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Amino acid transportation, sensing and signal transduction in the mammary gland: key molecular signalling pathways in the regulation of milk synthesis

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Abstract

The mammary gland, a unique exocrine organ, is responsible for milk synthesis in mammals. Neonatal growth and health are predominantly determined by quality and quantity of milk production. Amino acids are crucial maternal nutrients that are the building blocks for milk protein and are potential energy sources for neonates. Recent advances made regarding the mammary gland further demonstrate that some functional amino acids also regulate milk protein and fat synthesis through distinct intracellular and extracellular pathways. In the present study, we discuss recent advances in the role of amino acids (especially branched-chain amino acids, methionine, arginine and lysine) in the regulation of milk synthesis. The present review also addresses the crucial questions of how amino acids are transported, sensed and transduced in the mammary gland.

Key words: **Mammary gland: Amino acids: Milk protein: Milk fat: Signalling pathways**

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Introduction

Milk is primarily composed of milk protein, fat, lactose, vitamins and minerals, which are important nutrient sources for neonates. Maternal nutrients are considered as building blocks for milk synthesis. In humans, breast-feeding decreases the risk of neonatal acute illnesses, diarrhoea and overweight/obesity (breast-feeding for more than 6 months)[1]. However, in pigs, sufficient and quality colostrum (first 24 h) supplies are important for neonates to gain higher weaning weight and have better growth performance later in life[2]. Calves fed adequate colostrum during the first week of their life exhibit significantly enhanced metabolic and immunological status[3]. Thus, understanding nutritional strategies to regulate milk synthesis and its underlying mechanism is important for human beings and other mammals.

Amino acids are not only basic components of proteins, but also act as functional regulators in a variety of biological processes. The functions of amino acids have been extensively studied in the gut, liver, muscle and adipose tissue, especially in the field of protein[4] and fat metabolism[5]. In the mammary gland, amino acid uptake from blood is almost equal to milk output based on a nitrogen basis in dairy cows[6–8], goats[9], sows[10] and ewes[11]. However, the destinies of different amino acids in mammary cells are different[6–8,10–12]. The mammary amino acid uptake:output ratios could be larger (for example, valine, isoleucine, leucine and arginine), equal (for example, methionine and histidine) or less than 1 (for example, asparagine and proline) (mammary amino acid uptake:output ratios in cows, goats, sows and ewes are shown in Table 1)[6–8,10–12]. Those amino acids with uptake:output ratios greater than 1 can be metabolised to CO_2, urea, polyamine or simply other non-essential amino acids[13]. In addition, various amino acids (especially branched-amino acids, methionine and arginine) are involved in the regulation of milk synthesis. A variety of signalling molecules have been proposed to cooperate with amino acids to regulate biological functions in the mammary gland. Signalling pathways regulating mammary

Abbreviations: Akt, protein kinase B; BCAA, branched-chain amino acid; CAT-1, cationic amino acid transporter-1; ERK, extracellular signal-regulated kinase; FABP5, fatty acid-binding protein 5; GATOR, GTPase-activating protein activity toward Rags; GNG12, guanine nucleotide-binding protein subunit γ-12; GPRC, G-protein-coupled receptor; HEK 293, human embryonic kidney 293 cells; LAT1, L-type amino acid transporter 1; LAT2, L-type amino acid transporter 2; LeuRS, leucyl-tRNA synthetase; mTORC1, mammalian target of rapamycin complex 1; PI3K, inositol 1,4,5-trisphosphate 3-kinase; SAM, *S*-adenosylmethionine; SNAT, Na-coupled neutral amino acid transporter; SREBP-1, sterol regulatory element-binding protein 1.

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Table 1. Mammary amino acid uptake:output ratios in different mammals

Species	Uptake: output ratios	Amino acids	References
Cows	= 1	Methionine, histidine, tryptophan, phenylalanine and tyrosine	(6–8)
	> 1	Valine, isoleucine, leucine, lysine and arginine	
	< 1	Non-essential amino acids	
Goats	= 1	Methionine, histidine, tryptophan, phenylalanine and tyrosine	(12)
	> 1	Valine, isoleucine, leucine, lysine and arginine	
	< 1	Serine, glutamine, proline and asparagine	
Sows	= 1	Methionine, histidine and lysine	(10)
	> 1	Arginine, leucine, isoleucine, valine, tryptophan, phenylalanine, glutamate, glutamine, alanine and glycine	
	< 1	Proline, aspartate and asparagine	
Ewes	= 1	Methionine, phenylalanine, tyrosine, threonine and histidine	(11)
	> 1	Valine, isoleucine, leucine and arginine	
	< 1	Non-essential amino acids (except for serine)	

epithelial cell proliferation and differentiation have been well characterised previously[14]. However, the underlying mechanisms and signalling pathways by which amino acids regulate milk and fat synthesis were largely unknown until recently. The aim of the present review is to describe how amino acids are transported, sensed and transduced in the mammary gland, as well as their functions in the regulation of milk synthesis.

