The Relationship between Autophagy and Cell Death

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Select Historical Notes

Dying cells were first recognized as playing a normal role in animals by Glucksmann (1951).

Lockshin and Williams (1964) coined the term "Programmed Cell Death" after observing that insect flight muscles die as part of a developmental program. This programmed cell death has the morphology of type II cell death, also known as autophagic cell death.

Tata (1966) then showed that programmed cell death in frogs required gene transcription and protein synthesis.

Morphological Forms of Programmed Cell Death

Type I = Apoptosis

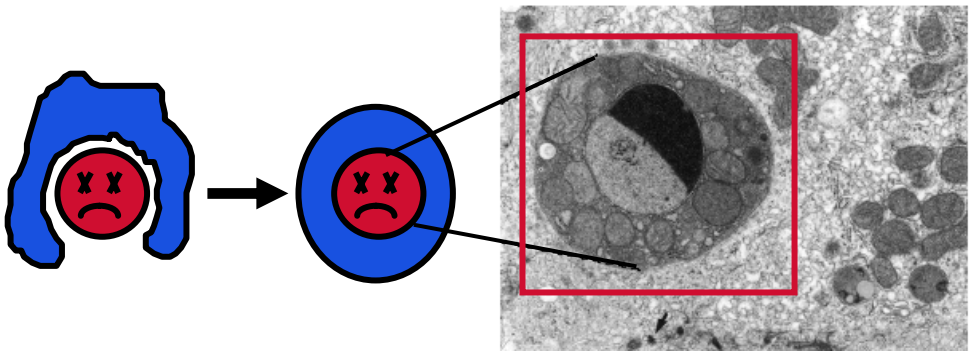
Type II = Autophagic Cell Death

Type III = Non-lysosomal

Kerr, Wyllie, and Currie, 1972; Schweichel and Merker, 1973; Clarke, 1990

Morphological Forms of Cell Death

Type I = Apoptosis



- Condensation of nucleus and cytoplasm, and association of chromatin with the nuclear margin.

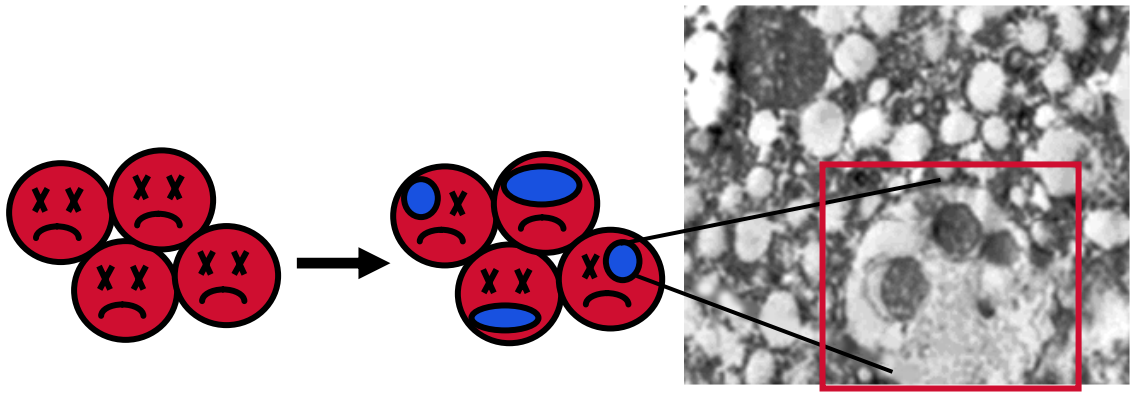
- Fragmentation of the cell.

- Engulfment of the cell by a phagocyte where the lysosome completes degradation.

Kerr, Wyllie, and Currie, 1972; Schweichel and Merker, 1973; Clarke 1990

Morphological Forms of Cell Death

Type II = Autophagic Cell Death



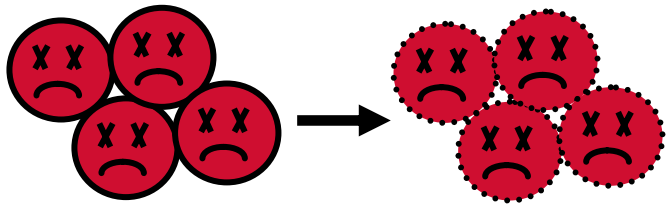
- Degrade with either little or no association with phagocytes.

- Use autophagy and lysosomes within the dying cell for degradation.

Schweichel and Merker, 1973; Clarke 1990

Morphological Forms of Cell Death

Type III = Non-lysosomal



- No use of lysosomes.

- Swelling of cavities with membrane borders such as endoplasmic reticulum and mitochondria.

- Not well studied, and uncertain if this is a physiological form of cell death.

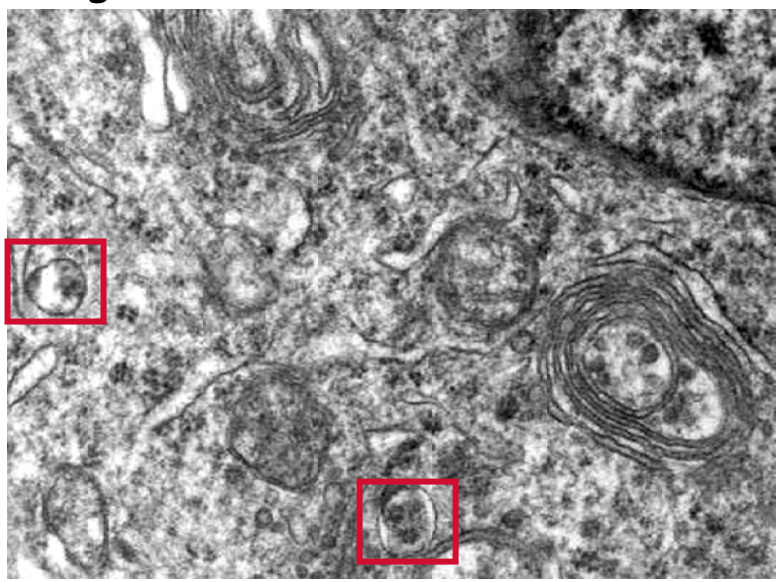
Schweichel and Merker, 1973; Clarke 1990

Why is Macro-Autophagy (Autophagy) Interesting to Cell Death Researchers?

