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 about 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. Driven by the overt recognition of such a potential obstacle, a panel of leading experts in the field attempts here to define 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.

Keywords | chaperone-mediated autophagy; LC3-associated phagocytosis; microautophagy; mitophagy; xenophagy

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Introduction

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 particular, AuTophaGy-related (Atg) genes, 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 employed the term autophagy (from the Ancient Greek ēυσφόρoς, 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). In parallel, 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 (Galluzzi et al, 2015b, 2017a; Amaravadi et al, 2016), as well as aging (Melendez et al, 2003; Lapierre et al, 2015; Lopez-Otín et al, 2016). 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 a barrier 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 done elsewhere (Galluzzi et al, 2017b). 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 experience in the field. Although such a semantic issue may appear trivial at first glance, we are concerned that it may constitute a significant obstacle to the optimal development of autophagy research, both at preclinical and translational levels. This problem has been overtly recognized and discussed throughout the past year. Starting from such a constructive exchange and driven by the success obtained by a similar initiative in the cell death field (Galluzzi et al, 2012, 2015a), 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 agree on two main features that characterize bona fide, functional autophagic responses, irrespective of type: (i) they involve cytoplasmic material; and (ii) 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 in order to discriminate autophagic responses from branches of vesicular trafficking that originate at the plasma membrane, which also culminates 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) (Munz, 2017; Foot et al, 2017). However, some forms of autophagy (notably macroautophagy and endosomal microautophagy, see below for definitions) and the endocytic pathway interact at multiple levels, and the molecular machinery responsible for the fusion of late endosomes (also known as multi-vesicular 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 (see below for a definition) (Lim & Yue, 2015; Moscat et al, 2016). Moreover, considerable 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 the main chaperone in CMA, can be efficiently redirected to proteasomal degradation upon interaction with ubiquitin 2 (UBQLN2) (Hjerpe et al, 2016). Thus, the proteasome system shares some substrates with different forms of autophagy. However, these two catabolic pathways differ radically in their final products. Proteasomal degradation results in short peptides (of 8–12 residues) that are not necessarily degraded further, but may feed into additional processes including (but not limited to) antigen presentation at the plasma membrane (Neefjes et al, 2011). In contrast, lysosomal proteases fully catalyze 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 and endosomal microautophagy

Microautophagy is a form of autophagy during which cytoplasmic entities destined for degradation are directly taken up by the vacuole (in yeast and plants) via direct membrane invagination (Farre & Subramani, 2004; Utenweiler & Mayer, 2008). In cells from Drosophila melanogaster and mammals, a similar mechanism involves late endosomes. This process, which also occurs in yeast cells, is commonly known as “endosomal microautophagy” (Sahu et al, 2011; Uyttewaal et al, 2015; Mukherjee et al, 2016). In yeast, microautophagy has been involved in the degradation of multiple substrates, including peroxisomes (a process called “micropexophagy”, historically the first form of yeast 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) (Sahu et al, 2011; Uyttewaal et al, 2015; Mukherjee et al, 2016). Of note, some proteins internalized by multivesicular bodies through
direct membrane invagination can be spared from degradation and released in the extracellular microenvironment 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 the 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 (Sahu et al., 2011; Liu et al., 2015b; Uytterhoeven et al., 2015; Mukherjee et al., 2016). 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 phosphatidylinerine on (and hence deform) the outer endosomal membrane (Uytterhoeven et al., 2015; Morozova et al., 2016). Along similar lines, chaperone ATPase HSP104 (Hsp104) reportedly underlies microautophagic responses to lipid droplets in Saccharomyces cerevisiae (Veeva et al., 2015). However, the strict requirement of chaperones from the HSP70 protein family in other variants of microautophagy has not yet been documented. Of note, the yeast orthologue of mammalian NBR1, autophagy cargo receptor (NBR1; which is known to operate as a macroautophagy receptor, see below) reportedly underlies an ESCRT-dependent and ubiquitin-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. Irrespective, we propose to define microautophagy and endosomal microautophagy as types of autophagy in which the cargo is directly internalized in small vesicles that form at the surface of the lysosome/vacuole or late endosomes (multivesicular bodies), 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 (i) its dependence on ESCRT systems and (ii) its independence from a specific splicing variant of lysosomal-associated membrane protein 2 (LAMP2A, see below) (Table 1).

