Autophagy and Cell Death: No Longer at Odds

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Autophagy has been associated with both cell survival and cell death, but the role of autophagy in cell death has been controversial. In this issue, Berry and Baehrecke (2007) report that autophagy is involved in physiological cell death during *Drosophila* development and is controlled by similar mechanisms as those that control its function in cell survival.

Autophagy is a conserved catabolic process that degrades long-lived proteins, organelles, and bulk cytoplasm (reviewed by Baehrecke, 2002; Maiuri et al., 2007). Autophagy is induced under conditions of stress such as starvation, hypoxia, heat, and drug treatment. A morphological hallmark of autophagy is the presence of autophagosomes—cytoplasmic vesicles that have a double membrane and contain cytoplasmic cargo to be degraded. Autophagosomes fuse with lysosomes to form autolysosomes in which the cytoplasmic cargo is digested. Finally, lysosomal permeases release the digested material back into the cytosol for recycling. In this capacity, autophagy sustains cell viability under starvation conditions for days and weeks.

Paradoxically, autophagy has also been implicated in a type of programmed cell death (type II PCD) called autophagic cell death that is different from apoptosis (type I PCD) (Schweichel and Merker, 1973). Autophagic cell death is also defined by the presence of autophagosomes and autolysosomes in dying cells. However, in contrast to autophagy—which is very well characterized and requires more than 20 autophagy (*atg*) genes (Xie and Klionsky, 2007)—little is known about the mechanisms and genes that regulate autophagic cell death under physiological conditions. In this issue, Berry and Baehrecke (2007) close this gap in our knowledge. These authors show that autophagic cell death occurs under physiological conditions during development and is controlled by similar mechanisms as autophagy in cell survival.

Key regulators of autophagy include the class I phosphoinositide 3-kinase (PI3K) (Blommaart et al., 1997). PI3-K regulates cell growth by sensing the availability of nutrients through growth factors such as insulin and is also regulated by the Ras pathway. Activated PI3K signals via Akt kinase to the TOR (target of rapamycin) kinase, which inhibits autophagy and promotes cell growth (Figure 1). Berry and Baehrecke tested whether these genes also regulate autophagic cell death. As an experimental model, they used salivary glands in *Drosophila* larvae, which secrete digestive enzymes during larval stages when the animal is heavily feeding. During pupal stages the animal stops feeding, and several larval structures including the salivary glands, the fat body, and the midgut are degraded and recycled to allow tis-

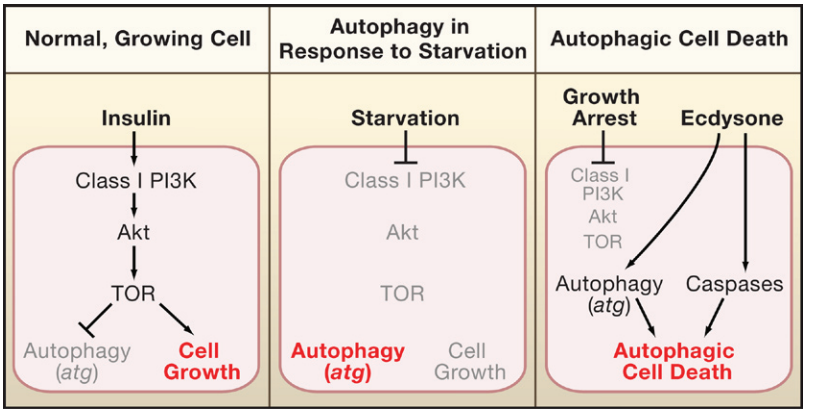

Figure 1. Cellular Responses to Different Environmental Conditions
The cellular response is highlighted in red. Active factors are in black and inactive factors in gray.
(Left) In normal, growing cells, insulin promotes cell growth and inhibits autophagy through class I PI3K, Akt, and TOR signaling.
(Middle) In starving cells, PI3K, Akt, and TOR are not activated; thus, autophagy is derepressed.
(Right) Autophagic cell death of salivary glands is under control of the steroid hormone ecdysone, which induces expression of autophagic *atg* genes and apoptotic components such as caspases. Because salivary glands die during pupariation when the animals stop feeding, growth arrest occurs and blocks PI3K, Akt, and TOR activity.

