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Review

Autophagy and cell death in model organisms

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Autophagy evolved in unicellular eukaryotes as a means for surviving nutrient stress. During the course of evolution, as multicellular organisms developed specialized cell types and complex intracellular signalling networks, autophagy has been summoned to serve additional cellular functions. Numerous recent studies indicate that apart from its pro-survival role under nutrient limitation, autophagy also participates in cell death. However, the precise role of this catabolic process in dying cells is not fully understood. Although in certain situations autophagy has a protective function, in other types of cell death it actually contributes to cellular destruction. Simple model organisms ranging from the unicellular *Saccharomyces cerevisiae* to the soil amoeba *Dictyostelium discoideum* and the metazoans *Caenorhabditis elegans* and *Drosophila melanogaster* provide clearly defined cell death paradigms that can be used to dissect the involvement of autophagy in cell death, at the molecular level. In this review, we survey current research in simple organisms, linking autophagy to cell death and discuss the complex interplay between autophagy, cell survival and cell death.
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Autophagy is a self-degradation process that is essential for survival, differentiation, development, and homeostasis. There are at least three forms of autophagy – chaperone-mediated autophagy, microautophagy, and macroautophagy – that differ with respect to their mechanisms, physiological functions and cargo specificity. In the best-studied form of autophagy, macroautophagy (herein referred to as autophagy), parts of the cytoplasm, long-lived proteins and intracellular organelles are sequestered within cytoplasmic double-membrane vesicles called autophagosomes or autophagic vacuoles. These characteristic vacuoles are finally delivered to lysosomes for bulk degradation (Figure 1).

Autophagy was discovered in mammalian cells and has been extensively investigated in yeast.[1] These studies have identified many genes encoding proteins involved in autophagy (ATG proteins).[2] ATG proteins participate in the induction of autophagy, the formation, expansion and maturation of autophagosomes, and in the retrieval of autophagic proteins from mature autophagosomes.[3] Fusion processes occur through the t- and v-SNARE complexes, and other molecules, such as the Rab GTPases and components of the vacuolar protein-sorting (VPS) complex. Several protein kinases regulate autophagy, the best characterized being the mammalian target of rapamycin (mTOR), which negatively regulates the pathway.[4] Downstream of TOR kinase, numerous proteins encoded by ATG genes (more than 20 genes in yeast) are essential for the execution of autophagy.[5] The autophagic process is evolutionarily conserved and most yeast ATG genes have homologues in higher organisms (Table 1).

Autophagy has also been linked to cell death pathways. Indeed, excess cytoplasmic vacuolation is the main feature of type II programmed cell death or autophagic cell death. Both protective and destructive contributions of autophagy during cell death have been reported.[6–8] In the following sections we consider the involvement of autophagic mechanisms in cell death pathways, in simple model organisms.

Autophagy in Cell Survival

Autophagy probably evolved as a cellular mechanism for surviving nutrient shortage in the extracellular environment. Autophagy transpires at low basal levels in all cells to serve homeostatic functions such as cytoplasmic, protein and organelle turnover. Autophagy is rapidly upregulated when cellular energy demands are increased and cannot be met by the nutrient supply, for example during starvation. In addition, autophagy acts as a pro-survival process in response to different forms of stress, including growth factor depletion, hypoxia, endoplasmic reticulum (ER) stress, microbial

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Keywords: apoptosis; autophagy; *Caenorhabditis elegans*; excitotoxicity; necrosis; neurodegeneration
Abbreviations: APAF, apoptotic protease activating factor; ATG, autophagy-related genes; Bak, Bcl-2 homologous antagonist/killer; Bax, Bcl-2-associated X protein; Bcl-X_L, Basal cell lymphoma-extra large; BEC, beclin; BH3, Bcl-2 homology region 3; CED, cell death abnormality; DEG, degenerin ion channel family; DIF, differentiation-inducing factor; EGL, egg laying defective; ER, endoplasmic reticulum; IP3R, 1,4,5-triphosphate receptor; JNK, Jun N-terminal kinase; LGG, LC3/GABARAP/GATE-16 family; MEC, mechanosensory abnormality; MEFs, mouse embryonic fibroblasts; polyQ, polyglutamine; Rab, ras in the brain; RIP, receptor-interacting protein; siRNA, small interfering RNA; SNARE, SNAP receptors; TOR, target of rapamycin; UNC, uncoordinated; UPS, ubiquitin-proteasome system; *Uth1*, youth 1; VPS, vacuolar protein-sorting; zDEVD-fmk, benzyloxycarbonyl-Asp-Glu-Val-Asp-fluoromethylketone
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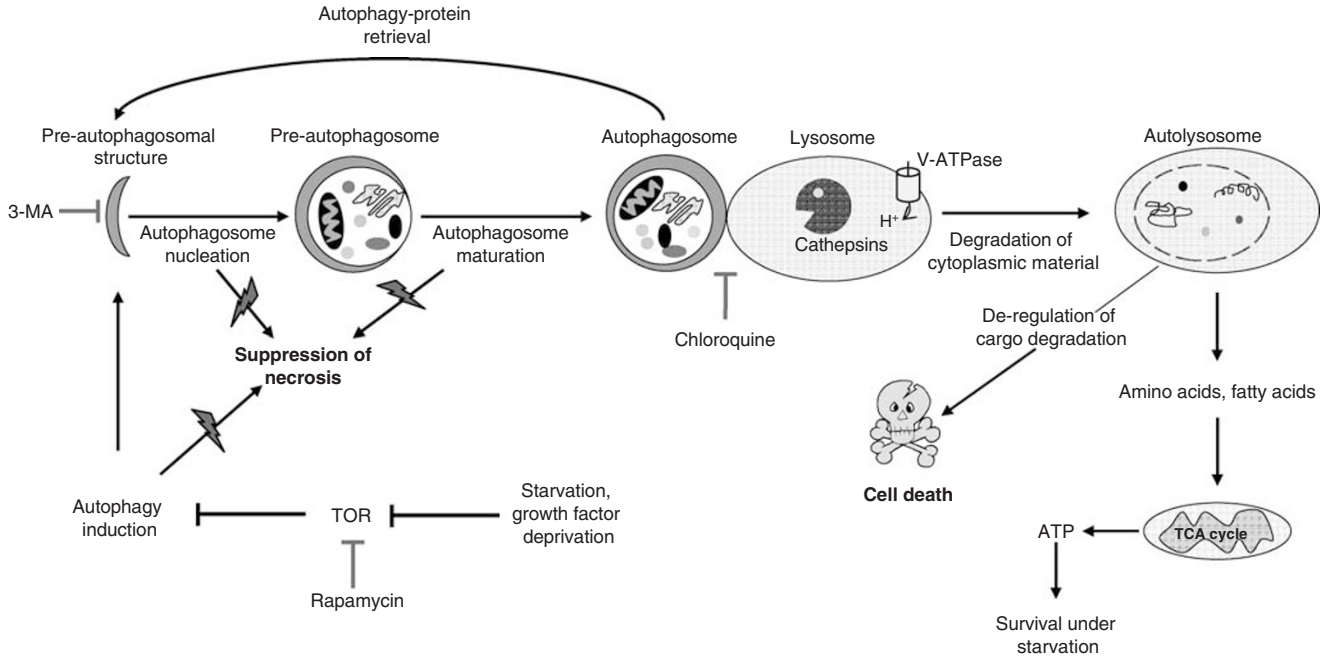

