IRSTI 34.15.37

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# New Insights into the Activation Mechanisms of AMPK

**Abstract:** The AMP-activated protein kinase (AMPK) is a sensor of energy status in the cell. Upon activation in response to metabolic stresses, AMPK maintains cellular energy balance by promoting energy generating catabolic pathways and suppressing ATP-consuming anabolic processes.Historically, AMP, ADP-induced allosteric change accompanied by Thr $^{172}$ at a subunit considered as the major mechanism of AMPK activation. In the last decade, our understanding of the mechanism of AMPK activation has significantly advanced. Recently several studies have showed that, glucose starvation – major type of energetic stress activates AMPK independently of AMP or ADP levels in the cell and regardless of phosphorylation status of Thr $^{172}$ at a subunit. More interestingly, small AMPK pools in the cellular compartments are regulated differently and distinctly targets downstream effectors.

**Keywords:** AMPK, AMP, LKB1, CaMKK $\beta$ , AXIN, mTOR

DOI: https://doi.org/10.32523/2616-7034-2020-131-2-38-42

$AMPK$ (5'-AMP-activated Protein Kinase) is a highly conserved key energy sensor which regulates metabolic homeostasis at cellular and whole-body level [1]. $AMPK$ is activated by increased levels of $AMP$ and $ADP$ during conditions of low cellular energy caused by glucose or nutrient deprivation, exercise, or hypoxia [2]. Functional $AMPK$ is a heterotrimeric complex composed of one catalytic $\alpha$ subunit (a serine/threonine kinase), one scaffolding $\beta$ subunit, and one regulatory $g-$ subunit. Vertebrates contain multiple $\alpha(\alpha 1, 2), \beta(\beta 1, 2),$ and $\gamma(\gamma 1-3)$ subunits and thus express twelve potential $AMPK\alpha\beta\gamma$ complexes whose distinct functions remain poorly defined [3, 4]. Upon activation, $AMPK$ phosphorylates a diverse set of targets that help cells to restore the balance of energy generation and consumption in two ways. On the one hand, activated $AMPK$ attenuates $ATP$-consuming anabolic pathways (such as ribosome biogenesis, fatty acid, lipid, and protein synthesis, gluconeogenesis, and cell growth and proliferation) in order to restore normal energy balance [1, 5]. For example, $mTORC1$ (Mechanistic Target of Rapamycin Complex 1) is one of the best characterized downstream targets of $AMPK$ which regulates protein synthesis [6, 7]. During energy insufficiency, $AMPK$ transiently inhibits $mTORC1$ and shuts off one of the most energy-intensive processes– protein synthesis machinery [8]. On the other hand, $AMPK$ redirects cell metabolism towards $ATP$-generating pathways (such as fatty acid oxidation, autophagy, glucose utilization, glucose uptake, and mitochondrial biogenesis) [1, 5]. One of the best examples for this is the $ACC2$ (acetyl-CoA carboxylase-2). $ACC2$ increases the local pool of malonyl-CoA which inhibits fatty acid transporter carnitine and prevents fatty acids from entering the mitochondria [9]. $AMPK$ phosphorylates and inactivates mitochondrial isoform of $ACC2$ , thus acutely increase the uptake of fatty acids into mitochondria and promote fatty-acid oxidation to generate $ATP$ [10]. Dysregulation of AMPK signaling has been implicated in many metabolic disorders and cancer. $AMPK$ is a double-edged sword in cancer biology, itplays cancer promoting and/or tumor suppressing roles depending on the context [5]. $AMPK$ activation during exercise or in response to pharmacologic agonists decrease blood glucose level by suppressing gluconeogenesis in the liver and facilitating cellular glucose uptake [11]. Therefore, $AMPK$ agonist metformin (GlucoPhage) is the most widely prescribed oral medicine for Type 2 diabetes.

