Autophagy wins the 2016 Nobel Prize in Physiology or Medicine: Breakthroughs in baker's yeast fuel advances in biomedical research

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Abstract

Autophagy is an ancient pathway in which parts of eukaryotic cells are self-digested within the lysosome or vacuole. This process has been studied for the past seven decades; however, we are only beginning to gain a molecular understanding of the key steps required for autophagy. Originally characterized as a hormonal and starvation response, we now know that autophagy has a much broader role in biology, including organellar remodeling, protein and organelle quality control, prevention of genotoxic stress, tumor suppression, pathogen elimination, regulation of immunity and inflammation, maternal DNA inheritance, metabolism, and cellular survival. Although autophagy is usually a degradative pathway, it also participates in biosynthetic and secretory processes. Given that autophagy has a fundamental role in many essential cellular functions, it is not surprising that autophagic dysfunction is associated with a wide range of human diseases. Genetic studies in various fungi, particularly *Saccharomyces cerevisiae*, provided the key initial breakthrough that led to an explosion of research on the basic mechanisms and the physiological connections of autophagy to health and disease. The Nobel Committee has recognized this breakthrough by the awarding of the 2016 Nobel Prize in Physiology or Medicine for research in autophagy.

On October 3, 2016, the Nobel Prize in Physiology or Medicine was awarded to Yoshinori Ohsumi for “discoveries of the mechanisms for autophagy.” Just a few weeks earlier, at an acceptance speech for the 2016 Paul Janssen Award, Yoshinori Ohsumi stated that although he performs research in a simple organism—baker’s yeast—he always hoped his research would have an impact upon human health. Indeed, Ohsumi’s discoveries, along with those of others working simultaneously on autophagy-related pathways in yeast, have provided a powerful molecular tool kit that thousands of scientists are now using worldwide to decipher the roles of autophagy in mammalian health and disease. The story of this year’s Nobel Prize represents a better-than-textbook example of why unbridled discovery in fundamental basic science is crucial for opening new frontiers in medicine.

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Autophagy Probably Evolved Billions of Years Ago

Derived from the Greek and meaning to eat (“phagy) oneself (“auto”), the term autophagy refers to a lysosomal degradation pathway of self-digestion (Fig. 1) (1). This process involves dynamic membrane rearrangements to sequester cargo for delivery to the lysosome, where the sequestered material is degraded and recycled. We now know that autophagy can be nonselective (such as during nutrient deprivation) or exquisitely selective (such as when damaged organelles or intracellular pathogens are degraded). The molecular machinery of autophagy (first discovered in yeast as described below) is present in the genome of every eukaryotic organism that has been sequenced to date, but not in that of any prokaryotes. Thus, the origins of autophagy are inextricably linked to coevolutionary events essential for the emergence of eukaryotic life.