- Autophagic vacuoles (autophagosomes) occur in starving cells as a survival mechanism.

- Autophagic vacuoles are associated with neoplastic tumor cells, protein aggregation diseases, and intracellular pathogens.

Ganglioneuroma

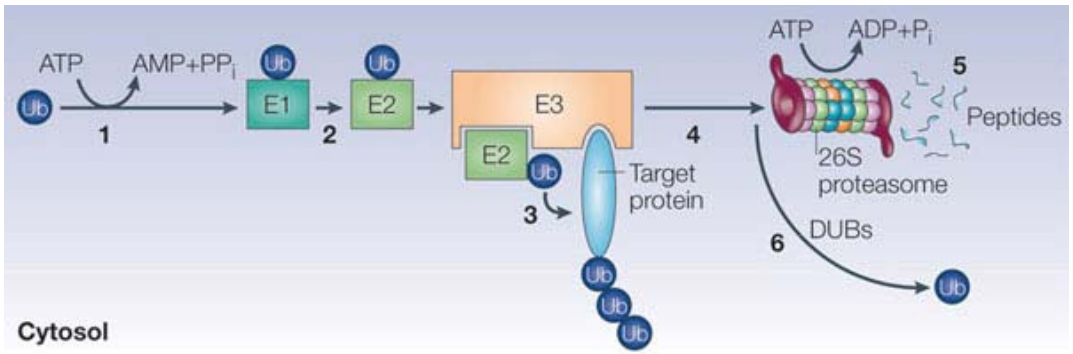


- Autophagic vacuoles occur in dying cells.

What is Autophagy?

Major Catabolic Pathways

Proteasome



Autophagy

Macro-autophagy

Micro-autophagy

Chaperone-mediated autophagy

Adapted from Welchman et al. Nat. Rev. MCB 2005 and Yoshimori BBRC 2003

Discovery of Genes that Regulate Autophagy

- Genetic screens in yeast for defects in either the formation of autophagosomes under starvation conditions, or protein degradation due to defects in trafficking to the lysosome.

  Tsukada and Ohsumi 1993; Thumm et al. 1994; Harding et al. 1995

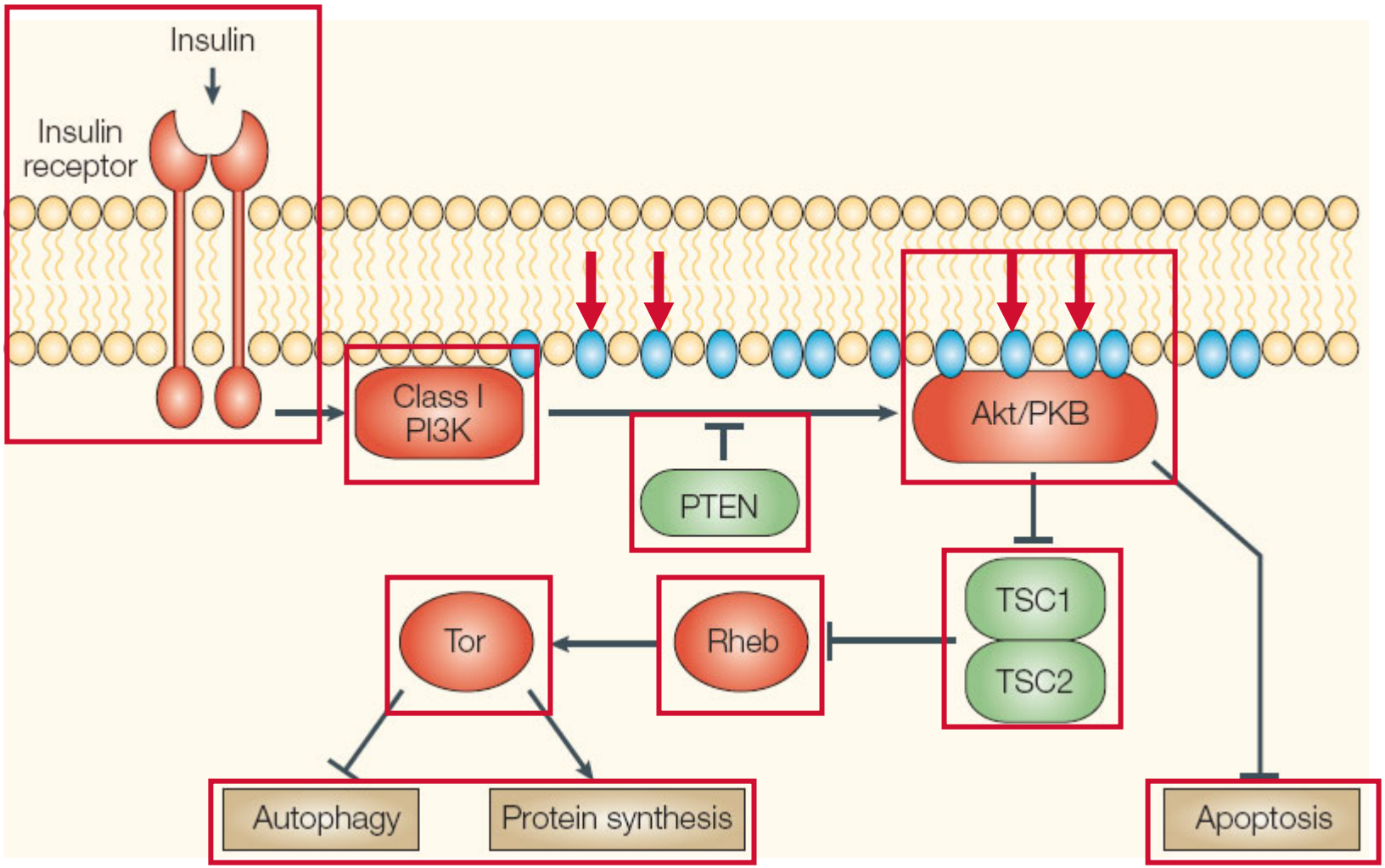
- Autophagy genes named Apg, Aut, and Cvt, and they are now refered to as Atg genes.

