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Review

The mammalian target of rapamycin pathway and its role in molecular nutrition regulation

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Mammalian target of rapamycin (mTOR) is a protein serine-threonine kinase that functions as a central element in signaling pathway involved in control of cell growth and proliferation. mTOR exists in at least two distinct multi-protein complexes, mTORC1 and mTORC2. mTOR kinase controls the translation machinery, in response to nutrients and growth factors, via activation of p70 ribosomal S6 kinase and inhibition of eukaryotic initiation factor-4E-binding protein. In this report, we review the mTOR signaling pathway and its interaction with food intake, insulin resistance, lifespan and adipogenic regulation during the molecular nutrition regulation.

Keywords: Eukaryotic initiation factor-4E-binding protein / Mammalian target of rapamycin / Nutrition regulation / Rapamycin / Ribosomal S6 kinase 1

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1 Introduction

Much of our knowledge of the mammalian target of rapamycin (mTOR) is based on the use of the drug rapamycin. Rapamycin has had a story book trajectory: emerging in the 1970s from the soil of Easter Island, playing the starring role in the discovery of a fundamental biological pathway and rising to its current status as an important drug [1], Rapamycin is a specific and potent inhibitor of mTOR that has been extensively employed to dissect TOR signaling in yeast and higher eukaryotes [2].

mTOR is an evolutionarily conserved serine-threonine protein kinase that belonged to the phosphoinositide 3-kinase (PI3K)-related kinase (PIKK) family [3], which contains a lipid kinase-like domain within their C-terminal region, and it plays an important role in regulating cell growth and proliferation. mTOR is a central signal integrator that receives signals arising from growth factors, nutrients, and cellular energy metabolism [3, 4].

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Abbreviations: **4E-BP**, elF4E-binding protein; **eIF**, eukaryotic initiation factor; **FKBP**, FK506-binding protein; **FRB**, FKBP-rapamycin binding; **mTOR**, mammalian target of rapamycin; **p70S6K**, p70 ribosomal S6 kinase; **RAFT**, rapamycin and FKBP target; **raptor**, regulatory associated protein of mTOR; **Rheb**, ras homologue enriched in brain; **rictor**, rapamycin-independent companion of mTOR; **S6K1**, ribosomal S6 kinase 1

Although the mechanisms through which mTOR signals and how the activity of mTOR is controlled are poorly understood, the signaling protein mTOR regulates both cell growth and cell-cycle progression and as such is recognized as an evolutionarily conserved central coordinator of these fundamental biological processes [5 – 7]. Within the past year, a spate of reports from several laboratories has dramatically advanced our understanding of the mTOR signaling pathway. In this review, we summarize some of the important recent developments in this fast-evolving field and some discoveries of mTOR-associated proteins and effectors. We also describe the roles of mTOR in control of cell growth and proliferation and lifespan during molecular nutrition regulation.

2 The mTOR protein

TOR was originally identified genetically by mutations in yeast, TOR1 – 1 and TOR2 – 1 that confer resistance to the growth-inhibitory properties of the immunophilin – immunosuppressant complex FK506-binding protein (FKBP)-rapamycin [8]. The TOR1 and TOR2 genes encode the two large (molecular weight ~280 kDa) and highly homologous (70% identical) TOR1 and TOR2 proteins [9, 10]. In metazoans, a single TOR gene is the rule rather than the exception. The mammalian TOR protein, mTOR [also known as FRAP (FKBP-rapamycin associated protein), RAFT (rapamycin and FKBP target), or RAPT (rapamycin target)] was subsequently discovered biochemically based on its FKBP-rapamycin binding properties [11 – 14]. Mammalian

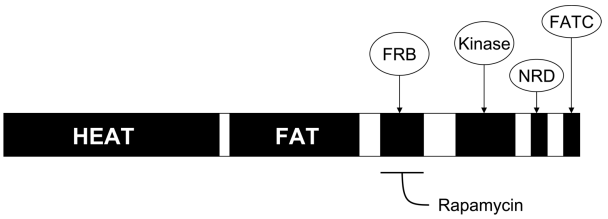

Figure 1. Domain structure of the TOR. The positions of the known functional domains of the TOR are depicted, including the C-terminal domain, the adjacent FRB domain, the central toxic effector domain, the repressor domain that is unique to mTOR, and the N-terminal HEAT repeats.

genomes, as well as those of other metazoans, encode a single TOR protein with a similar structure exhibiting ~42 and 45% amino acid sequence identity to the yeast TOR1 and TOR2 proteins, respectively. mTOR contains 2549 amino acids and comprises several conserved structural domains. Except for the putative regulatory domain (RD), the general domain structure of mTOR is similar to that of yeast TOR proteins (Fig. 1).

