Eaten alive: a history of macroautophagy

Zhifen Yang and Daniel J. Klionsky

Macroautophagy (hereafter autophagy), or 'self-eating', is a conserved cellular pathway that controls protein and organelle degradation, and has essential roles in survival, development and homeostasis. Autophagy is also integral to human health and is involved in physiology, development, lifespan and a wide range of diseases, including cancer, neurodegeneration and microbial infection. Although research on this topic began in the late 1950s, substantial progress in the molecular study of autophagy has taken place during only the past 15 years. This review traces the key findings that led to our current molecular understanding of this complex process.

The term 'autophagy' comes from the Greek words 'phagy' meaning eat, and 'auto' meaning self. Autophagy is an evolutionarily conserved process in eukaryotes by which cytoplasmic cargo sequestered inside double-membrane vesicles are delivered to the lysosome for degradation. When autophagy was initially discovered more than 40 years ago, it was perplexing as to why the cell would self-digest its own components. The simplest hypothesis was that autophagy serves as a cellular rubbish-disposal mechanism. However, we have since learnt that this 'self-eating' process not only rids the cell of intracellular misfolded or long-lived proteins, superfluous or damaged organelles, and invading microorganisms, but also is an adaptive response to provide nutrients and energy on exposure to various stresses. Autophagy has been connected to human pathophysiology, and continued expansion of our knowledge about autophagy has had implications for fields as wide-ranging as cancer, neurodegeneration, immune response, development and ageing. This timeline reviews the history of autophagy research with a focus on the key events that occurred over the past 15 years, when our molecular understanding of this process first began.

The development of the autophagy concept

More than four decades ago, Clark and Novikoff observed mitochondria from mouse kidneys within membrane-bound compartments termed 'dense bodies', which were subsequently shown to include lysosomal enzymes[1,2]. Ashford and Porter later observed membrane-bound vesicles containing semi-digested mitochondria and endoplasmic reticulum in the hepatocytes of rats that had been exposed to glucagon[3], and Novikoff and Essner observed that the same bodies contained lysosomal hydrolases[4]. One year later, in 1963, at the Ciba Foundation symposium on lysosomes, de Duve founded the field when he coined the term 'autophagy' to describe the presence of single- or double-membrane vesicles that contain parts of the cytoplasm and organelles in various states of disintegration. He pointed out that these sequestering vesicles, or 'autophagosomes', were related to lysosomes and occurred in normal cells. The origin of the membrane surrounding the autophagosome is still controversial; de Duve suggested that the sequestering membranes are derived from preformed membranes, such as smooth endoplasmic reticulum[5].

Cellular autophagy is observed in normal rat liver cells, but is enhanced in the livers of starved animals[6], and in 1967 de Duve and Deter confirmed that glucagon induces autophagy[7]. Ten years later, Pfeifer demonstrated the converse — that insulin inhibits autophagy[8]. Pioneering work by Mortimore and Schworer further demonstrated that amino acids, which are the end products of autophagic degradation, have an inhibitory effect on autophagy in rat liver cells[9]. These early lines of evidence are consistent with our current understanding of autophagy as an adaptive catabolic and energy-generating process. Subsequently, Seglen and Gordon carried out the first biochemical analysis of autophagy and identified the pharmacological reagent 3-methyladenine as an autophagy inhibitor[10]; they also provided the first evidence that protein kinases and phosphatases can regulate autophagy[11].

These early studies of autophagy from the 1950s to the early 1980s were based on morphological analyses. de Duve and others primarily examined the terminal stages of the process, the steps just before or after fusion with the lysosome. Subsequent studies by Seglen's laboratory began to use electro-injected radioactive probes to examine the early and intermediate steps of autophagy, leading to the identification of the phagophore (the initial sequestering vesicle that develops into the autophagosome; Fig. 1), as well as the amphisome (a non-lysosomal vesicle formed by the fusion of autophagosomes and endosomes[12]).

As early as the 1960s, de Duve suggested that most, if not all, living cells must employ a mechanism for nonspecific bulk segregation and digestion of portions of their own cytoplasm in the lysosome[5], but also hinted at the need of a selective proteolytic mechanism acting

