sekiguchi et al., 2014). The region in Kog1 is conserved in RAPTOR (amino acids 777-814 in Kog1 correspond to amino acids 595-632 in RAPTOR). Consistent with this, recent structural analyses of RAGAGTP-RAGCGDP in complex with mTORC1 (Anandapadamanaban et al., 2019) or with RAPTOR-Ragulator (Rogala et al., 2019) revealed that the region in RAPTOR comprising amino acids 546-650 binds RAGAGTP. Two additional regions of RAPTOR, located between the ARM and WD40 β-propeller domains, interact with RAGCGDP (Rogala et al., 2019). One region comprises amino acids 795-806 and the other amino acids 916-937. The last has been referred to as the “RAPTOR claw” due to its shape (Rogala et al., 2019). Interestingly, it has been suggested that the stress-activated MAP kinase-related kinase NLK (Nemo-Like Kinase) phosphorylates RAPTOR at Ser863 thereby disrupting RAG-RAPTOR interaction and inhibiting mTORC1 (Yuan et al., 2015). Ser863 is in a structurally unsolved and thus presumably disordered linker region (residues 841 to 949) between the ARM and WD40 β-propeller domains that contains several phosphorylation sites (Foster et al., 2010; Wang et al., 2009) (Fig. 4) (see below).
The nucleotide binding status of the RAGs is tightly regulated by conserved GAPs (GTPase activator proteins) and GEFs (guanine nucleotide exchange factors) (González and Hall, 2017; Nicastro et al., 2017; Wolfson and Sabatini, 2017) (Fig. 5). The heterotrimeric GATOR1 (GAP activity toward RAGs-1) complex is the GAP for RAGA/B, and thus negatively regulates mTORC1 activity (Bar-Peled et al., 2013; Panchaud et al., 2013a; Shen et al., 2018; Shen et al., 2019).

GATOR1 is tethered to the lysosomal surface by KICSTOR (KPTN, ITFG2, C12orf66, and SZT2-containing regulator of mTORC1) (Peng et al., 2017; Wolfson et al., 2017). The heteropentameric GATOR2 complex activates mTORC1 by binding and negatively regulating GATOR1 via an undefined mechanism (Bar-Peled et al., 2013; Panchaud et al., 2013b). The lysosomal amino acid transporter SLC38A9 (Jung et al., 2015; Rebsamen et al., 2015; Wang et al., 2015b; Wyant et al., 2017) acts as a GEF for RAGA (Shen and Sabatini, 2018). The Ragulator complex, which was initially described as the GEF for RAGA/B (Bar-Peled et al., 2012), is now proposed instead to activate mTORC1 by accelerating the release of GTP from RAGC (Shen and Sabatini, 2018), while the identity of the GEF for RAGC/D remains unclear. FLCN (folliculin) together with its binding partners FNIP1 and FNIP2 (folliculin-interacting protein 1 and 2) has been identified as the GAP for RAGC/D, and thus positively regulates mTORC1 (Petit et al., 2013; Tsun et al., 2013).

Upon amino acid starvation, the RAG heterodimer assumes an inactive configuration (RAGA/B loaded with GDP and RAGC/D with GTP) that is unable to recruit mTORC1 to the lysosomal surface, so that mTORC1 remains cytosolic and inactive. It has been proposed also that the “inactive” conformation of the RAG heterodimer recruits TSC2 (tuberous sclerosis complex 2) to the lysosome to inhibit mTORC1 (Demetriades et al., 2014; Demetriades et al., 2016; Menon et al., 2014). In budding yeast, glucose withdrawal triggers a Gtr-dependent formation of a vacuole-associated cylindrical filament of TORC1 molecules, termed a TOROID (TORC1 organized in inhibited domains). TOROID formation leads to TORC1 inactivation, and low-resolution cryo-EM reconstructions suggest that this oligomerization causes steric occlusion of the TORC1 active site (Prouteau et al., 2017). It is not known whether mTORC1 forms TOROID-like structures.

