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Autophagy: mechanism and physiological relevance 'brewed' from yeast studies

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TABLE OF CONTENTS

1. ABSTRACT

Autophagy is a highly conserved process of quality control occurring inside cells by which cytoplasmic material can be degraded and the products recycled for use as new building blocks or for energy production. The rapid progress and 'explosion' of knowledge concerning autophagic processes in mammals/humans that has occurred over the last 15 years was driven by fundamental studies in yeast, principally using Saccharomyces cerevisiae, leading to the identification and cloning of genes required for autophagy. This chapter reviews the role of yeast studies in understanding the molecular mechanisms of autophagic processes, focusing on aspects that are conserved in mammals/humans and how autophagy is increasingly implicated in the pathogenesis of disease and is required for development and differentiation.

2. INTRODUCTION

Autophagy is a term coined from the Greek "auto" (self) and "phagy" (to eat). Autophagy is a highly conserved process of quality control occurring inside cells by which cytoplasmic constituents including long-lived proteins, protein aggregates, organelles and invading pathogens can be degraded and the products recycled for reuse. Material targeted for destruction is delivered into the interior of the lysosome (mammalian cells) or vacuole (yeast), where they are degraded by resident hydrolases. Autophagy operates at a basal level but is upregulated in response to changes in the internal status of the cell and/or changes in the extracellular environment (e.g., starvation). It is, therefore, essential for the maintenance of cellular homeostasis, with a particular importance for nutrient metabolism (1), and for an efficient response to cellular

stresses (2). In non-dividing and terminally differentiated cells autophagy is of particular importance for the removal of damaged or superfluous cellular components since they cannot be removed by 'dilution' that might otherwise occur by rounds of cell division. Over the last decade, autophagy has been increasingly implicated either in the pathogenesis or response to a wide variety of diseases, including cancer, neurodegeneration, myopathies, diabetes, liver and heart disease, bacterial and viral infections, and ageing (3-4).

In this chapter, we focus on providing an overview of the contribution of yeast studies to our current understanding autophagy, emphasizing those aspects conserved in humans and implicated in disease. Many aspects that we touch on have been the subject of recent comprehensive reviews and we direct readers interested in the full details of specific aspects of autophagy to the cited sources. Those unfamiliar with the field of autophagy may like to consult a recently published "comprehensive glossary of autophagy-related molecules and processes" (5).

3. HOW THE ISOLATION OF YEAST MUTANTS 'OPENED THE AUTOPHAGIC BLACK BOX'

Autophagy was first described and studied in mammalian cells beginning in the 1950s, but molecular characterization of the autophagy machinery did not take place until the 1990s. Prior to this time autophagy was studied using morphological and biochemical methods. Indeed a major review dating from the mid-90s (6) is illustrative of the 'state of play' that pertained then. First, is the large amount of morphological and biochemical data that had been painstakingly collected by a relatively small number of laboratories in the preceding four decades, and second is the dearth of information concerning the molecular mechanism(s) involved. In a mechanistic sense little more than knowledge of the presence of autophagic vesicles and their fusion with lysosomes could be considered established. It was studies made in the yeast, Saccharomyces cerevisiae, which led to significant and rapid advances in our understanding of the molecular mechanism of autophagy. A timeline of seminal discoveries in autophagy is provided in Klionsky (7; see also 8).

3.1. Yeast autophagy mutants and gene identifcation

A landmark study conducted by Yoshinori Ohsumi's laboratory (9) demonstrated that the morphology of autophagy in yeast was similar to that already documented in mammals. The work in Ohsumi's laboratory was conducted as part of a study to investigate protein degradation in the vacuole and used mutant cells lacking three vacuolar proteinases (proteinase A, proteinase B, and carboxypeptidase Y). When such cells were transferred from a rich growth medium to a medium devoid of various nutrients, dramatic changes in the morphology of vacuoles could be readily observed and monitored by light microscopy. In nutrient-deficient ('starvation') conditions, spherical bodies (400- to 900-nm in diameter) named "autophagic bodies" were shown to accumulate over time. Examination by electron microscopy showed that these bodies are bounded by a single membrane. The contents of these bodies were reported to include cytoplasmic ribosomes, rough endoplasmic reticulum, mitochondria, lipid granules and glycogen granules. The conclusion from these observations was that the autophagic bodies contain sequestered portions of the cytoplasm. It was determined using genetic analysis that the accumulation of autophagic bodies in the vacuoles is the result of a lack of protease function in the mutant strains studied. The accumulation could also be observed following treatment of normal cells (i.e., those containing a normal complement of vacuolar hydrolase activities) using phenylmethylsulfonyl fluoride (PMSF), a serine protease inhibitor commonly used in the preparation of cell extracts. As the autophagic bodies disappeared rapidly after removal of PMSF, it was concluded that they represent an intermediate in the normal autophagic process. In subsequent morphological analyses by transmission and immunological electron microscopy (10) the precursor structures of autophagic bodies, "autophagosomes", were confirmed as being structures bound by double membranes. Collectively, these observations suggested that the autophagic process in yeast is essentially similar to that of the lysosomal system in mammalian cells.

