COMMENTARY

Rag GTPases regulate cellular amino acid homeostasis

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Amino acids are essential sources for synthesizing proteins, being precursors for hormones/neurotransmitters and key anaplerotic metabolites for the tricarboxylic acid cycle and gluconeogenesis. While mammalian cells can synthesize the majority of amino acids from other metabolic intermediates, among the 20 proteinogenic L-amino acids, 9 amino acids are required to be absorbed from nutrients, called essential amino acids. Our body possesses multiple layered systemic amino acid homeostatic regulations to ensure a constant and sufficient amino acid supply by maintaining plasma amino acid concentrations through intestine/kidney-mediated absorption and liver/muscle-mediated secretion. Hence, short-term fasting (up to 48 h) generally does not decrease plasma concentrations of amino acids except alanine, which is utilized for hepatic gluconeogenesis, and even long-term fasting (up to 6 wk) only decreases amino acids modestly (1). However, it has been known that chronic malnutrition, such as Kwashiorkor, significantly decreases amino acid concentrations in blood (2). In addition to the systemic regulations of amino acid homeostasis, cells also maintain intracellular concentrations of amino acids through multiple mechanisms, including transporter/endocytosis-mediated absorption, biosynthesis, and proteasome/lysosome-mediated proteolysis. Moreover, cellular amino acids can regulate their sensing pathways, which also modulate amino acid production, consumption, and temporal storage in organelles such as lysosomes. This storage mechanism may have important roles in adjusting concentrations of cytoplasmic proteinogenic amino acids (e.g., essential amino acids), preventing their oxidation and supplying building blocks for needed protein synthesis.

Recent studies mainly using HEK293T cells demonstrated that the mechanistic target of rapamycin complex 1 (mTORC1), which is activated by amino acids, controls the abundance of amino acids, particularly essential amino acids, in lysosomes in response to amino acid availability in a manner independent of mTORC2 activity or autophagy, which digests macromolecules to produce small molecular nutrients such as amino acids (3, 4). The Sabatini group had previously demonstrated that most essential amino acids were accumulated within the lysosome in response to short-term amino acid starvation or the treatment with the mTORC1 inhibitor Torin1 (3). Furthermore, the effect of Torin1 on the retention of amino acids within the lysosome was canceled when SLC38A9, an arginine sensor and nonpolar amino acid exporter on the lysosome, was ablated. Thus, it was proposed that while high mTORC1 promotes amino acid export from the lysosome through SLC38A9 when amino acids are readily available, low mTORC1 activity blocks amino acid egress from the lysosome, likely by reducing SLC38A9 activity when amino acids are scarce (3).

In PNAS, Bandyopadhyay et al. evaluate the trafficking of intracellular amino acids, such as leucine, in response to amino acid availability by multifaceted measurements (5). Using ^{3}H-leucine as a tracer, the authors determine amino acid secretion into the

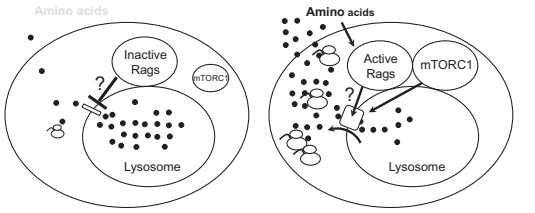

Fig. 1. In response to amino acid starvation, essential amino acids are stored in the lysosome in a manner dependent on the Rag-Ragulator system but not mTORC1. Under amino acid–sufficient conditions, amino acids are rapidly trafficked thorough lysosomes and utilized for protein synthesis.

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medium, incorporation into protein, and retention in the lysosome in response to amino acid starvation. Consistent with previous observations (3, 4), amino acid starvation leads to the accumulation of the essential amino acid leucine in the lysosome. Moreover, leucine starvation strongly inhibits the leucine secretion from the lysosomal reserve into the culture medium, revealing a mechanism of leucine autonomous homeostasis regulation. Surprisingly, inhibition of mTOR activity with Torin1 does not mimic the effect of leucine starvation, as Torin1 neither blocks the extracellular secretion nor facilitates the lysosomal accumulation of ^{3}H-leucine tracer in response to leucine availability. These observations suggest that, at least in their experimental settings using mouse embryonic fibroblasts and other cell types, lysosomal leucine storage or its secretion are regulated independently of cellular mTORC1 activity (Fig. 1).

Bandyopadhyay et al. further examine the role of mTORC1 activity in regulating lysosomal leucine storage using genetic approaches (5). The Rag and Rheb GTPases are key mTORC1 activators in response to amino acids and growth factors, respectively. The Rag GTPases form heterodimers (RagA/C, RagA/D, RagB/C, and RagB/D) and mediate amino acid signal to mTORC1 activation on the lysosome. The authors ablate sestrin2 or DEPDC5, upstream suppressors of Rag GTPases, or TSC2, a suppressor of Rheb, to activate mTORC1 and find that mTORC1 activation has little effect on starvation-induced leucine lysosomal storage or extracellular secretion. Interestingly, the authors observe that ablation of RagA/RagB or the Ragulator complex, which anchors Rag heterodimer to the lysosomal membrane, disrupts lysosomal leucine storage, suggesting that the Ragulator-Rag system inhibits export of amino acids from the lysosome in a manner independent of mTORC1 under amino acid starvation conditions. The authors also demonstrate that inhibition of protein synthesis by cycloheximide or leucenol reduces lysosomal leucine storage and promotes extracellular leucine secretion (5). These observations indicate that cytosolic free amino acids or newly synthesized proteins may contribute to the regulation of amino acid egress from the lysosome. Alternatively, the overall rate of protein synthesis might signal to lysosomal amino acid transporter machinery to control lysosomal amino acid pool. An appealing model is that amino acids might regulate their own homeostasis by activating Rag GTPases, which promote amino acids egress from the lysosome and activate mTORC1 to increase translation and amino acid utilization. The lysosomal protein SLC38A9 senses lysosomal luminal arginine, exports nonpolar essential amino acids to the cytosol, and is required for Rag activation. One may speculate whether SCL38A9 may similarly be involved in the regulation of the Rag-dependent lysosomal amino acid storage.