As discussed in the introduction to this review, it is thought that growth factor signalling co-evolved with multicellularity, at which time it was grafted onto the ancestral nutrient-activated TORC1 signalling pathway (Ben-Sahra and Manning, 2017; Guri and Hall, 2016; Kim and Guan, 2019). Growth factors such as insulin bind to RTKs (receptor tyrosine kinases) to activate PI3K
(phosphatidylinositol-4,5-bisphosphate 3-kinase) thereby generating PIP\(_3\) (phosphoinositide 3, 4, 5-trisphosphate) (Fig. 5). PIP\(_3\) then co-recruits PDK1 (phosphoinositide-dependent kinase-1) and AKT via their PIP\(_3\)-binding PH (pleckstrin homology) domains to the plasma membrane, where PDK1 phosphorylates Thr308 in the activation loop of AKT. Activated AKT in turn phosphorylates TSC2 on multiple sites to induce the release of the heterotrimeric TSC complex from the lysosome (Inoki et al., 2002; Menon et al., 2014). The TSC complex consists of TSC1, TSC2, and TBC1D7, and acts as a GAP towards RHEB (Dibble et al., 2012). Reduced TSC complex GAP activity at the lysosome leads to an increase in activated, GTP-loaded RHEB, which then binds the N-terminus and FAT domain of mTOR to allosterically realign residues in the catalytic site and activate mTORC1 (Chao and Avruch, 2019; Long et al., 2005; Yang et al., 2017).

**Amino acid sensors**

Leucine, arginine and glutamine are among the most effective amino acids for activation of mTORC1 (Fig. 5). The identity of the amino acid sensors upstream of TORC1 has begun to emerge recently (Wolfson and Sabatini, 2017). The cytoplasmic proteins SESTRIN2 (Chantranupong et al., 2014; Kim et al., 2015; Parmigiani et al., 2014; Saxton et al., 2016b; Saxton et al., 2016c; Wolfson et al., 2016) and CASTOR (cellular arginine sensor for mTORC1) (Chantranupong et al., 2016; Saxton et al., 2016a; Xia et al., 2016) bind and transmit the availability of leucine and arginine, respectively, to mTORC1 via the GATOR complexes. Under conditions of leucine and arginine deprivation, SESTRIN2 and CASTOR1 bind and most likely inhibit GATOR2 upstream of mTORC1. However, growth-promoting levels of leucine and arginine disrupt the interactions of SESTRIN2 and GATOR2 (Wolfson et al., 2016) and CASTOR1 and GATOR2 (Saxton et al., 2016a); this releases free GATOR2 and thereby activates mTORC1 (Fig. 5). SESTRINs may also inhibit mTORC1 by activating AMPK (Lee et al., 2016). However, budding yeast lacks SESTRIN and CASTOR orthologs (Wolfson and Sabatini, 2017). Whether and, if so, how arginine or leucine availability is transmitted to TORC1 in organisms lacking these proteins is not known. Leucine and glutamine can also activate mTORC1 via glutaminolysis and consequent production of \(\alpha\)-ketoglutarate upstream of RAGs (Duran et al., 2013; Durán et al., 2012), while glutamine also activates mTORC1 independently of the RAGs via the small GTPase ARF1 and the v-ATPase (Jewell et al., 2015).
It has been reported that LeuRS (leucyl-tRNA synthetase) acts as a cytoplasmic leucine sensor to activate mTORC1 via a RAG-independent mechanism. Leucine-bound LeuRS binds and activates the class III phosphoinositide kinase VPS34 that is present in non-autophagic structures. Active VPS34 stimulates PLD1 (phospholipase D1) thereby increasing phosphatidic acid levels which promote lysosomal activation of mTORC1 (Yoon et al., 2016; Yoon et al., 2011).

In some cell types, such as epithelial, glial and mesenchymal stem cells, leucine can activate mTORC1 via production of acetyl-CoA. Acetyl-CoA stimulates the acetyl transferase EP300 to acetylate RAPTOR at Lys1097, thereby promoting mTORC1 activity (Son et al., 2019). The acetylated residue is located in the WD40 β-propeller of RAPTOR, close to the ARM domain (Fig. 4). It is unclear whether RAPTOR acetylation affects mTORC1 structure.

Finally, methionine signals to mTORC1 through synthesis of the methyl donor SAM. SAM availability is transmitted to mTORC1 via SAMTOR (SAM sensor upstream of mTORC1), with SAM inhibiting the interaction between SAMTOR and GATOR1, thereby activating mTORC1 (Gu et al., 2017a).

