chondria. Perhaps the most significant finding is that tBid-induced oligomerization of Bax in isolated mitochondria was inhibited when these organelles were pretreated with protease K, an agent used for the general digestion of proteins. Taken together, these findings suggest that an OMM protein, rather than cardiolipin, is required for pore formation and protein efflux induced by mixtures of tBid and monomeric Bax. Several possible targets have been identified, among them the voltage-dependent anion channel in the OMM and the mitochondrial fission machinery.[37,38] However, similarly to cardiolipin, both have been questioned to play an important role in mediating the release of mitochondrial intermembrane space proteins by tBid and Bax.[36,39] To identify such a factor, and to unravel the precise steps of Bax oligomerization, will be an important task for future research.

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Atg5 and Bcl-2 provide novel insights into the interplay between apoptosis and autophagy

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Autophagy and apoptosis play important roles in the development and cellular homeostasis of eukaryotes. Apoptotic cell death is termed type I programmed cell death. Autophagy regulates both cell survival and cell death. While increased numbers of autophagosomes can be associated with cell death (called type II programmed cell death), it is often unclear if this association is causal. Recent data have revealed possible molecular mechanisms for crosstalk between autophagy and apoptosis. Atg5, previously considered to be an autophagy-specific gene involved in autophagosome precursor expansion and completion through an ubiquitin-like conjugation system, now appears to be an important mediator of apoptosis. Atg5 can be cleaved following death stimuli, and the cleavage product appears to promote mitochondria-mediated apoptosis. Bcl-2, the well-characterised apoptosis guard, appears to be important in autophagy, as it binds to

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Beclin 1/Atg6 and inhibits Beclin 1-mediated autophagy and autophagic cell death. Thus, Bcl-2 and Atg5 are proteins that regulate both apoptosis and autophagy.

Autophagy and Atg5

Macroautophagy (which we will call autophagy) is a major pathway mediating the degradation of long-lived proteins and cytoplasmic organelles in organisms from yeast to man. The process involves the formation of a double-layered autophagosome around a portion of cytosol, which can include organelles like mitochondria. The autophagosomes then ultimately fuse with lysosomes, where their contents are degraded.[1] In yeast, the understanding of autophagy has been significantly advanced by the identification of genes (called Atg genes), which influence various stages of the autophagy process.[2] In mammalian systems, there is basal autophagy that plays numerous roles, including regulating the accumulation of aggregate-prone proteins.[3–6] Under conditions of nutrient deprivation or growth-factor withdrawal, autophagy is induced to higher levels to release nutrients and energy from macromolecules. A key conserved pathway regulating autophagy is mediated by the mammalian target of rapamycin (mTOR). Inactivation of mTOR induces autophagy, while TOR activation inhibits the process. Autophagy appears to also be regulated by mTOR-independent pathways, mediated by IP_3.[7]

It is unclear where the membranes that form mammalian autophagosomes originate from. Some have proposed that they arise from ER or Golgi membranes, while others suggested that they can be assembled *de novo* from small pro-membrane structures.[8,9] Class III PI-3 kinase activity is critical for autophagosome-vesicle nucleation.[10] In *Saccharomyces cerevisiae*, this activity resides in a complex composed of Atg6 (orthologous to mammalian beclin-1), Atg14, Vps15 (an activator of Vps34) and the class III PI-3 kinase, Vps34. Atg14 serves a bridge between Atg6 and Vps34.[11] The PI-3 kinase complex phosphorylates PI and the phosphorylated PI then presumably recruits proteins containing FYVE or PX motifs required for autophagosome formation.[12,13]

