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Competing interests statement

The authors declare no competing financial interests.

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OPINION

Autophagy: dual roles in life and death?

Eric H. Baehrecke

Abstract | Autophagy is an evolutionarily conserved mechanism for the degradation of cellular components in the cytoplasm, and serves as a cell survival mechanism in starving cells. Recent studies indicate that autophagy also functions in cell death, but the precise role of this catabolic process in dying cells is not clear. Here I discuss the possible roles for autophagy in dying cells and how understanding the relationship between autophagy, cell survival and cell death is important for health and development.

The development of multicellular organisms involves an important balance between cell growth, cell division and cell death. The coordination of these cellular programmes ensures appropriate organ and body size¹, and the disruption of cell division and death can result in abnormal growth typical of cancer².

Other factors, including the cellular response to growth signals, the interactions between cells and the extracellular matrix, metabolic rate, nutritional status and nutrient uptake, might also deviate during abnormal growth³. Therefore, it is important to consider all these factors when investigating the mechanisms that regulate normal and abnormal growth and development.

Programmed cell death has an important role in all animals, by forming and deleting structures, controlling cell numbers and eliminating abnormal damaged cells⁴. Three forms of cell death have been defined in dying cells of developing animals on the basis of morphology, location and dependency on lysosomes^{5,6}. Type 1 programmed cell death (or apoptosis) is defined by the condensation of the nucleus and cytoplasm, the association of chromatin with the nuclear periphery, DNA fragmentation, membrane blebbing,

and the engulfment and lysosomal degradation of the dying cell by a phagocyte⁷. Type 2, or autophagic, cell death differs from apoptosis by the presence of autophagic vacuoles (autophagosomes) and autophagolysosomes in the dying cell that are used for self-degradation. This process is independent of phagocytes. Type 3 (or non-lysosomal) cell death involves the swelling of organelles and the lysosome-independent formation of 'empty spaces' in the cytoplasm, and has similarities to necrosis. Apoptosis and autophagic cell death have been observed in many types of developing animals, whereas non-lysosomal cell death does not seem to be as common in non-pathological conditions. Several other forms of cell death have been described on the basis of studies in derived cell lines (reviewed in REF 8), but it is not yet clear if these cell morphologies are relevant in physiological situations.

The degradation and recycling of the building blocks of organelles, proteins and other components of the cytoplasm is important for the maintenance of cellular homeostasis. Two general mechanisms are used for large-scale degradation of components of the cytoplasm; short-lived regulatory proteins are destroyed by the ubiquitin-proteasome system, and long-lived structures and proteins are targeted to the lysosome by autophagy⁹. Several forms of autophagy have been described^{10,11}, but here I focus on macroautophagy (hereafter referred to as autophagy) because of its association with type 2 autophagic cell death.

Many of the genes that regulate autophagy have been conserved in organisms that are as different as yeast and humans, and these organisms all seem to use autophagy as a cell survival mechanism during nutrient deprivation¹². By contrast, little is known about the possible role of autophagy in type 2 autophagic cell death. The presence of autophagic structures in dying cells of diverse organisms led to the hypothesis that autophagy can have a causative role in autophagic cell death^{4,6}. Recent work supports this hypothesis^{13,14}, but alternative models exist, and the precise function of this catabolic process in autophagic cell death is uncertain¹². Here I will briefly introduce the mechanisms that regulate autophagy during cell survival in higher animals, and discuss the roles that autophagy might have in cell death. Recent studies indicate that apoptosis and autophagic cell death are associated either through complementary pathways of activation, or by the presence of both active caspases and autophagy in a common dying cell. The relationship between caspase activation and autophagy might