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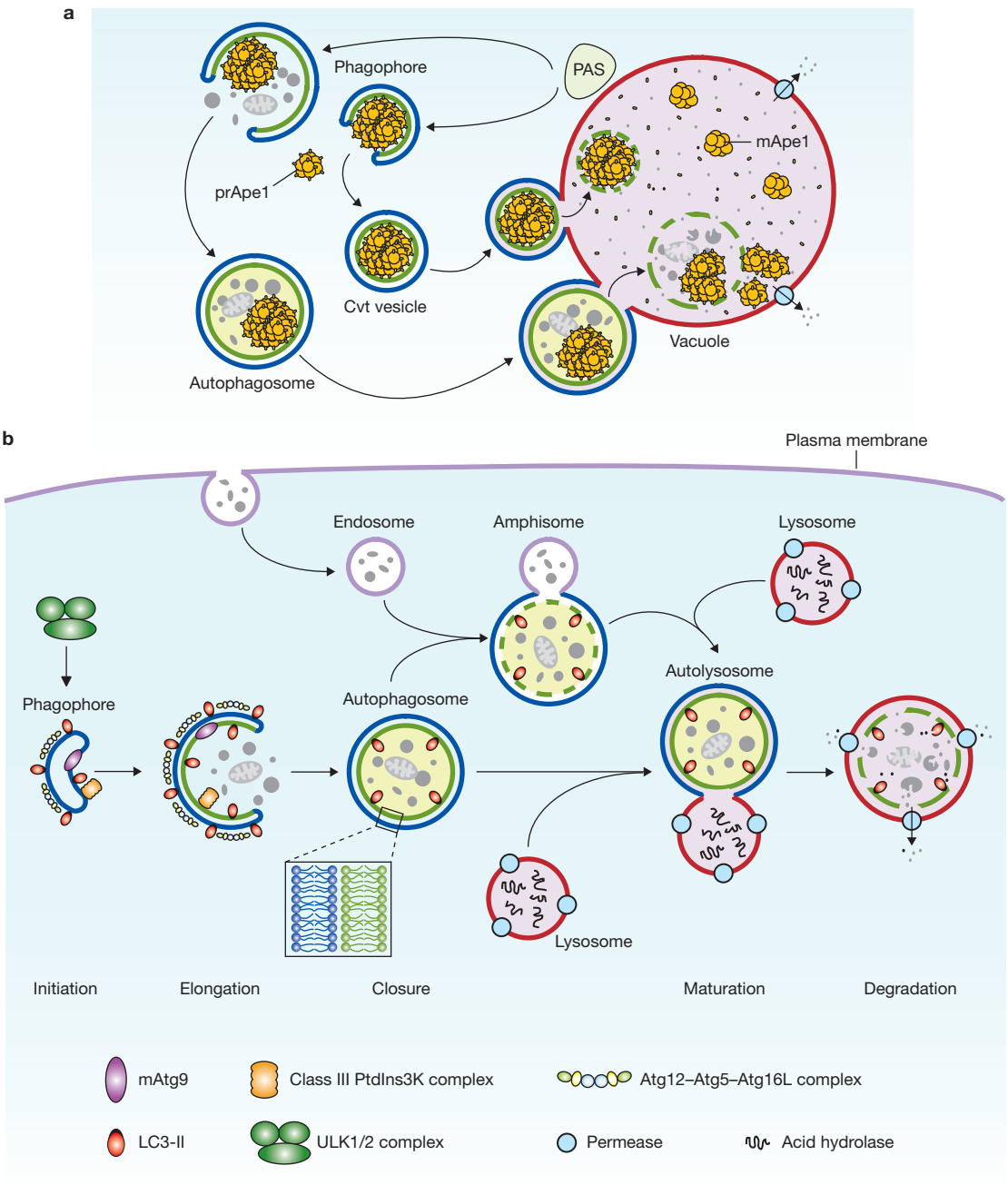

Figure 1 Schematic depiction of autophagy. (**a**) In yeast, both autophagy and the Cvt pathway engulf cargoes within distinct double-membrane vesicles, which are thought to originate from the phagophore assembly site (PAS). The PAS is defined as the initial site for autophagy-related (Atg) protein recruitment. The Cvt pathway is one example of selective autophagy, and the only example of a biosynthetic autophagy-related pathway. The Cvt vesicle (140–160 nm in diameter) appears to closely enwrap the specific cargo — the Cvt complex (consisting of the precursor form of aminopeptidase I — prApe1 — and the Atg19 receptor), and exclude bulk cytoplasm. The autophagosome (300–900 nm in diameter) engulfs cytoplasm, including organelles, and also the Cvt complex. The completed vesicles then fuse with the vacuole, the yeast analogue of the mammalian lysosome, and release the inner single-membrane vesicle (autophagic or Cvt body) into the lumen. Subsequent breakdown of the inner vesicles allows the maturation of prApe1 and the degradation of cytoplasm, and hence the recycling of the resulting macromolecules through vacuolar permeases. (**b**) Mammalian autophagy is initiated by the formation of the phagophore, followed by a series of steps, including the elongation and expansion of the phagophore, closure and completion of a double-membrane autophagosome (which surrounds a portion of the cytoplasm), autophagosome maturation through docking and fusion with an endosome (the product of fusion is known as an amphisome) and/or lysosome (the product of fusion is known as an autolysosome), breakdown and degradation of the autophagosome inner membrane and cargo through acid hydrolases inside the autolysosome, and recycling of the resulting macromolecules through permeases. So far, there is no evidence for a PAS that exists in mammalian cells, and so the mammalian phagophore could be equivalent to the yeast PAS, or derived from the PAS. The core molecular machinery is also depicted, such as the ULK1 and ULK2 complexes that are required for autophagy induction, class III PtdIns3K complexes that are involved in autophagosome formation, mammalian Atg9 (mAtg9) that potentially contributes to the delivery of membrane to the forming autophagosome and two conjugation systems, the LC3-II and Atg12–Atg5–Atg16L complex, which are proposed to function during elongation and expansion of the phagophore membrane.

