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INVITED MINI-REVIEW

Adenosine Monophosphate-Activated Kinase (AMPK) and its Key Role in Catabolism: Structure, Regulation, Biological Activity and Pharmacological Activation.

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Running Title: AMPK - A Key Regulator of Catabolism.

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Abbreviations:

4EBP1- 4E-binding protein 1

ACC1- acetyl CoA carboxylase 1

AD- Alzheimer's disease

AICAR- 5-aminoimidazole-4-carboxamide riboside

AICART- aminoimidazole-carboxamide ribonucleotide formyl-transferase

AIS- auto-inhibitory regulatory sequence

AMPK- adenosine monophosphate activated protein kinase

AS160- Akt substrate 160

α-CTD-*C*-terminal β/γ- subunit interacting domain

α-RIM- regulatory-subunit-interacting module

β-CTD- *C*-terminal domain of the β-subunit

CaM- calmodulin

CaMK- calmodulin-dependent kinase

CaMKKβ- calmodulin-dependent kinase kinase β

CBM- carbohydrate binding motif

CBS- cystathionine-β-synthase

CPT1- carnitine palmitoyltransferase 1

EMT- epithelial to mesenchymal transition

FASN- fatty acid synthase

GLUT4- glucose transporter 4

HD- Huntington's disease

HMGCoA- hydroxymethylglutaryl-CoA

HMGCR- hydroxymethylglutaryl-CoA reductase

IL-1- interleukin-1

LDL- low-density lipoprotein

LKB1- liver kinase B1

MAPKKK- mitogen activated protein kinase kinase kinase

MO25- mouse protein 25

mTOR- mammalian target of rapamycin

mTORC1- mammalian target of rapamycin complex 1

NES- nuclear export doomain

p38 MAPK- p38 mitogen activated kinase

PD- Parkinson's disease

PPARγ- proliferator-activated receptor-γ

Rheb- Ras homolog enriched in brain

S6K- S6 kinase

SGLT- sodium dependent glucose co-transporter

SREBP1- sterol regulatory element-binding protein-1

STRAD- STE20-related pseudokinase

TAK1- TGF-β activated kinase 1

TGF-β- transforming growth factor-β

TLR- toll like receptor

TSC1/2- tuberous sclerosis complex 1/2

TZDs- thiazolidinediones

ULK1- unc-51 like kinase 1

WPW- Wolff-Parkinson White syndrome

ZMP- AICAR 5'-monophosphate

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Abstract

Adenosine monophosphate-activated protein kinase (AMPK) is a cellular energy sensor, which once activated, plays a role in several processes within the cell to restore energy homeostasis. The protein enhances catabolic pathways such as β-oxidation and autophagy to generate ATP, and inhibits anabolic processes that require energy including fatty acid, cholesterol and protein synthesis. Due to its key role in the regulation of critical cellular pathways, deregulation of AMPK is associated with the pathology of many diseases, including cancer, Wolff-Parkinson White syndrome, neurodegenerative disorders, diabetes and the metabolic syndrome. In fact, AMPK is a target of some pharmacological agents implemented in the treatment of diabetes (metformin and thiazolidinediones), as well as other naturally-derived products such as Berberine, used in traditional medicine. Due its critical role in the cell and the pathology of several disorders, research into developing AMPK as a therapeutic target is becoming a burgeoning and exciting field of pharmacological research. A profound understanding of the regulation and activity of AMPK would enhance its development as a promising therapeutic target.

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General Introduction

Adenosine monophosphate-activated protein kinase (AMPK) belongs to a family of highly conserved, heterotrimeric, serine-threonine kinases, with orthologs present in all eukaryotes (Kahn et al., 2005). AMPK is an energy sensor that is activated during cellular stress (Hardie, 2007). During low energy conditions, it increases catabolic processes to generate ATP and inhibits anabolic processes that require ATP (Hardie, 2007). The protein is activated through phosphorylation of Thr172 in the α-subunit by three main kinases under different stimuli, namely liver kinase B1 (LKB1) (Woods et al., 2003), calmodulin-mediated kinase kinase β (CaMKKβ) (Anderson et al., 2008; Hawley et al., 2005; Hurley et al., 2005; Woods et al., 2005) and transforming growth factor-β (TGF-β) activated kinase 1 (TAK1) (Momcilovic et al., 2006). Along with phosphorylation, AMPK also requires allosteric activation through AMP-binding to the γ-subunit (Suter et al., 2006). Once activated, AMPK stimulates β-oxidation (Hardie, 1989), glucose uptake (Kurth-Kraczek et al., 1999) and autophagy (Egan et al., 2011; Kim et al., 2011), while inhibiting cellular proliferation and the synthesis of fatty acids (Hardie and Pan, 2002), cholesterol (Habegger et al., 2012) and protein (Gwinn et al., 2008). Hence, AMPK is a critical enzyme involved in the regulation and integration of catabolic and anabolic pathways.

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Due to its central role in regulating multiple processes within the cell, AMPK deregulation is associated with several pathological conditions, including diabetes and other metabolic diseases (Hardie, 2011), cancer (Faubert et al., 2013), neurodegeneration (Lim et al., 2012; Lopez-Lopez et al., 2007) and cardiac hypertrophy (Gollob et al., 2001a). The key role of AMPK in these conditions makes it a promising therapeutic target and this is discussed in the final section of the review.

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Structure and Regulation of AMPK

Structure of AMPK

AMPK belongs to a family of serine-threonine protein kinase complexes that exist as a heterotrimer, composed of a catalytic subunit (AMPKα) and two regulatory subunits (AMPKβ and AMPKγ) (Fig. 1A, B) (Carling et al., 2008; Hardie, 2007). The AMPKα- and AMPKβ-subunits have two isoforms each (α1, α2 and β1, β2), and the AMPKγ-subunit exists as three different isoforms (γ1, γ2 and γ3) (Hardie, 2011) (Table 1). These isoforms of AMPK-subunits interact with each other, to form one of 12 possible heterotrimers in different tissues (Hardie, 2007).

AMPKα is the catalytic subunit of the protein (Hardie, 2007). Complexes containing the α1 isoform were shown to be predominantly expressed in the liver and adipose tissue, whereas the AMPKα2 complexes were localized to the brain, heart and skeletal muscle (O'Neill, 2013; Steinberg and Kemp, 2009). Furthermore, the AMPKα1 isoform has been shown to localize intracellularly in the cytosol, while the AMPKα2 isoform is primarily found in the nucleus (Salt et al., 1998). The *N*-terminal of the protein harbors the kinase domain that extends from residues 1-280, comprising of Thr172 in the activation loop, which is phosphorylated for kinase activity (Carling et al., 1994; Halford et al., 2003; Suter et al., 2006) (Fig. 1B). The *C*-terminal of the protein has the auto-inhibitory regulatory sequence (AIS) of ~70 residues next to the kinase domain (Chen et al., 2009), and a *C*-terminal β/γ-subunit interacting domain (α-CTD) extending from residues 395-550 (Fig. 1B) (Jiang and Carlson, 1997; Xiao et al., 2007). Furthermore, the final 20 residues at the *C*-terminal end were shown to be conserved across diverse species (Brenman and Temple, 2007), and it was recently identified as the nuclear export sequence (NES) (Kazgan et al., 2010) (Fig. 1B).

To date, little is known about the nuclear and cytoplasmic pools of AMPK and how its intracellular distribution is controlled. However, it has been shown that post-translational modifications of the AMPKβ1-subunit affect enzyme activity and cellular distribution of the trimeric protein (Oakhill et

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al., 2010; Oakhill et al., 2011; Warden et al., 2001). AMPKβ exists in two isoforms, the first isoform is AMPKβ1 which is a 270 amino acid (30 kDa) protein, whereas AMPKβ2 is a protein consisting of 272 amino acids (34 kDa). Both isoforms are 71% identical and differ mostly at the *N*-terminal region (Thornton et al., 1998). Additionally, there have been studies showing that the carbohydrate-binding module (CBM) of the β2 subunit binds more tightly to oligosaccharides when compared to the β1 subunit (Koay et al., 2010). One of the main reasons for this was shown to be a single threonine insertion at position 101 in AMPK β2 after Trp99 (Koay et al., 2010). The latter residue has been shown to be essential for oligosaccharide-binding and glycogen association *in vitro* (McBride et al., 2009; Polekhina et al., 2005), and insertion of Thr101 in the CBM region of β1 subunit resulted in an increased affinity for oligosaccharides (Koay et al., 2010).

Similar to AMPKα, the expression of AMPKβ varies in different tissues (Chen et al., 1999; Thornton et al., 1998; Winder, 2001). AMPKβ1 is predominantly expressed in liver, pancreas, kidney, brown fat and brain, while AMPKβ2 is expressed in cardiac and skeletal muscle (Chen et al., 1999; Thornton et al., 1998; Winder, 2001). Furthermore, studies have demonstrated that each AMPKβ isoform has distinct roles in different tissues (Dasgupta et al., 2012; Dzamko et al., 2010). AMPKβ1 was shown to have a role primarily in the liver, with AMPKβ1-null mice demonstrating reduced fasting gluconeogenesis and enhanced hepatic insulin sensitivity (Dzamko et al., 2010). In addition, decreased AMPKβ1 activity led to defective proliferation and differentiation, un-regulated apoptosis and neuronal loss in the brains of these mice (Dasgupta and Milbrandt, 2009).

On the other hand, AMPKβ2-containing complexes play a crucial role in regulating glucose, glycogen and lipid metabolism in skeletal muscle during metabolic stress (Dasgupta et al., 2012; Steinberg et al., 2010). Studies using AMPKβ2-null mice demonstrated that these animals showed hyperglycemia, glucose intolerance and insulin resistance and were unable to maintain muscle ATP levels during exercise (Dasgupta et al., 2012; Steinberg et al., 2010). The AMPKβ-subunit contains

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the CBM region (residues 75-157) (Jiang and Carlson, 1996, 1997; McBride et al., 2009) and the C-terminal domain of the β- subunit (β-CTD), near the *C*-terminal of the subunit at approximately residues 186-270 (Iseli et al., 2005) (Fig. 1B).