Figure 1 Interfacing the core autophagic pathway with cell death mechanisms. Starvation or growth factor deprivation triggers autophagy by modifying TOR signaling. Autophagy involves the sequestration of portions of the cytoplasm within a double-membrane autophagic vacuole, called autophagosome. The autophagosome undergoes fusion with a lysosome, to form an autolysosome, in which hydrolases degrade the cytoplasmic material. Free fatty acids and amino acids generated in the process can be reutilized by the cell to maintain ATP levels and protein synthesis, promoting cell survival. Certain autophagy proteins are retrieved from autophagosomes and reused. The mechanism of autophagy protein retrieval is not well understood. Mutations that perturb various steps of the autophagy pathway result in suppression of necrotic cell death triggered by diverse stimuli. Pharmacological inhibitors are capable of inhibiting distinct steps of the autophagic pathway (red blocking arrows). TCA: tricarboxylic acid

infection and diseases characterized by the accumulation of protein aggregates.[9–12] This bulk form of macromolecule and organelle catabolism generates free amino and fatty acids, which are necessary metabolic substrates for adaptation to stress.

The role of autophagy in maintaining macromolecular synthesis and ATP production is likely a critical mechanism underlying its evolutionary conserved function. In single-cell organisms such as yeast, the response to starvation is one of the primary functions of autophagy. Genetic screens for mutants defective in survival on nitrogen-poor media, led to the identification of several autophagy genes.[13–15] Many of these genes are conserved and function in the autophagic catabolic process in diverse phyla such as plants, worms, flies and mammals.[5] In nitrogen-starved yeast, loss-of-autophagy results in death, defective sporulation (a process induced by nutrient starvation) and differentiation.[15]

Autophagy is also important for the response of *Dictyostelium discoideum*, a soil amoeba that feeds on bacteria, to starvation. Under nutrient limitation, *Dictyostelium* completes a complex developmental cycle to produce a multicellular organism, a process that requires autophagy. Mutations in the *Dictyostelium* autophagy gene orthologues *ATG5*, *ATG6*, *ATG7* and *ATG8* have subtle effects on growth in the presence of nutrients, but survival during nitrogen starvation is severely reduced.[16,17]

Loss-of-function mutations in ATG genes in plants reduce tolerance to nitrogen or carbon depletion, resulting in enhanced chlorosis, reduced seed set, accelerated leaf senescence and limited lateral root elongation.[18–20] Autophagy is rapidly induced during exposure of *Arabidopsis* seedlings to oxidative stress. Transgenic plants defective in autophagosome formation are hypersensitive to oxidative stress and accumulate a higher level of oxidized proteins because of a lower degradation rate. These findings suggest that autophagy plays a role in eliminating damaged proteins during oxidative stress.[21]

Physiological levels of autophagy promote optimal survival of *C. elegans* during starvation, whereas insufficient or excessive levels of autophagy render animals hypersensitive to starvation.[22] Autophagy is also upregulated during developmental transitions. In *C. elegans* the dauer larval stage is a quiescent, long-term survival diapause phase induced by nutrient deprivation, increased temperature and low insulin signalling. Inactivation of *C. elegans ATG* genes does not affect the initiation of dauer formation, but blocks dauer morphogenesis, inhibits autophagy in the seam cells (a cell type responsible for the formation of the specialized dauer cuticle) and prevents dauer survival.[23]

In mice, there is a massive increase in autophagy immediately after birth in various tissues. Neonates depend upon amino acids produced by autophagic degradation for the maintenance of energy homeostasis during neonatal starvation.[24] The role of autophagy in development is not restricted to developmental changes that occur in the presence of stress conditions. Impairment of autophagy gene function results in lethality during early development even in the presence of normal growth conditions. In *Drosophila*, RNAi against the