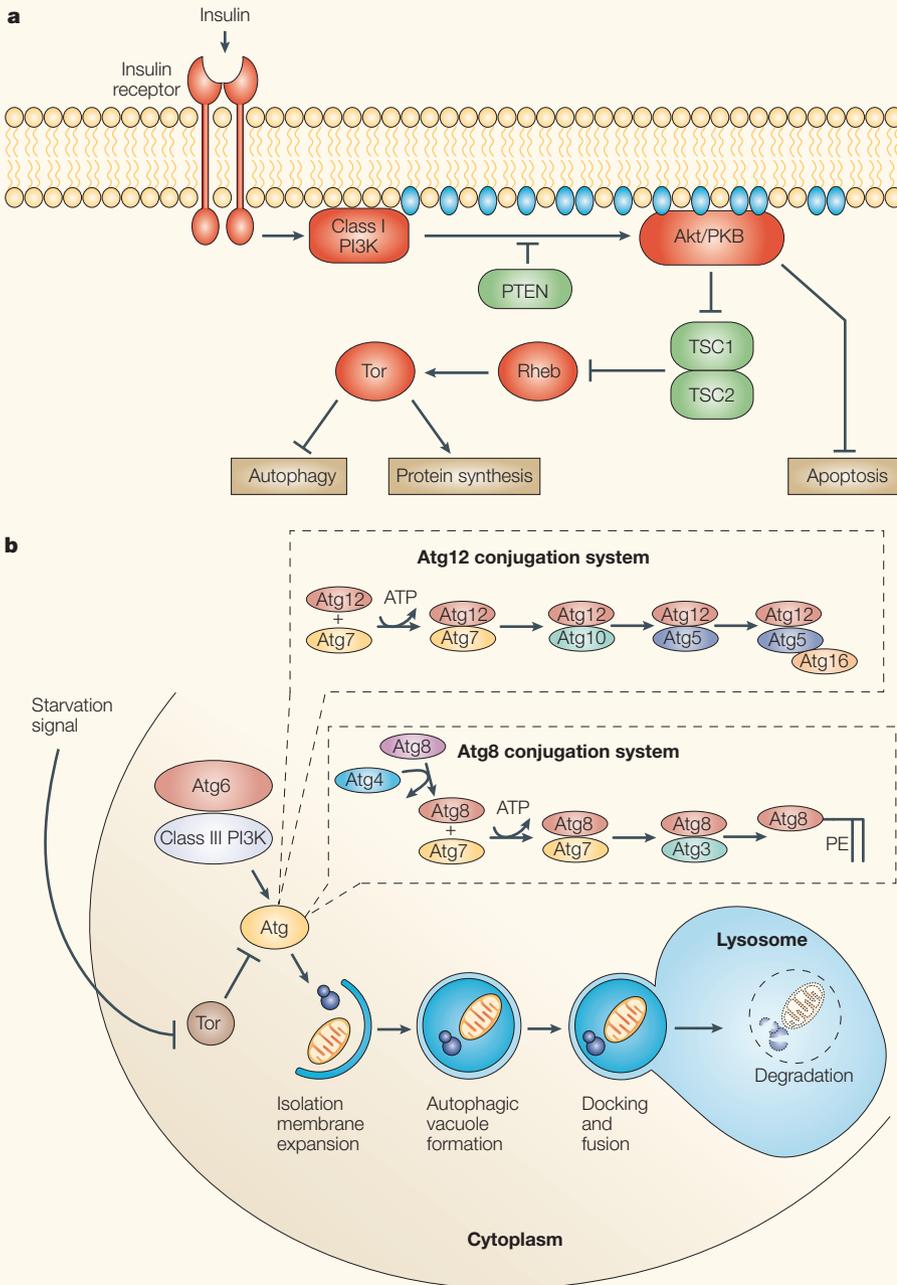


Figure 1 | **Genetic regulation of autophagy.** **a** | Class I phosphatidylinositol 3-kinase (PI3K) signalling has an important role in growth control by the regulation of apoptosis, protein synthesis and autophagy. In multicellular organisms, starvation reduces the levels of intercellular growth factor signals such as insulin. Insulin regulates growth by binding to the insulin receptor, and activating class I PI3K signalling and phosphorylation of plasma membrane lipids (blue). Positive regulators of autophagy (green) in this pathway include phosphatase and tensin homologue (PTEN), tuberous sclerosis complex 1 (TSC1) and TSC2 proteins, and negative regulators (red) include the insulin receptor, class I PI3K, Akt/protein kinase B (PKB), Rheb and Tor. **b** | Autophagy is used to degrade components of the cytoplasm by the induction of an isolation membrane that surrounds cytoplasmic cargo, forming an autophagic vacuole that docks and fuses with the lysosome, and, eventually, lysosomal hydrolases degrade the cargo. Starvation triggers autophagy by modifying Tor signalling. Tor represses autophagy, whereas Atg genes are required for autophagic vacuole formation. Atg6, also known as Beclin1, and class III PI3K are positive regulators of autophagic vacuole formation. Autophagic vacuole formation also involves the two Atg12 and Atg8 ubiquitin-like conjugation pathways. Atg4 encodes a cysteine protease that cleaves Atg8. Atg7 is similar to an E1-like protein, and Atg10 and Atg3 encode E2-like proteins. Atg5, Atg12 and Atg16 are physically associated with the isolation membrane, whereas Atg8 is directly conjugated to the lipid phosphatidylethanolamine (PE) that is inserted in the isolation membrane. Part **a** is modified with permission from *Nature* REF. 66 © Macmillan Magazines Ltd, and from *Science* REF. 9 © American Association for the Advancement of Science. Part **b** is modified with permission from *Nature Reviews Molecular Cell Biology* REF. 11 © Macmillan Magazines Ltd.