Branched-chain amino acids

Transportation and metabolism of branched-chain amino acids in the mammary gland

The plasma membrane transport system L is the most critical amino acid transporter system for branched-chain amino acids (BCAA) in mammary cells[15]. Transporters from the L system have been well characterised to regulate cell growth and proliferation by directly transporting branched or aromatic amino acids into the cytoplasm in a range of cell lines[16–18]. These transporters are heterodimeric proteins, which comprise of a catalytic subunit (L-type amino acid transporter 1 (LAT1), encoded by *SLC7A5*, or L-type amino acid transporter 2 (LAT2), encoded by *SLC7A6*) and a glycoprotein 4F2 heavy chain (4F2hc). Both LAT1 and LAT2 are highly expressed in the mammary tissues[15], but which subtype is predominately expressed in the mammary gland seems to be different among species[19–21]. In rats, the gene expression of LAT1 in the mammary gland is greater than that of LAT2 during the lactation period[19]. The gene expression of LAT2, but not LAT1, is significantly increased with the progression of lactation in sows[21]. In bovine mammary glands, more research has been focused on the effects of LAT1[20,22]. Depletion of LAT1 in the bovine mammary gland dephosphorylates and inhibits the activity of mTORC1 (mammalian target of rapamycin complex 1), thereby blunting cell viability and β-casein synthesis[20]. Additionally, the inhibition of mTORC1 can be rescued by re-expressing LAT1[20]. However, whether LAT2 also plays an important role in the bovine mammary gland has not been determined and remains to be studied.

BCAA catabolism in mammary tissue is similar to that in other tissues (such as muscle, liver and intestine)[23]. Leucine, isoleucine and valine share a number of BCAA catabolic enzymes, such as branched-chain aminotransferase (BCAT) and branched-chain α-keto acid dehydrogenase (BCKD)[24]. In the porcine mammary gland, the protein levels of BCAT are higher than those in the small intestine, skeletal muscle and liver[23], which indicates that BCAA are actively metabolised in the mammary gland. Mammary BCAA catabolism primarily produces glutamine and aspartate[23]. Notably, as BCAA share the same catabolic enzymes, excessive supplementation of either BCAA might affect the metabolism of other BCAA.

Potential signalling pathway of branched-chain amino acids in the mammary gland

Leucine. In the mammary gland, leucine regulates various biological processes, such as cell proliferation and milk synthesis (α_s-casein, β-casein and κ-casein) (as shown in Table 2). Numerous studies have demonstrated that mTOR functions as a critical regulator of these processes. Until recently, it was not clear of how leucine regulates mTOR until recently (Fig. 1). Before scientists started studying the effect of leucine on mTOR signalling in mammary cells, most pioneering studies were conducted in human embryonic kidney 293 (HEK 293) cells, which is a classical cell line for research investigating the cellular signalling pathway. In HEK 293 cells, it has been demonstrated that leucine regulates the mTOR signalling pathway primarily through mTOR complex 1 (mTORC1) which consists of regulatory-associated protein of mTOR (Raptor), mammalian lethal with SEC13 protein 8 (mLST8), 40 kDa proline-rich protein kinase B (Akt) substrate (PRAS40) and DEP domain-containing mTOR-interacting protein (DEPTOR). After being absorbed into the cytosol, leucine beings to regulate mTORC1 activation by first dephosphorylating Sestrin2[25,26]. Dephosphorylated Sestrin2 induces the dissociation of GTPase-activating protein activity toward Rags 2 (GATOR2), which further inhibits the function of GTPase-activating protein activity toward Rags 1 (GATOR1, a negative regulator of RagA/B)[27]. Finally, activated RagA/B promotes the translocation of mTORC1 to lysosomes for further activation[27].

Recent advances also strongly suggest that the leucine-regulated mTOR signalling pathway is also conserved in the mammary gland. In bovine mammary glands, overexpression of Sestrin2 depresses mTORC1 activity and synthesis of casein, indicating that Sestrin2 plays a major physical role in mammary gland cells[28]. *Danio rerio* SH3-domain binding protein 4 (SH3BP4) has been previously reported to abrogate mTORC1 activation by hydrolysing GTP to GDP of RagB in HEK 293 cells[29]. In the mammary gland, SH3BP4 is also proposed to play a vital role between Sestrin 2 and Rag GTPase[28]. Interestingly, not only leucine but also other essential amino acids and

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Table 2. Effects of branched-chain amino acids (BCAA) on mammary gland function and its potential signalling pathways

Amino acids	Cell lines/animal species	Functions	Potential signalling pathways	References
BCAA (–)	Cow mammary gland (*in vivo*, 105 ± 12 d of lactation for 5 d)	Milk yield ↓	Inhibit mTORC1/eIF2Bε/eIF2α signalling pathway	(94)
Leucine (+)	Bovine mammary epithelial cells/bovine mammary tissue slices	Milk protein synthesis ↑	Activate mTOR/S6K1 signalling pathway	(38)
Leucine (+)	Mouse mammary gland (*in vivo*, from parturition to day 17 of lactation)	–	Activate Akt/mTOR signalling pathway	(39)
Leucine (+)	Mouse mammary epithelial cells	Proliferation ↑	LAT1 and leucyl-tRNA synthetase	(34)
Leucine (+)	Mouse mammary gland (*in vivo*, from parturition to day 17 of lactation)	β-Casein synthesis ↑	Activate mTOR signalling pathway	(39)
Leucine (+)	Bovine mammary epithelial cells	α_s-, β-, κ-casein synthesis ↑	Activate mTOR signalling pathway	(98)
Leucine (+)	Bovine mammary epithelial cells	Expression of casein genes (CSN1S1, 2, 3) ↓	Activate JAK2/STAT5 and mTOR signalling pathway Inhibit AMPK phosphorylation	(99)
Leucine (-)	Mid-lactation Holstein cows (*in vivo*, 108 ± 11 d of lactation for 5 d)	Milk protein yield ↓ Synthesis of α_{s1}, β, κ-casein ↓	–	(100)
Leucine (-)	Bovine mammary epithelial cells/bovine mammary tissue slices	Milk protein synthesis ↓ Protein synthesis rates ↓	Inhibit mTOR/S6K1 signalling pathway	(38)
Isoleucine (+)	Bovine mammary epithelial cells/bovine mammary tissue slices	Milk protein synthesis ↑	Increase mTOR/S6K1 signalling pathway	(38)
Isoleucine (+)	Mouse mammary gland (*in vivo*, from parturition to day 17 of lactation)	–	Increase Akt/mTOR signalling pathway	(39)
Isoleucine (–)	Bovine mammary epithelial cells/bovine mammary tissue slices	Milk protein synthesis ↓ Protein synthesis rates ↓	Inhibit mTOR/S6K1 signalling pathway	(38)
Valine (+)	Porcine mammary epithelial cells	Milk fat synthesis ↑	Akt/mTOR/SREBP-1 pathway	(40)
Valine (+)	Porcine mammary epithelial cells	α-Lactalbumin and β-casein synthesis ↑	mTOR and Ras/ERK signalling pathways	(101)

