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Review

The ever-evolving role of mTOR in translation

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ABSTRACT

Control of translation allows for the production of stoichiometric levels of each protein in the cell. Attaining such a level of fine-tuned regulation of protein production requires the coordinated temporal and spatial control of numerous cellular signalling cascades impinging on the various components of the translational machinery. Foremost among these is the mTOR signalling pathway. The mTOR pathway regulates both the initiation and elongation steps of protein synthesis through the phosphorylation of numerous translation factors, while simultaneously ensuring adequate folding of nascent polypeptides through co-translational degradation of misfolded proteins. Perhaps most remarkably, mTOR is also a key regulator of the synthesis of ribosomal proteins and translation factors themselves. Two seminal studies have recently shown in translatome analysis that the mTOR pathway preferentially regulates the translation of mRNAs encoding ribosomal proteins and translation factors. Therefore, the role of the mTOR pathway in the control of protein synthesis extends far beyond immediate translational control. By controlling ribosome production (and ultimately ribosome availability), mTOR is a master long-term controller of protein synthesis. Herein, we review the literature spanning the early discoveries of mTOR on translation to the latest advances in our understanding of how the mTOR pathway controls the synthesis of ribosomal proteins.

Contents

1. mTOR complexes and substrates

The mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase with a pivotal role in exerting control over translation. mTOR exists in two multiprotein complexes termed mTOR complex 1 (mTORC1) and 2 (mTORC2) [1]. Of the two

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complexes, mTORC1, plays the most prominent role in translational control. mTORC1 and mTORC2 can be easily differentiated based on the proteins comprising each complex and their respective substrates. While mTORC1 and mTORC2 share a number of common components such as the small GTPase β-like protein (GβL) [2,3] and the dishevelled, Egl-10, pleckstrin [DEP]-domain-containing mTOR interacting protein (DEPTOR) [4], each complex is also defined by unique components. For instance, the scaffolding protein RAPTOR (shorthand for regulatory associated protein of mTOR) [5,6] and the substrate competitor PRAS40 (proline-rich Akt substrate of 40 kDa) [7–11] are found exclusively within mTORC1, while RICTOR (rapamycin-insensitive companion of TOR) [12], mSIN1 (mammalian stress-activated protein kinase interacting protein 1) [13,14] and PRR5/PROTOR (proline-rich protein 5/protein observed with RICTOR) are mTORC2-specific protein components [15,16]. mTORC1 and mTORC2 phosphorylate distinct substrates thus carrying different cellular functions. mTORC1 controls various important cellular functions, *e.g.*: translation (described in this review in detail), transcription of ribosomal RNA (rRNA) and transfer RNA (tRNA), ribosome biogenesis, lysosome biogenesis, lipid synthesis and macroautophagy (or protein breakdown). mTORC2 regulates co-translational protein degradation, cytoskeletal rearrangement and cell survival (reviewed elsewhere within this mTOR compendium). These two complexes can be functionally differentiated by their sensitivity to the drug rapamycin. Short term rapamycin treatment selectively inhibits mTORC1, while prolonged rapamycin treatment impairs mTORC1 and mTORC2 activities [17]. Between the mTOR complexes: mTORC1 is the one with the most defined role in translation. mTORC1 relays its effects on translation through the phosphorylation of numerous different substrates/target proteins (see Fig. 1 for a pathway diagram of mTORC1 substrates in translation). The role of each of these substrates and the mechanism by which mTORC1 regulates their activity are discussed in detail here.

2. mTOR and cap-dependent translation initiation

The small molecular weight translational repressors 4E-BPs (eukaryotic initiation factor 4E-binding proteins) were among the first mTORC1 targets to be discovered [18,19] and for this reason they are among the most well-defined downstream targets of mTORC1. 4E-BPs bind to the mRNA cap-binding protein eIF4E and preclude the formation of the eIF4F complex (comprising eIF4E, the scaffolding protein eIF4G and the ATP-dependent RNA helicase eIF4A) that recruits the 43S pre-initiation complex (PIC) to the 5′end of the mRNA [20]. The 4E-BP family comprises three members: 4E-BP1, 4E-BP2 and 4E-BP3 [21–27]. These three proteins are encoded by 3 distinct genes but show a high degree of homology and are regulated for the most part in a similar manner (exceptions will be detailed throughout this review). The binding of 4E-BPs to eIF4E is regulated through the sequential phosphorylation of four main phospho-residues in the following order: Thr^{46}, Thr^{37}, Thr^{70} and Ser^{65} [28–30]. In its hypophosphorylated state, 4E-BP1 associates tightly with eIF4E. Phosphorylation of Ser^{65} and Thr^{70} (located nearest to the eIF4E-binding site) on the dorsal face of the protein contribute to its release from eIF4E [31,32]. Phosphorylation of N-terminal priming sites (Thr^{37} and Thr^{46}) may also play a role in the release of 4E-BPs from eIF4E [33,34].

