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RESEARCH HIGHLIGHT

Leucyl-tRNA synthetase: double duty in amino acid sensing

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The cellular response to amino acids is controlled at the molecular level by TORC1. While many of the elements that participate in TORC1 signaling are known, we still have no clear idea how cells sense amino acids. Two recent studies found that leucyl-tRNA synthetase (LRS) is a leucine sensor for TORC1, in both yeast and mammalian cells.

Eukaryotic cells strictly depend on nutrient availability to sustain growth. In particular, amino acids are required as an energy supply, as building blocks for protein synthesis and as signaling molecules that activate the serine/threonine kinase Target of Rapamycin (TOR). Importantly, TOR regulates energy metabolism and protein synthesis. TOR, highly conserved from yeast to human, forms two functionally and structurally distinct complexes termed TORC1 and TORC2 [1-4]. TORC1 regulates protein synthesis, ribosome biogenesis, nutrient uptake and autophagy in response to growth factors, amino acids and cellular energy. TORC2 regulates actin cytoskeleton organization, cell survival and lipid synthesis. It has been known for many years that amino acids activate TORC1, which then regulates the cellular response to amino acids [2]. However, the task of dissecting the complex mechanism by which amino acids are sensed by the cell and how this input is transduced to TORC1 is still, despite a large amount of attention, far from finished. Indeed, the molecular mechanism by which cells sense nutrients remains one of the major open questions in biology.

Several elements have been proposed to participate in amino acid sensing, including the small GTPase Rag/Gtr, the Ragulator complex, MAP4K3, hVps34, PAT1, p62 and V-ATPase [5-13]. In mammalian cells, the accepted model for the activation of mTORC1 in response to amino acids is that mTORC1 is targeted and anchored to the surface of the lysosome [5]. Recruitment of mTORC1 to the lysosome involves the binding of mTORC1 to activated Rag that is attached to the lysosome via the so-called Ragulator complex. Activated Rag is a heterodimer of GTP-loaded RagA or B in complex with GDP-loaded RagC or D (i.e., the conformation RagA/B GTP-RagC/D GDP) [8]. The transition from RagA/B GDP-RagC/D GTP to RagA/B GTP-RagC/D GDP is stimulated by amino acids [5, 6], with leucine having the most robust effect of all 20 amino acids. Thus, the change in guanine nucleotide-binding status of the Rag heterodimer is a key step in amino acid sensing upstream of mTORC1. However, the factors mediating the transition (what are the GEFs and GAPs?) and how amino acids are originally sensed are unknown. Two recent studies by Han *et al*. [14] and by Bonfils *et al*. [15] propose leucyl-tRNA synthetase (LRS) as an evolutionarily conserved amino acid sensor that activates the Rag GTPases (Gtr in yeast).

As reported in the April 13 issue of *Cell*, Han *et al*. [14] demonstrate in a biochemical study performed with HEK293 cells that leucine-bound LRS activates mTORC1. First, the authors show that leucine-bound LRS co-localized, co-fractionated and co-precipitated with mTORC1. The interaction of LRS with mTORC1 correlated with the localization of LRS to the lysosomal surface. Specifically, LRS interacted with RagD, the member of the Rag heterodimer that is GDP bound upon addition of leucine to cells. The GTP-to-GDP transition of RagD is required for the lysosomal recruitment and subsequent activation of mTORC1. Second, the authors found a here-to-fore unknown GAP domain in LRS that catalyzes the hydrolysis of GTP to GDP in RagD upon leucine addition. Surprisingly, LRS is necessary for the activation of mTORC1 by any amino acid (not just leucine), as LRS inhibition prevented the activation of mTORC1 in the presence of all amino acids. These findings suggest the following model for how leucine is sensed in the cell to activate mTORC1. Leucine binding to LRS activates LRS GAP activity toward RagD, which then promotes lysosomal recruitment and

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activation of mTORC1.

