Molecular Mechanism of Intramammary Amino Acid Regulation of Milk Protein Synthesis Post-print

Authors: Zhang Muchen, Zhao Shengguo, Zheng Nan, Wang Jiaqi, Gao Haina, Wang Shanshan

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

Abstract

As an important "biological factory" for maintaining mammalian life activities, the mammary gland utilizes amino acids and other nutrients taken up from the circulating blood as substrates to synthesize milk proteins. Studies have confirmed that amino acids can also act as signaling factors that regulate the transcription and translation processes of milk protein genes through multiple signal cascade transduction pathways within the mammary gland, thereby affecting milk protein synthesis in the mammary gland. The Janus kinase–signal transducer and activator of transcription (JAK-STAT) signaling pathway and the mammalian target of rapamycin (mTOR) signaling pathway are the major regulatory pathways in the transcription and translation processes of milk protein genes. This review summarizes the molecular mechanisms of JAK-STAT and mTOR signaling pathways in the mammary gland and the research progress on amino acids regulating milk protein synthesis through these pathways, aiming to further elucidate the mechanism by which amino acids regulate milk protein synthesis.

Full Text

Preamble

Molecular Mechanisms in Regulation of Milk Protein Synthesis by Amino Acids in the Mammary Gland

ZHANG Muchen[1], GAO Haina[1,2], ZHAO Shengguo[1]*, WANG Shanshan[1,3], ZHENG Nan[1], WANG Jiaqi[1]

[1]Ministry of Agriculture-Milk Risk Assessment Laboratory, Ministry of Agriculture-Milk and Dairy Product Inspection Center, State Key Laboratory

of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100193, China;
[2]College of Animal Science and Technology, Gansu Agricultural University, Lanzhou 730070, China;
[3]College of Animal Science, Inner Mongolia Agricultural University, Hohhot 010018, China

Abstract: As an important "biological factory" that sustains mammalian life activities, the mammary gland utilizes nutrients such as amino acids absorbed from circulating blood as substrates to synthesize milk protein. Research has confirmed that amino acids also function as signaling factors that regulate milk protein gene transcription and translation through multiple intracellular signaling cascade pathways in the mammary gland, thereby influencing milk protein synthesis. The Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling pathway and the mammalian target of rapamycin (mTOR) signaling pathway represent the primary regulatory routes in milk protein gene transcription and translation. This review summarizes the molecular mechanisms of the JAK-STAT and mTOR signaling pathways in the mammary gland and research progress on how amino acids regulate milk protein synthesis through these pathways, aiming to further elucidate the mechanisms by which amino acids modulate milk protein synthesis.

Keywords: amino acid; milk protein; regulation; JAK-STAT; mTOR

*Corresponding author: Assistant Researcher, E-mail: zhaoshengguo1984@163.com

---

For multicellular eukaryotes including mammals, the availability of nutrients in the local environment is a primary determinant of cell survival. These nutrients are sensed through various intracellular signaling pathways and integrated into cellular anabolic programs that support cell growth and proliferation. In variable environments, animals readily adapt their growth and development processes through nutrient-sensing mechanisms, remote growth factor regulatory systems, and hormonal networks [1].

The mammary gland serves as a crucial biological factory sustaining mammalian life activities, capable of absorbing various small molecular nutrients such as glucose and amino acids from circulating blood via transporters into mammary epithelial cells. These nutrients serve as precursors for synthesizing essential milk components including lactose, milk fat, and milk protein [2]. The mammary gland exhibits the highest net utilization rate of amino acids among all tissues in dairy cows during lactation [3], using absorbed amino acids as substrates to synthesize over 90% of milk proteins—including casein, β-lactoglobulin, and α-lactalbumin—based on milk protein gene templates [4]. Recent studies have demonstrated that amino acids not only represent the most important precursors for milk protein synthesis but also function as signaling molecules that regulate milk protein gene transcription and translation processes through multiple intracellular signaling cascade pathways [5], thereby influencing milk

protein synthesis at both transcriptional and translational levels. The most significant pathways involved are the Janus kinase-signal transducer and activator of transcription (JAK-STAT) and mammalian target of rapamycin (mTOR) signaling pathways (Figure 1 [Figure 1: see original paper]).

