

Tor comes to the fore in autophagy

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Healthy cells are expert recyclers, rapidly breaking down worn-out or surplus macromolecules and reusing their building blocks. Several pathways, such as the proteasomal degradation route for protein breakdown, specifically pick out damaged or expendable molecules.

These selective degradation pathways usually operate in cells that have ready access to nutrients. But when cells encounter severe crises such as nutrient shortages, they activate an emergency recycling mechanism. In these cells, a bulk degradation pathway indiscriminately breaks down macromolecules and entire cytoplasmic organelles and ribosomes.

This process is commonly referred to as autophagy (Greek for “self-eating”) or sometimes as macroautophagy because other more specialized forms of autophagy are more restricted and selective (1, 2). It can be thought of as the rough equivalent of a wood chipper that shreds chairs, tables, and credenzas wholesale for use in plywood or as fireplace fuel.

Autophagy also represents an important quality-control mechanism that eliminates long-lived proteins and damaged organelles in some cells, such as neurons (2), whose total destruction by processes such as apoptosis would harm the organism.

Defects in autophagy have been linked to human disorders, such as neurodegenerative diseases and cancer (1, 2), highlighting that this recycling mechanism is essential for keeping cells healthy and alive. However, how a cell’s nutrient status is communicated to the autophagy machinery was unknown for quite some time.

In a 1998 landmark paper published in the *Journal of Biological Chemistry* and now recognized as a Classic here, Takeshi Noda and Yoshinori Ohsumi (Fig. 1) at the National Institute for Basic Biology in Japan uncovered this missing link, reporting that the target of rapamycin (Tor)² protein in yeast suppresses autophagy in cells that are growing in nutrient-rich conditions (3).

Researchers had known since the early 1990s that two Tor proteins of budding yeast (*Saccharomyces cerevisiae*), Tor1 and Tor2, respond to the nutritional state of the cell and regulate cell cycle progression (4). Noda and Ohsumi’s discovery that Tor proteins also control autophagy represented a major milestone in unraveling how bulk degradation is regulated in eukaryotic cells.

Ohsumi had begun studying autophagy in yeast also in the early 1990s (5). He chose this organism in part because it has a large vacuole (a structure analogous to the lysosome in mammalian cells) that is easy to study by light microscopy. In 1992, his team discovered that when yeast mutant cells that cannot degrade proteins via the proteasomal pathway are starved of nutrients, their vacuoles quickly fill up with conspicuous spherical structures (6).

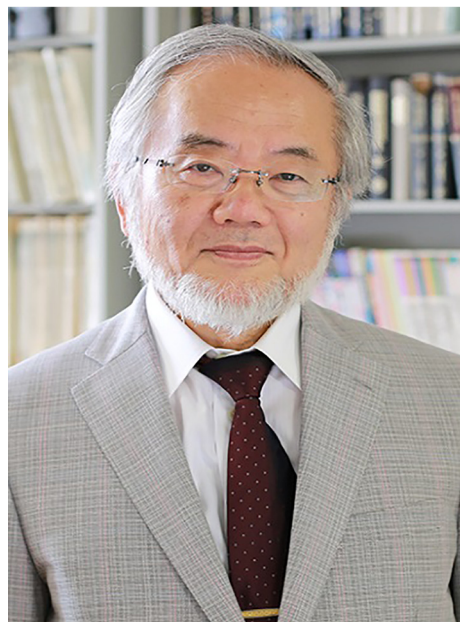


Figure 1. Yoshinori Ohsumi and colleagues discovered that the Tor protein controls autophagy in yeast cells. Photo from Wikimedia, used under Creative Commons.

These membrane-bound structures contained a sundry mix of cellular components, including ribosomes, mitochondria, lipids, and cytosolic enzymes, suggesting that they were signs of autophagy; the researchers therefore named them *autophagic bodies* (Fig. 2).

Noda, the first author of the 1998 JBC paper, was a graduate student in the Ohsumi laboratory. During peer review of the 1992 paper (6), he says, the referees asked for additional experiments to verify that what Noda and Ohsumi saw under the microscope was indeed autophagy.

The extra work paid off. “We clearly showed that cytoplasmic enzymes are incorporated into the isolated vacuole during this process,” Noda says, convincing the referees and securing the paper’s publication.

The referees’ requests also prompted Noda to develop more specific and sensitive methods. “After the experience with the first paper, I realized that we needed a quantitative assay for measuring autophagy activity,” he says.

In a follow-up paper (8), Noda and others in the Ohsumi lab developed an assay that measures the activity of a genetically engineered alkaline phosphatase (ALP) that becomes active only when translocated to the yeast vacuole as happens during autophagy.

“Having this system in hand, I got interested in studying the regulatory mechanism of autophagy in more detail,” says Noda.