sue remodeling and morphogenesis for the transformation of the worm-like larvae into the adult fly. The PCD of the salivary glands is triggered by the steroid hormone ecdysone and occurs extremely rapidly. The death of these glands exhibits the morphology of autophagic cell death. However, ecdysone also induces caspase activation in salivary glands, a hallmark of apoptosis (Figure 1), and it was unclear whether caspases contribute to autophagic cell death. Thus, salivary glands provide an excellent model to test the role of growth, autophagy (*atg* genes), and caspases for autophagic cell death under physiological conditions.

Berry and Baehrecke first tested the role of growth and growth arrest in PCD in the salivary gland. Indeed, a marker of PI3K activity and hence growth is no longer detectable in salivary glands at the onset of pupariation, suggesting that growth arrest precedes salivary gland PCD. To establish a causal link between growth arrest and PCD in the salivary gland, p110 (the active subunit of PI3K), Akt and active Ras (Ras^{V12}) were expressed in salivary glands to inhibit growth arrest. Under these conditions, the salivary glands continued to grow and salivary gland tissue remained 24 hr after pupariation. Interestingly, the increase of salivary gland size is due to increase in cell volume, whereas the total number of cells remains constant. Thus, the authors conclude that growth arrest correlates with autophagic cell death. Expression of cell-cycle regulators such as Myc or CyclinD/Cdk4, which prevents apoptosis, did not prevent salivary gland PCD. Given that cell-cycle arrest correlates with apoptosis, this result further distinguishes autophagic cell death from apoptotic cell death.

Despite prolonged survival, salivary glands expressing p110, Akt, and Ras^{V12} contain activated caspases and exhibit DNA fragmentation, both hallmarks of apoptosis. Thus, although maintaining growth conditions blocks autophagic cell death, the apoptotic component of autophagic cell death remains intact. Therefore, Berry and Baehrecke tested a requirement of caspases in autophagic cell death. Salivary glands are at least partially, if not completely, degraded in caspase mutants or in glands expressing the caspase inhibitor p35, suggesting that caspases are not strictly required for PCD in salivary glands. Nevertheless, combined expression of the active subunit of PI3K p110 and the caspase inhibitor p35 results in stronger persistence of salivary glands compared to expression of either alone. Thus, both growth arrest and caspases contribute to autophagic cell death of salivary glands. Furthermore, this observation also implies that additional caspase-independent factors are required for salivary gland PCD.

Good candidates for the caspase-independent factors are the *atg* genes, which are essential for starvation-induced autophagy and which are upregulated in dying salivary glands (Gorski et al., 2003). Berry and Baehrecke found that mutants or RNA interference of seven different *atg* genes display incomplete degradation of salivary glands 24 hr after pupariation, providing the first in vivo evidence that autophagy and *atg* genes are required for autophagic cell death. These data also indicate that autophagic cell death is not a failed survival attempt of dying cells, because if it were then the salivary glands would die in *atg* mutants. Overexpression of Atg1 induces premature onset of salivary gland destruction consistent with a previous report in the fly fat body (Scott et al., 2007). Thus, autophagy is both necessary and sufficient for autophagic cell death of salivary glands. Interestingly, salivary glands lacking *atg* also contain active caspases and the combined inhibition of both autophagy and caspases results in more intact salivary glands than inhibition of either alone. Thus, both autophagy and caspases contribute to autophagic cell death of salivary glands (Figure 1).

