

## Review articles

# Autophagy as a universal intracellular process. A comment on the 2016 Nobel Prize in Physiology or Medicine

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**ABSTRACT.** The 2016 Nobel Prize in Physiology or Medicine was awarded to molecular biologist Yoshinori Ohsumi for his work in the field of autophagy (Greek for “self eating”). This fact has once again directed the attention of many scientists to a common cellular phenomenon occurring in all eukaryotes from yeast to mammals, namely the process by which the cell digests and then recycles its components. Although the phenomenon of autophagy was discovered in mammals, a method for monitoring it by light microscopy was established in the unicellular eukaryote, the budding yeast *Saccharomyces cerevisiae*. The article describes the achievements of the Nobel Laureate, the mechanism of autophagy and its role in the cell physiology of organisms including the unicellular pathogen, the protozoan *Toxoplasma gondii*.

**Key words:** autophagy, Nobel Prize 2016, *T. gondii*

The phenomenon of autophagy is pictured as the mythical snake Uroboros, eating his own tail. The term *autophagy* was first coined in 1963 by Christian de Duve, the discoverer of lysosomes, who initiated his pioneering study on the relationship between autophagic vacuoles and lysosomes at the laboratory in the Rockefeller Institute, now Rockefeller University [1]. This was preceded by the discovery by Aaron Ciechanover, Avram Hershko and Irwin Rose of the proteasome, a ubiquitin-mediated cellular system used to degrade proteins, for which they were awarded the 2004 Nobel Prize in Chemistry. The proteasome efficiently and specifically degrades ubiquitinated proteins one-by-one, whereas autophagy eliminates larger protein complexes and old or damaged organelles.

### The 2016 Nobel Prize in Medicine or Physiology

The 2016 laureate is Japanese biologist Professor Yoshinori Ohsumi, a worker at the Tokyo Institute of Technology in Yokohama, who was studying *Saccharomyces cerevisiae* vacuoles, a model for mammalian cell lysosomes in 1988. The vacuoles, constituting about 25% of total cell volume

contained a relatively large quantity of low molecular weight solutes and ions, but quite small amounts of proteins, which was surprising considering that cell differentiation involves a large amount of protein degradation. To determine the lytic function of the vacuole, Ohsumi developed first yeast knockout strains defective for two proteases [2–4]. The mutants were not able to sporulate as normal cells under starvation conditions. After 30 minutes of starvation, he observed a few spherical bodies in the vacuole which increased in number, and almost completely filled the vacuole after three hours. These *autophagic bodies* were surrounded by a thin membrane and contained various cytosol components, including organelles. Further genetic manipulation identified 15 genes involved in budding yeast autophagy (AuTophagy, ATG or Atg) and the results were published in *Journal of Cell Biology* and *FEBS Letters* [5–7]. In a further study on human cells, he identified the protein cascade controlling the autophagy process. Prof. Ohsumi is known to be an open-minded man who is willing to help other scientists and students, and is regarded as a deserving Nobel laureate. Interestingly, he has stated that he chose not to publish his key discoveries in journals like *Science*

or *Nature*, because he is more interested in the science than becoming famous.

## Autophagy

Autophagy is a conserved catabolic process in eukaryotic cells involved in the targeted degradation of cellular organelles and the cytoplasm. Autophagy is induced in response to a large body of physiological and pathological stimuli and helps to maintain homeostasis within the cytosol i.e. the balance between the synthesis and degradation of proteins, nucleic acids, and many cellular organelles. The process is complex and highly regulated.

Autophagy is initiated by the formation of a double membrane structure named the phagophore or isolation membrane, mediated by the class III P13-K complex consisting of Vps15, Vps34, Atg14 and beclin1. The phagophore then elongates and engulfs the material to be degraded. Following this, the autophagosome itself is formed, accompanied by delipidation of LC-II by Atg4, and the outer membrane of the autophagosome fuses with the lysosome. Finally, lysosomal hydrolases degrade the inner membrane and the lysosome content. Simple degradation products like amino acids, lipids and sugars are used for the synthesis and production of ATP [8–11].

Studies on yeast have yielded significantly greater understanding of the autophagy process and have revealed differences between autophagy in yeasts and mammals. For example, the pre-autophagosomal structure is not found in mammals.

It is likely that autophagosomes in mammalian cells originate from different cell membrane structures, such as the endoplasmic reticulum, plasma membrane or mitochondrial outer membrane [8].

Autophagy plays a very important role in numerous physiological processes including blastocyst implantation and fetus development, erythrocyte maturation, inhibition of cancerogenesis, anti-aging activities and elimination of pathogenic microorganisms. However, autophagy also participates in several pathological processes including neurodegeneration, seen in Alzheimer's or Parkinson's disease, as well as muscular dystrophy and tuberculosis [11]. Although autophagy is considered a rescue mechanism, excessive autophagy can lead to cell pathology and ultimately to cell death, i.e. programmed cell death II. It is sometimes very difficult to generalize the role of autophagy in cancer and cell death. The main differences between autophagy and apoptosis are specified in Table 1.

