

# Molecular definitions of autophagy and related processes

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## **Abstract**

Over the past two decades, the molecular machinery that underlies autophagic responses has been characterized with ever increasing precision in multiple model organisms. Moreover, it has become clear that autophagy and autophagy-related processes have profound implications for human pathophysiology. However, considerable confusion persists on the use of appropriate terms to indicate specific types of autophagy and some components of the autophagy machinery, which may have detrimental effects on the expansion of the field. Here, a panel of leading experts in the field defines several autophagy-related terms based on specific biochemical features. The ultimate objective of this collaborative effort is to formulate recommendations that facilitate the dissemination of knowledge within and outside the field of autophagy research.

## Introduction

A few months ago, the Nobel Assembly at Karolinska Institute awarded the 2016 Prize in Physiology or Medicine to the cell biologist Yoshinori Ohsumi for his early identification and characterization of the autophagy machinery in yeast (Tsukada & Ohsumi, 1993). This came as an overt recognition to a field symbolically initiated by the Belgian cytologist and biochemist Christian De Duve, who in 1963 coined the term autophagy (from the Ancient Greek αὐτόφαγος, meaning "self-eating") for describing the presence of single- or double-membraned intracellular vesicles that contain parts of the cytoplasm and organelles in various states of disintegration (Yang & Klionsky, 2010). Our understanding of autophagy, which is highly conserved during evolution (**Table 1**), has tremendously expanded over the past decades, on both mechanistic and pathophysiological grounds (Choi *et al*, 2013; Noda & Inagaki, 2015). Alongside, we have begun to appreciate the considerable potential of pharmacological agents or dietary interventions that activate or inhibit autophagy as novel therapies for multiple human disorders and pathophysiological conditions, including neurodegenerative (Menzies *et al*, 2015), infectious (Deretic *et al*, 2013), autoimmune (Deretic *et al*, 2013; Zhong *et al*, 2016), cardiovascular (Shirakabe *et al*, 2016), rheumatic (Rockel & Kapoor, 2016), metabolic (Kim & Lee, 2014), pulmonary (Nakahira *et al*, 2016) and malignant diseases (Amaravadi *et al*, 2016; Galluzzi *et al*, 2016b; Galluzzi *et al*, 2015b), as well as aging (Lopez-Otin *et al*, 2016; Melendez *et al*, 2003). Nevertheless, there is not a single drug currently licensed by the US Food and Drug Administration (FDA) - or equivalent regulatory agency – that was developed with the primary aim of modulating autophagy (although many FDA-approved drugs indeed activate or inhibit autophagy to some extent) (Poklepovic & Gewirtz, 2014; Rosenfeld *et al*, 2014; Vakifahmetoglu-Norberg *et al*, 2015). Such an obstacle in the translation of robust preclinical data from multiple model organisms into clinically viable therapeutic interventions reflects the persistence of several obstacles of pharmacological, biological and technological nature. Discussing

these issues in a comprehensive manner goes well beyond the scope of the current article, and has been recently done elsewhere (Galluzzi *et al*, 2017). An analysis of the literature also reveals considerable confusion about the use of several autophagy-related terms, affecting not only less-experienced investigators but also researchers with many years of expertise in the field. Although such semantic issues may appear trivial at first glance, we are convinced that it constitutes a significant obstacle to the optimal development of autophagy research, both at preclinical and translational levels. Driven by the success obtained by a similar initiative in the cell death field over the past decade (Galluzzi *et al*, 2015a; Galluzzi *et al*, 2012), leading experts in autophagy decided to gather and tentatively define several autophagy-related terms based on precise, biochemical features of the process.

## Processes

**Autophagy.** Perhaps surprisingly, the relatively broad term “autophagy” itself has been used with rather variable and sometimes misleading connotations. We agreed on two main features that characterize *bona fide*, functional autophagic responses, irrespective of type: (1) they involve cytoplasmic material; and (2) they culminate with (and strictly depend on) lysosomal degradation. Thus, although autophagy substrates (see below for a definition) can be endogenous, such as damaged mitochondria and nuclear fragments, or exogenous, such as viruses or bacteria escaping phagosomes, autophagy operates on entities that are freely accessible to cytosolic proteins (notably, components of the autophagy machinery). This feature is important to discriminate autophagic responses from branches of vesicular trafficking that originate at the plasma membrane, which also culminate in lysosomal degradation. Such endocytic processes (which have cumulatively been referred to as “heterophagy” in the past) include phagocytosis (*i.e.*, the uptake of particulate material by professional phagocytes – such as macrophages and immature dendritic cells – or other cells), receptor-mediated endocytosis (*i.e.*, the uptake of extracellular material driven by plasma membrane receptors), and pinocytosis (*i.e.*, the relatively non-specific uptake of extracellular fluids and small molecules) (Foot *et al*, 2017; Munz, 2016). However, some forms of autophagy (notably macroautophagy, see below for a definition) and the endocytic pathway interact at multiple levels, and the molecular machinery responsible for the fusion of late endosomes (also known as multivesicular bodies) or autophagosomes (see below for a definition) with lysosomes is essentially the same (Tooze *et al*, 2014).

The strict dependency of autophagic responses on lysosomal activity is important to discriminate them from other catabolic pathways that also involve cytoplasmic material, such as proteasomal degradation (Bhattacharyya *et al*, 2014). The 26S proteasome degrades a large number of misfolded cytoplasmic proteins that have been ubiquitinated, as well as properly folded proteins that expose specific



degradation signals, such as the so-called “N-degrons” (Sriram *et al*, 2011). When ubiquitinated proteins accumulate, however, they tend to assemble into aggregates that are degraded by macroautophagy upon binding to autophagy receptors or adaptors (see below for a definition) (Lim & Yue, 2015; Moscat *et al*, 2016). Moreover, a cross-talk between the proteasome and chaperone-mediated autophagy (CMA, see below for a definition) has been described (Massey *et al*, 2006; Schneider *et al*, 2014), and cytosolic proteins bound to heat shock protein family A (Hsp70) member 8 (HSPA8), which serves as main chaperone in CMA, can be efficiently redirected to proteasomal degradation upon interaction with ubiquilin 2 (UBQLN2) (Hjerpe *et al*, 2016). Thus, the proteasome system shares some substrates with different forms of autophagy. However, these two processes differ radically in their final products. Proteasomal degradation results in short peptides (of 8-12 residues) that are not necessarily degraded further, but may be feed into additional processes including (but not limited to) antigen presentation at the plasma membrane (Neefjes *et al*, 2011). In contrast, lysosomal proteases fully catabolize polypeptides to their constituting amino acids, which eventually become available for metabolic reactions or repair processes. Moreover, lysosomal hydrolases also degrade lipids, sugars and nucleic acids (Settembre *et al*, 2013). In summary, *bona fide* functional autophagic responses direct cytoplasmic material of endogenous or exogenous origin to degradation within lysosomes (or late endosomes, in specific cases).

**Microautophagy.** Microautophagy is a form of autophagy during which cytoplasmic entities destined to degradation are directly taken up by the vacuole (in yeast and plants) via direct membrane invagination (Farre & Subramani, 2004; Uttenweiler & Mayer, 2008). In cells from *Drosophila melanogaster* and mammals, a similar mechanism is in place but it involves late endosomes. This process, which also occurs in yeast cells, is commonly known as “endosomal microautophagy”

(Mukherjee *et al*, 2016; Sahu *et al*, 2011; Uytterhoeven *et al*, 2015). In yeast, microautophagy has been involved in the degradation of multiple substrates, including peroxisomes (a process called “micropexophagy”, historically the first form of microautophagy to be described) (Farre & Subramani, 2004), portions of the nucleus (Kvam & Goldfarb, 2007), damaged mitochondria (Kissova *et al*, 2007), and lipid droplets (Vevea *et al*, 2015). In plants, microautophagy has been shown to mediate the degradation of anthocyanins (Chanoca *et al*, 2015). Finally, endosomal microautophagy degrades cytosolic proteins, either in bulk or selectively (only proteins containing a KFERQ-like motif recognized by HSPA8) (Mukherjee *et al*, 2016; Sahu *et al*, 2011; Uytterhoeven *et al*, 2015). Of note, some proteins internalized by late endosomes through direct membrane invagination are not degraded but excreted within exosomes (Record *et al*, 2014).

Arguably, microautophagy is the least studied form of autophagy, but a molecular signature of the process has begun to emerge. Thus, several forms of yeast microautophagy (*e.g.*, micropexophagy) require some components of macroautophagy machinery for cargo targeting and internalization, including (but perhaps not limited to) Atg7, Atg8 and Atg9 (Farre *et al*, 2008; Krick *et al*, 2008). Conversely, endosomal microautophagy relies on multiple endosomal sorting complexes required for transport (ESCRT) systems (Liu *et al*, 2015b; Mukherjee *et al*, 2016; Sahu *et al*, 2011; Uytterhoeven *et al*, 2015). In addition, the selective uptake of KFERQ-containing proteins by late endosomes in the course of endosomal microautophagy depends on HSPA8, reflecting its ability to directly interact with phosphatidylserine on (and hence deform) the outer endosomal membrane (Morozova *et al*, 2016; Uytterhoeven *et al*, 2015). Along similar lines, chaperone ATPase HSP104 (Hsp104) reportedly underlies microautophagic responses to lipid droplets in *Saccharomyces cerevisiae* (Vevea *et al*, 2015). However, the strict requirement of chaperones from the HSP70 protein family in other variants of microautophagy has not been documented yet. Of note, the yeast orthologue of mammalian NBR1, autophagy cargo receptor (NBR1; which is known to operate as a receptor for macroautophagy)

reportedly underlies an ESCRT-dependent and ubiquitination-dependent microautophagic pathway in *Schizosaccharomyces pombe* (Liu *et al*, 2015b). It will be interesting to determine whether NBR1 and other components of this pathway also contribute to microautophagy in mammalian cells. Irrespectively, we propose to define microautophagy and endosomal microautophagy as forms of autophagy in which the cargo is directly internalized by the lysosome/vacuole or late endosomes, respectively, via ESCRT-independent (microautophagy) or ESCRT-dependent (endosomal microautophagy), mechanisms. In addition, selective endosomal microautophagy can be defined as an HSPA8-dependent autophagic response, but it can be differentiated from CMA based on: (1) its dependence from ESCRT systems and (2) its independence from a specific splicing variant of lysosomal associated membrane protein 2 (LAMP2A, see below).