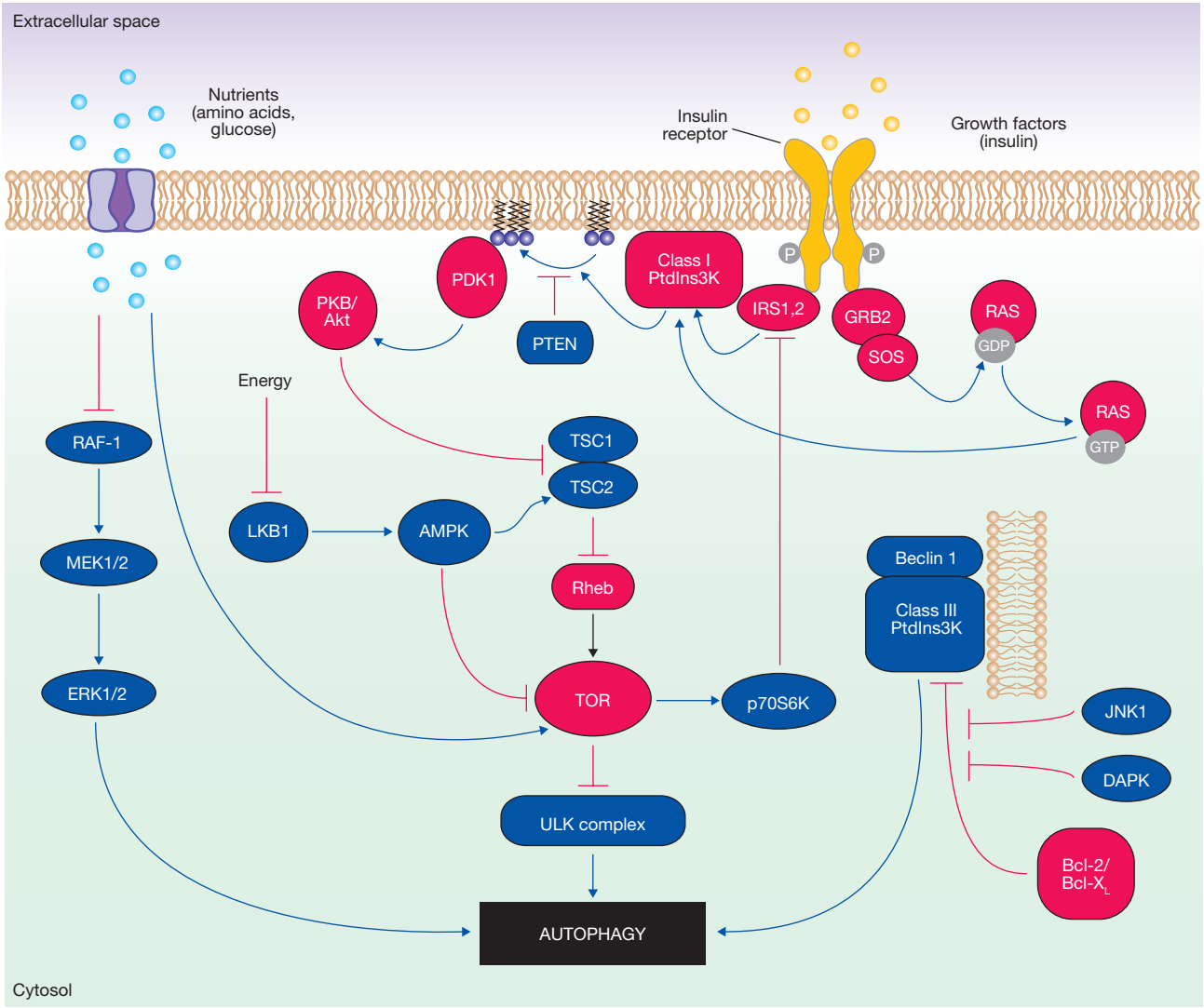

Figure 2 Signalling regulation of mammalian autophagy. In the figure, the blue components represent the factors that stimulate autophagy, whereas the red ones correspond to inhibitory factors. Autophagy is regulated by a complex signalling network of various stimulatory (blue arrows) and inhibitory (red bars) inputs. TOR plays a central role in autophagy by integrating the class I PtdIns3K signalling and amino acid-dependent signalling pathways. Activation of insulin receptors stimulates the class I PtdIns3K complex and small GTPase Ras, leading to activation of the PtdIns3K–PKB–TOR pathway. PKB phosphorylates and inhibits the tuberous sclerosis complex 1/2 (TSC1–TSC2), leading to the stabilization of Rheb GTPase, which in turn activates TOR, causing inhibition of autophagy. Amino acids inhibit the Raf-1–MEK1/2–ERK1/2 signalling cascade, leading to inhibition of autophagy. Energy depletion causes the AMP-activated protein kinase (AMPK) to be phosphorylated and activated by LKB1. AMPK phosphorylates and activates TSC1–TSC2, leading to inactivation of TOR and autophagy induction. p70S6K kinase is a substrate of TOR that may negatively feed back on TOR activity, ensuring basal levels of autophagy that are important for homeostasis. JNK1 and DAPK phosphorylate and disrupt the association of anti-apoptotic proteins, Bcl-2 and Bcl-X_L, with Beclin 1, leading to the activation of the Beclin 1-associated class III PtdIns3K complex and stimulation of autophagy. Beclin 1 is shown bound to the phagophore membrane.