differ depending on the organism, cell type and cell-specific conditions, and understanding this relationship is crucial to decipher the mechanisms that regulate cell death. It is important to distinguish between the possible role for autophagy in cell nutrition, survival and death, as these functions could profoundly impact on growth, development and health.

Autophagy regulates cell survival

Starvation triggers the degradation of components in the cytoplasm by autophagy. This involves the formation of double-membrane autophagic vacuoles, also known as autophagosomes, which transport cytoplasmic cargo to the lysosome for degradation⁹. Genetic screens in yeast led to the identification of the autophagy (Atg) genes¹⁵⁻¹⁷, and many of these genes are conserved and function in this catabolic process in animals, including worms, flies and mammals^{12,18}.

Autophagy is regulated by the class I and class III phosphatidylinositol 3-kinase (PI3K) signalling pathways¹⁹. Recent studies have shown that many components of the class I PI3K pathway are involved in the regulation of autophagy in animal cells²⁰⁻²³ (FIG. 1a). Class I PI3K is a negative regulator of autophagy that is activated by the insulin receptor and the insulin receptor substrate proteins, thereby providing a link to nutrient availability. Activation of class I PI3K leads to the phosphorylation of plasma membrane lipids that recruit and activate Akt/protein kinase B (PKB), a downstream negative regulator of autophagy. The tumour suppressor phosphatase and tensin homologue (PTEN) prevents the accumulation of phosphates on lipids and the activation of Akt/PKB, and therefore positively regulates autophagy. Activated Akt/PKB represses the positive regulators of autophagy, the tuberous sclerosis complex 1 (TSC1) and TSC2 proteins, that repress the small G protein Rheb. Rheb regulates the target of rapamycin (Tor), and Tor inhibits autophagy. The biochemical mechanism by which Tor kinase inhibits autophagy is not completely understood, but it involves a protein complex that is associated with the kinase Atg1, which has been studied primarily in yeast²⁴ (for a review, see REF. 25).

The isolation membrane that encloses cargo is derived from a pre-autophagosomal structure that seems to be under positive control of the class III PI3K signalling complex and includes the autophagy regulator Atg6 (also known as Beclin1)^{26,27} (FIG. 1b). Isolation membrane expansion involves two ubiquitin-like pathways (reviewed in REF. 11), and the proteins that regulate this

process, including *Atg3*, *Atg4*, *Atg5*, *Atg7*, *Atg8*, *Atg10*, *Atg12* and *Atg16*, seem to be conserved in higher animals. Once cargo is packaged into the autophagosome, the outer membrane fuses with the lysosome, and the cargo is subsequently degraded by lysosomal hydrolases. An important mystery that remains to be solved is how cargo is selected for degradation by the autophagosome.

The class I PI3K signalling pathway has a complex role in the maintenance of cellular homeostasis, which involves regulating several processes including protein synthesis, nutrient uptake, the cell cycle, inhibition of apoptosis, as well as autophagy^{28,29}. So, the regulation of autophagy is probably intimately linked with other fundamental cellular processes. As growth involves a balance between anabolic and catabolic processes such as protein synthesis and degradation, respectively, it is logical that these processes are controlled by a common pathway. This raises the issue of the relationship between the PI3K pathway, autophagy and cell death. Several studies have shown that PI3K and Akt signalling enables cell survival (reviewed in REF. 30). For example, growth factors suppress apoptosis by the Akt-dependent phosphorylation of BAD, thereby preventing this pro-apoptotic member of the BCL2 family from activating cell death³¹. Given the similarity between the outcomes of the suppression of apoptosis and autophagy-mediated cell survival, why and how does autophagy occur and function in type 2 programmed cell death?