***Chaperone-mediated autophagy.*** CMA involves the direct delivery of cytosolic proteins targeted for degradation to the lysosome (Kaushik & Cuervo, 2012). The distinctive feature of CMA is that neither vesicles nor membrane invaginations are required for substrate delivery to lysosomes, since substrates reach the lysosomal lumen through a protein-translocation complex at the lysosomal membrane (Kaushik & Cuervo, 2012). CMA only degrades soluble proteins bearing a KFERQ-like motif bound to HSPA8 (Dice, 1990), but not entire organelles, other macromolecules such as lipids, nucleic acids, or proteins integral to membranes (Chiang *et al*, 1989; Salvador *et al*, 2000; Wing *et al*, 1991). CMA has been shown to operate on a multitude of cytosolic proteins, hence exerting major regulatory functions in different pathophysiological scenarios such as metabolic regulation (Kaushik & Cuervo, 2015; Schneider *et al*, 2014), genome integrity preservation (Park *et al*, 2015), aging (Rodriguez-Muela *et al*, 2013; Schneider *et al*, 2015), T-cell activation (Valdor *et al*, 2014), neurodegeneration (Orenstein *et al*, 2013), and oncogenesis (Kon *et al*, 2011). Moreover, linear sequence analysis of the cytosolic

proteome suggests that approximately 30% of its components may be degraded by CMA (Dice, 1990). Importantly, the translocation of CMA substrates across the lysosomal membrane relies on a dedicated molecular machinery that critically involves a specific splicing isoform of LAMP2, namely, LAMP2A (Cuervo & Dice, 1996). Thus, chaperone-bound autophagy substrates bind LAMP2A monomers on the cytosolic side of the lysosome, which stimulate LAMP2A oligomerization (Bandyopadhyay *et al*, 2008).

While unfolding and dissociating from chaperones (Salvador *et al*, 2000), CMA substrates are translocated into the lysosomal lumen through oligomeric LAMP2A complexes that are stabilized by a lysosomal pool of heat shock protein 90 alpha family class A member 1 (HSP90AA1; best known as HSP90) (Bandyopadhyay *et al*, 2008), and a cytosolic pool of glial fibrillary acidic protein (GFAP) (Bandyopadhyay *et al*, 2010). Lysosomal HSPA8 operates as an acceptor for CMA substrates, possibly as it prevents cytosolic retrotranslocation (Agarraberes *et al*, 1997). Eventually, LAMP2A pores are dismantled within lipid-rich microdomains of the lysosomal membrane by a mechanism that relies on HSPA8, followed by cathepsin A (CTSA)-catalyzed LAMP2A degradation (Kaushik *et al*, 2006). The CMA-supporting activity of GFAP is negatively regulated by phosphorylation, which is catalyzed by a pool of AKT serine/threonine kinase 1 (AKT1) that resides on the lysosomal surface (Arias *et al*, 2015). In this setting, inhibition of AKT1 by PH domain and leucine rich repeat protein phosphatase 1 (PHLPP1) counteracts the tonic activity of mechanistic target of rapamycin (MTOR) complex 2 (MTORC2), resulting in CMA activation (Arias *et al*, 2015). It remains to be determined to which extent CMA is conserved in lower organisms, since the splice variant of LAMP2 that is essential for CMA (*i.e.*, LAMP2A) appeared relatively late in evolution (in birds). Recent studies propose that selective endosomal microautophagy, which shares with CMA the dependence on KFERQ-like motives and HSPA8, might constitute the alternative to CMA in *D. melanogaster* (Mukherjee *et al*, 2016). Irrespective of this unknown, we propose to define CMA as a HSPA8-dependent autophagic response

that relies on LAMP2A-mediated cargo translocation across the lysosomal membrane. In this context, it should be noted that other splicing isoforms of LAMP2 (including LAMP2B and LAMP2C) are dispensable for CMA but involved in macroautophagy (see below) (Eskelinen *et al*, 2005). This implies that genetic interventions aimed at specifically inhibiting CMA should not be directed to HSPA8 (which is also required for multiple forms of microautophagy), nor to *LAMP2* as a gene.

**Macroautophagy.** Macroautophagy is the variant of autophagy best characterized thus far, at least in part owing to its easily distinguishable morphological features. Indeed, whereas microautophagy and CMA are not associated with major morphological changes in vesicular compartments, macroautophagic responses involve dedicated vesicles that can occupy (at a specific moment) a considerable part of the cytoplasm, an impressive phenomenon that attracted attention as early as in the late 1950s (Yang & Klionsky, 2010). These double-membraned vesicles, which are commonly known as autophagosomes (see below for a definition), can sequester large portions of the cytoplasm including entire organelles or parts thereof. This endows macroautophagy with a considerable catabolic potential that - in specific settings – can contribute to regulated cell death (RCD) (Galluzzi *et al*, 2016a) or cellular atrophy leading to neurodegeneration (Cherra *et al*, 2010a; Cherra *et al*, 2010b; Zhu *et al*, 2013). The molecular machinery that executes and regulates macroautophagy in organisms encompassing yeast, nematodes, flies and mammals has been the subject of intense investigation throughout the past two decades (Antonioli *et al*, 2016; Noda & Inagaki, 2015). Although a detailed description of these pathways is not warranted here, a few functional modules of the macroautophagy apparatus are particularly important for this discussion. Indeed, the molecules that are part of these functional modules, their interactors and the processes they control have been extensively employed thus far to identify macroautophagic responses, though not always with precision. Efficient

macroautophagic responses involving the formation of autophagosomes, their fusion with lysosomes and lysosomal degradation have been associated with the activity of two ubiquitin-like conjugation systems (Antonioli *et al*, 2016; Noda & Inagaki, 2015). One relies on autophagy related 7 (ATG7) and ATG10, which promote the conjugation of a multiprotein complex containing ATG5, ATG12 and autophagy related 16 like 1 (ATG16L1) (Mizushima *et al*, 1998). Another one is mediated by ATG4, ATG3 and ATG7, which cooperate to catalyze the proteolytic maturation of microtubule associated protein 1 light chain 3 beta (MAP1LC3; best known as LC3B) and other orthologues of yeast Atg8, and their conjugation to phosphatidylethanolamine (Ichimura *et al*, 2000; Marino *et al*, 2010; Rockenfeller *et al*, 2015). Lipidated LC3 (often referred to as LC3-II) is generated onto forming autophagosomes and allows for substrate uptake upon binding to several autophagy receptors (Kabeya *et al*, 2000; Stolz *et al*, 2014; Wild *et al*, 2014). Importantly, recent data suggest that the ATG conjugations systems and Atg8-like proteins are not strictly required for the formation of autophagosomes, as classically thought (although their absence greatly reduces the efficiency of the process), but also contribute to autophagosome extension around large substrates and closure, the fusion of autophagosomes with lysosomes, and the degradation of the inner autophagosomal membrane (Nguyen *et al*, 2016; Tsuboyama *et al*, 2016).

In response to commonly studied stimuli including starvation, autophagosome formation is initiated by the formation and activation of a supramolecular complex containing ATG13, ATG101, RB1 inducible coiled-coil 1 (RB1CC1; best known as FIP200) and unc-51 like autophagy activating kinase 1 (ULK1, the mammalian orthologue of yeast Atg1) at ATG9-containing membranes, followed by ULK1-dependent ATG9 phosphorylation (Joachim *et al*, 2015; Karanasios *et al*, 2016; Orsi *et al*, 2012; Papinski *et al*, 2014; Stanley *et al*, 2014). This event initiates the elongation of pre-autophagosomal membranes upon incorporation of phospholipids from various sources including the endoplasmic reticulum (ER) (Hamasaki *et al*, 2013; Lamb *et al*, 2013) and mitochondria (Hailey *et al*, 2010;

Hamasaki *et al*, 2013), and allows for the recruitment of a multiprotein complex with Class III phosphatidylinositol 3-kinase (PI3K) activity, which contains beclin 1 (BECN1), phosphatidylinositol 3-kinase catalytic subunit type 3 (PIK3C3; best known as VPS34), phosphoinositide-3-kinase regulatory subunit 4 (PI3KR4; best known as VPS15) (Kihara *et al*, 2001a; Kihara *et al*, 2001b), the sensors of membrane curvature ATG14 (best known as ATG14L) (Matsunaga *et al*, 2009; Zhong *et al*, 2009), and nuclear receptor binding factor 2 (NRBF2) (Lu *et al*, 2014a). On activation, VPS34 produces phosphatidylinositol 3-phosphate (PI3P), which further supports the expansion of autophagosomal membranes until closure by engaging PI3P-binding ATG proteins and members of the WIPI family (Proikas-Cezanne *et al*, 2015). Both the ULK1 and autophagy-specific Class III PI3K complexes are highly regulated. One of the main regulators of macroautophagy is MTOR complex 1 (MTORC1), which robustly suppresses autophagosome formation by catalyzing the inactivating phosphorylation of ATG13 and ULK1 (Jung *et al*, 2009; Nazio *et al*, 2013; Nicklin *et al*, 2009). Such an inhibitory network is disrupted upon MTORC1 inactivation by AMP-activated protein kinase (AMPK), which responds to dwindling ATP levels and consequent AMP accumulation (Inoki *et al*, 2002). AMPK also catalyzes the activating phosphorylation of ULK1 (Egan *et al*, 2011; Kim *et al*, 2011; Lee *et al*, 2010) and BECN1 (Kim *et al*, 2013b). In mammalian cells, ULK1 directly phosphorylates BECN1, in thus far resembling AMPK in its VPS34-stimulatory effects (Russell *et al*, 2013). The autophagy-specific Class III PI3K complex is regulated by several interactors, including the VPS34 activator autophagy and beclin 1 regulator 1 (AMBRA1, originally “Activating Molecule in Beclin 1-Regulated Autophagy”), as well as the VPS34 inhibitor BCL2, which also interacts with ATG12 (Fimia *et al*, 2007; Liang *et al*, 1999; Pattingre *et al*, 2005; Rubinstein *et al*, 2011; Zalckvar *et al*, 2009).

