Yoshinori Ohsumi's Nobel Prize for mechanism of autophagy: from basic yeast biology to therapeutic potential

R. A. Frake[1], D. C. Rubinsztein[1*]

[1]Department of Medical Genetics, Cambridge Institute for Medical Research, Wellcome Trust/MRC Building, Cambridge Biomedical Campus, Hills Road, Cambridge, CB2 0XY, UK

*Corresponding author: D. C. Rubinsztein, phone: +44 (0)1223 762608, Fax: +44(0)1223 331206, E-mail: dcr1000@cam.ac.uk

Abstract

At the start of October 2016 Japanese cell biologist Yoshinori Ohsumi was awarded the Nobel Prize in Physiology or Medicine 'for his discoveries of mechanisms for autophagy', autophagy being an intracellular degradation pathway that helps maintain cytoplasmic homeostasis. This commentary discusses Ohsumi's Nobel prize-winning work in context, before explaining the clinical relevance of autophagy.

Key words

autophagy, Yoshinori Ohsumi, yeast genetics

Introduction

Macroautophagy (hereafter referred to as autophagy) is an important means of maintaining cellular homeostasis by trafficking cytoplasmic material for enzymatic degradation in the lysosome. In mammalian cells, the process involves formation of a cup-shaped, double-membraned phagophore (or isolation membrane), which closes around cytoplasmic material to form a spherical, double-membraned autophagosome (Figure 1A). The autophagosome outer membrane ultimately fuses with a lysosome to form an autolysosome, resulting in degradation of the inner autophagosome membrane and sequestered cargo. Autophagy is both a constitutive process and subject to dynamic regulation by a range of physiological signals. Notably, autophagy is induced by nutrient starvation. The substrates of autophagy are extremely diverse, ranging from organelles such as mitochondria, through to aggregate-prone proteins and invading microorganisms.

Characterisation of autophagy in yeast

Yoshinori Ohsumi's first contribution to autophagy research was demonstrating that autophagy in yeast is similar to that in mammalian cells (Figure 1B). He and colleagues observed that when the yeast *Saccharomyces cerevisiae* undergoes nutrient starvation, cytoplasmic components are delivered to the vacuole, equivalent to the mammalian lysosome, in single-membraned autophagic bodies.(1) Later, the group used electron microscopy to identify and characterise double-membraned autophagosomes as the precursors of autophagic bodies in yeast.(2) Autophagic bodies are the remains of autophagosomes after fusion with the vacuole; these intermediate structures are visible in yeast, but not in mammalian cells.

Early descriptions of autophagy in mammalian cells

By the time Ohsumi began working in yeast, the study of autophagy in mammalian cells had already been underway for several decades. An earlier recipient of the Nobel Prize in Physiology or Medicine, Christian de Duve is considered by many the founding father of autophagy research.(3) de Duve coined the term ‘autophagy’ in 1963 to describe single- and double-membraned vacuoles containing cytoplasmic components observed by electron microscopy.(4) Per Seglen and colleagues made major contributions to the emerging field of autophagy. For example, Seglen helped characterise the cup-shaped phagophore, which closes to form the autophagosome.(5)

Regulation of mammalian autophagy

The response of autophagy to various physiological stimuli was also investigated in these early days, with de Duve and colleagues reporting that administering glucagon to rats upregulates autophagy in the liver.(6) This observation fits with autophagy induction in response to nutrient starvation as a means of producing amino acids for gluconeogenesis and other metabolic pathways; a hypothesis that was borne out almost a decade later, when amino acid depletion was shown to upregulate autophagy in both cultured human cells and rat liver.(7, 8) Analysis of the signalling events linking nutrient starvation to autophagy induction was to follow. A breakthrough came when Alfred Meijer’s group, working in hepatocytes isolated from starved rats, noted that phosphorylation of ribosomal protein S6 paralleled the decrease in autophagy observed when amino acids were added to the culture medium. The immunosuppressive drug rapamycin, which indirectly inhibits S6 phosphorylation by antagonising mTOR (mammalian target of rapamycin) function, was then shown to induce autophagy.(9) This work uncovered the role of mTOR signalling in autophagy regulation and established rapamycin as the first drug in the expanding class of autophagy inducers.