mTORC1, mammalian target of rapamycin complex 1; eIF, eukaryotic initiation factor; mTOR, mammalian target of rapamycin; S6K1, S6 kinase 1; Akt, protein kinase B; LAT1, L-type amino acid transporter 1; CSN1S1, 2, 3, casein αs1, 2, 3; JAK2, Janus kinase 2; STAT5, signal transducers and activators of transcription 5; AMPK, AMP-activated protein kinase; SREBP-1, sterol regulatory element-binding protein 1; ERK, extracellular signal-regulated kinase.

non-essential amino acids can also regulate mTORC1 through Sestrin2 in the mammary gland[30], which is inconsistent with observations in HEK 293 cell lines and warrants further investigation.

In the mammary gland, the other potential leucine-mediated mTORC1 signalling pathway is through guanine nucleotide-binding protein subunit γ-12 (GNG12) and leucyl-tRNA synthetase (LeuRS). GNG12 regulates mTORC1 via interaction with Regulator[31], which affects the translocation of mTORC1 to lysosomal membranes[32]. LeuRS acts as a vital intracellular leucine sensor that can directly bind to Rag GTPase and activate mTORC1[33]. In mouse mammary cells, LeuRS activates the mTOR signalling pathway and increases cell proliferation[34]. In bovine mammary cells, GNG12 enhances cell growth and milk protein synthesis by activating the mTORC1 signalling pathway[31].

Valine and isoleucine. In addition to L-leucine, L-isoleucine and L-valine are also proposed to regulate milk synthesis. Dietary supplementation of L-isoleucine and L-valine during the whole lactation period enhances milk synthesis in sows and supports increased weaning weight of their litters[35,36]. Similarly, L-isoleucine and L-valine deficiency during the mid-lactation period inhibits milk synthesis in dairy cows[37]. Furthermore, recent advances indicate that both L-isoleucine and L-valine can activate the mTOR signalling pathway and have the potential to enhance milk protein synthesis[38–40]. Additionally, L-valine enhances fatty acid synthesis through activation of the mTOR/sterol regulatory element-binding protein 1 (SREBP-1) pathway[40]. SREBP-1 is a transcription factor and is proposed to be an important regulator of mammary gland fat synthesis in sheep[41], cows[42] and mice[43]. Lipin 1, a phosphatidic acid phosphatase, is a critical link between mTOR and SREBP-1[44]. When mTORC1 is activated, it phosphorylates lipin 1, which releases SREBP-1 and activates SREBP-1-regulated lipogenetic gene expression[44]. As L-valine regulates lipogenesis through activation of mTORC1, this finding strongly suggests that L-leucine and L-isoleucine might also participate in lipogenesis progression. The isotope tracing experiment showed that the carbon from L-leucine can incorporate into milk fat on goat mammary explants, which provides direct evidence that L-leucine is involved in milk fat production[45].

Recently, mTORC1 stimulation was observed before amino acid absorption into the cytostome[46–48]. T1R1/T1R3, a G-protein-coupled receptor (GPCR) in the cell membrane, is an important player in this process[46]. It has been demonstrated that amino acids regulate milk protein synthesis through T1R1/T1R3 in the mouse mammary gland[47,48]. Activation of the G-protein-coupled receptors (GPRC) T1R1/T1R3 increases phospholipase Cβ (PLCβ) activity and further enhances an influx of intracellular Ca^{2+}[49,50]. Extracellular signal-regulated kinase (ERK) 1 and 2 (ERK1/2), which are increased with Ca^{2+} concentration, regulate the activation of mTORC1 directly via the phosphorylation of Raptor (regulatory-associated protein of mTOR)[51]. The other possible pathway by which ERK1/2 activates mTORC1 signalling is through the inactivation of tuberous sclerosis complex 2 (TSC2),

