



## Review:

# Sensors for the mTORC1 pathway regulated by amino acids<sup>\*</sup>

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**Abstract:** The mechanistic target of rapamycin complex 1 (mTORC1) controls cell growth and metabolism in response to various environmental inputs, especially amino acids. In fact, the activity of mTORC1 is highly sensitive to changes in amino acid levels. Over past decades, a variety of proteins have been identified as participating in the mTORC1 pathway regulated by amino acids. Classically, the Rag guanosine triphosphatases (GTPases), which reside on the lysosome, transmit amino acid availability to the mTORC1 pathway and recruit mTORC1 to the lysosome upon amino acid sufficiency. Recently, several sensors of leucine, arginine, and S-adenosylmethionine for the amino acid-stimulated mTORC1 pathway have been coming to light. Characterization of these sensors is requisite for understanding how cells adjust amino acid sensing pathways to their different needs. In this review, we summarize recent advances in amino acid sensing mechanisms that regulate mTORC1 activity and highlight these identified sensors that accurately transmit specific amino acid signals to the mTORC1 pathway.

**Key words:** Mechanistic target of rapamycin complex 1 (mTORC1); Amino acid; Sensor; Lysosome; Rag GTPases  
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## 1 Introduction

The mechanistic target of rapamycin complex 1 (mTORC1), evolutionally conserved from yeast to mammalian, acts as a central signaling hub that integrates nutrients, growth factors, and energy to regulate many anabolic and catabolic processes, ultimately controlling cell growth and metabolism (Chantranupong et al., 2015; Saxton and Sabatini, 2017). Deregulation of mTORC1 frequently happens in pathophysiological conditions, especially in cancer.

The *target of rapamycin (TOR)* gene was originally identified in yeast mutants resistant to rapamycin which binds to the peptidyl-prolyl-isomerase FKBP12 (Heitman et al., 1991; Koltin et al., 1991). Subsequently, four groups independently discovered a protein which is a homolog of the yeast TOR directly interacting with FKBP12-rapamycin in the mammal, and named the protein FKBP12-rapamycin-associated protein, rapamycin and FKBP12 target 1, rapamycin target 1, and mammalian TOR (mTOR), respectively (Brown et al., 1994; Chiu et al., 1994; Sabatini et al., 1994; Sabers et al., 1995). Early functional studies showed that mTOR carries out kinase activity to directly phosphorylate ribosomal protein S6 kinase 1 (S6K1) and eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1), which can be regulated by amino acids (Burnett et al., 1998; Hara et al., 1998). However, alterations in amino acid sufficiency or rapamycin

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failed to change the kinase activity of mTOR, which implies that mTOR might function as a complex.

In addition to mTOR, mTORC1 is composed of regulatory associated protein of mTOR (Raptor) (Hara et al., 2002; Kim et al., 2002), mammalian lethal with SEC13 protein 8 (mLST8, also named as GβL) (Kim et al., 2003), proline-rich Akt substrate of 40 kDa (PRAS40) (Oshiro et al., 2007; Sancak et al., 2007; Vander Haar et al., 2007; Wang et al., 2007), and dishevelled, Egl-10 and pleckstrin (DEP) domain containing mTOR-interacting protein (DEPTOR) (Peterson et al., 2009). Recently, study of the architecture of human mTORC1 revealed that mTORC1 is a dimer of heterotrimer mTOR-Raptor-mLST8 (Aylett et al., 2016; Yang et al., 2016). Co-crystal structure of PRAS40 and mTOR-mLST8 demonstrated that PRAS40 also binds with FKBP12-rapamycin complex binding domain and mLST8 WD40 domain to achieve its inhibition of mTORC1 (Yang et al., 2017).

Amino acids serve as building blocks for protein and polypeptide synthesis, and molecular signal transducers to regulate diverse metabolic processes in animals (Layman et al., 2015). Defects in amino acid sensing pathways such as the mTORC1 pathway are linked to human diseases including immunodeficiency syndrome, cancers, and Birt-Hogg-Dube syndrome (Shimobayashi and Hall, 2016). In addition, amino acids have been proved to efficiently activate mTORC1. This increases the phosphorylation of substrates S6K1 and 4EBP1 to stimulate anabolic processes such as protein synthesis, and unc-51 like autophagy activating kinase 1 (ULK1) complex to suppress autophagy (Jewell et al., 2013). However, different amino acids regulate mTORC1 in distinct ways. Here, we discuss the precise mechanism of specific amino acid sensing in the regulation of the mTORC1 pathway.

## 2 Regulation of the mTORC1 pathway

### 2.1 Key sites of amino acid signaling to mTORC1

#### 2.1.1 Plasma membrane

Amino acid transporters at the plasma membrane, which are responsible for amino acid exchange between the extracellular and intracellular microenvironments, play a role in the regulation of mTORC1 activity (Taylor, 2014). The solute carrier family