At this time, Noda came across a recently published paper from the group of Michael Hall at the University of Basel, Switzerland, reporting that Tor in yeast controlled cellular processes that are typically induced in response to starvation (9).

JBC Associate Editor Paul Fraser at the University of Toronto nominated the paper as a Classic.

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² The abbreviations used are: Tor, target of rapamycin; ALP, alkaline phosphatase.

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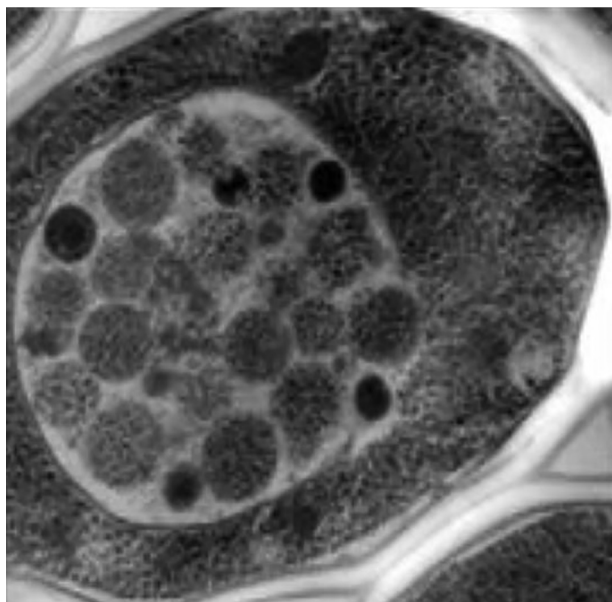


Figure 2. When yeast cells are depleted of nutrients, autophagic bodies (small dark spherical structures) start to accumulate in the cells' vacuoles (light gray structure). Noda and Ohsumi showed that the Tor protein inhibits autophagy in nutrient-rich conditions. Image from Ref. 7.

“As autophagy is induced by starvation, I was eager to use our ALP assay to test the hypothesis that Tor might also regulate autophagy,” says Noda.

Noda and Ohsumi grew yeast cells in nutrient-rich conditions in which autophagy is typically repressed. The researchers then added the Tor inhibitor rapamycin to the cell cultures. Using their ALP-based autophagy assay, they detected autophagy in the Tor-inhibited cells, and using light microscopy, they also observed the tell-tale autophagic bodies in the vacuoles (3).

The authors firmed up these observations with a yeast strain that lacked the WT *TOR1* and *TOR2* genes. The strain carried a *TOR2* gene encoding a temperature-sensitive Tor2 variant that functioned normally at 30 °C but became inactivated at 37 °C. Using this *TOR2*-mutant strain, the authors found that it exhibits autophagy when grown at 37 °C, confirming that the WT Tor protein represses autophagy.

Interestingly, Noda and Ohsumi also found that Tor's effect on autophagy was independent of its known activity in cell cycle progression, suggesting that it plays more multifaceted roles than previously thought.

“Our discovery of Tor's role in autophagy revised prevailing views at the time, showing that Tor regulates not only anabolic processes, but also catabolic ones,” says Noda. “This coupling of anabolic and catabolic processes has been a key game changer in the research field during the past decades,” he notes.

They next turned to the question of how Tor might control autophagy. The Ohsumi lab had previously generated 14 autophagy-deficient (*apg*) yeast strains (10), and Noda found that rapamycin did not restore autophagy in these mutants, indicating that the *APG* genes (now called *ATG*) all act downstream of Tor (3).

The Classic paper could not resolve the question how Tor might regulate the ATGs, but there was a promising hint—Tor was known to have a kinase domain essential for cell cycle regulation

(11). Although it was not definitively known whether this domain had actual kinase activity (3), its presence suggested that Tor might control autophagy by phosphorylating and thereby inhibiting autophagy-associated proteins such as the ATGs.

This was borne out by a 2000 study published by the Ohsumi lab, reporting that Tor phosphorylates the autophagy-related yeast protein Atg13 (12). This phosphorylation interfered with the ability of Atg13 to bind to and activate another kinase, Atg1, required for autophagy induction. Subsequent work by the Ohsumi lab with the facile yeast system helped pinpoint many other key players in autophagy (13).

Despite great strides in clarifying autophagy mechanisms in both yeast and mammalian cells (which use the mTOR protein to suppress autophagy (1)), more work is needed. In particular, to prevent self-destruction, “cells must avoid excessive degradation during autophagy,” Noda says, meaning they need mechanisms that terminate autophagy at the right time.

However, the details of these proposed mechanisms remain to be worked out. “We are currently investigating this question and already got some good candidate molecules [that might terminate autophagy],” says Noda.

For his work that helped uncover the molecular mechanisms in autophagy, Ohsumi was awarded the Nobel Prize in Physiology or Medicine in 2016.

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