RESEARCH HIGHLIGHT

# Amino acid availability governs mTOR ubiquitination

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**How mTORC1, a master regulator of cell growth, senses amino acid availability to regulate cell growth is an intriguing but mysterious question. In a recent paper published in *Cell Metabolism*, Ge et al. report that amino acid depletion promotes K27-linked poly-ubiquitination of mTOR through activating a novel tRNA-GCN2-FBXO22 signaling axis to suppress mTORC1 kinase activity.**

The availability of nutrients, including amino acids and glucose, serves as a critical signaling cue for cell growth.[1–3] To this end, the mechanistic target of rapamycin complex 1 (mTORC1), an evolutionarily conserved central nutrient sensor, integrates nutrient availability and growth factor signal to regulate cell growth and cell metabolism.[3,4] As a kinase, mTORC1 phosphorylates a variety of substrates including S6K1, 4EBP1, and ULK1 to promote anabolic metabolism and suppress catabolic metabolism to regulate cell growth.[3,4]

How amino acid availability regulates mTORC1 kinase activity is an elusive, but fundamental question in the field.[1,3] Recently, several amino acid sensors including leucine sensors Sestrin2 and SAR1B, arginine sensor CASTOR1 have been identified to bind individual amino acids to regulate mTORC1 lysosomal localization through the GATOR1/2-Rag GTPases axis.[3] However, how the remaining amino acids regulate mTORC1 activity is still relatively unknown. In a recent study published in *Cell Metabolism*, Ge et al.[5] reported that amino acid depletion promotes the ubiquitination of mTOR to inhibit mTORC1 activity. Specifically, the accumulation of uncharged tRNAs upon amino acid depletion activates GCN2, which phosphorylates the E3 ligase FBXO22 to ubiquitinate mTOR at K2066, leading to reduced mTORC1 substrate recruitment.

Ubiquitination is a reversible post-translational modification in which a ubiquitin (Ub) is attached to a substrate protein by the E1 activating enzyme, the E2 conjugating enzyme, and the E3 ligase.[6] The authors identified mTOR as a binding partner of the E3 ligase FBXO22 by mass spectrometry and further demonstrated that the ubiquitination of mTOR was enhanced after increased expression of FBXO22 via gene-specific transcriptional activation. In vitro ubiquitination assay validated that FBXO22 could directly ubiquitinate mTOR. By mutating each of the 7 lysine (K) residues in Ub to arginine (R), they showed that FBXO22 ubiquitinated mTOR largely in a K27-linked manner.

To pinpoint the possible ubiquitination site(s), Ge et al. demonstrated that overexpression of FBXO22 promoted the ubiquitination of the K2066 residue detected by mass spectrometry. Furthermore, the endogenous K2066R knock-in (KI) mutant significantly blocked the ubiquitination of mTOR by FBXO22. Although FBXO22 mediates ubiquitination of mTOR present in both mTORC1 and mTORC2, it did not affect the stability of mTOR protein and the formation of mTORC1 and mTORC2. Because K2066 is located within the FRB (FKBP12-rapamycin-binding) domain of mTOR, the authors analyzed the possible model of Ub-mTORC1 by molecular docking, and found that K27-linked-Ub chain likely poses a steric hindrance on mTORC1–substrate binding. To further validate this possibility, they found that FBXO22 overexpression impaired the interactions of mTORC1 substrates S6K1, 4EBP1, and ULK1 with mTOR, in wild-type (WT) but not in K2066R KI cells. These findings suggest that the ubiquitination of mTOR at K2066 blocks mTORC1 substrate recruitment.

mTORC1 activity can be regulated by amino acids, growth factors, and energy levels.[4] Interestingly, the ubiquitination of mTOR was specifically regulated by amino acid availability but not serum or glucose, in WT cells, but not in K2066R KI cells. Notably, depletion of any individual one of the 13 tested amino acids could increase mTOR ubiquitination. More importantly, mTOR-K2066R KI mutant could impair mTORC1 inhibition induced by individual depletion of most tested amino acids except Leu, Arg and Gln, implying that Leu, Arg, and Gln likely regulate mTORC1 independent of mTOR ubiquitination. Consistently, although knockout (KO) of *DEPDC5*, a GATOR1 component, could alleviate mTORC1 inhibition induced by depletion of some amino acids such as Leu and Arg, depletion of all individual amino acids still suppressed mTORC1 activity to a degree and promoted mTOR ubiquitination in *DEPDC5* KO cells. These results suggest that some amino acids regulate mTORC1 through GATOR1/2-Rags pathway, while others might go through FBXO22-mediated mTOR ubiquitination.

Interestingly, the authors found that nuclear FBXO22 could translocate to the cytoplasm after amino acid depletion by immunofluorescence assay. Notably, the deletion of nuclear localization sequence (NLS) of FBXO22 induced its cytoplasmic translocation, promoting mTOR ubiquitination. The key lingering question is how the localization of FBXO22 could be regulated by amino acid depletion. In this regard, GCN2, a tRNA-regulated protein kinase, can be activated by uncharged tRNA accumulated upon amino acid depletion and have been shown to regulate mTOR acitivity.[1] Through multiple assays, the authors found that GCN2 was associated with FBXO22. Depletion of *GCN2* abolished amino acid depletion-induced mTOR ubiquitination and FBXO22 translocation. Mechanistically, GCN2 could directly phosphorylate FBXO22 at Thr127, a process that can be regulated by amino acid availability and GCN2-specific inhibitors. Interestingly, Thr127 is located immediately after an NLS motif of FBXO22. As such, FBXO22 phospho-deficient mutant T127A could prevent GCN2-induced cytoplasmic retention of FBXO22, but phospho-mimetic mutant T127E significantly promoted cytoplasmic localization. These results suggest that GCN2-mediated T127 phosphorylation of FBXO22 is important for FBXO22 translocation and mTOR ubiquitination. Moreover, they found that uncharged $tRNA^{Ser(GCU)}$ mutant promoted mTOR ubiquitination and suppressed mTOR activity in WT, but not