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 organelles, other macromolecules such as lipids, nucleic acids, or proteins integral to membranes (Chiang et al., 1989; Wing et al., 1991; Salvador et al., 2000). 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 (Schneider et al., 2014; Kaushik & Cuervo, 2015), genome integrity preservation (Park et al., 2015), aging (Cuervo & Dice, 2000; 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 ~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 the formation of an oligomeric LAMP2A translocation complex (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 lysosomal pool of glial fibrillary acidic protein (GFAP) (Bandyopadhyay et al., 2010). Lysosomal HSPA8 operates as an acceptor for CMA substrates, possibly by preventing cytosolic retrotranslocation (Agarraberes et al., 1997). Eventually, LAMP2A complexes 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, dephosphorylation 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 what 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 (i.e., in birds) (Eskelinen et al., 2005). It has been suggested that selective endosomal microautophagy, which shares with CMA the dependence on KFERQ-like motives and HSPA8, constitutes an alternative to CMA in D. melanogaster (Mukherjee et al., 2016). Irrespective of this unknown, we propose to define CMA as an 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 (Table 1).

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, can
Table 1. Main autophagy-related proteins in common model organisms.¹

<table>
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<tr>
<th>Homo sapiens</th>
<th>Mus musculus</th>
<th>Drosophila melanogaster</th>
<th>Caenorhabditis elegans</th>
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¹Correction added on 3 July 2017, after first online publication: the protein MAP1LC3C has been deleted.
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, 2016) or cellular atrophy leading to neurodegeneration (Cherra et al, 2010a,b; Zhu et al, 2013). The molecular machinery that executes and regulates

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*Excluding non-coding pseudogenes, as per https://www.ncbi.nlm.nih.gov/gene/; common aliases are indicated between brackets.
Molecular definitions of autophagy
Lorenzo Galluzzi et al

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Macroautophagy in organisms encompassing yeast, nematodes, flies, and mammals has been the subject of intense investigation throughout the past two decades (Noda & Inagaki, 2015; Antonioli et al., 2016). 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 (Noda & Inagaki, 2015; Antonioli et al., 2016). One relies on ATG7 and ATG10, which promote the conjugation of ATG5 to ATG12 in the context of a multiprotein complex containing autophagy-related 16-like 1 (ATG16L1) (Mizushima et al., 1998). Another one is mediated by ATG3 and ATG7, which together with the ATG12-ATG5:ATG16L1 complex conjugates phosphatidylenolamine to microtubule-associated protein 1 light chain 3 beta (MAP1LC3B; best known as LC3B) and other orthologues of yeast Atg8 upon ATG4-dependent proteolytic maturation (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, robust data suggest that the ATG conjugation 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 assembly and activation of a multiprotein 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 (Orsi et al., 2012; Papinski et al., 2014; Stanley et al., 2014; Joachim et al., 2015; Karanasios et al., 2016). This event initiates the elongation of pre-autophagosomal membranes upon incorporation of phospholipids from various sources including the endoplasmic reticulum (ER), recycling endosomes, and mitochondria (Lamb 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 regulator subunit 4 (PIK3R4; best known as VPS15) (Kihara et al., 2001a, b), the sensor of membrane curvature ATG14 (also known as ATG14L or BARKOR) (Itakura et al., 2008; Sun et al., 2008; Matsunaga et al., 2009; Zhong et al., 2009; Fan et al., 2011), and nuclear receptor binding factor 2 (NRF2) (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; Nicklin et al., 2009; Nazio et al., 2013) Moreover, mTORC1 inhibits macroautophagic responses by preventing the nuclear translocation of transcription factor EB (TFEB, a master transcriptional regulator of lysosomal biogenesis and macroautophagy) upon phosphorylation on S142 (Settembre et al., 2011, 2012). Such a multipronged inhibitory network is disrupted upon mTORC1 inactivation by AMP-activated protein kinase (AMPK), which responds to reduced ATP levels and consequent AMP accumulation (Inoki et al., 2002). AMPK also catalyzes activating phosphorylation events on ULK1 (Lee et al., 2010; Egan et al., 2011; Kim et al., 2011) and BECN1 (Kim et al., 2013b). In mammalian cells, ULK1 directly phosphorylates BECN1, resembling AMPK in its VPS34-stimulatory effects (Russell et al., 2013), and ATG14 (Park et al., 2016; Wold et al., 2016). 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 BECN1 inhibitor BCL2, which also interacts with ATG12 (Liang et al., 1999; Pattingre et al., 2005; Fimia et al., 2007; Zalckvar et al., 2009; Rubinstein et al., 2011).