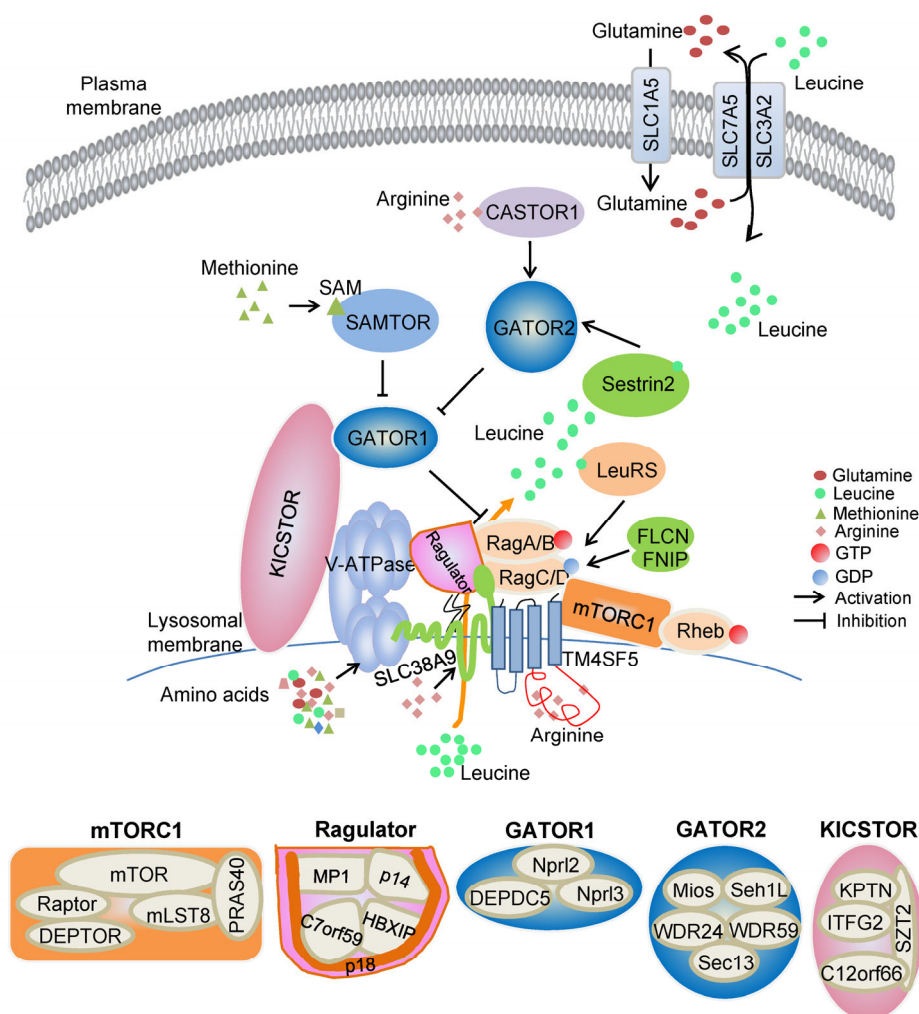
(SLC) 1 member 5 (SLC1A5), a high-affinity glutamine transporter, is required for mTORC1 activation. SLC7A5 forms a heterodimer with SLC3A2 and the heterodimer functions as the bidirectional antiporter for the exchange of intracellular L-glutamine which is accumulated by SLC1A5 for extracellular branched chain amino acids such as leucine, contributing to activation of the mTORC1 pathway eventually (Nicklin et al., 2009) (Fig. 1).

#### 2.1.2 Golgi apparatus

Golgi apparatus was reported as a potential platform for the activation of mTORC1 by amino acids (Thomas et al., 2014; Fan et al., 2016). Mechanistically, small guanosine triphosphatases (GTPases) Ras-related protein Rab-1A (Rab1A) and Ras homolog enriched in brain (Rheb) in coordination activate mTORC1 independently of Rag. Rab1A mediates the amino acid signal to mTORC1 on the Golgi apparatus, where Rheb localizes and activates mTORC1 (Thomas et al., 2014). However, how amino acid signal is transmitted to Rab1A is unclear, and amino acid sensors for Rab1A- and Golgi-dependent mTORC1 pathway need further investigation.

#### 2.1.3 Lysosome

The roles of lysosome in nutrient degradation, recycling, and signaling cooperate to regulate fundamental cellular processes such as nutrient sensing (Perera and Zoncu, 2016). Classically, amino acid signals transmitted to mTORC1 originate from the lysosomes (Sancak et al., 2010; Zoncu et al., 2011). Cell-free reconstitution of intact lysosomes and mTORC1 induced colocalization of lysosomes and mTORC1 upon amino acid sufficiency. However, lysosomes which have defects in amino acid accumulation inhibit translocation of mTORC1. These results suggested that the lysosome is the major site of amino acid signaling to mTORC1. RNA interference (RNAi) of lysosomal proteins in *Drosophila* cells discovered that vacuolar H<sup>+</sup>-adenosine triphosphatase (V-ATPase) is required for amino acid-dependent lysosomal recruitment and activation of mTORC1. V-ATPase interacts with Ragulator, Rag GTPases, and SLC38A9, involving both amino acid sensing and efflux from lysosome (Zoncu et al., 2011; Abu-Remaileh et al., 2017; Wyant et al., 2017). Together, lysosomal amino acid signaling to mTORC1 requires a V-ATPase-associated inside-out mechanism.