Table 1 Autophagy-related genes in higher eukaryotes

Organism						Protein function
Saccharomyces cerevisiae	***Arabidopsis thaliana***	***Dictyostelium discoideum***	***Caenorhabditis elegans***	***Drosophila melanogaster***	***Mus musculus***	
Regulation of autophagy induction						
TOR	*AtTOR*	*DdTOR*	*let-363*	*DrTOR*	*mTOR*	Protein kinase; negative regulator of autophagy
ATG1	*AtATG* (multiple isoforms)	*DdATG1*	*unc-51*	*DrATG1*	*munc51.2*	Ser/Thr protein kinase
ATG11	*AtATG11*	—	—	—	—	Peripheral membrane protein; interacts with ATG1; required for Cvt pathway
ATG19	—	—	—	—	—	Cargo receptor; required for Cvt pathway
Autophagosome nucleation						
ATG6	*AtATG6* (two predicted splice variants)	*DdATG6*	*bec-1*	*DrATG6*	*beclin-1*	Component of class III PI3-kinase complex
VPS34	*AtVPS34*	—	*vps-34*	—	—	Class III PIE-kinase
ATG14	—	—	—	—	—	Subunit of the PI-3-K catalytic subunit
Autophagosome expansion and completion						
ATG3	*AtATG3*	*DdATG3*	Y55F3AM.4	*DrAUT1*	*mAPG3*	E2 like enzyme; conjugates ATG8 to PE
ATG4	*AtATG4* (multiple isoforms)	*DdATG4*	Y87G2A.3	*APG4/AUT2*	*mAPG4*	Cys protease; cleaves at C terminus of ATG8
ATG5	*AtATG5*	*DdATG5*	*atgr-5*	*DrATG5*	*mAPG5*	Conjugated to ATG12 through internal lysine
ATG7	*AtATG7*	*DdATG7*	*atgr-7*	*DrATG7*	*mAPG7*	E1-like enzyme; activates ATG8 and ATG12
ATG8	*AtATG8* (multiple isoforms)	*DdATG8*	*lgg-1*	*DrATG8*	*mAPG8*	Ubiquitin-like protein conjugated to PE
ATG10	*AtATG10* (two predicted splice variants)	—	D2085.2	*DrATG10*	*mAPG10*	E2-like enzyme; conjugates ATG12 to ATG5
ATG12	*AtATG12* (multiple isoforms)	*DdATG12*	*lgg-3*	*DrATG12*	*mAPG12*	Ubiquitin-like protein conjugated to ATG5
ATG13	Two potential genes with restricted similarity	—	—	—	—	Phosphoprotein; dephosphorylated under starvation conditions
ATG16	—	—	K06A1.5	—	*mAPG16*	Component of ATG12-ATG5 complex
ATG17	—	—	—			Interacts with ATG1,
Retrieval of autophagic proteins						
ATG2	*AtATG2*	*DdATG2*	—	*DrATG2*	*mAPG2*	Interacts with ATG9
ATG9	*AtATG9*	*DdATG9*	*atgr-9*	*DrATG9*	*mAPG9*	Integral membrane protein; interacts with ATG2 and ATG23
ATG18	*AtATG18*	—	*atgr-18*	—	—	Required for ATG2 localization

Apart from the genes that have been directly implicated in autophagy, those identified through interactions with autophagy-related proteins or by sequence similarity searches are included.

homologue of the yeast *Aut1* (*ATG3*) gene causes inability to induce autophagy in fat body cells before pupariation and animals die during metamorphosis.[25] Thus, when the supply of environmental nutrients is limited, autophagy can generate a source of metabolic substrates to maintain cellular ATP production, protein, and fatty acid synthesis, to meet cellular and organism energy and metabolic demands.

Autophagy is also involved in the response to intracellular stress conditions (e.g., accumulation of abnormal protein aggregates, damaged or superfluous organelles and intracellular pathogens). In the absence of autophagy, the turnover of cytosolic proteins is impaired, increasing their propensity to become damaged, misfolded and subsequently ubiquitinated and aggregated.[26,27] Basal levels of autophagy may be particularly essential for post-mitotic cells, which cannot redistribute and dilute damaged organelles, proteins and aggregates through cell division. In contrast to the ubiquitin–proteasome system (the other major protein degradation system), autophagic breakdown of substrates is not limited by steric considerations and therefore it is uniquely capable of degrading whole organelles such as mitochondria (mitophagy), peroxisomes (pexophagy), and ER (reticulophagy). Thus, basal and induced autophagy are important for the physiological control of the number and quality of organelles in diverse phyla and function to eliminate superfluous, aged and damaged organelles and proteins.

Autophagy in Cell Death

Although primarily a homeostatic response, autophagy has been adapted to serve additional cellular functions. The presence of autophagic structures in dying cells, in diverse organisms has implicated autophagy in the cell death process. Cell death is of paramount importance both for the development and also during the adult life of animals, by forming and deleting structures, controlling cell numbers, eliminating abnormal or damaged cells and contributing in many pathological situations. Three major types of cell death have been defined based on morphological criteria.[28] Type 1 programmed cell death (or apoptosis) is characterized by dependence on caspases, chromatin condensation and fragmentation and overall cell shrinkage. Blebbing of the plasma membrane leads to the formation of apoptotic bodies, which are ingested by phagocytes. Type 2 (or autophagic cell death) is characterized by increased number of autophagosomes that are used for self-degradation. This process is independent of phagocytes. In type 3 (or necrotic cell death), several intracellular organelles dilate and the plasma membrane breaks down, causing spillage of cytoplasmic content and inflammation.

Based on morphologic criteria, it has been widely recognized that autophagic cell death occurs primarily during developmental periods that require massive cell elimination (e.g., insect metamorphosis). Both caspase activation and autophagic vacuole formation occur during autophagic cell death of *Drosophila* salivary glands and midguts.[29–31] However, in certain cases, autophagy *per se* is neither sufficient nor required for cell death. In addition, during salivary gland regression in *Drosophila*, the caspase inhibitor p35 blocks metamorphic cell death, suggesting that this cell death programme is executed primarily by apoptosis, rather than autophagy.[29] Furthermore, this process is associated with the transcriptional upregulation of pro-apoptotic molecules such as *grim*, *reaper*, *hid* the caspases *dronc* and *dcp-1* and the *ced-4*/*apaf*-1 homologue dark/ark and the downregulation of an apoptosis inhibitor *diap 2*.[29,32–34] Direct induction of autophagy by ATG1 overexpression in *Drosophila*, demonstrates that autophagy is sufficient to induce cell death. Importantly, the ensuing cell death is caspase-dependent and displays apoptotic features, supporting the concept that in animal cells autophagy can induce apoptosis, instead of being a distinct form of cell death.[35] Whether in certain cases autophagy occurs in parallel to the apoptotic pathway, or autophagosome formation is a consequence of caspase activation, or autophagy triggers apoptosis, remains to be clarified.