The CBM region in the β-subunit plays a role in the binding of glycogen to AMPK, and allows the protein to detect changes in glycogen levels in the cell (McBride et al., 2009). Additionally, this region is also involved in the interaction of the α-subunit kinase domain with the regulatory fragment, which consists of the *C*-terminal domains of the α and β-subunits and all domains of the γ subunit (Momcilovic et al., 2008; Xiao et al., 2011; Xiao et al., 2013). More recently, the CBM region was also shown to be involved in protecting AMPK from cellular phosphatases (Xiao et al., 2013), such as human protein phosphatase-2c α (PP2Cα) (Davies et al., 1995). In fact, the CBM region binds to the *N*-lobe of the kinase domain and is also connected to the regulatory fragment (Xiao et al., 2013). It has been suggested that an AMPK-activator, namely compound 991, promotes the interaction between the kinase and CBM region to protect active AMPK against dephosphorylation (Xiao et al., 2013). Furthermore, replacing ADP/AMP with ATP resulted in the displacement of the "α-hook" (discussed in detail below; also referred to as regulatory-subunit-interacting motif; α-RIM; residues $\alpha1_{359\text{–}365}$, $\alpha2_{365\text{-}371}$) (Chen et al., 2013; Xiao et al., 2013; Xin et al., 2013). Xiao et al speculated that this alteration leads to the dissociation of the kinase domain and CBM from the regulatory fragment (Xiao et al., 2013). In this form, the kinase is no longer allosterically activated and is susceptible to dephosphorylation, and thus, inactivation (Xiao et al., 2011; Xiao et al., 2013). The second domain, β-CTD, was shown to bind with both the α- and γ-subunits in mammalian cells and acts as an anchoring region (Iseli et al., 2005) (Fig. 1B). Furthermore, the *N*-terminal has a more variable sequence that regulates *N*-myristoylation, which is important for membrane-targeting and -binding (Oakhill et al., 2010) (Fig. 1B). This study also showed that this post-translational modification of the protein is crucial for AMPK activation *via* AMP-binding, as loss of the myristoyl group abolished AMP activation and reduced the extent of α-Thr172 phosphorylation (Oakhill et al., 2010).

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The AMPKγ-subunit acts as an allosteric activator of the AMPK complex (Hardie, 2011). It contains four tandem repeats of a structural module called the cystathionine-β-synthase (CBS) motif (Fig. 1B) (Bateman, 1997), which is involved in AMP/ADP-binding, a critical step in AMPK activation (Hardie, 2011). A recent study revealed that three of the four AMP-binding sites (*i.e.*, CBS) can be occupied by other nucleoside phosphates, such as ATP, ADP or AMP (Xiao et al., 2011). One of these sites (CBS site 4) was believed to have AMP permanently bound to it (Kemp et al., 2007), but more recent studies have shown that the AMP can be exchanged for an ATP at this site (Chen et al., 2012; Zhu et al., 2011). The two sites, namely CBS sites 1 and 3, that are sometimes referred to as exchangeable sites, bind AMP or ATP, and this allows the protein to respond to alterations in AMP/ATP levels (Oakhill et al., 2012; Xiao et al., 2007; Xiao et al., 2011). However, unlike AMP-binding, ATP-binding does not result in a conformational change of the protein structure (Carling et al., 1989; Xiao et al., 2011). AMP/ADP binds to the weaker of the two exchangeable sites (*i.e.*, CBS site 3) which interacts with the α-hook region (Xiao et al., 2011; Xiao et al., 2013). The Glu368$_{(\alpha 2\text{-hook})}$ residue has been shown to form salt bridges with Lys170(γ) and possibly Arg70(γ). These latter sites are suggested to mediate the initial signaling distinguishing nucleotide-binding (Xiao et al., 2011; Xiao et al., 2013). This interaction leads to restricted flexibility of the α-linker region (residues 300–370), promoting the interaction of the kinase domain with the regulatory fragment (Xiao et al., 2011; Xin et al., 2013). This conformation protects the activation loop from dephosphorylation by phosphatases (Xiao et al., 2011; Xiao et al., 2013). Alternatively, ATP-binding leads to a steric clash of the regulatory fragment with the α-hook, causing the α-hook to dissociate, and this increases the flexibility of the linker that promotes dissociation of the kinase domain (Xiao et al., 2011; Xiao et al., 2013). In this form, the activation loop is no longer protected by packing against the regulatory fragment and is accessible to attack by phosphatases (Xiao et al., 2011). Thus, the shift between AMP/ADP- or ATP-binding determines the change in conformation between dephosphorylation-sensitive and -insensitive states (Xiao et al., 2011).

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Regulation of AMPK Activity

The AMPK protein is activated by two crucial mechanisms. First, Thr172 in the activation loop of the α-subunit is phosphorylated by one of the three main upstream kinases: LKB1 (Woods et al., 2003), CaMKKβ (Anderson et al., 2008; Hawley et al., 2005; Hurley et al., 2005; Woods et al., 2005) and TAK1 (Momcilovic et al., 2006) (Fig. 2). Second, AMP-binding to the γ-subunit of AMPK leads to allosteric activation of AMPK (Carling et al., 2008) (Fig. 2).

LKB1 phosphorylates the Thr172 residue on the AMPKα-subunit (Hurley et al., 2005) during low cellular energy conditions and was found to be the key upstream kinase (Woods et al., 2003) (Fig. 2). Alternatively, activation by CaMKKβ occurs as a response to increased cytosolic calcium levels (Hawley et al., 2005) (Fig. 2). Furthermore, along with activating AMPK, AMP-binding also protects phosphorylated Thr172 from the activity of phosphatases (Davies et al., 1995) (Fig. 2). The mechanisms through which TAK1 activates AMPK are still uncharacterized (Momcilovic et al., 2006).

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Activation of AMPK by LKB1

The key upstream kinase of AMPK, namely LKB1, acts as a tumor suppressor in several cancers (Gan and Li, 2014) and is also mutated (loss-of-function) in Peutz-Jeghers syndrome (Hemminki et al., 1998) , with most of the subjects carrying just one of allele of this mutation. LKB1 has several phosphorylation sites which are either phosphorylated by upstream kinases (Collins et al., 2000), such as ataxia telangiectasia mutated kinase (Sapkota et al., 2002b), cAMP-dependent kinase (Collins et al., 2000; Sapkota et al., 2001), p90RSK (Sapkota et al., 2001), and protein kinase C zeta (Song et al., 2008), or by auto-phosphorylation (Sapkota et al., 2002a; Xie et al., 2009).

In addition to phosphorylation, activation of LKB1 requires STE20-related pseudokinase (STRAD) and mouse protein-25 (MO25)-binding to LKB1 to form the LKB1/STRAD/MO25 complex

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(Boudeau et al., 2003) (Fig. 2). Formation of this complex stabilizes LKB1, activates its kinase activity (Boudeau et al., 2003), and allows the protein to translocate from the nucleus to the cytoplasm (Baas et al., 2003; Baas et al., 2004). This translocation might have an effect on phosphorylation of nuclear AMPK, but further studies are required to confirm this. When in the cytoplasm, the LKB1/STRAD/MO25 complex phosphorylates proteins of the AMPK family (Hawley et al., 2003) (Fig. 2). It has been proposed that, under physiological conditions, the net phosphorylation at Thr172 is determined by the balance between the rates of phosphorylation and dephosphorylation, and under basal conditions, the rate of dephosphorylation is high, resulting in low net phosphorylation (Hawley et al., 2003). However, under high AMP conditions, AMP-binding to the AMPK γ-subunit causes a conformational change in the protein structure, and inhibits the dephosphorylation of Thr172 on the AMPKα-subunit, allowing it to remain phosphorylated (Davies et al., 1995; Sanders et al., 2007; Suter et al., 2006; Xiao et al., 2013). Furthermore, it has been shown that AMP-binding to AMPK enhanced the LKB1-mediated phosphorylation at Thr172 (Gowans et al., 2013). More recent studies have reported that activation of AMPK by LKB1 may take place on the lysosomal surface, suggesting a role of the late endosome/lysosome in controlling metabolic programs (Zhang et al., 2014).

Activation of AMPK by CaMKKβ

CaMKKβ is another key upstream kinase of AMPK and it belongs to the Ca^{2+}/calmodulin-dependent protein kinase (CaMK) family (Berridge, 2001) (Fig. 2). The two most well characterized substrates of CaMKKβ are CaMKI and CaMKIV, but more recently, AMPK was also shown to be a target (Hawley et al., 2005). Unlike LKB1, CaMKKβ does not phosphorylate AMPK in response to low ATP levels, but as a reaction to increased cytosolic calcium (Ca^{2+}) levels (Green et al., 2011) (Fig. 2). Generally, calcium is known to regulate many processes within the cell by forming a complex with calmodulin (CaM) (Berridge, 2001; Chin and Means, 2000). Studies have shown that even though

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CaMKKβ possesses autonomous activity, it requires Ca^{2+}/CaM-binding to phosphorylate AMPK at Thr172 (Hurley et al., 2005).

Activation of AMPK by TAK1

TAK1 is one of the most recently discovered activators of AMPK and is a member of the mitogen-activated protein kinase kinase kinase (MAPKKK) family (Herrero-Martin et al., 2009) (Fig. 2). Its activity is regulated by various cytokines, including: interleukin-1 (IL-1) (Landstrom, 2010), transforming growth factor-β (TGF-β) (Yamaguchi et al., 1995), and by toll-like receptors (TLR) (Landstrom, 2010), CD40 and B cell receptors (Landstrom, 2010). Once activated, TAK1 then stimulates further downstream kinases, including the p38 mitogen-activated protein kinase (p38 MAPK) (Shim et al., 2005), c-jun *N*-terminal kinase (JNK) (Shim et al., 2005) and I-kappa B kinase complex (IKK) (Ninomiya-Tsuji et al., 1999; Shim et al., 2005). Interestingly, TAK1 was also shown to directly phosphorylate Thr172 on the AMPKα-subunit (Herrero-Martin et al., 2009) (Fig. 2). Furthermore, mice carrying a cardiac-specific dominant negative mutation for TAK1 showed signs of the Wolff-Parkinson-White syndrome, which were similar to those signs associated with mutations in human AMPKα/γ (Xie et al., 2006).

