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Amino Acid Sensing: Architecture of mTORC1 on the Lysosome Surface

Wei Peng[1,2] and Jenna L. Jewell[1,3,4,*]
[1]Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA
[2]Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA
[3]Harold C. Simmons Comprehensive Cancer Center, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA
[4]Hamon Center for Regenerative Science and Medicine, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA
*Correspondence: Jenna.Jewell@UTSouthwestern.edu
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Amino acid signaling through the Rag GTPases promotes mTORC1 lysosomal localization and subsequent activation. Two new cryo-EM structures examine the architecture of the Rag GTPase heterodimers complexed with mTORC1.

Nutrient sensing by cells is essential for organismal function, and disruption of this sensing mechanism can lead to disease in humans. The mammalian target of rapamycin complex 1 (mTORC1) senses nutrient availability to sustain normal cell growth, as well as regulate cell proliferation and autophagy [1,2]. The core components of mTORC1 are regulatory-associated protein of mTOR (Raptor), which is involved in mTOR substrate recognition, the positive mTOR regulator mLST8 (also known as GβL), and the serine/threonine kinase mTOR, and this complex is hyperactivated in many human diseases, such as cancer, obesity, type 2 diabetes, neurodegeneration, and metabolic disorders. In two recent papers published in *Science*, the groups of Roger Williams and David Sabatini have reported cryo-EM structures that provide a more detailed picture of how mTORC1 senses nutrients [3,4].

Growth factors, amino acids, energy status, and stress regulate mTORC1 [1,2]. Of these stimuli, amino acids are the most potent and essential for mTORC1 activation and for its localization to the lysosomal membrane. Elevated amino acids promote mTORC1 lysosomal localization, where it is activated by growth factors through the small G protein Rheb. The last 15 amino acids of Rheb, which contain a CAAX (C = cysteine, A = aliphatic amino acid, X = terminal amino acid) box, are thought to anchor Rheb to the lysosomal surface. Growth factors activate Rheb by inhibiting tuberous sclerosis complex (TSC), a GTPase-activating protein (GAP) for Rheb that promotes the generation of the GDP-bound, inactive form. GTP-bound, active Rheb interacts with mTORC1, which allosterically activates mTOR kinase activity [5]. Thus, growth factors increase GTP-bound Rheb, resulting in mTORC1 activation at the lysosome.

The identification of the Rag GTPases in 2008 was the first clue in understanding the molecular mechanisms of how some amino acids (leucine, arginine, and methionine) promoted mTORC1 lysosomal localization and subsequent activation [6,7]. Mammals have four Rag proteins: RagA, RagB, RagC, and RagD. RagA and RagB have high sequence similarity and are thought to be functionally redundant, and similarly RagC and RagD are related in sequence and functionally redundant. RagA or RagB (RagA/RagB) forms a heterodimer with RagC or RagD (RagC/RagD), with the possibility of forming four distinct complexes. The dimerization between RagA/RagB and RagC/RagD is crucial for Rag GTPase protein stability and mTORC1 activation. The amino-terminal GTPase domain of Rag GTPases is similar to that of other Ras-like GTPases, and the Rag GTPases share a carboxy-terminal roadblock domain (CRD) that mediates RagA/RagB heterodimerization with RagC/RagD [8,9]. As with other small GTPases, guanine nucleotide binding to the Rag GTPases is important for their function. Only the heterodimer of GTP-loaded RagA/RagB ($RagA^{GTP}$/$RagB^{GTP}$) and GDP-loaded RagC/D ($RagC^{GDP}$/$RagD^{GDP}$) interacts with the mTORC1 component Raptor at the lysosome.

After the discovery of the Rag GTPases, the pentameric Ragulator complex was shown to anchor the Rag GTPase–mTORC1 complex to the lysosomal surface (reviewed in [1,2]). The tumor suppressor folliculin (FLCN) and its binding partners FNIP1 and FNIP2 were also reported to interact with the Rag GTPases and display GAP activity towards RagC/RagD, resulting in mTORC1 activation. Other components that are involved in amino acid signaling to mTORC1 via the Rag GTPases are the vacuolar H^+-ATPase (V-ATPase), the multimeric GATOR complexes (referred to as GATOR1 and GATOR2), KICSTOR complex, SLC38A9, Sestrin2, SAMTOR, and CASTOR1. Importantly, somatic gain-of-function missense mutations in RagC are found in ~17% of follicular lymphoma patients, and ~50% of these follicular lymphoma patients also have mutations in V-ATPase [10].