Once autophagosomes have enclosed autophagy substrates, they can fuse with late endosomes or lysosomes to form amphiphysins 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 effecter pleckstrin homology and RUN domain containing M1 (PLEKH1M1) (McEwan et al., 2015), the PI3P-binding protein tectonin beta-propeller repeat containing 1 (TECP1R) (Chen et al., 2012), ectopic P-granules autophagy protein 5 homolog (EPGS) (Tian et al., 2010), inositol polyphosphate-5-phosphatase E (INPP5E) (Hasegawa et al., 2016), syntaxin 17 (STX17), and other soluble N-ethylmaleimide-sensitive factor activating protein receptor (SNARE) proteins (Fader et al., 2009; Nair et al., 2011; Itakura et al., 2012), as well as homotypic fusion and vacuole protein sorting (HOPS) complexes (McEwan et al., 2015). ATG14, LAMP2B (but not LAMP2A) as well as phosphorylated and lipidated LC3 are also involved in the formation of autolysosomes (Eskeninen et al., 2005; Diao et al., 2015; Wilkinson et al., 2015; Nguyen et al., 2016). Conversely, RUN and cysteine-rich domain containing beclin 1 interacting protein (RUBCN; best known as RUBICON) negatively regulates the fusion of autophagosomes with lysosomes upon interacting with VPS34 (Matsunaga et al., 2009). Degradation of autophagy substrates proceeds as the lysosomal lumen is acidified (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, and VPS34 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 (Qu et al., 2003; Yue et al., 2003; Kuma et al., 2004; Komatsu et al., 2005; Can et al., 2006; Saitoh et al., 2008, 2009; 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) (Zhu et al., 2007; Nishida et al., 2009; Chang et al., 2013; Niso-Santano et al., 2015; Vicinanza et al., 2015) 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) (Nishida et al., 2009; Chu, 2011; Chang et al., 2013; 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) (Table 1).

Throughout the past decade, the terms “canonical” and “non-canonical” have been extensively employed to (i) refer to non-degradative functions of macroautophagy (e.g., unconventional secretion) (Ponpuak et al., 2015), or (ii) 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 (Niso-Santano et al., 2015; Huang & Liu, 2016), 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 alike, 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 *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. Comprehensive 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).

**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,b). 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 (Table 1).

Mitophagy 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 homeostasis, especially in highly metabolic tissues such as the brain) (Palikaras & Tavernarakis, 2014). Two physiological settings exemplify the macroautophagic removal of functional mitochondria: (i) 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 (Sandoval et al., 2008; Mortensen et al., 2010; Novak et al., 2010; Griffiths et al., 2012; Fader et al., 2016); (ii) the first steps of embryonic development (Ali 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 Parkinson disease (autosomal recessive, juvenile) 2, parkin (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 autophagy receptors, 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 sequestosome 1 (SQSTM1, best known as p62) (to a limited extent), optineurin (OPTN), calcium binding and coiled-coil domain 2 (CALKO2; best known as NDP52), and LC3 (Wong & Holzbaur, 2014; Heo et al., 2015; Lazarou et al., 2015; Moore & Holzbaur, 2016; Wei et al., 2017). Cardiolipin, a mitochondrial lipid, has also been proposed to directly interact with LC3 upon mitochondrial damage caused 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 (TG2M) have also been involved in the regulation or execution of mitophagy, although their exact role remains to be elucidated (Orvedahl et al., 2011; Rossin et al., 2015; Lee et al., 2017; 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 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; Yamashita et al., 2014; Sargent et al., 2016). 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-2α) 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 Nucleophagy can be defined as an autophagic response selectively targeting portions of the nucleus. In yeast, two
distinct forms of nucleophagy have been described: (i) a microautophagic form that relies on the autophagy receptor Nv1), the vacuolar protein Vac8 and members of the oxy-sterol-binding protein (OSBP) family (Roberts et al, 2003; Kvam & Goldfarb, 2004), which has been dubbed “piecemeal microautophagy of the nucleus”; and (ii) a variant that does not require Nv1, 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 (Rello-Varona et al, 2012; Dou et al, 2015). Lamin B1 (LMNB1) has been identified as the nuclear protein responsible for a variant of nucleophagy in mammalian cells (Dou et al, 2015).