**Fig. 1 Sensors for the mTORC1 pathway regulated by amino acids**

Schematic shows amino acid-sensing pathway upstream of mTORC1, including several complexes that control Rag GTPases, and amino acid sensors Sestrin2, LARS, CASTOR1, SLC38A9, and TM4SF5, as well as SAM sensor SAMTOR. mTORC1: mechanistic target of rapamycin complex 1; SLC1A5: solute carrier family (SLC) 1 member 5; CASTOR1: cellular arginine sensor for mTORC1 protein 1; SAM: *S*-adenosylmethionine; SAMTOR: SAM sensor upstream of mTORC1; V-ATPase: vacuolar H<sup>+</sup>-adenosine triphosphatase; FLCN: folliculin; FNIP: FLCN-interacting protein; LARS: leucyl-tRNA synthetase; TM4SF5: transmembrane 4 L six family member 5; Raptor: regulatory-associated protein of mTOR; DEPTOR: dishevelled, Egl-10 and pleckstrin (DEP) domain containing mTOR-interacting protein; mLST8: mammalian lethal with SEC13 protein 8; PRAS40: proline-rich Akt substrate of 40 kDa; MP1: mitogen-activated protein kinase (MAPK) kinase 1 binding partner 1; HBXIP: hepatitis B virus X-interacting protein; GATOR1/2: GAP activity toward the Rag GTPases 1/2; Npr12: nitrogen permease regulator 2-like protein; DEPDC5: DEP domain containing 5; Mios: meiosis regulator for oocyte development; Seh1L: Sec13-like protein; WDR24: WD repeat-containing protein 24; KICSTOR: named for kaptin (KPTN), integrin- $\alpha$  FG-GAP repeat-containing protein 2 (ITFG2), C12orf66, and seizure threshold 2 (SZT2)-containing regulator of mTORC1; GTP: guanosine triphosphate; GDP: guanosine diphosphate

## 2.2 Tuberous sclerosis complex-Rheb GTPase

The Rheb and Ras-related guanosine triphosphate (GTP)-binding (Rag) proteins in coordination regulate mTORC1 activity on the lysosomal surface in response to a range of environmental cues including amino acids, growth factors, and energy levels (Saito

et al., 2005; Buerger et al., 2006; Kim et al., 2008; Sancak et al., 2008). Tuberous sclerosis complex (TSC), composed of TSC1, TSC2, and TBC1D7, functions as a GTPase-activating protein (GAP) for GTP-loading Rheb which binds directly with and allosterically stimulates mTORC1 (Manning et al., 2002; Tee et al., 2003; Long et al., 2005; Dibble et al.,

2012; Yang et al., 2017). Growth factors promote protein kinase B (PKB)/Akt-mediated phosphorylation of TSC2, contributing to dissociation of TSC2 from the lysosome to activate Rheb and mTORC1 (Inoki et al., 2002; Potter et al., 2002; Menon et al., 2014). In contrast, energy starvation stimulates adenosine monophosphate (AMP)-activated protein kinase (AMPK)-induced phosphorylation of TSC2, enhancing the interaction of TSC2-Rheb to inhibit Rheb and mTORC1 (Inoki et al., 2003). In addition to these inputs, amino acids are thought to be presented to mTORC1 in a completely different way. Under amino acid sufficiency, the Rag GTPases recruit mTORC1 to the lysosomal surface (Kim et al., 2008; Sancak et al., 2008). Surprisingly, arginine and growth factors collectively promote TSC dissociation from the lysosome thereby activating Rheb and mTORC1 (Carroll et al., 2016). Under amino acid starvation, TSC relocates to the lysosome through the recruitment of Rag GTPases and stimulates GTP hydrolysis from Rheb, resulting in complete inactivation of mTORC1 (Demetriades et al., 2014). In conclusion, TSC-Rheb is essential for mTORC1 mediated by various microenvironmental factors, including amino acids.

### 2.3 Rag GTPases

The landmark discovery in understanding the regulation of mTORC1 pathway by amino acids is the identification of Rag GTPases that mediate amino acid signaling to mTORC1. The Rag GTPases have four Rag proteins in the mammal: functionally equivalent RagA or RagB (RagA/B) forms a heterodimer with functionally equivalent RagC or RagD (RagC/D) (Schürmann et al., 1995; Sekiguchi et al., 2001). In yeast, Gtr1, a homolog of RagA/B, interacts with Gtr2, a homolog of RagC/D (Hirose et al., 1998; Nakashima et al., 1999). Using chemical purification of the Raptor in HEK293 cells (Sancak et al., 2008) and RNAi screen of 132 GTPases in *Drosophila* cells (Kim et al., 2008), Rag GTPases were identified as participating in mTORC1 mediated by amino acids. Loss of the Rag GTPases in the mammal and *Drosophila* cells caused mTORC1 inactivity in response to amino acid sufficiency (Kim et al., 2008; Sancak et al., 2008). Neonatal mice with a constitutively active allele of RagA which stimulates the activity of mTORC1 in nutrient starvation failed to induce autophagy and survive the fasting periods (Efeyan et al., 2013).

Overall, the Rag GTPases play a central role in the amino acid-mediated mTORC1 pathway (Fig. 1).