- Many Atg genes are conserved in higher animals, and function in cell survival.

How is Autophagy Regulated?

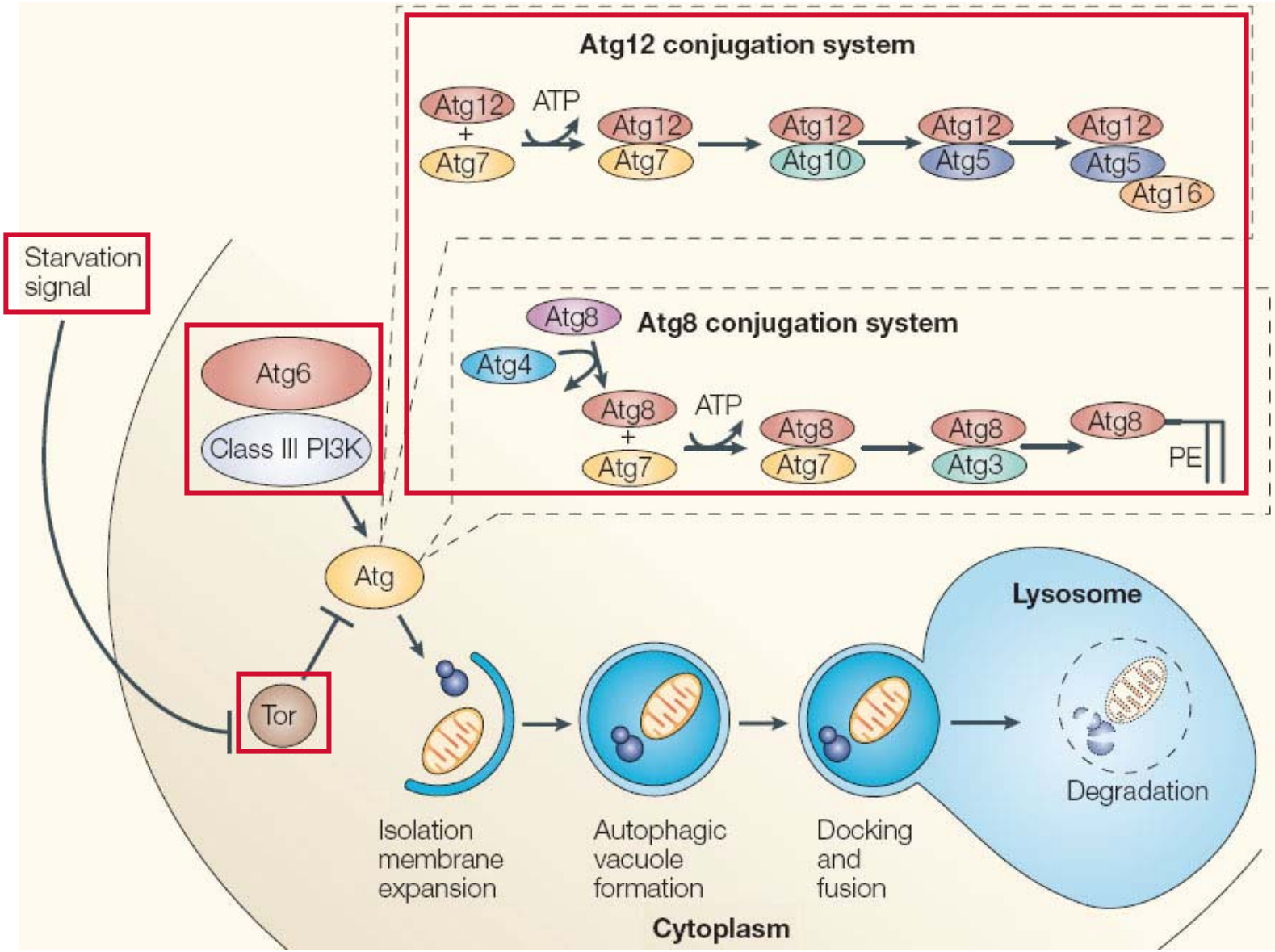
- class I PI3K/TOR signaling
- class III PI3K/Atg6 signaling
- Atg conjugation pathways

