

Invited Mini Review

Upstream signalling of mTORC1 and its hyperactivation in type 2 diabetes (T2D)

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Mammalian target of rapamycin complex 1 (mTORC1) plays a major role in cell growth, proliferation, polarity, differentiation, development, and controls transitioning between anabolic and catabolic states of the cell. It collects almost all extracellular and intracellular signals from growth factors, nutrients, and maintains cellular homeostasis, and is involved in several pathological conditions including, neurodegeneration, Type 2 diabetes (T2D), obesity, and cancer. In this review, we summarize current knowledge of upstream signaling of mTORC1 to explain etiology of T2D and hypertriglyceridemia, in which state, the role of telomere attrition is explained. We discuss if chronic inhibition of mTORC1 can reverse adverse effects resulting from hyperactivation. In conclusion, we suggest the regulatory roles of telomerase (TERT) and hexokinase II (HKII) on mTORC1 as possible remedies to treat hyperactivation. The former inhibits mTORC1 under nutrient-rich while the latter under starved condition. We provide an idea of TOS (TOR signaling) motifs that can be used for regulation of mTORC1. [BMB Reports 2017; 50(12): 601-609]

INTRODUCTION

The mammalian target of rapamycin (mTOR), is conserved in all eukaryotes including plants, worms, flies, and mammals (1). mTOR comprises two complexes, mTORC1 and mTORC2, among which mTORC1 is directly regulated by nutrient status of the cell. mTORC2 is indirectly regulated by RTK and activation occurs after activation of mTORC1 (2, 3). Mammalian mTORC1 is mainly composed of mTOR, Raptor, and GβL; the complex serves as a staple hub for upstream signaling (4).

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Nutrient-rich conditions facilitate translocation of mTORC1 to the lysosome, and thus regulate activation of this complex (5, 6). This complex receives intracellular and extracellular signals and controls cell size, growth, and proliferation by performing the anabolic function of protein synthesis, lipid synthesis, and mitochondrial metabolism (7-9).

mTORC1 activity is regulated by a plethora of upstream signaling elements. It collects upstream signals from growth factors, stress, energy, oxygen, glucose, and amino acids and promotes synthesis of proteins, lipogenesis, lysosome biogenesis, and activates energy metabolism (7, 8, 10). Newly discovered elements TERT and HKII inhibit mTORC1 activity under amino acid and glucose starvation, respectively (Fig. 1) (11, 12).

Diabetes is associated with obesity linked with prolonged intake of high energy diet. Dietary proteins generate circulating amino acids that activate the mTORC1 (13-15). The condition causes mTORC1 hyperactivation that over a prolonged period leads to insulin resistance, hypertriglyceridemia, and hyperlipidemia (16).

Loss of chromosomal DNA by telomere attrition has deleterious effect on numerous cellular functions. Telomere shortening leads to DNA damage, cellular senescence, and apoptosis that is linked with aging disorders (17). Short telomeres play an important role in pathogenesis and disease progression of T2D. Short telomeres increase probability of beta-cell senescence, reduce insulin secretion, and exhibit mitochondrial dysfunction (18, 19).

The purpose of this review is to discuss signaling molecules and external factors that affect mTORC1 regulation. We summarize current knowledge of how mTORC1 hyperactivation leads to T2D diabetes and hypertriglyceridemia. We added the role of telomerase attrition in T2D. Finally, we discussed a possibility if pharmacological inhibition of mTORC1 can reverse incidence of T2D. We suggested that inhibition of mTORC1 as well as restriction of mTORC1 may be useful in treating hyperactivation of this complex in T2D.