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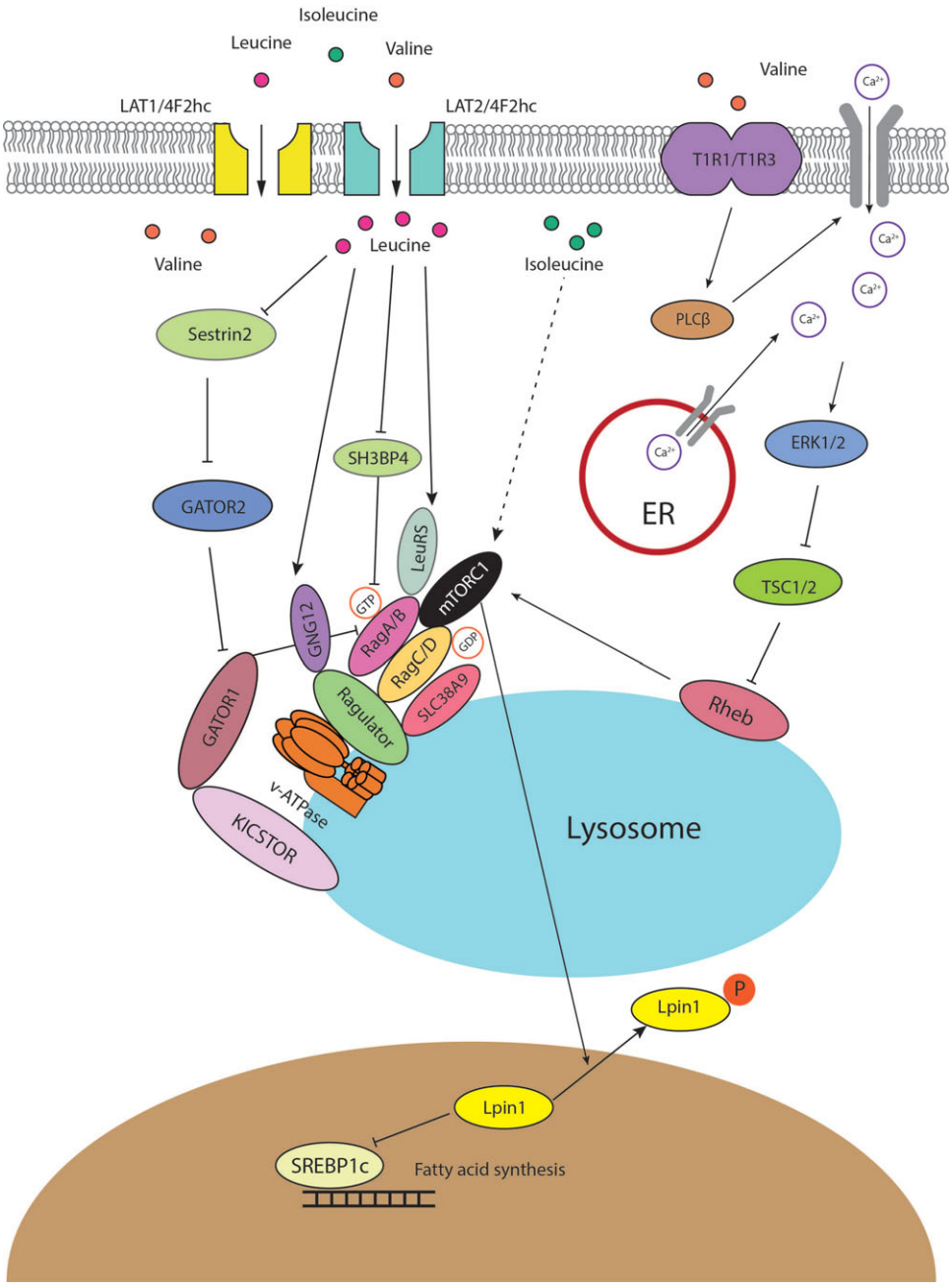

Fig. 1. Branched-chain amino acid (BCAA) and mammalian target of rapamycin complex 1 (mTORC1) signalling networks in the mammary gland. Note: L-type amino acid transporter 1/4F2 heavy chain (LAT1/4F2hc) and L-type amino acid transporter 2 (LAT2)/4F2hc derived from transporter system L are highly expressed and play a dominant role in BCAA transportation in the mammary gland. All three BCAA activate mTORC1 pathways in mammary glands. Leucine induces dephosphorylation of Sestrin2 and further promotes mTORC1 activation through GTPase-activating protein activity toward Rags (GATOR) 2, GATOR1 and RagA/B. In addition, GCG12, SH3-domain binding protein 4 (SH3BP4) and leucyl-tRNA synthetase (LeuRS) are crucial regulators in leucine-related mTORC1 activation. Extracellular valine activates G-protein-coupled receptors (GPRC) T1R1/T1R3, increases phospholipase Cβ (PLCβ) activity and further enhances an influx of intracellular Ca^{2+}. Increased Ca^{2+} regulates the mTORC1 signalling pathway through extracellular signal-regulated kinase 1/2–tuberous sclerosis complex 1/2–Rheb (ERK1/2–TSC1/2–Rheb) signalling. Intracellular isoleucine activates mTORC1 through an unknown mechanism. In the mammary gland, activated mTORC1 not only increases milk protein synthesis but also milk fat synthesis through lipin 1 (Lpn1)–sterol regulatory element-binding protein 1c (SREBP-1c) pathways. ER, endoplasmic reticulum. Please refer to the main text for details.

which is an inhibitor of mTORC1[52]. As T1R1/T1R3 can be widely activated by L-amino acids[46], it is supposed that all of the BCAA (leucine, valine and isoleucine) could activate mTORC1 through this signalling pathway in the mammary gland. However, in bovine mammary glands, valine, not isoleucine and leucine, regulates the mTOR signalling pathway through the membrane GPRC receptor T1R1/T1R3[53], which might be due to insufficient supplementation with isoleucine and leucine. Future experiments are warranted to verify these results in the mammary cells of other species.