Dictyostelium offers a particularly advantageous platform to isolate and dissect the mechanisms of autophagic cell death because the *Dictyostelium* genome does not encode components of the apoptotic pathway. In addition, caspase activity is not required for *Dictyostelium* cell death[36] and the single *Dictyostelium* paracaspase gene that has been identified is not required, either for autophagic cell death or for necrotic cell death.[37,38] Therefore, apoptosis does not interfere with autophagic cell death in this organism. *Dictyostelium* displays developmental cell death,[39] which can be mimicked under *in vitro* monolayer conditions, where *Dictyostelium* cells are subjected to starvation and the differentiation-inducing factor (DIF-1).[40] This type of cell death is autophagic, characterized by vacuolation[41] and requires an inositol 1,4,5-triphosphate receptor (IP3R), which governs Ca^{2+} fluxes from the endoplasmic reticulum (ER) stores into the cytosol.[42] Random mutagenesis provides a very powerful tool for the dissection of cell death in *Dictyostelium*.[43] A null mutation in the autophagy gene *ATG1* blocks autophagy and vacuolization, in the above model, but not cell death.[44] This non-vacuolar cell death demonstrates classical characteristics of necrotic cell death such as rapid generation of reactive oxygen species, ATP depletion, perinuclear clustering of mitochondria, and plasma membrane rupture.[45]

The intrinsic pathway that leads to caspase-dependent apoptosis is characterized by mitochondrial outer membrane permeabilization and the release of mitochondrial cytochrome *c*, which results in the formation of a caspase-activating complex encompassing caspase-9 and APAF-1. Expression of the human pro-apoptotic Bax protein in yeast induces growth arrest and loss of yeast cell plating efficiency. In yeast, Bax can be translocated to the outer mitochondrial membrane to form a high conductance channel and to facilitate cytochrome *c* release. This observation indicates that a primitive form of apoptosis exists in yeast. As mitochondrial damage is a critical, life-threatening condition for the cell, the autophagic process may play an important role in assisting cells to remove injured mitochondria and to regulate their turnover. The yeast gene *Uth1* encodes an outer mitochondrial membrane protein involved in mitochondrial biogenesis and stress responses. In the absence of Uth1p, mutants exhibit resistance to autophagy induced by rapamycin.[46] Uth1p is therefore the first mitochondrial protein shown to be required for the autophagic degradation of mitochondria. In addition, *Uth1* mutants survive and proliferate when expressing the human pro-apoptotic cell death gene *bax*.[47] The totality of these findings suggests that Uth1p may provide a direct association between mitophagy and cell death.

Beclin-1 is essential for the initiation of autophagy, perhaps through its interaction with the class III phosphatidylinositol-3-kinase Vps34.[48] The antiapoptotic proteins Bcl-2 and Bcl-X_L bind Beclin-1 and inhibit its autophagic activity. This interaction involves a BH3 domain within Beclin-1 and a BH3 receptor domain within Bcl-X_L. Disruption of this interaction by BH3-only proteins or BH3 mimetics induces autophagy.[49] Interestingly, the beclin-1–BCL-2/BCL-X_L complexes that normally inhibit autophagy are specifically located in the ER and not in the mitochondria. These findings suggest that BH3-only proteins represent a link between self-killing and self-eating. Beclin-1 was also found to interact with Bcl-2 and to protect neuronal cells against virus-induced apoptotic cell death.[50] This interaction is also conserved in *C. elegans* where BEC-1 (the nematode orthologue of Beclin-1) interacts with CED-9, (the *C. elegans* orthologue of Bcl-2).[51] Nematodes lacking *bec-1*, display increased caspase-dependent apoptosis and an elevated number of apoptotic cell corpses in embryonic tissues, revealing a role for BEC-1 in apoptosis. Taken together, these observations indicate that Beclin is a central node coordinating the involvement of autophagy in cell death (Figure 2).

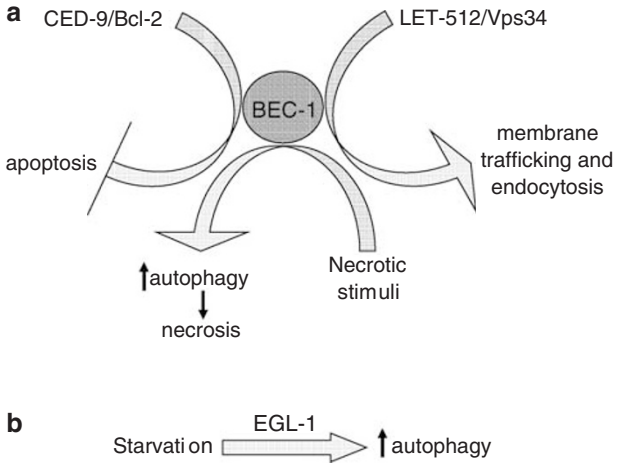

Figure 2 Distinct intracellular cell death pathways and autophagy share common proteins. (**a**) The autophagy protein BEC-1 participates, through different complexes, in the regulation of apoptosis, the induction of autophagy after necrotic insults, and in physiological autophagy functions. BEC-1 regulates apoptosis through complex formation with the antiapoptotic CED-9, providing a direct link between autophagy and apoptosis. Disruption of *bec-1* triggers apoptosis in *C. elegans*. BEC-1 is also required for autophagy induction in response to diverse necrotic stimuli. Inactivation of *bec-1* suppresses necrosis.[52,53] In addition, BEC-1 interacts with LET-512, the *C. elegans* ortholog of the yeast phosphatidylinositol (PtdIns) 3-kinase Vps34, and this interaction is necessary for membrane-trafficking and endocytosis. (**b**) The EGL-1 protein, an activator of programmed cell death, modulates starvation-induced autophagy in *C. elegans*. Depletion of EGL-1 results in limited induction of autophagy after starvation