UPSTREAM REGULATORS OF mTOR COMPLEX 1

Amino acids

Amino acids regulate mTORC1 through different signaling elements. Among amino acids, leucine plays an important role

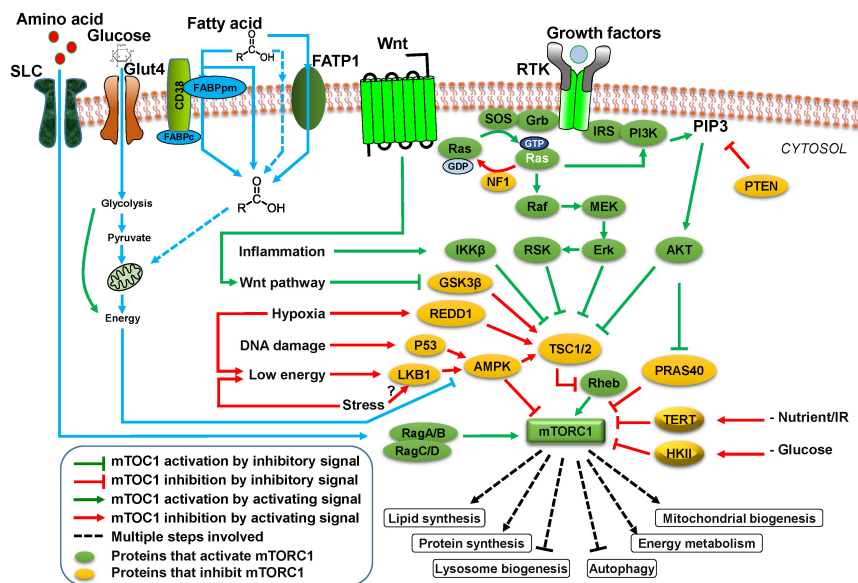


Fig. 1. mTOR signaling pathway and its upstream elements. Amino acids, glucose, fatty acids, Wnt ligand, growth factors, hormones, stresses, energy status, and oxygen are key factors that affect mTORC1 activity. Upon activation, mTORC1 facilitates the synthesis of protein, lipids, and energy metabolism. It inhibits autophagy.

in mTORC1 activation by inhibiting Sestrin 1/2 (20, 21). mTORC1 is stimulated by leucine and glutamine in Rag GTPase-dependent and independent fashion, respectively. Glutamine stimulates mTORC1 in RagA and RagB double knockout cells while existence of v-ATPase (proton pump) is required. Glutamine-mediated stimulation of mTORC1 requires adenosine diphosphate ribosylation factor (ARF)-1 GTPase for appropriate regulation (22).

Rag family of GTPases is one of the crucial links between amino acids and mTORC1. First group consisting of RagA and RagB binds to GTP while the second group of RagC and RagD has affinity for GDP. Upon nutrient provision, each member of the group can make heterodimer only with a member of another group (RagA-RagC or RagA-RagD; RagB-RagC or RagB-RagD) (23, 24). Sestrin1/2 interacts with GATOR2 to inhibit mTORC1 signaling (Fig. 1) (21, 25, 26). In this context, SLC38A9 is one of the strong candidates for sensing arginine at lysosome (27).

Growth factors

In higher eukaryotes, cell growth and proliferation rely on long-range communication to coordinate distribution of nutrients (1, 28). Phosphatidylinositol 3-kinase (PI 3-kinase)-dependent pathway regulates mTORC1 and is affected by insulin. Thus mTORC1 mediates crosstalk between amino acids and insulin signaling (Fig. 1) (29). Communication is mediated by growth factors such as insulin, PDGF, VEGF, EGF, and IGF1 (30). Insulin and IGF, when binding to receptors, activate mTORC1 through PI3K and AKT. Insulin and other growth factors bind RTK stimulating recruitment and activation of PI3K. Phosphatidylinositol-3,4,5-trisphosphate (PIP3) is generated by action of PI3K activity, that activates

AKT (30).

AKT phosphorylates TSC2 and inactivates its complex with TSC1 (31). The active complex has GTPase activity and converts Rheb-bound GTP into GDP and thus inactivates Rheb-mediated activation of mTORC1 (32). ERK inhibits TSC1/2 to relieve activity of mTORC1 activity as well as activates RSK (ribosomal s6 kinase) resulting in phosphorylation-mediated increased activity of RAPTOR (30). AKT phosphorylates TSC2 and inactivates its complex formation with TSC1 (31).