Class I PI3K Regulates Growth Autophagy, and Apoptosis



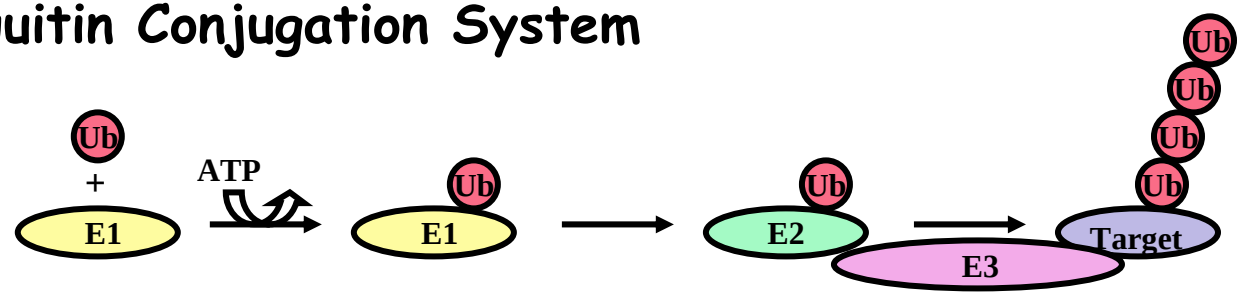
Baehrecke, Nat. Rev. Mol. Cell Biol. (2005); adapted from Klionsky, Nature (2004)

Class III PI3K Regulates Autophagy

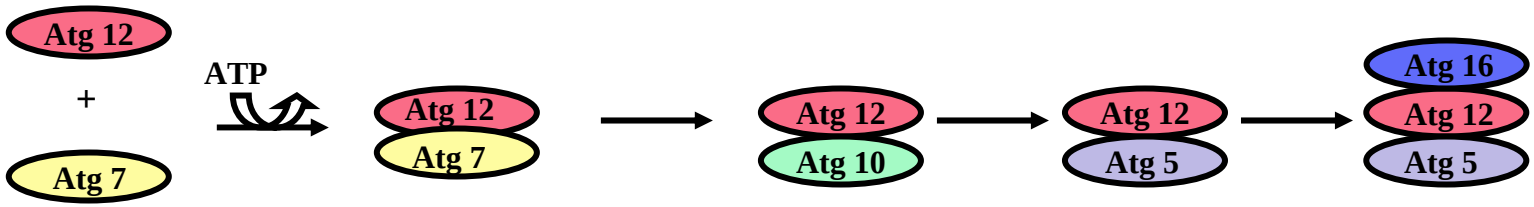


Baehrecke, Nat. Rev. Mol. Cell Biol. (2005); adapted from Ohsumi, Nat. Rev. Mol. Cell Biol. (2001)

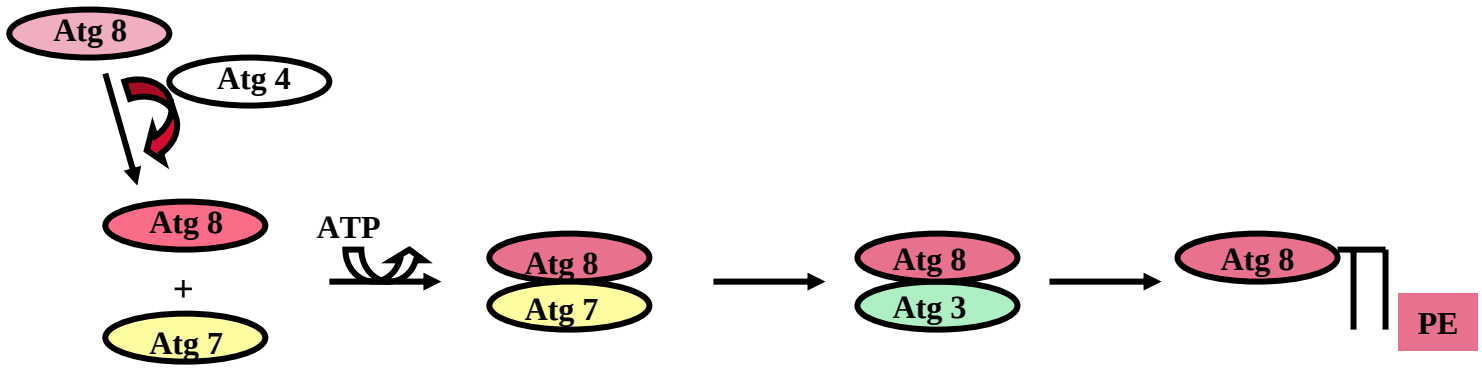
Ubiquitin Conjugation System



Atg 12 Conjugation System



Atg 8 Conjugation System



adapted from Ohsumi, Nat. Rev. Mol. Cell Biol. (2001)

Where has type II autophagic cell death morphology been observed?

- Animals from diverse taxa including insects, amphibians and mammals.

- Autophagic cell death occurs during insect and amphibian metamorphosis when many immature tissues (salivary glands, muscles, intestines, tails…) are destroyed and replaced with adult structures.

- Autophagic cell death occurs throughout mammalian development including regression of the corpus luteum, involution of mammary and prostate glands, and regression of Mullerian duct structures during male genital development.

Why autophagy in dying cells? …isn't apoptosis adequate?

- Physical barriers/structures may limit phagocyte access to dying cells, and require self-degradation.

- Phagocyte limitations (# or size) may require self-degradation.

- Autophagy may be used to mobilize nutrients during death.

What are the possible role(s) of autophagy in dying cells?

- Attempting to promote cell survival.

- Inducing cell death and/or degradation.

Evidence in Support of Autophagy Prolonging Cell Survival in Dying Cells

- Autophagy is known to promote cell survival under nutrient deprivation conditions.

- Atg6- ES cells die by apoptosis following either serum withdrawal or UV treatment.

Yue et al. 2003

- Death of HeLa cells increases when nutrients are depleted and Atg gene function is reduced.