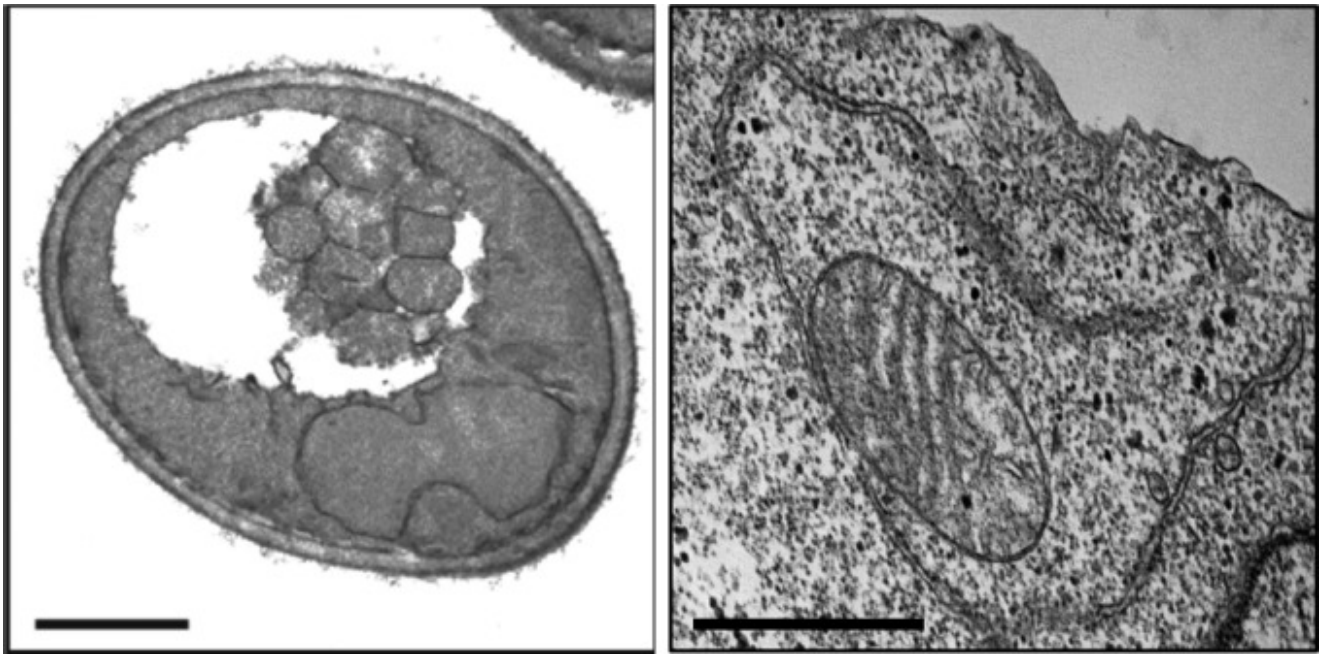

Fig. 1.

Ultrastructure of autophagy in yeast and mammalian cells. (*Left*) Electron micrograph of a yeast (*S. cerevisiae*) cell with autophagic bodies in the vacuole during nitrogen starvation. Reproduced with permission from ref. 54. (*Right*) Electron micrograph of a forming autophagosome in an amino acid-starved *beclin 1*-deficient human breast cancer cell expressing exogenous Beclin 1. Courtesy of X. H. Liang, N. Furuya, Y. Wei, and B. Levine. (Scale bars, 1 μm.)

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Autophagy Was First Described in Mammalian Cells in the 1950s

The history of autophagy research began when scientists looked at mammalian cells under the electron microscope. According to Christian de Duve (Nobel Laureate, 1974, discoverer of the lysosome) (2), the word "autophagy" was invented at the Ciba Foundation Symposium on Lysosomes in London in 1963. At the meeting, Alex Novikoff described his studies characterizing "cytolysosomes," acid phosphatase-positive structures that contained degrading mitochondria, endoplasmic reticulum membranes, ribosomes, and other cytoplasmic contents, and de Duve then proposed the term autophagy to denote this function of lysosomes in self-eating (this process is now termed macroautophagy, but we use autophagy for simplicity). In 1957, autophagosome-like structures (i.e., double-membrane vesicles) were found to be enriched in the developing newborn rat kidney (3), and later, large-scale autophagy was described during insect metamorphosis (4). In 1962, Ashford and Porter (5) and Novikoff and Essner (6) showed that autophagosomes were increased in rat livers exposed to glucagon and starvation, respectively. In 1977, Pfeifer (7) and Mortimore and Schworer (8) demonstrated that insulin and amino acids, respectively, suppressed hepatic autophagy. In 1982, Seglen and Gordon (9) performed the first biochemical analysis of autophagy and identified the pharmacological reagent 3-methyladenine as an autophagy inhibitor. Subsequently, the groups of Seglen (10), Meijer (11), and Codogno (12) first noted that protein kinases, phosphatases, and heterotrimeric G proteins can regulate autophagy. In 1988, Seglen's team identified the phagophore (the initial sequestering vesicle that develops into the autophagosome) (13). In 1992, Meijer's group (14) showed that peroxisomes preferentially undergo autophagic degradation in rat hepatocytes when deprived of their substrates [consistent with earlier studies on peroxisomal degradation in methylotrophic yeasts (15)].

These key early findings established the existence of autophagy in mammalian cells; its regulation by nutrient status, nutrient-sensing hormones, kinases, and phosphatases; its potential for selective cargo degradation; and its increase in certain stress and developmental conditions. Remarkably, a review article by de Duve and Wattiaux in 1966 (16) presaged much of what we know today about the functions of autophagy. These included a role in "nutrition under unfavorable conditions of food supply through piecemeal self digestion," "cellular differentiation and metamorphosis," "intracellular scavenging as part of the self-rejuvenation of long-lived cells," and the "self-clearance of dead cells." However, there was

one major problem—how could this (or other) functionality of autophagy be proved by morphological observations? Herein lies the basis for the importance of describing the conserved genetic machinery of autophagy in yeast.