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Table 3. Effects of methionine on mammary gland function and its potential signalling pathways

Items	Cell lines/animal species	Functions	Potential signalling pathways	References
Methionine (+)	Cows (*in vivo*, periparturient period)	Antioxidation ↑	Activate NFE2L2 signalling pathway	(102)
Methionine (+)	Cow mammary gland epithelial cells	Functional phosphoproteins ↑	Post-translational modification of proteins	(103)
Methionine (+)	Porcine mammary epithelial cells/mammary tissue slices	Mammary gland protein synthesis ↑	Activate mTOR/S6K1/4E-BP1 signalling pathway	(104)
Methionine (+)	Bovine mammary epithelial cells	Milk fat synthesis ↑	Activate FABP5/SREBP-1c signalling pathway	(65)
Methionine (+)	Bovine mammary epithelial cells	Milk fat synthesis ↑ Protein synthesis ↑ Cell proliferation ↑	Activate SNAT2/PI3K signalling pathway	(57)
Methionine (+)	Bovine mammary epithelial cells	–	Activate T1R1/T1R3/Ca^{2+}/mTOR signalling pathway	(53)
Methionine (+)	Bovine mammary epithelial cells	Amino acid transporter LAT1/4F2hc expression ↑	Activate mTORC1 signalling pathway	(22)
Methionine (+)	Holstein cows (*in vivo*, periparturient period)	Glucose and amino acid transporter ↑	Activate Akt signalling pathway	(64)
Methionine (+)	Bovine mammary epithelial alveolar cells	Regulate histone methylation	–	(105)
Methionine (+)	Mouse mammary gland (*in vivo*, from parturition to day 17 of lactation)	–	Increase Akt and mTOR signalling pathway	(39)

NFE2L2, nuclear factor erythroid 2-like 2; mTOR, mammalian target of rapamycin; S6K1, S6 kinase 1; 4E-BP1, eukaryotic initiation factor 4E-binding protein 1; FABP5, fatty acid-binding protein 5; SREBP-1c, sterol regulatory element-binding protein 1c; SNAT2, Na-coupled neutral amino acid transporter 2; PI3K, inositol 1,4,5-trisphosphate 3-kinase; mTORC1, mammalian target of rapamycin complex 1; Akt, protein kinase B.

Methionine

Transportation system of methionine in the mammary gland

In the mammary gland, three amino acid transporter systems are involved in methionine transportation, namely systems A, ASC and L[54]. System A consists of Na-coupled neutral amino acid transporter 1 (SNAT1; detected in pigs) and Na-coupled neutral amino acid transporter 2 (SNAT2; detected in rats and cows)[55]. System ASC primarily contains Na-dependent alanine cotransport 1 (ASCT1) (detected in humans, mice, cows and pigs) and Na-dependent alanine cotransport 2 (ASCT2) (detected in rats and cows)[55]. System L is composed of two heteromeric Na^+-independent transporters LAT1/4F2hc (detected in humans, rats, mice, cows) and LAT2/4F2hc (detected in rats and cows)[56]. In bovine mammary glands, SNAT2 inhibition strongly prevents the activation of mTORC1 caused by decreased methionine transportation[57]. The functional evaluation of the importance of specific methionine transporters is still insufficient and warrants further research to demonstrate which transporter system may play a dominant role in the mammary gland.

Potential signalling pathway of methionine in the mammary gland

The effects of methionine on milk synthesis in the mammary gland have been demonstrated for many years (Table 3). It has been shown through meta-analyses that methionine is one of the first two limiting amino acids in dairy cows[58,59] and is an important limiting amino acid in lactating sows[60,61]. Intriguingly, the effect of methionine on milk fat synthesis is not linked to the use of methionine carbon in fatty acid synthesis since its ratio of mammary uptake to milk output is always at 1 in dairy cows[62]. Advanced research in HEK 293 cells has demonstrated that when methionine is deficient, the cellular methyl donor *S*-adenosylmethionine (SAM) level will be reduced, which further increases the association of SAMTOR (SAM sensor) with GATOR2 and inhibits mTORC1 signalling[63]. However, to the best of our knowledge, whether SAMTOR also acts as a conserved SAM sensor in the mammary gland has not been determined and merits further research.

Two other potential methionine-regulated mTORC1 signalling pathways have been verified in the mammary gland (Fig. 2). One possible approach is the inositol 1,4,5-trisphosphate 3-kinase (PI3K)/Akt signalling pathway. Activated PI3K/Akt/mTORC1 significantly stimulates milk protein synthesis[57,64]. Furthermore, methionine also plays a crucial role in milk fat synthesis. Fatty acid-binding protein 5 (FABP5) is a crucial regulator that activates SREBP-1c for milk fatty synthesis[65], which can be partly activated by PI3K[66,67]. All of this information indicates that methionine may regulate milk lipid synthesis through the PI3K/Akt/FABP5/SREBP-1c signalling pathway. The other novel and crucial signalling pathway is the T1R1/T1R3 signalling pathway. Similar to isoleucine and valine, methionine also increases the influx of intracellular Ca^{2+} and regulates the effects of mTORC1 through T1R1/T1R3 in the mammary gland[53].

PI3K/Akt was previously demonstrated to be regulated by hormones, but not amino acids, in cell models. One possible cause of this discrepancy is that an unknown link between methionine and PI3K exists in the mammary gland. As IGF-1/PI3K/Akt/mTOR is the canonical pathway in cells[68,69], the other possible cause is that dietary methionine deficiency might indirectly inhibit PI3K activation via decreased IGF-1 secretion[70–72].