1. JAK-STAT Pathway

Janus kinases (JAKs) are a class of non-receptor cytoplasmic tyrosine protein kinases associated with cytokine receptors. In mammals, this family comprises Jak1, Jak2, Jak3, and Tyk2, all of which share similar JAK homology (JH) domains. These domains primarily include the kinase region JH1, a pseudokinase region JH2 with inhibitory activity, and receptor-binding regions JH6 and JH7 [6]. Studies investigating signal transduction pathways mediated by erythropoietin receptors identified a class of transcriptional regulators in JAK substrates—signal transducers and activators of transcription (STATs)—that play critical roles in cellular signal cascade transduction and gene transcription activation. The mammalian STAT family consists of STAT1, STAT2, STAT3, STAT4, STAT5 (STAT5a and STAT5b), and STAT6 [7]. STAT proteins generally contain functionally important segments including a tyrosine residue essential for activation, an SH2 domain for cytokine receptor binding, a DNA-binding domain, and a transcriptional activation region [7].

This pathway can be activated by over 50 cytokines and growth factors including interleukins, interferons, erythropoietin, prolactin, and growth hormone [8–10]. It mediates cascades that transmit various extracellular signals from the cell membrane to the nucleus, thereby regulating transcription of specific nuclear genes and playing crucial regulatory roles in cell proliferation, differentiation, migration, and apoptosis [9] (Figure 1). The specific mechanism proceeds as follows: signal transduction initiates when extracellular cytokines or growth factors bind to their corresponding transmembrane receptors, promoting transactivation of receptor-associated JAKs. This brings JAKs into close spatial proximity and induces conformational changes that distance their kinase domains from the inhibitory pseudokinase domains. Activated JAKs subsequently phosphorylate "latent"STAT monomer molecules, leading to STAT dimerization and nuclear translocation. These dimers bind to specific DNA sequences, some located near transcription start sites but more often associated with enhancers or other cis-acting elements at distal locations. A typical example is the IFN-γ activation site (GAS), and binding of STAT dimers to these sites regulates transcription of corresponding specific genes [9] (Figure 1).

2. Amino Acids Regulate Milk Protein Gene Transcription Through the JAK2-STAT5 Pathway in the Mammary Gland

Research indicates that JAK2 and STAT5, and their coordinated signaling cascade, play crucial roles in milk protein synthesis in the mammary gland. *In vivo*

experiments demonstrate that knockout of the STAT5a gene in mice results in impaired mammary alveolar development and failure to lactate after the first pregnancy, accompanied by reduced STAT5b protein concentration and weakened tyrosine phosphorylation. Knockout of the STAT5b gene also reduces mammary alveolar developmental capacity [11]. Both JAK2 and STAT5 genes are essential for maintaining proliferation and differentiation of mammary epithelial cells during pregnancy in mice; JAK2-deficient mice cannot form mammary secretory alveoli at parturition, and hormone-induced mammary epithelial cell proliferation is reduced by 95% [2]. *In vivo* studies using transgenic animals show that the β-lactoglobulin and whey acidic protein genes contain GAS-specific sequences near their promoters that are essential for promoting secretion of these milk proteins [11–13]. *In vitro* culture of bovine mammary tissue reveals that prolactin can activate STAT5, thereby activating GAS element-containing promoter sequences in the β-casein gene (CSN2) and promoting β-casein gene transcription [14]. Inhibition of STAT5a expression in bovine mammary epithelial cells reduces intracellular β-casein gene mRNA abundance, while overexpression of STAT5a increases it [15]. Additionally, studies in mouse mammary tissue show that prolactin activates JAK2, which subsequently phosphorylates STAT5, and activated STAT5 promotes transcription of multiple milk protein genes, ultimately increasing milk protein secretion [16]. Growth hormone and other lactation-related factors such as insulin-like growth factor exhibit similar mechanisms in regulating milk protein gene expression in bovine mammary glands [17].

Recent investigations into the molecular mechanisms by which amino acids affect milk protein synthesis have revealed that various amino acids can regulate milk protein gene transcription by influencing the transcriptional levels of key signaling molecules (JAK2, STAT5) in the JAK-STAT pathway, thereby affecting milk protein synthesis (Figure 1).

