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The engulfment receptor Draper is required for autophagy during cell death

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Autophagy is a process to degrade and recycle cytoplasmic contents. Autophagy is required for survival in response to starvation, but has also been associated with cell death. How autophagy functions during cell survival in some contexts and cell death in others is unknown. Drosophila larval salivary glands undergo programmed cell death requiring autophagy genes, and are cleared in the absence of known phagocytosis. Recently, we demonstrated that Draper (Drpr), the Drosophila homolog of *C. elegans* engulfment receptor CED-1, is required for autophagy induction during cell death, but not during cell survival. *drpr* mutants fail to clear salivary glands. *drpr* knockdown in salivary glands prevents the induction of autophagy, and Atg1 misexpression in *drpr* null mutants suppresses salivary gland persistence. Surprisingly, *drpr* knockdown cell-autonomously prevents autophagy induction in dying salivary gland cells, but not in larval fat body cells following starvation. This is the first engulfment factor shown to function in cellular self-clearance, and the first report of a cell-death-specific autophagy regulator.

Key words: autophagy, Draper, programmed cell death, engulfment, development

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Programmed cell death is required for animal development and tissue homeostasis. Improper cell death leads to pathologies including autoimmunity and cancer. Several morphological forms of cell death occur during animal development, including apoptosis and autophagic cell death. Autophagic cell death is characterized by the presence of autophagosomes in dying cells that are not known to be engulfed by phagocytes. Autophagic cell death is observed during several types of mammalian developmental cell death, including regression of the corpus luteum and involution of mammary and prostate glands.

During macroautophagy (autophagy), cytoplasmic components are sequestered by autophagosomes and delivered to the lysosome for degradation. Autophagy is a cellular response to stress required for survival in response to starvation. Whereas autophagy has been associated with cell death, it is unknown how autophagy is distinguished during cell death and cell survival. Autophagy is induced in Drosophila in response to starvation in the fat body where it promotes cell survival, while autophagy is induced by the steroid hormone ecdysone in salivary glands where it promotes cell death. This allows studies of autophagy in different cell types and in response to different stimuli.

Drosophila larval salivary glands die with autophagic cell death morphology and autophagy is required for their degradation. Expression of the caspase inhibitor p35 enhances salivary gland persistence in *Atg* mutants, suggesting that caspases and autophagy function in parallel during salivary gland degradation. Either activation of caspases or Atg1 misexpression is sufficient to induce ectopic salivary gland clearance. We queried genome-wide microarray data from purified dying salivary glands and noted the induction of engulfment genes, those required for a phagocyte to consume and degrade a dying cell. We also noted few detectable changes in engulfment genes in Drosophila larvae during starvation.

We found that Drpr, the Drosophila orthologue of *C. elegans* engulfment receptor CED-1, is enriched in dying salivary glands, and *drpr* null mutants have persistent salivary glands. Interestingly, whereas knockdown of *drpr* in phagocytic blood cells fails to influence salivary gland clearance, expression of *drpr*-RNAi in salivary glands prevents gland clearance. Drosophila *drpr* is alternatively spliced to produce three isoforms. We found that *drpr-I*-specific knockdown prevents salivary gland degradation and Drpr-I expression in salivary glands of *drpr* null mutants rescues salivary gland persistence. Therefore, *drpr* is autonomously required for salivary gland clearance. However, how Drpr is induced or activated during hormone-regulated cell death remains to be determined.

drpr knockdown fails to influence caspase activation, and caspase inhibitor p35 expression in *drpr* null mutants enhances salivary gland persistence, suggesting that Drpr functions downstream or parallel to caspases in dying salivary glands. Interestingly, we found that *drpr* knockdown in salivary glands prevents the formation of GFP-LC3 puncta. Further, Atg1 misexpression in salivary glands of *drpr* null mutants suppresses salivary gland persistence. *drpr* is therefore required for autophagy induction in salivary glands, and Atg1 functions downstream of Drpr in this tissue. We found that several other engulfment genes are required for salivary gland degradation. However, the Drpr signaling mechanism leading to autophagy induction in salivary glands remains to be elucidated.

We tested whether *drpr* is a general regulator of autophagy. The Drosophila fat body is a nutrient storage and mobilization organ akin to the mammalian liver, and is a well-established model to study starvation-induced autophagy. We found that *drpr*-RNAi expression in fat body clone cells fails to prevent GFP-Atg8 puncta formation in response to starvation. Similarly, *drpr* null fat body clone cells form Cherry-Atg8 puncta after starvation. Strikingly, *drpr*-RNAi expression in salivary gland clone cells inhibits the formation of GFP-Atg8 puncta. Therefore, *drpr* is cell-autonomously required for autophagy induction in dying salivary gland cells, but not for autophagy induction in fat body cells after starvation. These findings suggest that distinct signaling mechanisms regulate autophagy in response to nutrient deprivation compared to steroid hormone induction. Little is known about what distinguishes autophagy function in cell survival versus death. It is possible that varying levels of autophagy are induced during specific cell contexts and that high levels of autophagy could overwhelm a cell—leading to cell death. Autophagic degradation of specific cargo, such as cell death inhibitors, could also contribute to cell death.

Given recent interest in manipulation of autophagy for therapies, it is possible that factors such as Drpr could be used as biomarkers to distinguish autophagy leading to cell death versus cell survival. While it is generally accepted that augmentation of protein clearance by autophagy during neurodegeneration would be beneficial, the role of autophagy in tumor progression is less clear. For example, monoallelic loss of the human *Atg6* homolog *beclin 1* is prevalent in human cancers, suggesting that autophagy is a tumor-suppressive mechanism. Thus, autophagy enhancers have been proposed for cancer prevention. However, autophagy occurs in tumor cells as a survival mechanism, and autophagy inhibitors have been proposed for anti-cancer therapies. Understanding how autophagy is regulated in different contexts is critical for appropriate therapeutic strategies.

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