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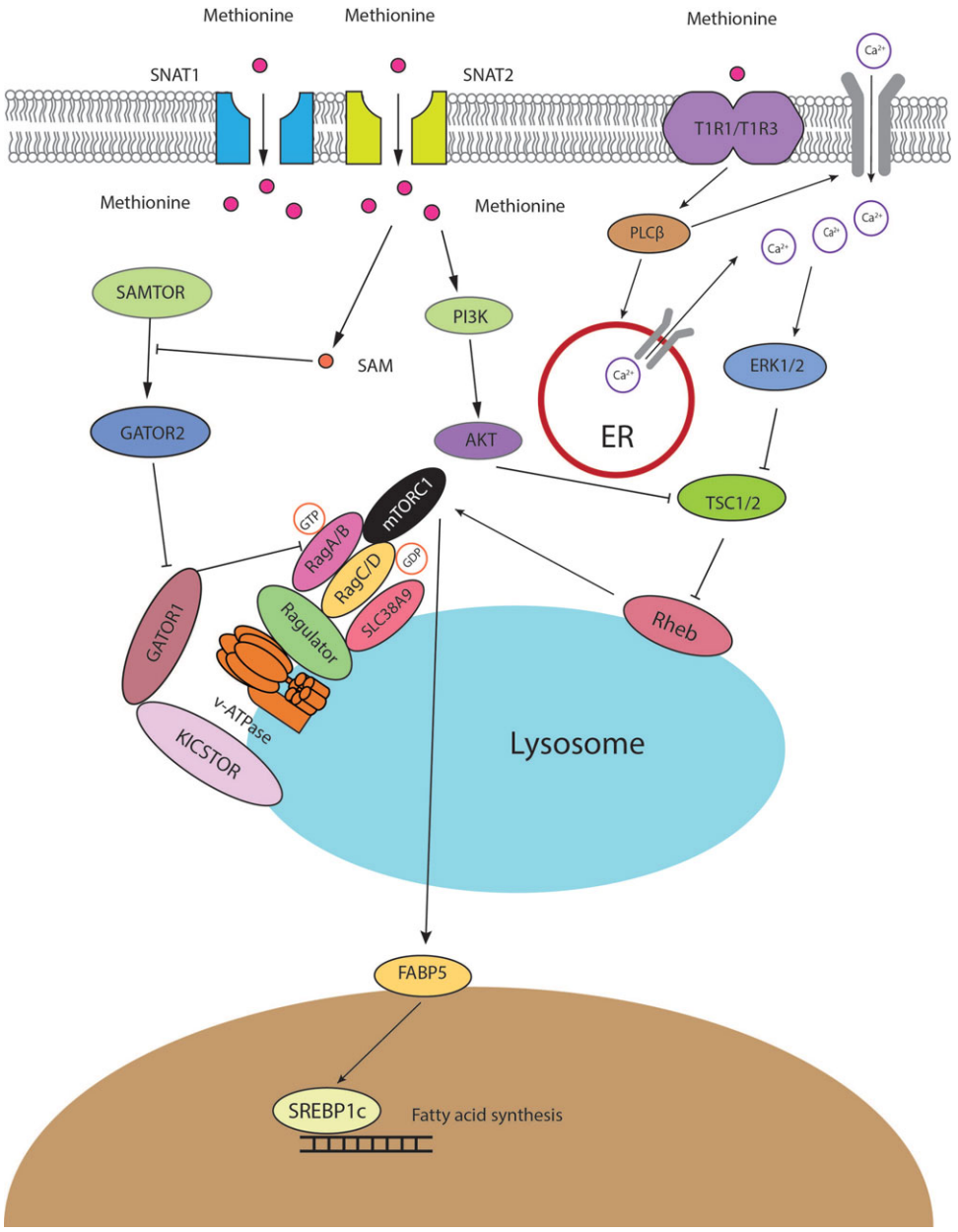

Fig. 2. Methionine and mammalian target of rapamycin complex 1 (mTORC1) signalling networks in the mammary gland. Note: sodium-coupled neutral amino acid transporter 1 (SNAT1) and SNAT2 originate from transporter system A and are crucial methionine transporters in the mammary gland. Intracellular methionine increases cellular *S*-adenosylmethionine (SAM) levels, which decreases the association of SAMTOR (SAM sensor) with GTPase-activating protein activity toward Rags 2 (GATOR2) and inhibits the mTORC1 signalling pathway. In addition, intracellular methionine regulates mTORC1 through the inositol 1,4,5-trisphosphate 3-kinase/protein kinase B/Rheb (PI3K/Akt/Rheb) signalling pathway. Extracellular methionine activates the G-protein-coupled receptors (GPCR) T1R1/T1R3, increases phospholipase Cβ (PLCβ) activity and further enhances the influx of intracellular Ca^{2+}. Increased Ca^{2+} regulates the mTORC1 signalling pathway through extracellular signal-regulated kinase 1/2–tuberous sclerosis complex 1/2–Rheb (ERK1/2–TSC1/2–Rheb) signalling. Activated mTORC1 increases milk protein synthesis and regulates milk fat synthesis through sterol regulatory element-binding protein 1 (SREBP-1) and fatty acid-binding protein 5 (FABP5). ER, endoplasmic reticulum. Please refer to the main text for details.