Early studies examining the effects of leucine supplementation on protein synthesis in mammals found that leucine infusion in rats increased skeletal muscle protein synthesis [18]. When different doses of leucine were infused into the duodenum of lactating Holstein cows, the 40 g/d dose significantly increased milk protein content, while groups receiving no supplementation or doses below or above this level showed significant decreases. Research on leucine' s effects on casein synthesis in bovine mammary epithelial cells and its underlying mechanisms demonstrated that adding 0.9 mmol/L leucine to culture medium significantly increased -casein (CSN3) gene mRNA levels, with this effect diminishing at concentrations below or above this level. The trends in JAK2 and STAT5 gene mRNA levels under different treatments paralleled those of casein genes, both reaching maxima at 0.9 mmol/L [19]. Furthermore, *in vivo* studies indicate that the amount of arginine absorbed by the goat mammary gland from blood far exceeds the arginine content secreted in milk protein [20]. Subsequent *in vitro* studies on arginine' s effects on lactation capacity in bovine mammary epithelial cells found that when arginine was added at 0, 69.5, 139, or 278 mg/L without altering other amino acids in the medium, αs2-casein (CSN1S2) and

-casein gene mRNA abundance showed increasing trends with supplementation. In high-concentration groups (556, 1,112, 2,224 mg/L), αs2-casein and -casein gene mRNA abundance showed decreasing trends with increasing dose. Both casein synthesis levels reached maximum values at 556 mg/L, significantly higher than other groups. All arginine supplementation groups significantly upregulated JAK2 and STAT5 gene mRNA abundance compared to the non-supplemented group, showing a trend of initial increase followed by decrease with increasing arginine concentration, peaking at the 556 mg/L arginine supplementation group. This demonstrates that arginine can regulate milk protein gene transcription by affecting the JAK2-STAT5 pathway [21].

Lysine and methionine are considered the primary limiting amino acids for dairy cows fed diets with straw as the main roughage source [2]. Nan et al. [22] investigated different lysine-to-methionine ratios in bovine mammary epithelial cells and reported that lysine and methionine effects on milk protein synthesis capacity were dose-dependent. With increasing concentrations of lysine and methionine in culture medium, total casein secretion showed initial increases followed by decreases, with optimal effects at 1.2 mmol/L and 0.5 mmol/L, respectively. Treatment with these optimal concentrations significantly increased mRNA levels of αs1-casein (CSN1S1), αs2-casein, β-casein, -casein, JAK2, and STAT5 compared to non-supplemented controls. The authors then treated cells with the optimal concentration ratio (lysine:methionine = 3:1), which also significantly promoted JAK2 and STAT5 gene transcription. Nan et al. [22] thus concluded that lysine and methionine effects on lactation are closely associated with the JAK2-STAT5 pathway. Wang Lina [15] demonstrated that amino acids interact with the STAT5a gene to regulate lactation in bovine mammary epithelial cells, with optimal amino acid concentrations significantly promoting JAK2 and STAT5a gene transcription, increasing total and phosphorylated STAT5a protein levels, and ultimately significantly enhancing β-casein gene transcription and β-casein secretion. Other studies showed that replacing 15% of free methionine in culture medium with methionyl-methionine dipeptide significantly increased αs1-casein gene mRNA expression and synthesis in cultured mammary tissue, while significantly increasing cellular methionine uptake. JAK2 and STAT5 gene mRNA levels were also significantly higher than in the non-replacement group, suggesting that methionine dipeptide promotes casein synthesis by enhancing intracellular substrate availability and activating the JAK2-STAT5 pathway [23]. Additionally, reports indicate that amino acids may also regulate milk protein synthesis by interacting with other lactogenic hormones to jointly act on the mammary JAK2-STAT5 pathway [24].