The groundbreaking advances in the field flowed from building on the morphological events that could be followed in yeast using light microscopy. A screen was conducted based on the use of a proteinase-deficient (proteinase A; pep4) strain to identify mutants defective in the accumulation of autophagic bodies within vacuoles (11). A single mutant, apg1, was isolated and subsequently used to generate a strain carrying an apg1 mutation but having normal vacuolar proteinases. Nitrogen starvation did not induce protein degradation in this strain, and it exhibited a dramatic loss of viability compared to wild-type cells under such starvation conditions. By using the loss of viability phenotype as a first screening test, 75 other apg mutants were selected, that were subsequently shown to fall into approximately 15 complementation groups. Genetic analyses of representative mutants from each of these groups then revealed that they carry single recessive chromosomal mutations and were defective in protein degradation in the vacuoles that is normally induced by nitrogen starvation.

Contemporaneous independent screens conducted in the laboratories of Michael Thumm (12) and Daniel Klionsky (13) and others (14) produced further mutants in autophagic processes and contributed significantly to the identification of genes encoding components of the autophagy mechanism. Although the mutants isolated by the different laboratories were originally designated with different gene names there was an early agreement to adopt a unified nomenclature of ATG (autophagy-related) (14). Since the report of the first cloned gene, now designated ATG1, published in 1997 (15) the number has continued to increase and recently the thirty-fifth gene with an ATG designation (ATG35) has been described (16). Of particular note have been recent screens carried out for mutants defective in mitophagy (a process by which damaged mitochondria can be selectively targeted), which led to the identification of genes specific for mitophagy, such as

ATG32 (17-18). In addition, some ATG gene names, including ATG25, ATG28, and ATG30, are assigned for genes in other yeast species for which there is no identifiable equivalent in S. cerevisiae. Finally, although ATG26 exists in S. cerevisiae, mutants in this gene do not have a phenotype in autophagy (19).

3.2. Mammalian autophagy genes and proteins

The identification of ATG genes in yeast was pivotal for the rapid expansion of the molecular analysis of autophagy into mammalian cells and other model organisms. A detailed listing of these genes and the encoded proteins can be found in Tanida (20).

The first mammalian autophagy genes to be identified, ATG5 and ATG12, are components of a ubiquitin-like (UBL) conjugation cascade that is conserved from yeast to humans (21). Of particular note and continuing importance to the field was the identification of the mammalian homologue of yeast Atg8 (another UBL conjugation cascade component), commonly referred to as LC3 (MAP1LC3; microtubule-associated protein 1 light chain 3) (22). The ability to tag the N-terminal end of LC3 with GFP (or other fluorescent proteins) facilitated the development of the now very widely used LC3-based fluorescence assay for monitoring autophagic puncta in mammalian cells (23).

Predictably, autophagy is more complex in mammals than in yeast. This is reflected in part by several ATG proteins having multiple family members and possibly representing developmental or tissue-specific regulation not relevant to yeast. For example, while Atg8 in yeast is represented by a single gene, the ATG8 family in humans contains at least 8 members divided into two subfamilies based on amino acid sequence homology. The LC3 subfamily comprises LC3A (including 2 splice variants of LC3A), LC3B (the "characterized" LC3 usually presumed to be followed in autophagy assays) and LC3C. The GABARAP/GATE subfamily comprises GABARAP, GABARAPL1, GATE-16 (aka GABARAPL2), and GABARAP-L3. Aside from LC3B the other family members remain largely unstudied and it has been unclear whether each member has a distinct and crucial role in autophagy. Recent work has indicated that the two subfamilies act differently at early stages of autophagosome biogenesis. Thus, the LC3 subfamily is required for elongation of the phagophore membrane, whereas the GABARAP/GATE subfamily is required for a later stage in autophagosome maturation (24).

