mTORC1 Signaling Mechanism in Amino Acid Deficiency-Induced Autophagy: Research Advances (Postprint)

Authors: Bai Xue, Wang Jianping, Ding Xuemei, Bai Shiping, Zeng Qiufeng, Zhang Keying

Date: 2017-10-11T00:00:00+00:00

Abstract

Autophagy is an essential physiological process for maintaining organismal homeostasis. Upon deficiency of nutrients such as amino acids or glucose within the organism, cells initiate autophagy. Autophagy is regulated by multiple signaling pathways, among which the mammalian target of rapamycin complex 1 (mTORC1) signaling pathway represents a critical one that can phosphorylate autophagy-related gene 13 (Atg13) to suppress autophagy initiation. This review will focus on recent advances regarding the mTORC1 signaling pathway induced by amino acid starvation, including the roles of small G proteins, AMP-activated protein kinase (AMPK), microRNA (miRNA), and aminoacyl-tRNA synthetases.

Full Text

Advances in the Signaling Mechanisms of Amino Acid Deficiency-Induced Autophagy via the Mammalian Target of Rapamycin Complex 1

Xue Bai[1],[2], Jianping Wang[1],[3], Xuemei Ding[1],[3], Shiping Bai[1],[3], Qiufeng Zeng[1],[3], Keying Zhang[1],[3]*

[1]Institute of Animal Nutrition, Sichuan Agricultural University, Chengdu 611130, China
[2]College of Life Science and Technology, Southwest Minzu University, Chengdu 610000, China
[3]Key Laboratory for Animal Disease-Resistance Nutrition of the Ministry of Education, Chengdu 611130, China

Abstract: Autophagy is a crucial physiological process for maintaining organismal homeostasis, which is activated when cells experience nutrient deficiencies such as amino acid or glucose starvation. This process is regulated by multiple signaling pathways, among which the mammalian target of rapamycin complex 1 (mTORC1) pathway plays a pivotal role by phosphorylating autophagy-related gene 13 (Atg13) to inhibit autophagy initiation. This review summarizes recent research progress on amino acid deficiency-induced autophagy through the mTORC1 signaling pathway, focusing on the roles of small G proteins, adenosine monophosphate-activated protein kinase (AMPK), microRNA (miRNA), and aminoacyl-tRNA synthetase.

Keywords: amino acid; autophagy; mTORC1; mechanism

Preamble

During the first few hours after birth, newborn mammals abruptly lose their maternal food supply and must initiate a series of metabolic responses to survive starvation. A key physiological mechanism involved in this adaptation is autophagy. As one of the primary pathways for protein degradation in cells, autophagy activity is influenced by changes in amino acid levels within the metabolic pool. For instance, plasma concentrations of essential amino acids decrease significantly in piglets 1–2 days after early weaning, coinciding with a marked increase in autophagy levels. Current research identifies the target of rapamycin (TOR) pathway as a critical sensor of amino acid signals. This review examines the mechanisms through which amino acid deficiency induces autophagy via the mammalian target of rapamycin complex 1 (mTORC1) signaling pathway, providing insights for studying nutrient-sensing pathways and offering new research directions for managing early weaning stress in piglets.

1 Overview of Autophagy

Autophagy is a lysosome-dependent physiological process that degrades intracellular macromolecules and organelles, playing vital roles in defense, homeostasis maintenance, longevity, and cancer prevention. Through autophagy, damaged or senescent organelles and less essential biomolecules (such as long-lived proteins) are enveloped by autophagosomes and delivered to lysosomes for degradation, thereby maintaining cellular homeostasis. The degradation products, including amino acids and nucleotides, can be recycled to provide raw materials and energy for synthesizing new macromolecules. Autophagy is classified into three types: macroautophagy, microautophagy, and chaperone-mediated autophagy. Macroautophagy, the most extensively studied and commonly referred to form, is generally considered non-selective, though it appears to preferentially target specific organelles such as mitochondria and peroxisomes.