In a parallel study published on the same day in *Molecular Cell*, Bonfils *et al*. [15] also suggest a role for LRS in TORC1 activation in yeast cells. However, in contrast to the observations by Han *et al*. in human cells, yeast LRS interacts with Gtr1 (RagA/B homologue) instead of Gtr2 (RagD homologue). According to the model proposed by Bonfils *et al.*, leucine-bound LRS interacts with Gtr1 through the CP1 editing domain in LRS, resulting in GTP-bound Gtr1. Disruption of the LRS-Gtr1 interaction converts Gtr1 to the GDP-bound form and causes loss of TORC1 activity. Thus, although LRS appears to be evolutionarily conserved as an amino acid sensor upstream of the Rag heterodimer (Gtr1-Gtr2 complex in yeast) and TORC1, it seems that its mechanism of action is substantially different in yeast and mammals. In mammals, LRS is a GAP for RagD, whereas in yeast, it has GEF-like activity toward Gtr1 (RagA/B). How the GEF-like activity of LRS in yeast is functionally related to the previously described Gtr1 GEF Vam6 is unknown [16].

These two reports raise new questions and complexity regarding the current model of amino acid sensing and TORC1 activation. Several previous reports identified a number of elements located at the surface of the lysosome that are involved in the activation of TORC1 by amino acids. Those include the Rag/Gtr complex itself, MP1, p14, p18, p62, PAT1 and V-ATPase. Furthermore, it has been proposed that amino acid sensing in mammalian cells involves a so far incompletely understood 'inside-out' mechanism at the lysosome [13]. According to this inside-out model, activation of mTORC1 by amino acids involves the sensing of amino acids accumulated inside the lysosome, and this sensing is dependent on the vacuolar H+-ATPase. Supporting this model, mTORC1 is inhibited upon overexpression of PAT1, a lysosomal amino acid transporter that extrudes amino acids from the lysosomal lumen. However, the role of PAT1 with respect to mTORC1 activation seems to be controversial, as inhibition of PAT1 has also been shown to prevent mTORC1 activation by amino acids [11]. At face value, PAT1 as a TORC1 activator appears to be consistent with amino acids being sensed by cytoplasmic LRS.

The apparently discrepant findings supporting the cytoplasmic sensing model versus the lumenal sensing model can be explained in different ways. First, it could be that none of the proposed mechanisms (LRS, V-ATPase, MAP4K3, *etc*.) are exclusive. In other words, the concept of a unique amino acid sensor activating TORC1 is too simple — rather, multiple sensing mechanisms exist. This possibility is supported by the finding that LRS acts toward mTORC1 only as a GAP for RagD, leaving open the possibility that another amino acid sensor stimulates GTP loading of RagA/B. A different mechanism involving another amino acid sensor could be responsible for the GDP-to-GTP exchange in the RagA/B subunit (which is the dominant subunit in the heterodimer). In yeast, while LRS mediates GTP loading of Gtr1, another amino acid sensor other than LRS could be responsible for the GTP-to-GDP transition in Gtr2. A second possible explanation is that all the implicated factors located on the surface of the lysosome (Rag/Gtr complex, MP1, p14, p18, p62, PAT1 and V-ATPase) are indeed necessary for the activation of TORC1, but do not participate in the sensing process itself. Many of the factors on the surface of the lysosome could form a scaffold or platform for TORC1 signaling without directly participating in the amino acid sensing/regulation process. This would explain why proteins like p62, the Ragulator complex or V-ATPase, which do not respond directly to amino acids, are necessary for TORC1 activation. It would also explain why in some cases, as for PAT1, both overexpression and knockdown of a protein results in the inhibition of TORC1. Alteration of a lysosomal scaffold/platform could impair the normal response of TORC1 to amino acids. In any case, it seems clear that the lysosome plays a critical role in the regulation of mTORC1 by amino acids. To determine whether the actual amino acid sensing is in the lysosomal lumen, in the cytosol, in both locations or even in other organelles, will require further work. Certainly, the finding that LRS is a leucine sensor for TORC1 opens a new direction in the dissection of the longstanding question of how cells sense nutrients.

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