Arginine and lysine

Transportation system of arginine and lysine in the mammary gland

Arginine and lysine are both cationic amino acids and have the same amino acid transporter systems in the mammary gland[73]. Four cationic amino acid transporter (CAT) systems have been identified in the mammary gland as follows: (1) y^+ system: CAT-1 (detected in humans, cows, pigs and rats) and CAT-2 (detected in pigs); (2) y^+L system: y^+LAT1 (detected in pigs and cows) and y^+LAT2 (detected in pigs); (3) $b^{0,+}$ system: $b^{0,+}$AT (detected in pigs); and (4) $B^{0,+}$ system: $ATB^{0,+}$ (detected in pigs, humans, rats)[74–77]. Among all transporters, CAT-1 seems to play a central role in arginine uptake in the mammary gland. In mammary MCF-7 cells, when 50 % cellular CAT-1 was knocked down, arginine uptake was inhibited by 35–40 %[78,79]. Furthermore, blocking $ATB^{0,+}$ also inhibits arginine uptake in the MCF-7 cell line[80]. Recently, $ATB^{0,+}$ has also been considered

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Table 4. Effects of arginine on mammary gland function and its potential signalling pathways

Items	Treatments	Functions	Potential signalling pathways	References
Arginine (+)	Porcine mammary epithelial cells	Cell proliferation ↑ Protein synthesis ↑	Activate mTOR signalling pathway	(86)
Arginine (+)	Bovine mammary epithelial cells	Milk synthesis ↑ Cell proliferation ↑ Amino acid transporter ↑	–	(106)
Arginine (+)	Lactating dairy cows (*in vivo*, 20 ± 2 d of lactation for 7 d)	Milk protein synthesis ↑ Amino acid transporters ↑	–	(107)
Arginine (+)	Lactating sows (*in vivo*, from parturition to day 21 of lactation)	Mammary tissue angiogenesis ↑	–	(91)
Arginine (+)	Lactating cows (mid-lactation for 14 d)	Increase casein ↑	–	(108)
Arginine (+)	Bovine mammary epithelial cells	Inflammatory response ↓ Casein secretion ↑	–	(109)
Arginine (+)	Primary bovine mammary epithelial cells	Milk protein ↑ Fat synthesis ↑	Inhibit GCN2/eIF2α pathway	(110)
Arginine (–)	Primary bovine mammary epithelial cells	Mammary gland autophagy ↑	Activate GCN2 signalling pathway	(111)
Arginine (–)	Mid-lactation Holstein cows (*in vivo*, 108 ± 11 d of lactation for 5 d)	Milk protein yield ↓ Synthesis of α_{s1}, β, κ-casein ↓	–	(100)
Arginine (+)	Bovine mammary epithelial cells	Casein synthesis ↑	mTOR signalling pathway	(87)
Arginine (+)	Bovine mammary epithelial cells	Casein synthesis ↑	–	(112)

mTOR, mammalian target of rapamycin; GCN2, general control non-derepressible 2; eIF, eukaryotic initiation factor.

as the most crucial lysine transporter in the mammary gland. In bovine mammary gland epithelial cells, lysine activates the mTOR signalling pathway, which is inhibited by blockade of the lysine transporter $ATB^{0,+}$[81]. In the sow mammary gland, the transportation of lysine is partly inhibited by excessive arginine supplementation[82]. This evidence indicates that arginine and lysine have the same critical transporter system (CAT-1 and $ATB^{0,+}$) in the mammary gland.

Potential signalling pathway of arginine in the mammary gland

Positive effects of arginine on placental growth and fetal survival and growth have been demonstrated in pigs, rats, mice and sheep[83], whereas its functions in the mammary gland were not determined until recently (Table. 4). In the mammary gland, arginine catabolism produces proline, ornithine, urea, glutamate, glutamine, CO_2 and polyamines (putrescine, spermidine and spermine)[84]. In sows, supplementation with 0·5 or 1·0 % L-Arg-HCl activates the milk synthesis and increases the litter weight of sucking piglets[85]. Similar to other amino acids, arginine also regulates milk protein synthesis through mTORC1[86,87]. The central regulator linking arginine to the mTORC1 signalling pathway is cellular arginine sensor for mTORC1 (CASTOR1)[88,89]. Sufficient arginine dissociates GATOR2 from CASTOR1 and further activates the mTOR signalling pathway[88,89] (Fig. 3). The other crucial function of arginine in the mammary gland is primarily achieved through its metabolite NO[90]. Briefly, NO increases the mammary blood vessel density and diameter, which might enhance the transportation of nutrients to the mammary gland and support milk synthesis[91].

Potential signalling pathway of lysine in the mammary gland

Similar to methionine, lysine is one of the first two limiting amino acids both in dairy cows[59,92] and sows[60,61]. In the bovine mammary gland, the use of lysine is dose dependent as it can be the first limiting amino acid. In case of low supply, lysine is mainly utilised in milk protein synthesis with the ratio of lysine uptake to lysine output close to 1[8]. However, when sufficient amount of lysine is provided through the diet, it can also be used to either synthesise non-essential amino acids[62,93] or be oxidised into CO_2 as BCAA[81]. When lysine is deficient, milk protein synthesis is inhibited[94], with a decrease in mTORC1 activity in dairy cows[95]. However, the function of lysine in the mammary gland has largely not been determined. Recent advances have found that lysine increases milk fat synthesis through the GPRC6A/PI3K/FABP5 signalling pathway[67]. GPRC6A is a G protein-coupled receptor that is specific for cationic amino acid sensing[96]. As a Gαi/Gαq receptor, GPRC6A has the potential to regulate cellular cAMP levels and activate the MAPK signalling pathway[97]. Thus, both GPRC6A/PI3K/Akt/mTOR and GPRC6A/ERK/mTOR can be the potential signalling pathways for lysine to regulate milk protein and fat synthesis in the mammary gland.