Importantly, the guanine nucleotide-loading status of the Rag heterodimer controls Rag activity and regulates mTORC1 lysosomal localization and activity. When cells are rich in amino acids, activated Rag heterodimer in which RagA/B is loaded with GTP while RagC/D is loaded with guanosine diphosphate (GDP) (RagA/B<sup>GTP</sup>-RagC/D<sup>GDP</sup>) enhances the Rag GTPases-Raptor interaction, and recruits mTORC1 to the lysosome (Kim et al., 2008; Sancak et al., 2008, 2010). Instead, GDP-loaded RagA/B in complex with GTP-loaded RagC/D (RagA/B<sup>GDP</sup>-RagC/D<sup>GTP</sup>) inhibits mTORC1 activity. A recent study has shed light on a “locking mechanism” of the nucleotide-binding status of the Rag heterodimer (Shen et al., 2017). In brief, loading GTP with one subunit of the Rag heterodimer inhibits the GTP-loading and triggers GDP-loading with another subunit. If unexpected GTP loading with both subunits occurs, the subunit with the later-loaded GTP induces faster hydrolysis. In addition, nutrient-induced alteration of the nucleotide-loading state of Rag heterodimer as an affinity switch governs the dynamic interactions between Rag GTPases, mTORC1, and Ragulator (Lawrence et al., 2018). RagA/B<sup>GTP</sup>-RagC/D<sup>GDP</sup> exhibits higher affinity for mTORC1, but reduces lysosomal association with Ragulator, leading to a decrease of the lifetime of mTORC1 on the lysosome. However, although RagA/B<sup>GDP</sup>-RagC/D<sup>GTP</sup> shows higher affinity for Ragulator, it is unable to capture mTORC1 dispersed in cytoplasm.

### 2.4 Regulation of the Rag GTPases

The guanine nucleotide-loading status of GTPases is regulated by multiple factors including GAPs that stimulate GTP hydrolysis, guanine nucleotide exchange factors (GEFs) that facilitate GDP dissociation, and the guanine nucleotide dissociation inhibitors that prevent GDP dissociation.

#### 2.4.1 GEFs for the Rag GTPases

To identify the interacting proteins of the Rag GTPases, Ragulator was discovered as a pentameric complex of p18, p14, mitogen-activated protein kinase (MAPK) kinase 1 binding partner 1 (MP1), C7orf59, and hepatitis B virus X-interacting protein (HBXIP), also known as late endosomal and lysosomal adaptor

and MAPK and mTOR activator (LAMTOR) complex of LAMTOR1 to LAMTOR5, respectively (Sancak et al., 2010; Bar-Peled et al., 2012). With the absence of Ragulator components in cells, the Rag GTPases fail to reside on the lysosome, and recruit mTORC1 to the lysosomal surface, leading to the mTORC1 pathway inactivation (Sancak et al., 2010; Bar-Peled et al., 2012). Myristoyl and palmitoyl modifications of LAMTOR1 are required for Ragulator specific localization to the lysosome (Nada et al., 2009). Structure of the complex Rag GTPases and Ragulator (Rag-Ragulator) demonstrated that C-terminal domains of Rags directly bind with LAMTOR2-LAMTOR3, and LAMTOR1 bridges LAMTOR2-LAMTOR3 with LAMTOR4-LAMTOR5 and enhances Rag-Ragulator interaction (de Araujo et al., 2017; Su et al., 2017; Yonehara et al., 2017; Zhang et al., 2017). Thus, Ragulator tethers the Rag GTPases to the lysosomal membrane.

The nucleotide loading state of the Rag GTPases is also important for its interaction with Ragulator. Nucleotide-free Rag GTPases bind to Ragulator more strongly than nucleotide-loading. Ragulator accelerates both GTP and GDP dissociation from RagA/B (Bar-Peled et al., 2012). Moreover, silencing LAMTOR2, RagB is unable to load GTP under a leucine stimulation condition (Lee et al., 2018). These results indicate that Ragulator is a GEF for RagA/B. However, a recent study has shed light on the mechanism activating the Rag GTPases by SLC38A9 and Ragulator which function as GEFs for RagA and RagC, respectively (Shen and Sabatini, 2018). First, Ragulator accelerates GTP release from RagC, not RagB, thus opening up the nucleotide-binding pocket. Second, SLC38A9 promotes GDP release from RagA.

#### 2.4.2 GATOR functions as a GAP for RagA/B

GAP activity toward the Rag GTPases (GATOR) complex is an octamer of two subunits including GATOR1 (DEP domain containing 5 (DEPDC5), nitrogen permease regulator 2-like protein (Nprl2), Nprl3) and GATOR2 (meiosis regulator for oocyte development (Mios), Sec13, Sec13-like protein (Seh1L), WD repeat-containing protein 24 (WDR24), WDR59). Lack of components of GATOR1 and GATOR2 caused mTORC1 to be insensitive to amino acid deprivation and sufficiency, respectively, indicating that GATOR1 and GATOR2 are functionally contrary regulators for mTORC1 (Bar-Peled et al., 2013).

GATOR1 binds to, and stimulates GTP hydrolysis of RagA/B, suggesting that GATOR1 is a GAP for RagA/B (Bar-Peled et al., 2013). The architecture of GATOR1 and GATOR1-Rag visualized by cryo-electron microscopy delineated that Nprl2 connects DEPDC5 and Nprl3, and DEPDC5 directly binds to RagA (Shen et al., 2018). However, further biochemical assays showed that Nprl2-Nprl3, not DEPDC5, receives amino acid signals from GATOR2 and executes the GAP activity to RagA. Interestingly, an inhibitory mode of GATOR1-Rag interaction has been identified such that a high-affinity binding of DEPDC5 and RagA prevents Nprl2-Nprl3-mediated GAP activity toward RagA.