Once autophagosomes have enclosed autophagy substrates, they can fuse with late endosomes or lysosomes to form amphisomes or autolysosomes (see below for definitions). The molecular machinery

that is responsible for these fusion events involve dozens of proteins, most of which are shared with the endocytic pathway (Amaya *et al*, 2015; Antonioli *et al*, 2016). In this setting, an important role is mediated by the activation of the GTPase RAB7A, member RAS oncogene family (RAB7A), which is required for autophagosome maturation (Gutierrez *et al*, 2004; Jager *et al*, 2004; Liang *et al*, 2008), the RAB7 effector pleckstrin homology and RUN domain containing M1 (PLEKHM1) (McEwan *et al*, 2015), the PI3P-binding protein tectonin beta-propeller repeat containing 1 (TECPR1) (Chen *et al*, 2012), ectopic P-granules autophagy protein 5 homolog (EPG5) (Tian *et al*, 2010), syntaxin 17 (STX17) and other soluble N-ethylmaleimide-sensitive factor activating protein receptor (SNARE) proteins (Fader *et al*, 2009; Itakura *et al*, 2012; Nair *et al*, 2011), as well as homotypic fusion and vacuole protein sorting (HOPS) complexes (McEwan *et al*, 2015). ATG14L, LAMP2B, LAMP2C (but not LAMP2A) as well as phosphorylated and lipidated LC3 are also involved in the formation of autolysosomes (Diao *et al*, 2015; Eskelinen *et al*, 2005; Nguyen *et al*, 2016; Wilkinson & Hansen, 2015; Wilkinson *et al*, 2015). Degradation of autophagy substrates proceeds as the lysosomal lumen is acidic (owing to the activity of an ATP-dependent proton pump commonly known as V-type ATPase) (Mindell, 2012), upon disassembly of the inner autophagosomal membrane supported by the ATG conjugation systems (Tsuboyama *et al*, 2016). Finally, MTORC1 reactivation inhibits macroautophagy as it promotes so-called “autophagic lysosome reformation” (ALR), a process whereby proto-lysosomal vesicles extruding from autolysosomes mature to regenerate the lysosomal compartment (Yu *et al*, 2010).

Several of the proteins mentioned above including ATG3, ATG5, ATG7, ATG9, ATG13, ATG16L1, ULK1, BECN1, VPS34 and have been considered as strictly required for macroautophagic responses (irrespective of their functions in autophagy-independent processes) (Codogno *et al*, 2012). At least in part, such a view originated from the embryonic or post-natal lethality caused in mice by the genetic ablation of any of these components of the macroautophagy machinery at the whole-body level (Gan *et*



*al*, 2006; Komatsu *et al*, 2005; Kuma *et al*, 2004; Qu *et al*, 2003; Saitoh *et al*, 2009; Saitoh *et al*, 2008; Sou *et al*, 2008), which is likely to reflect the key role of macroautophagy in development and adult tissue homeostasis (although such a general phenotype might also stem from autophagy-independent functions of these proteins). In addition, both pharmacological and genetic interventions targeting these and other components of the macroautophagy apparatus have been associated with autophagic defects in hundreds of experimental settings, *in vitro* and *in vivo*. However, the discovery of *bona fide* macroautophagic responses occurring independently of ATG3, ATG5, ATG7, ULK1, BECN1, VPS34 and its product (PI3P) (Chang *et al*, 2013; Nishida *et al*, 2009; Niso-Santano *et al*, 2015; Vicinanza *et al*, 2015; Zhu *et al*, 2007) casted doubts on the exclusive requirement of these factors for all forms of macroautophagy (Klionsky *et al*, 2016). The existence of ATG3-, ATG5-, ATG7-, ULK1-, BECN1-, VPS34- and PI3P-independent forms of macroautophagy lent further support to the hypothesis that the molecular mechanisms underlying macroautophagic responses exhibit considerable degree of redundancy (at least in mammals) (Chang *et al*, 2013; Chu, 2011; Nishida *et al*, 2009; Niso-Santano *et al*, 2015; Vicinanza *et al*, 2015). This notion had previously been postulated based on the observation that some components of the macroautophagy apparatus have multiple functional homologues. For instance, the human genome codes for at least six distinct Atg8-like proteins, namely, microtubule associated protein 1 light chain 3 alpha (MAP1LC3A; best known as LC3A), LC3B, microtubule associated protein 1 light chain 3 gamma (MAP1LC3C; best known as LC3C), GABA type A receptor-associated protein (GABARAP), GABA type A receptor associated protein like 1 (GABARAPL1), and GABA type A receptor associated protein like 2 (GABARAPL2; best known as GATE-16) (Shpilka *et al*, 2011).

Throughout the past decade, the terms “canonical” and “non-canonical” have been extensively employed to (1) refer to non-degradative functions of macroautophagy (*e.g.*, unconventional secretion) (Ponpuak *et al*, 2015), or (2) discriminate between those macroautophagic responses that critically rely

on ATG3, ATG5, ATG7, ULK1, BECN1 and VPS34-mediated PI3P production and those that do not (Codogno *et al*, 2012; Ktistakis & Tooze, 2016). Although this latter use of the adjectives “canonical” and “non-canonical” may be advantageous as it refers to molecular signatures that are shared by various instances of macroautophagy, we fear that it might be rather misleading, for at least two reasons. First, they implicitly convey the notion that some macroautophagic responses are frequent and observable in many distinct experimental settings, while others are relatively exceptional. The literature describes hundreds of scenarios in which macroautophagy can be slowed-down by the inhibition of ATG3, ATG5, ATG7, ULK1, BECN1, and VPS34-dependent PI3P production, but only a few instances of ATG3-, ATG5-, ATG7-, ULK1-, BECN1-, VPS34- and PI3P-independent macroautophagic responses (Nishida *et al*, 2009; Niso-Santano *et al*, 2015; Vicinanza *et al*, 2015). However, this imbalance might stem from an observational bias linked to the stimuli used to elicit autophagy (starvation, rapamycin or targeted cellular damage) and/or to the biomarkers used so far to monitor macroautophagic responses (such as LC3 lipidation) (Klionsky *et al*, 2016). Second, and perhaps most important, a real consensus on the set of features that would characterize “canonical” versus “non-canonical” macroautophagy has never been reached. Thus, while some authors have used the term “non-canonical” for ATG5-dependent, BECN1-independent cases of macroautophagy (Huang & Liu, 2016; Niso-Santano *et al*, 2015), others have employed the same expression for ULK1-independent, ATG5- and BECN1-dependent macroautophagic responses (Martinez *et al*, 2016). To avoid confusion, we propose to avoid terms such as “canonical” and “non-canonical”. Rather, we encourage the use of explicit expressions such as “ATG5-dependent”, “BECN1-independent” and similar, provided that such a dependence/independence has been experimentally verified. Of note, this recommendation does not intend to imply the existence of distinct pathways that fully depend or not on specific components of the macroautophagy apparatus, but to support the description of a specific instance of macroautophagy based on experimental validation.

As for the definition of *bona fide* macroautophagic responses, relying upon specific components of the underlying molecular apparatus may also be relatively misleading. We propose therefore a functional definition of macroautophagy as a type of autophagic response (*i.e.*, a response that involves the lysosomal degradation of a cytosolic entity, see above) that relies on autophagosomes, which can be subtyped based upon dependence on specific proteins. Recent guidelines provide robust methods to monitor the formation of functional autophagosomes and autophagic flux (Klionsky *et al*, 2016). We surmise that a common molecular signature of macroautophagic responses may be difficult to identify, at least in part owing to the high degree of redundancy and interconnectivity of the process (at least in mammalian cells).