Despite extensive investigation of the topic, the mechanism behind AMPK activation by TAK1 remains elusive. Some studies have suggested that TAK1 indirectly phosphorylates AMPK *via* activating LKB1 (Inokuchi-Shimizu et al., 2014). However, TAK1 has also been shown to directly activate AMPK (Scholz et al., 2010). Additionally, a recent study suggests AMPK acts as an activator of TAK1, and that these two proteins reciprocally regulate their activation (Kim et al., 2012b).

Once activated, AMPK regulates several different metabolic processes in cells (Hardie, 2011). In most cells, including skeletal and cardiac muscle, liver and adipose tissue, AMPK stimulates fatty

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acid oxidation, mitochondrial biogenesis, glucose transporter type 4 (GLUT4) translocation and glucose uptake, while inhibiting protein synthesis, gluconeogenesis and fatty acid and cholesterol synthesis (Ruderman et al., 2013; Srivastava et al., 2012; Steinberg and Kemp, 2009). Insulin secretion from pancreatic β-cells can be inhibited by AMPK (Steinberg and Kemp, 2009) and it has been shown that AMPK can increase food intake *via* activating signaling in the hypothalamus (Minokoshi et al., 2004). AMPK also initiates autophagy, a catabolic process that is activated during starvation or stress (Egan et al., 2011; Kim et al., 2011). Overall, AMPK plays a major role in regulating energy homeostasis through modulation of cellular metabolism (Habegger et al., 2012; Hardie, 1989), protein synthesis and cellular proliferation (Shackelford and Shaw, 2009).

Biological Functions of AMPK

Regulation of Metabolism and Glucose Uptake

AMPK plays a critical role in regulating β-oxidation, fatty acid and cholesterol synthesis (Hardie, 2007, 2011) (Fig. 3). Catabolism of fatty acids *via* β-oxidation results in the generation of acetyl CoA, which then enters the citric acid cycle to generate ATP (Eaton et al., 1996). On the other hand, both fatty acid and cholesterol synthesis are anabolic processes, and hence, require ATP (Horton et al., 2002; Wakil et al., 1983). Fatty acid synthesis is a multi-step energy consuming process that generates fatty acids from acetyl CoA and malonyl CoA (Wakil et al., 1983) (Fig. 3). Similarly, cholesterol is synthesized *in vivo* from acetyl CoA to generate hydroxymethylglutaryl CoA (HMGCoA) (Horton et al., 2002). The HMGCoA is then converted into mevalonate which is the committed step in cholesterol synthetic pathway (Horton et al., 2002) (Fig. 3).

AMPK regulates these processes by phosphorylating and inhibiting the enzymes, acetyl CoA carboxylase 1 (ACC1) (Bianchi et al., 1990; Hardie and Pan, 2002) and hydroxymethylglutaryl CoA reductase (HMGCR) (Habegger et al., 2012) (Fig. 3). The enzymes ACC1/ACC2 and HMGCR are rate-limiting steps in the synthesis of fatty acids and cholesterol, respectively (Bianchi et al., 1990;

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Habegger et al., 2012; Hardie and Pan, 2002). Importantly, ACC1/ACC2 is required for the carboxylation of acetyl CoA to malonyl CoA (Bianchi et al., 1990; Thampy, 1989; Wakil et al., 1958). Malonyl CoA subsequently inhibits carnitine palmitoyltransferase 1 (CPT1) (Bremer, 1963), an enzyme essential for enabling activated long chain fatty acids to enter the mitochondrion for metabolism *via* the β-oxidation pathway (Fig. 3). This process eventually results in increased ATP synthesis (Bremer, 1963; Fritz and Yue, 1963). Hence, inhibition of ACC1 by AMPK results in increased activity of CPT1, leading to enhanced β-oxidation and ATP synthesis. Furthermore, inhibition of acetyl CoA carboxylation to malonyl CoA by AMPK, interrupts fatty acid synthesis (Wakil et al., 1983) (Fig. 3). Similarly, upon phosphorylation, HMGCR is inactivated and leads to reduced cholesterol synthesis (Habegger et al., 2012) by preventing the reduction of HMGCoA to mevalonate (Horton et al., 2002) (Fig. 3).

AMPK has also been shown to suppress fatty acid synthesis by inhibiting the activity of sterol regulatory element-binding protein-1 (SREBP1) (Li et al., 2011) (Fig. 3). SREBP1 is a transcription factor which up-regulates the expression of fatty acid synthase (FASN) and ACC1 (Hardie, 1989). Both these latter enzymes are crucial for the *de novo* synthesis of fatty acids, and by inhibiting SREBP1, AMPK inhibits the expression of FASN and ACC1 (Hardie, 1989) (Fig. 3). These effects result in decreased *de novo* fatty acid synthesis which requires ATP hydrolysis (Foretz et al., 1999).

Apart from regulating energy expenditure, studies have shown that AMPK translocates the GLUT4 from intracellular storage vesicles to the plasma membrane, resulting in increased glucose uptake into the cell (Fig. 3) (Kurth-Kraczek et al., 1999). Translocation and fusion of the GLUT 4 vesicles is regulated by the Rab family of G proteins when bound to GTP (active form) (Steinberg and Kemp, 2009). Rabs are inactivated by Rab GTPase-activating proteins (Rab GAPs), such as Akt substrate 160 (AS160; also known as TBC1D4) and TBC1D1 (Cartee and Wojtaszewski, 2007; Roach et al., 2007; Sano et al., 2003; Steinberg and Kemp, 2009). Phosphorylation of AS160 promotes its

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association with the 14-3-3 scaffolding protein (Chen et al., 2008; Kramer et al., 2006; Pehmoller et al., 2009; Steinberg and Kemp, 2009), resulting in inhibition of its Rab GAP activity (Fig. 3). Chen *et al* observed regulatory interactions among different phosphorylation sites of TBC1D1. For example, phosphorylation at Ser235 prevented subsequent phosphorylation at Ser237 (Chen et al., 2008). Phosphorylated TBC1D1 was also shown to bind the 14-3-3 scaffolding protein resulting in its inactivation (Chen et al., 2008). Moreover, these authors also showed that AMPK activators such as 5-aminoimidazole-4-carboxamide riboside (AICAR) and phenformin, phosphorylated TBC1D1 (Chen et al., 2008). In contrast, TBC1D4 was phosphorylated in response to insulin, but not AMPK activators (Chen et al., 2008). Hence, inactivation of Rab GTPases by AMPK prevents the hydrolysis of Rab GTP (Steinberg and Kemp, 2009), which then enhances GLUT4 translocation to the plasma membrane, and increases glucose uptake into the cell (Pehmoller et al., 2009).

Collectively, AMPK plays an important role in energy homeostasis *via* increasing catabolic pathways including β-oxidation, and inhibiting anabolic processes such as fatty acid and cholesterol synthesis.

AMPK Regulates Signaling *via* the Mammalian Target of Rapamycin

AMPK plays a role in controlling protein synthesis and cellular growth by inhibiting the mammalian target of rapamycin (mTOR) pathway (Gwinn et al., 2008) (Fig. 4). The mTOR complex 1 (mTORC1) plays a critical role as a nutrient/energy/redox sensor and controls protein synthesis (Kim et al., 2002). The complex, mTORC1, is composed of mTOR1 and the regulatory-associated protein of mTOR (raptor), which phosphorylates eukaryotic translation initiation factor 4E-binding protein (4EBP1) and ribosomal S6 kinase (S6K) (Fig. 4) (Holz et al., 2005; Wullschleger et al., 2006).

AMPK has been shown to suppress mTORC1 by a dual mechanism, namely: **(1)** by inhibiting PI3K/AKT signaling (Menon et al., 2014; Shaw, 2009), and **(2)** directly inhibiting raptor (Gwinn et al., 2008) (Fig. 4). AKT inhibits the tuberous sclerosis 1/2 (TSC1/2) complex by

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phosphorylating TSC2 serine residues. A recent study by Menon *et al* showed that the AKT-mediated phosphorylation of TSC2 does not affect its GTPase-activated protein (GAP) activity (Menon et al., 2014). Rather, this phosphorylation of TSC2 by AKT was shown to stimulate the dissociation of TSC complex from Ras homolog-enriched in brain (Rheb) at the lysosomal surface and the activation of Rheb (Menon et al., 2014). In contrast, when AMPK is activated, it phosphorylates TSC2 at distinct serine sites (*e.g.,* Ser1387) than those targeted by other kinases (Shaw, 2009). This AMPK-mediated phosphorylation of TSC2 results in activation of the TSC1/2 complex, which leads to inactivation of Rheb (Inoki et al., 2003; Shaw, 2009). This inactivation of Rheb results in inhibition of mTORC1 signaling (Inoki et al., 2003). Furthermore, AMPK also directly inhibits mTORC1 by phosphorylating raptor (Gwinn et al., 2008). This phosphorylation allows 14-3-3-binding to raptor, which reduces mTORC1 activity (Gwinn et al., 2008) (Fig. 4).

Inhibition of mTORC1 by AMPK leads to suppression of 4EBP1 and S6K phosphorylation (Holz et al., 2005; Wullschleger et al., 2006). When phosphorylated, 4EBP1 is unable to bind and inhibit the translational initiation factor eIF4E, resulting in an accumulation of free eIF4E and increased protein translation (Gingras et al., 1999; Haghighat et al., 1995) (Fig. 4). Furthermore, S6K up-regulates the translation of proteins such as, HIF-1α, MYC and cyclin D1 (Guertin and Sabatini, 2007) that are involved in cell cycle progression and cell growth. Hence, suppression of mTORC1 by AMPK results in decreased protein synthesis and cellular proliferation (Fig. 4) (Shackelford and Shaw, 2009). The suppressive effect of AMPK on cell proliferation could lead to its development as an important therapeutic target in proliferative disorders such as cancer and atherosclerosis (Motoshima et al., 2006).