The autophagic process comprises several distinct stages: (1) **Autophagy in-**

duction, where cells receive signals to initiate the process; (2) **Isolation membrane formation**, where the endoplasmic reticulum generates a double-layered lipid membrane in the cytoplasm; (3) **Vesicle elongation**, where this double-membrane structure expands into a cup-shaped phagophore; (4) **Autophagosome formation**, where the phagophore seals to encapsulate cytoplasmic components destined for degradation; and (5) **Autolysosome fusion**, where the inner membrane of the autophagosome is degraded by lysosomal enzymes, merging the two compartments and allowing lysosomal enzymes to break down the contents, with the resulting products (such as amino acids) being made available for cellular reuse. Research has identified 35 autophagy-related genes (Atg) and their encoded proteins involved in autophagosome formation, including Atg1/ULK1, Atg3, Atg4, Atg5, Atg6/Beclin1, Atg7, Atg8/light chain 3 (LC3), Atg10, Atg12, Atg13, and Atg16. Currently, autophagy can be directly observed via electron microscopy, which remains the "gold standard" for detection, or indirectly assessed by measuring autophagy-related factors such as the autophagosomal surface protein markers LC3-I and LC3-II. Researchers can also manipulate autophagy levels by overexpressing or silencing autophagy-related genes. For example, Tian et al. introduced green fluorescent protein (GFP)-LC3 into mice and used in vivo imaging to detect GFP fluorescence intensity in ischemic brain tissue after experimental stroke, yielding results consistent with Western blot and immunofluorescence histochemistry.

Autophagy is intimately linked to various physiological activities in animals and humans and plays a critical role in cancer initiation and progression. Whether autophagy is beneficial or harmful depends primarily on the efficiency of autophagosome-lysosome fusion. Gottlieb et al. demonstrated that impaired fusion exerts negative effects on cells. When autophagosomal contents cannot be degraded by lysosomes, the plasma membrane fuses with autophagosomes, expelling them as exosomes while releasing large amounts of matrix metalloproteinases and causing catastrophic leakage of lysosomal enzymes, triggering massive inflammatory secretion and cell death. Additionally, excessive autophagy can lead to type II programmed cell death, causing various pathological consequences. As a primary regulator of metabolic processes, autophagy must be tightly controlled, though the signal transduction mechanisms and their impact on cell survival remain incompletely understood. Current evidence indicates that the phosphatidylinositol 3-kinase (PI3K)-protein kinase B (AKT)-mTOR and AMPK-tuberous sclerosis complex 1/2 (TSC1/2)-mTOR pathways are crucial regulators of autophagy. In yeast, mTORC1 phosphorylates the autophagy factor Atg13, disrupting the Atg1-Atg13-Atg17 complex and thereby inhibiting autophagy at the initiation stage. Beclin1, Beclin2, and UVRAG collectively form the class III PI3K complex to regulate autophagy, while death-associated protein kinase (DAPK), casein kinase II, mitogen-activated protein kinase (MAPK), and calcium also participate in the intricate regulatory network, though their mechanisms are not yet fully elucidated.

2.1 Amino Acids and mTORC1

TOR is a critical serine/threonine protein kinase in organisms. In mammals, the TOR gene is termed mTOR, which serves as an essential regulator of growth and development and exists in two protein complex forms: C1 and C2. mTORC1 functions as a key cellular sensor that detects various signals reflecting physiological status, including nutrient levels (such as glucose and amino acids), growth factors (such as insulin), and environmental stress (such as hypoxia). Current understanding of amino acid signal transduction remains limited, though studies suggest that amino acids promote TSC1-TSC2 complex formation and transmit signals to mTORC1 by regulating the guanosine nucleotide status of Ras-related GTP-binding proteins. The Ragulator-Rag complex also plays a crucial role in receiving amino acid signals and targeting mTORC1 to the lysosomal surface for activation.

2.2 Amino Acids and Autophagy

Both in vivo and in vitro studies have demonstrated a negative correlation between certain essential amino acid concentrations and autophagy levels. In aged rats after starvation, correlation coefficients between plasma branched-chain amino acids (valine, phenylalanine, methionine, and leucine) and autophagy were -0.779, -0.566, -0.527, and -0.531, respectively. Chen found that compared with non-weaned controls, 14-day-old early-weaned piglets exhibited significantly reduced plasma concentrations of phenylalanine, serine, lysine, and 1-methylhistidine on day 4 post-weaning, accompanied by significantly elevated expression of the autophagy-related protein LC3-II in the liver. In vitro experiments have also confirmed that glutamine, arginine, and leucine deficiency can induce autophagy in porcine intestinal epithelial cells and human embryonic kidney cells, with autophagy levels returning to normal upon amino acid repletion. These findings collectively demonstrate that amino acid deficiency indeed induces autophagy. Notably, as autophagy increases in weaning-stressed piglets, declining trends in immune-related indicators such as white blood cells, red blood cells, and hemoglobin concentration are alleviated, suggesting that enhanced autophagy activity helps mitigate weaning stress.