**Canonical activation of $AMPK$ by $AMP$ and $ADP$.** $AMP$ and $ADP$-mediated activation of $AMPK$ has long been considered to be the major mechanism, as it was named. It is the best studied and most appreciated mechanism.Upon energetic demand, $ATP$ is hydrolyzed to $ADP$ and $AMP$ , thus increasing the intracellular $AMP : ATP$ or $ADP : ATP$ ratio. $AMP$ and $ADP$

activate $AMPK$ by several mechanisms. First, direct binding of ADP or AMP to the three nucleotide binding sites on $\gamma$-subunit facilitates $\alpha$-subunit phosphorylation at the activation loop site (Thr$^{1}$72) by $LKB1$ (considered as a constitutively active kinase) or $CaMKK\beta$ [12, 13]. Second, binding of $AMP$ but not $ADP$ to the $g-$ subunit, causes an allosteric conformational change that activates $AMPK$ which contains an $\alpha-$ subunit already phosphorylated at Thr$^{1}$72 [14]. Third, binding of either $ADP$ or $AMP$ to the $\gamma$-subunit protects $\alpha$-subunit from de-phosphorylation at Thr$^{1}$72, thus maintains active state of $AMPK$ [15]. It is important to note that, for $AMPK$ activation, $AMP:ATP$ or $ADP:ATP$ ratio is more important than $AMP$ or $ADP$ concentration in the cell. Because $ATP$ antagonize the binding and allosteric activation of AMPK by AMP and ADP. Some of the pharmacologic agonists of AMPK act on AMPK by mimicking $AMP$ and/or $ADP$. For example, potent and the most commonly used drug $AICAR$ (5aminoimidazole4 carboxamide ribonucleotide) is converted $toAICAR$ 5'-monophosphate( $ZMP$ )e in the cell which mimics $AMP$ and allosterically activates $AMPK$ [16]. Cordycepin – an adenosine analog derived from *Cordyceps militaris*, recently shown to activate $AMPK$ by mimicking $AMP$ [17].

Canonical activation of $AMPK$ by $AMP$ or $ADP$ binding and new insights into the non-canonical activation of $AMPK$ will be discussed below.

**Non-canonical activation of $AMPK$.** Almost all cellular stimuli which increase cytosolic $Ca^{2+}$ concentration such as ER stress activate $AMPK$ through $CaMKK\beta$ ($Ca^{2+}$/calmodulin-dependent kinase) by phosphorylating at $AMPK$ Thr$^{172}$ [18]. Intracellular $Ca^{2+}$ forms a complex with $CaM$ and binds to $CaMKKb$ to activate it in a not well-defined mechanism. A recent study revealed that replication stress elevates intracellular $Ca^{2+}$ pool and activates $AMPK$ in the nucleus via $CaMKKb$ during $DNA$ replication and safeguards chromosome stability [19].

Crystallographic structure of $AMPK$ and biochemical methods have helped finding the new approaches to activate $AMPK$. Anarray of small molecule pharmacologic agents can allosterically activate $AMPK$ dependently or independently of $AMPK$ $Thr^{172}$ phosphorylation. The four $\gamma$-sites on $\gamma$-subunit in which three of them are binding sites for adenylate nucleotide, $\beta$-CBM (carbohydrate-binding module) on $\beta$-subunits, and $ADaM$ (Allosteric Drug and Metabolite) site on intact $AMPK\alpha\beta\gamma$ complex are attractive targets for small molecule AMPK-activators [20, 21]. One example of $\beta-CBM$-mediated agonist is aspirin active metabolite salicylate. Salicylate binds to the interface of $\beta-CBM$ and kinase domain of $\alpha$-subunit and prevents $AMPK\alpha$ $Thr^{172}$ from dephosphorylation [22]. Researchers found that some of the metabolic benefits of aspirin such as increased fatty acid oxidation, lowered cholesterol are mediated by salicylate-induced activation of $AMPK$ [22]. $A769662$, another recently discovered drug mimics both effects of $AMP$ to allosterically activates $AMPK$ and prevents it from $Thr^{172}$ dephosphorylation [23].