**Downstream targets of mTORC1**

TOR promotes cell growth by stimulating anabolic processes such as ribosome biogenesis and protein, lipid, and nucleotide synthesis, while repressing catabolic processes such as autophagy (Ben-Sahra and Manning, 2017; Saxton and Sabatini, 2017; Shimobayashi and Hall, 2014).

mTORC1 promotes protein synthesis by phosphorylating: (i) S6K at Thr389 in its hydrophobic motif, to increase translation initiation and elongation, and: (ii) 4EBP, to promote cap-dependent translation. mTORC1 also induces purine synthesis via the tetrahydrofolate cycle (Ben-Sahra et al., 2016) and pyrimidine synthesis by phosphorylating and activating CAD (carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase) via S6K (Ben-Sahra et al., 2013; Robitaille et al., 2013). Furthermore, mTORC1 promotes lipogenic gene expression by activating the SREBP (sterol-regulatory element-binding protein) transcription factor (Ben-Sahra and Manning, 2017). mTORC1 also inhibits autophagy by phosphorylating the autophagy-inducing kinase ULK1 (Kim & Guan, 2011) and TFEB (transcription factor EB) (Martina et al., 2012; Rocznia-Ferguson et al., 2012; Settembre et al., 2012). Phosphorylated TFEB remains cytosolic and inactive, thus failing to induce expression of genes required for autophagy and lysosome...
biogenesis (Puertollano et al., 2018a) (Fig. 5).

S6K has several substrates, including ribosomal protein S6 and insulin receptor substrate 1 (IRS1). Phosphorylation of IRS1 by S6K inhibits IRS1, thereby forming a negative feedback loop acting on PI3K and mTORC2 (Shimobayashi and Hall, 2014). mTORC2 regulates cytoskeletal remodeling, proliferation, and survival by phosphorylating and activating AGC kinase family members such as AKT at Ser473, PKC (protein kinase C) and SGK (serum/glucocorticoid-regulated kinase) (Guri and Hall, 2016).

**Yin-Yang: regulation of mTORC1 by AMPK**

If the energy status of cells is compromised, it would not be a sensible idea for them to grow or divide, even if nutrients were still available. It therefore makes sense that AMPK should switch off mTORC1. Indeed, AMPK activation switches off the mTORC1 complex by twin mechanisms:

1. AMPK phosphorylates TSC2 at Thr1271 and Ser1387 (residue numbering from human isoform 1 (NP_000539); these sites are referred to as Thr1227 and Ser1345 in the original paper (Inoki et al., 2003)). Mutation of these two sites was found to reduce the ability of the glycolytic inhibitor 2-deoxyglucose to inhibit S6K and 4EBP phosphorylation. This phosphorylation is sometimes assumed to promote the GAP activity of the TSC complex toward RHEB, although this has not been directly demonstrated.

2. AMPK directly phosphorylates the RAPTOR component of mTORC1 at two sites, Ser722 and Ser792. Once again, mutation of these two sites was found to reduce the ability of the AMPK activators, AICAR or phenformin, to inhibit S6K and 4EBP phosphorylation (Gwinn et al., 2008), although the detailed mechanism for this inhibitory effect remains unclear. Ser722 and Ser792 lie in a structurally uncharacterised, and likely disordered, region within the RAPTOR ARM domain (residues 687-805) (Fig. 4) - note that some publications incorrectly place Ser792 in the RAPTOR β-propeller. Curiously, PKA (cyclic AMP-dependent protein kinase) phosphorylates RAPTOR on Ser791, but not Ser792, and is reported to either inhibit (Jewell et al., 2019) or activate (Liu et al., 2016) mTORC1 - the reasons for this discrepancy are not clear.

These mechanisms may be at least partly conserved across eukaryotes. Although there appear to be no direct orthologs of TSC2 in either budding yeast or plants, there is evidence that phosphorylation of the RAPTOR orthologs in *S. cerevisiae* (Hughes Hallett et al., 2015) and plants...
(Nukarinen et al., 2016) also leads to inactivation of TORC1 in those organisms. While these effects were dependent upon the AMPK orthologs, neither of the two well-defined sites for AMPK in mammalian RAPTOR (Gwinn et al., 2008) are conserved in S. cerevisiae, and only one is conserved in plants. The detailed mechanisms for these effects may therefore be different.

These results therefore show that activation of mammalian AMPK inhibits mTORC1 via two mechanisms, equivalent to the fail-safe method of using both “belt and braces” to hold up one’s pants! A major effect of mTORC1 activation is to promote translation, particularly of mRNAs encoding proteins required for rapid cell growth, including ribosomal proteins. Since protein synthesis accounts for as much as 20% of total energy turnover in rapidly growing cells (Buttgereit and Brand, 1995), switching it off would have a major effect to conserve energy.