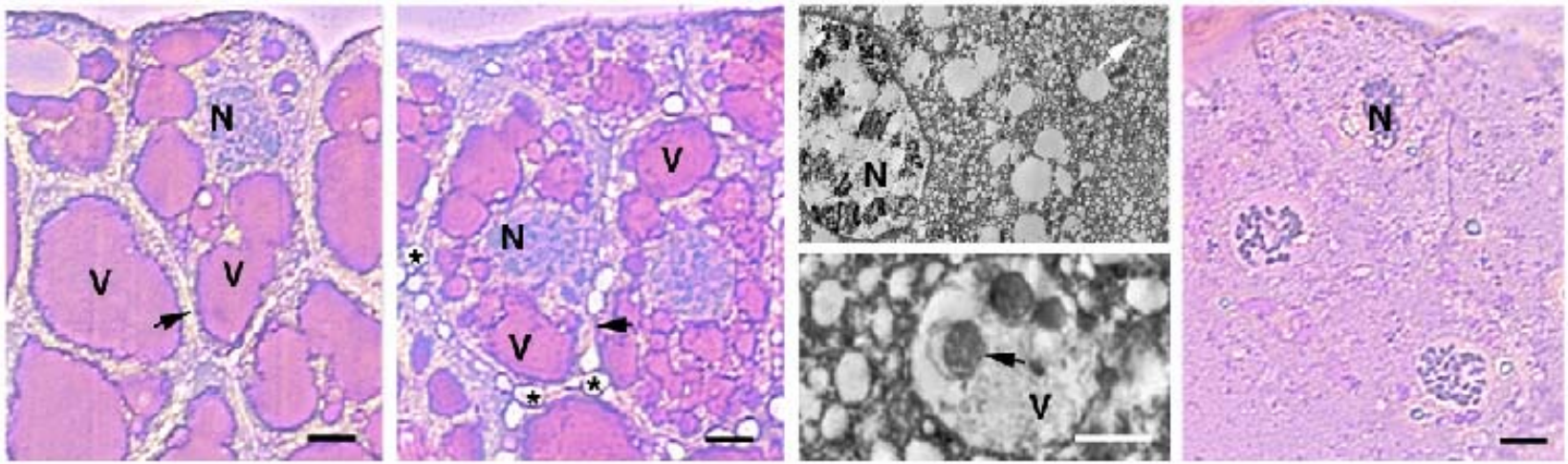
Boya et al. 2005

- Bax-/Bak- hematopoetic cells exhibit increased death following IL-3 withdrawal when Atg gene function is reduced, and cell death is caused by a metabolic crisis.

Lum et al. 2005 and others…

Studies of Drosophila salivary glands suggest that both autophagy and caspases may be involved in steroid-triggered autophagic cell death during development.

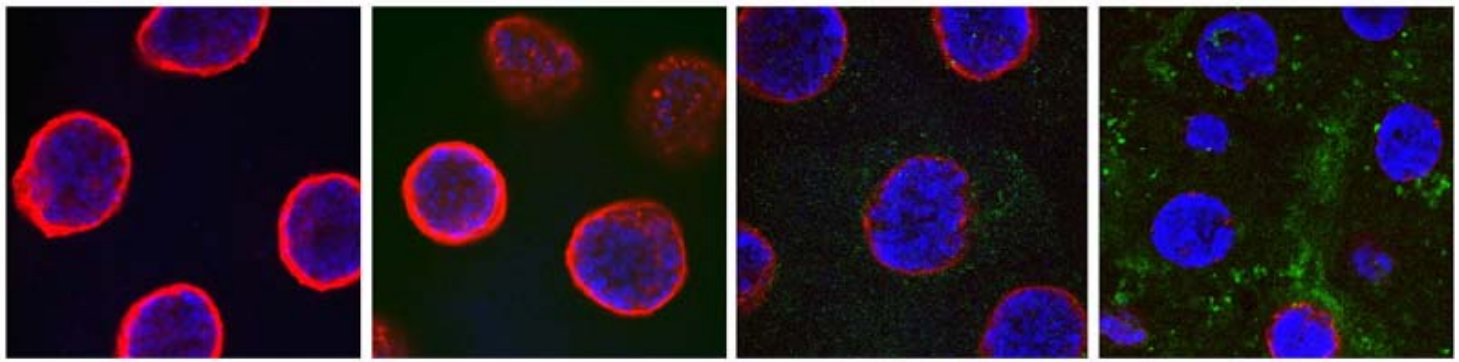
Dying Salivary Glands Have Autophagic Vacuoles



12 h 13 h 14 h 14.5 h

Lee and Baehrecke, Development 2001.