AMPK as an Initiator of Autophagy

AMPK is an important initiator of autophagy, a catabolic process that is activated during starvation or stress (Choi, 2012). Autophagy regulates the digestion of cellular macromolecules and even whole

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organelles, by engulfing the organelles and proteins in a double membrane vesicle (autophagosomes), which fuse with the lysosomes to hydrolyze its contents (Choi, 2012; Sahni et al., 2014). Autophagy acts as a mechanism of avoiding cell death, but under some conditions, can lead to autophagic cell death (Choi, 2012).

Once AMPK is activated, it phosphorylates an initiator of autophagy Unc 51-like kinase (ULK1) at certain sites, namely Ser317 and Ser777 (Kim et al., 2011) (Fig. 5). Phosphorylation of ULK1 at these sites is important for initiation of autophagy (Kim et al., 2011). Another study has shown that AMPK also phosphorylates ULK1 at Ser467, Ser555, Thr574, and Ser637 (Egan et al., 2011). Additionally, ULK1 is also phosphorylated by mTORC1 at Ser757, which has an inhibitory effect on the phosphorylation of ULK1 at Ser317 and Ser777, resulting in suppression of the autophagic pathway (Kim et al., 2011) (Fig. 5). As discussed earlier, when active, AMPK suppresses mTORC1 activity, this leads to decreased phosphorylation of ULK1 at Ser757 (Kim et al., 2011). The removal of the inhibitory phosphorylation at Ser757, allows ULK1 to be phosphorylated at Ser317 and Ser777 by AMPK, which results in the activation of autophagy (Kim et al., 2011) (Fig. 5). Thus, AMPK initiates autophagy directly by activating ULK1 and also by removing the inhibitory effect of mTORC1 on ULK1 (Fig. 5). Together, the suppression of mTORC1 and activation of ULK1 by AMPK, leads to inhibition of protein synthesis (an ATP-consuming process) and activation of autophagy in the cell, leading to prolonged survival.

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AMPK in Pathological Conditions

Diabetes and Metabolic Diseases

As AMPK is a potent regulator of cellular metabolism, it plays a crucial role in type 2 diabetes and several other metabolic diseases (Hardie, 2011). Previous studies have indicated that an elevation of plasma fatty acid levels could lead to impaired insulin-mediated glucose uptake (Frayn et al., 1993), which requires the translocation of GLUT4 to the plasma membrane (Cushman et al., 1998).

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Considering that AMPK is involved in regulating both fatty acid metabolism (Hardie, 1989) and GLUT4 translocation to the membrane (Kurth-Kraczek et al., 1999), reduced activity of AMPK activity would be expected to inhibit fatty acid oxidation and decrease glucose uptake into the cell. Furthermore, AMPK activators have been shown to improve insulin resistance and glucose uptake in rodent models of the metabolic syndrome (Habegger et al., 2012; Song et al., 2002). These activators also prevented the development of diabetes, lipid deposition and degranulation of pancreatic islet β cells (Yu et al., 2004). Furthermore, AMPK is expressed in adipose tissue and is involved in metabolic regulation in these cells (Wang et al., 2014). Hence, due to the correlation between excess adiposity and metabolic diseases (Abate et al., 1995; Premanath et al., 2014) this suggests another possible pathway through which AMPK plays a role in these disorders. These studies illustrate a crucial role of AMPK in metabolic diseases and diabetes, and signify its importance as a molecular target for novel therapeutics

Wolff-Parkinson-White Syndrome

Wolff-Parkinson White (WPW) syndrome is a genetic disorder caused by mutations in the *PRKAG2* gene, which codes for the AMPKγ2-subunit (Gollob et al., 2001a; Gollob et al., 2001b; Gollob and Roberts, 2002). As discussed earlier, the γ-subunit is essential for the allosteric activation of the protein (Bateman, 1997; Hardie, 2007). Previous studies have demonstrated that *PRKAG2* gene mutations such as His142Arg, Arg302Gln, His383Arg, Thr400Asn,Tyr487His, Asn488Ile, Gln506Lys, Arg531Gly, Ser548Pro, andInsLeu351 occur in the CBS region of the gene and affect the AMP-binding ability of the subunit (Gollob and Roberts, 2002). However, this notion has been challenged by a recent study in Chinese patients that has discovered a new missense mutation, namely Gly100Ser (G100S) in the *PRKAG2* gene, but unlike the other mutations, this one was not in the CBS region (Zhang et al., 2013a). Over-expression of this mutated protein resulted in thicker heart wall, increased glycogen storage and decreased AMPK enzymatic activity (Zhang et al., 2013b). Cardiomyocytes from patients with WPW syndrome exhibit a variable phenotype consisting

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of cardiac hypertrophy, pre-excitation, and conduction abnormalities, which are probably caused by the derangement of AMPK's function (Light, 2006). *PRKAG2*-induced cardiac syndrome is believed to be a result of increased glycogen storage (Gollob, 2003). Studies have shown an accumulation of glycogen-like substances in the myocardium, which leads to increased thickness of the myocardium, *i.e.*, cardiac hypertrophy (Arad et al., 2003). Apart from causing cardiac hypertrophy, increased glycogen storage in cardiomyocytes interferes with electrical conductance (Gollob, 2003). This problem leads to the development of a less efficient accessory pathway (Gollob, 2003), which is another electrical conduction system that connects the atrium to the ventricle (Ho, 2008). Furthermore, studies have suggested the increased glucose uptake in these cells leads to an increase in cytosolic glucose-6-phosphate and consequent activation of glycogen synthesis (Luptak et al., 2007). However, the effect of *PRAKG2* mutation on cardiac growth was shown to be independent of glycogen storage (Kim et al., 2014). In contrast, the electrical defects in these cardiomyocytes seem to be secondary consequences of the high glycogen content (Kim et al., 2014).

Initially, WPW was believed to be due to a loss of function mutation (Sidhu et al., 2005). However, studies have shown that the increased glycogen storage was due to a gain of function mutation in the *PRKAG2* gene (Banerjee et al., 2007; Gollob et al., 2001b). Recent evidence from transgenic mouse studies suggest that some *PRKAG2* mutations, such as N488I, lead to increased AMPK activity and accumulation of glycogen in myocytes, contributing to cardiac hypertrophy (Arad et al., 2003). Another mutation located at R302Q within the AMPKγ2-subunit, was also identified and shown to amplify glycogen storage in cardiac muscle (Gollob et al., 2001b; Gollob, 2003). However, there is conflicting data that shows mice expressing the γ2 R302Q variant have reduced AMPK activity when measured with the SAMS peptide (Sidhu et al., 2005), a well-established AMPK substrate widely used for determination of AMPK activity (Davies et al., 1989). A similar γ2 mutation, T400N, when expressed in transgenic mice, resulted in a biphasic effect of γ2-subunit on cardiac muscle AMPK activity, with increased activity in samples from young mice, but reduced activity later in life

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(Banerjee et al., 2007). These conflicting findings suggest that the lower AMPK activity observed in older mice might be due to secondary effects of the mutation acquired later in life (Banerjee et al., 2007). These studies suggest deregulation of AMPK activity caused by *PRKAG2* mutations leads to the development of WPW, but the mechanisms by which these mutations in the *PRKAG2* gene cause WPW syndrome are not clearly defined.

Furthermore, more recent data has shown that knockdown of cardiac sodium-dependent glucose co-transporter (SGLT) in mice with the *PRKAG2* Thr400Asn mutation (TGT400N) attenuates the phenotype consisting of cardiomyopathy (Ramratnam et al., 2014). Transgenic mice with cardiac SGLT1 over-expression (TGSGLT1-ON) replicate phenotypic features of the cardiomyopathy, suggesting role of SGLT1 in the pathophysiology of the *PRKAG2* mutation (Ramratnam et al., 2014).

Neurodegenerative diseases

Several studies have shown AMPK over-activation in neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson’s disease (PD) and Huntington's disease (HD) (Chou et al., 2005; Lim et al., 2012; Lopez-Lopez et al., 2007). Additionally, neurodegenerative diseases (*e.g.*, PD) are also associated with mitochondrial dysfunction (Milakovic and Johnson, 2005; Panov et al., 2002; Ruan et al., 2004). Mitochondrial dysfunction can lead to high lactate levels in PD brains (Harms et al., 1997), which can result in activation of AMPK (Chen et al., 2010). A recent study demonstrated that exposure of neuronal cells to lactate caused an AMPK-dependent accumulation of α-synuclein in the cytoplasm (Jiang et al., 2013), a protein associated with PD development.

In HD, it has been demonstrated that the AMPKα1-subunit abnormally translocates to the nucleus in the striatal neurons expressing the mutant *huntingtin* gene (Ju et al., 2011). This resulted in the suppression of Bcl2 (an anti-apoptotic protein) expression, leading to neurotoxicity (Ju et al., 2011). Furthermore, this study also showed that the pharmacological agent, CGS21680, that ameliorates HD

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symptoms, acts in part, by preventing AMPKα1 translocation to the nucleus (Ju et al., 2011). In the case of AD, the role of AMPK in progression of this disease is still not clear. In human patients it was observed that AMPK is abnormally activated and translocated from the nucleus to the cytoplasm in AD tangles (Vingtdeux et al., 2011). It was demonstrated that AMPK activation, either directly or indirectly, inhibits tau phosphorylation in rat cortical neurons (Greco et al., 2009). Conversely, other studies have demonstrated that AMPK phosphorylates tau at multiple sites and disrupts the binding of tau to microtubules (Thornton et al., 2011; Vingtdeux et al., 2011). From these findings, it is evident that AMPK is activated during Alzheimer's disease. However, it is not clear if it is the primary cause or a cellular response in order to repair the damage, for example through the activation of autophagy.