Furthermore, additional ATG proteins for which there is no counterpart in yeast will likely be identified in the future as participating in autophagy in mammalian cells, such as the recently identified ATG101 protein (25).

In the near future it is likely that systems biology approaches will make an increasing contribution to our understanding of human autophagic processes. In this context, a systematic proteomic analysis of human autophagy was recently reported (26) that provides new information on the autophagy interaction network within mammalian/human cells and a resource for further mechanistic analysis.

4. PROCESSES DESCRIBED IN YEAST

As the molecular characterization of autophagy developed in the 1990s and into the succeeding decade it became clear that autophagy comprises several processes and that the ATG genes identified to date encode proteins needed for several autophagic processes. In yeast these include a process said to be "non-specific" (starvation-induced macroautophagy) and also several modifications of this process that are said to be "selective" (27) and targeted towards degradation of specific organelles including peroxisomes (28), mitochondria (29) and the nucleus (30). In addition, the cytoplasm-to-vacuole targeting (Cvt) pathway is unique in that it is a selective autophagy-related route that is used for biosynthetic purposes. We now present a brief description of these autophagic processes. For full details, readers are referred to recent comprehensive reviews we cite as references.

4.1. Macroautophagy

Cytoplasmic components or organelles are sequestered by formation of double-membrane vesicles, "autophagosomes", in the cytoplasm (31). Autophagosome formation is a multi-step process utilizing a machinery composed of many ATG gene products. These proteins assemble in a hierarchical order at a single site that is proximal to the vacuole called the phagophore assembly site/pre-autophagosomal structure (PAS). The PAS generates a phagophore, which expands around the cargo and eventually fuses at the ends to complete formation of the double-membrane autophagosome. At the vacuole, the outer membrane of the autophagosome fuses with the vacuolar membrane and releases a single-membrane autophagic body into the lumen. The action of the resident complement of acid hydrolases typically degrades the autophagic body and its contents. The molecular 'building blocks' so recovered are reused in biosynthetic pathways.

4.2. Microautophagy

Microautophagy-like processes differ morphologically from macroautophagy in that the sequestration event occurs directly at the vacuolar membrane. There are two general categories of microautophagic processes. Microautophagy refers to the invagination and scission of a portion of the limiting membrane, which may occur primarily for homeostatic purposes; membrane that is delivered to the vacuole through macroautophagy and other targeting pathways is removed from the organellar membrane and degraded. This type of microautophagy does not directly utilize the autophagy-related machinery. There are also microautophagy-like procesesses that are used to target peroxisomes, mitochondria and the nucleus (denoted micropexophagy, etc.) (27). In this case, the vacuolar membrane may form an invagination or an arm-like projection so as to sequester and internalize cytoplasmic components in single-membrane vesicles (derived from the vacuolar membrane). These vesicles are then degraded in the vacuolar lumen similar to autophagic bodies.

4.3. Cytoplasm-to-vacuole targeting (Cvt)

The Cvt pathway shares morphological features with macroautophagy and is used to deliver at least three resident hydrolases to the vacuole. In this context the Cvt pathway represents a biosynthetic, rather than a degradative, use of an autophagic process. Precursors of the hydrolases are sequestered from the cytosol into double-membrane Cvt vesicles that are substantially smaller than the autophagosomes that form during non-specific autophagy. These vesicles subsequently fuse with the vacuolar membrane to release single-membrane Cvt bodies into the vacuolar lumen. Subsequent action of the vacuolar hydrolase complement degrades the membrane and activates the precursors in part by proteolytic cleavage (32-33).

4.4. Vacuolar import and degradation (Vid)

In yeast, glucose starvation induces production of key gluconeogenic enzymes including fructose-1,6-bisphosphatase (FBPase), malate dehydrogenase and phosphoenolpyruvate carboxykinase. When glucose is added to long-term starved cells, these enzymes are degraded in the vacuole by a selective autophagic pathway whereby the proteins are first imported into novel Vid (vacuole import and degradation) vesicles, which then merge with the endocytic pathway. Following the fusion of endosomes with the vacuole, the cargo enzymes are released and then degraded in the vacuolar lumen (34). The model for Vid sequestration is that uptake of the target proteins takes place by translocation across the completed vesicles, which are composed of a single membrane; however, the mechanism by which this might occur is not known.