**Reticulophagy** Reticulophagy is the preferential autophagic degra- dation of portions of the ER. According to some authors, reticulophagy (also called ER-phagy) occurs independently of both the micro- and macro- autophagy machinery, at least in yeast (Schuck et al, 2014), but is regulated by the Rab family GTPase Ypt1 (Lipatova et al, 2013). Other authors, however, provided evidence suggesting that reticulophagy constitutes a specific form of macroautophagy that 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).

**Ribophagy** Ribophagy is a specific autophagic response targeting ribosomes. In yeast, ribophagy involves ribosomal de-ubiquitination by the mRNA-binding ubiquitin-specific protease Ubp3 and its cofactors Bre5, Doa1 (also known as Ufd3), and Cde48 (Kraft et al, 2008; Ossareh-Nazari et al, 2010) and requires Atg11 (Walilullah et al, 2017). 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). Ribophagy driven by nutrient starvation in yeast is accompanied by bulk RNA degradation within the vacuole (Huang et al, 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** 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; Lu et al, 2013), 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; Komatsu et al, 2007; Pankiv et al, 2007; Kirk et al, 2009b), NBR1 (an orthologue of which participates in plant aggrephagy) (Kirk et al, 2009a,b), 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) (Simonsen et al, 2004; Filimonenko et al, 2010) 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, subgroup 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 co-chaperones 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).

**Lipophagy** Lipophagy is 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 worms and in yeast. In *C. elegans*, lipophagy involves lysosomal lipases such as LIPL-4, which play key signaling roles in longevity (Lapierre et al, 2011; O’Rourke & Ruvkun, 2013; Polick et al, 2015). In yeast, it involves a microautophagic process (Wang et al, 2014; Vevea et al, 2015). However, there are contradicting reports on the molecular requirements for *S. cerevisiae* lipophagic responses to intracellular lipid accumulation (Wang et al, 2014; Vevea et al, 2015). Thus, while some authors propose that lipophagy in yeast does not involve Atg7 but requires ESCRT components (Vevea et al, 2015), other authors favor the interpretation that lipophagic responses in yeast depend on Atg7 and several other components of the macroautophagy machinery (even though they manifest with a microautophagic appearance and proceed 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, 2016), demonstrating the existence of intimate cross talk between different forms of autophagy in the control of intracellular homeostasis. Moreover, several autophagy genes including *bec-1* (the worm orthologue of BECN1) are 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 Bacterial xenophagy is the macroautophagic removal of cytoplasmic bacteria, that is, 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 strategies to actively inhibit autophagic responses in the host (Galluzzi et al, 2017b). In mammalian cells, cytoplasmic bacteria are rapidly recognized by various autophagy receptors including p62, OPTN, NDP52, and TAX1-binding protein 1 (TAX1BP1), via a mechanism that relies on receptor phosphorylation by TANK1-binding kinase 1 (TBK1) (Thurston et al, 2009; Wild et al, 2011; Tumbarello et al, 2015) 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 (Cooney et al, 2010; Travassos et al, 2010; Chauhan et al, 2015). 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) (von Muhlinen et al, 2012; Verlhac et al, 2015). 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 (Thurston et al, 2012; Kim et al, 2013a; Li et al, 2013) and/or various members of the TRIM protein family as receptors or receptor regulators (see below for a definition) (Kimura et al, 2015, 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 (Wu et al, 2007; Yano et al, 2008; Kim et al, 2012), C. elegans (Jia et al, 2009; Zou et al, 2014) and Dictyostelium discoideum (Jia et al, 2009).