3. mTOR Signaling Pathway

Changes in growth factors, energy status, environmental stress, and amino acid supply in the cellular environment of mammalian cells can all regulate intracellular mTOR activity. mTOR is typically activated through phosphorylation of its component proteins, subsequently promoting multiple downstream parallel

effector pathways that facilitate anabolic processes such as protein translation while inhibiting catabolic processes like autophagy. This prevents futile cycles caused by mismatched synthesis and degradation activities, thereby regulating cell growth and metabolism [1]. mTOR is an atypical serine/threonine kinase that forms two distinct complexes with various proteins: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTORC1 comprises the scaffold subunit regulatory-associated protein of mTOR (raptor), two endogenous kinase inhibitors (DEP-domain-containing mTOR-interacting protein, DEPTOR; proline-rich AKT substrate 40 kDa, PRAS40), and the positive regulatory protein mammalian ortholog of LST8 (mLST8) [1].

mTORC1 is regulated by Ras homolog enriched in brain (Rheb) [25], a small G protein that localizes to the lysosomal surface [26] and potently stimulates mTORC1 kinase activity by promoting phosphorylation of specific serine or threonine residues in the mTORC1 complex. Rheb is negatively regulated by the tuberous sclerosis complex (TSC), a heterotrimeric complex composed of TSC1, TSC2, and TBC1D7 [27]. The TSC2 component functions as a GTPase-activating protein for Rheb, converting Rheb from its active GTP-bound state to an inactive GDP-bound state. The TSC complex acts as a central hub upstream of mTORC1, integrating signals including cell division signals, growth factors, energy levels, oxygen availability, and genotoxic stress, all of which can influence mTORC1 signaling by modulating TSC complex activity [1] (Figure 1). Recent studies have identified multiple upstream molecules that mediate amino acid effects on mTOR complexes, including SLC38A9, leucyl-tRNA synthetase, Ragulator, Rag GTPases, and GATOR [25–28], which are currently under further investigation.

Studies on amino acid effects on skeletal muscle protein synthesis revealed that amino acids stimulate protein synthesis in rats and newborn infants, with leucine being the most prominent effector [28]. This process occurs through mTORC1-mediated regulation of muscle protein translation [28]. The specific mechanism involves mTORC1 activation by amino acids, which primarily regulates the phosphorylation status of translation control factors including eukaryotic translation initiation factor 4E binding protein 1 (4EBP1), ribosomal protein S6 kinase 1 (S6K1), and eukaryotic elongation factor 2 (eEF2). Activated 4EBP1 releases eukaryotic translation initiation factor 4E (eIF4E), which then forms the eIF4F complex with eukaryotic translation initiation factor 4G (eIF4G), eIF4A, and eIF4B. This complex binds the 40S ribosomal subunit to form the 43S preinitiation complex, initiating translation. Simultaneously, mTOR-phosphorylated and activated S6K1 phosphorylates ribosomal protein S6 (rpS6), enhancing rpS6 activity (rpS6 is a component of the 40S complex, and its enhanced activity promotes 40S complex activity) and inhibiting eEF2 kinase (eEF2K) activity, thereby preventing eEF2K-mediated inhibition of eEF2 and regulating mRNA translation [24].

4. Amino Acids Regulate Post-Transcriptional Translation of Milk Protein Genes Through the mTOR Pathway in the Mammary Gland

In vivo studies demonstrate that the mTOR signaling pathway plays an indispensable role in both initiation and elongation stages of milk protein synthesis translation, and is influenced by amino acid supply levels. Doepel et al. [3] reported that abomasal infusion of all essential amino acids increased milk yield and milk protein yield in dairy cows. Rius et al. [29] found that intravenous leucine infusion in lactating cows promoted mammary mTOR and S6K1 phosphorylation and milk protein synthesis. Tracking studies of physiological changes in bovine mammary glands from pregnancy to full lactation revealed that compared to pregnancy, lactation significantly upregulated transcription levels of amino acid transporter genes, particularly carriers for essential amino acids. Additionally, FRAP1—the gene encoding mTOR in bovine mammary glands—showed expression changes throughout lactation that matched the milk protein secretion curve [24]. Compared to pregnant cows, lactating cows showed significantly increased expression of mTOR and its downstream eIF4E, and throughout lactation, mRNA levels of ribosomal 40S and 60S subunits and eEF2 gene, as well as protein synthesis rates, were all upregulated [24].