Glucose, fatty acid, and energy status

All cellular processes need energy in the form of ATP. Being a major regulator of growth and proliferation, it is logical that mTORC1 activity must be under the control of energy status of the cell (33). Glycolysis, citric acid cycle, β -oxidation, and oxidative respiration all lead to conversion of nutrients into ATP (34-36). Upon nutrient scarcity, ATP level of cells quickly fall and AMPK is stimulated (37), subsequently activates and inactivates TSC2 (38) and Raptor (39), respectively. This mechanism provides an AMPK-facilitated pathway for mTORC1 to sense ratio of AMP/ATP.

mTORC1 signaling inhibits fatty acid oxidation. Ketone bodies are produced as a result of acetyl-CoA released from β -oxidation, that either enter TCA cycle or, under nutrient deficiency into the liver (40). Glucose passes through transporters and after being converted into energy, inhibits AMPK, subsequently reviving mTORC1 (41, 42). Fatty acids are transported through specific transporters such as fatty-acid-transport protein (FATP) and fatty-acid-binding protein (FABP) families, eventually entering the citric acid cycle to produce energy and activate mTORC1 (Fig. 1) (43-45).

Glucose entering cells after passing through glycolysis are subjected to Krebs cycle inside the mitochondria. When energy status of the cell is stabilized, AMPK & TSC1/2 are inhibited and mTORC1 activity is revived.

mTORC1 REGULATION BY ITS INTERACTING PARTNERS

mTORC1 is affected by a plethora of factors such as energy

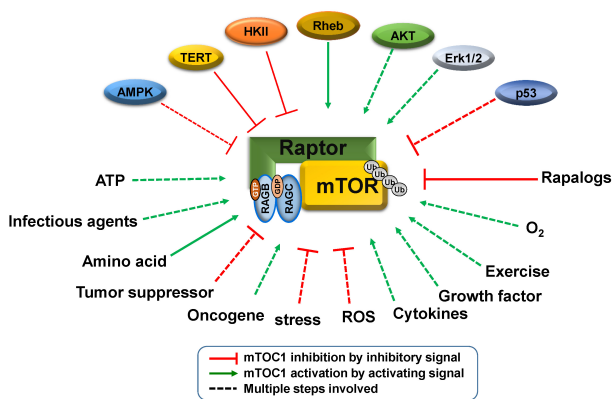


Fig. 2. mTORC1 interacting proteins and external factors that affect activity of this complex. mTORC1 is regulated by a plethora of gene products. AMPK, TERT, HKII, and p53 have inhibitory effect while Rheb, AKT, and ERK activate the effect on this complex. Almost all kinds of stress and nutrients regulate activity of mTORC1. Cellular nutrients, energy status, and growth factors affect mTORC1 activity.

status, O₂ level, cytokines, ROS and many more (30). All these factors affect mTORC1 signaling that leads to cellular growth conditions by regulating metabolic processes (Fig. 2).

mTORC1 interacting elements such as p70S6K and 4E-BP1 bind to RPTOR (Regulatory-associated protein of mTOR) through their TOS motif mTOR signaling motif) (46). Immunoprecipitation study revealed that HK-II links to and restricts the autophagy suppressor, mTOR complex 1 (mTORC1), and this binding is promoted in hypoglycemic condition (47). Similarly, TERT restricts mTORC1 under amino acid starvation (11).

mTORC1 suppression by p53 requires TSC1 and TSC2. Formation of TSC1/TSC2 complex is mandatory for p53-dependent mTORC1 inhibition. p53 stimulation has potential to inhibit activity of mTORC1 through a pathway analogous to the withdrawal of energy. This uncommon regulatory pathway is crucial for and contributes to tumor suppressive roles of p53 (48). There can be an alternative approach: TERT has a regulatory (like a rheostat) effect on mTORC1. TERT binds and restricts activated mTORC1 and it is detached from the inactivated complex (11). Some regulatory proteins need to have a TOS consensus sequence [F (D/E) (F/I/L/M) (D/E) (L/I)] to bind to Raptor leading to presence in mTORC1 (46, 47).