Although it is therefore clear that AMPK inhibits mTORC1, very recently it has been reported, rather counter-intuitively, that it activates mTORC2 (Kazyken et al., 2019). Treatment of serum-deprived mouse embryo fibroblasts, HEK293 cells or primary mouse hepatocytes with AMPK activators such as AICAR, biguanides or A-769662 was found to increase phosphorylation of the mTORC2 site on AKT, Ser473. Although these activators all have known “off-target” (i.e. AMPK-independent) effects, and more specific AMPK activators are now available, their effects were reduced, although not eliminated, in cells with AMPK knocked out or knocked down, suggesting that they were at least partly mediated by AMPK. The effects were associated with phosphorylation of Ser1261 on mTOR and unidentified site(s) on RICTOR, although Ser1261 phosphorylation did not appear to be required for enhanced phosphorylation of AKT. The authors proposed that the activation of mTORC2 by AMPK represents part of the mechanism by which the latter increases cell survival during energetic stress, and in some circumstances may therefore paradoxically promote tumorigenesis (Kazyken et al., 2019).

In addition, there seems to be a dual “belt and braces” system to turn off mTORC1 when cells are facing shortage of glucose supply. Besides the above-mentioned mechanisms involving phosphorylation of mTORC1-related factors by AMPK, glucose deprivation can inactivate mTORC1 independently of AMPK. Mutations of RAGA/B that abolish GTPase activity completely abrogated inhibition of mTORC1 by glucose starvation, despite intact activation of AMPK, suggesting that RAGs or RAG-interacting partners may play a more direct role in controlling mTORC1 in response to nutrients (Efeyan et al., 2013; Kalender et al., 2010). Indeed, in low
glucose AXIN translocates to the surface of the lysosome and interacts with the v-ATPase and Ragulator, thereby facilitating the release of mTORC1 from the lysosomal surface (Zhang et al., 2014). Additional evidence for AMPK-independent regulation is that mTORC1 suppression after glucose starvation occurs several hours later in AXIN-null compared to AXIN-wild type cells in which AMPKα1/α2 had been knocked out (Zhang et al., 2014). This additional device highlights the importance of inhibiting mTORC1 when glucose is absent.

**Antagonistic effects of AMPK and mTORC1 on autophagy and lysosome biogenesis**

Autophagy, of which mitophagy (discussed above) is a special case, is the process by which cellular contents that are surplus to requirements are engulfed into lysosomes where they are broken down to recycle their components for catabolism or re-use. By phosphorylating the autophagy-initiating kinase ULK1 at distinct sites, AMPK activates while mTORC1 inhibits autophagy (Egan et al., 2011b; Kim et al., 2011). AMPK can therefore promote autophagy not only by direct phosphorylation of ULK1, but also indirectly by inactivating mTORC1 via mechanisms discussed in the previous paragraph.

One key downstream target of ULK1 is BECLIN-1, which forms a complex with VPS34, a class III phosphoinositide kinase that generates phosphoinositide-3-phosphate (PI3P) on intracellular membranes. PI3P recruits to those membranes proteins containing PI3P-binding domains, which mediate subsequent membrane-trafficking events. VPS34 occurs in several distinct complexes; AMPK appears to activate complexes involved in autophagy by phosphorylating BECLIN-1, while inhibiting those involved in other membrane-trafficking events by phosphorylating VPS34 itself; this switch depends on the presence of ATG14L in the former complex (Kim et al., 2013). Thus, AMPK may divert membrane traffic (an energy-requiring process) toward the autophagy/mitophagy pathway and away from other trafficking events that might be a luxury in cells experiencing glucose starvation or energy stress.

As well as their acute effects on autophagy, in the longer term AMPK and mTORC1 also act antagonistically via effects on the related transcription factors EB and E3 (TFEB and TFE3), which induce genes involved in lysosome biogenesis and autophagy. mTORC1 directly phosphorylates TFEB and TFE3, and this promotes their retention in the cytoplasm, inhibiting their transcriptional
functions (Puertollano et al., 2018b). By contrast, AMPK activation promotes dephosphorylation and nuclear translocation of TFEB, an effect that appears to be at least partially independent of mTORC1 (Collodet et al., 2019). One possible mechanism for increased transcription at TFEB/TFE3-regulated promoters in response to AMPK activation is the increased expression of CARM1 (coactivator-associated arginine methyltransferase-1) due to down-regulation of a E3 ubiquitin ligase containing SKP2 (S-phase kinase-associated protein-2) (Shin et al., 2016). Another transcription factor, FOXO3a, is phosphorylated by AMPK at several sites (Greer et al., 2007), and this enhances its ability to repress SKP2 expression. The final link in this proposed chain of events is that CARM1 is recruited to promoters of genes involved in autophagy and lysosome biogenesis by TFEB, leading to methylation of Arg17 on histone H3 and consequent activation of transcription at those sites (Shin et al., 2016).