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Enter Ohsumi and Other Yeast Researchers: The Dawn of the Genetic Era of Autophagy Research

In the early 1990s, several laboratories began studying nonselective and selective autophagy in yeasts. In 1992, Ohsumi and colleagues (17) described the presence of "autophagic bodies" in the vacuole (the analog of the lysosome) of protease-deficient *Saccharomyces cerevisiae* devoid of nutrients—the first morphological characterization of nonselective autophagy in yeast. In the same issue of the *Journal of Cell Biology*, Klionsky et al. (18) described the import of aminopeptidase I into the vacuole of *Saccharomyces cerevisaie*—the first characterization of selective autophagy in yeast. Shortly thereafter, Dunn and colleagues (19) described a form of selective autophagy in the methylotrophic yeasts, *Pichia pastoris* and *Hansenula polymorpha*, involving the selective degradation of peroxisomes in the vacuole.

These morphological and biochemical characterizations set the stage for nearly parallel efforts in three laboratories to isolate mutants in *S. cerevisiae* and clone the genes encoding the yeast autophagy machinery, now referred to as the autophagy-related (*ATG*) genes (20); similar efforts were undertaken in several laboratories working with the methylotrophic yeasts (21). Ohsumi's group (22) performed a genetic screen to isolate mutants that accumulate autophagic bodies in the vacuole and show decreased viability during nitrogen starvation (originally known as the *apg* mutants); Thumm et al. (23) performed a genetic screen to isolate yeasts deficient in protein uptake for degradation in the vacuole (originally known as the *aut* mutants); and Klionsky's group (24) performed a screen for mutants deficient in the delivery of a resident vacuolar hydrolase from the cytoplasm to the vacuole (originally known as the *cvt* mutants).

In October 1996, the first yeast autophagy gene, *APG5* (now known as *ATG5*), was reported by Ohsumi and colleagues (25). Shortly after, in February 1997, Thumm's group (26) reported the cloning of *AUT1* (now known as *ATG3*), which encodes an E2-conjugating enzyme involved in the modification of the ubiquitin-like protein Atg8. In 1998, Mizushima et al. (27) described a protein conjugation system in yeast, the Atg12–Atg5 system that requires a ubiquitin activating E1-like enzyme, Atg7, and in 1999, three additional groups (28–30) reported in back-to-back papers the cloning and characterization of yeast *ATG7*. In 2000, Ichimura et al. (31) reported that the Atg7 E1-like enzyme conjugates Atg8 to phosphatidylethanolamine, establishing a role for protein lipidation in membrane dynamics during autophagy.

Taken together, between 1996 and 2000, the various laboratories working with yeasts cloned genes encoding components that function at different stages of autophagy including initiation (involving the Atg1 serine/threonine kinase complex and the Vps34 lipid kinase complex) as well as phagophore expansion and autophagosome maturation (involving the two ubiquitin-like protein conjugation systems). From this period until the present, extensive studies (particularly in *S. cerevisiae*) using molecular genetic, biochemical, and structural approaches have continued to teach us about the workings of the core autophagy machinery and have identified additional components critical for various types of selective autophagy.

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The Dawn of the Genetic Era of Mammalian Autophagy Research

Studies in yeast were crucial to elucidate the molecular mechanisms of autophagosome formation. Early yeast genetic screens also established that autophagy is essential for survival during starvation and for the yeast differentiation process of sporulation. However, as yeasts are unicellular organisms, the discovery of the molecular machinery of autophagy in more complex eukaryotes (especially mammals) was critical to define the functions of autophagy in health and disease.

Throughout the 1990s, several vertebrate genes were cloned that shared a high degree of sequence homology with essential yeast autophagy genes, including bovine *PIK3C3/VPS34* (32), rat *MAP1LC3/LC3* (33), mouse and human *Ulk1/ULK1* (34, 35), human *ATG5* (36, 37), human *ATG12* (37), and human *VPS30/ATG6* (*beclin 1*) (38). In 1999, Levine's group (39) provided the first demonstration of an autophagic function of a mammalian protein; Beclin 1, the human ortholog of yeast Vps30/Atg6, rescued autophagy in *VPS30/ATG6*-deficient yeasts and in autophagy-deficient breast cancer cells lacking one copy of *beclin 1*. Over the next several years, it was demonstrated that virtually all of the yeast autophagy genes are functionally conserved throughout metazoan evolution.