Molecular mechanisms of autophagy

The morphology and regulation of autophagy were therefore beginning to be understood, yet none of the molecular machinery was known. At this point the Ohsumi and colleagues performed their first pioneering screens. The group used yeast lacking vacuolar proteases, which normally accumulate autophagic bodies under nutrient starvation, to identify fifteen autophagy-defective mutant yeast strains that failed to form autophagic bodies. In this way, they initially identified fifteen novel autophagy genes (now referred to as ATG genes).(10) Shortly afterwards, other groups such as those of Daniel Klionsky and Michael Thumm published genetic screens in yeast, which contributed to the growing list of ATG genes.(11-13) ATG1 was the first such gene to be characterised by Ohsumi and colleagues. The kinase activity of the encoded protein (Atg1p) was found to be essential for autophagy, since genetic reconstitution with 'kinase-dead' mutant ATG1 cannot rescue the autophagy-deficient phenotype of ATG1 mutant yeast. Moreover, the phosphorylation status of Atg1p was shown to be regulated by nutrient availability, leading the authors to speculate (as has since been established) that the protein plays a key role in autophagy induction in response to nutrient starvation.(14) The Ohsumi group also demonstrated that inhibiting TOR with rapamycin induces autophagy in yeast, as previously shown in mammalian cells,(9) and that TOR signalling is upstream of the Atg proteins.(15) This suggests significant commonalities between autophagy regulation in yeast and mammalian cells, which have subsequently been utilised my many in autophagy research. Indeed, much pioneering work has been conducted in mammalian systems by Tamotsu Yoshimori and Noboru Mizushima, both of whom trained with Ohsumi.

Ubiquitin-like conjugation systems in autophagy

Investigations then began into how the Atg proteins interact. Despite having no apparent homology to ubiquitin, Atg12p was shown to covalently bind Atg5p in much the same way as

ubiquitin tags substrates (Figure 2A). In wild-type yeast most Atg5p/Atg12p was found as Atg5p-Atg12p conjugate, yet this was not the case in ATG7 and ATG10 mutant yeast. The Ohsumi group generated a series of yeast strains with targeted mutations to discover how this conjugate forms; first the carboxyl-terminal glycine of Atg12p is activated by Atg7p, then Atg12p is transferred to Atg10p and finally onto Atg5p.(16, 17) Around the same time Klionsky's lab showed that Atg7p functions at the substrate sequestration step of autophagosome formation.(18) These studies lead to identification of the first mammalian ATG genes; human *ATG12* and *ATG5*, which conjugate *via* reactions analogous to those in yeast and are expressed across the full range of human tissues.(19) Next, Ohsumi and colleagues described Atg16p as the third member of the Atg5p-Atg12p complex. The ATG16 gene had been missed in their initial screen, but was identified *via* a secondary screen for Atg12p binding partners. Atg16p was shown to bind Atg5p directly (and thereby Atg12p indirectly) and play a key role in autophagy initiation, possibly by forming Atg5p-Atg12p/Atg16p multimers.(20)

Two years later a second ubiquitin-like conjugation system was discovered (Figure 2B), with Atg8p as the ubiquitin-like protein.(21) Ohsumi and colleagues had in fact already identified Atg8p as a marker of autophagic structures in yeast(22) and this new discovery explains how the hydrophilic protein Atg8p is able to associate with autophagic membranes. The reaction begins with Atg4p cleaving nascent Atg8p to reveal a carboxyl-terminal glycine.(21) Next Atg8p is activated by Atg7p (just as Atg12p), transferred to Atg3p and finally onto the membrane component phosphatidylethanolamine.(23) This lipidation reaction, the first example of a protein conjugating to a membrane phosphoglycerolipid, is central to membrane dynamics in autophagy. The Yoshimori lab then identified LC3 (short for microtubule-associated protein 1 light chain 3) as the mammalian homologue of Atg8p. Two forms were described; non-lipidated LC3-I in the cytosol and phosphatidylethanolamine-conjugated LC3-

II on autophagic membranes.(24) The amount of LC3-II was found to parallel autophagosome number and has gone on to become the canonical readout of autophagy in mammalian cells.(25)