**Yang-Yin: regulation of AMPK by TORC1 and/or upstream pathways**

There is one report that rapamycin treatment of budding yeast, in wild type strains but not in strains expressing a TOR1 mutation that confers rapamycin resistance, increases phosphorylation of Thr210 in Snf1 (equivalent to Thr172 in mammalian AMPK) (Orlova et al., 2006). Despite this, neither rapamycin nor the catalytic site inhibitor of mTOR, Torin1, affected AMPK activity in mouse embryonic fibroblasts (Zhang et al., 2014), and at this time there is no well-established direct mechanism by which AMPK is regulated by mTORC1. However, AMPK can be down-regulated by the upstream insulin signalling pathway that activates mTORC1. The insulin-stimulated protein kinase, AKT, phosphorylates Ser496 (human numbering, Q13131) in the α1 catalytic subunit of AMPK (Horman et al., 2006), and this down-regulates (while not completely abolishing) AMPK signalling by inhibiting the phosphorylation of Thr172 by LKB1 (Hawley et al., 2014). Ser496 in AMPK-α1 can also be phosphorylated by PKC (Heathcote et al., 2016), and PKA (Hurley et al., 2006). Ser496 occurs in a serine/threonine rich sequence just prior to the C-terminal α-helix of AMPK-α1 that has been termed the “ST loop” (Fig. 2). A similar sequence is present in the α2 isoform, although in that case the serine residue equivalent to Ser496 (Ser491) is a poor substrate for AKT and appears to be modified by autophosphorylation instead (Hawley et al., 2014) (it should therefore not be assumed, as is often done, that the regulation of the two isoforms by ST loop phosphorylation is identical). Relevant to this, Ser491 in AMPK-α2 has been reported to be
phosphorylated by S6K1 (Dagon et al., 2012), which is interesting because the latter is phosphorylated and activated by mTORC1. However, it is puzzling why there was no phosphorylation of Ser491 in the absence of S6K1 in this study (Dagon et al., 2012), when others have observed that Ser491 in α2 complexes undergoes rapid autophosphorylation (Hawley et al., 2014).

The ST loop may be subject to multisite phosphorylation, because GSK3 has been reported to phosphorylate sequentially within the ST loop of α1 at Thr490, Ser486 and Thr482 (human numbering, Q13131), which was proposed to promote Thr172 dephosphorylation (Suzuki et al., 2013). Interestingly, the ST loop is also present in AMPK-α orthologs from *C. elegans* and vertebrates but is absent in those from *D. melanogaster* and *S. cerevisiae*, suggesting that it is a regulatory sequence that has been inserted during evolution. In the currently available crystal structures of mammalian heterotrimeric AMPK, the ST loop has either been deliberately deleted or is not resolved. However, the residues at either end of the missing loop lie just 20 and 40 Å from Thr172, suggesting that, once phosphorylated, the loop might interact with the kinase domain and physically block access to Thr172 (Fig. 2). Indeed, there is experimental support for this model (Hawley et al., 2014).

Another potential “Yang-Yin” interaction involves the phosphorylation of AMPK by ULK1, the autophagy-regulating kinase that is inactivated/activated by phosphorylation at distinct sites by mTORC1/AMPK respectively (Egan et al., 2011a). ULK1 has been reported to phosphorylate Ser108 on AMPK-β1 but not -β2 (Dite et al., 2017). Phosphorylation of Ser-108 is known to stabilize the ADaM site (see above) by interacting with conserved threonine and lysine residues on the N-lobe of the α subunit kinase domain (Calabrese et al., 2014; Xiao et al., 2013), and is required for allosteric activation of AMPK by ADaM site ligands both with purified AMPK (Scott et al., 2014) and in intact cells (Dite et al., 2017). However, understanding the significance of this requires further study, partly because Ser108 is also rapidly modified by AMPK itself by cis-autophosphorylation (Scott et al., 2014), and partly because the natural ligands that bind to the ADaM site, if they exist, have not yet been identified.