5. THE FORMS OF AUTOPHAGY IN MAMMALIAN CELLS

In mammalian cells three forms of autophagic process have been described: macroautophagy, microautophagy and chaperone-mediated autophagy (CMA). These differ morphologically and mechanistically, and presumably crosstalk between them can occur depending on the particular circumstances, although the details remain to be elucidated. It should be noted that the vast majority of reports on autophagy in mammalian cells, unless specified otherwise, are addressing macroautophagy.

5.1. Macroautophagy

Macroautophagy is a conserved process from yeast to mammals, and thus in mammals it involves the sequestration of cytoplasmic constituents into autophagosomes, which then fuse with lysosomal membranes. Due to the relative size difference between the lysosome and the vacuole, macroautophagy in higher eukaryotes does not generate autophagic bodies. Nonetheless, fusion of the autophagosome outer membrane with the lysosome provides access to the lumenal hydrolases. In addition, it is clear in mammalian cells that the macroautophagic pathway converges with endocytosis; autophagosomes may fuse with endosomes to generate amphisomes that subsequently fuse with the lysosome. It is not known whether an equivalent intermediate structure exists in yeast, in part because of the difficulty of detecting the endosome by morphological analysis.

Macroautophagy is initiated by the formation of the phagophore (sometimes referred to as the isolation membrane). The origin of this membrane has been contentious, with opinion mostly divided between *de novo* assembly (as for yeast) or formation from endoplasmic reticulum(ER)/Golgi membranes. Very recent studies have suggested that ER/Golgi, mitochondria or plasma membrane may, at least under certain circumstances, provide membrane for the mammalian phagophore (3, 35-36).

Extracellular (nutrient starvation, hormone or pharmacological treatment) as well as intracellular stimuli (accumulation of misfolded proteins and protein aggregates, invasion by pathogens) are able to modulate the macroautophagic response. As macroautophagy occurs at a basal, constitutive level under normal conditions, there are mechanisms by which extracellular or intracellular signals are transmitted to the regulatory factors to promote or inhibit autophagy when needed (37-38). In brief, pathways that interpret the status of cellular energy (e.g., the AMP level via AMP-dependent protein kinase), nutrients (e.g., the glucose level via protein kinase A) and growth factors (e.g., insulin) control autophagy signalling. A central player in these pathways is the evolutionarily-conserved protein serine/threonine kinase, TOR (target of rapamycin), which has other known roles in regulating cell growth, proliferation, motility and survival, as well as transcription and protein synthesis (39). Although recent studies in flies and mammalian tissue culture cells have elucidated upstream signalling components, a complete and fully integrated picture of autophagy regulation is not currently available. Some aspects have been covered in focused reviews (38, 40-41).

5.2. Microautophagy

In mammalian cells dissection of the mechanism of microautophagy has progressed little over the past four decades (42), but in general this process is considered to proceed as described in yeast where the lysosomal membrane invaginates or makes protrusions so as to sequester and internalize the intended cargo. Very recently a selective microautophagy-like process has been identified that delivers soluble cytosolic proteins to the vesicles of late endosomes/multivesicular bodies (43). It is not known if microautophagy in mammals plays a role in lysosome membrane homeostasis, but similar issues regarding the additional membrane that results from fusion with the autophagosome make it likely that maintaining the properties of the lysosome limiting membrane is one function of this process.

5.3. Chaperone-mediated autophagy (CMA)

CMA differs from the other two forms of mammalian autophagy in that vesicular traffic is not involved in transfer of the “cargo” to the lysosomes. Cytosolic proteins containing the pentapeptide sequence motif, KFERQ, are target substrates for CMA. This motif is recognized by cytosolic HSC70 chaperones, which unfold

the substrate and promote, with the help of co-chaperones, its docking with a lysosomal membrane receptor, lysosome-associated membrane protein type 2A (LAMP-2A). CMA is responsible for the degradation of 30% of cytosolic proteins under conditions of prolonged nutrient deprivation. An equivalent process does not occur in yeast (although the closest may be the Vid pathway) and characterization of CMA has occurred entirely through studies in mammals (44).