Autophagy and cell death

Recent studies have identified an association between apoptosis and other forms of cell killing, including autophagic cell death. Cells that are exposed to extracellular death ligands, including tumour-necrosis factor (TNF) and Fas ligand, undergo death-receptor-mediated apoptosis³² (FIG. 2). These ligands are bound by trimeric death receptors that recruit adaptor proteins, including FADD and TRADD, and the receptor-interacting protein RIP. The adaptor proteins then recruit procaspase-8 and procaspase-10 to this death-receptor signalling complex, where they are activated and initiate apoptosis. Caspase-8 and caspase-10 either directly activate effector caspases, such as caspase-3, or activate the mitochondrial apoptosis pathway, which involves BCL2 family members, cytochrome *c* release, and the formation and activation of the apoptosome (a complex that comprises caspase-9, apoptotic protease-activating factor 1 (APAF1) and cytochrome *c*)³³.

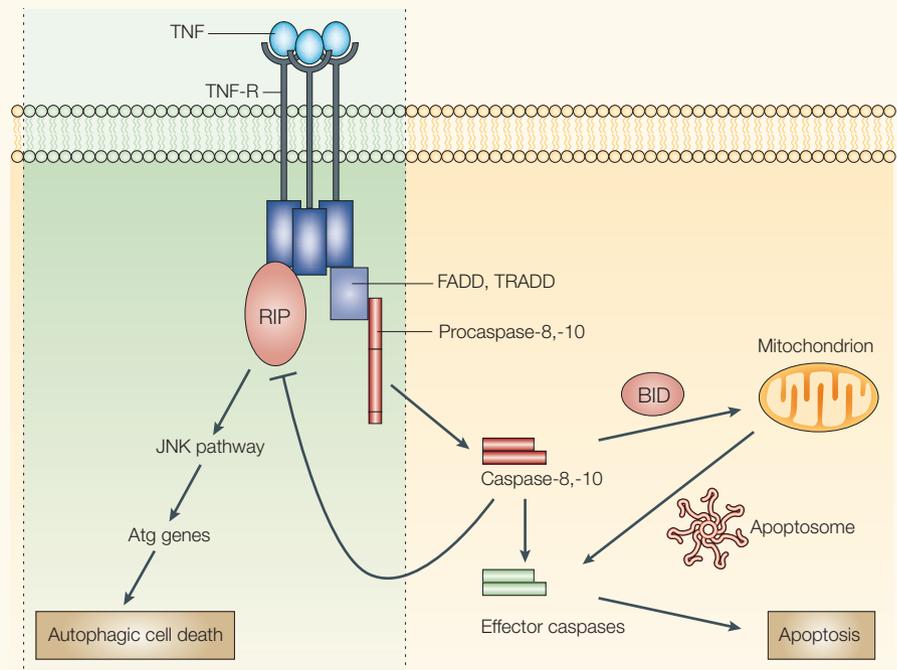


Figure 2 | Cell death can involve both caspases and autophagy. Caspases and autophagy are involved in complementary death pathways in cells. Binding of trimeric extracellular death ligands, such as tumour-necrosis factor (TNF), to trimers of their death receptors, such as the TNF receptor (TNF-R), recruits both the kinase receptor-interacting protein (RIP) and procaspase-8 and procaspase-10 through the adaptor proteins TNF-R-associated death domain (TRADD) and Fas-associated death domain (FADD) to form the death-receptor signalling complex. The proximity of caspase-8 and caspase-10 leads to the activation of either the mitochondrial cell death pathway involving BCL2 family members, such as BID, and the apoptosome (comprising caspase-9, apoptotic protease-activating factor 1 (APAF1) and cytochrome *c*) or the activation of effector caspases and apoptosis. If caspase-8 is inhibited, RIP, Jun N-terminal kinase (JNK) and *Atg* genes regulate autophagic cell death. A model has emerged in which type 2, or autophagic, cell death and apoptosis might complement each other, as active caspase-8 cleaves RIP and prevents it from activating autophagic cell death.