Although most cells show nutrient-regulated leucine storage, the MCF7 breast cancer cells are defective in lysosomal leucine storage even under amino acid starvation. Notably, MCF7 cells have low levels of RagA and RagB expression compared to MCF10a cells, a normal breast epithelial cell line. Importantly, overexpression of RagA or/and RagB not only restored the ability of starved MCF7 cells to store amino acids in lysosomes but also enhanced protein synthesis. These observations suggest that the ability to control lysosomal amino acid storage likely plays a fundamental role in coordinating protein synthesis with amino acid availability to support proper cell growth/proliferation and viability. This study discovered previously unrecognized mTORC1-independent functions of the Ragulator-Rag system in modulating lysosomal amino acid trafficking.

In PNAS, Bandyopadhyay et al. evaluate the trafficking of intracellular aminoacids, such as leucine, in response to amino acid availability by multifaceted measurements.

Amino acids activate Rag GTPases by regulating Rag guanine nucleotide-binding. RagA-GTP/RagC-GDP is active, while RagA-GDP/RagC-GTP is inactive to stimulate mTORC1. An important open question is whether the nucleotide-binding state of the Rag heterodimer regulates their activity for the lysosomal amino acid storage. Given the observations that the lysosomal amino acid storage occurs under amino acid scarcity, the inactive Rag heterodimer, which is not occupied by mTORC1, may inhibit leucine egress from the lysosome by directly or indirectly blocking the activity of lysosomal amino acid transporters such as SLC38A9 and LAT1 (3, 4, 6, 7). In line with the above notion, recent studies demonstrated that the inactive Rag heterodimer preferentially interacts with several proteins, including TSC2, SH3BP4, and ATXN3 (8–10). Identification of the key downstream effectors of Rag GTPases in regulating lysosomal amino acid storage warrants future study. It is conceivable that in addition to leucine and arginine, other amino acids may potentially regulate the lysosomal storage of amino acids. Finally, it is crucial to determine if the described lysosomal amino acid storage model is physiologically relevant, as amino acid concentrations in the plasma of rodents and humans are rarely dropped dramatically even under strict protein restriction (11–13). However, cancer cells can experience severe nutrient-deficient conditions in certain tumor microenvironments (14). New technology to accurately measure free amino acid concentrations in lysosome in cells and tissues in situ would be critical to deciphering the role of lysosomal amino acid storage in the regulation of cell growth/proliferation and survival in pathophysiological settings.

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1 P. Felig, O. E. Owen, J. Wahren, G. F. Cahill Jr., Amino acid metabolism during prolonged starvation. *J. Clin. Invest.* **48**, 584–594 (1969).
2 L. E. Holt Jr., S. E. Snyderman, Disturbances of amino acid metabolism. *Bull. N. Y. Acad. Med.* **36**, 431–450 (1960).
3 M. Abu-Remaileh *et al.*, Lysosomal metabolomics reveals V-ATPase- and mTOR-dependent regulation of amino acid efflux from lysosomes. *Science* **358**, 807–813 (2017).
4 G. A. Wyant *et al.*, mTORC1 activator SLC38A9 is required to efflux essential amino acids from lysosomes and use protein as a nutrient. *Cell* **171**, 642–654.e12 (2017).
5 U. Bandyopadhyay *et al.*, Leucine retention in lysosomes is regulated by starvation. *Proc. Natl. Acad. Sci. U.S.A.* **119**, e2114912119 (2022).
6 F. Beaumatin *et al.*, mTORC1 activation requires DRAM-1 by facilitating lysosomal amino acid efflux. *Mol. Cell* **76**, 163–176.e8 (2019).
7 R. Milkereit *et al.*, LAPTM4b recruits the LAT1-4F2hc Leu transporter to lysosomes and promotes mTORC1 activation. *Nat. Commun.* **6**, 7250 (2015).
8 C. Demetriades, N. Doumpas, A. A. Teleman, Regulation of TORC1 in response to amino acid starvation via lysosomal recruitment of TSC2. *Cell* **156**, 786–799 (2014).
9 Y. M. Kim *et al.*, SH3BP4 is a negative regulator of amino acid-Rag GTPase-mTORC1 signaling. *Mol. Cell* **46**, 833–846 (2012).

10 Y. Yao *et al.*, Amino acids enhance polyubiquitination of Rheb and its binding to mTORC1 by blocking lysosomal ATXN3 deubiquitinase activity. *Mol. Cell* **80**, 437–451.e6 (2020).
11 L. A. Cynober, Plasma amino acid levels with a note on membrane transport: Characteristics, regulation, and metabolic significance. *Nutrition* **18**, 761–766 (2002).
12 S. C. Kalhan *et al.*, Metabolic and genomic response to dietary isocaloric protein restriction in the rat. *J. Biol. Chem.* **286**, 5266–5277 (2011).
13 S. M. Hutson, A. E. Harper, Blood and tissue branched-chain amino and alpha-keto acid concentrations: Effect of diet, starvation, and disease. *Am. J. Clin. Nutr.* **34**, 173–183 (1981).
14 C. Commisso *et al.*, Macropinocytosis of protein is an amino acid supply route in Ras-transformed cells. *Nature* **497**, 633–637 (2013).