Viral xenophagy 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 (Nakamoto et al, 2012; Moy et al, 2014) and perhaps nematodes (Bakowski et al, 2014). Moreover, HIV-1+ 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 host virophagic responses, including the expression of Becn1 inhibitors (Orvedahl et al, 2007; Levine et al, 2011) 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 (Orvedahl et al, 2010; Mandell et al, 2014), proteins that participate in mitophagy, such as SMURF1 (Orvedahl et al, 2011), Fanconi anemia complementation group C (FANCC) (Suppeter et al, 2016), and PEX13 (Lee et al, 2017), as well as the phosphorylation of eukaryotic translation initiation factor 2A (EIF2A) (Tallozcy et al, 2002).

Proteaphagy Proteaphagy is a term 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. Mammalian cells subjected to starvation and other stressful conditions mount proteaphagic responses that mainly on p62 as a receptor (Cuervo et al, 1995; Cohen-Kaplan et al, 2016).

Lysophagy Lysophagy is 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.

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 zymogen granules in pancreatic acinar cells) (Grasso et al., 2011), granulaphagy (targeting stress granules) (Buchan et al., 2013), and ferritinophagy (targeting ferritin via the receptor nuclear receptor coactivator 4, NCOA4) (Dowdle et al., 2014; Mancias et al., 2014). Finally, macroautophagy has been invoked 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 yet to be proposed.

**Autophagic flux**

All forms of autophagy are multistep processes during which autophagy substrates are 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) (Li et al., 2012; Cuervo & Wong, 2014; 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+ cytoplasmic dots (as monitored by immunofluorescence microscopy) (Klionsky et al., 2016). Both LC3 lipidation and GFP-LC3+ cytoplasmic dots, indeed, are relatively reliable indicators of the pool 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 (Katayama et al., 2011; Kaizuka et al., 2016) 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 (Schweichl & Merker, 1973; Eskelinen et al., 2011). 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 (Schweichl & Merker, 1973; Kroemer et al., 2009). With the advent of modern molecular biology, it has become clear that macroautophagy has robust cytoprotective functions in the majority of pathophysiological and experimental settings (Menzies et al., 2015; Galluzzi et al., 2016). 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; Yousefi et al., 2006; Mroschik et al., 2015). 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 or components of the machinery for macroautophagy can also: (i) have little, if any, impact on RCD (so-called non-protective autophagy) (Saleh et al., 2016); or (ii) etiologically contribute to RCD (at least in specific developmental or pathophysiological scenarios) (Seay & Dinesh-Kumar, 2005; Masini et al., 2009; Sharma et al., 2014; Denton et al., 2015). 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., 2009, 2013; 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 Na+/K+-ATPase (called “autosis”) occurs in cells exposed to nutrient deprivation or a BECN1-derived peptide, 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 (i) specificity issues affect most, if not all, pharmacological agents employed so far for suppressing macroautophagic responses (Maycotte et al., 2012; Maes et al., 2014; Eng et al., 2016; Galluzzi et al., 2017b); and (ii) 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. Expressions such as “ATG5-dependent cell death” or “BECN1-dependent cell death” may be even more appropriate when the involvement of one or more
specific components of the macroautophagy apparatus has been experimentally validated in the absence of links to increased autophagic flux. Autosis can be functionally defined as a Na⁺/K⁺-ATPase-mediated form of autophagy-dependent cell death.

**Cytoplasm-to-vacuole targeting pathway**
The cytoplasm-to-vacuole targeting (Cvt) pathway delivers hydrolyses 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., 1996, 2000, 2001). Moreover, Ape1, Ape4, and Ams1 are imported into the vacuole as large oligomers, being reminiscent of the substrates of aggrephagy (Bertiapiglia 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 strictly speaking.