**Autophagic flux.** All forms of autophagy are multistep processes during which autophagy substrates are recognized, isolated (biochemically and/or physically) from the cytoplasmic milieu, and delivered to lysosomes for degradation. In physiological conditions, microautophagy, CMA and macroautophagy proceed at baseline levels, hence contributing to the preservation of cellular homeostasis as they avoid the accumulation of potentially cytotoxic entities that may accumulate as a result of normal cellular functions (*e.g.*, damaged mitochondria) (Cuervo & Wong, 2014; Li *et al*, 2012; Sica *et al*, 2015). In addition, all autophagic pathways described so far are sensitive to perturbations of intracellular or extracellular homeostasis. Thus, stimuli as different as nutritional, metabolic, chemical, physical and hormonal cues can alter (increase or decrease) the ability of microautophagy, CMA and macroautophagy to degrade autophagy substrates (Galluzzi *et al*, 2014; Green & Levine, 2014; Kaur & Debnath, 2015; Mukherjee *et al*, 2016; Tasset & Cuervo, 2016). The rate at which lysosomes degrade autophagy substrates is a good indicator of such a global efficiency in autophagic responses, which is commonly known as “autophagic flux” (Loos *et al*, 2014). The importance of this concept leaps to the

eye upon considering macroautophagic responses and some of the biomarkers that have been employed so far to measure them, such as LC3 lipidation (as monitored by immunoblotting) and the formation of GFP-LC3<sup>+</sup> cytoplasmic dots (as monitored by immunofluorescence microscopy) (Klionsky *et al*, 2016). Both LC3 lipidation and GFP-LC3<sup>+</sup> cytoplasmic dots, indeed, are relatively reliable indicators of the size of the autophagosomal compartment, which is known to expand in the course of productive macroautophagic responses (increased on-rate) (Klionsky *et al*, 2016). However, autophagosomes also accumulate when the formation of autolysosomes or lysosomal degradation is blocked (decreased off-rate), a situation in which autophagy substrates are not disposed of (Boya *et al*, 2005; Gonzalez-Polo *et al*, 2005). Moreover, it cannot be excluded that the autophagosomal compartment also mediates autophagy-independent functions. Although several techniques are currently available to monitor autophagic flux in real-time (Kaizuka *et al*, 2016; Katayama *et al*, 2011), and to discriminate between situations of increased on-rate and situations of decreased off-rate (Klionsky *et al*, 2016), this profound difference should be kept under critical consideration. In summary, the term “autophagic flux” refers to the rate at which the molecular machinery for autophagy identifies, segregates and disposes of its substrates (through lysosomal degradation).

***Autophagy-dependent cell death.*** Since the very beginning of the field, when microscopy was the main (if not the sole) experimental approach for the study of cell biology, scientists have been observing cells that die as they accumulate autophagosomes and autolysosomes in the cytoplasm (Eskelinen *et al*, 2011; Schweichel & Merker, 1973). Morphologically, these cells differ considerably from cells undergoing apoptosis or necrosis (be it regulated or accidental), which led investigators to adopt the term “autophagic cell death” or “type II cell death” based on observational/correlational (rather than interventional/causal) grounds (Kroemer *et al*, 2009; Schweichel & Merker, 1973). With the advent of

modern molecular biology, it has become clear that macroautophagy generally has robust cytoprotective functions in the majority of pathophysiological and experimental settings (Galluzzi *et al*, 2016a; Menzies *et al*, 2015). Indeed, pharmacological inhibitors of macroautophagy as well as genetic interventions targeting various components of the macroautophagy machinery generally accelerate (rather than retard) the demise of cells experiencing perturbations of homeostasis (Boya *et al*, 2005; Merschtik *et al*, 2015; Yousefi *et al*, 2006). Thus, RCD often occurs in the context of failing macroautophagic responses that are activated as an ultimate attempt of the cell to preserve homeostasis (Galluzzi *et al*, 2015a).

Importantly, there are numerous exceptions to this tendency, suggesting that functional macroautophagic responses can also: (1) have little, if any, impact on RCD (so-called “non-protective autophagy”) (Saleh *et al*, 2016); or (2) etiologically contribute to RCD (at least in specific developmental or pathophysiological scenarios) (Denton *et al*, 2015; Masini *et al*, 2009; Seay & Dinesh-Kumar, 2005; Sharma *et al*, 2014). For instance, disrupting any of several *Atg* genes in *D. melanogaster*, as well as blocking autophagy initiation by modulating growth signaling, results in a failure to remove larval salivary gland and midgut tissue during metamorphosis (Berry & Baehrecke, 2007; Denton *et al*, 2013; Denton *et al*, 2009; Xu *et al*, 2015). Interestingly, larval midgut degradation, which occurs independent of caspase-dependent apoptosis, does not require all components of the macroautophagy apparatus involved in starvation-induced autophagy in the *Drosophila* fat body (Xu *et al*, 2015).

Moreover, pharmacological and genetic data indicate that a specific form of autophagy-dependent cell death involving the plasma membrane  $\text{Na}^+/\text{K}^+$ -ATPase (called “autosis”) occurs in cells exposed to nutrient deprivation or a BECN1-derived peptide (Liu *et al*, 2013), as well as in the brain of newborn rodents experiencing ischemia/hypoxia (Liu *et al*, 2013; Xie *et al*, 2016). In summary, autophagy-

dependent cell death can be defined as a form of RCD that can be retarded by pharmacological or genetic inhibition of macroautophagy. In this context, it is important to note that (1) specificity issues affect most, if not all, pharmacological agents employed so far for suppressing macroautophagic responses (Eng *et al*, 2016; Galluzzi *et al*, 2017; Maes *et al*, 2014; Maycotte *et al*, 2012); and (2) multiple components of the macroautophagy machinery have autophagy-independent functions (Hwang *et al*, 2012; Maskey *et al*, 2013). Thus, we recommend to favor genetic approaches and to test the involvement of at least two different proteins of the macroautophagy apparatus in a specific instance of RCD before etiologically attributing it to macroautophagy. Autosis can be functionally defined as a  $\text{Na}^+/\text{K}^+$ -ATPase-mediated form of autophagy-dependent cell death.

***Non-selective and selective types of autophagy.*** Micro- and macroautophagic responses can involve disposable cytoplasmic components in a relatively non-selective manner. Upon lysosomal degradation, these autophagy substrates fuel bioenergetic metabolism or repair processes (Liu *et al*, 2015a; Sica *et al*, 2015). In addition, microautophagy, macroautophagy and CMA can operate in a specific manner, through a mechanism that involves the recognition of autophagy substrates by dedicated receptors (Farre & Subramani, 2016). In this setting, it is useful to remember that the specificity of autophagic responses is highly affected by the mechanisms of substrate delivery to lysosomes. Thus, whereas CMA appears as a highly selective type of autophagy (as it virtually operates only on cytosolic proteins containing KFERQ-like motives bound to HSPA8 and compatible with LAMP2A-mediated translocation), both microautophagy and macroautophagy can exhibit incomplete specificity under specific conditions (reflecting the relatively “leaky” processes of lysosomal invagination and autophagosome formation, respectively) (Sica *et al*, 2015; Zaffagnini & Martens, 2016). This notion should be kept under attentive consideration when specific instances of autophagy (see below) are

measured. The literature offers a collection of articles in which specificity was not addressed, as investigators focused on the degradation of a single substrate (*e.g.*, damaged mitochondria) but did not monitor to which extent other cytoplasmic entities were also degraded. Thus, it may be difficult to differentiate between non-selective micro- or macroautophagic responses and their specific counterparts, especially for some substrates like mitochondria. Indeed, mitophagy (see below for a definition) is arguably the best-characterized form of selective macroautophagy (at least in mammalian cells), but parts of the mitochondrial network are also degraded in the course of macroautophagic responses driven by bioenergetic needs (Gomes *et al*, 2011a; Gomes *et al*, 2011b). We propose to define specific instances of micro- and macroautophagy based on the enrichment of a precise autophagy substrate, coupled to requirement of specific molecular factors (such as autophagy receptors), which may be used to selectively monitor or experimentally manipulate the process.

*Mitophagy* can be defined as the specific removal of damaged or excess mitochondria by micro- or macroautophagy. Microautophagic responses preferentially targeting mitochondria have been observed in yeast cells submitted to nitrogen starvation (Kissova *et al*, 2007). In this system, the microautophagic response depends on SUN family protein UTH1 (Uth1), an integral factor of the inner mitochondrial membrane (Kissova *et al*, 2007). Whether Uth1 is the actual receptor for mitochondrial microautophagy, however, remains to be determined. Conversely, macroautophagic responses specific for mitochondria have been described in a wide panel of model organisms, including yeast, nematodes, flies, and mammals. This process contributes to the removal of superfluous mitochondria that have no functional defects *a priori*, as well as to the degradation of mitochondria that are damaged beyond repair, hence dysfunctional and potentially cytotoxic (which is critical for the maintenance of cellular hemostasis, especially in highly metabolic tissues such as the brain) (Palikaras & Tavernarakis, 2014). Two physiological settings exemplify the macroautophagic removal of functional mitochondria: (1) the maturation of reticulocytes and consequent formation of mature erythrocytes, a setting in which

mitophagy critically relies on BCL2 interacting protein 3 like (BNIP3L; best known as NIX) and the complete removal of mitochondria may also depend on unconventional secretion (Fader *et al*, 2016; ma *et al*, 2012; Mortensen *et al*, 2010; Novak *et al*, 2010; Sandoval *et al*, 2008); (2) the first steps of embryonic development (Al Rawi *et al*, 2011; Sato & Sato, 2011), in which paternal mitochondria undergo fission, mitochondrial 1 (FIS1)-dependent fragmentation (Rojansky *et al*, 2016; Wang *et al*, 2016), lose transmembrane potential (Rojansky *et al*, 2016; Wang *et al*, 2016) and are removed by a mitophagic response depending on endonuclease G (ENDOG; at least in *Caenorhabditis elegans*) (Zhou *et al*, 2016), prohibitin 2 (PHB2) (Wei *et al*, 2017), PTEN induced putative kinase 1 (PINK1), and parkin RBR E3 ubiquitin protein ligase (PARK2) (in mammals, but not in *D. melanogaster*) (Politi *et al*, 2014; Rojansky *et al*, 2016). In this scenario, CPS-6 (the worm orthologue of ENDOG) promotes mitophagy via a poorly characterized mechanism that involves the degradation of the mitochondrial genome (Zhou *et al*, 2016), whereas PHB2 and the PINK1-PARK2 system contribute to the generation of tags recognizable by LC3 or the autophagy receptor sequestosome 1 (SQSTM1, best known as p62), respectively (Geisler *et al*, 2010; Narendra *et al*, 2010; Wei *et al*, 2017).