on abnormal cellular proteins or organelles. In 1973, Bolender and Weibel provided some of the first evidence that a specific organelle (the smooth endoplasmic reticulum) can be engulfed by autophagy[13]. Four years later, Beaulaton and Lockshin suggested that mitochondria are selectively cleared during insect metamorphosis[14]. In 1983, Veenhuis demonstrated that superfluous peroxisomes are selectively degraded by autophagy in the yeast *Hansenula polymorpha*[15], and five years later Lemasters and colleagues showed that changes in mitochondrial membrane potential lead to the onset of autophagy[16]. Further evidence that autophagy can be selective was provided by subsequent studies in yeast and higher eukaryotes.

The molecular era

Insights into the molecular control of autophagy, starting in the late 1990s, revolutionized the ability to detect and genetically manipulate this process, which allowed the field to grow at an extraordinarily fast pace and uncovered the importance of autophagy in human health and disease.

Although autophagy was initially identified in mammals, a significant breakthrough in our understanding of how autophagy is controlled came from analysis in the genetically tractable yeast system. Pioneering work from Ohsumi's group showed that the morphology of autophagy in yeast was similar to that documented in mammals[17]. They then carried out the first genetic screen for yeast mutants that affected protein turnover

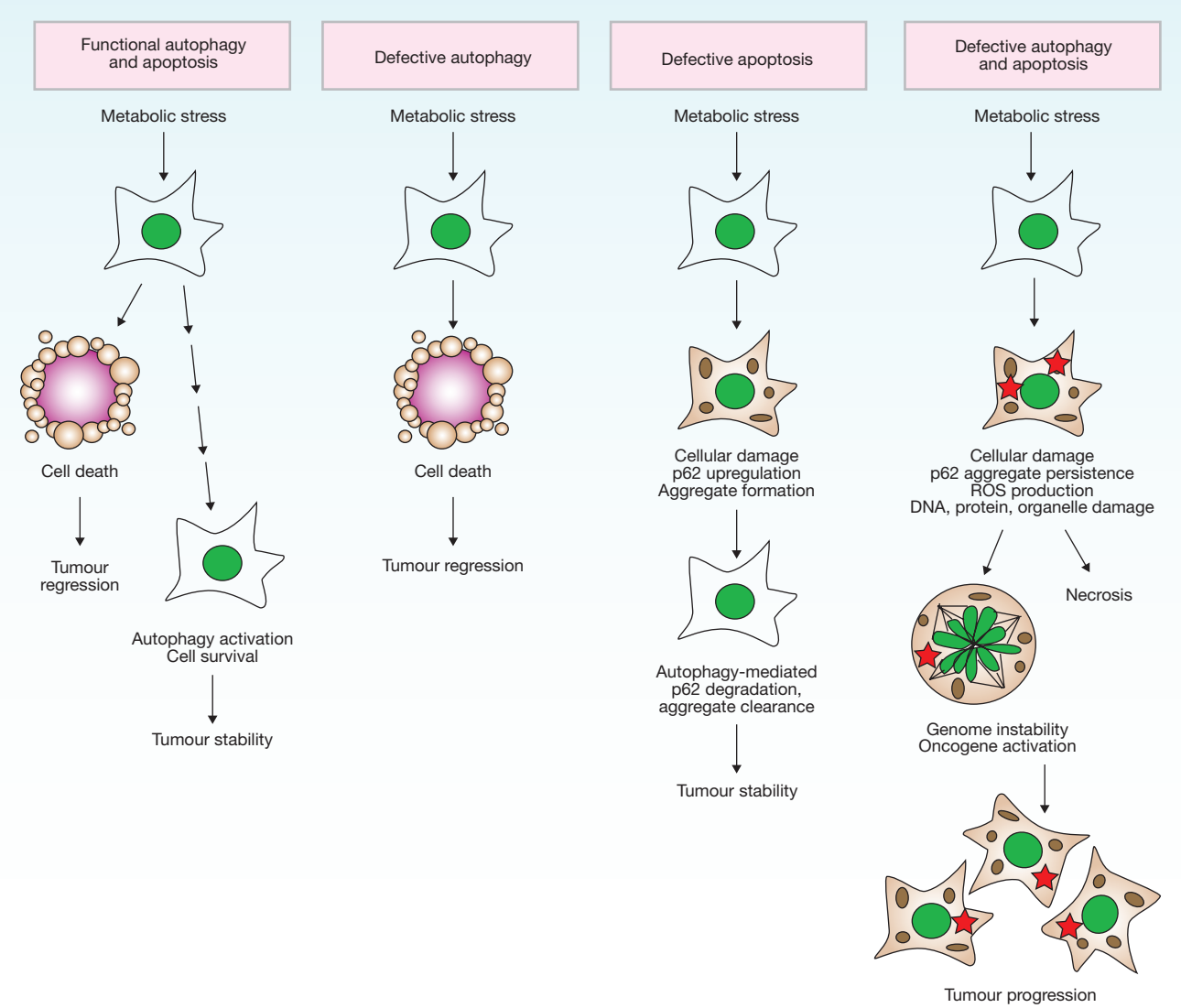

Figure 3 A model for the roles of apoptosis and autophagy in tumorigenesis. A common cellular response to metabolic stress is cell death mediated by apoptosis, which limits tumour growth. Tumours may trigger autophagy-mediated cell survival in certain metabolic-stressed tumour regions. In apoptotic-defective, metabolic-stressed tumour cells, activation of autophagy prevents death from necrosis, whereas defects in autophagy lead to accumulation of p62, damaged mitochondria, ROS and protein aggregates, resulting in genome damage and tumorigenesis. For additional information, see refs 68 and 119.