The mTOR contains a C-terminal region with strong homology to the catalytic domain of phosphatidylinositol 3-kinase (PI3K). However, mTOR functions as a protein kinase [15, 16]. Indeed, it is a founding member of a family of serine-threonine (Ser-Thr) protein kinases that are more similar to the lipid kinase PI3K than to members of the larger family of Ser-Thr or Tyr protein kinases. The FKBP-rapamycin complex, which inhibits mTOR function, interacts with the FRB (FKBP-rapamycin binding) domain in mTOR, adjacent to the catalytic kinase domain. The N-terminal region of mTOR contains up to 20 tandemly repeated HEAT motifs, grouped in two blocks and important for protein-protein interaction [17]. Each HEAT motif comprises approximately 40 amino acids that form a pair of antiparallel a helices. Other two domains, called FAT (FRAP–ATM–TRAPP) and FATC (FAT C terminus), have been proposed to mediate interactions in multi protein complexes [18, 19]. The FATC domain occurs only in combination with the FAT domain and may be important for catalytic activity of PIK-related kinases. In fact, the large size of mTOR suggests it may interact with other regulatory or scaffold proteins, which can modulate its activity and signaling pathway.

3 Two mTOR complexes

All the above-mentioned reviews [1, 3–8] pertain to growth control in single cells, yeast and cultured mammalian cells. However, control of cell growth is also important in determining overall organ and body size in multicellular systems [7]. Animals attain characteristic body size and proportions via the coordinate regulation of cell growth, cell proliferation, and cell death [20].

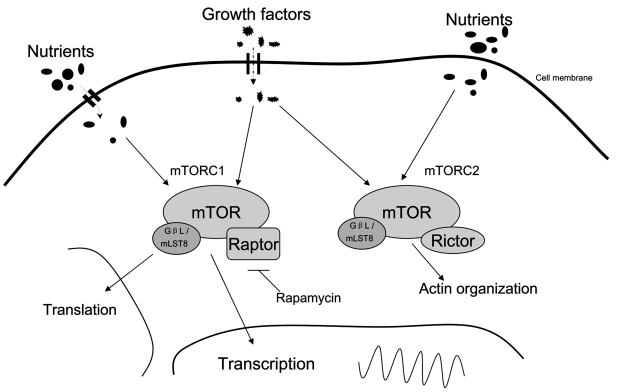

Figure 2. Mammalian TOR complexes.

The mTOR exists in at least two distinct multi-protein complexes-mTORC1 and mTORC2 [21]. mTORC1 is a heterotrimeric protein kinase that consists of the mTOR catalytic subunit and two associated proteins, raptor (regulatory associated protein of mTOR) and mLST8 (also known as GβL) (see Fig. 2). Raptor is a 150-kDa protein, containing a conserved N-terminal domain, three HEAT domains, and seven WD40 motifs. The mLst8 is composed of seven WD40 motif repeats and has sequence homology to the β subunit of heterotrimeric G proteins [22]. Raptor might have roles in mTOR assembly, recruiting substrates to mTOR, and in regulating mTOR activity. The strength of the association between mTOR and raptor is regulated by nutrients and other signals that regulate the mTORC1 pathway. The mTORC1 pathway regulates growth through downstream effectors, such as the regulators of translation 4EBP1 (eukaryotic translation initiation factor-4E-binding protein 1) and S6K1 (ribosomal S6 kinase 1) [23].

The mTORC2 also contains mTOR and mLST8 but instead of raptor two other proteins, rictor (rapamycin-independent companion of mTOR) and mSin1 (also known as mitogen-activated protein-kinase-associated protein 1). Both rictor and mSin1 are necessary for the phosphorylation of Akt (also known as protein kinase B) on its C-terminal hydrophobic motif. This second mTOR-containing complex is less understood than mTORC1 but recent work indicates that it should be considered part of the PI3K-Akt pathway as it directly phosphorylates Akt on one of the two sites that are necessary for Akt activation in response to growth factor signaling [21, 24].