In vitro studies on amino acid regulation of lactation mechanisms in bovine mammary epithelial cells have found that various amino acids can influence milk protein synthesis by regulating transcriptional levels and protein phosphorylation status of key mTOR pathway signaling molecules (mTOR, 4EBP1, S6K1, eEF2) (Figure 1), with variations depending on amino acid type, concentration, and ratio. Gao et al. [30] showed that when bovine mammary epithelial cells were cultured with different leucine or histidine concentrations, milk protein gene and mTOR pathway-related gene transcription levels differed. Compared to blank controls, addition of 0.45–10.80 mmol/L leucine or 0.15–4.80 mmol/L histidine for 6 h significantly upregulated αs1-casein and -casein gene mRNA abundance. Different leucine and histidine concentrations had varying effects on mRNA abundance of mTOR, raptor, GβL, rpS6, and 4EBP1. At leucine concentrations of 0.45–5.40 mmol/L, mTOR, raptor, and GβL gene mRNA abundance were significantly higher than in non-supplemented groups, peaking at 1.35 mmol/L. Compared to negative controls, raptor, GβL, 4EBP1, eEF2, eIF4E, and rpS6 gene mRNA abundance showed increasing trends with histidine concentration (0.15–9.60 mmol/L). Addition of 0.45–10.80 mmol/L leucine significantly promoted total casein secretion and phosphorylation levels of mTOR (Ser^{2481}), raptor (Ser^{792}), eEF2 (Thr^{56}), and eIF4E (Ser^{209}) in bovine mammary epithelial cells, while 0.15–9.60 mmol/L histidine significantly promoted secretion of αs2-casein, β-casein, and -casein and phosphorylation levels of mTOR (Ser^{2481}), raptor (Ser^{792}), S6K1 (Thr^{389}), 4EBP1 (Thr^{37}), eEF2 (Thr^{56}), and eIF4E (Ser^{209}) [31].

Appuhamy et al. [32] demonstrated that removal of all essential amino acids from culture medium for bovine mammary epithelial cells and mammary tissue slices

significantly reduced phosphorylation levels of mTOR (Ser^{2448}), S6K1 (Thr^{389}), and 4EBP1 ($Thr^{37}/^{46}$), while significantly increasing eEF2 (Thr^{56}) phosphorylation. Phosphorylation levels of mTOR, S6K1, and 4EBP1 positively correlated with mammary tissue protein synthesis rates, indicating that available essential amino acids regulate mammary protein synthesis through key control points of translation initiation and elongation. Appuhamy et al. [33] subsequently confirmed that leucine and isoleucine promoted mTORC1 phosphorylation and activation in cultured bovine mammary tissue and epithelial cells, thereby activating S6K1 and 4EBP1 phosphorylation and increasing milk protein synthesis rates. When culture medium lacked leucine and isoleucine, cultured mammary tissue synthesized 59% and 61% less milk protein, respectively, with mTOR phosphorylation levels decreasing by over 40%. Addition of mTOR upstream pathway inhibitors significantly reduced S6K1 phosphorylation and decreased milk protein secretion in bovine mammary epithelial cells [34].

Nan et al. [22] reported that when cells were treated with optimal concentrations of lysine (1.2 mmol/L) and methionine (0.5 mmol/L) that promote casein synthesis, mTOR gene mRNA abundance was significantly higher than in blank controls, with no significant effect on S6K1 gene mRNA abundance but a significant reduction in 4EBP1 gene mRNA expression. Different mixing ratios affected casein synthesis differently, with an optimal concentration ratio (lysine:methionine = 3:1) that significantly promoted mTOR and 4EBP1 gene transcription. Both optimal lysine alone and the optimal ratio significantly increased total and phosphorylated mTOR protein levels, suggesting that appropriate lysine and methionine concentrations can promote lactation by activating the mTOR signaling pathway in mammary epithelial cells.