One of the most exciting findings of $AMPK$ activation is that glucose starvation can activate lysosomal $AMPK$ in a nucleotide-independent mechanism in parallel to the nucleotide-dependent activation. Increased intracellular level of $ADP$ and $AMP$ during glucose starvation have been considered as the mechanism of glucose starvation-inducedAMPK activation. But, recently, several studies revealed that $AMPK$ activity is regulated by the translocation of upstream kinase to the subcellular pool of $AMPK$ in response to glucose starvation without any associated change of the intracellular $AMP:ATP$ or $ADP:ATP$ ratios [24]. In mammalian cells, nucleotide-independent activation of $AMPK$ occurs exclusively on lysosomal membrane where Ragulator complex (comprising p18, p14, MP1, C7orf59 and HBXIP) serves as a docking site for co-localization of LKB1 with AMPK [25, 26]. A pool of AMPK permanently resides on lysosomal membrane mediated by the myristoylation on $\beta$-subunit [20]. Glucose starvation first activates aldolase and converts $FBP$ (fructose- 1,6-bisphosphate) into triose phosphates. Triose phosphates bind to $v-ATPase$ (Vacuolar $ATPase$) complex on the lysosomes and undergoes conformational change which enables $v-ATPase$ to interact with cytosolic $AXIN/LKB1$ complex and Ragulator complex. This way $LKB1$ and $AMPK$ co-localize where constitutively active $LKB1$ phosphorylates $AMPKThr^{172}$ and activates it [26]. This novel mechanism was a paradigm shifting in our understanding of $AMPK$ activation. This study also advanced our understanding of $AMPK$-mediated inhibition of $mTORC1$ occurs on lysosomes during glucose starvation. It was known that $mTORC1$ is recruited to lysosomal membrane via binding to Ragulator and activated by binding of GTP-bound Rheb [25].

Activation of $AMPK$ on lysosome partially explains $AMPK$-mediated inhibition of $mTORC1$ in two ways. First, upon glucose depletion, $AMPK$ is activated on lysosomes and phosphorylates Raptor at inhibitory $Ser^{792}$ site. Second, AMPK also phosphorylates and activates $TSC$ complex which inactivates Rheb [7, 26, 27].

Newly emerging data shows that distinct pools of $AMPK$ within a cell are differently activated and regulates distinct cellular processes [5, 27]. The detailed mechanism and function of differential regulation of $AMPK$ at subcellular compartments (lysosomes, mitochondria, nuclei, ER etc.), as well as the distinct AMPK complexes remain to be elucidated and it might help us for better understanding of the fine-tuning role of $AMPK$ in cellular and organismal metabolic homeostasis as a response for energy and nutrient availability. It also shed lights on the conflicting functions of $AMPK$ in cancer biology as its tumor-suppressive and tumor-promoting roles responding to the distinct environmental cues in distinct cell types and different stages of the cancer.

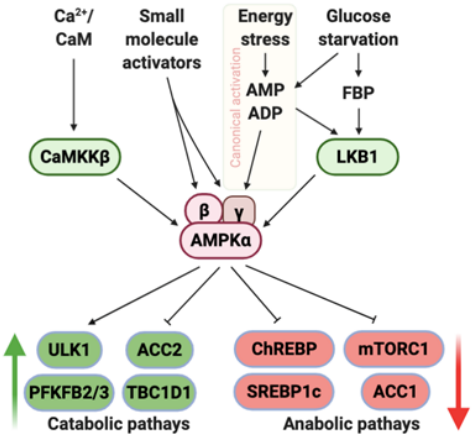


Figure 1 – $AMPK$ **Signaling. In response to diverse extracellular and intracellular cues,** $AMPK$ **is activated by canonical and non-canonical mechanisms. Upon activation, AMPK promotes catabolic pathways and suppresses anabolic processes by direct or indirect regulation of downstream effector pathways.**

## References

1 Hardie DG. $AMPK$ : positive and negative regulation, and its role in whole-body energy homeostasis//CurrOpin Cell Biol. -2015. -Vol. 33. -P. 1–7.

2 Huet C., et al. Glucose availability but not changes in pancreatic hormones sensitizes hepatic AMPK activity during nutritional transition in rodents//J. Biol. Chem. -2020. -Vol. 295(18). -P. 5836-5849.

3 Cheung PC., et al. Characterization of AMP-activated protein kinase $\gamma$-subunit isoforms and their role in AMP binding//Biochem. J. -2000. -Vol. 346. -P. 659–669

4 Thornton, C., et al. Identification of a novel AMP-activated protein kinase $\beta$ subunit isoform that is highly expressed in skeletal muscle//J. Biol. Chem. -1998. -Vol.273. -P. 12443–12450.

5 Gonzalez A., et al. AMPK and TOR: The Yin and Yang of Cellular Nutrient Sensing and Growth Control//Cell Metab. -2020. -Vol. 31(3). -P. 472–492.