4 The downstream targets of mTOR

Two main targets of mTOR are p70 ribosomal S6 kinase (p70S6K) and the eukaryotic translation initiation factor (eIF4E)-binding protein (4E-BP1). The mTOR kinase, in response to amino acids and growth factors, phosphorylates these substrates, inducing activation of p70S6K and inhibition of 4E-BP1 [25]. The p70S6K protein phosphorylates

the 40S ribosomal protein S6 and possibly the translation initiation factor eIF4B [26, 27]. It is also known that rapamycin suppresses the translation of 5′-TOP mRNA, encoding ribosomal proteins and components of the translational apparatus, through inhibition of p70S6K [28]. The 4E-BP1 is a translation inhibitor that is phosphorylated and inactivated in response to a growth signal. In fact, dephosphorylated 4E-BP1 binds and inhibits the translation initiation factor eIF4E. The mTOR kinase phosphorylates 4E-BP1, inducing its dissociation from eIF4E, which can bind the cap structure at the 5-termini of mRNA, thereby allowing cap-dependent translation [7].

4.1 S6K1

Mammalian cells contain two similar S6 kinase proteins (S6K1 and S6K2) encoded by two different genes [23]. Both proteins are phosphorylated and all of the phosphorylation sites are conserved between the two proteins. The S6 kinases regulate cell growth in *Drosophila* and mammals, and are direct targets of TOR. S6K2 was discovered much later than S6K1 [29], and therefore, S6K1 has been used for most of the studies on substrate phosphorylation and effects on cell growth.

S6K1 directly phosphorylates the 40S ribosomal protein S6, which correlates with enhanced translation of transcripts with 5′-terminal oligopyrimidine (5′-TOP) sequences that encode components of the translational machinery [30]. S6K phosphorylation and thus activation have been implicated in mediating the downstream effects of TOR on translation initiation in both flies and mammals. This idea is suggested by the observation that the TOR knockout phenotype can be suppressed by overexpressing S6K in flies [31]. Consistent with the effect of S6K on growth, flies carrying homozygous mutations in dS6K show a developmental delay and a reduction in body size [32]. Phosphorylation of ribosomal protein S6 by S6K is accompanied by up-regulation of a class of mRNA that contain an oligopyrimidine tract at their transcriptional start site, termed 5′-TOP [33].

4.2 4E-BP

The interaction between eIF4E and eIF4G is regulated by members of the eIF4E-binding proteins (4E-BPs), a family of translational repressor proteins [23]. The mammalian family consists of three low-molecular weight proteins, 4E-BP1, 4E-BP2, and 4E-BP3, encoded by three separate genes, whereas *Drosophila* expresses only one 4E-BP [34 – 36]. The 4E-BP, a protein that is phosphorylated upon insulin stimulation, is also targeted by mTOR to regulate translation and growth [37, 38]. The 4E-BP is an important regulator of overall translation levels in cells [25]. It has also been assumed to serve as a growth regulator. The 4E-BP can bind eIF4E, and block its normal function of recruiting the initiation complex (containing the 40S ribosomal subunit) to the m7GpppX cap structure present at the 5′-end of all eukaryotic cellular mRNA [39].

The activity of 4E-BP is regulated through phosphorylation by the protein kinase mTOR [23]. When TOR activity is low, 4E-BP is hypophosphorylated allowing it to bind efficiently to eIF4E and block translation. When TOR activity increases, it phosphorylates 4E-BP, causing its affinity for eIF4E to drop and allowing cap-dependent translation to occur. The 4E-BP has also been proposed to control the rate of growth of tissues during development [36, 40].

4.3 eIF4G

The eIF4G is a modular scaffolding protein that plays a key role in the assembly of the ribosome initiation complex. As described above, all eukaryotes have two related eIF4G proteins. The eIF4G consist of three functional and structural domains that are connected by hinge regions. The three domains interact with different initiation factors [41]. Both eIF4GI and eIF4GII are phosphoproteins [42, 43]. Phosphorylation sites have been mapped for both eIF4G, but phosphorylation of eIF4GI is much more robust than that of eIF4GII [43].

5 The mTOR signaling pathway and its role in molecular nutrition regulation

5.1 The mTOR signaling pathway and how nutrients sense this pathway

Evolutionary role of the mTOR signaling pathway controls cell growth, proliferation, and survival. All cells require a constant supply of nutrients to provide the substrates and metabolic energy necessary for cell growth, proliferation, and repair. A particularly important regulatory system in regard to cell growth is the mTOR-raptor (regulatory-associated protein of mTOR) signaling pathway, which monitors intracellular amino acid availability and cellular energy status and links this information with external signals originating from cell surface receptors (such as insulin signaling). This sensory input is then biochemically integrated and tightly coupled to a coordinated response that controls cell growth and proliferation as well as other aspects of cellular function (Fig. 3).