6. COMMONALITIES OF AUTOPHAGIC PROCESSES BETWEEN YEAST AND MAMMALS

In this section the focus is on macroautophagy since the detailed knowledge for this process is greatest in both yeast and mammals. As indicated above, autophagosome formation is regulated by a multi-component molecular machinery. There is general agreement that the process involves five complexes conserved from yeast to mammals although there are some additional components in mammals (for detailed discussion see 8, 20, 45). There probably remain components in both systems as yet unidentified. The five 'common' complexes are:

1. Atg1-Atg13-Atg17 kinase complex in yeast, and in mammals the unc-51-like kinase (ULK) complex triggers autophagy in response to induction signals and initiates the early steps in autophagosome formation (46).

2. Atg6/Beclin 1 class III phosphatidylinositol 3-kinase complex controls production of the phosphoinositide signals that facilitate assembly of autophagosomes (47). Beclin 1 acts as part of a core complex that contains vacuolar sorting protein 34 (VPS34), a class III phosphatidylinositol-3 kinase. In mammals Beclin 1 mediates the regulation of diverse cellular functions in addition to autophagy including cell death pathways. Several proteins have been identified that function in distinct mammalian Beclin 1-VSP34 complexes that control autophagic and other cellular events (48-49).

3. A ubiquitin-like (UBL) protein conjugation cascade is required for autophagosome maturation and cargo recruitment (31). In yeast the cascade is comprised of the E1 enzyme Atg7, two E2 enzymes (Atg10 and Atg3), and two UBLs (Atg8 and Atg12). Atg12 is conjugated to Atg5 via the activity of Atg7 (E1) and Atg10 (E2) to form an oligomeric Atg12–Atg5-Atg16 complex that subsequently dimerizes. This complex in conjunction with the activity of Atg3 promotes conjugation of Atg8 via its C-terminal Gly residue to phosphatidylethanolamine (PE). Additionally, the Atg12–Atg5-Atg16 complex may also determine the site of incorporation of Atg8–PE into phagophores.

4. A recycling system whose key component is Atg9 and which participates in transfer and recycling of components to the phagophore, thereby providing the source of lipids for phagophore expansion (50).

5. The molecular machinery that organizes and drives fusion between the autophagosome and the acidic degradative compartment (vacuole/lysosome) (51).

The conservation of mechanism and core apparatus used in macroautophagy has recently been exploited to develop a "network" view of autophagy. Protein interactome data from yeast were used to construct a composite yeast-human interaction network (52). Using this approach 14 novel and putative autophagy-associated human proteins were identified. One, FNBP1L, was confirmed as interacting with Atg3 (a conjugation cascade component) and then demonstrated to be essential for antibacterial autophagy, but not canonical macroautophagy.

The response of both yeast and mammalian cells to starvation is mediated by the TOR kinase. Nutrient starvation induces autophagy through inhibition of TOR, which functions in two distinct multi-protein complexes, TOR complex (TORC) 1 and 2. The structure and functions of these complexes are conserved also. TORC1 is inhibited by rapamycin and is thought to couple growth signals to cellular metabolism. Thus, rapamycin is an activator of autophagy commonly used in both mammals and yeast. TORC2 is relatively insensitive to rapamycin and appears to regulate spatial aspects of growth such as cell polarity (40).

Selectivity with respect to cargo for transport to the degradative compartment is now understood to represent an important aspect that applies to autophagic processes in both yeast and mammals. Key to this selectivity is a small but increasing number of proteins that serve as 'markers' for specific substrates for autophagic degradation. For example, p62 is a conserved, ubiquitously expressed protein found in metazoans, but not in fungi (or plants). Evidence supports a role for p62 as a receptor for ubiquitinated proteins to deliver them selectively into the autophagosome, mediated through interaction of p62 with LC3. p62 has also been implicated in formation of disease-related inclusions and their potential clearance by macroautophagy (53).

Many selective forms of macroautophagy are now recognized, with target organelles such as mitochondria (mitophagy) and peroxisomes (pexophagy). Thus, the same autophagic machinery for yeast macropexophagy is essential for peroxisome degradation in mammals (54). Defects in these processes can be associated with pathology, for example, defective mitophagy in Parkinson disease (55). In addition, mammalian cells are subject to infection by bacteria (both gram-positive and gram-negative), parasites (protozoa and fungi), and viruses (including both RNA and DNA viruses). Their removal from infected host cells involves directed cargo recognition, marking and sequestration (56-57). The specific degradation of such pathogens is referred to as xenophagy.

