

Nutrients are indispensable for life. Thus, perception of the nutrient environment is also essential for cells. To recognize cellular nutrient conditions, eukaryotic cells employ Tor (target of rapamycin) protein kinase. Tor forms two distinct protein complexes, TORC (Tor complex) 1 and TORC2. TORC1 regulates rapamycin-sensitive branches of the TOR pathway, such as protein synthesis, cell cycle and autophagy. TORC1 is thought to act as a nutrient sensor, because rapamycin, a TORC1 inhibitor, mimics a starved condition. On the other hand, TORC2, whose function is insensitive to rapamycin, 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 Tor receives nutrient 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 pathway (Figure 1).

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

Autophagy is mainly a response to nutrient starvation, and TORC1 negatively regulates autophagy. The Atg1 kinase and its regulators, i.e. Atg13, Atg17, Atg29, and Atg31 collaboratively function in the initial step of autophagy induction, downstream of TORC1. Atg1 is a Ser/Thr protein kinase, the activity of which is essential for autophagy and is largely enhanced following nutrient starvation or the addition of rapamycin. This regulation involves phosphorylation of Atg13.

We found that Atg13 is directly phosphorylated by TORC1. Phosphorylated Atg13 (during nutrient-rich conditions) loses its affinity to Atg1, resulting in repression of autophagy. On the other hand, under starvation conditions Atg13 is immediately dephosphorylated and binds to Atg1 to form Atg1 complex. Atg1 complex formation confers Atg1 activation and consequently induces autophagy. We determined phosphorylation sites of Atg13 and generated an unphosphorylatable Atg13 mutant (Atg13-8SA). Expression of Atg13-8SA induces autophagy bypassing inactivation of TORC1, the same as starvation treatment or rapamycin. These results demonstrate that Atg13 acts as a molecular switch for autophagy induction.

## II. Monitoring in vivo activity of TORC1 by phosphorylation state of Atg13

Since Atg13 has turned out to be a substrate of TORC1, *in vivo* activity of TORC1 can be monitored by the phosphorylation state of Atg13. Various conditions and mutants have been examined to determine what kind of nutrients TORC1 recognizes and how nutrient signals are transmitted to TORC1.

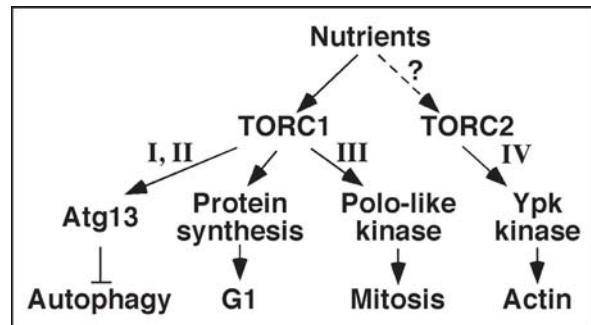


Figure 1. Tor signaling pathway of the budding yeast. Our group has found three branches of the Tor pathway.

## III. How and why TORC1 regulates mitotic entry?

TORC1 regulates protein synthesis, which is important for promotion of the cell cycle at G1 (G0). Little is known, however, about whether or not TORC1 is involved in other stages of the cell cycle.

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. Recently 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. Ypk2 kinase acts at the downstream of TORC2 to control actin organization

Genetic studies have shown that TORC2 controls polarity of the actin cytoskeleton via the Rho1/Pkc1/MAPK cell integrity cascade. However, the target (substrate) of TORC1 was not yet identified. We found that Ypk2, an AGC-type of protein kinase whose overexpression can rescue lethality of TORC2 dysfunction, is directly phosphorylated by TORC2.

### Publication List

#### [Original paper]

- Matsui, A., Kamada, Y., and Matsuura, A. (2013). The role of autophagy in genome stability through suppression of abnormal mitosis under starvation. PLoS Genetics 9, e1003245.