The first component of the mTOR signaling pathway is the tuberous sclerosis complex (TSC), which comprises two interacting proteins that form a stable heterodimeric complex [44 – 46]. TSC functions as a negative regulator of cell growth (in other words, as a tumor suppressor protein). TSC also integrates information from the insulin-signaling cascade and the AMPK (AMP-activated protein kinase) signaling pathway.

The immediate downstream target of TSC is ras homologue enriched in brain (Rheb), a member of the Ras super-

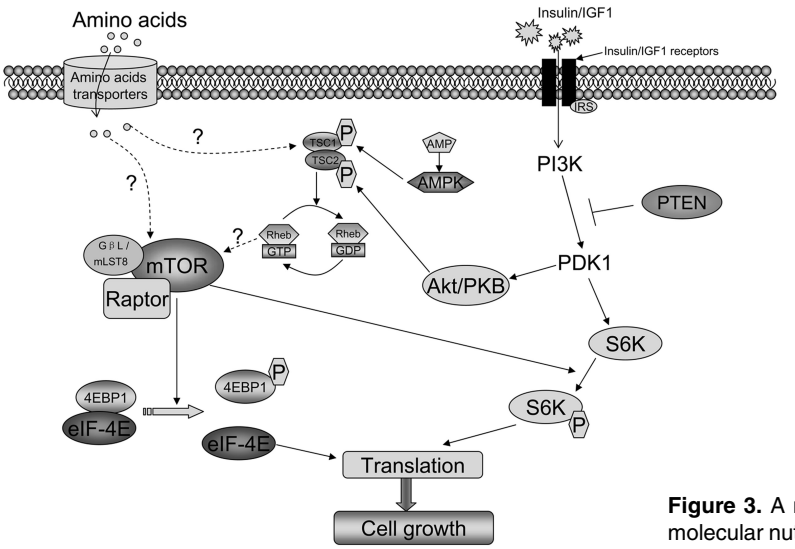

Figure 3. A model of the role of mTOR signaling pathway in molecular nutrition regulation.

family of small GTP-binding proteins that functions to activate mTOR kinase [46, 47]. This protein can be converted to a lipophilic protein through the enzymatic addition of a farnesyl group, and this modification appears to be functionally important because farnesyltransferase inhibitors can block insulin-mediated activation of the mTOR signaling pathway [47, 48].

The central component of the mTOR signaling pathway is mTOR itself, which is a relatively large (290 kDa) serine-threonine kinase that contains several regulatory domains [1, 2, 22, 23, 37, 49 – 51]. Information regarding the function and regulation of the mTOR-mLST8-rictor complex (called mTORC2) is less extensive, but recent studies have shown that this complex plays a role in actin organization [52] and mediates the phosphorylation and activation of Akt [21, 26, 53].

The strong similarity between starved and rapamycin-treated cells suggests that mTOR signaling pathway regulates cell growth in response to nutrients [54]. In fact, branched chain amino acids, particularly leucine, stimulate translation initiation through a rapamycin-sensitive pathway [55, 56]. It is known that mTOR integrates signals from nutrients and growth factors leading to cell growth. In fact, mTOR belongs to the PI3K pathway, activated by insulin and growth factors [57]. This pathway involves the serine/threonine kinase Akt, an upstream regulator of mTOR [58].

5.2 Hypothalamic mTOR activity in the control of food intake

When available fuels are deficient, mTOR activity is low. If mTOR signaling is linked to the regulation of energy balance in the CNS, it may restraint the organism to consume more energy. Indeed, mTOR signaling plays a important role in the brain mechanisms to alter food intake [59]. Central administration of leucine increases hypothalamic mTOR signaling and decreases food intake. Leptin increases hypothalamic mTOR activity, and rapamycin blunts leptin's anorectic effect. Consistent with this, a recent study showed that rats placed on a low-protein diet exhibited increased food intake and direct intracerebroventricular injection of either an amino acid mixture or leucine alone suppressed food intake, suggesting that increasing amino acids concentrations can act within the brain to inhibit food intake through mTOR-dependent pathway [60].

5.3 mTOR in regulation of insulin resistance and glucose metabolism

Insulin controls glucose homeostasis by regulating glucose use in peripheral tissues, and its own production and secretion in pancreatic β cells. These responses are largely mediated by insulin receptor substrates (IRS) through distinct signaling pathways. Recent studies have showed the existence of a negative feedback loop from nutrient-sensitive mTOR-S6K1 pathway to insulin-responsive IRS-PIK-Akt pathway [61 – 63]. Supporting this model, mice deficient for S6K1 are hypoinsulinemic, glucose intolerant, and have reduced pancreatic β cells mass [64]. Moreover, S6K1-deficent mice have enhanced insulin sensitivity [63]. Recently, a study showed that rapamycin stimulates insulin-mediated glucose uptake in man by activating the mTOR-S6K1 pathway and inhibiting IRS phosphorylation [65].