Recently, multiple structures have been unveiled for components involved in amino acid signaling to mTORC1 via the Rag GTPases. With the advancement of cryo-electron microscopy (cryo-EM), structures of mTORC1 [11,12], mTORC1–Rheb [5], multimeric GATOR1 complex and GATOR1–$RagA^{GMPPNP}$–RagC (where GMPPNP is a non-hydrolyzable analog of GTP) [9], Ragulator–RagA(CRD)–RagC(CRD) [13], and V-ATPase [14] have been revealed. Crystal structures of Sestrin2 [15] and CASTOR1 [16] have also been solved. Moreover, the crystal structures of the yeast equivalent of the RagA/RagB (Gtr1) and RagC/RagD (Gtr2) heterodimer have been determined ($Gtr1^{GMPPNP}$–$Gtr2^{GMPPNP}$ and

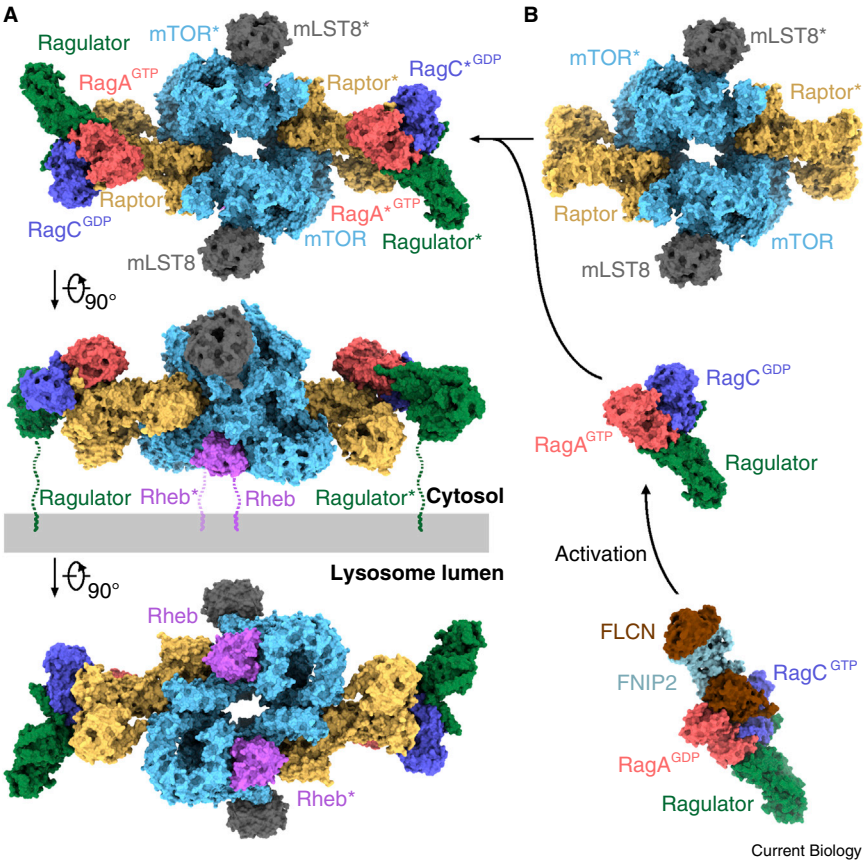

Figure 1. Architecture of the mTORC1 supercomplex at the lysosome.
(A) The mTORC1–Rheb–RagA^{GTP}–RagC^{GDP}–Ragulator complex at the lysosome. The Raptor–Rag–Ragulator complex (PDB accession code: 6U62 [4]) was superimposed with the mTORC1–Rheb complex (PDB accession code: 6BCU [5]) to generate the model. Individual complex subunits are colored and indicated by color-coded text. Dimeric mTORC1 subunits are indicated with or without an asterisk. Figures were generated using UCSF ChimeraX. (B) RagA–RagC regulation by FLCN–FNIP2 (a GAP for RagC). In response to nutrient signals, RagA–RagC changes from the inactive state of RagA^{GDP}–RagC^{GTP} (bottom, PDB accession code: 6NZD [20]) to the active state of RagA^{GTP}–RagC^{GDP} (middle, PDB accession code: 6U62 [4]). RagA^{GTP}–RagC^{GDP} binds to the mTORC1 component Raptor (top, PDB accession code: 6BCU [5]).

Gtr1^{GTP}–Gtr2^{GDP}) [8,17]. Although structures of mTORC1 and Rag GTPases are available, the mechanism of mTORC1 activation by these GTPases is elusive without a mTORC1–Rag complex structure. Therefore, determination of the architecture of mTORC1 complexed to the Rag GTPases would significantly enhance the mechanistic understanding of how mTORC1 senses amino acids through these GTPases.