Many paradigms of programmed cell death in plants display typical morphological features of autophagic cell death.[54] Upon infection by the tobacco mosaic virus, cells elicit the hypersensitive response, a form of programmed cell death, as a means of limiting pathogen spread. A high-throughput virus-induced gene silencing screen to identify genes involved in programmed cell death during the hypersensitive response to pathogen infection led to the isolation of an ATG6/Beclin-1 homologue. Silencing of this gene does not affect the death of infected cells or pathogen spread but causes cell death lesions to spread beyond the infection site, and even to uninfected leaves. These results suggest that Beclin-1 is required for restriction of cell death to the site of infection.[55] The mechanism by which autophagy prevents the spread of cell death is not clear. One possibility is that autophagy contributes to the degradation and removal of death-promoting signals, preventing their dispersal to uninfected cells. Alternatively, autophagy may protect healthy cells from damage caused by reactive oxygen intermediates produced during the defence response.

Autophagy–Apoptosis Interplay

The interplay between autophagy and apoptosis is complex and not fully understood. The two processes are regulated by common factors and share common components, whereas the activity of one can regulate the activity of the other. Several pro-apoptotic signals (e.g., those transduced by BH3-only proteins) induce autophagy, whereas signals that inhibit apoptosis (e.g., through Bcl-2 family members) also inhibit autophagy. An additional finding supporting a direct molecular link between autophagy and apoptosis is the observation that ATG5 can undergo calpain-mediated cleavage to generate a pro-apoptotic fragment that functions in the intrinsic mitochondrial death pathway.[56] The overlap between the molecular mechanisms mediating autophagic cell death and apoptosis is also reflected in the results of microarray studies and serial analysis of gene expression during developmental programmed cell death of the *Drosophila* salivary glands, where genes involved in both apoptosis and autophagy share similar expression profiles.[32,34]

Several apoptotic stimuli can promote compensatory autophagic cell death, in particular when the apoptotic pathway is impaired. Suppression of caspase-8 activity induces non-apoptotic cell death in mouse L929 cells.[57] These cells display loss of membrane integrity and accumulation of autophagosome-like vesicles in the cytoplasm. Execution of cell death requires activation of a pathway involving RIP and components of the Jun N-terminal kinase pathway, and is reduced by downregulation of the autophagy genes *ATG7* and *beclin-1*. Moreover, elimination of the pro-apoptotic proteins Bax and Bak sensitizes specific cell types to autophagic demolition. Bax- and Bak-deprived mouse embryonic fibroblasts (MEFs) treated with apoptotic stimuli (etoposide or staurosporine), or exposed to radiation, fail to undergo apoptosis and instead manifest increased autophagy accompanied by delayed cell death.[58,59] Knockdown of key genes in the autophagic pathway attenuated etoposide-induced cell death of $Bax^{-/-}Bak^{-/-}$ MEFs. These findings indicate that autophagy acts as a 'fail–safe' mechanism to ensure the demolition of the cells destined to die. Surprisingly, treatment with siRNA against Bcl-X_L protects these cells against non-apoptotic death, whereas overexpression of Bcl-2 or Bcl-X_L sensitizes wild type MEFs to autophagy.[59] Together, these data raise the possibility that the mode of programmed cell death is determined in part by the levels of pro- and antiapoptotic signals.

Autophagic death is also inflicted by exposure of apoptosis-defective mammalian cells to histone deacetylase inhibitors.[60] Furthermore, in murine leukaemia cells, treatment with Bcl-2, Bcl-X_L antagonists causes a rapid increase in autophagosome formation and apoptosis.[61] In addition, administration of the autophagy inhibitor wortmanin promotes apoptosis, whereas treatment with the caspase-3/7 suppressor zDEVD-fmk promotes autophagy. Thus, cells can compensate for potential deficiencies in programmed apoptotic death mechanisms by activating alternative autophagic death pathways. What are the mechanisms through which the inhibition of autophagy may favour cell death? One possibility is that inhibition of autophagy perturbs energetic homeostasis and triggers apoptosis. Moreover, inhibition of autophagy may impair the capacity of cells to remove damaged organelles and misfolded proteins, which in turn disrupts homeostasis and elicits apoptosis.

Autophagy–Necrosis Interplay

Several studies have revealed that autophagy is upregulated by necrosis-inducing stimuli. Traumatic brain injury in mice leads to elevation of Beclin-1 levels in neurons and in astrocytes.[62,63] Dying brain cells of adult mice subjected to

unilateral common carotid artery occlusion display cytoplasmic vacuolization, lysis of intracellular organelles and activated autophagy.[64] In addition, autophagy upregulation occurs in murine and chicken models of excitotoxicity.[65–67] Although in some cases autophagosomes accumulate mostly in neuronal axons, in others they gather in the perinuclear region.[66,67] Activation of RIP, a component of the Jun N-terminal kinase (JNK) pathway, in mammalian cells treated with caspase inhibitors also causes toxicity characterized by excess autophagosome formation.[57] The JNK pathway has been associated with caspase-independent necrotic-like damage.[68–70]

Although autophagy is upregulated in the above cases, it was not clear whether it protects cells or contributes to their destruction. Recent studies in *C. elegans* indicate a causative role of autophagy in necrotic cell death.[71,52] Impairment of autophagy by downregulation of the autophagy genes *bec-1*, *unc-51* and *lgg-1* or pharmacological treatment interfering with autophagy partially suppresses necrotic neuronal death induced by hyperactive MEC-4, DEG-1 and DEG-3 ion channels. In contrast, autophagy upregulation by knockdown of the negative autophagy regulator *Ce*TOR or under nutrient deprivation promotes neuron necrosis. Autophagy synergizes with lysosomal proteolytic pathways to facilitate necrotic cell death (Figure 3).[71]