Milk protein content and composition constitute major material foundations of milk's nutritional quality. Investigating methods and mechanisms for improving milk protein content and composition through dietary nutrition to guide efficient dairy livestock production is becoming a frontier in ruminant nutrition research. As fundamental components of proteins, amino acids represent a highlight in molecular nutrition research regarding their metabolic value and nutritional mechanisms. During milk protein synthesis, amino acids function not only as precursor nutrients but also as signaling molecules that regulate milk protein gene transcription through the JAK-STAT pathway and post-transcriptional translation through the mTOR-centered hub pathway, jointly influencing milk protein synthesis at two temporally and spatially associated levels. However, current research on the mammary JAK-STAT pathway has primarily focused on cytokines, and the specific regulatory mechanisms of nutrients on this pathway require further investigation. Moreover, the relationship between this pathway and the mTOR pathway remains unclear, and research on amino acid sensing and transport in mammary cells is still limited. This review will facilitate further research on amino acid nutrition in mammary tissue protein synthesis and regulation.

References

[1] BAR-PELED L, SABATINI D M. Regulation of mTORC1 by amino acids[J]. Trends in Cell Biology, 2014, 24(7): 400–406.

[2] LI Q Z. Dairy Mammary Gland Development and Lactation Biology[M]. Beijing: Science Press, 2014: 123–124.

[3] DOEPEL L, LAPIERRE H. Changes in production and mammary metabolism of dairy cows in response to essential and nonessential amino acid infusions[J]. Journal of Dairy Science, 2010, 93(7): 3264–3274.

[4] WANG J F, HUANG J L, LIANG G Y. Research on the synthesis mechanism and regulation pathways of milk protein in lactating ruminants[J]. Feed Industry, 2005, 26(7): 13–17.

[5] TOERIEN C A, TROUT D R, CANT J P. Nutritional stimulation of milk protein yield of cows is associated with changes in phosphorylation of mammary eukaryotic initiation factor 2 and ribosomal s6 kinase 1[J]. The Journal of Nutrition, 2010, 140(2): 285–292.

[6] LIONGUE C, O' SULLIVAN L A, TRENGOVE M C, et al. Evolution of JAK-STAT pathway components: mechanisms immune system development[J]. PLoS One, 2012, 7(3): e32777.

[7] WANG Y M, LEVY D E. Comparative evolutionary genomics of the STAT family of transcription factors[J]. JAK-STAT, 2012, 1(1): 23–36.

[8] STARK R, DARNELL E, Jr. The JAK-STAT pathway twenty[J]. Immunity, 2012, 36(4): 503–514.

[9] VILLARINO A V, KANNO Y, FERDINAND J R, et al. Mechanisms of Jak/STAT signaling in immunity and disease[J]. The Journal of Immunology, 2015, 194(1): 21–27.

[10] BROOKS A J, WEI D, O'MARA M L, et al. Mechanism of activation of protein kinase JAK2 by the growth hormone receptor[J]. Science, 2014, 344(6185): 1249783.

[11] LIU X, ROBINSON G W, WAGNER K U, et al. Stat5a is mandatory for adult mammary gland development and lactogenesis[J]. Genes & Development, 1997, 11(2): 179–186.

[12] SHILLINGFORD J M, MIYOSHI K, ROBINSON G W, et al. Jak2 is an essential tyrosine kinase involved in pregnancy-mediated development of mammary secretory epithelium[J]. Molecular Endocrinology, 2002, 16(3): 563–570.

[13] OLIVER C H, WATSON C J. Making milk: a new link between STAT5 and Akt1[J]. JAK-STAT, 2013, 2(2): e23228.

[14] LIU X, ROBINSON G W, GOUILLEUX F, et al. Cloning and expression of Stat5 and an additional homologue (Stat5b) involved in prolactin signal trans-

duction in mouse mammary tissue[J]. Proceedings of the National Academy of Sciences of the United States of America, 1995, 92(19): 8831-8835.

[15] WANG L N. The regulatory effect and mechanism of amino acids interacting with STAT5A gene on lactation in bovine mammary epithelial cells[D]. PhD Thesis. Harbin: Northeast Agricultural University, 2014: 2-61.

[16] WATSON C J, BURDON T G. Prolactin signal transduction mechanisms in the mammary gland: the role of the Jak/Stat pathway[J]. Reviews of Reproduction, 1996, 1(1): 1-5.

[17] YANG J, KENNELLY J J, BARACOS V E. The activity of transcription factor Stat5 responds to prolactin, growth hormone, and IGF-I rat and bovine mammary explant culture[J]. Journal of Animal Science, 2000, 78(12): 3114-3125.