The precise mechanism by which amino acids transmit signals to autophagy proteins remains incompletely understood. Some researchers propose that amino acids signal through mTOR to autophagy-related proteins, while others suggest a pathway from amino acids to eukaryotic initiation factor (eIF)-2α and then to autophagy proteins. Yan et al. blocked the mTOR signaling pathway with rapamycin in leucine-deficient medium and then re-supplemented leucine, finding that autophagy was no longer suppressed. This indicates that the mTOR signaling pathway is essential for leucine-mediated autophagy relief, and that mTORC1 inhibition is a necessary condition for autophagy induction.

3.1 The AMPK-mTOR Pathway

Current research suggests that the AMPK-mTOR pathway regulates autophagy by precisely modulating three serine sites on the autophagy initiation protein ULK1. During cellular starvation (glucose deprivation), AMPK phosphorylates ULK1 at serine residues 317 and 777 to trigger autophagy, whereas under nutrient-rich conditions, mTOR phosphorylates ULK1 at serine 757, disrupting AMPK' s regulation of ULK1. Amino acid starvation likely employs a similar regulatory mechanism. In piglets, AMPK activity is significantly activated while mTOR activity is markedly reduced in the liver 1–2 days after weaning, coinciding with increased autophagy. As the weaning period extends, autophagy levels decrease and the differences in AMPK and mTOR activities become less pronounced.

3.2 Small G Proteins

The molecular regulation of mTOR is extremely complex and diverse. Small G proteins, with molecular weights of only 20–30 kDa, possess GTPase activity like heterotrimeric G proteins but with weaker intrinsic activity that requires enhancement by GTPase-activating proteins (GAPs). These proteins are activated by GTP binding and inactivated upon GTP hydrolysis to GDP. The small G protein family includes the Ras, Rab, Rho, Arf, and Ran subfamilies, which form active ternary complexes with mTOR by binding to mTOR adaptor protein (Raptor) and mTOR itself, thereby recruiting downstream substrates to mTORC1.

Ras homolog enriched in brain (Rheb) is an upstream small G protein that regulates the mTORC1 signaling pathway and is essential for amino acid-mediated mTORC1 signaling. Interference with either Rheb or Raptor in leucine-starved cells induces significant autophagy that cannot be rescued by leucine repletion, demonstrating that both Rheb and Raptor are necessary molecules for transmitting amino acid signals to autophagy proteins. Current consensus holds that amino acids promote mTORC1-Raptor complex formation and translocation to the lysosomal surface, where interaction with Rheb activates mTORC1, though the precise signaling mechanism between amino acids and mTORC1 remains unclear. In contrast, interference with another upstream mTOR component, G protein β-subunit-like protein (GβL), still allows leucine starvation-induced autophagy, and leucine repletion can partially but not completely restore normal autophagy levels, indicating that GβL is not an essential factor for leucine signaling.

In 2008, American researchers discovered that amino acids activate mTORC1 through Rag GTPases, which are essential for amino acid-induced mTORC1 activation. However, Rag GTPases lack membrane localization sequences. The four Rag subunits—RagC and RagD forming heterodimers with RagA and RagB, respectively—convert from GDP-bound to GTP-bound forms when intracellular amino acid concentrations increase, though the mechanism of this GDP-to-GTP

conversion remains unclear. Subsequently, Ragulator and the GTP-bound Rag heterodimers interact with Raptor, facilitating mTORC1 complex translocation to the lysosomal surface where Rheb resides, thereby activating mTORC1. This suggests that Rag GTPases transmit amino acid signals to mTORC1 by altering nucleotide binding of Rag subunits. Recent studies indicate that Rag-Rheb-mediated mTOR signaling is required for amino acid-induced mTORC1 activation and operates independently of the classical TSC1/TSC2-Rheb regulatory pathway.

Harvard and MIT researchers generated a genetically engineered mouse expressing a constitutively active form of GTPase RagA. These mice exhibited suppressed autophagy induction during nutrient (amino acid) deficiency, resulting in nutritional crisis and death. In normal mice, RagA activation in the presence of amino acids switches on mTORC1 signaling to regulate growth, while amino acid deficiency inactivates RagA, leading to mTORC1 inactivation and autophagy initiation to help animals survive until the next feeding. In the engineered mice, however, persistent RagA activity maintained mTORC1 activation regardless of amino acid status, as evidenced by increased phosphorylation of the mTOR downstream effector ribosomal protein S6 kinase 1 (S6K1). Although recent years have witnessed new advances in understanding amino acid activation of mTORC1, the fundamental question of how intracellular amino acid concentrations are sensed by signaling molecules remains unanswered.