**Conclusions and Perspectives**

We have argued in this review that the AMPK and TOR pathways arose very early during...
eukaryotic evolution and may have been required to regulate cell growth in response to the availability of the energy or nutrients provided by some of the newly acquired subcellular compartments, such as mitochondria or lysosomes/vacuoles. The recent findings that lysosomes/vacuoles represent key hubs for nutrient sensing by both AMPK and TOR may reflect the fact that early unicellular eukaryotes utilized phagocytosis or pinocytosis for feeding, with nutrients being delivered initially to lysosomes or the vacuole, which in a unicellular eukaryote can therefore be considered to be equivalent to the gut. Just as the gut (and associated endocrine pancreas) of multicellular animals has become a hub for nutrient sensing and signaling, so perhaps did the lysosome or vacuole of unicellular eukaryotes.

AMPK can be regarded as representing the Yin or “dark” side of growth control that is activated by lack of energy or nutrients and switches off cell growth, while TOR represents the Yang or “bright” side that is activated by availability of nutrients and promotes cell growth. In general, TOR pathways promote anabolic activities, while AMPK pathways exercise a brake on them. These pathways clearly act in opposition to each other and it is not surprising, as discussed in this review, that there are complex interactions between them. As in Taoist philosophy, the exquisite balance between Yin and Yang ultimately ensures homeostasis and a healthy cell or organism.
Figure legends

Figure 1: Conservation of TOR and AMPK signalling components among eukaryotic species. Black boxes indicate presence, and white boxes absence, of the indicated genes/proteins in the corresponding organisms (Tatebe and Shiozaki, 2017; van Dam et al., 2011). Gray boxes indicate limited similarity to the human counterpart. There is no evidence that the S. cerevisiae Rheb regulates TORC1.

Figure 2: Schematic view of the structure of the AMPK heterotrimer. The diagram is a composite derived from the structures of the human α2β1γ1 (Xiao et al., 2013), α1β1γ1 (Calabrese et al., 2014) and α1β2γ1 (Li et al., 2015) complexes, and is an active conformation with Thr172 phosphorylated and three molecules of AMP bound to the γ subunit. The α subunit is shown in yellow (apart from the ST loop, in red), the β subunit in lilac and the γ subunit in blue. The α-linker is depicted as a yellow chain connecting the α-AID and the α-CTD, and it contacts AMP bound in the CBS3 site. The ST loop is not resolved in any of the structures and its exact positioning is speculative. The N-terminal regions of the β subunits, and the linker between the β-CBM and the β-CTD, (not shown) are either absent or are not resolved in any of the structures.
**Figure 3:** **Canonical and non-canonical mechanisms of AMPK activation.** Proteins shown in green promote activation of AMPK, while proteins shown in red promote inhibition (aldolase is a positive effector when unoccupied by FBP). Canonical activation by energy stress requires LKB1, occurs in the cytoplasm and is triggered by increases in AMP:ATP or ADP:ATP ratios. By contrast, non-canonical activation by glucose starvation involves translocation of AXIN:LKB1 to the lysosome, where a pool of AMPK myristoylated on the β subunit resides permanently, and can occur in the absence of any changes in adenine nucleotides. Non-canonical activation by Ca$^{2+}$ ions released from the ER or within the nucleus, triggered by hormones or DNA damage respectively, requires CaMKK2 and not LKB1. Note that the localized increase in Ca$^{2+}$ caused by activation of TRPV channels is not sufficient to activate CaMKK2. See main text for details.

**Figure 4:** **Human mTORC1 architecture.** A) Linear representation of the domain organization of mTOR, RAPTOR, and mLST8. The residue numbers indicate the domain boundaries. Grey areas in RAPTOR indicate regions presumed to be disordered linkers, comprising amino acids 687-805 and 841-949. B) Cryo-EM derived model of human mTORC1 (PDB: 6BCX) (Yang et al., 2017), with domains colored according to the primary structure scheme in A. Key residues for mTORC1 activation at the catalytic site (Asp2338, His2340, Asn2343, and Asp2357 (Yang et al., 2013)) are highlighted in red, while the two copies of the TOS peptide of 4EBP are shown in purple. A gray line indicates the RAG binding region. Gray dashed lines represent the two disordered linker regions in RAPTOR. AMPK, PKA and NLK phosphorylate RAPTOR at Ser722 plus Ser792, Ser791 and Ser863 respectively. EP300 acetylates RAPTOR at Lys1097 (residue highlighted in magenta). RHEB binds the N-terminus and FAT domain of mTOR, distal to the catalytic site (not shown). See main text for details.
Figure 5: Cross-talk between mTORC1 and AMPK signalling pathways in mammals.