The Controversial Role of AMPK in Cancer

Current evidence indicates that AMPK has properties of both a tumor suppressor and a tumor promoter (Bonini and Gantner, 2013). The role of AMPK in cancer is discussed in detail in a recent review by Jones and colleagues (Faubert et al., 2014). It has been suggested that AMPK inhibits tumor growth through its regulation of metabolic pathways (Shackelford and Shaw, 2009). As cancer cells generally have high energy expenditure due to their more rapid metabolism, it would seem that AMPK, as a master regulator of energy homeostasis, would play a key role in tumor progression (Faubert et al., 2013). Additionally LKB1, the key upstream kinase of AMPK, has been shown to inhibit cancer proliferation as well as metastasis, possibly through AMPK activation (Gan and Li, 2014).

Metastasis is an important step in the progression of cancer. It involves the detachment of tumor cells from their primary site, and their dissemination to distant sites *via* blood vessels and the lymphatic system (Geiger and Peeper, 2009). Cell polarity can also be observed in migrating cells and is responsible for directional migration (Marcus and Zhou, 2010). Loss of polarity may be

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responsible for the epithelial to mesenchymal transition (EMT) and subsequent tumor invasion that plays a key role in metastasis (Thiery, 2003).

It has been noted that AMPK regulates mammalian cell motility and its loss causes directional migration defects (Nakano et al., 2010), suggesting a potential role for AMPK in energy-dependent regulation of cell motility (Fig. 6). Interestingly, AMPK phosphorylates the microtubule plus end protein, CLIP-170, to regulate cell polarity *via* microtubule dynamics (Fig. 6) (Zheng and Cantley, 2007). CLIP-170 directly binds newly polymerized distal ends of growing microtubules and rapidly dissociates from the older microtubule lattice (Dragestein et al., 2008). Microtubules induce cell polarity by transporting protein complexes to the new end of the cell (Sawin and Nurse, 1998). Inhibition of AMPK leads to an accumulation of non-phosphorylated CLIP-170 and disturbed cell polarity (Nakano et al., 2010), suggesting a role of AMPK in maintaining polarity.

A recent study has shown that activation of AMPK reduces metastasis and invasion by downregulating the levels of integrin β1 (Park et al., 2012) that is known to participate in cell migration (Lee et al., 2010). Integrins mediate adhesion to the extracellular matrix, but also participate in the regulation of cytoskeletal organization of the cell (Banno and Ginsberg, 2008), making it important for cell migration and invasion. Hence, reducing integrin β1 levels would lead to reduced cell migration and invasion. In addition to regulating cell polarity, studies have shown suppression of metastasis by AMPK occurs through its ability to inhibit S6K (Taliaferro-Smith et al., 2009). Activation of AMPK resulted in the inhibition of the mTORC1 pathway (Gwinn et al., 2008), resulting in suppressed expression of proteins required for tumor growth and metastasis, including HIF-1α, MYC and cyclin D1 (Guertin and Sabatini, 2007).

Interestingly, there have also been studies showing that AMPK elevates tumor growth and metastasis. One investigation suggested that AMPK activation during energy stress prolongs cell survival by

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redox regulation (Jeon et al., 2012). Under these conditions, NADPH generation through the pentose phosphate pathway was down-regulated, but AMPK was able to induce alternative metabolic routes to maintain NADPH levels and inhibit cell death (Jeon et al., 2012). Inhibition of ACC1 by AMPK decreases NADPH consumption in fatty acid synthesis and increases NADPH generation by means of fatty acid oxidation (Jeon et al., 2012). Other investigations have suggested that the pro-survival role of AMPK can be attributed to the induction of autophagy and the maintenance of proliferative quiescence (Degenhardt et al., 2006). It has been suggested that AMPK activation, and therefore autophagy, can serve as a protective mechanisms conferring resistance to growth factor deprivation therapies (Chhipa et al., 2011). Overall, these studies suggest that AMPK promotes tumor growth by inhibiting cell death and prolonging cell survival through autophagy under stressful conditions (Chhipa et al., 2011; Degenhardt et al., 2006; Jeon et al., 2012).

From the investigations described above, it is evident that AMPK plays a primary role in regulating tumor growth and metastasis. While there is some controversy regarding the anti-tumor and pro-tumor effects of AMPK, the majority of studies have demonstrated that AMPK plays a crucial role in inhibiting tumor growth and metastasis.

Therapeutic Targeting of the AMPK Signaling Pathway

Due to the diverse roles of AMPK in the cell, as well as its regulation in several pathological conditions, AMPK acts as a promising therapeutic target for treatment of a number of pathological conditions (Hardie, 2013; Jeong et al., 2014; Russo et al., 2013). There are several known physiological, pharmacological, natural and hormone activators of AMPK, with some of these already being used clinically (Corton et al., 1995; Lee et al., 2006; Zhou et al., 2001a). Currently, pharmaceutical agents such as metformin and thiazolidinediones (TZDs) are used for the treatment of type 2 diabetes *via* their ability to activate the AMPK pathway (Owen et al., 2000). These agents are discussed in detail below.

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Metformin

Metformin (Fig. 7A) is used as a treatment for type 2 diabetes (Duncan and Seaton, 1962), as well as polycystic ovarian syndrome (Diamanti-Kandarakis et al., 2010) and metabolic syndrome (Bianchi et al., 2007). The efficacy of metformin in these metabolic disorders is attributed to its ability to reduce hepatic gluconeogenesis and improve insulin sensitivity (Viollet et al., 2012). Metformin activates AMPK indirectly by inhibiting complex I of the mitochondrial respiratory chain which promotes a switch from aerobic to anaerobic glycolysis, to increase the cellular AMP/ATP ratio (Hawley et al., 2010; Owen et al., 2000; Zhou et al., 2001b). Interestingly, recent findings by Fullerton *et al* show that phosphorylation of ACC1 by AMPK is required for metformin's effects on lipid metabolism and insulin sensitivity (Fullerton et al., 2013). In addition to its anti-diabetic effects, studies have also shown that metformin possesses anti-tumor properties including reduced cell growth, induction of apoptosis, and reduced migration and invasion in a variety of human cancer cell lines (Liu et al., 2011; Papanas et al., 2010; Rozengurt et al., 2010; Viollet et al., 2012).

Several *in vitro* and *in vivo* studies have shown that one of the potential pathways *via* which metformin mediates its anti-tumor effects is through AMPK activation and reduced mTOR activity (Ben Sahra et al., 2010; Brown et al., 2010; Tosca et al., 2010; Zakikhani et al., 2008). However, metformin also acts through AMPK-independent mechanisms (Foretz et al., 2010; Janjetovic et al., 2011a; Janjetovic et al., 2011b; Miller et al., 2013; Rattan et al., 2011) . Studies have shown that LKB1 is a crucial protein required for the anti-tumor effects of metformin, as cells lacking LKB1 failed to exhibit reduced tumor growth when treated with this agent (Huang et al., 2008; Legro et al., 2008; Rattan et al., 2011).

Currently, numerous phase II-III clinical trials are underway to investigate the potential of treatment with metformin in a variety of tumor-types, including breast cancer (Goodwin et al., 2011; Hadad et al., 2011; Martin-Castillo et al., 2010), endometrial cancer, colorectal cancer and prostate cancer.

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However, some of these studies are in the recruitment stage and are yet to determine the potential of metformin as an adjuvant to current cancer therapeutics.

Thiazolidinediones

TZDs (*e.g.*, Rosiglitazone; Fig. 7B) are another class of drugs used in the treatment of diabetes, which primarily target the peroxisome proliferator-activated receptor-γ (PPARγ) to improve insulin sensitivity (LeBrasseur et al., 2006). The effects of TZDs are partly mediated through AMPK activation (LeBrasseur et al., 2006). These drugs activate AMPK in a similar way to metformin, by inhibiting complex I of the mitochondrial respiratory chain, increasing the AMP/ATP ratio (Brunmair et al., 2004; Hawley et al., 2010). Additionally, TZDs also increase AMPK activation through PPARγ to stimulate adiponectin secretion from adipose tissue (LeBrasseur et al., 2006). Adiponectin was shown to increase AMPK phosphorylation and regulate glucose levels and stimulate FA oxidation in skeletal muscle (Yoon et al., 2006). Hence, TZDs are effective drugs to treat diabetes through their ability to activate AMPK which subsequently regulates glucose levels in cells and improves insulin sensitivity.

5-Aminoimidazole-4-carboxamide riboside

The compound, 5-aminoimidazole-4-carboxamide riboside (AICAR; Fig. 7C), is a well known activator of AMPK (Corton et al., 1995; Sullivan et al., 1994). In fact, it was the first compound to be identified as an activator of AMPK (Sullivan et al., 1994). Structurally, AICAR is similar to AMP, and upon intracellular metabolism, it is converted to AICAR 5′-monophosphate (ZMP), an analogue of AMP, by adenosine kinase (Corton et al., 1995). Activation of AMPK by ZMP is similar to that by AMP, where ZMP binds to the CBS domains on the γ-subunit of AMPK and leads to allosteric activation of the complex (Corton et al., 1995). Additionally, AICAR treatment either prevented and/or reversed some aspects of metabolic syndrome in animal models, including improved glucose tolerance, better systemic glucose disposal (Song et al., 2002), and reduced hepatic glucose output, as

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well as plasma triglyceride and free fatty acid levels (Bergeron et al., 2001). However, AICAR does have some disadvantages that make it unlikely to be used as a therapeutic agent, such as low bioavailability and a short half-life (Coughlan et al., 2014). Due its ability to mimic AMP, it can interfere with several other pathways in the cell that require AMP (Longnus et al., 2003), making AICAR an unpromising lead agent for drug development.

Similar to AICAR, the drugs pemetrexed and sorafenib also increase AMPK activity through inhibition of aminoimidazole-carboxamide ribonucleotide formyl-transferase (AICART) (Bareford et al., 2014; Racanelli et al., 2009). Inhibition of AICART results in increased ZMP and activation of AMPK (Bareford et al., 2014). These agents have demonstrated anti-tumor effects through regulation of AMPK and subsequently autophagy (Bareford et al., 2014). Currently, these drugs are in phase I clinical trials, and it has been suggested that combination treatment with pemetrexed and sorafenib represent a future therapeutic option for the treatment of solid tumors (Bareford et al., 2014).