(nonspecific macroautophagy)[18]. This work was followed by similar screens for mutants that affected peroxisome degradation (pexophagy)[19] and delivery of a resident vacuolar hydrolase (the cytoplasm to vacuole targeting (Cvt) pathway[20], as reviewed in ref. 21). The identification of the first autophagy-related (Atg) gene, *ATG1*, was published in 1997 (ref. 22). The recent genetic screens for mutants that affect selective mitochondrial degradation (mitophagy), led to the identification of *ATG32* and *ATG33* (refs 23 and 24).

Although the Cvt pathway, pexophagy, mitophagy and macroautophagy are morphologically and mechanistically similar and require most of the Atg components, they are different in important ways. Macroautophagy is generally considered to be nonselective, whereas the Cvt pathway, pexophagy and mitophagy are highly selective. Pexophagy, mitophagy and nonspecific macroautophagy are degradative, whereas the Cvt pathway is biosynthetic, delivering at least two resident hydrolases to the vacuole[25,26] (Fig. 1a). Overall, they share one subset of the Atg proteins that are essential for autophagosome formation and referred to as the 'core' molecular machinery (reviewed in ref. 27). The core machinery includes four major functional groups: (1) the Atg1–Atg13–Atg17 kinase complex, (2) the class III phosphatidylinositol 3-kinase (PtdIns3K) complex I, consisting of Vps34, Vps15, Atg6 and Atg14, (3) two ubiquitin-like protein conjugation systems (Atg12 and Atg8) and (4) Atg9 and its cycling system. Furthermore, in yeast the autophagy machinery is concentrated at a perivacuolar (the vacuole is the yeast equivalent of the lysosome) site termed the phagophore assembly site (PAS), and the concerted action of the autophagy machinery at the PAS leads to phagophore expansion and autophagosome formation[28,29]. A fifth set of core components includes proteins needed for the last steps of autophagy when the single-membrane intravacuolar vesicles (that result from fusion of the autophagosome or other sequestering vesicles with the vacuole limiting membrane) and their cargo break down, and permeases release these degradation products back into the cytosol for re-use[30–32].

The identification of the *ATG* genes in yeast led to molecular analysis of autophagy in higher eukaryotes. Mizushima, while in Ohsumi's laboratory, identified the first mammalian autophagy genes, *ATG5* and *ATG12*, and demonstrated that the Atg12–Atg5 conjugation system is conserved[33]. Perhaps the most critical finding in higher eukaryotes was the identification of the mammalian Atg8 homologue, MAP1LC3 (also known as LC3), by Yoshimori and colleagues, followed by the development of LC3-based assays for monitoring autophagy in mammals and other higher eukaryotes[34]. However, the increased synthesis or lipidation of LC3 are not sufficient for evaluating autophagy, and it is also critical to follow flux through the entire pathway, including in lysosomes[35]. In addition to the two conjugation systems, other mammalian Atg homologues have been identified and investigated (Fig. 1b, reviewed in ref. 36). Two Atg1 homologues, ULK1 and ULK2, are essential for autophagy induction and are found in a large complex that includes a mammalian homologue of Atg13 (mAtg13) and the scaffold protein, FIP200 (an orthologue of yeast Atg17). Formation of the human class III PtdIns3K complex, including human Vps34 (hVps34), Beclin 1 (a homologue of Atg6), Atg14L (an orthologue of Atg14) and p150 (a homologue of Vps15), is also conserved. Mizushima *et al.* used a green fluorescent protein-tagged Atg5 (ref. 37) to follow autophagosome formation, indicating that it proceeds in a step-wise manner, marked by the expansion of the sequestering vesicle (Fig. 1b).

The complexity of autophagy regulation in multicellular eukaryotes is becoming apparent from recent molecular analyses. For example, using *Caenorhabditis elegans*, Tian *et al.* identified four autophagy genes that are specific to multicellular animals, named *epg-2*, *epg-3/VMP1*, *epg-4/EI24* and *epg-5*: *epg-2* mediates cargo recognition and is specific to *C. elegans*, whereas the other three genes are conserved from worms to mammals[38]. Finally, two large-scale screens with human cells have identified numerous additional components that may interact with the known autophagy-related proteins, or participate in the signal transduction pathways that control this process[39,40].

The origin of the autophagosome membrane is still under considerable debate. For example, recent studies have suggested that the endoplasmic reticulum membrane[41–43], the mitochondrial outer membrane[44] and the plasma membrane[45] can contribute to autophagosome formation, suggesting that a range of organelles can provide the required membrane components (for details, see page 831 of this issue)[46].