More recently, 4E-BP was shown to control insulin sensitivity and glucose metabolism [66, 67]. Increased insulin resistance in 4E-BP1 and 4E-BP2 double knockout mice was associated with increased S61 activity and impairment of Akt signaling in muscle, liver, and adipose tissue [67]. Therefore, mTOR, including its two downstream targets 4E-BP and S6K were critical effectors in controlling glucose homeostasis and impaired function of them contributed to the development of specific forms of diabetes mellitus.

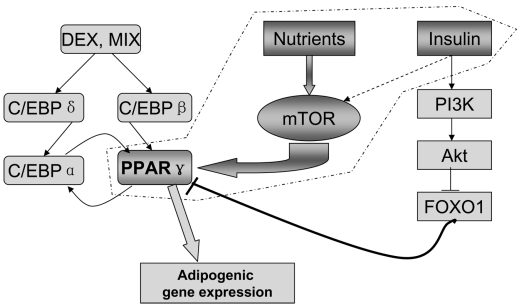

Figure 4. A proposed model of adipogenic regulation by mTOR (adapted from Ref. [78]). See Ref. [78] for details.

5.4 Regulation of lifespan by TOR in response to nutrients

Calorie restriction (CR) is the only intervention known to increase lifespan in yeast, worms, flies, and mammals. Kennedy's group [68] results showed ten gene deletions that increased replicative lifespan and six of these corresponded to genes encoding components of the nutrient-responsive TOR and Sch9 pathways. As inhibition of TOR activity induces autophagy in yeast and mammalian cells [7], autophagy may be a major target for TOR to regulate *C. elegans* lifespan as well as dauer formation [69]. As the TOR pathway primarily responds to nutrient availability, caloric restriction may extend lifespan by decreasing TOR activity. Interestingly, it has been reported that caloric restriction and reduced insulin signaling may exhibit their aging effects at least partly by their common stimulatory action on autophagy [70]. If insulin/IGF and nutrient signaling converge at raptor, TOR signaling could be a central pathway mediating caloric restriction [69]. Vellai *et al.* and Kapahi *et al.* also provided evidence for connections between the TOR pathway and lifespan in *C. elegans* or *Drosophila* [71 – 73].

6 Adipogenic regulation by mTOR pathway

Adipocyte differentiation is a developmental process that is critical for metabolic homeostasis and nutrient signaling. Adipose tissue plays major roles in energy homeostasis, lipid metabolism, and insulin actions. It also acts as an endocrine organ to regulate the secretion of a wide range of factors such as leptin, adiponectin, tumor necrosis factor-α, plasminogen activator inhibitor-1, and various cytokines, some of which are key regulators of energy homeostasis [74]. In adipocytes, mTOR is thought to regulate protein synthesis [75], adipose tissue morphogenesis [76], and leptin synthesis/secretion [77].

Chen group's [78] research data suggested that adipocytes might utilize the mTOR pathway to sense nutrient availability and modulate the activity of PPAR-γ, revealing the first molecular link between nutrients and adipogenesis/adipogenic functions. The mTOR pathway specifically regulates the transactivation activity of PPAR-γ, which is essential for a positive feedback control of C/EBP-a expression as well as the adipogenic gene expression program and thus critical for both initiating and maintaining adipogenesis. Furthermore, mTOR signaling may serve to transduce nutrient availability signals to control the activity of PPAR-γ (Fig. 4).

7 Conclusions

We now realize that TOR is a central regulator for cell growth, which integrates signaling from both growth factors and nutrients. Recent important discoveries include the finding of a negative regulator, the TSC1-TSC2 complex, and positive regulators, such as Rheb, mLST8.

The mTOR is part of at two distinct multi-protein complexes that complex signaling pathways involved in regulating cell growth and proliferation by controlling many major cellular processes.

The new research in the understanding of the upstream and downstream targets of mTOR provides rational explanations for the origins and progression of these diseases, like cancer.

The mTOR in control of food intake, insulin resistance and glucose metabolism provide novel therapies for metabolic disease, such as type 2 diabetes and obesity.

An interesting challenge to dissect the downstream effectors of the TOR and ILS pathways that regulate lifespan.

We still do not know the mechanism by which amino acids increase mTOR activity. How Akt-mediated phosphorylation influences the function of the TSC1 and TSC2 complex and how Rheb signal to mTOR are two other major unanswered questions.

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