Caspases and autophagy. Death receptor signalling can also activate caspase-independent cell death, and these dying cells have a vacuolated, necrosis-like morphology^{34–37}. Many of these caspase-independent cell deaths depend on specific gene functions that distinguish these types of cell death from non-physiological necrosis, and this prompted us to investigate whether autophagy occurs in such dying cells¹³. The inhibition of caspase-8 induced cell death in mouse L929 cells, and these dying cells contained autophagic vacuoles. As described in previous studies of necrosis-like cell death, this autophagic cell death depends on the function of RIP, as well as on components of the Jun N-terminal kinase pathway (FIG. 2). Importantly, the formation of autophagic structures and the induction of cell death can be blocked by silencing of the autophagy genes *Atg7* and *Atg6*. Similarly, embryonic fibroblasts derived from mice that lack the function of the BCL2 family members BAX and BAK are resistant to apoptosis but die by autophagic cell death that requires *Atg5* and

Atg6 function¹⁴. These studies indicate that caspase activity and autophagy might have complementary roles that regulate distinct forms of cell death.

By contrast, caspases might also function in dying cells with an autophagic morphology. Both the formation of autophagic vacuoles and the activation of caspases occur during autophagic cell death of *Drosophila melanogaster* salivary glands and midguts^{38–40}. Autophagic vacuoles are not observed in dying salivary glands until after caspases are activated, which indicates that autophagy might be involved in degrading cell structures after death proteases are activated. Alternatively, caspases and autophagy could function in parallel genetic pathways. Consistent with this model, expression of the caspase inhibitor p35 prevents DNA fragmentation and caspase cleavage of nuclear lamin in salivary glands, but does not block changes in cytoplasmic structures, which indicates that autophagy might occur independently of caspase activation^{38,39}. Similarly, autophagosomes and active caspases are

present during the death of luminal cells in three-dimensional cultures of human MCF-10 cells⁴¹. Even though the TNF-related apoptosis-inducing ligand (TRAIL) was required for the formation of autophagic vacuoles in these cultures, the inhibition of TRAIL alone did not completely block lumen formation⁴². Lumen formation was blocked when both BCL-X_L was expressed and TRAIL was inhibited, which indicates that the combined activity of caspases and autophagy are involved in proper lumen formation. Although empirical studies of *D. melanogaster* salivary glands and three-dimensional cultures of human MCF-10 cells are complicated by the presence of caspases and autophagy, these more physiologically relevant systems could indicate that such complexity is more common than studies of isolated cell lines indicate.

Autophagy and cell death: why? Which roles could autophagy serve in dying cells? One possibility is that autophagy does not have a role in cell death, and that autophagy is keeping the cell alive under stress conditions that precede their demise. The fact that autophagy is a known survival strategy is a compelling reason for this possibility, and this is further supported by data indicating that the inhibition of autophagy in starving cells activates apoptosis⁴³. However, these results do not exclude the possibility that autophagy could also have an active role in cell death. For example, it is counter-productive that the same steroid signal that activates transcription of cell death regulators, including caspases, would activate Atg genes for survival just 2 hours prior to the death of salivary glands in *D. melanogaster*⁴⁴. Although it is possible that autophagy is trying to save cells after the induction of caspases in these fly cells, this does not seem to be an efficient use of energy and resources.

Autophagy could also function by activating type 2 autophagic cell death. Such a death function could be achieved by several mechanisms, including either the autophagic digestion of a crucial threshold of cytoplasmic factors that are needed for survival, or the selective degradation of regulatory molecules or organelles that are essential for survival. Even though self-degradation seems to be an inefficient way to die, targeted degradation of survival factors such as cell-death inhibitors would be an appealing cell-death mechanism and is a known mechanism for caspase activation⁴⁵. Autophagy is considered a non-selective bulk degradation mechanism, but a recent study indicates that proteins can be preferentially

“Although the mechanisms that regulate autophagy in dying cells, and how autophagy might actually participate in death, are not clear, there are several possibilities for how autophagy might participate in cell death, and these hypotheses need to be tested.”

targeted for autophagic degradation⁴⁶, which indicates that selective degradation of a survival factor is possible, in principle.

There are several additional possibilities that could explain the presence of autophagic structures in dying cells. Whereas many studies emphasize autophagy in the context of the nutritional status of the isolated cell, it is also possible to consider both autophagy and programmed cell death in the context of the nutritional status and the use of resources, such as amino acids, by multicellular organisms. The non-feeding dauer larva stage of the nematode worm *Caenorhabditis elegans* uses autophagy to sustain prolonged periods without food²⁰. Similarly, autophagic cell death during the non-feeding metamorphosis stages in insects and amphibians, such as *D. melanogaster* and *Xenopus laevis*, could represent a mechanism for the use of the resources of obsolete larval cells to benefit the forming adult cells at a time when food cannot be consumed.

