Mammalian Target of Rapamycin

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The entry outlines the discovery of mTOR and describes mTOR complex 1 (mTORC1) and mTORC2.

mTOR mTORC1 mTORC2

1. Introduction

In 1964, a scientific expedition ventured to Rapa Nui (also known as Easter Island) to collect soil and plants samples [1][2][3]. These samples were brought back to Canada, and rapamycin was isolated from the bacterium Streptomyces hygroscopicus in 1972. Initially, rapamycin was characterized as an antifungal agent, and further studies identified rapamycin to be an immunosuppressant. The ability of rapamycin to inhibit cell growth was discovered later. Experiments demonstrated that rapamycin formed a complex with peptidyl-prolyl cis-trans isomerase FK506-binding protein 12 (FKBP12) [4]. Through genetic screens, the target of rapamycin (TOR) was first discovered in yeast, where mutations in TOR were resistant to rapamycin [5][6][7]. Biochemical experiments in mammalian cells revealed that the rapamycin-FKBP12 complex specifically targets and inhibits the mammalian target of rapamycin (mTOR) [8][9][10]. Through affinity purification, the FKBP12-rapamycin complex was shown to bind a large molecular weight protein called mTOR (also referred to FRAP, RAFT1). Currently, rapamycin and rapamycin analogs (rapalogs) are commonly used as cancer and transplant therapeutics. Decades later, the precise mechanism of how mTOR is regulated is still being elucidated. mTOR coordinates multiple physiological processes through downstream signaling networks.

2. mTOR

mTOR is an evolutionarily conserved Ser/Thr protein kinase that is classified in the phosphatidylinositide 3 kinase (PI3K)-related kinase family within the human phylogenetic kinome tree. mTOR functions as the catalytic subunit of two distinct complexes, referred to as mTORC1 and mTORC2. Rapamycin and rapalogs inhibit mTORC1 activity allosterically, while mTORC2 demonstrates short-term rapamycin insensitivity [11][12][13]. The rapamycin-FKBP12 complex binds to the FKBP12-rapamycin-binding (FRB) domain on mTOR reducing availability of the catalytic cleft, resulting in some substrates unable to access the active site. Prolonged treatment of rapamycin is thought to inhibit mTORC2 through the sequestration of mTOR in some cell types [14][15]. ATP-competitive inhibitors like Torin1 have also been developed, which directly target the catalytic site and inhibit the kinase activity of mTOR [16].

3. mTORC1

mTORC1 consists of three main core components: mTOR, regulatory protein associated with mTOR (Raptor) and mammalian lethal with Sec13 protein 8 (mLST8, also referred to as GβL) (Figure 1, Left) [17][18][19]. Raptor acts as a substrate recognizing subunit that facilitates mTOR phosphorylation through the TOR signaling (TOS) motif found in some mTORC1 substrates [20][21]. Mutations in the TOS motif were shown to render mTORC1 downstream targets, such as the phosphorylation of p70 ribosomal S6 kinase 1 (S6K1) and eIF4E-binding protein 1 (4EBP1, also known as PHAS-1), insensitive to amino acid changes [22]. mLST8 is a positive regulator of mTORC1, stabilizing the association between Raptor and mTOR, and stimulating mTOR kinase activity [19]. mTORC1 contains two additional negative regulators, Proline-rich Akt substrate 40 kDa (PRAS40) [23][24][25] and DEP-domain-containing mTOR-interacting protein (DEPTOR) [26]. PRAS40 acts as a direct inhibitor of substrate binding through the interaction with Raptor, repressing mTORC1 activity [24]. PRAS40 phosphorylation by mTORC1 relieves the negative regulation, increasing mTORC1 signaling [27]. The postsynaptic density 95, discs large, zonula occludens-1 (PDZ) domain of DEPTOR directly interacts with mTOR to inhibit activity [26]. Additionally, mTOR has been shown to promote its own activity via the E3 ubiquitin ligase Skp1, Cullin1, F-box (SCF) adaptor, βTrCP, mediated degradation of DEPTOR [28][29][30].