Autophagy and Neurodegenerative Conditions

Several progressive neurodegenerative disorders are associated with accumulation of expanded polyglutamine repeat (polyQ) proteins.[72] Disease severity is correlated with the extent of polyQ expansion; longer polyQ stretches result in earlier onset and more severe symptoms. The mechanisms of pathogenesis underlying both polyQ expansion and toxicity are subject to intense study and several animal models have been developed and brought to bear on this question.[73] The prominent autophagosome accumulation both in experimental models and in human brain autopsies has led to the concept that autophagy constitutes a form of non-programmed cell death that contributes to neurodegeneration. However, recent experimental evidence indicates that autophagy has a neuroprotective role by facilitating clearance of misfolded proteins and aggregates, a hallmark of many neurodegenerative diseases. These two roles of autophagy are not mutually exclusive, and it is possible that the autophagic response is initially activated as a housekeeping mechanism but may eventually contribute to neuropathology.

Misfolded proteins are targeted for degradation either through the ubiquitin–proteasome system (UPS) or the autophagic pathway. New findings now suggest that that these two pathways interact. Autophagy acts as a compensatory degradation system when the UPS is impaired in *Drosophila*. Histone deacetylase 6, a microtubule-associated deacetylase, provides an essential link between autophagy and the UPS in this system.[74] In *C. elegans*, expression of proteins with expanded polyQ in the nervous system results in neuronal dysfunction and degeneration. Amino-terminal huntingtin fragments with varying polyglutamine repeat lengths have been expressed in nematode sensory neurons. Fragments carrying the longest polyglutamine stretch (150 glutamine residues) inhibit neuronal function in an age-dependent fashion. Toxicity is characterized by the formation of protein aggregates and loss of neuronal function, which precedes physical neuron degeneration. Direct evidence that autophagy plays a role *in vivo* in preventing the accumulation of polyQ protein aggregates and protecting against polyQ aggregate-induced disease, is provided from analysis of *C. elegans* polyQ expansion disease models.[75] RNAi knockdown of different autophagy genes that act in distinct steps of the autophagy pathway increases the accumulation of protein aggregates in *C. elegans* muscle cells and sensory neurons and enhances their toxicity.

The abnormal accumulation of intracellular aggregates is a hallmark of disorders associated with expanded polyglutamine proteins. Whether the formation of these aggregates is protective or deleterious is not yet clear. mTOR is sequestered into polyglutamine aggregates in cell models, transgenic mice and human brains, leading to decreased mTOR activity.[76] Impaired mTOR signalling induces autophagy and provides a molecular basis supporting a protective role of aggregate formation, through the indirect stimulation of autophagic clearance of mutant huntingtin fragments. The therapeutic potential of rapamycin a specific inhibitor of mTOR,[77] was tested in a fly model of Huntington's disease. Flies expressing mutant huntingtin exhibited markedly slower neurodegeneration, when treated with rapamycin.[76] Subsequent studies in cell cultures and *Drosophila* have shown that rapamycin also protects against a range of pro-apoptotic insults and reduces paraquat toxicity.[78] A loss-of-function mutation of ATG1 abolished the beneficial effect of rapamycin. The proposed mechanism is based on a reduction of mitochondrial load and consequently of cytochrome *c* release. However, clearance of other non-mitochondrial pro-apoptotic proteins cannot be excluded. TOR, controls many cellular functions in addition to autophagy, including cell growth, protein translation, metabolism and cell death. Therefore, attempts to control autophagy through the manipulation of TOR activity need to take into consideration potential unpredictable and adverse effects.

A screen for autophagy enhancers using a library of FDA-approved drugs has identified the drugs minoxidil (an ATP-sensitive K^+ channel agonist); clonidine (an α_2-adrenergic and type I imidazoline receptor agonist); and verapamil (an L-type Ca^{2+} channel antagonist) as inducers of autophagy. This study reveals a new mTOR-independent pathway regulating autophagy, in which cAMP regulates IP_3 levels and influences calpain activity, which in turn cleaves and activates $G_{s\alpha}$. The therapeutic relevance of the two identified compounds that induce autophagy has been verified using cell, fly and zebrafish models of Huntington's disease.[79] Lithium also stimulates a novel mTOR-independent pathway that regulates autophagy and promotes aggregate clearance in cells and in *Drosophila* models of Huntington's disease.[80] Finally, mutations that affect the dynein machinery impair autophagosome–lysosome fusion, leading to decreased autophagic clearance of aggregate-prone proteins and enhanced toxicity of the mutation that causes Huntington's disease in fly and mouse models.[81]