The two recent *Science* papers have now built a three-dimensional model of mTORC1 bound to Rheb, Rag GTPases, and Ragulator on the lysosomal surface (Figure 1A). Anandapadamanaban *et al.* [3] determined crystal structures of heterodimers of the human RagA^{GTP}(Q66L) mutant (which is defective in GTP hydrolysis and constitutively GTP bound) and either RagC^{GDP}(T90N) or RagC^{GDP}(S75N); both of these RagC mutants are GDP bound and are typically found in follicular lymphoma patients [10], and were introduced to stabilize the Raptor–Rag interaction. Cryo-EM structures of mTORC1 bound to PRAS40-fused RagA^{GTP}(Q66L)-RagC^{GDP}(T90N) and RagA^{GTP}(Q66L)-RagC^{GDP}(T90N) were also determined to 5.5 Å and 6.2 Å, respectively [3]. The authors used PRAS40-fused RagA^{GTP}(Q66L)-RagC^{GDP}(T90N) in order to help stabilize the complex; however, the PRAS40-fused RagA^{GTP}(Q66L)-RagC^{GDP}(T90N) dimer bound to mTORC1 to the same extent as the non-PRAS40-fused RagA^{GTP}(Q66L)-RagC^{GDP}(T90N) dimer. In this study, the Rag GTPases were purified from bacteria, whereas mTORC1 was isolated from human cells. In the other paper, Rogala *et al.* [4] solved the Raptor–Rag–Ragulator supercomplex (to 3.2 Å) via cryo-EM. The RagA^{GTP}–RagC^{GDP}(S75N,T90N) heterodimer and pentameric Ragulator were purified from bacteria, and Raptor was purified from human cells. Wild-type RagA was used in this study because of its slow GTP hydrolysis rate and it was confirmed to be GTP bound. The GTPase domain and CRD of RagA and RagC are involved in the interaction between these Rag GTPases. A three-dimensional structure model of the mTORC1–Rheb–RagA–RagC–Ragulator dimer at the lysosome is presented in both papers (Figure 1A). In order to generate this working model, Anandapadamanaban *et al.* [3] used a previous Ragulator–RagA(CRD)–RagC(CRD) structure [13], and both Anandapadamanaban *et al.* [3] and Rogala *et al.* [4] used a 3.4 Å mTORC1–Rheb structure [5].

The central region of Raptor (amino acids 376–844) contains an α-solenoid domain that participates in the interaction with RagA–RagC. As observed in other GTPases, RagA and RagC contain nucleotide-sensitive secondary elements that undergo conformational changes in response to nucleotide binding within the GTPase domain, known as switches (switch I, interswitch, and switch II). Three helices (α24, α26, and α29) of the Raptor α-solenoid directly contact the switch I and interswitch elements of RagA. Rogala *et al.* [4] predict that GDP binding to RagA would rearrange the switch I and interswitch regions and disrupt binding sites for Raptor, possibly explaining why only GTP-bound RagA interacts with mTORC1. Raptor has less extensive contact with the GTPase domain of RagC compared with that of RagA, and the RagA–RagC CRDs contribute less than the GTPase domain to the Raptor interaction. In the RagA–RagC–mTORC1 structure, the GTPase domains of RagA and RagC need to open up in order to accommodate Raptor binding.

Interestingly, Rogala *et al.* [4] uncover a previously unidentified Raptor element that plays a crucial part in the Raptor–Rag interaction; this element, referred to as a 'Raptor claw', is localized in the complex between the GTPase domains of RagA and RagC. The GTPase domains

and CRDs of RagC and RagA all contact the Raptor claw. Moreover, the Raptor claw is likely to be an element that can detect the nucleotide state of RagC, ensuring that RagC is GDP bound, because the Raptor claw only fits well with $RagC^{GDP}$ and would significantly clash with $RagC^{GTP}$. Ragulator binds only to the CRDs of RagA and RagC, which is somewhat surprising because Ragulator has been reported as a RagA/RagB guanine nucleotide exchange factor (GEF). In contrast to the Rag GTPases, the Ragulator subunits (LAMTOR1–5) do not directly contact Raptor, indicating the essential role of Rag GTPases in linking mTORC1 with Ragulator. Taken together, the structural features described in both papers now explain why mTORC1 selectively interacts with $RagA^{GTP}$–$RagC^{GDP}$.

The yeast version of the $RagA^{GTP}$–$RagC^{GDP}$ complex ($Gtr1^{GTP}$–$Gtr2^{GDP}$) displays significant conformational differences in its GTPase domains compared with the human complex [17]. These differences lead to a spatial clash and incompatibility of Rag GTPases with mTORC1, on the basis of the mammalian structure model. Therefore, Gtr1–Gtr2 or yeast Raptor (KOG1) may adopt a different conformation when forming a complex. This is consistent with the fact that mammalian RagA can complement a Gtr1-deficient yeast strain, whereas mammalian RagC cannot complement Gtr2 deficiency [18]. Additional studies are needed in order to fully understand the molecular mechanisms involved in amino acid signaling to TOR in yeast.

Shortly after publication of the *Science* papers, additional structures of components involved in amino acid sensing by mTORC1 have been published, one from the Sabatini lab and the other a collaboration between the Hurley and Zoncu labs. In these studies, two new cryo-EM structures of $RagA^{GDP}$–$RagC^{GTP}$ bound to Ragulator and FLCN–FNIP2 have been solved (to 3.3 Å and 3.6 Å resolution; Figure 1B) [19,20] and these partially reveal the mechanism by which FLCN–FNIP2 act as a GAP for RagC. The catalytic residue in FLCN, R164, is essential for its GAP activity but is displaced in both structures. How $RagC^{GTP}$ transitions to $RagC^{GDP}$ for FLCN–FNIP2-regulated mTORC1 activation calls for further studies. Taken together, the new structural studies [3,4,19,20] provide extensive mechanistic details and significantly advance our understanding of how amino acids regulate mTORC1 at the lysosome.

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