Autophagy in health and disease

With Ohsumi's work having facilitated the molecular dissection of autophagy, several groups began to focus on clinically-relevant aspects of the process. The seminal work linking autophagy to cancer was performed by Beth Levine's group, who identified Beclin 1 as the human homologue of yeast Atg6p. *BECN1* is described as a tumour suppressor in breast cancer, with monoallelic deletions and decreased expression observed in patient samples. Genetic reconstitution of MCF7 human breast cancer cells, which do not express detectable Beclin 1, is reported to restore autophagy induction in response to nutrient starvation and promote a less malignant phenotype.(26) In this way, autophagy is suggested to inhibit tumorigenesis. Over time the picture has become more complicated, since while maintenance of cellular homeostasis by autophagy can protect against malignant transformation, autophagy also drives resistance to conditions that cause cell death (hypoxia, for instance) and thereby promotes metastatic tumour progression.(27) Such complexity is illustrated by the roles of established oncogenes and tumour suppressors in autophagy. XIAP and cIAP1 (overexpressed in several human cancers) induce autophagy by upregulating transcription of Beclin 1(28) and reduced expression of the proto-oncogene *MYC* impairs autophagosome formation.(29) On the other hand, the tumour suppressor *PTEN* positively regulates autophagy.(30)

Autophagy is also implicated in infection and immunity, with the degradation of intracellular bacteria by autophagy (referred to as xenophagy) being possibly the most ancient form of defence against invading microorganisms. Xenophagy was first studied in the labs of

Yoshimori and Vojo Deretic. The Yoshimori group demonstrated that autophagy could eliminate group A *Streptococcus* from infected HeLa cells(31) and this was followed almost immediately by Deretic and colleagues' report that autophagy induction in macrophages infected with *Mycobacterium tuberculosis* promotes clearance of the pathogen.(32) Autophagy is now thought to have additional immunologic roles, such as modulating inflammation and influencing lymphocyte development and function.(33) For instance, single-nucleotide polymorphisms in the core autophagy gene *ATG16L1* have been identified by genome-wide association studies as susceptibility loci for Crohn's disease,(34, 35) with *Atg16L1*-deficient mice showing much worse colitis pathology compared with wild-type controls.(36)

Finally, autophagy has been identified as a potential therapeutic target in neurodegenerative disease. The functional link between autophagy and neurodegeneration was first established when we published that upregulating autophagy promotes the degradation of polyglutamine-expanded huntingtin, the toxic species in Huntington's disease.(37) This finding has since been translated into animal models, with autophagy induction ameliorating neurodegenerative pathology in a mouse model of Huntington's disease.(38) A protective role for autophagy has subsequently been described in many more neurodegenerative diseases, predominantly *via* the clearance of intracytoplasmic aggregate-prone proteins, such as alpha-synuclein in Parkinson's disease and Tau in various neurodegenerative conditions.(39) Impaired autophagy has also been shown to predispose to neurodegeneration, as highlighted by mouse studies demonstrating autophagy is essential for neuronal survival. Mice lacking *Atg7* in the central nervous system suffer massive neuronal loss in the cerebral and cerebellar cortices(40) and those lacking *Atg5* develop progressive motor function deficits, which are attributed to neurodegeneration.(41) Several human neurodegenerative conditions have since been linked to autophagic dysfunction, some directly attributable to mutations in autophagy

genes. Most recently, a homozygous missense mutation in *ATG5* was identified that impairs autophagy and causes ataxia with developmental delay in affected patients.(42)

Concluding remarks

Building on what was already known about the morphology and regulation of autophagy in mammalian cells, the pioneering work Ohsumi performed in yeast gave researchers a toolkit with which to dissect the molecular machinery of autophagy. This has facilitated greater understanding of autophagy in human health and disease, especially in the fields of cancer, immunology and neurodegeneration. The hope is that autophagy will become an ever more tractable therapeutic target, with autophagy modulators moving into mainstream clinical practice.

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Figures

Figure 1: Autophagy pathway in mammalian cells and yeast. (a) In mammalian cells, the phagophore closes around cytoplasmic material to form a spherical, double-membraned autophagosome. The autophagosome outer membrane fuses with a lysosome to form an autolysosome, resulting in degradation of the inner autophagosome membrane and sequestered cargo. (b) The pathway is similar in yeast, except that the autophagosome outer membrane fuses with the vacuole. This fusion event forms single-membraned autophagic bodies within the vacuole that are degraded, together with the sequestered cargo.