**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 (Sanjuan et al., 2007; Martinez et al., 2015, 2016). 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., 2015, 2016). However, in the mammalian systems investigated thus far, LAP does not involve ULK1 signaling, AMBRA1 and ATG14 (which are also involved in macroautophagy), but critically depends on RUBICON and NAPDH oxidase 2 (which are dispensable for macroautophagy). LAP has been involved in the control of bacterial and fungal pathogens (Sanjuan et al., 2007; Zhao et al., 2008; Gong et al., 2011; Lam et al., 2013; Choi et al., 2014; Martinez et al., 2015; Selleck et al., 2015), 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 that never enter the cytoplasm, LAP 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 cyttoplasmic entities (including soluble proteins with extracellular functions, potentially cytotoxic protein aggregates, secretory granules, and invading pathogens) (Manjithaya et al., 2010; Dupont et al., 2011; Shravag et al., 2013; Lock et al., 2014; Gerstenmaier et al., 2015; Kimura et al., 2017), 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 organanel 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 following the fusion of secretory granules with lysosomes (Marzella et al., 1981). This process, which has been observed in secretory cells and is distinct from zymophagy, ensures the 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 (autophagic cargo)**
The terms autophagy substrates and autophagic cargo can be interchangeably used to describe a large and heterogeneous set of cytoplasmic entities (of endogenous or exogenous origin) that are targeted to lysosomal degradation by autophagy (Fig 1). 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 (which contribute to the preservation of enzymatic homeostasis) nor extracellular entities reaching lysosomes via the endocytic pathway (which never enter the cytoplasm) can be considered as bona fide autophagy substrates.

**Autophagy receptors and adaptors**
An autophagy receptor is any of the proteins that bind autophagy substrates, allow for their recognition by the autophagy machinery, and become 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) (Uytterhoeven et al., 2015; Morozova et al., 2016). In addition, dozens of proteins have been involved in the recognition of macroautophagy substrates (see above) (Rogov et al., 2014; Farre & Subramani, 2016). Most receptors for macroautophagy share an evolutionary conserved LC3-interacting region (LIR), which allows them to bring macroautophagy substrates in the proximity of LC3⁺ forming autophagosomes. This applies to p62, NBR1, OPTN, NDP52, BNIP3, BNIP3L, ATG34, FUNDCL1, PHB2, TRIM5, TAXIBP1, 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). Moreover, some receptors including yeast Atg19 and Atg32 as well as human p62, OPTN, and NDP52 have
been shown to 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, 2016). These proteins have been dubbed “receptor regulators”. It cannot be excluded that other autophagy receptors might have regulatory functions besides cargo recognition.

Although the term autophagy adaptor has also been used as a synonym of autophagy receptor, we recommend to employ this expression to indicate any of the 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 their fusion with lysosomes, and sorting nexin 18 (SNX18), which participates in autophagosome formation (Knaevelsrud et al., 2013; Olsvik et al., 2015).

**Phagophores (isolation membranes)**

Phagophores (also called isolation membranes) are the precursors of autophagosomes. Mammalian phagophores generally form near ER-mitochondria contact sites in the context of unique 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 (Ravikumar et al., 2010; Orsi et al., 2012; Puri et al., 2013). Irrespective of the exact source of

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**Figure 1. Autophagy substrates.**
A wide and heterogeneous set of cytoplasmic entities—be they of endogenous/intracellular or exogenous/extracellular origin—can be targeted to lysosomal degradation by non-selective or selective forms of autophagy. ER, endoplasmic reticulum.
lipids (which remains a matter of debate), forming mammalian phagophores recruit the ULK1 complex and ATG14 (Karanasios \textit{et al}, 2013), which facilitates the assembly of the autophagy-specific Class III PI3K complex (Matsunaga \textit{et al}, 2010). This enables the association of the PI3P-binding proteins DFCP1 and WIP2 (Polson \textit{et al}, 2010), the formation of ATG12-ATG5:ATG16L1 complexes, and consequent local LC3 lipidation (Dooley \textit{et al}, 2014). Either mammalian phagophores or omegasomes, or both, stain positively for ULK1, ATG13, ATG101, FIP200, VPS34, BECN1, VPS15, ATG5, ATG12, ATG16L1, DFCP1 as well as for lipidated LC3 family members (Antonioli \textit{et al}, 2016). In yeast, phagophores are formed at the so-called “phagophore-assembly site” or “pre-autophagosomal structure” (PAS), that is, a site within the cytoplasm enriched in Atg9+ 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 (Yamamoto \textit{et al}, 2012; Stanley \textit{et al}, 2014).

\textbf{Autophagosomes}

Transient, double-membrane organelles (mean diameter in mammals 0.5–1.5 μm) 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 \textit{et al}, 2016). LC3 is abundant at both the inner and outer membrane of forming autophagosomes. However, it is efficiently removed by Atg4 family members from the surface of closed autophagosomes (Lamb \textit{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 \textit{et al}, 2016).