Berberine

Berberine (Fig. 7D) is a clinically important pharmacological agent derived from the *Berberis* plant species that has exhibited AMPK activation properties (Imanshahidi and Hosseinzadeh, 2008; Tillhon et al., 2012). It is used in traditional Chinese and Korean medicine to treat bacterial and fungal infections as well as type 2 diabetes (Tillhon et al., 2012). Berberine treatment using several rodent models resulted in improved glucose tolerance (Lee et al., 2006), reduced body weight (Lee et al., 2006), increased expression of both the insulin receptor and low-density lipoprotein (LDL) receptor (Kong et al., 2004), lower total and LDL cholesterol levels, and reduced triglyceride levels (Kong et al., 2004). Studies have shown that Berberine is able to lower blood glucose, triglyceride, and cholesterol levels to a similar extent as metformin (Yin et al., 2008). Collectively, these results suggested that Berberine activates the AMPK pathway (Kong et al., 2004; Lee et al., 2006). Similarly

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to metformin, Berberine was shown to act upon AMPK through inhibiting complex I of the mitochondrial respiratory chain (Hawley et al., 2010; Turner et al., 2008).

More recent studies have also demonstrated that the anti-tumor and anti-metastatic effects of Berberine are also mediated through activation of the AMPK pathway (Kim et al., 2012a; Park et al., 2012). Another investigation showed that Berberine-induced AMPK activation inhibits the metastatic potential of melanoma cells *via* reduction of ERK activity and COX-2 protein expression (Kim et al., 2012a). The ERK/COX-2 pathway is involved in the regulation cell proliferation, angiogenesis and metastasis in many cancers (Muller-Decker and Furstenberger, 2007).

Salicylates

Salicylates are natural compounds secreted by many plants as a defense against infections, and synthetic derivatives including acetyl salicylate (aspirin; Fig. 7E) or diester salicylate, are used pharmaceutically (Higgs et al., 1987). Studies have shown that salicylate binds to a site in an AMPK cleft between the kinase domain of the α-subunit and the CBM domain of the β-subunit (Hawley et al., 2012). Furthermore, it was only able to activate AMPK complexes containing the β1 isoform and requires β-Ser108 phosphorylation (Hawley et al., 2012). However, high-fat-fed wild-type and AMPKβ1 knockout mice treated with salicylate showed improved glucose tolerance, reduced fasting glucose and insulin levels, suggesting AMPK-independent activity (Hawley et al., 2012). Hence, even though salicylates seem promising as an AMPK-mediated anti-diabetic treatment, further research is required to determine the role of AMPK in these outcomes.

Resveratrol

Resveratrol (Fig. 7F) is a polyphenol found in red wine that has been shown to stimulate AMPK activity in many different cell lines (Dasgupta and Milbrandt, 2007; Park et al., 2007; Um et al., 2010). The mechanism by which resveratrol activates AMPK is thought to be attributed to an

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increase in AMP levels due to inhibition of the mitochondrial F_1 ATPase (Gledhill et al., 2007; Hawley et al., 2010). Resveratrol treatment stimulates glucose uptake (Park et al., 2007) and mitochondrial biogenesis (Baur et al., 2006) in muscle cells, and reduces liver lipid accumulation (Zang et al., 2006). However, it is still unclear as to whether these effects are mediated through AMPK activation (Ruderman et al., 2010).

Conclusions

AMPK is an important protein involved in systemic energy homeostasis. It leads to the activation of catabolic pathways such as β-oxidation and autophagy and suppression of anabolic pathways (for example, fatty acid synthesis) in order to provide an alternate source of energy to the cell under stressful conditions. In conclusion, AMPK is a critical cellular regulators involved in both physiological and pathological conditions. Due to the role of AMPK in energy homeostasis, it is implicated in a number of disorders such as diabetes and cardiac hypertrophy, and is a promising therapeutic target. Indeed, the design of AMPK activators for therapeutic applications represents a thriving field for future research.

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Wrote or contributed to the writing of the manuscript: Krishan, Richardson, and Sahni.

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Footnotes

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FIGURE LEGENDS

Figure 1. Structure of AMPK. (A) Crystal structure of AMPK. The α-subunit is indicated in yellow, orange and cyan, the β-subunit is in green with the CBM in bright green, and the γ-subunit is in pink. For this structure, an AMPK complex phosphorylated on Thr172, was crystallized in the presence of AMP and the kinase inhibitor staurosporine. Additionally AMPK activator 991 shown in magenta is bound in a pocket generated at the interface between the CBM and the kinase domain. Only AMP-binding at CBS site 3 and 4 is shown (marked as AMP-3 and AMP-4, respectively). Reprinted with permission from Macmillan Publishers Ltd: [Nature] (Xiao et al., 2013), (http://creativecommons.org/licenses/by/3.0/legalcode). **(B)** The α-subunit is a 550 amino acid long catalytic subunit, and is composed of the kinase domain, α-linker region, the C-terminal β/γ- subunit interacting domain (α-CTD) and a nuclear export signal (NES). The α-linker region contains the auto-inhibitory sequence (AIS) and "α-hook" domain. The kinase domain is phosphorylated at Thr172 in the activation loop. The β-subunit is 270 amino acids long and has a carbohydrate-binding motif (CBM) and the C-terminal domain of the β- subunit (β-CTD). The *N*-terminal contains a more variable sequence including the *N*-myristoylation site. The γ-subunit contains 4 tandem repeats of the CBS, AMP-binding pockets (*i.e.,* CBS 1-4).

Figure 2. Regulation of AMPK activity. AMPK is phosphorylated by three kinases, LKB1, CaMKKβ and TAK1. LKB1 forms a complex with STRAD and MO25, which then allows it to translocate from the nucleus to the cytoplasm. Once in the cytoplasm, LKB1 can phosphorylate AMPK at Thr172. CaMKKβ phosphorylates AMPK as a response to increased cytosolic Ca^{2+} levels. TAK1 is activated by IL-1, TGF-β, TLR, CD40 and B cell receptors. Furthermore, AMP-binding to the γ-subunit of AMPK prevents the dephosphorylation of Thr172 in the α-subunit.

Figure 3. Regulation of energy homeostasis by AMPK. AMPK phosphorylates acetyl CoA carboxylase 1 (ACC1) and inactivates it. ACC1 is required to convert acetyl CoA to malonyl CoA for

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fatty acid (FA) synthesis. Malonyl CoA inhibits carnitine palmitoyltransferase 1 (CPT1), a protein that enables the entry of FAs into the mitochondria for β-oxidation. Phosphorylation of ACC1 by activated AMPK prevents acetyl CoA from being converted to malonyl CoA, and thus, prevents the inhibition of β-oxidation. Furthermore, inhibition of malonyl CoA synthesis also prevents FA synthesis. AMPK also suppresses ACC1 levels by inhibiting sterol regulatory element-binding protein-1 (SREBP1), a transcription-factor involved in ACC1 transcription. Similarly, inhibition of hydroxymethylglutaryl CoA reductase (HMGCR) *via* phosphorylation by AMPK prevents cholesterol synthesis by interrupting the conversion of hydroxymethylglutaryl CoA (HMGCoA) to mevalonate. In addition to this, AMPK also regulates glucose uptake by increasing GLUT4 translocation to the membrane.

Figure 4. Regulation of mTOR complex 1 (mTORC1) by AMPK. AMPK inhibits mTOR signaling *via* a dual mechanism. First, AMPK inhibits raptor, and prevents the formation of mTORC1. Second, AMPK also interferes with PI3K/AKT signaling by phosphorylating and activating the tuberous sclerosis 1/2 (TSC1/TSC2) dimer, which inhibits Ras homolog enriched in brain (Rheb). Rheb is an activator of mTORC1. Hence, activation of TSC1/TSC2 dimer by AMPK, results in suppression of Rheb, which is then unable to activate mTORC1. Suppression of mTORC1 leads to inhibition of ribosomal S6 kinase (S6K) and eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1) phosphorylation. Non-phosphorylated 4EBP1 can bind to translation factor eIF4E, and prevent protein synthesis. This leads to decreased protein translation, which then results in decreased cell proliferation.

Figure 5. Regulation of autophagy by AMPK. AMPK regulates autophagy through a dual mechanism. First, AMPK phosphorylates Unc 51-like kinase (ULK1) at Ser317/777, which results in initiation of autophagy. Second, AMPK also indirectly enhances ULK1 phosphorylation at these sites by suppression of mTORC1. mTORC1 phosphorylates ULK1 at Ser757, but this phosphorylation has

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an inhibitory effect on the phosphorylation of Ser317/777. Hence, suppression of mTORC1 removes the inhibitory phosphorylation on ULK1 and allows it to be phosphorylated at Ser317/777.

Figure 6. AMPK regulates cell polarity and directional migration. AMPK phosphorylates the microtubule plus end protein, CLIP-170, which is involved in binding of newly polymerized distal ends of growing microtubules and rapidly dissociating from the older microtubule lattice. This results in directional migration of cells.

Figure 7. Activators of AMPK. Line drawings of the structures of some of the pharmacological and natural compounds that activate AMPK.

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TABLES

Table 1: AMPK-subunits. The gene encoding the subunits, their chromosomal location, molecular weight (MW) and transcript size.