The housekeeping functions of the autophagic process include the elimination of invading pathogens and the delivery of antigens to the immune system [12].

## Autophagy and *Toxoplasma gondii*

### *Immunity to T. gondii and autophagy*

The fusion of lysosomes with the pathogen-containing endosome is a critical effector mechanism and acts as the starting point for killing pathogens. *Toxoplasma gondii* and many other intracellular parasites survive within host cells by

Table 1. Comparative characteristics of autophagy vs. apoptosis

Organelle/activity	Autophagy	Apoptosis
Plasma membrane	Disrupted, sometimes blebbing	Blebbing
Nucleus	Intact	Fragmentation
– condensation of chromatin	No	Yes
Organelle size	Enlargement	No
Mitochondria	Membrane depolarization and swelling	Membrane depolarization (often)
Lysosome activity	Increased	?
Activation of caspases	Caspase independent*	Caspase dependent
Essential molecules:		
mTOR	Yes	No
Bcl-2 & cytochrome	No	Yes
Newly appearing cell structures	Autophagosomes and autolysosomes	Apoptotic body

\*The problem is discussed in the paper by Wu et al. [28]

preventing the lysosomal compartment from fusing with the parasitophorous vacuole (PV) containing the parasite. During active penetration of the parasite into the host cell, the PV membrane undergoes a number of deep modifications, namely the exclusion of many host cell proteins and the incorporation of many parasite proteins [13]. However, it is unknown whether the nonfusogenic status of the PV could change during infection.

Many studies on laboratory mice have confirmed that IFN- $\gamma$  is an essential cytokine for antitoxoplasmic protection. Together with TNF- $\alpha$  and nitric synthase 2 (NOS2), it controls the parasite in the brain and the eye preferentially occupied by the parasite [14]. Autophagy proteins Atg7 and Atg3, as well as the Atg12-Atg5-Atg16L1 complex, are involved in the conjunction of microtubule-associated protein light 3 (LC3-I) with phosphatidylethanolamine (PI), and are required for the proper targeting of IFN- $\gamma$  effectors on the membrane of the parasitophorous vacuole [15]. The LC-II protein, a ubiquitin-like protein obtained by conversion from LC-I, is incorporated into the lipid layers of the vesicles, where it serves as a general marker for autophagic membranes and for monitoring the autophagic process as it develops. Interferon  $\gamma$  stimulates ubiquitin and p62 recruitment to the *T. gondii* parasitophorous vacuole (PV), and this step is crucial for the immune response (CD8+) acquired after PV disruption by IFN- $\gamma$  inducible GTPases [16].

CD40, which is expressed on various cells including antigen-presenting cells, and its ligand CD40L or CD154, expressed mainly on activated T lymphocytes; both of which are active in mice and humans and confer anti-*T. gondii* immunity. This immune pathway restricts the multiplication of the parasite during acute toxoplasmosis, not only in peripheral tissues but also in the brain [17,18]. Macrophages play a key role in *T. gondii* immunity, and although the cells allow intracellular parasites to survive and multiply in them while resting, they acquire toxoplasmicidal activity following CD40 stimulation [19]. Parasite-containing vacuoles fuse with late endosomes and lysosomes, and colocalization of LysoTracker Red (acidotrophic dye) and typical late endosomal/lysosomal markers as Rab7, LAMP-1 and -2, CD63 can be observed at this time [20]. CD40 stimulation induces the killing of parasites enclosed in the PV by vacuole-lysosome fusion, as confirmed experimentally by pharmacologic inhibition of lysosomal enzymes,

vacuolar ATPase and kinase PI3K; this process inhibited the killing of *T. gondii* induced by CD40 stimulation [19]. Vacuole-lysosome fusion mediated by CD40 likely contributes to host protection because CD40 stimulation *in vivo* induces macrophage toxoplasmicidal activity and reduces the parasite load [17,19].

The CD40-mediated fusion of vacuole and lysosomes is based on autophagy, a highly conserved and ubiquitous process in eukaryotic cells enabling them to adapt to environmental changes. Autophagy directs cytoplasmic material to the lysosomes. According to a model presented by Subauste [21], the reaction of CD40-CD40L recruits TRAF6 (TNF Receptor Associated Factor 6) to the intracytoplasmic tail of CD40 that in turn enhances autocrine production of TNF- $\alpha$ . TRAF6 and TNF- $\alpha$  signaling then combine to trigger autophagy. Beclin 1 (Atg6) together with hVps34 recruit autophagosomes to the PV that contains the pathogen. The subsequent recruitment of Rab7 initiates fusion with the lysosomes and the killing of *T. gondii*. It is likely that autophagy bestows protective properties against *T. gondii*, particularly in neural tissue, where it is possible that two arms of immunity function: one dependent on IFN- $\gamma$ /NOS2 and another on CD40. Several studies suggest that the latter mechanism is particularly effective in humans. Defects in the CD40-dependent pathway are likely relevant to those patients who develop cerebral and/or ocular toxoplasmosis and, for instance, are diagnosed as suffering from X-linked hyper IgM syndrome, characterized by a lack of functional CD40 [22].