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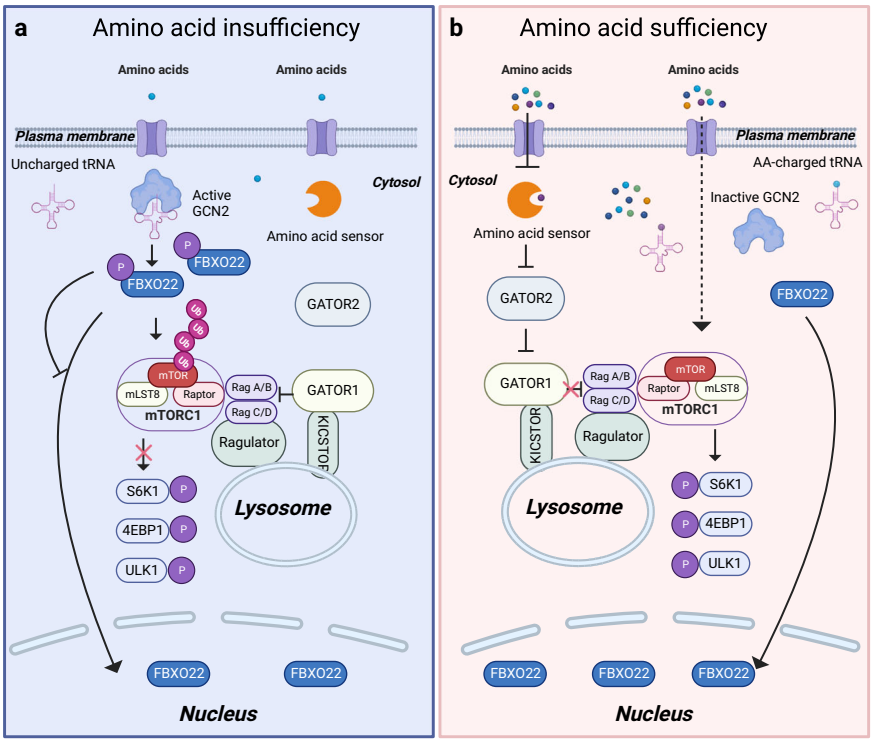


**Fig. 1 The regulation of mTORC1 by GATOR1/2-Rag GTPases and the tRNA-GCN2-FBXO22 axis. a** When amino acid levels decrease, uncharged tRNAs activate GCN2 to phosphorylate FBXO22, which promotes K27-linked ubiquitination of mTOR and impedes mTORC1 substrate recruitment. At the same time, amino acids cannot bind to amino acid sensors to suppress GATOR1 and activate mTORC1. **b** When amino acids levels increase, some amino acids like Leu and Arg bind to different amino acid sensors to activate GATOR2 and inhibit GATOR1 function, subsequently leading to mTORC1 activation to phosphorylate substrates. At the same time, tRNAs are charged with amino acids and cannot activate GCN2 to suppress mTORC1 kinase activity. Created with BioRender.com.

K2066R KI cells. Consistently, threonyl-tRNA synthetase and prolyl-tRNA synthetase inhibitors could drive uncharged tRNA accumulation and promote GCN2 activation and mTOR ubiquitination, subsequently leading to mTORC1 inhibition.

To further investigate the physiological role of mTOR ubiquitination, the authors generated mTOR ubiquitination-deficient $Mtor^{KR/KR}$ mouse strain. Although no apparent phenotypes were observed under standard growth conditions, $Mtor^{KR/KR}$ mice exhibited decreased mTOR ubiquitination and increased mTORC1 activity under fasting conditions. Interestingly, $Mtor^{KR/KR}$ neonates died shortly after fasting, which could be rescued by administration of the mTORC1 inhibitor rapamycin. These phenotypes are reminiscent of those of $RagA^{GTP/GTP}$ and *Szt2* KO mice,[7,8] supporting the relevance of mTOR ubiquitination in the control of mTOR activity. To further pinpoint that individual amino acid deprivation modulates mTOR ubiquitination in vivo, the authors showed that dietary threonine deprivation could inhibit mTORC1 activity via mTOR ubiquitination in WT, but not $Mtor^{KR/KR}$ mutant mice.

Overall, these findings provide a novel mechanism by which amino acid deprivation regulates mTOR ubiquitination through activating the tRNA-GCN2-FBOX22 axis. Hence, this study adds a new block of the amino acid sensing puzzle, complementing the established GATOR1/2-Rag GTPases axis (Fig. 1). Additionally, it prompts further inquiries and raises new questions for exploration in this field. For example, how amino acids coordinate with different amino acid sensing pathways including GATOR1/2-Rag GTPases and the tRNA-GCN2-FBOX22 axis to modulate mTORC1 activity, and is there crosstalk among various amino acid sensing pathways? The ubiquitination of mTOR by FBOX22 regulates its interaction with canonical substrates such as S6K, 4EBP1 and ULK1. Recently, TFEB was identified to be phosphorylated by mTORC1 via a substrate-specific mechanism that is mediated by Rag GTPases,[9] and thus it is curious to know whether mTOR ubiquitination regulates non-canonical mTOR substrate TFEB. Further investigations are warranted to expand our current knowledge of how mTORC1 integrates different arms of amino acid sensing pathways to precisely govern cell metabolism and cell growth and how aberrant amino acid sensing leads to human diseases including cancer.

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## COMPETING INTERESTS

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## ADDITIONAL INFORMATION

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