ROLE OF mTORC1 IN DIABETES AND HYPERTRIGLYCERIDEMIA

Diabetes mellitus (DM) is a heterogeneous metabolic disorder of chronic hyperglycemia (49). Among total diabetic patients, the ratio of patients with insulin dependent diabetes mellitus (Type 1, IDDM) is only 5-10% with a major cause of the

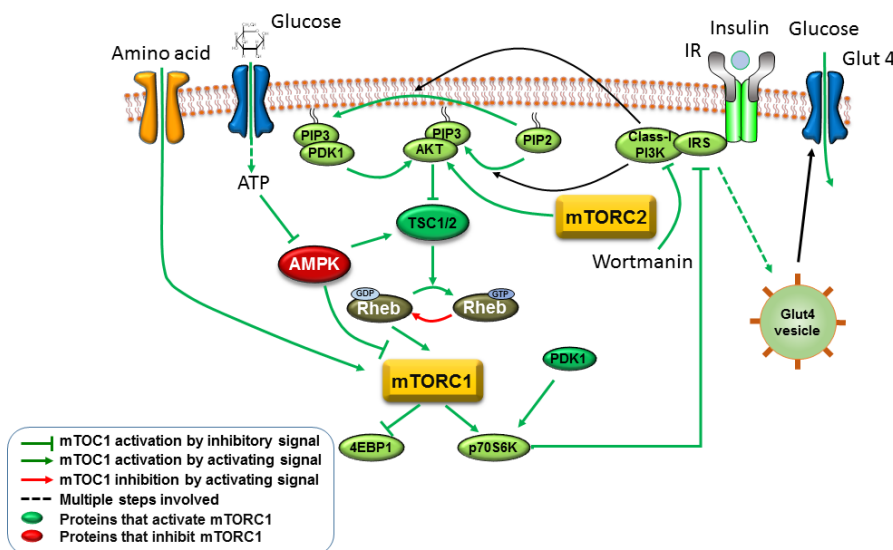


Fig. 3. Insulin resistance by inhibition of IRS under constitutive activation of mTORC1. mTORC1 is activated from high energy diet through uptake of amino acids and glucose. High activity of mTORC1 over a long period inhibits IRS through p70S6K. Thus, IRS loses its capability for transfer of glucose transporters to the cell surface.

destruction of β -cells of the pancreas by cell-mediated autoimmune responses (50). Whereas 90-95% patients are those with T2D having insulin deprivation or resistance are termed as non-insulin dependent diabetes mellitus (NIDDM) or adult-onset diabetes (50). Rapid increase in T2D prevalence worldwide has been associated with a Western, obesogenic lifestyle (51).

It is important to consider that diabetes is linked to more than one organ and diabetic cardiomyopathy is reported to cause heart attack, a leading cause of morbidity and mortality in diabetic patients. Metabolic profiles of diabetic patients are highly disturbed, having increased level of glucose and lipids, causing hyperglycemia and hyperlipidemia, respectively, as a result of insulin resistance (52-54).

In mammals, the liver is the main organ that controls physiology of the whole body in response to nutrients (55). Hyperactivation of mTORC1 regulates insulin and growth factor signaling through insulin receptor substrates (IRS) (56). mTORC1 has been associated with Type 1 and T2D (56). Class I PI3-Kinases are key components of the insulin signaling pathway (57, 58). Prolonged activation of mTORC1, under high energy diet, inhibits IRS through p70S6K (Fig. 3) (59). This pathway renders IRS incapable of transferring glucose transporters onto the cell surface, increasing blood glucose level (60-62). This finally leads to T2D.

Hypertriglyceridemia is triggered by activation of hepatic mTORC1/S6k activation (63). Intake of surplus energy in the form of fat and protein is the root cause of metabolic imbalances and metabolic disorders that promote obesity (64). Amino acids produced from dietary proteins, directly enter in the cytoplasmic circulation contribute to activation of mTORC1-p70S6K pathway through several signaling arrays (13-15). Additionally, other intracellular and extracellular signals, such as growth factors, oxygen (O_2), tension, and energy levels, induce mTORC1 signalling (65). The factors are shown above (Fig. 2).