The selective removal of depolarized mitochondria also involves the PINK1-PARK2 system and PHB2 (Clark *et al*, 2006; Park *et al*, 2006), which generate ubiquitin and non-ubiquitin tags at damaged mitochondrial membranes to allow recognition by p62 (to a limited extent), optineurin (OPTN), calcium binding and coiled-coil domain 2 (CALCOCO2; best known as NDP52) and LC3 (Heo *et al*, 2015; Lazarou *et al*, 2015; Moore & Holzbaur, 2016; Wei *et al*, 2017; Wong & Holzbaur, 2014). Cardiolipin, a mitochondrial lipid has also been proposed to directly interact with LC3 upon mitochondrial damage by a variety of stimuli (Chu *et al*, 2013; Kagan *et al*, 2016). FUN14 domain containing 1 (FUNDC1), a protein of the outer mitochondrial membrane, operates as autophagy receptor in response to hypoxia (Liu *et al*, 2012). Finally, SMAD specific E3 ubiquitin protein ligase 1 (SMURF1), peroxisomal biogenesis factor 3 (PEX3), PEX13, various members of the Fanconi anemia



(FA) protein family and transglutaminase 2 (TGM2) have also been involved in the regulation or execution of mitophagy, although their exact role remains to be elucidated (Lee *et al*, 2016; Orvedahl *et al*, 2011; Rossin *et al*, 2015; Sumpter *et al*, 2016). Atg32 is the main receptor for macroautophagic responses targeting dispensable mitochondria in yeast (Kanki *et al*, 2009; Okamoto *et al*, 2009), and BCL2 like 13 (BCL2L13) has been suggested to play analogous functions in mitophagy in mouse and human cells (Murakawa *et al*, 2015). In *C. elegans*, macroautophagic responses specific for mitochondria are coordinated with mitochondrial biogenesis owing to the coordinated activity of the BNIP3 homologue DCT-1 and the transcription factor SNK-1 (Palikaras *et al*, 2015).

*Pexophagy* is a macroautophagic response preferentially targeting peroxisomes. In yeast, a large supramolecular complex is responsible for the selective recognition of peroxisomes by the molecular machinery for macroautophagy and their actin-dependent transport to the vacuole (Reggiori *et al*, 2005). This complex includes the peroxisomal proteins Pex3 (Burnett *et al*, 2015), Pex14 (Zutphen *et al*, 2008) as well as Atg37 (Nazarko *et al*, 2014), which are bound by Atg30 (Burnett *et al*, 2015), Atg11 (Burnett *et al*, 2015; Torggler *et al*, 2016) and Atg36 (Motley *et al*, 2012; Tanaka *et al*, 2014). In mammalian cells, pexophagy proceeds upon the PEX2- and PEX3-dependent ubiquitination of multiple peroxisomal proteins including PEX5 and ATP binding cassette subfamily D member 3 (ABCD3; best known as PMP70), which are recognized by the autophagy receptors p62 and NBR1 (Deosaran *et al*, 2013; Sargent *et al*, 2016; Yamashita *et al*, 2014). Of note, mammalian pexophagy is highly responsive to oxidative stress, possibly as a consequence of cytoplasmic ATM activation or endothelial PAS domain protein 1 (EPAS1; best known as HIF-2 $\alpha$ ) signaling (Walter *et al*, 2014; Zhang *et al*, 2015). Of note, the selective degradation of peroxisomes in yeast has also been shown to occur through a selective form of microautophagy termed micropexophagy (Farre & Subramani, 2004).

*Nucleophagy* can be defined as an autophagic response selectively targeting portions of the nucleus. In yeast, two distinct forms of nucleophagy have been described: a microautophagic form that relies on the autophagy receptor Nvj1, the vacuolar protein Vac8 and members of the oxysterol-binding protein (OSBP) family (Kvam & Goldfarb, 2004; Roberts *et al*, 2003), which has been dubbed “piecemeal microautophagy of the nucleus”; and a variant that does not require Nvj1, Vac8 but does involve components of the macroautophagy machinery, such as Atg3 and Atg4 (but not Atg6, the yeast orthologue of BECN1) (Krick *et al*, 2008; Mijaljica *et al*, 2012), and the autophagy receptor Atg39 (Mochida *et al*, 2015). Nucleophagy also occurs in mammalian cells (Park *et al*, 2009), in which it contributes to the maintenance of genomic integrity (Dou *et al*, 2015; Rello-Varona *et al*, 2012). Lamin B1 (LMNB1) has been identified as the nuclear protein responsible for a variant of nucleophagy in mammalian cells (Dou *et al*, 2015).

The term *reticulophagy* refers to the preferential autophagic degradation of portions of the ER. According to some authors, reticulophagy (also called ER-phagy) occurs independently of the both the micro- and macroautophagy machinery, at least in yeast (Schuck *et al*, 2014), but is regulated by the Rab family GTPase Ypt1 (Lipatova *et al*, 2013). Recent data, however, support the notion that reticulophagy constitutes a specific form of macroautophagy, which relies on the autophagy receptors Atg39 and Atg40 (in yeast), or their mammalian orthologue family with sequence similarity 134 member B (FAM134B) (in human and mouse cells) (Khaminets *et al*, 2015; Mochida *et al*, 2015). In *S. cerevisiae*, reticulophagy also involves Atg11 (Mochida *et al*, 2015) and Sec63 complex subunit SEC62 (Sec62) (Fumagalli *et al*, 2016).

The term *ribophagy* describes specific autophagic responses targeting ribosomes. In yeast, ribophagy involves macroautophagy, ribosomal de-ubiquitination by the mRNA-binding ubiquitin-specific protease Ubp3 and its cofactors Bre5, Doa1 (also known as Ufd3) and Cdc48 (Kraft *et al*, 2008;

Ossareh-Nazari *et al*, 2010), and requires Atg11 (Waliullah *et al*, 2016). Conversely, the autophagic removal of dispensable ribosomes is negatively regulated by listerin E3 ubiquitin protein ligase 1 (Ltn1)-dependent ubiquitination (Ossareh-Nazari *et al*, 2014), and possibly by NEDD4 family E3 ubiquitin-protein ligase Rsp5 (Shcherbik & Pestov, 2011). Ubp3 has also been involved in the autophagic and proteasomal removal of translation and RNA turnover factors during nitrogen starvation (Kelly & Bedwell, 2015). Interestingly, some plants exhibit a microautophagic variant of ribophagy (Niki *et al*, 2014). To the best of our knowledge, ribophagic responses in mammalian cells have not yet been described.

*Aggrephagy* can be defined as an autophagic response specific for protein aggregates. Aggrephagy has been described in a variety of model organisms, including yeast (Lu *et al*, 2014b), worms (Jia *et al*, 2007), flies (Simonsen *et al*, 2008), plants (Toyooka *et al*, 2006) and mammals (Bjorkoy *et al*, 2005; Hara *et al*, 2006; Komatsu *et al*, 2006). The macroautophagic disposal of protein aggregates is particularly relevant for the preservation of cellular homeostasis, especially in the context of neurodegenerative disorders (Menzies *et al*, 2015). Besides relying on the macroautophagy machinery and often on substrate ubiquitination, mammalian aggrephagy involves the autophagy receptors p62 (which can form insoluble aggregates itself) (Bjorkoy *et al*, 2005; Kirkin *et al*, 2009b; Komatsu *et al*, 2007; Pankiv *et al*, 2007), NBR1 (an orthologue of which participates in plant aggrephagy) (Kirkin *et al*, 2009a; Kirkin *et al*, 2009b), OPTN (Korac *et al*, 2013), and toll interacting protein (TOLLIP) (Lu *et al*, 2014b), as well as the p62-binding proteins WD repeat and FYVE domain containing 3 (WDFY3; best known as ALFY) (Filimonenko *et al*, 2010; Simonsen *et al*, 2004) and TGM2 (D'Eletto *et al*, 2012). However, it is worth noting that the redundancy between these factors and their specific roles in the degradation of different substrates has not been extensively explored. In yeast, the ubiquitin-binding protein Cue5 (the orthologue of mammalian TOLLIP) operates as autophagy receptor for aggrephagic responses (Lu *et al*, 2014b). In *D. melanogaster* the control of proteostasis by aggrephagy

impinges on forkhead box, sub-group O (FOXO)-dependent transcription (Demontis & Perrimon, 2010). Importantly, LC3 can accumulate at protein aggregates in a p62-dependent but autophagosome-independent manner (Kuma *et al*, 2007; Shvets & Elazar, 2008). This adds to the potential sources of bias deriving from the use of GFP-LC3 aggregation as a standalone biomarker for macroautophagy (see above). HSPA8 as well as other chaperones and cochaperones have been involved in a specific form of aggrephagy commonly known as “chaperone-assisted selective autophagy” (CASA) (Arndt *et al*, 2010). CASA differs from endosomal microautophagy and CMA in its dependence on multiple components of the macroautophagy apparatus, *de facto* constituting a selective form of macroautophagy (Arndt *et al*, 2010).