Perhaps the simplest explanation for autophagy in dying cells is that type 2 cell deaths require both the death and the degradation machineries, which, in the case of apoptosis, are contained in distinct apoptotic and phagocytic cells, respectively. For example, during insect and amphibian metamorphosis, many larval cells are destroyed by autophagic cell death while adult structures are being formed, and it is possible that the number of phagocytes that are available to degrade the large number of dying cells is limited. In addition, physical requirements, including basement membranes and a need for maintaining epithelial integrity and polarity, might present a barrier that isolates phagocytes from dying cells. This could provide an explanation for autophagic cell death during cavitation and lumen formation to enable the recycling of valuable resources.

Survival versus cell-death mechanisms.

Assuming that autophagy has an active role in cell death, it is important to determine the regulatory mechanisms that distinguish between the function of autophagy in survival and in death (FIG. 3). During starvation, autophagy might need to be coordinated with the suppression of apoptosis, the inhibition of protein synthesis and other processes to enable cell survival. The class I PI3K signalling pathway can explain some of these relationships, but there are gaps in our understanding of the coordinated regulation of autophagy, cell survival and cell death. If autophagy functions in the context of death, this indicates that the suppression of death and autophagy might not always be strictly associated. Perhaps one way that autophagy can effectively kill a cell is to rapidly consume components of the cytoplasm, including mitochondria, while maintaining protein synthesis and other energy-consuming processes.

Several proteins have been identified that seem to be involved in autophagic cell death. The transcription regulators BR-C, E74A and E93 are required for steroid-triggered autophagic cell death of *D. melanogaster* salivary glands³⁸, and mutations in these DNA-binding proteins alter the mRNA levels of several classes of genes, including Atg genes, in these dying cells⁴⁴. The death-associated protein kinase (DAPk) and DAPk-related protein-1 (DRP-1) regulate membrane blebbing during apoptosis, but can also promote autophagic vacuole formation in dying cells⁴⁷. Last, *D. melanogaster spinster* mutants have excess neurons and display mating defects⁴⁸, and the expression of the human homologue of spinster in cancer cells causes necrosis-like cell death with increased staining of acidic compartments⁴⁹. Human Spinster binds to the anti-apoptotic BCL2 and BCL-X_L proteins, and expression of BCL-X_L inhibits Spinster-induced cell death. Spinster is also localized to the late endosome and lysosome, which indicates that it might function in lysosome biogenesis and in the late stages in autophagy. Although we know little about how these genes regulate autophagic cell death, studies of their function might provide insight into what distinguishes the use of autophagy in survival and death.

Autophagy and abnormal development

Studies of knockout mice indicate that non-apoptotic cell death might compensate when apoptosis gene function is compromised. Even though mice with mutations in the apoptosis genes *Apaf1*, *caspase-3* and *caspase-9* exhibit reduced brain cell

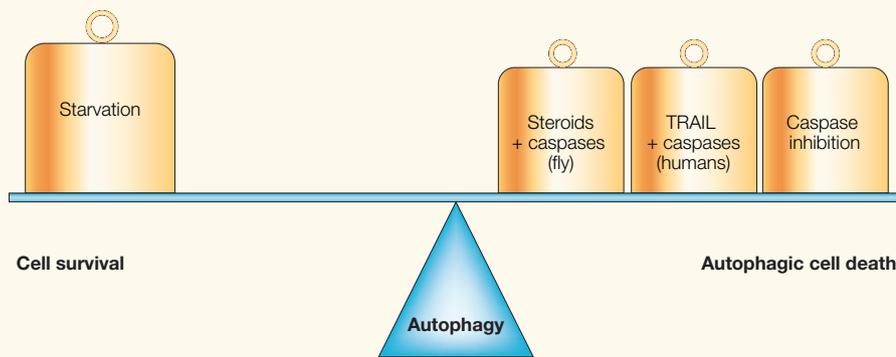


Figure 3 | Possible roles of autophagy in cell survival and death. Autophagy might act as a balancing mechanism between cell survival and cell death. Autophagy functions in cell survival by enabling the use of intracellular resources under starvation conditions. Autophagy has also been associated with cell death. Caspases and autophagy are involved in complementary death pathways in cells where autophagy is required for cell death when caspases are inhibited. In addition, signals such as steroids in flies and tumour-necrosis-factor-related apoptosis-inducing ligand (TRAIL) in human cells activate autophagic cell death that involves both active caspases and autophagy.

death^{50–53}, cells still die in these mutants and exhibit a vacuolated cell morphology^{54,55}. Although the mechanisms that regulate these cell deaths are not clear, recent studies indicate that cells that are unable to die by apoptosis can die while displaying an autophagic morphology^{13,14}. This death depends on the function of the PI3K pathway, Atg5, Atg6 and Atg7, and indicates that some cells can compensate for defects in cell death by using an autophagic cell-death mechanism.