and Processed Caspase3



Lamin Dmo

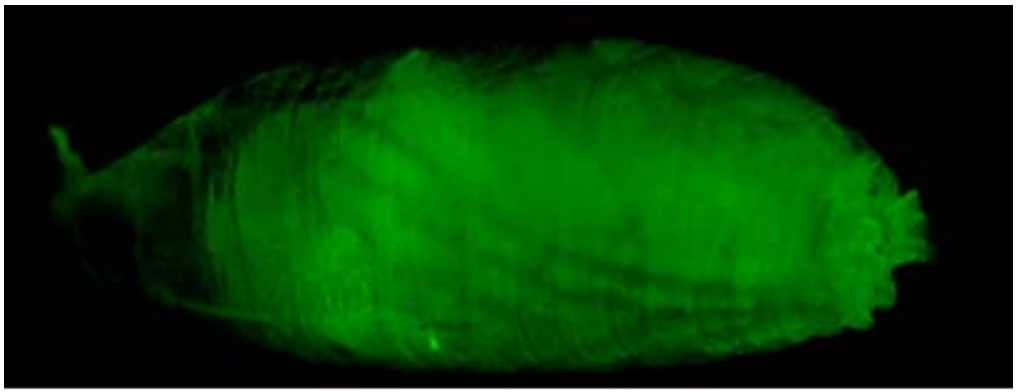
Processed Caspase3

DNA

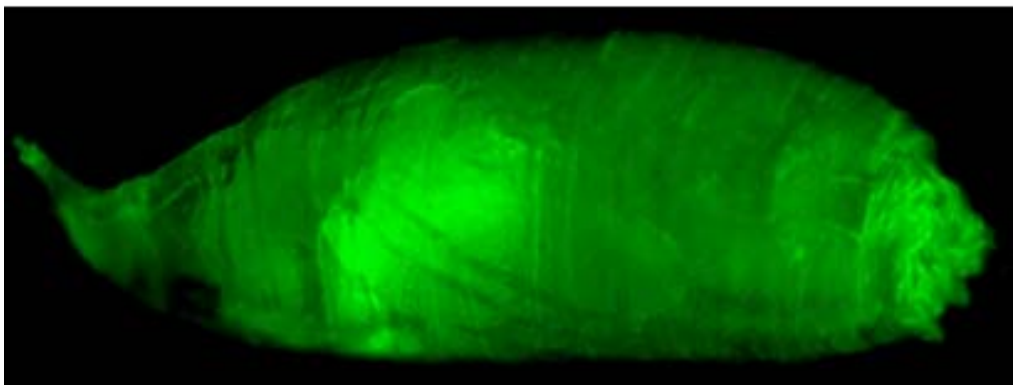
8 h 10 h 12 h 14 h

Martin and Baehrecke, Development 2004.

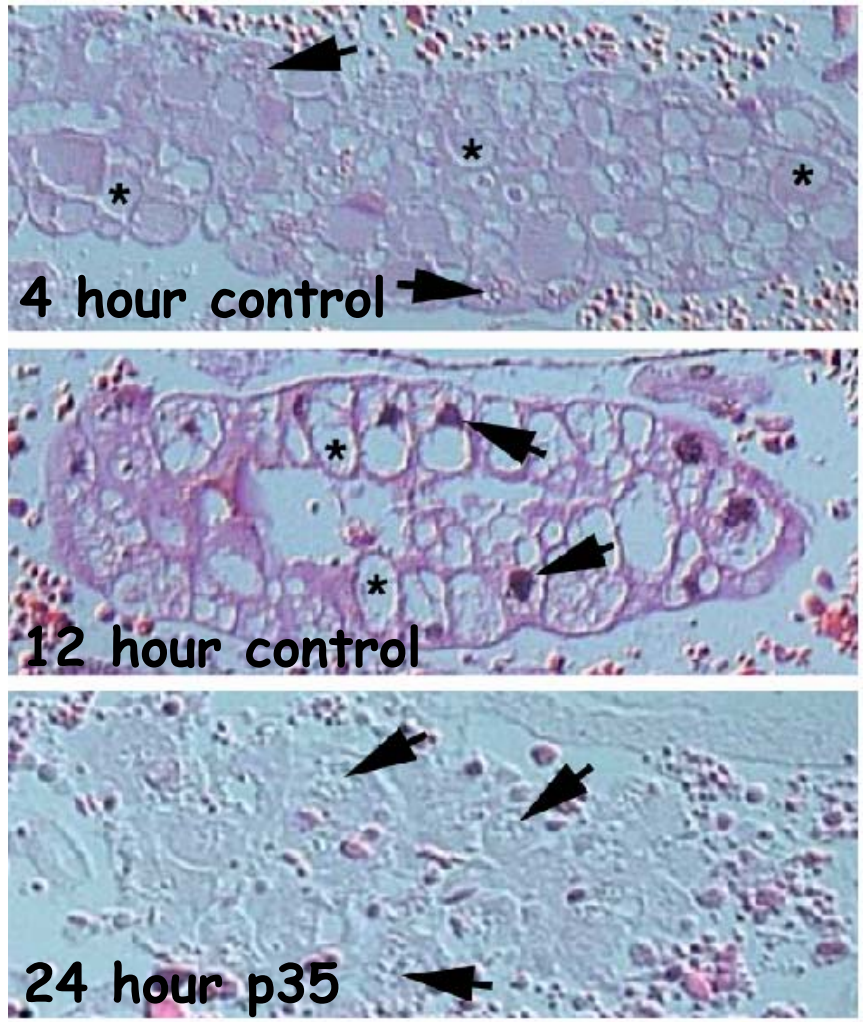
p35 Inhibits Salivary Gland Cell Death but not all changes in dying cells