Expression of the sodium-coupled neutral amino acid transporter (SNAT2) provokes the mTORC1-p70S6K pathway and increases serum triglycerides (TGs) while reducing adipose lipoprotein lipase (LPL). Similarly, expression of hepatic Rheb (Ras-homolog enriched in brain) or active-S6K produces the same metabolic effects, while expression of dominant-negative-p70S6K inhibits increase of hepatic TG in liver-specific SNAT2-expressing mice. Hypertriglyceridemia and adipose LPL up-regulation are transduced between liver and adipose tissue using a neuronal passage comprising afferent vagal and efferent sympathetic nerves (65). Unsaturated AA's stimulate mTORC1 involved in developing insulin resistance and obesity (66). Activation of mTORC1 for a lengthy time promotes insulin resistance and potentially exacerbating obesity triggering lipid deposits (7).

Association of the mTORC1-p70S6K pathway with lipid metabolism is the point of interest that contributes to fatty acid biosynthesis (66). mTORC1 is mandatory for denovo lipid

synthesis in murine liver (67). mRNA and protein expression of main gluconeogenic enzymes, in specimens of human liver, revealed that levels of only pyruvate carboxylase protein have strong relation with glycaemia. Pyruvate carboxylase specific antisense oligonucleotide (ASO) does not disturb de novo synthesis of fatty acid, lipolysis, or fatty acid oxidation of liver cells (68). High-fat diet increases endogenous glucose production (69).

Excessive synthesis of very-low-density lipoproteins (VLDL), accompanying greater release of triglyceride & apolipoprotein B100 (apoB100), is central to excess plasma VLDL-TG levels in insulin-resistant diabetic patients (70). Prolonged hyperinsulinemia predisposes liver for insulin resistance that leads to inability of insulin to trigger an increased signal at insulin receptor substrate-2 (71). Up-regulation of sterol regulatory element-binding protein 1c (SREBP-1c) occurs leading to increased lipid synthesis (71). Thus, hyperinsulinemia may be a pivotal cause of hepatic insulin resistance associated with steatosis. Failure of insulin action on skeletal muscle and the liver leads to hyperglycemia (7).

At least three outcomes are implicated with over nutrition-mediated prolonged hyperactivation of mTORC1. As the first outcome, IRS stops responding to insulin signaling, leading to high blood glucose and consistent production of glucose in liver cells (59). Second, there is increased hyperlipidemia and hypertriglyceridemia, causing insulin resistance and overproduction of hepatic glucose that at later stages are converted into fatty acids and cellular lipids deposits (72, 73). Third, liver communicates to brain through vagal nerve and then to white adipose tissues through sympathetic nerves. As a result of this inter-tissue communication, lipoprotein lipases in the blood stream decreases and triglyceride level increases (63).

TELOMERE ATTRITION AND DIABETES

Telomeres are maintained by a plethora of factors including epigenetic, genetic, environmental, and several unknown events (74, 75). Specific diets such as grains, vegetables, and fruits have antioxidant and anti-inflammatory properties and may positively reduce telomere shortening (76-79). Increasing body mass index (BMI), through increased inflammation, has inverse relation with telomere length (80). Telomere length has been decreasing by 7 base pairs per unit increase in BMI (kg/m^2). It decreases with increase of C-reactive protein (CRP), revealing that inflammation plays a major role in telomere attrition (81). Several studies have revealed that erosion of the telomerase system is associated with both types of diabetes. Short leukocyte telomere length (LTL) is associated with T1D (82). Increasing of oxidative stress, increases aging and obesity that is directly proportional to telomere attrition in T1D and T2D (83). Chronic hyperglycemia and nutritional overload act together to increase oxidative stress leading to increase in activity of the protein kinase C pathway. This affects insulin

signaling, as well as secretes pro-inflammatory cytokines (84).

In brief, we can say that short telomeres implicate with diabetes, and may play an important role in pathogenesis and severity of T2D. Shorter telomeres increase probability of beta-cell senescence, leading to reduced cell mass and decreased insulin level (85). Mice with short telomeres reveal disturbed metabolism through mitochondrial dysfunction (85). In this context, deletion of TERC exhibited a prominent effect. Additionally, telomere shortening may attenuate calcium-mediated insulin exocytosis (85). Finally, inhibition of p53 activity can reverse cellular senescence of adipocyte and insulin resistance (86).