3.3 Aminoacyl-tRNA Synthetase (aaRS)

aaRS participates in RNA translation by catalyzing tRNA aminoacylation. Organisms possess 20 types of aaRS, each corresponding to one of the 20 proteinogenic amino acids (e.g., leucine corresponds to LeuRS). The canonical functions of aaRS include amino acid activation and tRNA aminoacylation. Recent discoveries in yeast and mammals have revealed that leucyl-tRNA synthetase (LRS) can directly sense intracellular leucine concentrations and transmit amino acid stimuli to Rag GTPases.

On one hand, LRS assembles into mTORC1 by directly interacting with RagD, a regulatory protein within the complex. On the other hand, LRS binds to inactive RagD-GTP and promotes its conversion to active RagD-GDP through its GAP domain, with the activated heterodimer directly stimulating mTORC1. Other branched-chain amino acids, such as isoleucine, can also activate mTORC1 through this mechanism.

3.4 MicroRNA (miRNA)

Wu et al. identified 84 differentially expressed miRNAs in leucine-deficient C2C12 cells using miRNA microarray technology. Target prediction revealed that miR-20a and miR-106b are associated with the mTOR-autophagy pathway and the autophagy-related gene Atg1. Co-transfection of miR-20a and miR-106b decreased renilla luciferase expression by 40–45% compared to

non-starved controls. Overexpression of these miRNAs for 36 hours did not alter Atg1 mRNA levels but reduced its protein expression by 40% as shown by Western blot. The researchers concluded that miR-20a and miR-106b influence autophagy by post-transcriptionally suppressing Atg1 expression.

Autophagy is a dynamic process that continuously changes with physiological status; therefore, results obtained from static methods may not accurately reflect true autophagic flux in cells. More importantly, we currently lack quantitative criteria to determine whether a given level of autophagy is beneficial or detrimental. Consequently, research should integrate in vivo and in vitro findings to accurately and comprehensively elucidate autophagy' s role in various biological processes. While autophagy has become a major focus in biological and medical research, and the relationship between mTOR signaling and metabolic regulation has been partially investigated in animal nutrition, few reports have addressed how mTOR signaling regulates autophagy, particularly regarding nutrient sensing mechanisms. Therefore, the regulatory effects and response mechanisms of amino acids—especially essential amino acids—on animal metabolism, including autophagy and signaling pathways, warrant further intensive study.

References

[1] LEVINE B, KROEMER G. Autophagy in the pathogenesis of disease[J]. Cell, 2008, 132(1): 27-42.

[2] CHEN Q. Study on the induction of autophagy by early weaning stress in piglets[D]. Master' s Thesis. Wuhan: Huazhong Agricultural University, 2013.

[3] ZONCU R, EFEYAN A, SABATINI D M. mTOR: From growth signal integration to cancer, diabetes and ageing[J]. Nature Reviews Molecular Cell Biology, 2011, 12(1): 21-35.

[4] SHINTANI T, KLIONSKY D J. Autophagy in health and disease: a double-edged sword[J]. Science, 2004, 306(5698): 990-995.

[5] TIAN F, DEGUCHI K, YAMASHITA T, et al. In vivo imaging of autophagy in a mouse stroke model[J]. Autophagy, 2010, 6(8): 1107-1114.

[6] JUNG C H, JUN C B, RO S H, et al. ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery[J]. Molecular Biology of the Cell, 2009, 20(7): 1992-2003.

[7] GOTTLIEB RA, CARREIRA RS. Autophagy in health and disease. 5. mitophagy as a way of life[J]. American Journal of Physiology-Cell Physiology, 2010, 299(2): C203-C210.

[8] JUNG C H, RO S H, CAO J, et al. mTOR regulation of autophagy[J]. FEBS Letters, 2010, 584(7): 1287-1295.

[9] SURYAWAN A, DAVIS T A. The abundance and activation of mTORC1 regulators in skeletal muscle of neonatal pigs are modulated by insulin, amino

acids, and age[J]. Journal of Applied Physiology, 2010, 109(5): 1448-1454.

[10] SANCAK Y, BAR-PELED L, ZONCU R, et al. Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids[J]. Cell, 2010, 141(2): 290-303.

