

Nutrients are indispensable for life. Among various nutrients amino acids are the major nitrogen source; therefore, perception of the amino acid environment is essential for cells. The cellular amino acid sensing system employs Tor (target of rapamycin) protein kinase. Tor forms two distinct protein complexes, TORC1 (Tor complex1) and TORC2. TORC1 is involved in amino acid sensing, regulating protein synthesis, the cell cycle, and autophagy. On the other hand, TORC2 is responsible for actin organization and cell integrity. So far, it is not clear whether TORC2 also perceives nutrient signals.

The aim of our research group is to reveal the molecular mechanisms of how TORC1 receives amino acid signals and how the TORC1/2 pathways regulate each phenomenon. We have been studying Tor signaling in the budding yeast *Saccharomyces cerevisiae*, and have found three novel branches of the TOR signaling pathways (Figure 1).

Figure 1. Tor signaling pathway of the budding yeast. Our group has found three branches of the Tor pathway.

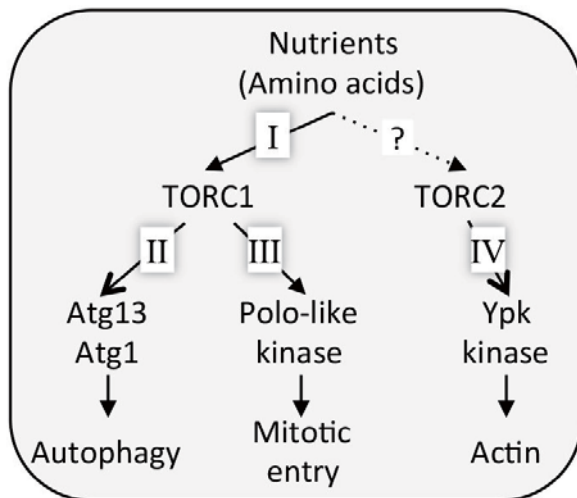


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### I. How do amino acids regulate TORC1?

TORC1 is regulated by amino acids. Amino acids are fundamental nutrients, and 20 species of amino acids building proteins are not interchangeable with each other. Therefore, TORC1 should sense each amino acid individually. Mammalian mTORC1 is believed to be controlled by Rag GTPases and their regulators. However, Rag-factors are dispensable for amino acid sensing by TORC1 in the budding yeast, suggesting an alternative mechanism of TORC1 regulation.

Genetic investigation was done to discover the involvement of (aminoacyl)-tRNA in TORC1 regulation. Biochemical *in vitro* TORC1 assay also revealed that tRNA directly inhibits TORC1 kinase activity. Reducing cellular tRNA molecules

desensitizes TORC1 inactivation by nitrogen starvation *in vivo*. Based on these results, a TORC1 regulatory model is proposed that free tRNA released from protein synthesis under amino acid starvation inhibits TORC1 activity. Therefore, TORC1 employs a tRNA-mediated mechanism to monitor intracellular amino acids.

### II. TORC1 phosphorylates Atg13, the molecular switch of autophagy

TORC1 negatively regulates autophagy, a protein degradation system induced by nutrient starvation.

We found the TORC1-mediated regulatory mechanism of autophagy. Under nutrient-rich conditions, TORC1 directly phosphorylates Atg13, a component of the Atg1 kinase complex. Atg1 is a Ser/Thr protein kinase, the activity of which is essential for autophagy and is largely enhanced in response to TORC1 inactivation. Activation of Atg1 requires formation of the Atg1 complex. Phosphorylation of Atg13 by TORC1 plays a pivotal role in Atg1 complex formation; phosphorylated Atg13 loses its affinity to Atg1 resulting in disassembly of the Atg1 complex and repression of autophagy. On the other hand, dephosphorylation of Atg13 triggers formation of the Atg1 complex, activation of Atg1 kinase, and consequently induction of autophagy.

### III. TORC1 regulates mitotic entry via polo-like kinase

TORC1 regulates protein synthesis, which is important for promotion of the cell cycle at the G1 phase.

We demonstrated that TORC1 is also involved in another stage of the cell cycle, mitotic entry. Cdc5, the yeast polo-kinase mediates this regulation, and the nuclear localization of Cdc5 at G2/M transition is controlled by TORC1. In addition, we discovered a physiological role of TORC1 in mitosis; autophagy negatively controlled by TORC1 plays an important part in maintenance of genome stability under starvation conditions.

### IV. Ypk kinase acts directly downstream of TORC2 to control actin organization

TORC2 has an essential function controlling polarity of the actin cytoskeleton. We found that Ypk2, a member of the AGC kinase family is directly phosphorylated by TORC2. The activated allele of *YPK2* can rescue a lethality caused by TORC2 dysfunction, suggesting that Ypk kinase is the major downstream protein of the TORC2 pathway.

#### Publication List:

[Original paper]

- Kamada, Y. (2017). Novel tRNA function in amino acid sensing of yeast Tor complex1. *Genes Cells* 22,135-147.