Subunit	Gene	Location	MW (Da)	Transcript	
AMPKα1	*PRKAA1*	5p12	64009	1929 bp	(Krishan et al., 2014; Strausberg et al., 2002)
AMPKα2	*PRKAA2*	1p31	62320	1703 bp	(Strausberg et al., 2002)
AMPKβ1	*PRKAB1*	12q24.1-q24.3	30382	813 bp	(Gao et al., 1996; Mitchelhill et al., 1997)
AMPKβ2	*PRKAB2*	1q21.1	30302	819 bp	(Gao et al., 1996)
AMPKγ1	*PRKAG1*	12q12-q14	37579	996 bp	(Gao et al., 1996)
AMPKγ2	*PRKAG2*	7q36.1	63066	1962 bp	(Ota et al., 2004)
AMPKγ3	*PRKAG3*	2q35	54258	1599 bp	(Gao et al., 1996)

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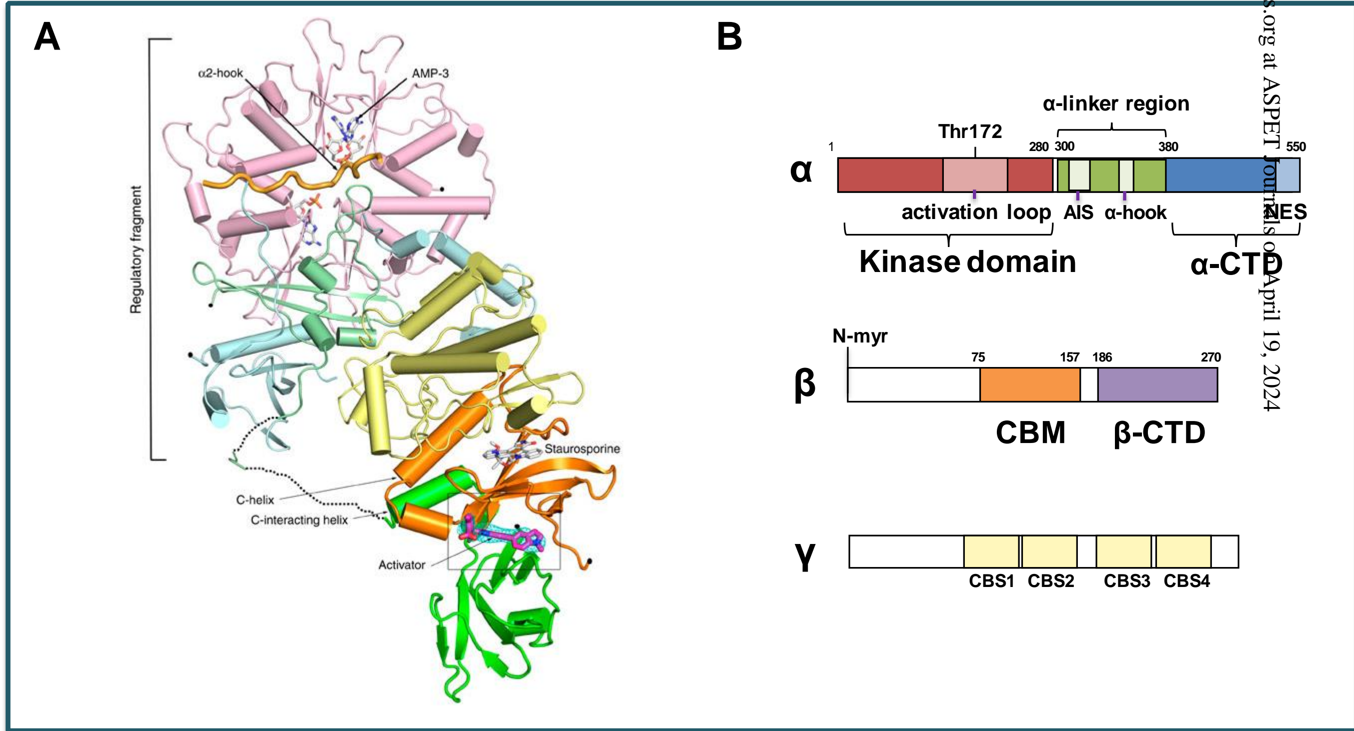

Figure 1

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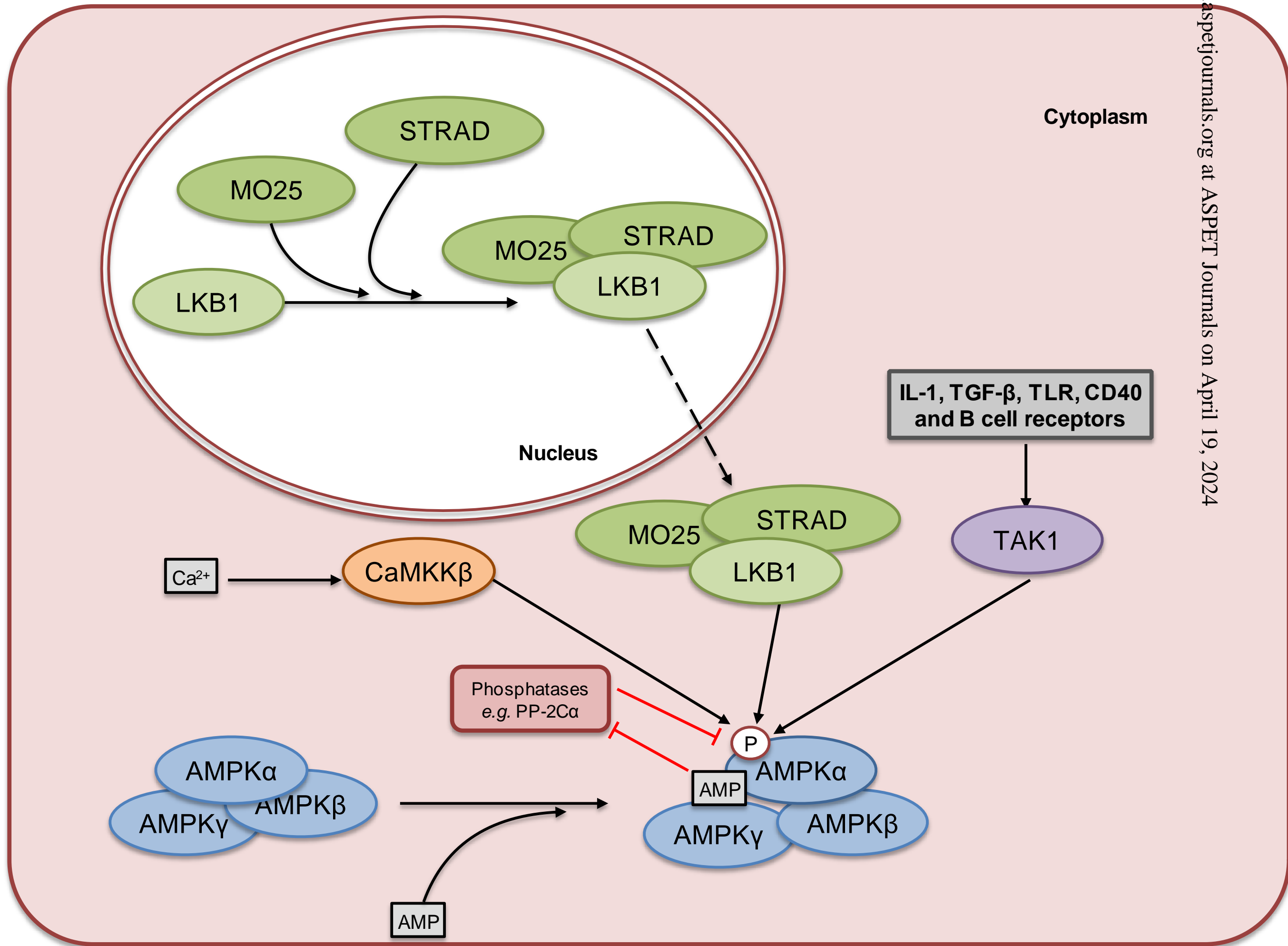