24 hour Control SG-GAL4 x UAS-GFP

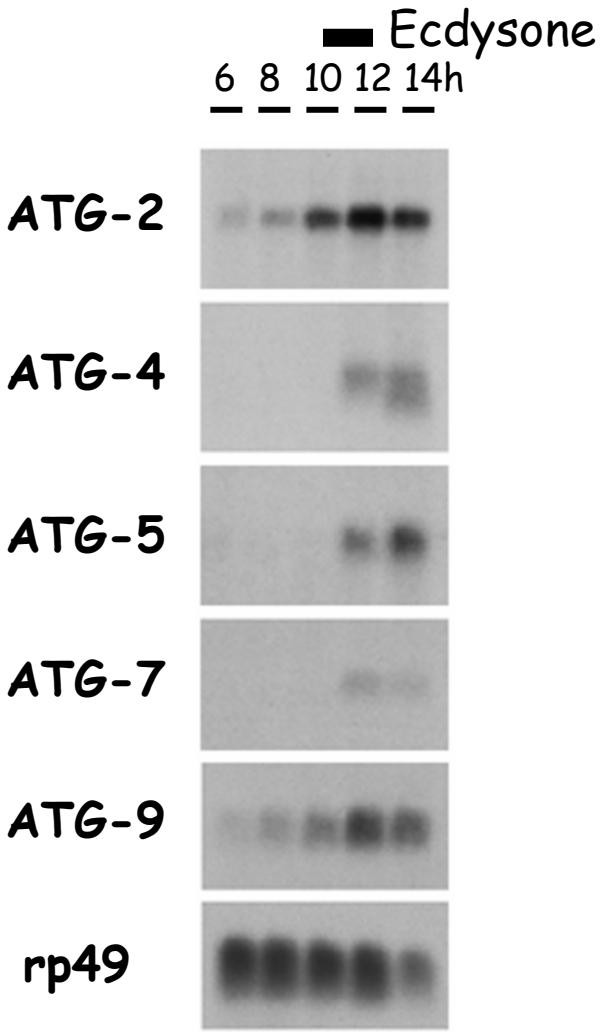


24 hour SG-GAL4 x UAS-GFP UAS-p35



Lee and Baehrecke, Development 2001.

Atg genes are induced following the rise of ecdysone that triggers autophagic cell death



Lee et al. Curr. Biol. 2003.

… but autophagy occurs in mutants that lack the function of the caspase regulator ark (Apaf1), and these mutants possess altered salivary gland cell death.

Akdemir et al. Development 2006.

… but an independent studied showed that ark (Apaf1) mutant salivary glands do die.

Mills et al. J. Cell Biol. 2006.

Similar to fly salivary glands, both autophagy and caspases appear to be involved in autophagic cell death during lumen formation in 3-dimensional cultures of mammary MCF-10A cells.

Debnath et al. Cell 2002; Mills et al. PNAS 2004.

Evidence in Support of Autophagy Promoting Cell Death

- z-VAD and Caspase 8-knockdown-triggered autophagic cell death of L929 cells is inhibited by reducing *Atg* gene function.

Yu et al. 2004

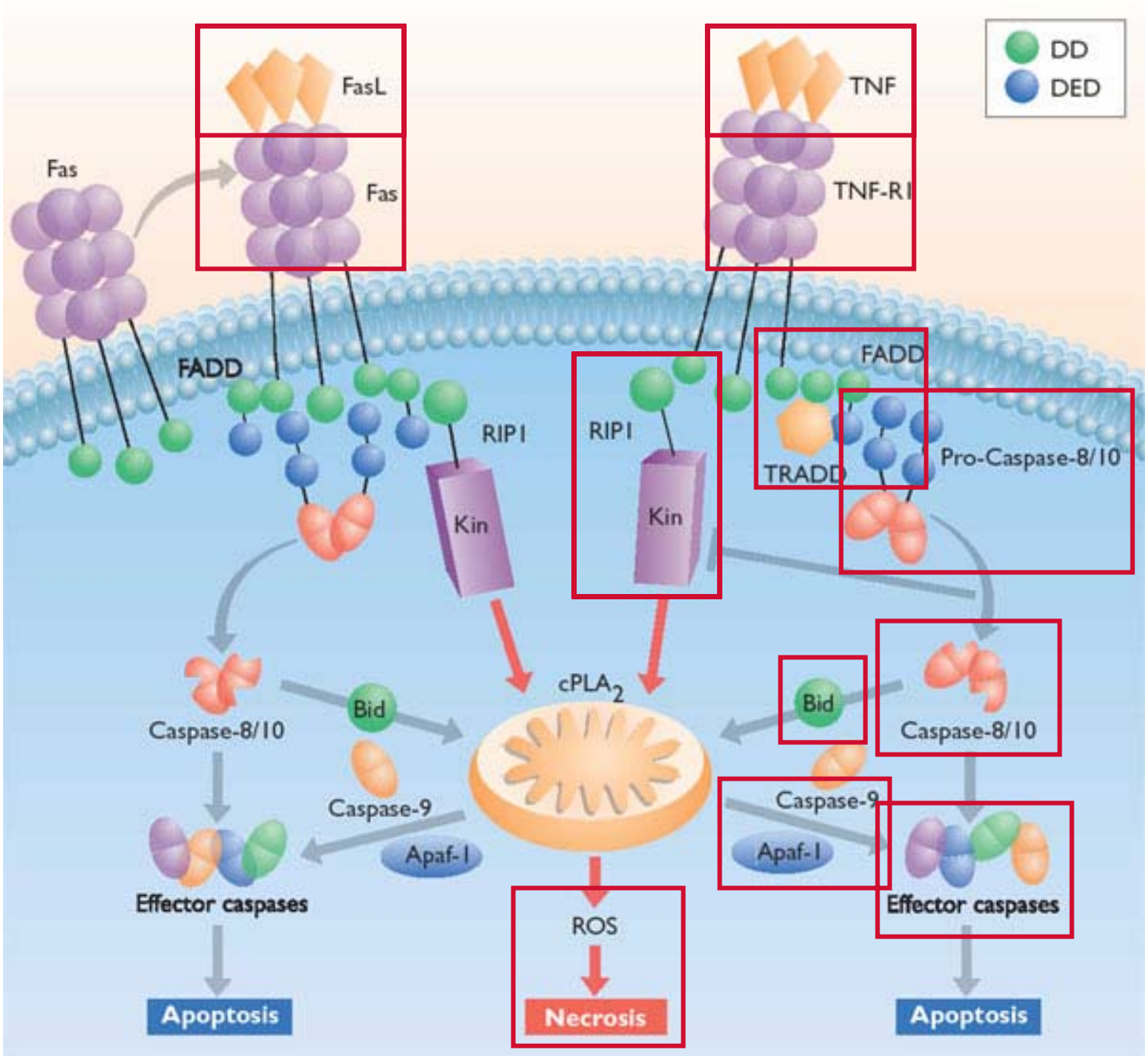
- Bax-/Bak- fibroblasts die with an autophagic cell death morphology when treated with either etoposide or stauosporine, and death is inhibited when *Atg* gene function is reduced.