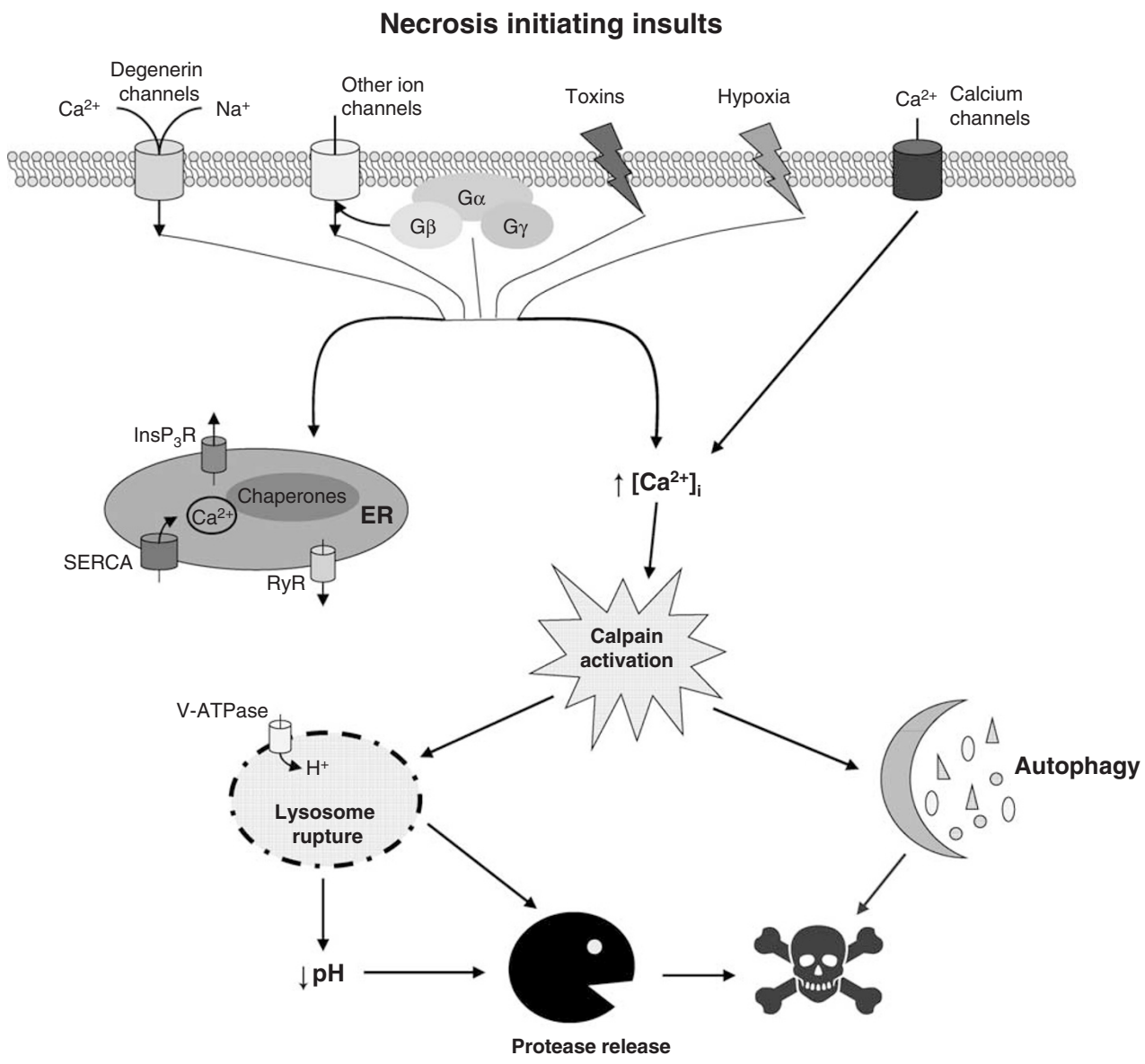

Figure 3 Autophagy is required for and is induced during neurodegeneration in *C. elegans*. Diverse genetic and environmental insults lead to increase of cytoplasmic calcium concentration. Intracellular calcium stores also contribute to the elevation of calcium concentration beyond tolerable levels. The channels and molecules known to be involved in calcium homeostasis are shown. Increased calcium concentration activates cytoplasmic calpain proteases, which facilitate lysosomal rupture and release of acidic lysosomal contents into the cytoplasm, which consequently becomes acidified. The valuolar H^+ ATPase (V-ATPase) pump is responsible for lysosomal and subsequent cytoplasmic acidification. Low pH conditions favour activation of cathepsin proteases and contribute to cellular destruction. Autophagy is upregulated upon induction of necrosis directly and/or through calpain activation and synergizes with lysosomal cathepsin proteases to mediate cell death. $[Ca^{2+}]_i$: cytoplasmic calcium concentration; ER: endoplasmic reticulum; InsP3R: inositol triposphate receptor; RyR: ryanodine receptor; SERCA: sarco-endoplasmic reticulum Ca^{2+}-ATPase; V-ATPase: vacuolar H^+-ATPase

Single Proteins: Multiple Roles

In addition to the direct involvement of autophagy in cell death pathways, accumulating evidence suggests that components of the autophagic machinery may participate in cellular processes other than autophagy. Likewise, recent studies have shown that caspases, apart from their well known role in cell death execution, also regulate differentiation processes in *Drosophila* and participate in T and B lymphocyte activation and proliferation in humans.[82,83] Moreover, EGL-1, the sole pro-apoptotic BH3-only protein in *C. elegans* which is required for developmental cell death in the nematode is also a mediator of starvation-induced autophagy.[49] In addition, APAF-1, an essential factor for cytochrome *c*-driven caspase activation during mitochondrial apoptosis is also implicated in DNA damage-induced cell-cycle arrest.[84] Likewise, *ATG5*, a gene product required for the formation of autophagosomes, is proteolytically activated to become a pro-apoptotic molecule that translocates from the cytocol to mitochondria and triggers cytochrome *c* release and caspase activation.[56]

Mutations in the *unc-51* gene, the *C. elegans* orthologue of yeast *ATG1* result in various abnormalities in axonal elongation and axonal structures. This finding suggests a distinct additional role for *unc-51* in axonal elongation.[85] In addition, the identification of *Drosophila ATG4* gene in a genetic screen for modifiers of developmental phenotypes of Notch-signalling mutants revealed an unexpected link between autophagy and the Notch-signalling pathway.[86] Thus, a general caveat associated with overexpression or knockout of single ATG genes is that such interventions could have unpredictable indirect effects beyond those related with autophagy.

Concluding Remarks and Outlook

Despite the considerable recent advances, our understanding of the dual role of autophagy in cell survival and cell death remains incomplete. It is still not fully understood what factors determine whether autophagy is cytoprotective or cytotoxic and how autophagy contributes to death (Figure 4). In the absence of tight regulation, autophagy may exceed a crucial threshold, inadvertently causing the catabolism of cytoplasmic factors, regulatory molecules and organelles that are essential for survival. Moreover, autophagy may accelerate the apoptotic destruction of the cell by initiating the self-digestion of cells destined to die. Autophagy may also provide the cell with high ATP levels necessary for the energy-dependent apoptotic process. In several cases, morphologic features of both autophagic and apoptotic cell death, or autophagic and necrotic cell death are observed in the same cell.