Proteins shown in green promote activation of mTORC1 (blue box), while proteins shown in red promote its inhibition. Inputs into mTORC1 from AMPK signaling are shown in gray, because AMPK and mTORC1 would not be simultaneously active. Dashed lines indicate indirect interactions. Amino acids and growth factors activate mTORC1, which then promotes cell growth by stimulating anabolic processes. Growth factor-stimulated PI3K activates mTORC2 (yellow box) by promoting its association with the ribosome. Active mTORC2 then promotes cell proliferation and survival. See main text for details.

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Toyama, E.Q., Herzig, S., Courchet, J., Lewis, T.L., Jr., Loson, O.C., Hellberg, K., Young, N.P.


Figure 1

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Figure 2

- α subunit
- β subunit
- γ subunit

- CBS2
- CBS4
- CBS3
- CBS1

- α-subunit
- β-subunit
- γ-subunit

- α-linker
- β-linker
- γ-linker

- AMP in CBS1
- AMP in CBS4
- AMP in CBS3 (under α-linker)

- glycogen-binding site
- catalytic site
- ADaM site

- possible position of phosphorylated ST loop, masking Thr172

- proposed movement of α-AID on binding of ATP to CBS3

- accessible from rear face of γ subunit
Figure 3

**Catabolic Processes**
- TXNIP
- TBC1D1
- PFKFB-2/-3
- ACC2
- ULK1

**Anabolic Processes**
- ACC1
- HMGR
- GYS1/2
- PRPS-1/-2

**DNA Damage Replicative Stress**
- DNA Fork Resection

**Hormones**
- TRPV
- IP3 receptor
- ER

**Energy Stress**
- AMP/ADP
- Ca2+

**Cytoplasm**
- LKB1
- AXIN

**Nucleus**
- CaMKK2
- Ca2+

**Lysosome**
- aldolase
- V-ATPase
- Ragulator

**LKB1**
- AMPK
- P
- T172

**Subset of AMPK Targets**
- TXNIP
- TBC1D1
- PFKFB-2/-3
- ACC2
- ULK1

**Energy Metabolism**
- GLUCOSE STARVATION
- FBP down

**Subcellular Localization**
- Cytoplasm
- ER
- Lysosome
- Nucleus
Figure 4

A

mTOR
1 933 1266 2549

Horn
Bridge
FATKIN

RAPTOR
1 400 1011 1335

RNC
ARM
WD40

mLST8
1 326

WD40

B

active site residues

RAG binding

TOS peptide

TOS peptide

Lys1097

Ser722

Ser791

Ser792

Ser863

P

P

P

P
Figure 5

**GROWTH FACTORS**

- IRS1
- PI3K
- PDK1
- AKT
- TSC

**ENERGY STRESS**

- AMPK
- Ca²⁺

**GLUCOSE STARVATION**

- AMPK

**AMINO ACIDS**

- Leu
- Arg
- Met
- SAM

**GLUTAMATE**

- Gln

**GTPases**

- RAGA/B
- RAGC/D
- KICSTOR
- GATOR1
- GATOR2
- CASTOR
- SESN2
- SAMTOR
- FLCN

**GTP**

- GTP
- RHEB

**PI3K**

- IRS1
- PI3K

**AKT**

- AKT
- S6K
- 4EBP
- ULK1
- TFEB

**mTORC1**

- RICTOR
- mSin1
- mLST8
- Raptor
- mTOR

**mTORC2**

- RICTOR
- mSin1
- mLST8
- mTORC2

**MAPK**

- RAS

**PKC**

- PKC

**SGK**

- SGK

**AMPK**

- AMPK
- P-P

**GROWTH FACTORS**

- GROWTH FACTORS

**ANABOLIC PROCESSES**

- anabolic processes (cell growth)

**CELL PROLIFERATION AND SURVIVAL**

- cell proliferation and survival

**GLUCOSE**

- glucose

**STARVATION**

- starvation

**GLUCOSE**

- glucose

**GLUCOSE**

- glucose

**GLUCOSE**

- glucose