The evolutionary conservation of autophagy genes provided an unparalleled opportunity to perform reverse genetics in a wide variety of model organisms—ranging from flies to plants to worms to mice—to probe the function of autophagy in diverse biological processes. In 2003, Heintz's group (40) and Levine's group (41) independently reported the first autophagy gene knockout mouse; homozygous deletion of *beclin 1* is embryonically lethal and heterozygous deletion of *beclin 1* results in increased spontaneous tumorigenesis [consistent with the earlier reported tumor suppressor activity of *beclin 1* in breast cancer cells and its common allelic loss in human ovarian and breast cancer (39, 42)]. The same year, Levine's group showed that autophagy genes were essential for life span extension and dauer development in nematodes (43). In 2004, Mizushima's group (44) reported the knockout of the first autophagy ubiquitin-like conjugation system gene, *Atg5*; these mice (and additional targeted mutant mice lacking other autophagy conjugation system genes) survived to birth but died shortly thereafter, presumably due to an inability to adapt to a postnatal starvation

period. Also in 2004, three groups reported a role for the autophagy machinery in degrading medically important bacteria that invade intracellularly, including *Mycobacterium tuberculosis* (45), group A *Streptococcus* (46), and *Shigella flexneri* (47).

These early studies were soon followed by a plethora of additional genetic analyses in various metazoan organisms, which unequivocally established a crucial role for the autophagy machinery in differentiation and development, nutrient and energy homeostasis, stress adaptation, tumor suppression, innate and adaptive immunity, metabolism, regulation of inflammation, and protection against cardiac and skeletal diseases, neurodegeneration, and aging (48–50). These roles stem from the ability of autophagy to degrade unwanted or harmful substrates (largely via selective autophagy), to recycle cytoplasmic constituents during environmental stress (largely through nonselective autophagy), and from other homeostatic functions of the autophagy machinery. Importantly, it is now clear that mutations in genes encoding core components that are required for autophagosome formation are associated with human disease (51, 52).

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Yeast and Mammalian Autophagy Researchers Unite

The first Gordon Research Conference on autophagy, held in 2003, brought together researchers from North and South America, Europe, the Middle East, and Asia (Fig. 2). Its participants included nearly all of the living autophagy researchers mentioned above, as well as many other senior leaders and rising stars in the field. Yoshinori Ohsumi gave the keynote lecture and threw the first pitch at a “yeast versus mammals” softball game held during an afternoon break. The transnational and transkingdom nature of the meeting united participants with diverse expertise but who shared a common passion for autophagy, thus laying the groundwork for enduring scientific collaborations that markedly accelerated the pace of discovery in the field. As one of many examples, Yoshimori’s group (53) had discovered that GFP-LC3 was a useful marker of autophagosomes; he generously shared his reagents, and fluorescent-tagged versions of Atg8/LC3 orthologs have been used worldwide ever since to monitor autophagosome numbers in a wide range of species.

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Fig. 2.

Attendees of the first Gordon Research Conference on “Autophagy in Stress, Development, and Disease” (Colby College, Maine, June 22–27, 2003). In the front row (positions 10–12) are Daniel J. Klionsky, the vice chair; Beth Levine, the chair; and Yoshinori Ohsumi, the 2016 Nobel Laureate in Physiology or Medicine.

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The High Noon of Autophagy Research

Thanks to the elucidation of the genetic machinery and molecular mechanisms for autophagy in yeast (and all of the subsequent work in more complex eukaryotes that has built upon this foundation), we have entered an exciting new era in autophagy research. We have made major inroads into understanding how autophagy is regulated, what the core autophagy machinery looks like structurally and how it works, what autophagy is doing to keep us healthy (or in more rare circumstances, make us ill), and how we can manipulate autophagy pharmacologically or via lifestyle interventions to prevent and/or treat aging and many common diseases. We are also learning that the genetic machinery of autophagy participates in other trafficking and signaling pathways to control mammalian physiology, thus further

expanding the long-term biomedical impact of the original yeast genetic screens. Although not directly linked to the yeast screens for genes required for autophagosome formation, the recent revolution in identifying genes required for selective autophagy (many of which are mutated in human diseases, particularly cancer and neurodegenerative disorders) also holds great promise for strategies to augment autophagy to protect against disease.

To be sure, there are many questions yet to be answered about the basic mechanisms of autophagy (and other autophagy gene-dependent processes), its roles in disease, and its potential as a pathway for drug targeting. Nonetheless, it is quite possible that in our lifetime, or at least that of our children, we will witness the realization of Yoshinori Ohsumi's hope—the eloquent molecular dissection of a previously underappreciated cell biology process in yeast will likely ultimately fuel groundbreaking advances in human health.

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Footnotes

The authors declare no conflict of interest.

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This article is part of a series of articles in PNAS highlighting the discoveries and profiling the award winners of the Nobel Prize. Articles about the 2016 Nobel Laureates in Physics and in Chemistry can be found at 10.1073/pnas.1620134114 and 10.1073/pnas.1619330114.

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