The term *lipophagy* describes the selective autophagic degradation of neutral lipid droplets. Originally discovered in the mammalian system, where it involves the molecular machinery for macroautophagy (Singh *et al*, 2009), lipophagy also occurs in yeast, in which it involves a microautophagic process (Vevea *et al*, 2015; Wang *et al*, 2014). However, there are contradicting reports on the molecular requirements for *S. cerevisiae* lipophagic responses to intracellular lipid accumulation (Vevea *et al*, 2015; Wang *et al*, 2014). Thus, while some authors propose that lipophagy in yeast does not involve Atg7 but requires ESCRT components (Vevea *et al*, 2015), other authors privilege the interpretation that lipophagic responses in yeast depends on Atg7 and several other components of the macroautophagy machinery (even though it manifests with a microautophagic appearance and proceeds in the absence of autophagosomes) (Wang *et al*, 2014). In mammalian cells, lipophagy is coordinated by transcriptional programs depending on nuclear receptor subfamily 1 group H member 4 (NR1H4; best known as FXR), cAMP responsive element binding protein 1 (CREB) and peroxisome proliferator activated receptor alpha (PPARA) (Lee *et al*, 2014; Seok *et al*, 2014). Interestingly, the CMA-dependent degradation of lipid droplet-associated proteins such as perilipin 2 (PLIN2) and PLIN3 precedes and facilitates lipolysis (Kaushik & Cuervo, 2015; Kaushik & Cuervo, 2016), demonstrating

the existence of an intimate crosstalk between different forms of autophagy in the control of intracellular homeostasis. Moreover, *bec-1* (the worm orthologue of *BECN1*) is required for the accumulation of neutral lipids in the intestine of developing *C. elegans* (Lapierre *et al*, 2013), pointing to a broader implication of autophagy in systemic lipid homeostasis.

*Bacterial xenophagy* is the macroautophagic removal of cytoplasmic bacteria, *i.e.*, bacteria that escape the phagosomal compartment upon phagocytosis, and damaged bacteria-containing phagosomes. As mentioned above, bacterial xenophagy must be conceptually discriminated from efficient phagocytosis, a setting in which bacteria never gain direct access to the cytosolic milieu (Huang & Brumell, 2014). Xenophagic responses targeting bacteria constitute a first, cell-autonomous line of innate defense against prokaryotic infections (Deretic *et al*, 2013). Accordingly, multiple bacteria have evolved strategy to actively inhibit autophagic responses in the host (Galluzzi *et al*, 2017). In mammalian cells, cytoplasmic bacteria are rapidly recognized by multiple autophagy receptors including p62, OPTN and NDP52, via a mechanism that relies on receptor phosphorylation by TANK1-binding kinase 1 (TBK1) (Thurston *et al*, 2009; Wild *et al*, 2011) and ubiquitination by ring finger protein 166 (RNF166) (Heath *et al*, 2016). Additional proteins that direct the formation and expansion of autophagosomes to sites of bacterial invasions include (but may not be limited to) WD repeat domain, phosphoinositide interacting 2 (WIPI2) and its interactor TECPR1, which are recruited in a TBK1-dependent manner (Ogawa *et al*, 2011; Thurston *et al*, 2016), as well as the pattern recognition receptors nucleotide binding oligomerization domain containing 1 (NOD1) and NOD2, which physically interact with ATG16L1 and immunity related GTPase M (IRGM) upon recognition of bacterial muramyl dipeptide (Chauhan *et al*, 2015; Cooney *et al*, 2010; Travassos *et al*, 2010). Besides operating as a receptor for the recruitment of forming autophagosomes to invading bacteria, NDP52 supports autophagosome maturation upon interaction with LC3A, LC3B, LC3C, GABARAPL2 and myosin VI (MYO6) (Verlhac *et al*, 2015; von Muhlinen *et al*, 2012). Ubiquitin D (UBD; best known as FAT10) has also been involved in the

rapid and transient recognition of phagosome-escaping bacteria, and FAT10 deficiency has been associated with increased susceptibility to *Salmonella typhimurium* infection in mice (Spinnenhirn *et al*, 2014). The molecular mechanisms through which FAT10 supports xenophagy, however, remain to be clarified. Interestingly, xenophagic responses targeting damaged phagosomes and their bacterial cargo have been described. This particular variant of xenophagy relies on galectin 8 (LGALS8) or galectin 3 (LGALS3), both of which tag damaged endosomes (Chauhan *et al*, 2016), as well as on NDP52 (Kim *et al*, 2013a; Li *et al*, 2013; Thurston *et al*, 2012) and/or various members of the TRIM protein family as receptors or receptor regulators (see below for a definition) (Kimura *et al*, 2015; Kimura *et al*, 2016). Although xenophagic responses have mainly been studied in the mammalian system, there are *bona fide* instances of xenophagy in *D. melanogaster*, in which it also operates at the boundary of innate pattern recognition (Kim *et al*, 2012; Wu *et al*, 2007; Yano *et al*, 2008), *C. elegans* (Jia *et al*, 2009; Zou *et al*, 2014), and *Dictyostelium discoideum* (Jia *et al*, 2009).

*Viral xenophagy (virophagy)* is a macroautophagic response targeting fully formed cytoplasmic virions or components thereof. The first description of endogenous membranes engulfing cytoplasmic viruses dates back to the late 1990s (Schlegel *et al*, 1996), and it is now clear that virophagy occupies a position similar to that of bacterial xenophagy in the first line of defense against pathogens (Paul & Munz, 2016). In line with this notion, several defects in the molecular machinery for macroautophagy – such as the genetic inhibition of *Atg5* in mice – render animals more susceptible to succumb to infection (Orvedahl *et al*, 2010). This holds true not only in mammalian systems, but also in plants (Liu *et al*, 2005), flies (Moy *et al*, 2014; Nakamoto *et al*, 2012) and perhaps nematodes (Bakowski *et al*, 2014). Moreover, HIV-1<sup>+</sup> patients who remain clinically stable for years in the absence of therapy (so-called “long term non-progressors”) display high baseline levels of autophagy in peripheral blood mononuclear cells (Nardacci *et al*, 2014). Accordingly, multiple viruses have evolved strategies to avoid virophagic responses, including the expression of BECN1 inhibitors (Orvedahl *et al*, 2007) or

proteins that inhibit the autophagosomal-lysosomal fusion (Gannage *et al*, 2009). Besides relying on the core macroautophagy machinery, efficient virophagic responses involve p62 and tripartite motif containing 5 (TRIM5) as receptors (Mandell *et al*, 2014; Orvedahl *et al*, 2010), proteins that participate in mitophagy, such as SMURF1 (Orvedahl *et al*, 2011), Fanconi anemia complementation group C (FANCC) (Sumpter *et al*, 2016) and PEX13 (Lee *et al*, 2016), as well as the phosphorylation of eukaryotic translation initiation factor 2A (EIF2A) (Talloczy *et al*, 2002).

The term *lysophagy* refers to the specific macroautophagic disposal of damaged lysosomes in mammalian cells. Several lysosomotropic agents as well as monosodium urate (MSU) and silica have been shown to promote lysosomal damage followed by ubiquitination and recruitment of the macroautophagy machinery (Hung *et al*, 2013; Maejima *et al*, 2013), a process that may be directed by the common marker of endovesicular damage LGALS3 (Kawabata & Yoshimori, 2016). Most of the molecular details underlying lysophagy, however, remain to be determined. Similarly, if and how a lysophagy-like mechanism contributes to the preservation of vacuolar homeostasis in yeast and plants remains obscure.

The expression *proteaphagy* has recently been coined to indicate macroautophagic responses specific for inactive proteasomes. In *Arabidopsis thaliana*, proteaphagy relies on the proteasomal component regulatory particle non-ATPase 10 (RPN10), which operates as a *bona fide* autophagy receptor to bridge ubiquitinated proteasome subunits to ATG8 (Marshall *et al*, 2015). In yeast, Rpn10 is dispensable for proteaphagy (Waite *et al*, 2016) but a similar function is mediated by Cue5 (Marshall *et al*, 2016), drawing an interesting parallelism with aggrephagy (see above). Besides involving Atg7, optimal proteaphagic responses in *S. cerevisiae* rely on the co-chaperone Hsp42 (Marshall *et al*, 2016). Thus, it is tempting to speculate that the macroautophagic disposal of inactive proteasomes may

proceed upon their accumulation in aggregates, at least in yeast. In mammalian cells, p62 is the main receptor for proteaphagy (Cohen-Kaplan *et al*, 2016).

*Other specific forms of autophagy.* Additional instances of selective macroautophagy have been described, mostly based on cargo selectivity. These include (but are likely not limited to): *myelinophagy* (targeting myelin in Schwann cells) (Gomez-Sanchez *et al*, 2015), *zymophagy* (targeting secretory granules in pancreatic  $\beta$  cells) (Grasso *et al*, 2011), and *granulophagy* (targeting stress granules) (Buchan *et al*, 2013). Finally, macroautophagy has been involved in the degradation of specific proteins owing to their ability to physically interact with members of the Atg8 protein family. This applies, for instance, to the centriole and centriolar satellite protein OFD1, whose degradation by macroautophagy has a major impact on the regulation of ciliogenesis (Tang *et al*, 2013). A term to indicate such a protein-specific variant of macroautophagy has not yet been proposed.

***Cytoplasm-to-vacuole targeting pathway.*** The cytoplasm-to-vacuole targeting (Cvt) pathway delivers hydrolases including aminopeptidase 1 (Ape1), Ape4 and alpha-mannosidase (Ams1) to the yeast vacuole (Umekawa & Klionsky, 2012). The molecular machineries for the Cvt pathway and macroautophagy share a large number of components, including several Atg proteins (Scott *et al*, 2001; Scott *et al*, 1996; Scott *et al*, 2000). Moreover, Ape1, Ape4 and Ams1 are imported into the vacuole as large oligomers, being reminiscent of the substrates of aggrephagy (Bertipaglia *et al*, 2016). The Cvt pathway, however, contributes to the preservation of normal enzymatic activity within the vacuole, especially in vegetative conditions, *de facto* mediating biosynthetic, rather than catabolic, functions (Umekawa & Klionsky, 2012). Thus, the Cvt pathway does not represent an instance of autophagy *sensu stricto*.