Several studies have indicated links between autophagy, abnormal growth and diseases including cancer, muscular disorder, neurodegeneration, pathogenesis and ageing (reviewed in REF. 56). The presence of autophagic structures in neoplastic cells of various tumours indicates that autophagy could enhance cancer cell survival^{29,57}. This could be accomplished by several mechanisms, including optimizing the use of nutrients in rapidly growing cells, the degradation of organelles such as depolarized mitochondria that activate cell-death pathways, or the prevention of the accumulation of free-radical-induced damage by removing organelles that are sources or targets of such damage. Autophagy might also suppress tumour growth by limiting cell size and, possibly, causing cell death. Mutations in both positive and negative regulators of autophagy that are components of the class I PI3K pathway are associated with human cancers^{30,58}, but it is not clear whether any of these mutations either enhance or suppress autophagy in neoplastic cells. Many of these mutations increase either class I PI3K or Akt activity, and this indicates that autophagy might be suppressed

during neoplastic growth. The diverse biological functions that are regulated by class I PI3K signalling, and the lack of information with regard to autophagy in different types of tumours, make it difficult to draw firm conclusions about how mutations in this pathway are altering autophagy and tumour development at this time. Importantly, one allele of the autophagy gene *Atg6* is deleted in several forms of human cancer, and *Atg6*-knockout mice that are heterozygous mutant have epithelial and haematopoietic malignancies^{59,60}. Even though it is not clear why these mice develop neoplastic growth, they provide a fascinating possible link between autophagy and cancer. It should also be noted that *Atg6* was identified as a BCL2-interacting protein⁶¹, thereby providing an interesting association with cell death, which also requires further investigation.

Concluding remarks

Recent studies indicate that autophagy might have an active role in programmed cell death. Autophagic cell death compensates for apoptosis in some cell types where crucial apoptosis regulators are either lacking or inhibited^{13,14}. By contrast, active caspases are present and autophagy occurs in other cell types that die with an autophagic cell-death morphology^{38,39,41,42}. Although the mechanisms that regulate autophagy in dying cells, and how autophagy might actually participate in death, are not clear, there are several possibilities for how autophagy might participate in cell death, and these hypotheses need to be tested.

Most of our understanding of autophagy is based on studies in yeast and recent

analyses of related genes in higher animals¹². The PI3K pathway is an important regulator of autophagy and several other processes involved in the maintenance of homeostasis. Similar to autophagy, the PI3K pathway is generally thought to function in cell survival, which raises the question of whether this pathway has any role in autophagic cell death. Genetic screens in yeast that resulted in the isolation of the *Atg* genes have provided insight into the mechanisms that regulate autophagy. Even though the mechanisms that regulate autophagy in the context of cell survival seem to be conserved, the study of autophagy in cell death needs to be analysed in higher animals. Perhaps the greatest limitation to studies of autophagy in programmed cell death is the lack of an ideal genetic model system. In addition, the difficulty of visualizing small and dynamic autophagosomes presents yet another challenge. The recent development of better markers of autophagy, such as Atg-green-fluorescent-protein fusion proteins⁶², should improve our ability to study autophagic cell death in animals, and the recent interest in non-apoptotic cell death mechanisms will probably result in a better understanding of this process.

It is reasonable to be sceptical about the role of autophagy in cell death, as substantial data indicate that this catabolic process functions in cell survival¹². This apparent paradox is reminiscent of caspases, which are well known for their function as death proteases. However, recent studies have shown that caspases function in differentiating cells in *D. melanogaster*^{63,64}, in T and B lymphocyte activation and proliferation in humans⁶⁵. So, cell life and death seem to be regulated by common degradation pathways with capabilities to be implemented in seemingly opposing outcomes. Matters of life and death are now further complicated by the observation that both caspases and autophagy might be required in the death of a common cell. Determining the relationship between autophagy and cell death could have important implications for human health, but much work needs to be done before we fully understand the relationship between autophagy, cell survival, cell death and disease.

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Competing interests statement

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