Several factors are involved in GATOR1-Rag interaction. KICSTOR (named for kaptin (KPTN), integrin- $\alpha$  FG-GAP repeat-containing protein 2 (ITFG2), C12orf66 and seizure threshold 2 (SZT2)-containing regulator of mTORC1) was discovered to bind and recruit GATOR1 complex on the lysosomal membrane regardless of amino acid levels. Loss of SZT2 released the inhibitory function of GATOR1 by dissociation from Rags, which contributes to mTORC1 persistent activation upon amino acid deprivation, showing that KICSTOR negatively regulates mTORC1 through promoting GATOR1-Rag interaction (Peng et al., 2017; Wolfson et al., 2017). In contrast to KICSTOR, tyrosine kinase Src positively regulates mTORC1 through disrupting GATOR1-Rag interaction (Pal et al., 2018).

Ubiquitination plays a role in GATOR1-Rag interaction. E3 ubiquitin ligases S-phase kinase-associated protein 2 (Skp2) and RING finger protein 152 (RNF152) mediate K63-linked ubiquitination of RagA and recruit GATOR1 to the lysosomal surface in an amino acid-dependent manner (Deng et al., 2015; Jin et al., 2015). Deng et al. (2015) suggested that amino acid starvation increases RNF152-mediated RagA ubiquitination and its interaction with GATOR1 which inhibits RagA GTPase activity, eventually leading to mTORC1 inactivity. Surprisingly, Jin et al. (2015) revealed that, conversely, replenishing amino acid after starvation promotes Skp2-mediated RagA ubiquitination and its interaction with GATOR1. This is likely a negative feedback to control mTORC1 hyperactivity. In addition, E3 ubiquitin ligase Kelch-like protein 22 (KLHL22)-induced K48-linked polyubiquitination and degradation of DEPDC5 release the

inhibitory function of GATOR1, contributing to activating mTORC1 under a condition of amino acid repletion (Chen et al., 2018).

#### 2.4.3 FLCN-FNIP functions as a GAP for RagC/D

Folliculin (FLCN), an evolutionally conserved protein, interacts with FLCN-interacting protein 1/2 (FNIP1/2) and is involved in the mTORC1 pathway (Baba et al., 2006; Hasumi et al., 2008; Takagi et al., 2008). Subsequent studies showed that FLCN-FNIP shifts to the lysosome and binds with the Rag GTPases in the absence of amino acids. Loss of FLCN-FNIP in cells suppressed lysosomal recruitment of mTORC1 and desensitized the mTORC1 pathway to amino acids, indicating that FLCN is necessary for mTORC1 regulation by amino acids (Petit et al., 2013; Tsun et al., 2013). Tsun et al. (2013) further suggested that FLCN-FNIP functions as a GAP for RagC/D and promotes GTP hydrolysis of RagC/D.

### 3 Sensors for the mTORC1 pathway regulated by amino acids

What properties should a sensor have in an amino acid-stimulated mTORC1 pathway? First, sensors should bind directly with substrates at a physiologically relevant concentration (Table 1). Second, sensor-substrate interaction should be necessary for its function in the mTORC1 pathway. Third, sensors

should directly or indirectly signal amino acid availability to the mTORC1 pathway. Several sensors have been identified for the mTORC1 pathway stimulated by leucine, arginine, and methionine (Fig. 1).

#### 3.1 Sensors for arginine-mediated mTORC1

Arginine can significantly regulate mTORC1 signaling, and deprivation of arginine strongly suppresses mTORC1 in various cell types. Recently, arginine sensors have been found for the mTORC1 pathway, including CASTOR1, SLC38A9, and TM4SF5.

##### 3.1.1 CASTOR1

Cellular arginine sensor for mTORC1 protein 1/2 (CASTOR1/2), initially named GATS protein-like 3/2, was found to be a GATOR2-interacting protein and cytosolic arginine sensor (Huttlin et al., 2015; Chantranupong et al., 2016). Sequence analysis revealed that CASTOR1 has two conserved aspartate kinase, chorismate mutase, tyrA (ACT) domains which have important roles in small molecule binding, such as amino acids (Grant, 2006; Chantranupong et al., 2016), while structural analysis suggested that CASTOR1 has four ACT domains (Gai et al., 2016; Saxton et al., 2016b; Xia et al., 2016). Co-immunoprecipitation assay suggested that CASTOR1 and CASTOR2 could form homodimers and heterodimers, but only CASTOR1 dimers, including CASTOR1-CASTOR1 and CASTOR1-CASTOR2, interact with GATOR2

**Table 1** Affinities of sensors for their substrates including leucine, arginine, SAM, and methionine and corresponding intracellular and lysosomal concentrations of those substrates

Amino acid/ metabolite	Binding protein	$K_d/K_i/IC_{50}$ ( $\mu\text{mol/L}$ )	Intracellular concentration in HEK293T ( $\mu\text{mol/L}$ )		Lysosomal concentration in HEK293T ( $\mu\text{mol/L}$ )	
			Amino acid repletion	Amino acid starvation	Amino acid repletion	Amino acid starvation
Leucine	Sestrin1	10–15	192.55	20.25	53.99	48.09
	Sestrin2	20				
	LARS	45				
Arginine	CASTOR1	30	344.29	38.68	119.42	73.66
	SLC38A9	100–200				
	TM4SF5	10.5–37.9				
SAM	SAMTOR	7	33.24	Unknown	3.63	Unknown
Methionine	Unknown	Unknown	157.48	19.49	118.18	53.77