***LC3-associated phagocytosis.*** LC3-associated phagocytosis (LAP) describes the recruitment of some (but not all) components of the macroautophagy apparatus (notably, LC3) to single-membraned phagosomes that contain extracellular pathogens or dead cell corpses destined to lysosomal degradation (Martinez *et al*, 2016; Martinez *et al*, 2015; Sanjuan *et al*, 2007). Multiple molecular determinants of LAP are also required for macroautophagic responses. This applies to ATG3, ATG5, ATG7, ATG12, ATG16L1, BECN1, VPS34 and UVRAG (Martinez *et al*, 2016; Martinez *et al*, 2015). However, in the mammalian systems investigated thus far, LAP does not involve ULK1 signaling, AMBRA1 and ATG14L (which are also involved in macroautophagy), but critically depends on LC3- and RUN and cysteine rich domain containing beclin 1 interacting protein (RUBCN) and NADPH oxidase 2 (which are dispensable for macroautophagy). LAP has been involved in the control of bacterial and fungal pathogens (Choi *et al*, 2014; Gong *et al*, 2011; Lam *et al*, 2013; Martinez *et al*, 2015; Sanjuan *et al*, 2007; Selleck *et al*, 2015; Zhao *et al*, 2008), in entosis (a variant of RCD that ensues engulfment by non-phagocytic cells) (Florey *et al*, 2011), as well as in the optimal disposal of dead cells (Martinez *et al*, 2016). However, since the substrates of LAP are extracellular entities, it cannot be considered as a *bona fide* autophagic response.

***Secretory autophagy.*** Multiple components of the molecular apparatus for macroautophagy including (but presumably not limited to) ATG4B, ATG5, ATG7, ATG16L1, BECN1, ULK1, LC3, p62, some SNAREs and specific members of the TRIM protein family also participate in the conventional or unconventional secretion of cytoplasmic entities (including soluble proteins with extracellular functions, potentially cytotoxic protein aggregates, secretory granules and invading pathogens) (Dupont *et al*, 2011; Gerstenmaier *et al*, 2015; Kimura *et al*, 2017; Lock *et al*, 2014; Manjithaya *et al*, 2010;

Shravage *et al*, 2013), which led to the introduction of the term “secretory autophagy” (Ponpuak *et al*, 2015). Although these non-degradative functions of the macroautophagy machinery are essential for multiple intracellular and organismal processes, including viral clearance, inflammation and hematopoiesis, they should not be considered as *bona fide* autophagic responses. Along these lines, we encourage the use of molecularly oriented expressions such as “ATG5-dependent secretion” over potentially misleading terms including “secretory autophagy”.

***Crinophagy.*** The term crinophagy refers to the degradation of secretory material ensuing the fusion of secretory granules with lysosomes (Marzella *et al*, 1981). This process, which has been observed in all secretory cells and is distinct from zymophagy, ensures degradation and recycling of excess/obsolete secretory granules, for instance those that persist after a hormone-induced wave of secretion is over (Weckman *et al*, 2014). Strictly speaking, crinophagy should not be considered as a form of autophagy as the content of secretory granules is not accessible from the cytoplasm (it is contained in secretory granules, similar to endosomal or phagosomal cargoes).

## Components of the autophagy machinery

***Autophagy substrates (autophagy cargo).*** The terms autophagy substrates and autophagic cargo can be interchangeably used to describe the cytoplasmic entities targeted to lysosomal degradation by autophagy. From a conceptual standpoint, autophagy substrates should be differentiated from autophagy receptors (see below). Indeed, both autophagy substrates and receptors are subjected to lysosomal degradation, but only the latter function as part of the autophagy apparatus (Boya *et al*, 2013; Noda & Inagaki, 2015; Zaffagnini & Martens, 2016). Of note, neither hydrolytic enzymes delivered to the vacuole via Cvt, nor extracellular entities reaching lysosomes via the endocytic pathway can be considered as *bona fide* autophagy substrates.

***Autophagy receptors and adaptors.*** The expression *autophagy receptor* refers to any of the proteins that bind autophagy substrates, allow for their recognition by the autophagy machinery, and get degraded within lysosomes in the course of functional autophagic responses (Stolz *et al*, 2014). Based on this definition, HSPA8 is the main receptor for endosomal microautophagy but not for CMA (during CMA, the cytoplasmic pool of HSPA8 is not degraded) (Morozova *et al*, 2016; Uytterhoeven *et al*, 2015). In addition, dozens of proteins have been involved in the recognition of macroautophagy substrates (see above) (Farre & Subramani, 2016; Rogov *et al*, 2014). Most receptors for macroautophagy share an evolutionary conserved LC3-interacting region (LIR), which allows them to bring macroautophagy substrates in the proximity of LC3<sup>+</sup> forming autophagosomes. This applies to p62, NBR1, OPTN, NDP52, BNIP3, BNIP3L, ATG34, FUNDC1, PHB2, Atg19 and Atg32 (Birgisdottir *et al*, 2013; Chourasia *et al*, 2015; Wei *et al*, 2017). Many macroautophagy receptors also contain ubiquitin-binding domains, allowing them to recruit ubiquitinated substrates to forming autophagosomes (Khaminets *et al*, 2016). Recent data indicate that some receptors including yeast

Atg19 and Atg34 as well as human p62, OPTN and NDP52 also bind to the Atg12-Atg5-Atg16 (ATG12-ATG5-ATG16L1) complex to stimulate conjugation of Atg8 family members at the autophagic cargo (Fracchiolla *et al*, 2016). Along similar lines, multiple members of the TRIM protein family not only target autophagy substrates to forming autophagosomes upon LC3 binding, but also physically and functionally interact with upstream components of the autophagy apparatus, including the ULK1 and VPS34 complexes (Kimura *et al*, 2015; Kimura *et al*, 2016). These proteins have been dubbed “receptor regulators”. It cannot be excluded that other autophagy receptors might have regulatory functions besides cargo recognition. Finally, *autophagy adaptors* are proteins that interact with Atg8 family members but are not involved in cargo recognition (and hence not degraded during macroautophagic responses) (Stolz *et al*, 2014). Two examples of autophagy adaptors outside of the ATG protein family (many members de facto behave as adaptors) are FYVE and coiled-coil domain containing 1 (FYCO1), which is involved in the interaction of autophagosomes with the cytoskeleton, and sorting nexin 18 (SNX18), which participates in autophagosome formation and autophagosome-lysosome fusion (Knaevelsrud *et al*, 2013; Olsvik *et al*, 2015).

***Phagophores (isolation membranes).*** Phagophores (also called isolation membranes in yeast) are the precursors of autophagosomes. Mammalian phagophores generally form near ER-mitochondria contact sites in the context of peculiar structures staining positively for zinc finger FYVE-type containing 1 (ZFYVE1; best known as DFCP1) known as omegasomes (Axe *et al*, 2008). In mammals, phagophore biogenesis has been suggested to involve ATG9-containing vesicles that derive from the Golgi apparatus, late endosomes or the plasma membrane (Orsi *et al*, 2012; Puri *et al*, 2013; Ravikumar *et al*, 2010). Irrespective of the exact source of lipids (which remains a matter of debate), forming mammalian phagophores recruit the ULK1 complex and ATG14L (Karanasios *et al*, 2013), which

facilitates the assembly of the autophagy-specific class III PI3K complex (Matsunaga *et al*, 2010), and the consequent association of the PI3P-binding proteins DFCP1 and WIPI2 (Polson *et al*, 2010), ATG12-ATG5-ATG16L1 complexes, followed by local LC3 lipidation (Dooley *et al*, 2014). Thus, both mammalian phagophores and omegasomes stain positively for ULK1, ATG13, ATG101, FIP200, VPS34, BECN1, VPS15, ATG5, ATG12, ATG16L1, DFCP1 as well as for lipidated LC3 family members (Antonoli *et al*, 2016). In yeast, phagophores are formed at the so-called phagophore-assembly site or pre-autophagosomal structure (PAS), *i.e.*, a site within the cytoplasm enriched in Atg9<sup>+</sup> vesicles with a diameter of 30-60 nm that fuse together owing to the tethering activity of Atg1 (the yeast counterpart of ULK1), Atg13, Atg17, Atg19 and Atg31 (Stanley *et al*, 2014; Yamamoto *et al*, 2012).

**Autophagosomes.** Transient, double-membraned organelles (mean diameter in mammals 0.5-1.5  $\mu$ m) that mediate cargo sequestration and delivery to lysosomes in the course of macroautophagic responses (Shibutani & Yoshimori, 2014). Autophagosomes originate from, and hence share some biomarker proteins with, closing phagophores (see above). Since autophagosomes are devoid of hydrolytic activity, both ubiquitinated and non-ubiquitinated autophagy substrates, as well as autophagy receptors, can be detected in this compartment (Klionsky *et al*, 2016). Moreover, LC3 is abundant at both the inner and outer membrane of autophagosomes (Lamb *et al*, 2013). In the course of functional macroautophagic responses, autophagosomes rapidly fuse with late endosomes or lysosomes (see below), and hence may be difficult to detect as a stable pool. This can be experimentally circumvented by inhibiting fusion or lysosomal acidification (Klionsky *et al*, 2016).

***Amphisomes.*** Single or double-membraned organelles that originate from the fusion of autophagosomes and (late) endosomes (Gordon & Seglen, 1988). Amphisomes contain common autophagosomal markers including lipidated LC3, as well as classical endosomal markers like RAB5, RAB7 and RAB11 (the latter of which is also required for autophagosome formation) (Chandra *et al*, 2015; Fader *et al*, 2009). Moreover, amphisomes have been proposed to contain small amounts of the lysosomal V-type ATPase, which would be responsible for progressive acidification of their lumen (Bader *et al*, 2015).