How is the phosphorylation of 4E-BPs regulated? Phosphorylation of 4E-BPs is modulated by numerous environmental cellular cues known to activate the mTORC1 pathway (*e.g.* amino acids, hormones, growth factors and pH). Pharmacological agents that impinge either directly (*e.g.* rapamycin, ATP-mimic mTOR inhibitors) or indirectly onto the mTORC1 pathway [35–39] can also be used to regulate 4E-BPs phosphorylation. Rapamycin blocks the phosphorylation of Ser^{65} but it does not effectively prevent $Thr^{37/46}$ or Thr^{70} phosphorylation [28,40]. Overall, rapamycin is a poor inhibitor of 4E-BP1 function in that it has a weak effect on eIF4F assembly and, therefore, protein synthesis. Recent years have observed a heightened use of a distinct class of mTOR inhibitors with an alternative mode of action and improved inhibitory properties (relative to rapamycin). ATP-mimics (or ATP-competitor drugs) with specificity for the mTOR active-site (*e.g.* Torin-1, PP242, AZD8055, Ku-0063794 and respective derivatives) [35–38] are now widely used in biochemical and phenotypic studies of mTOR signalling. In stark contrast to rapamycin, ATP-mimic mTOR inhibitors potently inhibit the phosphorylation of all the aforementioned residues on 4E-BPs, effectively abolishing eIF4F assembly thus having a profound inhibitory effect on general protein synthesis [41,42]. Polysome profile analysis of HEK293T cells cultured in the presence/absence of an ATP-mimic mTOR inhibitor reveals that these drugs even when compared to rapamycin, cause a marked decrease in the number of polysome-associated mRNAs accompanied by an increase in RNA absorbance at the 80S fraction (Fig. 2).

What is the physiological significance of mTORC1 regulation of 4E-BPs? 4E-BPs (specifically 4E-BP1 and 4E-BP2) play a key role in cell proliferation and oncogenic transformation downstream of mTORC1 [43,44]. Simultaneous genetic ablation of the *eif4ebp1* and *eif4eb2* genes (that encode 4E-BP1 and 4E-BP2) renders the proliferation of mouse embryonic fibroblasts partially resistant to the inhibitory effects of ATP-mimic mTOR inhibitors [43].

How do 4E-BPs differ? 4E-BP1 and 4E-BP2 are highly conserved (they display 50% identity at the amino acid level). 4E-BP3 is evolutionary more divergent (displaying 44% and 46% identity to 4E-BP1 and 4E-BP2, respectively) and indeed lacks a RAIP motif, present in 4E-BP1/2, which reduces the capacity of mTORC1 signalling by insulin to phosphorylate it [45]. Thr^{37}, Thr^{46}, Ser^{65} and Thr^{70} residues are conserved in all three 4E-BPs, and phosphorylation of these sites in 4E-BP1 and 4E-BP2 is under to the control of mTORC1 considerably less is known about the control of 4E-BP3 phosphorylation [45]. 4E-BP2 (but not 4E-BP1) is also subject to a distinct post-translational modification: asparagine deamidation, *i.e.*, the conversion of asparagine to aspartate or isoaspartate [46,47]. Asparagine deamidation of residues Asn99 and Asn102 within an asparagine-rich sequence in 4E-BP2 increases its association with RAPTOR. This increase is accompanied by a reduction in the binding of 4E-BP2 to eIF4E. Deamidation occurs during a stage of post-natal brain development that is associated with low mTORC1 activity, suggesting that 4E-BP2 deamidation (and the resulting decreased eIF4E binding) may act to compensate for reduced mTORC1 signalling in the brain at this developmental stage [47].

3. mTOR translatome

Which mRNAs are translationally regulated by the mTORC1/4E-BP/eIF4E pathway? eIF4E, the target of 4E-BPs, has been originally proposed to facilitate the translation of mRNAs with long, highly structured 5′untranslated regions (UTR) through the recruitment of the ATP-dependent RNA helicase eIF4A by eIF4G [48,49]. One typical example is the ornithine decarboxylase 1 (ODC1) mRNA: the 5′UTR of ODC1 (isoform 1) is long (561 nucleotides-long) and predictably highly structured with an estimated Gibbs free energy (ΔG) value of −299.00 kcal/mol (Table 1). Translation of ODC1 mRNA *in vitro* is enhanced in extracts from eIF4E-overexpressing cells, indicating that eIF4E plays an important stimulatory role in the translation of this mRNA [50–52]. Consistent with the fact that eIF4E plays a critical role in ODC1 translation, insulin, a well-described activator of the PI(3)K/mTORC1/4E-BP1/eIF4E pathway,

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