Structural analysis of the Atg proteins should reveal the mechanism of autophagy; the structure of the mammalian Atg8 homologues, including the γ-aminobutyric acid receptor-associated protein (GABARAP)[47] and LC3 (ref. 48) were the first to be reported. Recently, Miller *et al.* reported the structure of *Drosophila melanogaster* Vps34 in a complex with PtdIns3K inhibitors, which may help in the design of new drugs that specifically target this kinase[49].

Signalling regulation of autophagy

The key breakthrough in our understanding of the signalling pathways that regulate autophagy occurred following the identification of the target of rapamycin kinase (TOR)[50,51], which modulates cell growth, cell-cycle progression and protein synthesis. In 1995, Meijer's group showed that rapamycin, an inhibitor of TOR, induces autophagy in rat hepatocytes, and relieves the inhibitory effect of amino acids on autophagy[52]. They also demonstrated that amino acids stimulate ribosomal protein S6 phosphorylation, an effect inhibited by rapamycin, providing a connection between amino acid-dependent and TOR-dependent regulation (Fig. 2). The TOR signalling pathway is critical because of its ability to integrate the information from nutrient, metabolic and hormonal signals. Research in the yeast system initially lagged behind the mammalian field, but in 1998 Ohsumi's laboratory reported that rapamycin also induces autophagy in yeast[53].

In 1997, Meijer's group found that amino acid-induced S6 phosphorylation was prevented by the PtdIns3K inhibitors wortmannin and LY294002 in rat hepatocytes[54] and thus by analogy with rapamycin, should induce autophagy. Unexpectedly, these PtdIns3K inhibitors (and 3-methyladenine) blocked autophagy in the absence of amino acids. One explanation for this apparent contradiction was the presence of two classes of phosphoinositides and phosphatidylinositol kinases. Indeed, Codogno's group, in collaboration with Meijer's laboratory, showed that the class III PtdIns3K product, phosphatidylinositol 3-phosphate (PtdIns(3)P), is essential for autophagy, whereas the class I PtdIns3K products, phosphatidylinositol (3,4)-bisphosphate (PtdIns(3,4)P_2) and phosphatidylinositol (3,4,5)-trisphosphate (PtdIns(3,4,5)P_3), have inhibitory effects[55]. In agreement with these results, overexpression of PTEN, which hydrolyzes PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3, stimulates autophagy[56]. The PtdIns3K inhibitors inhibit both classes of PtdIns3K enzymes, and thus downregulate both autophagy and S6 phosphorylation.

Insulin had been shown to inhibit autophagy and we now know that the initial steps in insulin signal transduction occur at the plasma membrane and lead to the activation of the class I PtdIns3K and the production of PtdIns(3,4,5)P_3 to promote the membrane recruitment and activation of protein kinase B (PKB; also known as AKT) through 3-phosphoinositide-dependent protein kinase 1 (PDK1; Fig. 2). Subsequent studies demonstrated that activation of this pathway, by expressing an active form of PKB, or expressing a constitutively active form of PDK1, has an inhibitory effect on autophagy[56,57]. Moreover, TOR is a downstream target: rapamycin reverses the inhibition of autophagy that results from activation of the class I PtdIns3K pathway.

Although TOR was considered central to autophagy regulation, TOR-independent pathways have been recently reported (Fig. 2). For example, Beclin 1 can be activated by the stress-responsive c-Jun amino-terminal kinase 1 (JNK1) and death-associated protein kinase (DAPK)[58,59].

Health and disease

Cancer. Accumulating evidence reveals that alterations in autophagy occur in various human diseases. Cancer was one of the first diseases genetically linked to impaired autophagy: a landmark discovery by Levine's laboratory found that Beclin 1, a phylogenetically conserved protein essential for autophagy, is also a haploinsufficient tumour suppressor[60]. Beclin 1 was originally isolated as a Bcl-2 (B-cell lymphoma 2)-interacting protein. Binding of Beclin 1 to the anti-apoptotic protein Bcl-2 decreases Beclin 1-associated hVps34 PtdIns3K activity and thereby inhibits autophagy[61]. *beclin 1* monoallelic deletion on chromosome locus 17q21 occurs in 40–75% of human ovarian, breast and prostate cancers[62]. Mice with heterozygous loss of *beclin 1* show an accelerated rate of spontaneous tumour development [63,64], and *Atg4C*-deficient mice display a similar propensity[65]. These observations suggest that autophagy is important for tumour suppression.

White and colleagues have provided evidence to explain the apparent paradox as to why autophagy, which functions primarily as a cell-survival pathway, also functions in tumour suppression (Fig. 3). First, in apoptosis-defective cells, autophagy prevents death from necrosis, a

process that might exacerbate local inflammation and promote tumour growth[66]. Second, stressed autophagy-defective tumour cells accumulate p62 (also known as sequestosome 1), damaged mitochondria, reactive oxygen species (ROS) and protein aggregates, which might cause DNA damage, oncogene activation and tumorigenesis[67,68]. However, autophagy may also promote tumour cell survival during metabolic stress in the tumour microenvironment, under conditions of hypoxia and low nutrients. Accordingly, genetic or pharmacological inhibition of autophagy was shown to enhance the cytotoxicity of cancer chemotherapy agents and to promote tumour regression[69,70]. Thus, autophagy can act both positively and negatively with regard to cancer cell survival; autophagy probably functions to prevent cancer initially, but once a tumour develops, the cancer cells utilize autophagy for their own cytoprotection.