7. THE FUNCTIONS OF AUTOPHAGY IN HEALTH AND DISEASE

7.1. Autophagy and pathogenesis

The demonstration of connections between autophagy and disease quickly followed the identification of the mammalian homologues of yeast ATG genes. The

Table 1. Autophagy in pathogenesis (after 64; see also 76-77)

Cancer
Neurodegenerative diseases:
Alzheimer disease
Amyotrophic lateral sclerosis
Huntington disease
Parkinson disease
Spinocerebellar ataxia
Infection/immunity/immune-mediated diseases:
Bacteria
Viruses
Parasites
Antigen presentation through MHC class II molecules
Systemic lupus erythematosus
Crohn disease
Myopathies
Heart disease
Ageing
Liver disease
Kidney disease

forerunner of such demonstrations was the 1999 report from the laboratory of Beth Levine that BECN1 (ATG6) is mono-allelically deleted in a large proportion of human breast and ovarian cancers (58). Subsequent studies showed that mice having only one functional BECN1 gene copy display higher rates of spontaneous tumor formation, leading to the designation of BECN1 as a tumor suppressor gene (59-60).

Over the ensuing decade many studies have connected autophagy to various pathophysiological conditions (Table 1; 3, 61-64). Autophagy also contributes to both the innate and adaptive immune responses in mammals (65-66). In terms of adaptive immunity, autophagy regulates the development and survival of lymphocytes as well as the modulation of antigen processing and presentation (67).

Autophagy is recognized as contributing to cell death, and this can have an impact upon outcomes in situations of disease (2). In most cases, autophagy probably functions initially as a cytoprotective mechanism that contributes to the avoidance of cell death, but if cellular damage is too extensive, or if apoptosis is compromised, excessive autophagy may lead to cell death (designated type II programmed cell death). Although far from being fully elucidated, it is increasingly recognized that there is crosstalk between apoptosis (type I programmed cell death) and autophagy (68). It is possible both processes or elements of them occur simultaneously, and a recent illustration of molecular crosstalk is the finding that apoptotic caspases can cleave Beclin 1, thereby destroying its pro-autophagic activity. Interestingly, the resulting C-terminal cleavage fragment of Beclin 1 acquires a new function, being able to amplify mitochondrially-mediated apoptosis (69).

7.2. Other physiological roles for autophagy

The knockout of yeast ATG genes showed a role for autophagy in spore formation (11). Studies in other lower eukaryotes have also revealed an essential role for autophagic processes in differentiation and development. The identification and cloning of mammalian ATG genes facilitated the production and analysis of systemic and tissue-specific knockout mice. Their phenotypic characterization has made a significant contribution to our understanding of the functions of autophagy in mammalian development and differentiation. In summary, autophagy has an essential role in two stages of early development: the pre-implantation period after fertilization of the oocyte, and the postnatal period immediately following loss of the placental food supply (70). Of note are studies that show a role for Beclin 1 in embryonic cavitation, and a role for Ambra1 (a vertebrate-specific, positive regulator of macroautophagy that interacts with Beclin 1) in neural development (reviewed in 70).

7.3. Potential for clinical manipulation of autophagy

One potential practical use for the modulation of autophagy may be as an anti-cancer treatment. The outcome of the induction of autophagy can be variable in terms of cell survival and will depend not only on the genotype of the cell, but also on the environment (for example, metabolic stress induced by the treatment regime). Signals that enable unrestricted cell proliferation also inhibit autophagy, which is normally induced to sustain cells during nutrient limitation. Thus, on the one hand, autophagy could promote survival of non-cancerous cells in the setting of metabolic stress in the tumor microenvironment. On the other hand, in some more advanced cancers, autophagy could benefit the progression of the tumor, because it might enable tumor cells to resist damage induced by cytotoxic anti-cancer treatments and enhance their survival in conditions of nutrient limitation and decreased energy production (71).

Proper functioning of autophagy has been related to ageing and longevity. The efficiency of both macroautophagy and CMA decrease with age (72). The cellular consequences of a decline in autophagic activity (including inefficient removal of damaged intracellular structures, and the inability to adapt to extracellular or intracellular stresses) could clearly contribute to the general deterioration of cellular function that is a hallmark of ageing (see 73). The ability to 'restore' autophagy by therapeutic intervention might thus offer clinical benefits (74).