Many studies confirm that adaptive immunity against *T. gondii* comprises a spectrum of mechanisms and autophagy is one of them. The levels of the autophagic markers Beclin 1 and LC-II transiently increase in HeLa cells infected with *T. gondii* and treated with rapamycin at 18 and 24 hours before decreasing. Ultrastructural observation revealed that tachyzoite proliferation coincides with a decline in autophagosome number by 18 hours. Hence, autophagy appears to be initiated at the early stage of infection to prolong cell survival, but it may be suppressed in the host cells by the subsequent proliferation of tachyzoites [23]. Both autophagy and apoptosis are cell processes which play a critical role in controlling the fate of *T. gondii* infection. In human umbilical cord mesenchymal stem cells, the autophagy process has been found to be initiated at the early stage of *T. gondii* infection

but the subsequent proliferation of tachyzoites may suppress autophagy and induce apoptosis in the host cell [24]. Autophagy proteins are evolutionary conserved and have acquired unique functions in the immune system cells, independent of their roles in the degradation process.

#### *T. gondii* and autophagy

The absence of apoptotic death in yeasts and protozoa suggests autophagy may play a role in programmed cell death. The studies on autophagy machinery in apicomplexan parasites have been performed mainly on *Toxoplasma gondii*, a more convenient object for genetic manipulation than other parasites of this taxon. Besteiro et al. [25,26] using TgAtg8 as an autophagosome marker have revealed that the machinery of autophagy is functional in *T. gondii* tachyzoites, both extracellularly during starvation and intracellularly during normal development. A mutant lacking TgAtg3, a key autophagy protein, was unable to lipidate and then associate TgAtg8 to the autophagosomal membrane. In addition, it displayed a fragmented mitochondrion and growth arrest, and the mitochondrial material was expelled to the parasitophorous vacuole. Acute starvation or antiparasitic drug treatment induces rapid and selective degradation of the single mitochondrion of *T. gondii* (mitophagy) and leads to parasite death [27,28]. The Atg3 protein seems crucial for maintaining mitochondrial homeostasis and for parasite growth.

Despite many differences, autophagy and apoptosis are highly interconnected in determining the fate of cells. Caspases, primary drivers of apoptosis, play a key role in the crosstalk between apoptosis and autophagy. They can degrade essential autophagy proteins and convert some pro-autophagic proteins into pro-apoptotic proteins [29]. As an inducible and irreversible cell death pathway, autophagy seems a very promising model for the development of novel antiparasitic drugs and the treatment of life-threatening human disorders including neurodegenerative diseases such as dementia and Parkinson's disease, as well as cancers and Crohn's disease [30].

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The exciting story of 2016 Nobel Prize winner Yoshinori Ohsumi's research career, during which he discovered autophagy and uncovered its mechanism in yeast, will also be described. The course is aimed at a non-specialist audience and is divided into three sections (weeks). During the first week, we'll cover basic cell biology and discuss the fundamentals of autophagy and its role in the cell. In the second week, Professor Ohsumi's four-decade research career, during which he discovered autophagy in yeast and uncovered its molecular mechanism, will be discussed in detail. Start your review of Autophagy: Research Behind the 2016 Nobel Prize in Physiology or Medicine. Home. Science. Learn the fundamentals/applications of autophagy through the inspirational story of Yoshinori Ohsumi's research, for which he was awarded the 2016 Nobel Prize in Physiology or Medicine and an array of other prizes. Ver el video. Reproducir video para Autophagy: Research Behind the 2016 Nobel Prize in Physiology or Medicine. Reproducir video para Autophagy: Research Behind the 2016 Nobel Prize in Physiology or Medicine. Hay una sesi3n disponible: Ya se inscribieron 7,474! The Nobel prize in medicine has been awarded to a Japanese cell biologist for discoveries on how cells break down and recycle their own components. Yoshinori Ohsumi, 71, will receive the prestigious 8m Swedish kronor (718,000) award for uncovering mechanisms for autophagy, a fundamental process in cells that scientists believe can be harnessed to fight cancer and dementia. Intense research is underway to develop drugs that can target autophagy to treat various diseases. Speaking to reporters in Tokyo on Monday, Ohsumi said: "As a boy, the Nobel Prize was a dream, but after starting my research, it was out of my picture."