6 Kim DH., et al. $mTOR$ interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery// Cell. -2002. -Vol. 110. -P. 163–175.

7 Inoki K., et al. TSC2 mediates cellular energy response to control cell growth and survival//Cell. -2003.-Vol. 115(5). -P.577–590.

8 Bolster DR., et al. AMP-activated protein kinase suppresses protein synthesis in rat skeletal muscle through down-regulated mammalian target of rapamycin (mTOR) signaling// J Biol Chem. -2002.-Vol. 277(27). -P. 23977–23980.

9 Abu-Elheiga L., et al. Continuous fatty acid oxidation and reduced fat storage in mice lacking acetyl-CoA carboxylase 2// Science. -2001. -Vol.291(5513). -P. 2613–2616.

10 Winder, WW., et al. Inactivation of acetyl-CoA carboxylase and activation of AMP-activated protein kinase in muscle during exercise//Am. J. Physiol. -1996. -Vol.270. -P. 299–304.

11 Steinberg GR., Carling D. AMP-activated protein kinase: the current landscape for drug development//Nat Rev Drug Discov. -2019. -Vol. 18(7). -P.527–551.

12 Hawley, SA., et al. 5'-AMP activates the AMP-activated protein kinase cascade, and Ca2+/calmodulin activates the calmodulin-dependent protein kinase I cascade, via three independent mechanisms//J. Biol. Chem. -1995. -Vol.270. -P.27186–27191.

13 Woods, A., et al. . Ca2+/calmodulin-dependent protein kinase kinase-beta acts upstream of AMP-activated protein kinase in mammalian cells//Cell Metab. -2005. -Vol.2. -P. 21–33.
14 Ross FA., et al. Differential regulation by AMP and ADP of AMPK complexes containing different g subunit isoforms// Biochem. J. -2016. -Vol.473. -P.189–199.
15 Davies SP., et al. 5'-AMP inhibits dephosphorylation, as well as promoting phosphorylation, of the AMP- activated protein kinase. Studies using bacterially expressed human protein phosphatase-2C alpha and native bovine protein phosphatase-2AC// FEBS Lett. -1995. -Vol.377. -P.421–425.
16 Corton JM., et al. 5-aminoimidazole-4-carboxamide ribonucleoside. A specific method for activating AMP-activated protein kinase in intact cells//Eur J Biochem. -1995. -Vol. 229. -P.558–565.
17 Hawley SA., et al. Mechanism of Activation of AMPK by Cordycepin//Cell Chem Biol. -2020. -Vol. 27(2). -P. 214–222.e4.
18 Hawley SA., et al. Calmodulin-dependent protein kinase kinase-beta is an alternative upstream kinase for AMP-activated protein kinase//Cell Metab. -2005. -Vol. 2. -P.9–19.
19 Li S., et al. Ca2+-Stimulated AMPK-Dependent Phosphorylation of Exo1 Protects Stressed Replication Forks from Aberrant Resection//Mol Cell. -2019. -Vol. 74(6). -P. 1123–1137.e6.
20 Oakhill JS., et al. $\beta$-Subunit myristoylation is the gatekeeper for initiating metabolic stress sensing by AMP-activated protein kinase (AMPK)//PNAS. -2010. -Vol. 107(45). -P. 19237–19241.
21 Xiao B., et al. Structural basis of AMPK regulation by small molecule activators//Nat. Commun. -2013. -Vol.4. -P.3017.
22 Hawley SA., et al. The ancient drug salicylate directly activates AMP-activated protein kinase//Science. -2012. -P. -Vol.336. -P.918–922.
23 Goransson O., et al. Mechanism of action of A-769662, a valuable tool for activation of AMP-activated protein kinase//J. Biol. Chem. -2007. -Vol.282. -P. 32549–32560.
24 Zhang CS., et al. Fructose-1,6-bisphosphate and aldolase mediate glucose sensing by AMPK// Nature. -2017. -Vol. 548. -P.112–116.
25 Sancak Y., et al. Ragulator-Rag complex targets mTORC1 to the lysosomal sur- face and is necessary for its activation by amino acids//Cell. -2010. -Vol.141. -P.290–303.
26 Zhang C.S., et al. The lysosomal v-ATPase-Ragulator complex is a common activator for AMPK and mTORC1, acting as a switch between catabolism and anabolism//Cell Metab. -2014. -Vol.20, -P.526–540.
27 Gwinn D.M., et al. AMPK phosphorylation of raptor mediates a metabolic checkpoint. Mol//Cell. -2008. -Vol. 30. -P.214–226.