CHRONIC INHIBITION AND RESTRICTION OF mTORC1 BY TERT AND HKII

Rapamycin partially protects against insulin resistance, *in vivo* (87). It was suggested that rapamycin may serve as a promising drug to control hyperactive mTORC1 and insulin resistance in obesity. But prolonged suppression of mTORC1-p70S6K signaling by rapamycin treatment upsets lipid and glucose metabolic rate (88). Chronic intake of rapamycin causes hyperlipidemia and stimulates glucose intolerance (89-91). In conclusion, rapamycin induces a diabetic condition, promoting insulin resistance and dropping β -cell function and mass (91, 92).

TERT binds and restricts mTORC1, but binding decreases

when mTORC1 is alternatively inhibited by use of rapamycin (11, 93, 94). This indicates that TERT predominately binds to the activated form of mTORC1. Recently, mTORC1 inhibiting effect of TERT has been explored (11, 12). In this context, we may choose an example of TERT with a regulatory effect on mTORC1. Similarly, the restricting effect of HKII (47) can be used to control hyperactivation of mTORC1. Similarly, HKII binds and inhibits mTORC1 upon glucose starvation where its TOS motif is required for this activity (47). It is important to note that restriction of mTORC1 by TERT and HKII follows opposite nutrient status. It appears that TERT will work under high nutrient status and HKII will be effective under starvation. In addition to TERT and mTORC1, the function of TOS motifs of Deptor and PRAS 40 can be tested for regulatory effect on kinase activity of the complex (Table 1).

We conclude that mTORC1 hyperactivation may lead to insulin resistance, hypertriglyceridemia, and diabetes. Chronic inhibition of mTORC1 exacerbates the pathological condition. Therefore, we propose that hyperactivation of mTORC1 can be controlled by using a specific domain or full length TERT and/or HKII. Further studies are required to identify functional domains of TERT and HKII. This may lead to an effective drug discovery that can regulate mTORC1 activity.

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Table 1. Raptor- interacting proteins

Protein	TOS motif	Bioinformatics	Co-IP with Raptor	Effect on mTORC1
	TOS motif		F (D/E)(F/I/L/M) (D/E)(L/I) (62)	
Raptor	No	—DLLGR <u>F</u> LDLGPWAV— ^{a,b}	NA	Agonist (95)
S6K	Yes	—MAGV <u>F</u> DIDLDQPE— ^c	Yes (97)	Substrate (60)
4EBP1	Yes	—EESQ <u>F</u> EMD—	Yes (97)	Substrate (46)
HKII	Yes	—RRGD <u>F</u> DIDVAVV—	Yes (62)	Antagonist (47)
Sqstm1	No	—LGIE <u>V</u> DIDVEHGG—	Yes (5)	Agonist (5, 6)
TRAF6	No	—YDVE <u>F</u> DPPL ^L ESKY—	Yes (6)	Agonist (5)
mTOR	No	—LIYV <u>F</u> DVESRELE—	Yes (99)	Agonist (99)
TERT	No	—SSGL <u>F</u> DVFLRFMC—	Yes (94)	Antagonist (11, 12)
Rag A (GTP)	No	—LIYV <u>F</u> DVESRELE—	Yes (21)	Agonist (23, 100)
Rag C (GDP)	No	—PDMN <u>F</u> EVFIHKVD—	Yes (21)	Agonist (23, 100)
Rag B (GTP)	No	—NTKT <u>F</u> DVEHSHVR—	Yes (21)	Agonist (23, 100)
Rag D (GDP)	No	—TDIN <u>F</u> EVFIHKVD—	Yes (21)	Agonist (23, 100)
Deptor	No	—GAQQ <u>R</u> ELERMAEV—	Yes (102)	Antagonist (101)
PRAS40	No	—NGGL <u>F</u> VMDEDATL—	Yes (102, 103)	Antagonist (102)
mLST8	No	—LWCV <u>E</u> TGEIKREY—	Yes (102)	Not yet found (95)
Rheb	No	—SIKS <u>F</u> EVIVIHG—	Yes (104)	Agonist (103)
Hsp90	No	—RRAP <u>F</u> DLFENRKK—	Yes (105)	Agonist (104)

^aThe amino acid differing from the TOS motif are underlined.

^bYellow highlighted motifs differ from TOS motif with respect to one or more amino acids.

^cGreen highlighted motifs exactly match with TOS motif.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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