Neurodegeneration. Early studies by Rubinsztein's laboratory demonstrated that autophagy affects the degradation of certain aggregate-prone proteins, such as those involved in Huntington's disease[71]. Induction of autophagy by inhibition of TOR attenuates the accumulation of mutant huntingtin aggregates and protects against neurodegeneration in fly and mouse models of Huntington's disease[72]. Subsequent studies provided compelling evidence that activation of autophagy is a beneficial physiological response in nearly all neurodegenerative diseases[73,74]. In addition, two parallel mouse studies using neuronal-specific knockouts of *Atg5* or *Atg7* demonstrate that basal autophagy controls the constitutive turnover of soluble, cytosolic proteins to prevent the accumulation of abnormal neuroproteins that may cause symptoms of neurodegeneration[75,76].

Furthermore, autophagy may eliminate protein aggregates through a selective mechanism[77,78]. One possible autophagy receptor is p62/SQSTM1, a multifunctional adaptor protein that contains an LC3-interacting region (LIR) and a ubiquitin-associated (UBA) domain[79]. Mounting evidence suggests that p62 and NBR1, another apparent autophagy receptor with similar domains[80], serve as cargo receptors to selectively deliver polyubiquitylated, misfolded, aggregated proteins and damaged, potentially deleterious organelles for clearance through autophagy in both mammals and *Drosophila*[79–83] (for more details see also the Perspective by Peter and colleagues on page 836 of this issue)[84]. Importantly, as an *in vivo* LC3-interacting protein that is constantly degraded by autophagy, p62 has been widely used as a marker for autophagic flux[36].

Innate and adaptive immunity. Although as early as 1984 Rikihisa reported that autophagy is induced during *Rickettsia* infection[85], it was not until the emergence of new tools to detect autophagy in infected cells that it became clear autophagy affects diverse aspects of immunity. In 2004, Yoshimori's group, and simultaneously the laboratories of Deretic and Colombo, provided landmark studies showing autophagy is an important defence mechanism against certain invading bacterial pathogens, such as *Mycobacterium tuberculosis* and *Streptococcus pyogenes*[86,87]. Other studies soon extended the list of invading microorganisms that interact with autophagy[88–90]. Recently, Kurata's group, in collaboration with Yoshimori's laboratory, provided the first evidence that an intracellular pattern-recognition receptor, PGRP-LE, affects recognition and delivery of invading *Listeria monocytogenes* to the autophagy-mediated host defence system in *Drosophila*[91]. Randow's laboratory provided further evidence that a human autophagy receptor, NDP52 (nuclear dot protein 52 K), detects ubiquitin-coated *Salmonella enterica* and directs this bacteria into autophagosomes by simultaneously binding to LC3 (ref. 92).

Furthermore, the sequestration of intracellular pathogens during autophagy is not limited to bacteria and parasites. More than a decade ago, Liang *et al.* demonstrated that enforced neuronal expression of Beclin 1 protected mice against alphavirus replication and encephalitis[93]. Subsequent studies with herpes simplex virus confirmed the role of autophagy in engulfing newly assembled viruses inside the host cells[94], whereas autophagy inhibition is essential for viruses to evade innate immunity and cause disease[95]. It is worth noting that as with certain bacteria, viruses may have evolved strategies to utilize the autophagic machinery to establish their own replicative niche. Finally, in addition to a role in innate immunity, autophagy also promotes the adaptive immune response. In particular, Münz's laboratory provided the first demonstration that autophagy is involved in efficient MHC class II presentation of an endogenously synthesized viral protein (Epstein-Barr virus nuclear antigen 1; EBNA1)[96]; the involvement of autophagy in facilitating the processing and presentation of MHC class I antigen was recently demonstrated by Desjardins's laboratory[97].

Ageing and longevity. A common feature of all ageing cells is a progressive accumulation of damaged proteins and organelles (such as defective mitochondria) and decreased autophagic activity could be important for this. Early studies from Bergamini's laboratory showed that autophagy function declines with age *in vivo* in rodents and *in vitro* in isolated hepatocytes[98,99]. They also carried out the first critical analysis showing that caloric restriction, the only intervention known to effectively slow down ageing, prevents the decline of autophagic activity with age[99,100]. The first experimental study implicating autophagy genes in ageing was performed by Levine's group, which showed that knockdown of *bec-1* (*C. elegans* Beclin 1 orthologue-1), inhibits a lifespan-extending phenotype in mutants lacking the insulin signalling gene, *daf*-2 (ref. 101). Subsequent studies in *Drosophila* confirm a critical role for autophagy in promoting longevity, based on the observation that Atg7-deficient flies are short-lived[102], whereas promoting basal levels of autophagy enhances longevity in adult flies[103]. Recent advances in understanding the molecular links between autophagy and ageing control are reviewed on page 842 of this issue[104] and suggest that various signalling pathways and environmental factors may converge on autophagy to regulate ageing.