The isolation membrane that is thought to be formed as a result of the activity of the Beclin–Vps34 complex is elongated with the help of an ubiquitin-like conjugation system. Atg12 is first activated by Atg7, then transferred to Atg10 and finally covalently attached to Atg5, a process requiring ATP.[14] The Atg12–Atg5 conjugate localises to autophagosome precursors and dissociates just before or after completion of autophagic-vacuole formation.[15,16] A second ubiquitin-like modification involving the protein microtubule-associated protein 1 light chain 3 (MAP-LC3 or LC3) is required for completion of autophagosome formation. The cytosolic precursor of LC3 is cleaved at its C terminus by Atg4 to form LC3-I.[17] LC3-I is covalently conjugated to phosphatidylethanolamine to form LC3-II, a process requiring the activities of Atg7 and Atg3.[18] LC3-II is specifically targeted to the Atg12–Atg5-associated, elongated autophagosome precursors and remains associated with autophagosomes even after fusion with lysosomes, subsequent to which LC3-II is delipidated and recycled.[19]

In addition to autophagy playing protective roles in many contexts, increased number of autophagosomes are seen in certain cell-death scenarios.[20] In many cases, it is unclear if the relationship between cell death and autophagy is causal – for instance, increased numbers of autophagosomes can result if there is decreased autophagic flux due to blockage of autophagosome-lysosome fusion (as well as in situations of increased autophagy). The causal relationships between cell death and autophagy are likely to be complex and context-dependent. However, recent data have added a new ingredient to our understanding of the interplay as it now appears that Atg5 and Bcl-2 each play critical roles in both autophagy and apoptosis. These new findings will be the focus of this short review.

Atg5 and Apoptosis

Atg5 is a critical protein required for autophagy at the stage of autophagosome-precursor synthesis (see above) and its deletion in yeast or mammalian cells/mice effectively blocks autophagy.[21,22] Besides regulating autophagosome formation, Atg5 may be important in apoptosis. Pyo *et al*.[23] reported that Atg5 interacts with FADD (Fas-associated protein with death domain) and this interaction mediates interferon-γ (IFN-γ)-induced cell death. Downregulation of Atg5 in HeLa cells reduced cell death and vacuole formation induced by IFN-γ, and ectopic expression of Atg5-increased cell death. Atg5 did not modulate cell death caused by etoposide, staurosporine or cisplatin. However, FADD-induced cell death was not affected by the reduced expression of Atg5 and by treatment with 3-MA, a class III PI-3 kinase inhibitor widely used to block autophagosome formation. Also, only cell death but not vacuole formation was blocked by caspase inhibition. These data suggested that Atg5 may participate both in autophagy and certain forms of cell death, but that the two processes could be dissociated. Thus, there may be no causal link between these processes in the context of IFN-γ-induced cell death. Instead, Atg5 appears to be a common participant in both processes.

More recently, Yousefi *et al*.[24] showed that Atg5-overexpressing cells exhibit sensitisation to various death stimuli and that silencing of Atg5 reduces drug-induced cell death. There are interesting details that differ between the Yousefi *et al*.[24] and Pyo *et al*.[23] studies – for instance, Pyo *et al*.[23] reported that Atg5-knockdown did not affect staurosporine-induced cell death, while Yousefi *et al*.[24] reported a major protective effect of Atg5-knockdown in this context. Some of these differences may be due to subtle methodological differences. The key finding of Yousefi *et al*.[24] was the identification of a 24 kDa truncated form of Atg5 (comprising residues 1–193) in human neutrophils following withdrawal of granulocyte macrophage colony-stimulating factor (GM-CSF) and in Jurkat cells in response to anti-CD95 antibody, a Fas ligand mimic. Their subsequent studies led to the conclusion that Atg5 is cleaved by calpains 1 and 2 to form this 1–193 cleavage product. Intriguingly, this 24 kDa fragment translocated to mitochondria and caused cytochrome c release (Figure 1). Bcl-2 could block the cell death induced by this Atg5 fragment. The truncated form of Atg5, but not full-length Atg5, bound to Bcl-xl, leading the authors to hypothesise that this binding may inactivate Bcl-xl anti-apoptotic activity by displacing Bcl–xl–Bax complexes, thereby promoting Bax–Bax complex formation. The death-inducing activity of the