Figure 2: Ubiquitin-like conjugation systems in yeast autophagy. (a) The first conjugation reaction, the carboxyl-terminal glycine of Atg12p is activated by Atg7p. Atg12p is then transferred to Atg10p and finally onto Atg5p. The final Atg5p-Atg12p/Atg16p complex is formed when Atg16p binds Atg5p. (b) In the second conjugation reaction, Atg4p first cleaves nascent Atg8p to reveal a carboxyl-terminal glycine. Atg8p is then activated by Atg7p, transferred to Atg3p and finally onto the membrane component phosphatidylethanolamine (PE). Homologous proteins undergo equivalent conjugation reactions in mammalian autophagy.

Figure 3: Timeline of autophagy research. Schematic showing how the autophagy field has developed; from when the term was first coined, to current research on autophagy in human health and disease.

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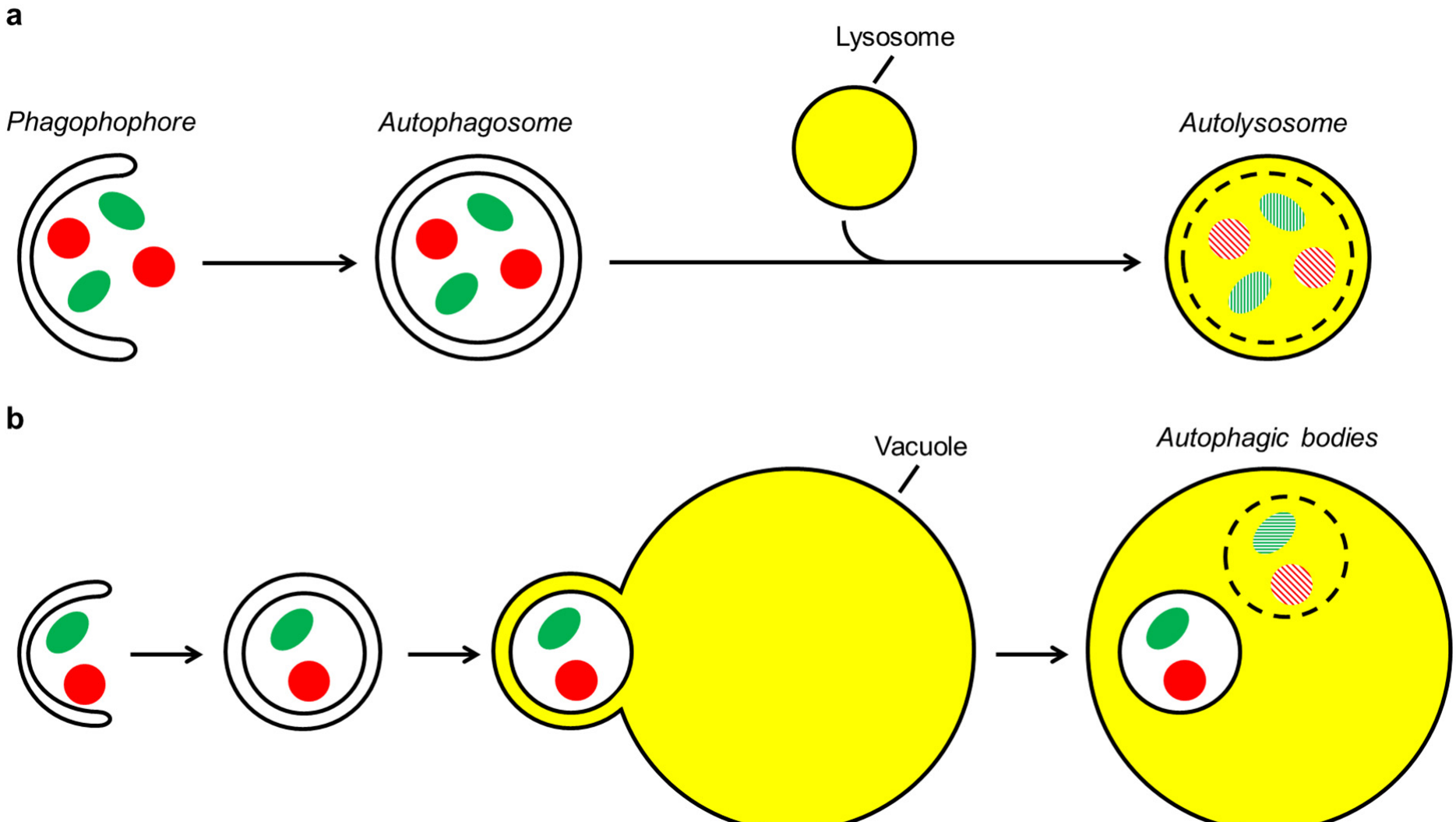
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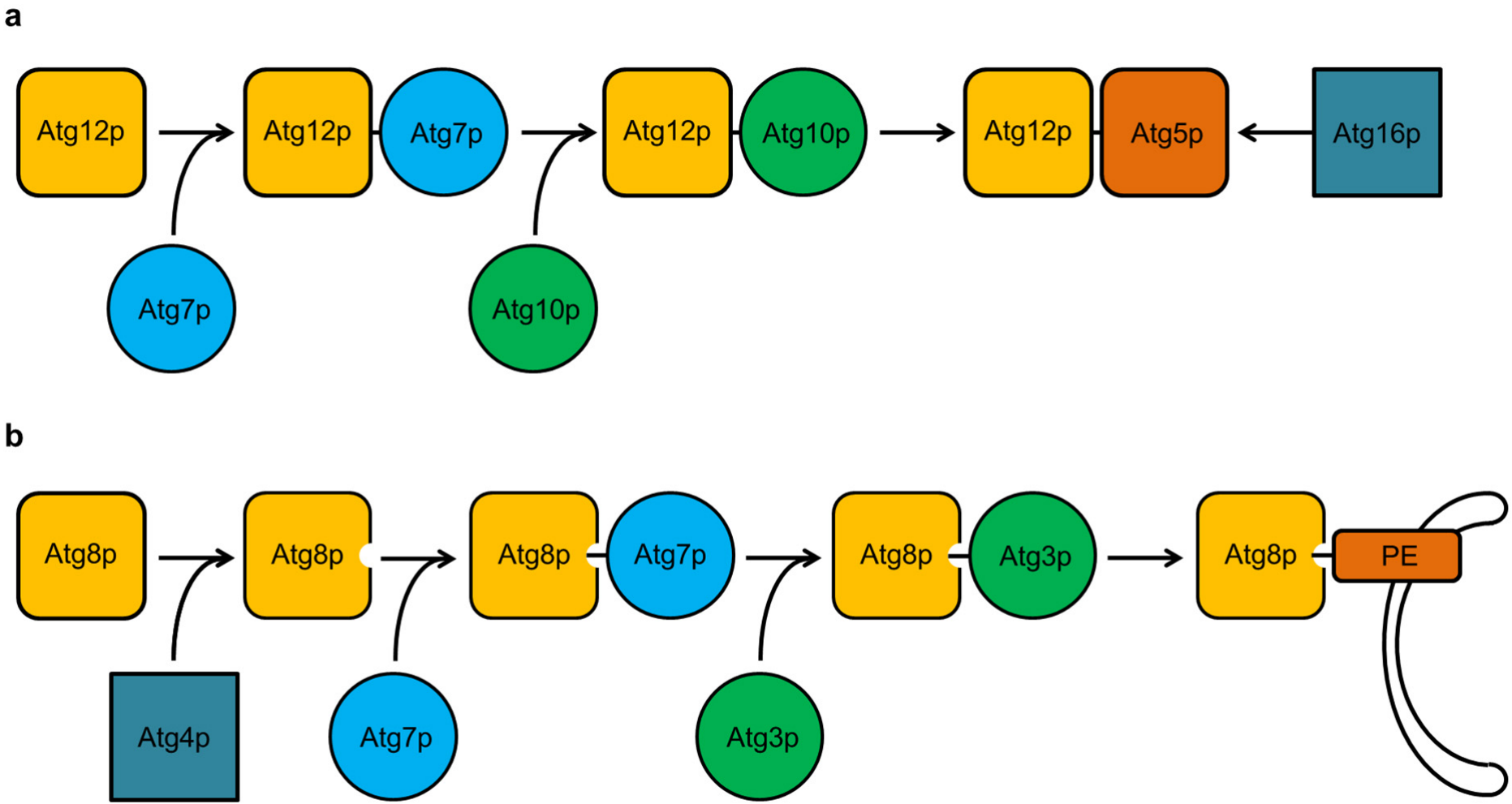
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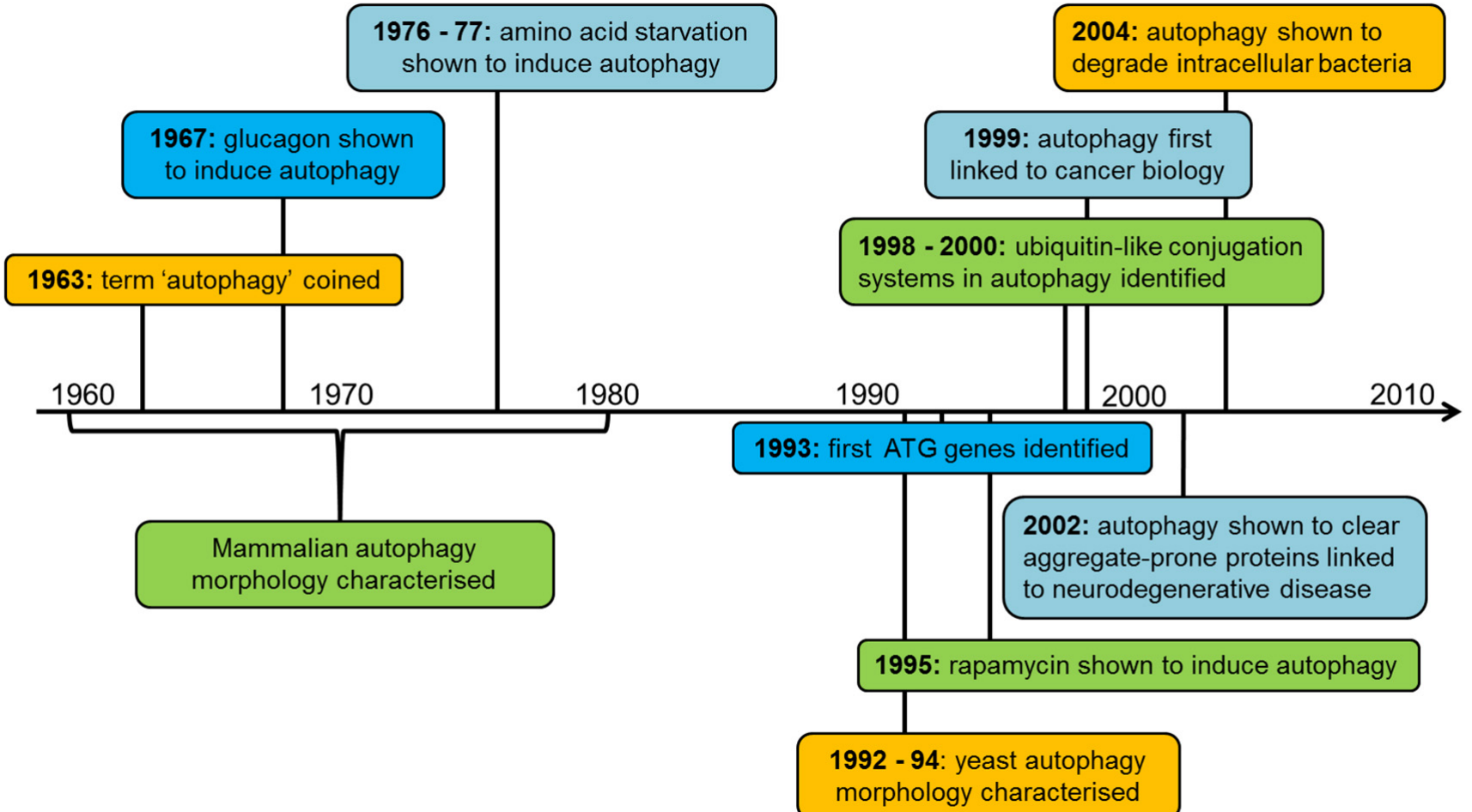
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