Studies of autophagy in model organisms have revealed a great deal on the complex interplay between autophagy and cell death pathways. Autophagy has been shown to protect against cell demise in conditions of starvation, nutrient withdrawal, and neurodegeneration,[26,27,87] but is also a critical contributing factor in certain types of cell death.[57,59] It is likely that the dual role of autophagy in cell death is context-dependent. The presence of autophagic structures in dying cells is equally consistent with a causal, neutral, or inhibitory role of autophagy in cell death. The question of whether this is the result of increased autophagic activity or decreased autophagosome–lysosome fusion also needs to be taken into account. Thus to manipulate autophagy for therapeutic purposes, it is essential to consider both its cytoprotective

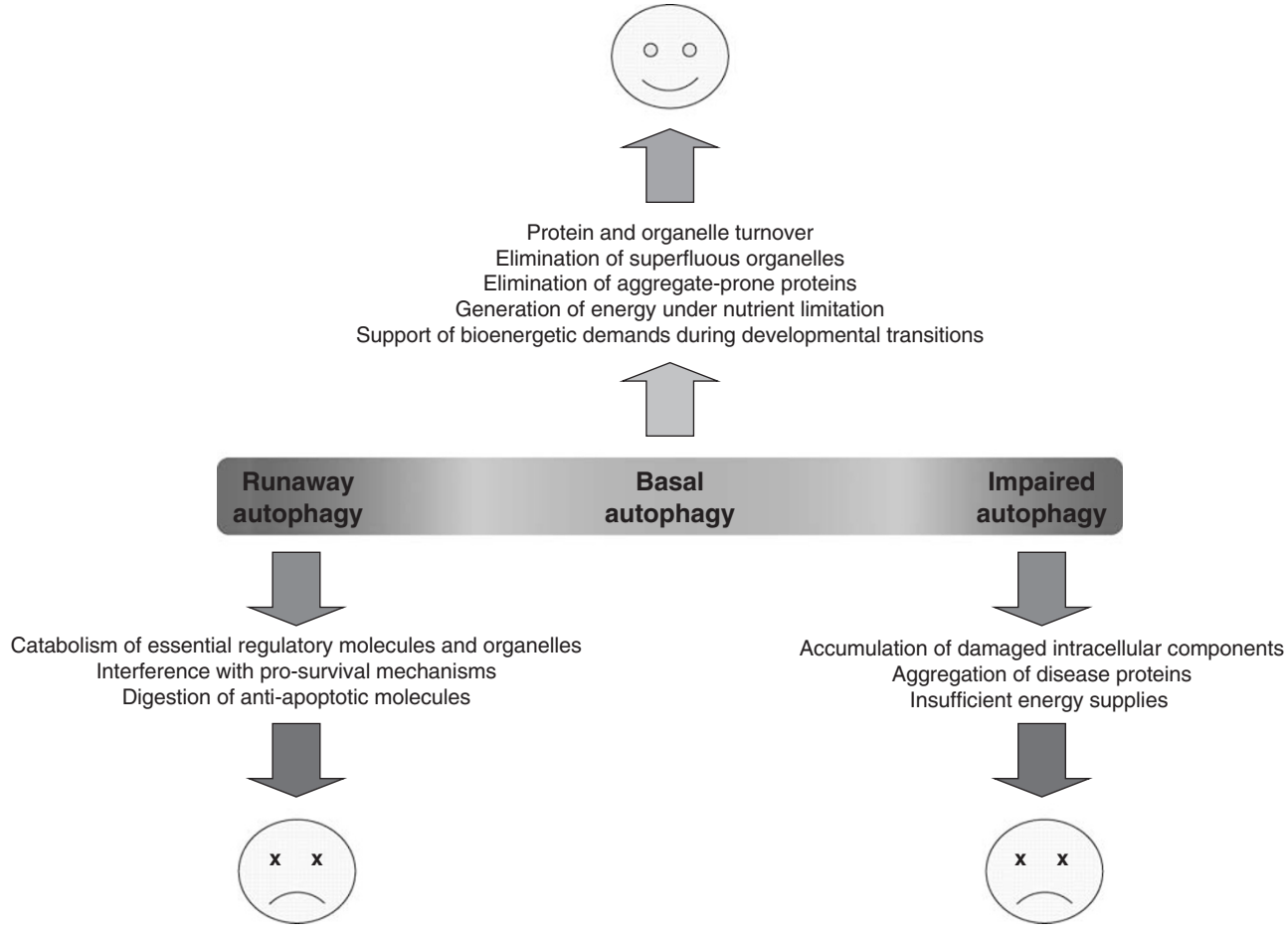

Figure 4 The role of autophagy in cell survival and death. Basal autophagy acts as a cytoprotective mechanism and serves homeostatic functions such as cytoplasmic, protein and organelle turnover. Moreover, autophagy provides metabolic substrates when cell energetic demands are increased, during developmental transitions or nutrient/growth factor deprivation. Uncontrolled, 'runaway' autophagy can digest vital amounts of cell components and survival factors, thus leading to the demise of the cell. Similarly, impaired autophagy can be deleterious by allowing damaged proteins and organelles to accumulate and by failing to provide energy for essential cell functions

and cytotoxic roles, and the additional caveat that blockage of one type of cell death may trigger compensatory cell-destructive pathways. Simple model organisms, with their genetic malleability, provide ideal platforms for investigating these possibilities. Given the evolutionary conserved core autophagic and cell death mechanisms, this endeavour is likely to yield novel and valuable insights on role of autophagy in human disease.

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