***Autolysosomes.*** Single-membraned organelles that form in the course of macroautophagy upon fusion of autophagosomes or amphisomes and lysosomes (Klionsky *et al*, 2014). Autolysosomes are positive for lysosomal enzymes and classical endo/lysosomal markers, including LAMP1, LAMP2 and the V-type ATPase, but may display low levels of autophagosomal markers such as lipidated LC3, especially if autophagic flux is high (and unless lysosomal hydrolases are pharmacologically or genetically inhibited) (Klionsky *et al*, 2014). Along similar lines, autophagic substrates and receptors are rapidly degraded within autolysosomes in conditions of elevated autophagic flux, implying that it may be difficult to reveal their presence in this compartment. Once the degradation of autophagy cargos is completed, autolysosomes contribute to the regeneration of the lysosomal pool via ALR (see above) (Yu *et al*, 2010). Of note, the term *autophagolysosome* indicates a specific type of autolysosome that forms in the course of some xenophagic responses (Klionsky *et al*, 2014). In this setting, autophagosomes can engulf entire phagosomes in the absence of membrane fusion, followed by the delivery of a double-membraned cargo (secluded by the inner autophagosomal membrane plus the phagosomal membrane) to lysosomes (Klionsky *et al*, 2014). We support the proper semantic and conceptual discrimination between autolysosomes and autophagolysosomes, and at the same time

discourage the incorrect use of these terms as interchangeable synonyms (which is rather common in the literature).

## Concluding remarks

Throughout the past two decades, our understanding of autophagy in mechanistic and pathophysiological terms has progressed tremendously. In parallel, we unveiled a considerable therapeutic potential for molecules that target autophagy and autophagy-related processes such as LAP. Such a potential remains largely unexploited in the clinic, for reasons that relate to the complex nature of autophagic responses themselves, to the specificity of pharmacological agents developed so far, to the limitations of currently available models, as well as to the imprecise use of autophagy-related terms. Here, we attempted to provide semantic and conceptual recommendations that may help with this latter issue, at least to some extent. Our aim is not to provide a rigid vocabulary, but a working framework that can be revised and modified as the field evolves to address the current outstanding questions (Lindqvist *et al*, 2015) (**Figure 1**). These recommendations are intended to facilitate the dissemination of results and ideas within and outside the field, and eventually benefit scientific progress in this and other areas of biological/biomedical investigation.

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**Table 1. Main autophagy-related proteins in common model organisms\***

<i>Homo sapiens</i>	<i>Mus musculus</i>	<i>Drosophila melanogaster</i>	<i>Caenorhabditis elegans</i>	<i>Saccharomyces cerevisiae</i>
ACBD3 (PMP70)	ACBD3	Pmp70	PMP-2	-
ACBD5	ACBD5	-	-	Atg37
AMBRA1	AMBRA1	-	-	-
ATG2A, ATG2B	ATG2A, ATG2B	Atg2	ATG-2	Atg2
ATG3	ATG3	Atg3	ATG-3	Atg3
ATG4A, ATG4B, ATG4C, ATG4D	ATG4A, ATG4B, ATG4C, ATG4D	Atg4a, Atg4b	ATG4.1, ATG-4.2	Atg4
ATG5	ATG5	Atg5	ATG-5	Atg5
ATG7	ATG7	Atg7	ATG-7	Atg7
ATG9A, ATG9B	ATG9A, ATG9B	Atg9	ATG-9	Atg9
ATG10	ATG10	Atg10	ATG-10	Atg10
ATG12	ATG12	Atg12	LGG-3	Atg12
ATG13	ATG13	Atg13	EPG-1 (ATG-13)	Atg13
ATG14L	ATG14L	Atg14	EPG-8	Atg14
ATG16L1	ATG16L1	Atg16	ATG-16.1, ATG-16.2	Atg16
ATG101	ATG101	Atg101	EPG-9	-
BCL2	BCL2	Debcl	CED-9	-
BCL2L13	BCL2L13	-	-	-
BECN1	BECN1	Atg6	BEC-1	Atg6
BNIP3 (NIP3)	BNIP3	-	DCT-1	-
BNIP3L (NIX)	BNIP3L	-	-	-
CALCOCO2 (NDP52)	CALCOCO2 (NDP52)	-	-	-
-	-	-	EPG-2	-
EI24 (EPG4)	EI24 (EPG4)	-	EPG-4	
EPG5	EPG5	Epg5	EPG-5	-
ENDO G	ENDO G	EndoG, Tengl1, Tengl2, Tengl3, Tengl4	CPS-6	Nuc1
FAM134B	FAM134B			Atg40
FANCC	FANCC	-	-	-
FUNDC1	FUNDC1	-	T06D8.7	-
GFAP	GFAP	-	-	-
HSP90AA1	HSP90AA1	Hsp83	DAF-21	Hsc82, Hsp82
HSPA8 (HSC70)	HSPA8 (HSC70)	Hsc70-1, Hsc70-2, Hsc70-3, Hsc70-4, Hsc70-5, Hsc70-6,	HSP-70	Ssa1, Ssa2, Ssa3, Ssa4

Hsc70Cb				
LAMP1	LAMP1	Lamp1	LMP-1, LMP-2	-
LAMP2	LAMP2	-	-	-
LGALS3	LGALS3	-	-	-
LGALS8	LGALS8	-	-	-
MAP1LC3A, MAP1LC3B, MAP1LC3C, GABARAP, GABARAPL1, GABARAPL2	MAP1LC3A, MAP1LC3B, MAP1LC3C, GABARAP, GABARAPL1, GABARAPL2	Atg8a, Atg8b	LGG-1, LGG-2	Atg8
MTOR	MTOR	Tor	LET-363	Tor1
NBR1	NBR1	-	-	-
NRBF2	NRBF2	-	-	Atg38
OPTN	OPTN	-	-	-
PARK2	PARK2	park	PDR-1	
PEX2	PEX2	Pex2	PRX-2	Pex2
PEX3	PEX3	Pex3	PRX-3	Pex3
PEX5	PEX5	Pex5	PRX-5	Pex5
PEX13	PEX13	Pex13	PRX-13	Pex13
PEX14	PEX14	Pex14	PRX-14	Pex14
PHB2	PHB2	Phb2	PHB-2	Phb2
PIK3C3 (VPS34)	PIK3C3 (VPS34)	Pi3K59F	VPS-34	Vps34
PIK3R4 (VPS15)	PIK3R4 (VPS15)	Vps15	VPS-15	Vps15
PINK1	PINK1	Pink1	PINK-1	-
PLEKHM1	PLEKHM1	-	Y51H1A.2	-
PSMD4 (RPN10)	PSMD4 (RPN10)	Rpn10	RPN-10	Rpn10
RAB7A, RAB7B	RAB7A, RAB7B	Rab7	RAB-7	Ypt7 (Rab7)
RAB11A	RAB11A	Rab11	RAB-11.1, RAB-11.2	Ypt31, Ypt32
RB1CC1 (FIP200)	RB1CC1	Atg17	EPG-7	Atg11, Atg17
RNF166	RNF166	-	-	-
RUBCN	RUBCN	-	-	-
SMURF1	SMURF1	Smurf	-	-
SNX4	SNX4			Snx4 (Atg24)
SQSTM1 (p62)	SQSTM1 (p62)	ref(2)P	SQST-1, SQST-2, SQST-3, SQST-4	-
STX17	STX17	Syx17	VF39H2L.1	-
TBK1	TBK1	LOC108141996	-	-
TECPR1	TECPR1	-	-	-
TGM2	TGM2	Tg	-	-
TOLLIP	TOLLIP	-	TLI-1	Cue5

TRIM5	TRIM5	-	-	-
ULK1 (ATG1), ULK2	ULK1 (ATG1), ULK2	Atg1	UNC-51	Atg1
UVRAG	UVRAG	Uvrag	T23G11.7, Y34BA.2	Vps38
VCP	VCP	TER94	CDC-48.1, CDC-48.2	Cdc48
WDFY3 (ALFY)	WDFY3 (ALFY)	bchs	WDFY-3	-
WIPI1, WIPI2, WDR45B (WIPI3), WDR45 (WIPI4)	WIPI1, WIPI2, WDR45B (WIPI3), WDR45 (WIPI4)	Atg18a, Atg18b	ATG-18, EPG-6	Atg18, Atg21
VMP1	VMP1	-	EPG-3	-
WAC	WAC	Wac	-	-
ZFYVE1 (DFCP1)	ZFYVE1 (DFCP1)	-	-	-

Yeast proteins with no known orthologues in *C. elegans*, *D. melanogaster*, *M. musculus* or *H. sapiens*: Atg19, Atg20, Atg23, Atg26, Atg27, Atg29, Atg30, Atg31, Atg32, Atg33, Atg34, Atg36, Atg39, Bre5, Doa1, Hsp104, Ubp3, Uth1.

\*Excluding non-coding pseudogenes, as per <https://www.ncbi.nlm.nih.gov/gene/>; common aliases are indicated between brackets.

## Legends to Figures

**Figure 1. Outstanding questions in autophagy research.** Besides clarifying the source of lipids for the formation of the phagophore (P) and the autophagosome (AP), an issue that remains matter of debate amongst investigators focusing on macroautophagy, it will be important to determine which cellular and/or organismal functions are modulated by (1) microautophagy, chaperone-mediated autophagy (CMA) or macroautophagy as entire processes, (2) the supramolecular complexes that are responsible for these pathways of lysosomal degradation, and (3) the single proteins that build up such complexes. Moreover, it will be important to elucidate the mechanisms that underlie microautophagic responses in eukaryotic cells, as well as the spatial and temporal coordination between the molecular and supramolecular entities that drive autophagic responses. L, lysosome.

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