[11] KIM J, KUNDU M, VIOLLET B, et al. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1[J]. Nature Cell Biology, 2011, 13(2): 132-141.

[12] XU D, HE G. Research progress in amino acid sensing and metabolic regulation[J]. Chinese Journal of Animal Nutrition, 2015, 27(2): 342-351.

[13] GAO Y, WU G. Research progress on amino acid signal transduction mechanisms in the mTORC1 pathway[J]. Chinese Journal of Cell Biology, 2012, 34(8): 812-818.

[14] EFEYAN A, ZONCU R, CHANG S, et al. Rag GTPase-mediated regulation of mTORC1 by nutrients is necessary for neonatal autophagy and survival[J]. Nature, 2013, 493(7434): 679-683.

[15] HOSOKAWA N, SASAKI T, IEMURA S, et al. Atg101, a novel mammalian autophagy protein interacting with Atg13[J]. Autophagy, 2009, 5(7): 973-979.

[16] WANG H, QIU L, LI Y. Correlation analysis between plasma essential amino acid concentration and autophagy level[J]. Journal of Shanxi Medical University, 2010, 41(4): 288-291.

[17] ZHU Y, XUE X, ZHOU T, et al. Study on glutamine, arginine, and leucine deficiency-induced autophagy in porcine intestinal epithelial cells[C]//Proceedings of the 11th National Animal Nutrition Symposium of the Chinese Association of Animal Science and Veterinary Medicine. Changsha: Chinese Association of Animal Science and Veterinary Medicine, 2012.

[18] YAN X H, SUN Q M, JI J, et al. Reconstitution of leucine-mediated autophagy via the mTORC1-Barkor pathway in vitro[J]. Autophagy, 2012, 8(2): 213-221.

[19] DODD K M, TEE A R. Leucine and mTORC1: a complex relationship[J]. American Journal of Physiology-Endocrinology and Metabolism, 2012, 302(11): E1329-E1342.

[20] KIM E, GORAKSHA-HICKS P, LI L, et al. Regulation of TORC1 by Rag GTPases in nutrient response[J]. Nature Cell Biology, 2008, 10(8): 935-945.

[21] ZHANG T L, PÉLI-GULLI M P, YANG H, et al. Ego3 functions as a homodimer to mediate the interaction between Gtr1-Gtr2 and Ego1 in the EGO complex to activate TORC1[J]. Structure, 2012, 20(12): 2151-2160.

[22] JEONG J H, LEE Y M, KIM D H, et al. Crystal structure of the Gtr1p (GTP)-Gtr2p(GDP) protein complex reveals large structural rearrangements

triggered by GTP-to-GDP conversion[J]. The Journal of Biological Chemistry, 2012, 287(35): 29648–29653.

[23] BAR-PELED L, SCHWEITZER L D, ZONCU R, et al. Ragulator is a GEF for the Rag GTPases that signal amino acid levels to mTORC1[J]. Cell, 2012, 150(6): 1196–1208.

[24] SANCAK Y, PETERSON T R, SHAUL Y D, et al. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1[J]. Science, 2008, 320(5882): 1496–1501.

[25] TEE A R, MANNING B D, ROUX P P, et al. Tuberous sclerosis complex gene products, Tuberin and Hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb[J]. Current Biology, 2003, 13(15): 1259–1268.

[26] HAN J M, JEONG S J, PARK M C, et al. Leucyl-tRNA synthetase is an intracellular leucine sensor for the mTORC1-signaling pathway[J]. Cell, 2012, 149(2): 410–424.

[27] RUI G, LI L, YI L, et al. Crystal structure of the Gtr1p-Gtr2p complex reveals new insights into the amino acid-induced TORC1 activation[J]. Genes & Development, 2011, 25(16): 1668–1673.

[28] SEGEV N, HAY N. Hijacking leucyl-tRNA synthetase for amino acid-dependent regulation of TORC1[J]. Molecular Cell, 2012, 46(1): 4–6.

[29] WU H, WANG F L, HU S L, et al. MiR-20a and miR-106b negatively regulate autophagy induced by leucine deprivation via suppression of ULK1 expression in C2C12 myoblasts[J]. Cellular Signaling, 2012, 24(11): 2179–2186.

[30] KLIONSKY D J, ABELIOVICH H, AGOSTINIS P, et al. Guidelines for the use and interpretation of assays for monitoring autophagy in higher eukaryotes[J]. Autophagy, 2008, 4(2): 151–175.

Note: Figure translations are in progress. See original paper for figures.