SAM: *S*-adenosylmethionine; LARS: leucyl-tRNA synthetase; CASTOR1: cellular arginine sensor for mTORC1 protein 1; SLC38A9: solute carrier family 38 member 9; TM4SF5: transmembrane 4 L six family member 5; SAMTOR: SAM sensor upstream of mTORC1;  $K_d/K_i/IC_{50}$ : dissociation constant or inhibition constant or half maximal inhibitory concentration. Data from Chen et al. (2011), Han et al. (2012), Chantranupong et al. (2016), Wolfson et al. (2016), Abu-Remaih et al. (2017), Gu et al. (2017), Wyant et al. (2017), and Jung et al. (2019)

in an arginine-sensitive manner. Under arginine depletion condition, GATOR2 binds to the CASTOR1 dimers. In contrast, re-addition of arginine triggers dissociation of GATOR2 from CASTOR1 dimers (Chantranupong et al., 2016). In addition, dimerization of CASTOR1 is important for its interaction with GATOR2 and the mTORC1 pathway in response to arginine (Saxton et al., 2016b). Further binding assay revealed that arginine binds directly to CASTOR1 dimers to disrupt CASTOR1-GATOR2 interaction (Chantranupong et al., 2016). Loss of CASTOR1 resulted in the mTORC1 being insensitive to arginine deprivation. Also, a CASTOR1 mutant that could not bind to arginine has a defect in transmitting arginine sufficiency to the mTORC1. Altogether, CASTOR1 is an arginine sensor for the mTORC1 pathway.

### 3.1.2 SLC38A9

According to the transporter classification database, SLC38A9 belongs to the amino acid/auxin permease family which is characterized by 10 or 11 transmembrane domains (Saier et al., 2016). In addition to the 11 transmembrane domains, SLC38A9 has a distinct about 110-residue cytosolic N-terminal domain (SLC38A9N) responsible for its interaction with Rag-Ragulator (Jung et al., 2015; Rebsamen et al., 2015; Wang et al., 2015). Overexpression of SLC38A9 or SLC38A9N renders mTORC1 activity resistant to amino acid starvation, especially arginine or leucine starvation. Lacking SLC38A9 confers arginine, but not leucine, insensitivity on mTORC1 signaling. Biochemical analysis suggested that SLC38A9 interacts with Rag-Ragulator, which is dependent on SLC38A9N and sensitive to arginine. Interestingly, SLC38A9N also binds to Rag-Ragulator, and the interaction is insensitive to amino acid. Altogether, these results demonstrated that SLC38A9 is a candidate for an arginine sensor.

The following study validated that SLC38A9 is a bona fide arginine sensor for mTORC1 (Wyant et al., 2017). Mutants of SLC38A9 that could not bind to arginine or Rag-Ragulator fail to convey arginine sufficiency to mTORC1 in the SLC38A9-null cells. In vitro binding assay showed that arginine strengthens the interaction of SLC38A9 with Rag-Ragulator at a lysosomal arginine concentration of 100–200  $\mu\text{mol/L}$ . However, in cells, arginine blunts the interaction of SLC38A9 with Rag-Ragulator (Wang et al., 2015).

Recent work has shed light on the relationship between SLC38A9, Ragulator, and Rag GTPases (Shen and Sabatini, 2018). SLC38A9 preferentially binds to RagA/B<sup>GDP</sup>-RagC/D<sup>GTP</sup> heterodimer. Consequently, arginine deprivation which promotes the formation of RagA/B<sup>GDP</sup>-RagC/D<sup>GTP</sup> enhances interaction of SLC38A9 with Rag-Ragulator in cells. In vitro binding assay indicated that arginine promotes the interaction of Rag GTPases with SLC38A9. GDP-loaded RagA associates more strongly with SLC38A9 than GTP-loaded RagA. These results elucidated that the nucleotide state of Rag is the dominant effect on the mTORC1 pathway.

Apart from being an arginine sensor for the mTORC1 pathway, SLC38A9 is also an effluxer for several essential amino acids, especially for leucine. Interestingly, lysine promotes the interaction of SLC38A9 and Rag-Ragulator, indicating that lysine could regulate mTORC1 through SLC38A9 (Wyant et al., 2017). In addition, SLC38A9 plays a crucial role in the lysosomal cholesterol-activated mTORC1 pathway (Castellano et al., 2017). Altogether, SLC38A9 is a complicated and multifunctional protein, and future work may focus on the exploration of high-resolution structures and other crucial functions of SLC38A9.

### 3.1.3 TM4SF5

Interestingly, a very recent study identified transmembrane 4 L six family member 5 (TM4SF5) as a potential lysosomal arginine sensor for the mTORC1 pathway (Jung et al., 2019). TM4SF5 is an N-glycosylated protein with four transmembrane domains, two extracellular loops, an intracellular loop, and two cytosolic terminal tails (Wright et al., 2000). TM4SF5, especially the extracellular loops which are in the lysosomal lumen, binds to arginine with an affinity that is compatible with the lysosome arginine concentration. Furthermore, the TM4SF5 mutant that could not bind to arginine has a defect in activating the mTORC1 pathway upon arginine sufficiency. Loss of TM4SF5 could partially inhibit the mTORC1 pathway upon arginine sufficiency. Thus, TM4SF5 is a lysosomal arginine sensor. Surprisingly, TM4SF5 interacts with another arginine sensor SLC38A9, and Jung et al. (2019) established a new model that TM4SF5 senses and signals lysosomal arginine to SLC38A9 which mediates arginine efflux, contributing to activation of the mTORC1 pathway. However,

the relationship between TM4SF5 and other components of mTORC1 such as Rag GTPases, GATOR, Regulator, and FLCN-FNIP remains elusive.