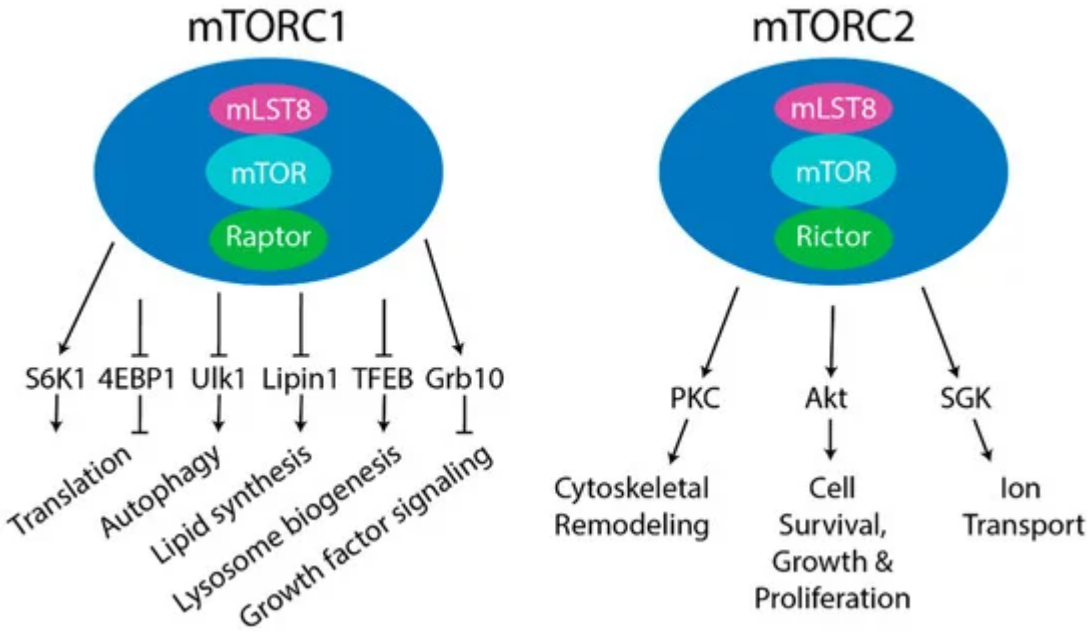

Figure 1. Components of mTOR complex 1 (mTORC1) and mTORC2. Left- Core components of mTORC1 are mammalian target of rapamycin (mTOR) (kinase), Raptor (substrate recognizing component), and mLST8 (positive regulator). Other reported mTORC1 components are PRAS40 (negative regulator) and DEP-domain-containing mTOR-interacting protein (DEPTOR) (negative regulator). Five main downstream pathways are shown. The phosphorylation of S6 kinase 1 (S6K1) and 4EBP1 by mTORC1 regulates protein translation. The phosphorylation of ULK1 by mTORC1 regulates autophagy. mTORC1 also regulates lipid synthesis by phosphorylating S6K1 or Lipin1 to control SREBP, lysosome biogenesis by phosphorylating TFEB, and growth factor signaling by phosphorylating Grb10. Right- Core components of mTORC2 are mTOR (kinase), Rictor (substrate recognizing component), and mLST8 (positive regulator). Other complex components include mSin1 (positive regulator), Protor1/2 (positive regulator), and DEPTOR (negative regulator). mTORC2 regulated processes include

cytoskeletal remodeling by phosphorylating PKC; cell survival, growth, and proliferation by phosphorylating Akt; and ion transport by phosphorylating SGK.

When localized to the lysosome, mTORC1 directly interacts with and is activated by the small GTPase Ras homolog enriched in brain (Rheb) [24][31]. However, some mTORC1 mediated process, such as protein translation, presumably occur in the cytoplasm [32]. Additionally, mTORC1 has been observed in other subcellular locations such as the mitochondria [33], stress granules [34], and at the plasma membrane [35]. mTORC1 components have also been reported at multiple locations within the cell [36]. For example, mTOR and Raptor were detected in the nucleus [37]. A more complete discussion of this topic has been reviewed previously [36].