Conclusion

Amino acids play crucial roles in the synthesis of milk protein and fat in the mammary gland. The dominant amino acid transposers (BCAA, methionine, lysine and arginine) of the mammary gland are summarised in the present review. In addition, our review has focused on a number of canonical and novel signalling molecules involved in amino acid signalling pathway in the mammary gland. Remarkably, mTORC1 acts as the central node of the amino acid-regulated signalling pathway and can be activated intracellularly and extracellularly (through a G-protein-coupled receptor (GPCR)). Currently, the amino acid signalling pathway in the mammary gland still warrants further investigation. Achieving a better understanding of the amino acid signalling pathway might help us to optimise the amino acid profiles in maternal diets for human beings and other mammals in the future.

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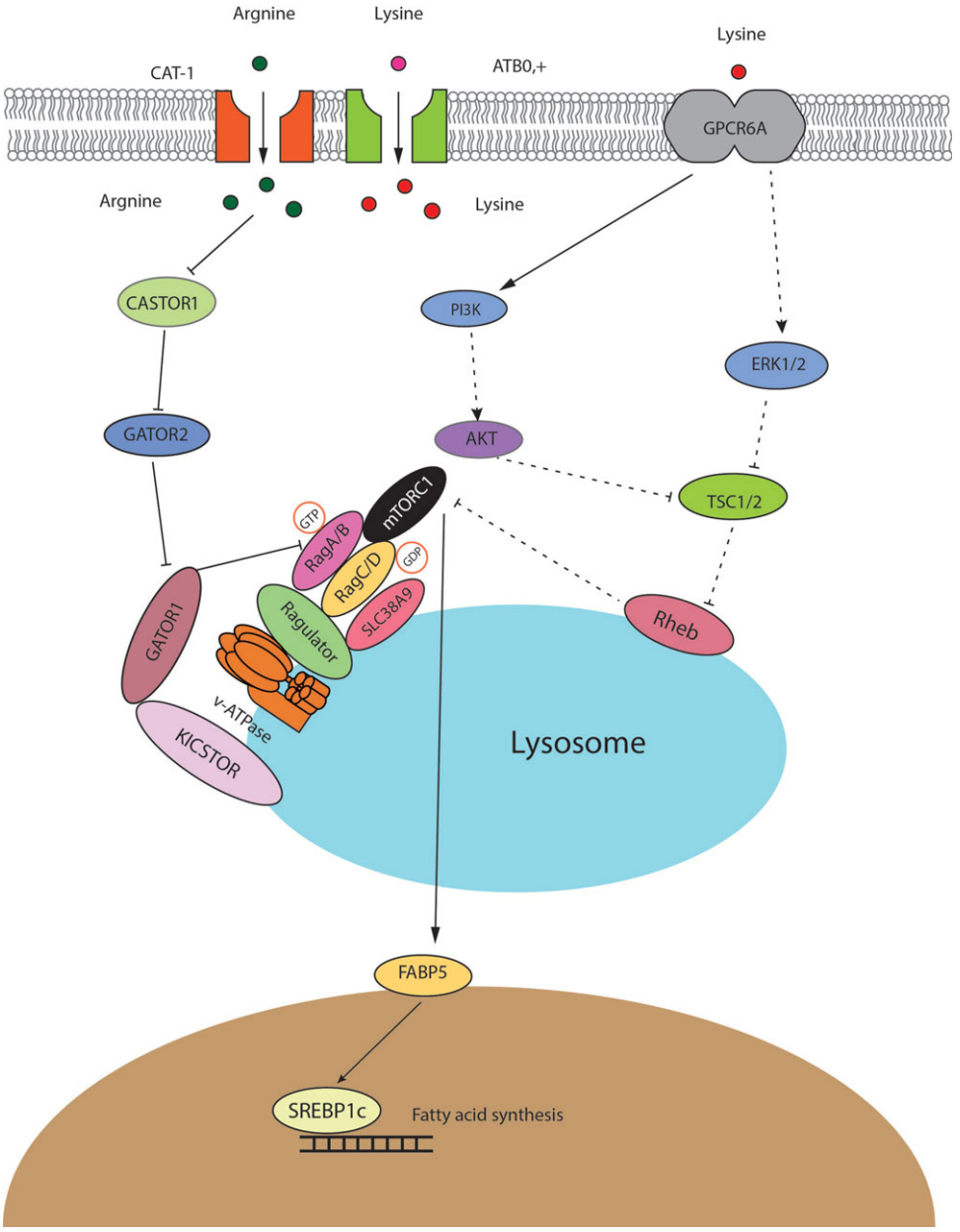

Fig. 3. Lysine and arginine regulate the mammalian target of rapamycin complex 1 (mTORC1) signalling network in the mammary gland. Note: cationic amino acid transporter-1 (CAT-1) and $ATB^{0,+}$ are critical cationic amino acid transporters for arginine and lysine transportation in the mammary gland. The intracellular arginine regulator mTORC1 acts through the cellular arginine sensor for mTORC1–GTPase-activating protein activity toward Rags 2–GTPase-activating protein activity toward Rags 1–RagA/B (CASTOR1–GATOR2–GATOR1–RagA/B) signalling pathway, whereas extracellular lysine regulates mTORC1 through the G-protein-coupled receptor (GPCR) GPCR6A. As a Gαi/Gαq receptor, GPCR6A can activate milk protein synthesis through the GPRC6A–inositol 1,4,5-trisphosphate 3-kinase–protein kinase B–tuberous sclerosis complex 1/2–Rheb (GPRC6A–PI3K–Akt–TSC1/2–Rheb) and GPRC6A–extracellular signal-regulated kinase 1/2 (ERK1/2)–TSC1/2–Rheb pathways. Dashed lines represent potential signalling pathways that have not been verified in the mammary gland. FABP5, fatty acid-binding protein 5; SREBP-1, sterol regulatory element-binding protein 1. Please refer to the main text for details.

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