Current efforts to avoid the decline of autophagy function with age include the practice of using an anti-lipolytic drug that mimics the beneficial effect of caloric restriction on autophagy[105]. Spermidine, a naturally occurring polyamine, also promotes longevity by inducing autophagy, although its lifespan-extending effect has not been investigated clinically[106].

Development and cell death. Since the discovery of the Atg machinery in yeast, Tsukada and Ohsumi noted that yeast autophagy mutants cannot sporulate during starvation[18]. Many subsequent studies in various organisms confirmed the role of autophagy in development. For example, autophagy mutants of *Dictyostelium discoideum* are defective in multicellular development[107], inactivation of *C. elegans* autophagy genes disrupts normal dauer formation[101], mutation of *Drosophila Atg1* or *Atg3* results in premature death from the larval to the pupal stage[107] and loss of *beclin 1* in mice results in early embryonic lethality[63,64]. Given

this, it has long been presumed that autophagy supplies nutrients during developmental remodelling processes that occur during starvation. However, caution must be exercised in drawing conclusions about these phenotypes. For example, *Atg7*$^{-/-}$ *Drosophila* show normal metamorphosis[102], and *Atg5*$^{-/-}$ and *Atg7*$^{-/-}$ mice (generated by mating heterozygotes) survive embryogenesis and appear normal at birth[109,110]. These studies, however, might overlook the role of autophagy during very early development. Indeed, Mizushima's laboratory provided compelling evidence using oocyte-specific *Atg5*$^{-/-}$ mice that autophagy is induced shortly after fertilization and is essential during a short period of early embryogenesis, the oocyte-to-embryo transition, but not for later embryo development[111] (see also the review by Mizushima and Levine on the role of autophagy in mammalian cell differentiation and development on page 823 of this issue)[112].

In addition to the well-documented role of autophagy in cell survival, a function for autophagy in cell death has long been proposed. Autophagic cell death was originally described in tissues undergoing active development. In the early 1960s and 1970s, ultrastructural studies revealed that in *Drosophila* autophagic vacuoles accumulate during an early stage in the destruction of most larval tissues[113,114]. Autophagy is often called 'type II programmed cell death', in contrast to apoptosis, or 'type I programmed cell death'. Yu *et al.* and Shimizu *et al.* provided the first evidence that when apoptosis is compromised, activation of autophagy leads to cell death[115,116]. Notably, the complex crosstalk between 'self-digestion' by autophagy and 'self-killing' by apoptosis may be key in diverse aspects of development and disease pathogenesis. Autophagic cell death is especially important for development because certain developmental programmes require massive cell elimination. Although there is no definitive evidence that autophagy is necessary for developmental cell death in mammals, Berry *et al.* have provided compelling evidence that in *Drosophila* autophagy is indeed required for developmental degradation of salivary gland cells[117]. McPhee *et al.* provided further evidence that an engulfment receptor, Draper, is required for the induction of autophagy during degradation of salivary glands, but not starvation-induced autophagy in the fat body, which is associated with survival[118]. This suggests that Draper functions to separate autophagy associated with cell death from autophagy leading to cell survival[118]. However, since it is difficult to separate the independent roles of autophagy and apoptosis during the rapid destruction that occurs in the salivary gland, the physiological role of autophagy in developmental cell death is rather complicated, and the observations of 'autophagic cell death' may not be correct, even if autophagy occurs in dying cells. Thus, there is little direct evidence that autophagy drives physiological cell death, and most researchers now refer to cell death 'with autophagic features', reflecting the fact that autophagy is primarily a cell survival mechanism.

Finally, it is worth noting that the putative function of autophagy in cell death is not restricted to developmental programmed cell death but also extends to cell death that occurs during various pathological conditions, such as cancer, neurodegeneration, immunity and ageing. There is no doubt that the process of autophagy, which has the capacity to degrade entire organelles, can be extremely detrimental to cellular physiology if not properly regulated. Therefore, a full understanding of the paradoxical roles of autophagy in promoting life and death will be critical for a practical assessment of autophagy and its use as a therapeutic intervention.

CONCLUSIONS

Three critical points emerge from this historical survey. First, our current knowledge of autophagy, especially in human physiology, represents only the tip of the iceberg. Autophagy may function primarily as a cytoprotective mechanism, for example, to maintain nutrient and energy homeostasis during starvation conditions, or to clear defective proteins, damaged organelles and invasive pathogens that cause various diseases. However, activation of autophagy can also be harmful: autophagy might allow cancer cells to become resistant to chemotherapy, or excessive autophagy might cause undesirable cell death. Thus, defining the precise roles of autophagy in specific disease contexts, and determining whether stimulation or inhibition of autophagy is more beneficial are future goals. Second, we need a greater understanding of the regulatory pathways that control autophagy. In particular, how does the cell determine the specificity and magnitude of autophagy based on complex signalling inputs? Finally, there are still many fundamental questions about the molecular actions of the Atg proteins, the membrane source(s) for autophagosome formation, the mechanism of sequestering vesicle formation and the selective nature of autophagy. Our knowledge about autophagy is growing rapidly. Perhaps soon, we will be able to manipulate autophagy to fight disease and promote health.

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