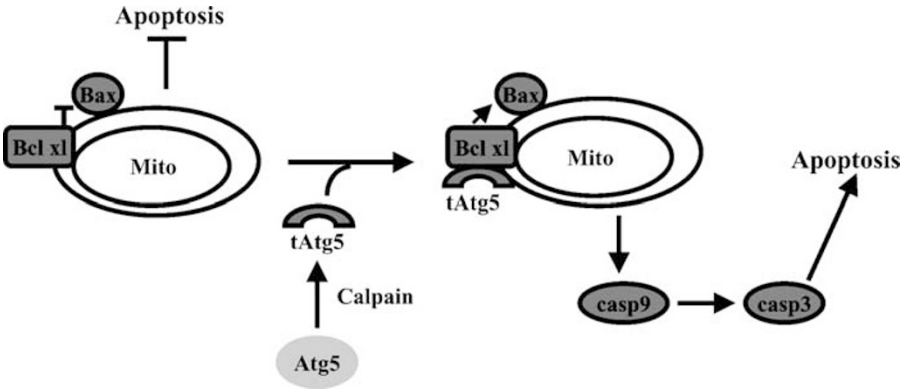

Figure 1 Atg5 is cleaved by calpain and induces apoptosis. Atg5, an autophagy protein is cleaved by calpain 1, 2 into truncated Atg5 (tAtg5), tAtg binds to Bcl-xl and leads to apoptosis

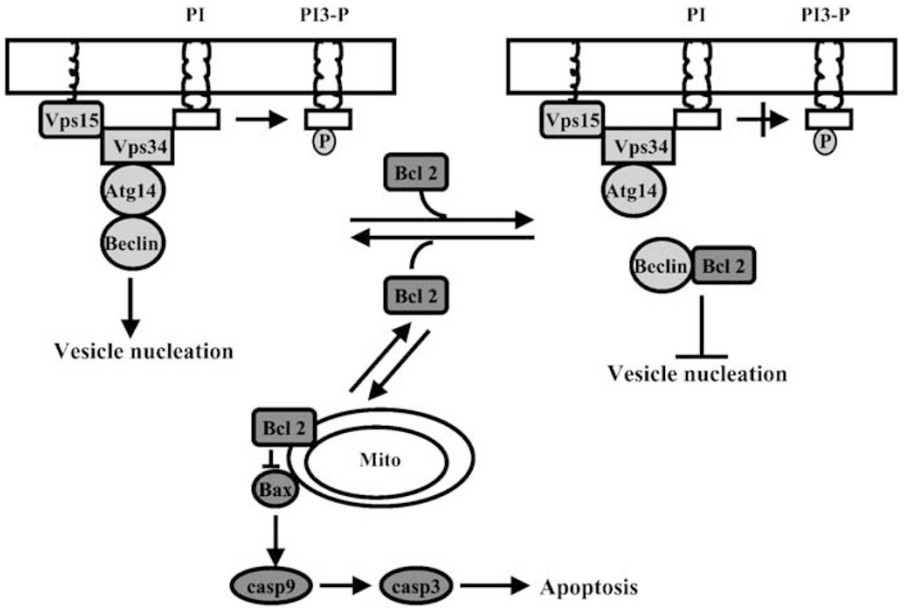

Figure 2 Bcl-2 regulates autophagy and apoptosis. Bcl-2/Bcl-xl binds to Beclin 1 (possibly affecting Beclin 1-Vps34 complex) and inhibits autophagy

truncated form of Atg5 (1–193) was observed in the absence of autophagy. This is consistent with the idea suggested by the data of Pyo *et al*.[23] that these two processes can be dissociated and that Atg5 may be an independent but key player in both. It is possible that the low levels of Atg5-cleavage product may have significant effects on cell death without having any significant impact on the total levels of intact Atg5 that participates in autophagy.