[18] DARDEVET D, SORNET C, BAYLE G, et al. Postprandial stimulation of muscle protein synthesis in old rats can be restored by a leucine-supplemented meal[J]. The Journal of Nutrition, 2002, 132(1): 95-100.

[19] DAI W T, LI A J, ZHENG N, et al. Effects of leucine levels on proliferation of bovine mammary epithelial cells and expression of genes related to -casein synthesis[J]. Journal of Animal Nutrition, 2015, 27(5): 1559-1566.

[20] BEQUETTE B J, BACKWELL F R C, CALDER A G, et al. Application of a U-13C-labeled amino acid tracer in lactating dairy goats for simultaneous measurements of the flux of amino acids in plasma and the partition of amino acids to the mammary gland[J]. Journal of Dairy Science, 1997, 80(11): 2842-2853.

[21] WANG M Z, XU B L, WANG H R, et al. Effects of Arginine concentration on the in vitro expression of Casein and mTOR pathway related genes in mammary epithelial cells from dairy cattle[J]. PLoS One, 2014, 9(5): e95985.

[22] NAN X M, BU D P, LI X Y, et al. Ratio of lysine to methionine alters expression of genes involved in milk protein transcription and translation and mTOR phosphorylation in bovine mammary cells[J]. Physiological Genomics, 2014, 46(7): 268-275.

[23] YANG J X, WANG C H, XU Q B, et al. Methionyl-Methionine promotes α-s1 casein synthesis in bovine mammary gland explants by enhancing intracellular substrate availability and activating JAK2-STAT5 and mTOR-Mediated signaling pathways[J]. The Journal of Nutrition, 2015, 145(8): 1748-1753.

[24] BIONAZ M, LOOR J J. Gene networks driving bovine mammary protein synthesis during the lactation cycle[J]. Bioinformatics and Biology Insights, 2011, 5: 83-98.

[25] HUANG K Z, FINGAR D C. Growing knowledge of the mTOR signaling network[J]. Seminars in Cell & Developmental Biology, 2014, 36: 79-90.

[26] ABRAHAM R T. Making sense of amino acid sensing[J]. Science, 2015, 347(6218): 128-129.

[27] DIBBLE C, ELIS W, MENON S, et al. TBC1D7 is a third subunit of the TSC1-TSC2 complex upstream of mTORC1[J]. Molecular Cell, 2012, 47(4): 535-546.

[28] REBSAMEN M, POCHINI L, STASYK T, et al. SLC38A9 is a component of the lysosomal amino acid sensing machinery that controls mTORC1[J]. Nature, 2015, 519(7544): 477-481.

[29] RIUS A G, APPUHAMY J A D R N, CYRIAC J, et al. Regulation of protein synthesis in mammary glands of lactating dairy cows by starch and amino acids[J]. Journal of Dairy Science, 2010, 93(7): 3114-3127.

[30] GAO H N, HU H, WANG J Q, et al. Leucine or histidine affects casein synthesis in bovine mammary epithelial cells via the mammalian target of rapamycin signaling pathway[J]. Journal of Animal Nutrition, 2015, 27(4): 1124-1134.

[31] GAO H N, HU H, ZHENG N, et al. Leucine and histidine independently regulate milk protein synthesis in bovine mammary epithelial cells via mTOR signaling pathway[J]. Journal of Zhejiang University Science B, 2015, 16(6): 560-572.

[32] APPUHAMY J A D R N, BELL A L, NAYANANJALIE W A D, et al. Essential amino acids regulate both initiation and elongation of mRNA translation independent of insulin in MAC-T cells bovine mammary tissue slices[J]. The Journal of Nutrition, 2011, 141(6): 1209-1215.

[33] APPUHAMY J A D R N, KNOEBEL N A, NAYANANJALIE W A D, et al. Isoleucine and leucine independently regulate mTOR signaling and protein synthesis in MAC-T cells and bovine mammary tissue slices[J]. The Journal of Nutrition, 2012, 142(3): 484-491.

[34] KIMBALL S R, JEFFERSON L S. New functions for amino acids: effects on gene transcription and translation[J]. American Journal of Clinical Nutrition, 2006, 83(2): 500S-507S.

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