In conclusion, this study demonstrates that growth arrest, autophagy, and caspases contribute to physiologically occurring autophagic cell death. Several questions remain; for instance, how is the life-or-death decision of autophagy controlled? Good candidates for the decision makers are caspases. It is noteworthy that caspase activity has been observed in other models of autophagic cell death (mammary lumen formation, embryonic cavitation, and amphibian development). Thus, it is possible that caspase activity in autophagy may tip the balance from survival to death.

Another question is, why do salivary glands die by an autophagic process despite containing active caspases? Caspases do not always induce apoptosis and have important functions outside of apoptosis (Kuranaga and Miura, 2007). Participation in autophagic cell death may be another example of a nonapoptotic function of caspases. However, it is unknown how caspases trigger nonapoptotic responses.

It is now clear that autophagy participates in cell death under some circumstances and promotes cell survival in others. Although this presents a dichotomy, any resulting confusion is largely resolved if autophagy is simply viewed as a catabolic process that provides energy and resources to many cellular and biological processes. In some cases, this may promote cell survival; in others it may facilitate cell death. This context-dependent behavior of autophagy may also be relevant for human health. Autophagy sometimes prevents and sometimes promotes cancer (Levine, 2007). Because drug treatment can induce autophagy, it will be important to determine which types of cancer use autophagy for survival and which ones undergo autophagic cell death. Thus, the study by Berry and Baehrecke demonstrates the importance of a careful case-by-case evaluation of autophagy.

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Synapse Remodeling, Compliments of the Complement System

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A growing body of evidence indicates that some proteins known for their immune functions also have distinct nonimmune functions in the normal uninjured central nervous system. In this issue, Stevens et al. (2007) demonstrate an unexpected requirement for molecules of the complement cascade in the remodeling of synaptic connections in the developing visual system.

Traditionally, immune molecules have been associated with neurons only in the context of pathological conditions such as brain injury, neuroinflammation, and autoimmune disorders. However, our definition of neuroimmunology is expanding, based on mounting evidence that certain proteins that were originally identified in the immune system also have nonimmune functions in the central nervous system (e.g., Boulanger et al., 2001; Goddard et al., 2007; Huh et al., 2000; Loconto et al., 2003; Oliveira et al., 2004; Syken et al., 2006). The work now presented by Stevens et al. (2007) reinforces this emerging concept and introduces a new set of players—members of the complement cascade.

The complement cascade is an arm of the innate immune system and is composed of over 30 small proteins and protein fragments that are normally found in inactive forms in the blood. The complement cascade can be triggered via three basic mechanisms: the classical, lectin, and alternative pathways. The classical pathway is initiated by binding of the complement protein C1q. All three pathways converge on complement C3, which triggers a sequence of proteolytic events that amplify the signal and can lead to formation of the cell-killing membrane attack complex. In these cascades, both C1q and C3 selectively bind to pathogens and potentially toxic cellular debris and mark them for destruction and clearance by phagocytosis.

In the current study, Stevens et al. found using a gene chip screen that mRNA encoding C1q is upregulated by purified neurons from the developing mouse eye (retinal ganglion cells) in vitro in response to astrocytes. Punctate C1q immunoreactivity was detected at postnatal day 5 (P5) in the developing retina and in the dorsal lateral geniculate nucleus (dLGN), where retinal ganglion cell axons from both eyes initially send exuberant, overlapping projections. These retinal projections undergo activity-dependent remodeling during the first two postnatal weeks, such that selective strengthening of some connections and weakening of others results in the establishment of the adult pattern of distinct, nonoverlapping eye-specific layers. Imaging of the dLGN during this remodeling (at P5) showed that some C1q protein was colocalized with either the postsynaptic marker PSD95 or the presynaptic marker SV2, whereas less C1q was detected at sites of close apposition between the two markers. Because such close apposition is a hallmark of mature stable synapses, this pattern is consistent with the presence of C1q at nascent or retracting synaptic connections. Importantly, the timing of C1q expression coincided closely with the