8. PERSPECTIVE

Returning in the end to yeast, autophagy—similar to many cellular processes—is highly conserved from yeast to human. Thus, information gained from studying autophagy in yeast will likely continue to provide information applicable to higher eukaryotes. The application of genomic, proteomic and bioinformatic approaches will continue to allow the identification of proteins associated with diseases by first uncovering their roles in yeast (75).

9. ACKNOWLEDGEMENTS

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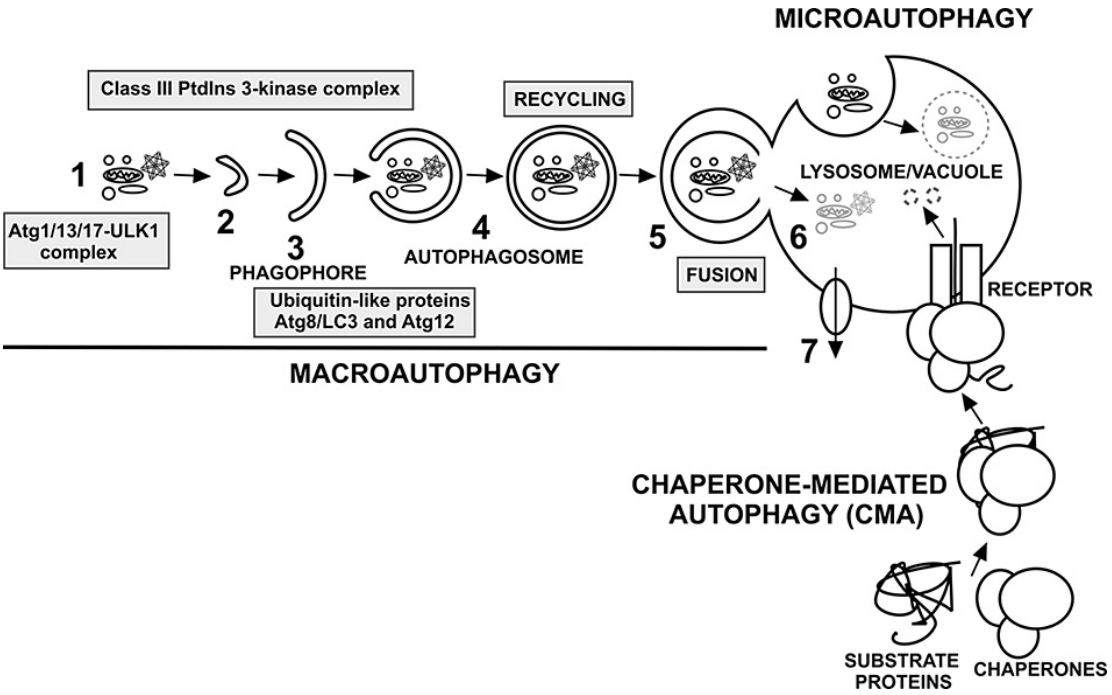

Figure 1. Three main types of autophagy in yeast and mammalian cells. Three mechanistically and / or morphologically different main types of autophagy have been described in yeast and mammalian cells: Macroautophagy, microautophagy and chaperone-mediated autophagy (CMA). Macroautophagy requires autophagosome formation which is a multi-step process. The five 'common' complexes involved in macroautophagy (see section 6) are indicated at the step in which they participate (Boxes shaded gray). An induction signal (1) leads to the commencement of phagophore formation (2). (Note that the source of membrane for the phagophore is not shown (refer to section 5.1.) The phagophore expands (3) to eventually sequester the cargo by fusion of the phagophore ends thereby forming the double-membrane autophagosome (4). At the acid compartment (lysosome/vacuole), the outer membrane of the autophagosome fuses with the membrane and, in the case of yeast, releases a single-membrane vesicle into the lumen (5). For micro- and macroautophagy, the resident complement of acid hydrolases degrades the vesicle and its contents, or, in the case of CMA, the translocated substrates (6). The molecular 'building blocks' so recovered are recycled to the cytosol by efflux across the membrane (7).

to understanding mammalian/human autophagy have not been cited; where possible we have chosen to cite recent reviews in which details of such contributions can be found. We thank Dalibor Mijaljica for drawing Figure 1.

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