### 3.2 Sensors for leucine-mediated mTORC1

Leucine is an essential amino acid that serves as not only a building block for protein synthesis but also a signal molecule that regulates protein metabolism, including autophagy (Grinde and Seglen, 1981; Wu et al., 2012; Yan et al., 2012) and the mTORC1 pathway (Hara et al., 1998; Gao et al., 2015). Leucine regulates the mTORC1 pathway through leucine sensors, such as leucyl-tRNA synthetase (LARS) and Sestrin2 (Han et al., 2012; Wolfson et al., 2016), and leucine metabolite acetyl-coenzyme A which promotes EP300-mediated acetylation of the Raptor (Son et al., 2019).

#### 3.2.1 LARS

LARS catalyzes the ligation of leucine and tRNA<sup>Leu</sup> to form leucyl-tRNA<sup>Leu</sup> (Park et al., 2005). Interestingly, LARS was identified as a regulator for leucine-stimulated TORC1 from yeast to the mammal (Bonfils et al., 2012; Han et al., 2012). In the mammal, LARS specifically and directly binds to RagD GTPase, and functions as a GAP for RagD in a leucine-dependent fashion (Han et al., 2012). In addition, LARS likely functions as a leucine sensor for the mTORC1. The Michaelis constant ( $K_m$ ) of LARS for leucine activation is about 45  $\mu\text{mol/L}$  (Chen et al., 2011). Silence of LARS renders mTORC1 resistant to leucine and unable to translocate to the lysosome. LARS mutant without leucylation activity leads to mTORC1 being less sensitive to leucine (Han et al., 2012). Interestingly, He et al. (2018) found that LARS signals intracellular leucine to mTORC1 through direct leucylation on lysine 142 (K142) of RagA. K142 leucylation regulates the interaction of RagA with Regulator and GATOR1, and promotes the formation of GTP-loaded RagA which has higher GTPase activity. This result indicated that aminoacyl-tRNA synthetases act as potential amino acid sensors by direct lysine aminoacylation on the specific target proteins in response to amino acid sufficiency.

#### 3.2.2 Sestrins

Sestrins, highly conserved proteins, have been proved to play crucial roles in oxidative damage and

mTOR signaling (Ho et al., 2016). Sestrin1 and Sestrin2 were initially identified as p53 target genes (Buck-binder et al., 1994; Velasco-Miguel et al., 1999; Budanov et al., 2002), involved in cellular stress and mTOR signaling (Budanov and Karin, 2008; Lee et al., 2010). Sestrin1 and Sestrin2 were previously considered to inhibit the mTORC1 pathway through the activation of AMPK and TSC (Budanov and Karin, 2008). However, subsequent studies found that Sestrin2 still suppresses the mTORC1 pathway in the absence of AMPK and TSC (Parmigiani et al., 2014; Peng et al., 2014), indicating that AMPK and TSC are not necessary for Sestrin2-mediated inhibition of mTORC1. Indeed, Sestrin2 negatively regulates mTORC1 in a GATOR- and Rag GTPase-dependent manner (Chantranupong et al., 2014; Parmigiani et al., 2014; Peng et al., 2014). Sestrin2 binds to GATOR2, and functions upstream of the Rag GTPases and GATOR1 in the amino acid-sensing mTORC1 pathway (Chantranupong et al., 2014).

Amino acid deprivation obviously promotes Sestrin2-GATOR2 interaction, but leucine restimulation disrupts the interaction in cells and in vitro (Wolfson et al., 2016). As well as leucine, methionine and isoleucine stimulation both disrupt Sestrin2-GATOR2 interaction at concentrations about 50 and 100  $\mu\text{mol/L}$ , respectively, much higher than the 5  $\mu\text{mol/L}$  of leucine (Wolfson et al., 2016). The following evidence demonstrates that Sestrin2 is a leucine sensor for the mTORC1 pathway. First, Sestrin2 binds directly to leucine at an affinity that is compatible with the concentration at which mTORC1 is activated. Second, cells lacking Sestrins confer leucine insensitivity on the mTORC1. Third, Sestrin2 mutants that cannot bind with leucine are unable to transmit leucine sufficiency to the mTORC1 (Wolfson et al., 2016). The crystal structure of Sestrin2-leucine further supports the concept that Sestrin2 is a leucine sensor (Saxton et al., 2016c). However, it was argued that the crystal structure of Sestrin2 in the presence of leucine is highly similar to the absence of leucine (Kim et al., 2015; Lee et al., 2016; Saxton et al., 2016c). The authors analyzed the apo-Sestrin2 structure (Kim et al., 2015) and the 3.0 Å structure of Sestrin2 which was purified without adding exogenous leucine (Saxton et al., 2016a). However, both structures have leucine present, indicating that the apo-Sestrin2 structure is elusive.