\textbf{Amphisomes}

Single- or double-membrane 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) (Fader \textit{et al}, 2009; Chandra \textit{et al}, 2015). 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 \textit{et al}, 2015).

\textbf{Autolysosomes}

Single-membraned organelles that form in the course of macroautophagy upon fusion of autophagosomes or amphisomes and lysosomes (Klionsky \textit{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 (unless lysosomal hydrolases are pharmacologically or genetically inhibited) (Klionsky \textit{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 \textit{et al}, 2010). Of note, the term \textit{autophagolysosome} indicates a specific type of autolysosome that forms in the course of some xenophagic responses (Klionsky \textit{et al}, 2014).

\begin{small}

\textbf{Box 1: Key recommendations}

- \textit{Bona fide} autophagic responses deliver cytoplasmic material (of endogenous or exogenous origin) to lysosomes (or vacuoles) for degradation.
- Microautophagy is a LAMP2A-independent autophagic response that proceeds upon direct membrane invagination at the surface of the lysosome/vacuole.
- Endosomal microautophagy is an ESCRT-dependent, LAMP2A-independent autophagic response that relies on direct membrane invagination at the surface of late endosomes, occurring either as a bulk process or following HSPA8-mediated cargo recognition.
- Chaperone-mediated autophagy (CMA) is an HSPA8- and LAMP2A-dependent autophagic response that involves the translocation of substrates across the lysosomal membrane.
- Macroautophagy is a type of autophagic response that relies on the formation of autophagosomes and can be subtyped based upon dependence on specific factors (including—but not limited to—ATG proteins).
- Selective instances of autophagy should be defined based on the enrichment of a precise substrate, coupled to the requirement of specific molecular factors (such as autophagy receptors).
- Autophagic flux refers to the global efficacy of autophagic responses, which is generally well represented by the rate at which lysosomes degrade autophagy substrates.
- Autophagy-dependent cell death is a form of regulated cell death that can be retarded by pharmacological or genetic inhibition of components of the macroautophagy apparatus.
- Autosis is a Na+/K+-ATPase-mediated type of autophagy-dependent cell death.
- Cytoplasm-to-vacuole targeting (Cvt), LC3-associated phagocytosis (LAP), crinophagy, and instances of protein secretion that depend on components of the macroautophagy apparatus are not \textit{bona fide} autophagic responses.
- Autophagy substrates are cytoplasmic entities (of endogenous/intracellular or exogenous/extracellular origin) delivered to lysosomal degradation by autophagy.
- Autophagy receptors are 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.
- Autophagy adaptors are proteins that interact with Atg8 family members, hence conferring additional functions to the autophagosome, but are not involved in cargo recognition.
- Phagophores (also called isolation membranes) are the precursors of autophagosomes.
- Autophagosomes are transient, double-membrane organelles that mediate cargo sequestration and delivery to lysosomes in the course of macroautophagic responses.
- Amphisomes are single- or double-membraned organelles that originate from the fusion of autophagosomes and (late) endosomes.
- Autolysosomes are single-membraned organelles that form in the course of macroautophagy upon fusion of autophagosomes or amphisomes with lysosomes.
- Autophagolysosomes are a specific type of autolysosome that forms in the course of xenophagic responses targeting intact or ruptured phagosomes.

\end{small}
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 (Box I). 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). 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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**Author contributions**

LG conceived and wrote the manuscript, centralized and integrated comments from co-authors, and revised the review upon editorial feedback. JMBSP designed the figure, performed bibliographic searches, and helped with table preparation. All authors corrected the article and provided valuable input to obtain a unified view. With the exception of LG and GK, authors are listed alphabetically, which does not reflect their relative contribution to the preparation of this article.

**Conflict of interest**

A.C.K. is an inventor on patents pertaining to Kras regulated metabolic pathways, redox control pathways in pancreatic cancer, targeting GOT1 as a therapeutical approach, and the autophagic control of iron metabolism. A.C.K. is on the SAB of Cornerstone Pharmaceuticals and is a founder of Vescor Therapeutics. The other authors declare that they have no conflict of interest.

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