LABORATORY OF BIOLOGICAL DIVERSITY

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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).

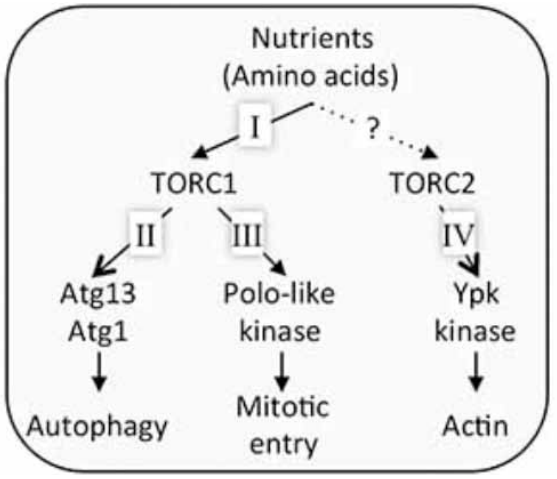

Figure 1. Tor signaling pathway of the budding yeast. Our group has found three branches of the Tor pathway.

I. How do amino acids regulate TORC1?

It is confirmed that TORC1 is the master regulator in amino acid sensing. However, little is known how TORC1 perceives amino acid signals.

Since both amino acid sensing and TORC1 have essential cellular functions, we assume that factor(s) transmitting amino acid signals to TORC1 should be encoded by essential gene(s). We identified several essential genes as candidates of TORC1-regulators.

II. TORC1 phosphorylates Atg13, the molecular switch of autophagy

TORC1 negatively regulates autophagy, a protein degradation system induced by nutrient starvation and rapamycin (a TORC1 inhibitor).

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 which consist of Atg1, Atg13, Atg17, Atg29, and Atg31. 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.

We further determined eight phosphorylation sites of Atg13 and generated an unphosphorylatable Atg13 mutant (Atg13-8SA). Expression of Atg13-8SA induces autophagy bypassing inactivation of TORC1, suggesting that Atg13 acts as a molecular switch for autophagy induction.

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. We generated a temperature-sensitive allele of *KOG1 (kog1-105),* which encodes an essential component of TORC1, and found that TORC1 plays an important role in mitotic entry (G2/M transition). Since Cdc5, the yeast polo-kinase is mislocalized and inactivated in *kog1-105* mutant cells, TORC1 mediates G2/M transition via regulating polo-kinase. 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 identified Ypk2, a member of the AGC kinase family which acts directly downstream of TORC2 using molecular genetics. 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. We also demonstrated that Ypk2 is directly phosphorylated by TORC2 through a biochemical assay.