Bcl-2 and Autophagy

In addition to its protective role in apoptosis, Bcl-2 appears to inhibit autophagy. While a number of studies hinted at a role for Bcl-2 in autophagy,[25–27] the strongest case has been made by Levine and colleagues, who have provided molecular mechanisms. The mammalian gene Beclin 1 (which corresponds to Atg6) was originally identified as a Bcl-2-binding protein from yeast two-hybrid experiments. Beclin 1 can complement autophagy in autophagy-defective yeast with Atg6 deficiency, and promote autophagy in human breast carcinoma cells, MCF-7 cells.[28] Interestingly, Bcl-2 inhibits starvation-induced autophagy as a function of its direct interaction with Beclin 1.[29] In yeast, mammalian cells and *in vivo* models, Bcl-2 effectively inhibits Beclin 1-mediated autophagy. However Bcl-2 mutants, which are not able to bind to Beclin 1, do not have this autophagy-inhibitory effect (Figure 2).

Bcl-2 appears to inhibit both autophagy under non-toxic conditions and autophagy-gene-dependent cell death. Conversely, Bcl-2 binding-defective Beclin 1 mutants induce excessive autophagy and autophagic death. This cell death is caspase-independent. Interestingly and mysteriously, only endoplasmic reticulum-targeted Bcl-2 but not mitochondria-targeted Bcl-2 inhibits autophagy.[29]

Is the Mitochondrion a Key Site for Crosstalk between Apoptosis and Autophagy?

Mitochondria are well-known regulators and mediators of apoptosis, for instance serving as the site of release of cytochrome *c*, which activates caspase 9 and then caspase 3.

It is interesting to note that both Atg5 and Bcl-2 mediate many of their effects at the level of the mitochondria, and that the toxicity of the Atg5 cleavage fragment can be abrogated by Bcl-2.

Another level of complexity is introduced into this cross-talk, since the major pathway for mitochondrial clearance is via autophagy. Indeed, levels of mitochondria accumulate when autophagy is blocked, while mitochondrial load decreases when autophagy is activated. Cells show increased susceptibility to subsequent propapoptotic insults after autophagy is blocked.[30,31] Conversely, after autophagy is induced in cells (or flies), cells show increased resistance to subsequent proapoptotic insults.[30] Our data suggest that this is likely to be due to the changes in mitochondrial load resulting from perturbation of autophagy.[30] When autophagy is induced, there are fewer mitochondria after a period of $\sim$72 h (or a period of autophagy perturbation sufficient to influence steady-state levels of mitochondria), and if cells are exposed to proapoptotic insults, there is understandably less cytochrome *c* release and subsequent caspase activation. (The converse occurs when autophagy is inhibited.). Since cells can tolerate major decreases in mitochondrial load without compromise to oxidative phosphorylation, there is a significant window where decreases in mitochondrial load may have beneficial effects with regard to survival after certain toxic insults. Clearly, this mechanism would also be relevant to Atg5 fragment toxicity.

In conclusion, studies investigating molecular mechanisms of cross-talk between apoptosis and autophagy are still in their infancy. However, these provide testable hypotheses and insights into both processes. For instance, the data suggest that it may be important to examine carefully the roles of Atg5 in different types of apoptosis (e.g. in development). This is possible, as both conditional and constitutive knockout mouse models are available for this gene. Interestingly, no developmental abnormalities have been reported in such models, although these may not have been studied carefully in the initial analyses.[22] Further insights into the mechanism by which Atg5 enhances apoptosis will also be revealed. Does the Atg5 fragment bind Bcl-2, in addition to Bcl-xl, and which domains of these proteins interact with Atg5? Does Atg5 fragment binding prevent Bcl-2 and Bcl-xl from sequestering pro-death Bcl-2 family members? Deeper insights into these and related interplays between autophagy and apoptosis are likely to have important implications for our understanding of both process in development, normal physiology and disease.

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Roles and mechanisms of action of the Nrf2 transcription factor in skin morphogenesis, wound repair and skin cancer

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The Nrf2 transcription factor plays a key role in the cellular defense against oxidative and xenobiotic stresses through its capability to induce the expression of genes, which encode detoxifying enzymes and antioxidant proteins. Most interest-

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