Figure 2

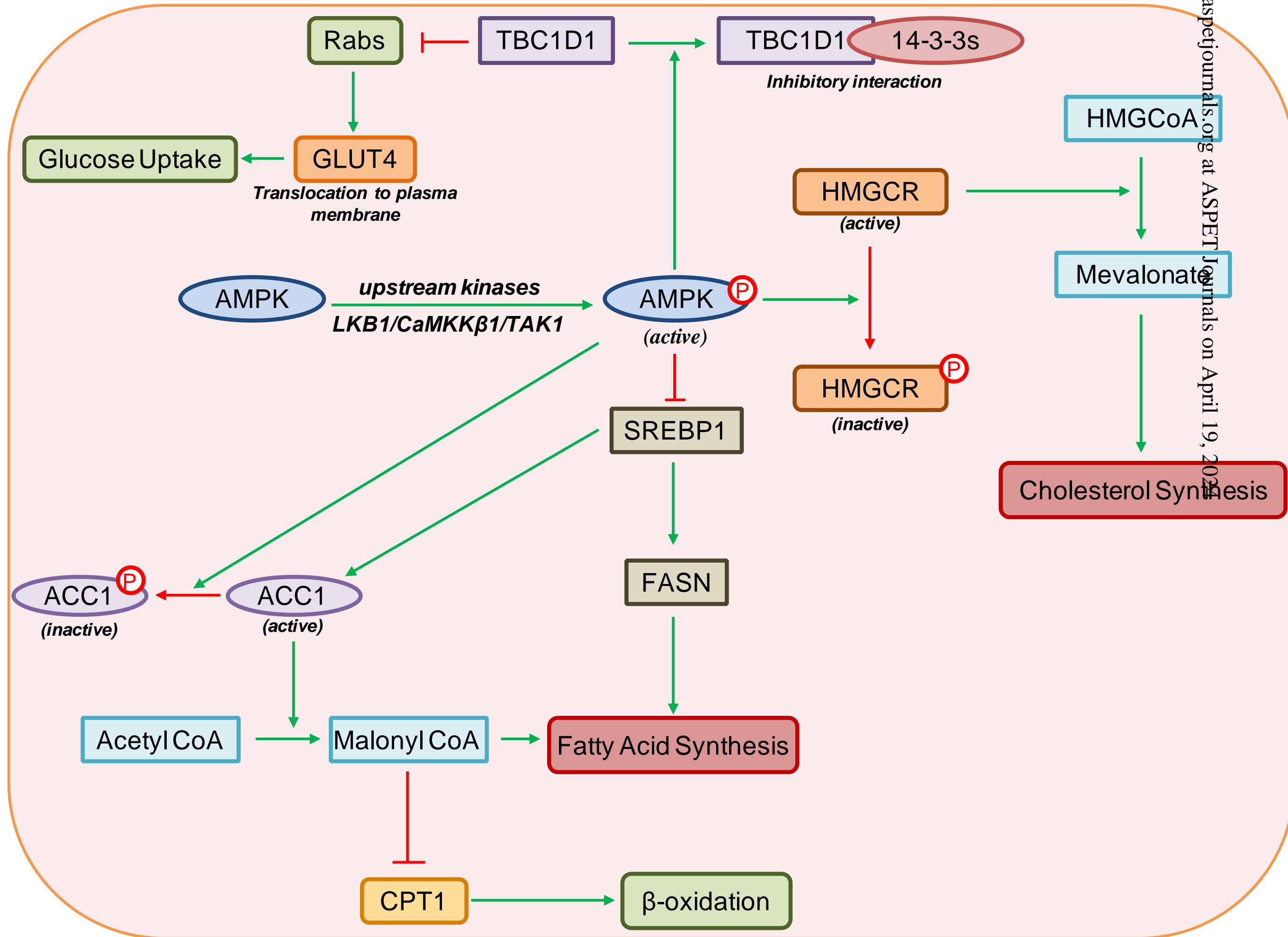

Figure 3

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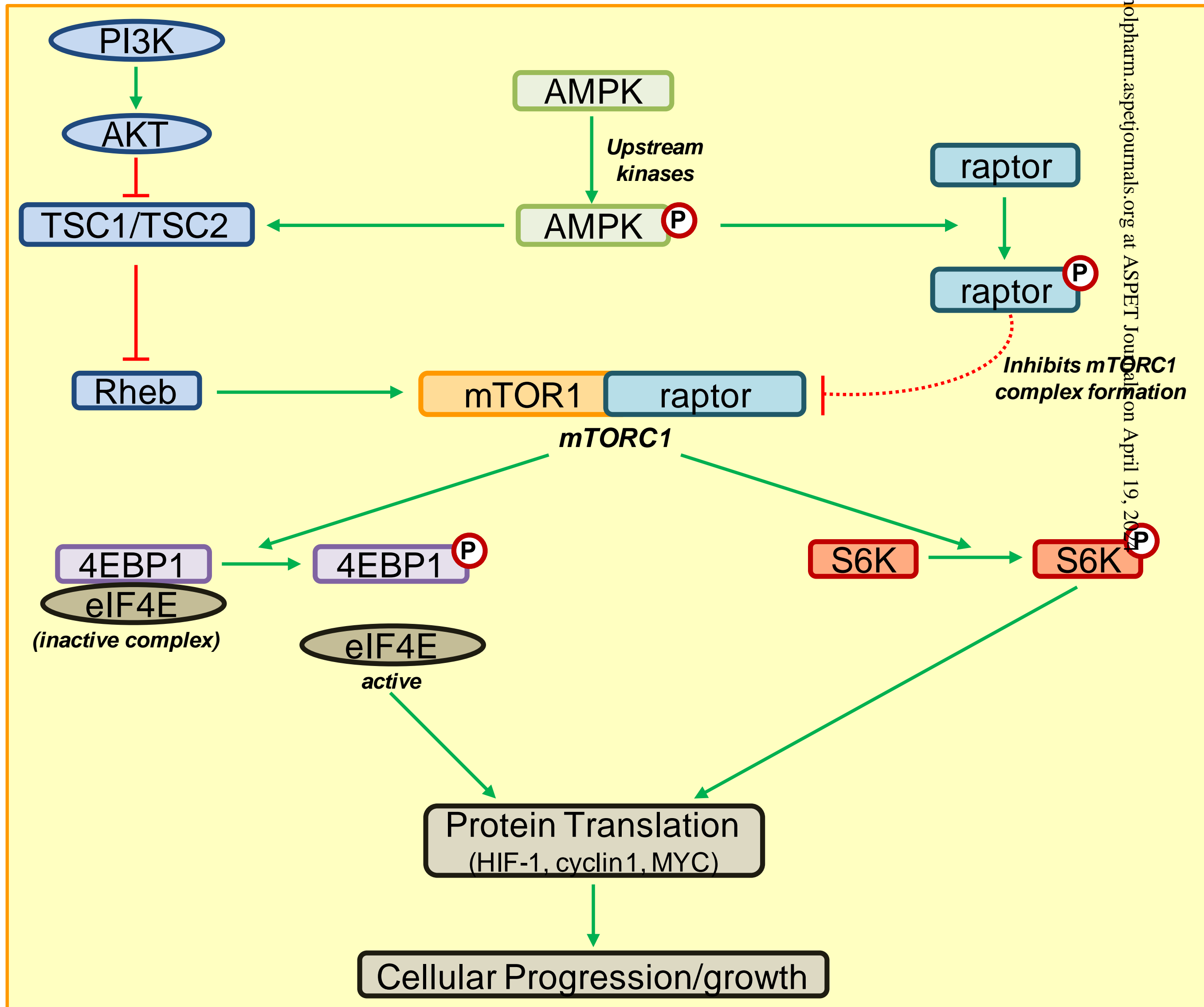

Figure 4

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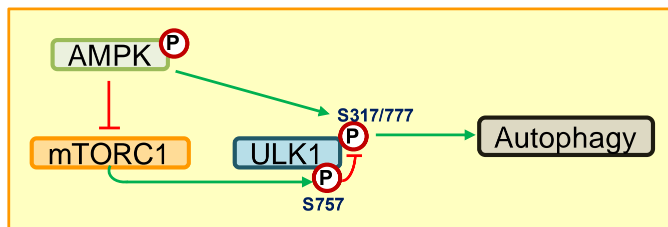

Figure 5

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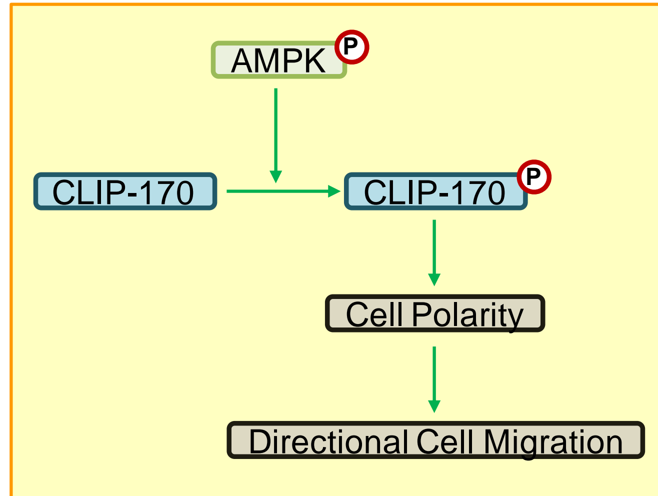

Figure 6

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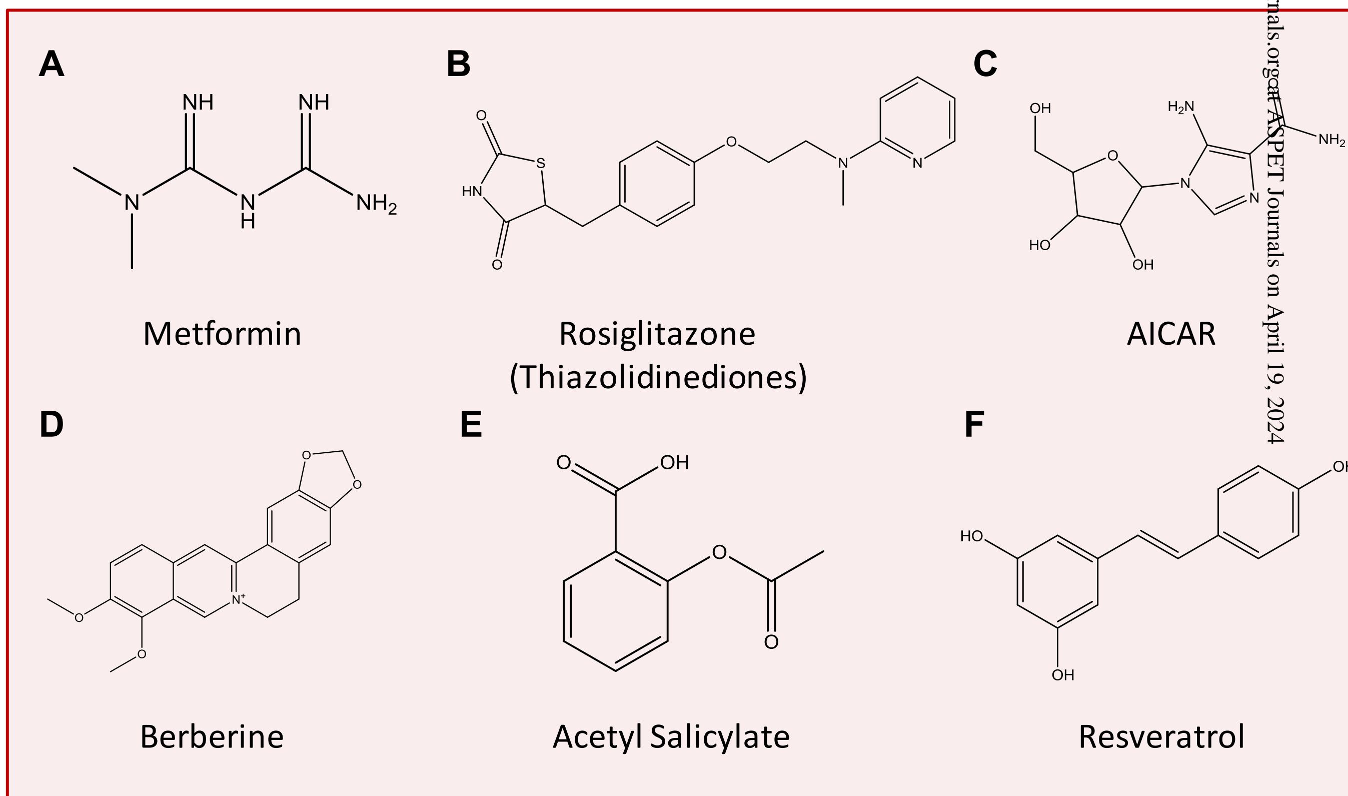

Figure 7

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