### 3.3 Sensors for methionine-mediated mTORC1

SAMTOR was originally named C7orf60, but was renamed *S*-adenosylmethionine (SAM) sensor upstream of mTORC1 according to its functions in the mTORC1 pathway (Gu et al., 2017). SAMTOR was identified as an interacting protein of GATOR1 and KICSTOR through the BioPlex database (<https://bioplex.hms.harvard.edu>). Overexpression of SAMTOR inhibits mTORC1, indicating that SAMTOR is a negative regulator for the mTORC1. Sequence analysis predicted that SAMTOR has a SAM-binding domain. Indeed, SAM binds SAMTOR and disrupts the interaction of SAMTOR with GATOR1 and KICSTOR (Gu et al., 2017). SAM is derived from methionine, and methionine starvation reduces SAM concentration (Gu et al., 2017; Quinlan et al., 2017). Accordingly, methionine starvation strengthens the interaction of SAMTOR with GATOR1 and KICSTOR, and SAM stimulation reverses the effect. In addition, Sutter et al. (2013) reported that methionine and SAM inhibit autophagy through TORC1, but SAM, not methionine, is sensed directly by TORC1. Thus, these results indicated that SAMTOR likely serves as a SAM sensor for the methionine-mediated mTORC1. Loss of SAMTOR renders mTORC1 insensitive to methionine deprivation. Importantly, SAMTOR mutants that cannot bind to SAM are incapable of transmitting methionine sufficiency to the mTORC1 (Gu et al., 2017).

## 4 Perspectives

mTORC1 is not equally responsive to individual amino acids; arginine and leucine are particularly efficient for mTORC1 activation (Hara et al., 1998). How arginine and leucine are sensed by the mTORC1 pathway has been partially elucidated through the discovery of their own specific sensors CASTOR1, SLC38A9, TM4SF5, Sestrin2, and LARS (Han et al., 2012; Rebsamen et al., 2015; Wang et al., 2015; Chantranupong et al., 2016; Wolfson et al., 2016; Wyant et al., 2017; Jung et al., 2019). However, knowing other amino acid sensors for the mTORC1 pathway and whether these sensors are limited to specific intracellular organelle, cell type, tissue, or even species remain elusive.

Glutamine activates mTORC1 both independently and dependently on the Rag GTPases (Durán et al., 2012; Stracka et al., 2014; Jewell et al., 2015). In the

Rag-independent manner, ADP ribosylation factor (Arf1) GTPase promotes mTORC1 translocation to lysosomes and activates mTORC1 in response to glutamine (Stracka et al., 2014; Jewell et al., 2015). Thus, regulators of Arf1 GTPase have the potential to be glutamine sensors. Lysine seems to have similar functions to those of arginine (Liu et al., 2012; Wyant et al., 2017). Lysine deprivation is less inhibitory to mTORC1 than that of leucine and arginine (Hara et al., 1998; Wyant et al., 2017). SLC38A9 contributes to conveying arginine and lysine sufficiency to the mTORC1 pathway, but it is more sensitive to arginine (Wyant et al., 2017). Instead, lysosomal amino acid transporter 1 (LAAT-1) transports lysosomal arginine and lysine, while it is more susceptible to lysine (Liu et al., 2012). It is possible that LAAT-1 is not only a transporter, but also a sensor for lysine.

In addition, increasing numbers of small GTPases containing Rags, Rheb, Arf1, Rab, Ras-related protein Ral-A, and Ras homolog gene family Rho are involved in mTORC1 activity (Nguyen et al., 2017). Classically, the Rag GTPases reside on the lysosomal surface and recruit mTORC1 to the lysosome in response to amino acid sufficiency (Sancak et al., 2008). It will be interesting to discover how other GTPases regulate mTORC1 subcellular localization, and how these different proteins are responsive to and integrate amino acid signals. The intracellular organelle-specific amino acid sensors may be linked to small GTPases which localize to various intracellular organelles including lysosome, mitochondria, endoplasmic reticulum, and Golgi apparatus.

### Contributors

Xiu-zhi LI and Xiang-hua YAN analyzed the literatures and wrote the manuscript. Both authors have read and approved the final manuscript.

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### Compliance with ethics guidelines

Xiu-zhi LI and Xiang-hua YAN declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by either of the authors.

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## 中文概要

**题目:** 参与受氨基酸调控的哺乳动物雷帕霉素靶蛋白复合物 1 信号通路的感受体的研究进展

**概要:** 哺乳动物雷帕霉素靶蛋白复合物 1 (mTORC1) 能够感受一系列细胞内外的环境因素 (如氨基酸), 从而控制细胞生长和代谢。在过去的几十年里, 众多蛋白被发现能够参与受氨基酸调控的 mTORC1 信号通路中。Rag GTPases 能够将氨基酸的信号传递给 mTORC1 并招募 mTORC1 到溶酶体表面。近年来, 参与 mTORC1 信号通路的氨基酸代谢物、亮氨酸以及精氨酸的感受体逐渐被发现。感受体的鉴定有助于理解细胞是如何通过调整内部氨基酸感应通路来满足自身需求。本文综述了氨基酸调控 mTORC1 信号通路的分子机制, 并探讨了感受体如何将特定氨基酸信号精确传递给 mTORC1 信号通路。

**关键词:** 哺乳动物雷帕霉素靶蛋白复合物 1 (mTORC1); 氨基酸; 感受体; 溶酶体; Rag GTPases



### Introducing editorial board member:

Xiang-hua YAN, PhD, an editorial board member of *Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)*. Research in YAN laboratory focuses on animal molecular nutrition, especially on autophagy regulated by nutrients and intestinal health in piglets. We study the molecules and mechanisms that govern animal growth, nutritional regulation, and microbial endocrinology, often using comparative proteomics screens in early-weaned piglet as entry points. We also seek to understand how amino acids (leucine) promote mTORC1 and organ growth, a surprising connection between protein synthesis and protein degradation (autophagy) uncovered by our analysis. Together, YAN lab's studies have provided important insights on the fundamental molecular mechanisms that govern the functional complexity of the amino acids and intestinal microbiota in swine production.