mTORC1 regulates a multitude of cellular processes, such as protein translation, autophagy, lysosome biogenesis, lipid synthesis, and growth factor signaling [38]. mTORC1 regulates translation via the phosphorylation of S6K1 at Thr 389 to activate S6K1 [17]. S6K1 then proceeds to promote translation initiation through the subsequent phosphorylation of factors such as eukaryotic translation initiation factor 4B (eIF4B) [39]. Additionally, mTORC1 phosphorylates 4EBP1 at multiple sites (Thr 37, Thr 46, Ser 65, Thr 70) to promote translation [40]. Once 4EBP1 is phosphorylated it dissociates from eIF4E, which allows the recruitment of the other translation initiation proteins eIF4G and eIF4A [41]. mTORC1 disrupts Unc-51 like autophagy activating kinase 1 (ULK1) interaction with 5′AMP-activated protein kinase (AMPK) through the phosphorylation of Ser 757 (equivalent to Ser 758 in human) on ULK1, to regulate autophagy [42]. Sterol-responsive element-binding protein (SREBP) promotes de novo lipid synthesis [43]. mTORC1 positively regulates SREBP through the phosphorylation and activation of S6K1 or through the multiple site phosphorylation and inhibition of Lipin1, another mTORC1 substrate [43][44][45]. mTORC1 negatively regulates transcription factor EB (TFEB), which promotes genes for lysosomal biogenesis and autophagy machinery at Ser 142 and Ser 211, preventing TFEB nuclear translocation [46][47][48]. Phosphorylation of growth factor receptor-bound protein 10 (Grb10) by mTORC1 at Ser 501 and Ser 503 negatively regulates growth factor signaling through IGF-1 receptor [49][50]. A more comprehensive review of mTORC1 substrates and downstream signaling pathways controlled by mTORC1 is elsewhere [38][39][51].

4. mTORC2

Similar to mTORC1, mTORC2 consists mTOR and mLST8. However, mTORC2 contains rapamycin insensitive companion of mTOR (Rictor) as the substrate recognizing component (Figure 1, Right) [12][13]. Additionally, mTORC2 is comprised of the negative regulator DEPTOR. mTORC2 contains mammalian stress-activated MAPK-interacting protein 1 (mSin1) [52][53][54], which is necessary for the assembly of mTORC2 on the plasma membrane [55]. Activation of mTORC2 depends on the pleckstrin homology (PH) domain of mSin1 that binds to phosphatidylinositol 3,4,5-triphosphate (PtdIns(3,4,5)P3, also referred to as PIP3) at the plasma membrane [56]. Lastly, mTORC2 consists of protein observed with Rictor 1/2 (Protor1/2, also known as PRR5) [57].

mTORC2 has been observed in multiple locations throughout the cell. Using a reporter of endogenous mTORC2 activity, a study showed mTORC2 associates with the plasma membrane, mitochondria, and on endosomal vesicles [58]. mTORC2 has also been reported to localize to the endoplasmic reticulum (ER) and ER associated

membranes, such as mitochondria-associated ER membranes (MAMs) [36]. mTORC2 can regulate MAM integrity through the mTORC2 substrate Rac-α Ser/Thr-protein kinase (Akt, also known as PKB) [59]. Lastly, evidence showed mTORC2 may shuttle to the nucleus, however the function of mTORC2 in the nucleus remains unknown [37].

mTORC2 regulates physiological processes through the phosphorylation and activation of downstream substrates like the protein kinase A, G and C (AGC) family. Protein kinase C α (PKCα) at Ser 657 was the first identified substrate of mTORC2 [13]. Other PKC family members have been shown to be phosphorylated and activated by mTORC2, including PKCδ, PKCξ (Thr 560), PKCγ, and PKCε to regulate cytoskeletal remodeling and cell migration [60][61][62]. mTORC2 phosphorylates Akt on Ser 473 to promote cell survival, proliferation and growth [63]. Akt mediates these processes through the subsequent phosphorylation of substrates such as Forkhead box O1/3 (FoxO1/3a) at Thr 32 and Ser 253, glycogen synthase kinase 3 β (GSK3-β) at Ser 9, and tuberous sclerosis complex 2 (TSC2) at Ser 939 and Thr 1462 [54][64][65][66]. Lastly, through the phosphorylation of serum/glucocorticoid-regulated kinase 1 (SGK1) at Ser 422, mTORC2 controls processes like ion transport and cell growth [67]. mTORC2 will not be discussed further in this review, and mTORC2 has been reviewed elsewhere [38][51][68].

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