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**$AMPK$ белсендіру механизмдері туралы жаңаша түсінік**

**Аңдатпа.** $AMP$-белсендірілген протеинкиназа ($AMPK$) – бұл жасушаның энергетикалық күйінің сенсоры. Метаболикалық күйзелістерге жауап ретінде белсендірілген кезде, $AMPK$ катаболикалық жолдармен энергия өндіруді ынталандыру және анаболикалық процестерді $ATP$ тұтынатын процестерді басу арқылы жасушалық энергия тепе-теңдігін сақтайды. Тарихи тұрғыдан қарағанда, $AMP, ADP$ - индукцияланған, $Thr^{172}$ $\alpha$ суббірлігімен бірге жүретін, $AMPK$ активтендірудің негізгі механизмі ретінде қарастырылатын аллостериялық өзгеріс. Соңғы онжылдықта $AMPK$ -ны іске қосу туралы түсінігіміз айтарлықтай алға жылжыды. Жақында бірнеше зерттеулер көрсеткендей, глюкозаның ашығуы - энергетикалық стресстің негізгі түрі-жасушадағы $AMP$ немесе $ADP$ деңгейіне қарамастан және $Thr^{172}$ $\alpha$ суббірлігінің фосфорлану дәрежесіне қарамастан $AMPK$-ны белсендіреді. Бір қызығы, жасуша компартменттеріндегі кішкентай $AMPK$ бассейндері әртүрлі жолмен реттеледі және төменгі эффекторларға нақты бағытталған.

**Түйін сөздер:** $AMPK$, $AMP$, $LKB1$, $CaMKK\beta$, $AXIN$, $mTOR$.

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**Новое понимание механизмов активации $AMPK$**

**Аннотация.** $AMP$-активированная протеинкиназа ($AMPK$) является датчиком энергетического статуса клетки. При активации в ответ на метаболические стрессы $AMPK$ поддерживает клеточный энергетический баланс, стимулируя выработку энергии катаболическими путями и подавляя $ATP$ -потребляющие анаболические процессы. Исторически сложилось так, что $AMP$, $ADP$-индуцированное аллостерическое изменение, сопровождаемое субъединицей $Thr^{172}$ $\alpha$, рассматривалось как основной механизм активации $AMPK$. В последнее десятилетие наше понимание механизма активации $AMPK$ значительно продвинулось вперед. Недавно несколько исследований показали, что глюкозное голодание - основной тип энергетического стресса - активирует $AMPK$ независимо от уровня $AMP$ или $ADP$ в клетке и независимо от статуса фосфорилирования субъединицы $Thr^{172}$ $\alpha$. Более интересно, что небольшие пулы $AMPK$ в клеточных компартментах регулируются по-разному и отчетливо нацелены на нижестоящие эффекторы.

**Ключевые слова:** $AMPK$, $AMP$, $LKB1$, $CaMKK\beta$, $AXIN$, $mTOR$.

## References


1 Hardie DG. AMPK: positive and negative regulation, and its role in whole-body energy homeostasis, *CurrOpin Cell Biol.* 33, 1–7 (2015).

2 Huet C., et al. Glucose availability but not changes in pancreatic hormones sensitizes hepatic AMPK activity during nutritional transition in rodents, *J. Biol. Chem.*, 295(18), 5836-5849(2020).

3 Cheung PC., et al. Characterization of AMP-activated protein kinase $\gamma$-subunit isoforms and their role in AMP binding, *Biochem. J.*, 346, 659–669(2000).

4 Thornton C., et al. Identification of a novel AMP-activated protein kinase $\beta$ subunit isoform that is highly expressed in skeletal muscle, *J. Biol. Chem.,* 273, 12443–12450(1998).

5 Gonzбlez A., et al. AMPK and TOR: The Yin and Yang of Cellular Nutrient Sensing and Growth Control, *Cell Metab*, 31(3), 472–492(2020).

6 Kim DH., et al. mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery, *Cell.*, 110, 163–175(2002).

7 Inoki K., et al. TSC2 mediates cellular energy response to control cell growth and survival. *Cell.* 115(5), 577–590(2003).

8 Bolster DR., et al. AMP-activated protein kinase suppresses protein synthesis in rat skeletal muscle through down-regulated mammalian target of rapamycin (mTOR) signaling, *J Biol Chem.*, 277(27), 23977–23980(2002).

9 Abu-Elheiga L., et al. Continuous fatty acid oxidation and reduced fat storage in mice lacking acetyl-CoA carboxylase 2, *Science,* 291(5513), 2613–2616(2001).

10 Winder, WW., et al. Inactivation of acetyl-CoA carboxylase and activation of AMP-activated protein kinase in muscle during exercise, *Am. J. Physiol,* 270, 299–304(1996).

11 Steinberg GR., Carling D. AMP-activated protein kinase: the current landscape for drug development, *Nat Rev Drug Discov*, 18(7), 527–551(2019).

12 Hawley SA., et al. 5'-AMP activates the AMP-activated protein kinase cascade, and Ca2+/calmodulin activates the calmodulin-dependent protein kinase I cascade, via three independent mechanisms, *J. Biol. Chem.* 270, 27186–27191(1995).

13 Woods, A., et al. Ca2+/calmodulin-dependent protein kinase kinase-beta acts upstream of AMP-activated protein kinase in mammalian cells, *Cell Metab.* 2, 21–33(2005).

14 Ross, FA., et al. Differential regulation by AMP and ADP of AMPK complexes containing different g subunit isoforms, *Biochem. J.* 473, 189–199(2016).

15 Davies, SP., et al. 5'-AMP inhibits dephosphorylation, as well as promoting phosphorylation, of the AMP- activated protein kinase. Studies using bacterially expressed human protein phosphatase-2C alpha and native bovine protein phosphatase-2AC. *FEBS Lett.* 377, 421–425(1995).

16 Corton JM., et al. 5-aminoimidazole-4-carboxamide ribonucleoside. A specific method for activating AMP-activated protein kinase in intact cells?, *Eur J Biochem.* 229, 558–565(1995).

17 Hawley SA., et al. Mechanism of Activation of AMPK by Cordycepin, *Cell Chem Biol.* 27(2), 214–222.e4(2020).

18 Hawley, SA., et al. Calmodulin-dependent protein kinase kinase-beta is an alternative upstream kinase for AMP-activated protein kinase. *Cell Metab.* 2, 9–19(2005).

19 Li, S., et al. Ca2+-Stimulated AMPK-Dependent Phosphorylation of Exo1 Protects Stressed Replication Forks from Aberrant Resection, *Mol Cell.* 74(6),1123–1137.e6(2019).

20 Oakhill JS., et al. $\beta$-Subunit myristoylation is the gatekeeper for initiating metabolic stress sensing by AMP-activated protein kinase (AMPK). *PNAS.* 107(45), 19237–19241(2010).

21 Xiao, B., et al. Structural basis of AMPK regulation by small molecule activators. *Nat. Commun.* 4, 3017(2013).

22 Hawley, SA., et al. The ancient drug salicylate directly activates AMP-activated protein kinase. *Science.* 336, 918–922(2012).

23 Goransson, O., et al. Mechanism of action of A-769662, a valuable tool for activation of AMP-activated protein kinase. *J. Biol. Chem.* 282, 32549–32560(2007).

24 Zhang, CS., et al. Fructose-1,6-bisphosphate and aldolase mediate glucose sensing by AMPK. *Nature.* 548, 112–116(2017).

25 Sancak, Y., et al. Ragulator-Rag complex targets mTORC1 to the lysosomal sur- face and is necessary for its activation by amino acids. *Cell.* 141, 290–303(2010).

26 Zhang, C.S., et al. The lysosomal v-ATPase-Ragulator complex is a common activator for AMPK and mTORC1, acting as a switch between catabolism and anabolism, *Cell Metab.* 20, 526–540(2014).

27 Gwinn, D.M., et al. AMPK phosphorylation of raptor mediates a metabolic checkpoint. Mol. *Cell.* 30, 214–226(2008).



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*Received 14.04.2020*