Shimizu et al. 2005

- Bcl-2 inhibits autophagy by interacting with Atg6/Beclin1, and this reduces cell death.

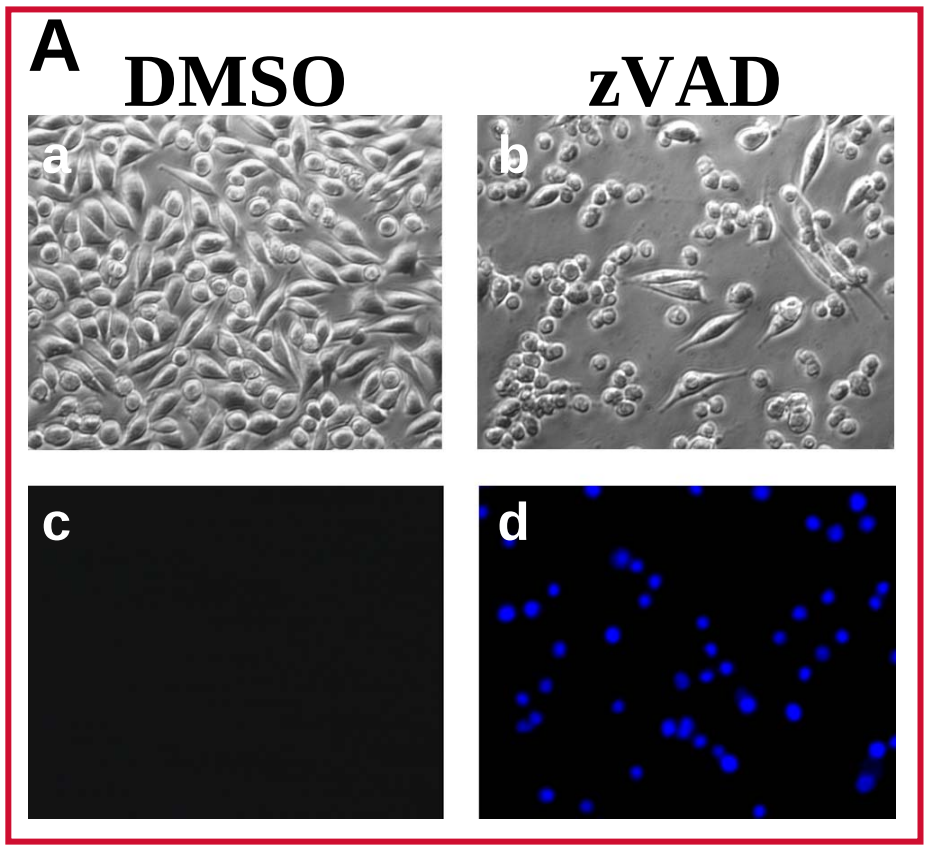
Pattingre et al. 2005

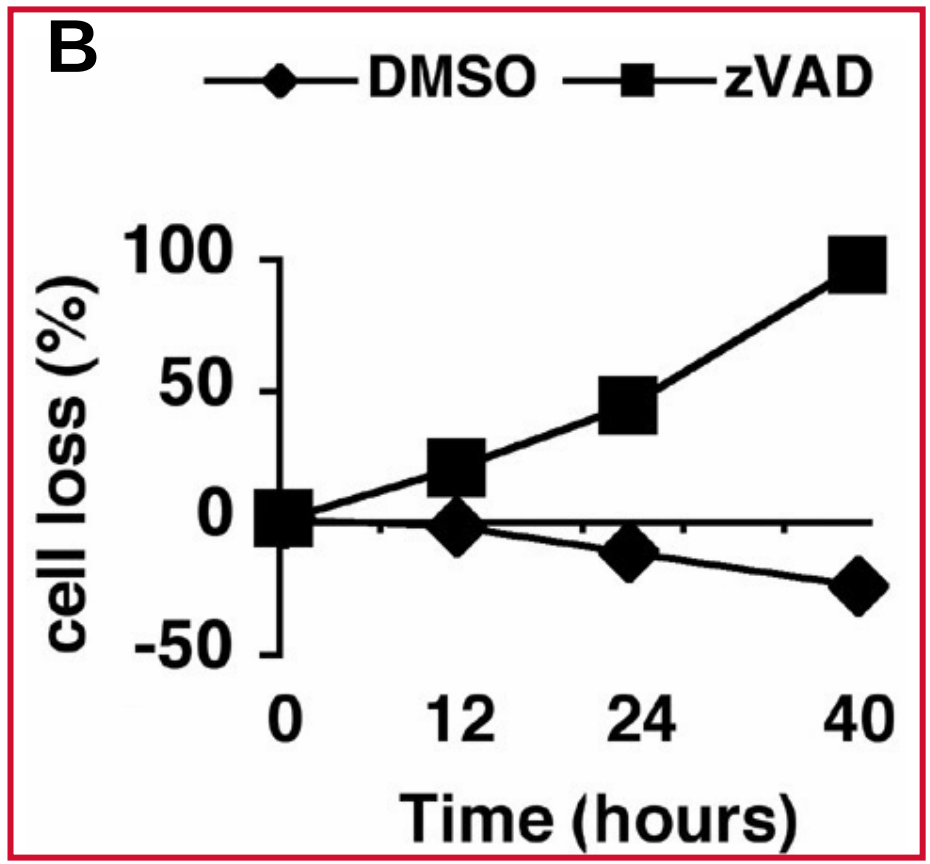
and others…



Jaattela and Tschopp, Nat. Immunol. 2003

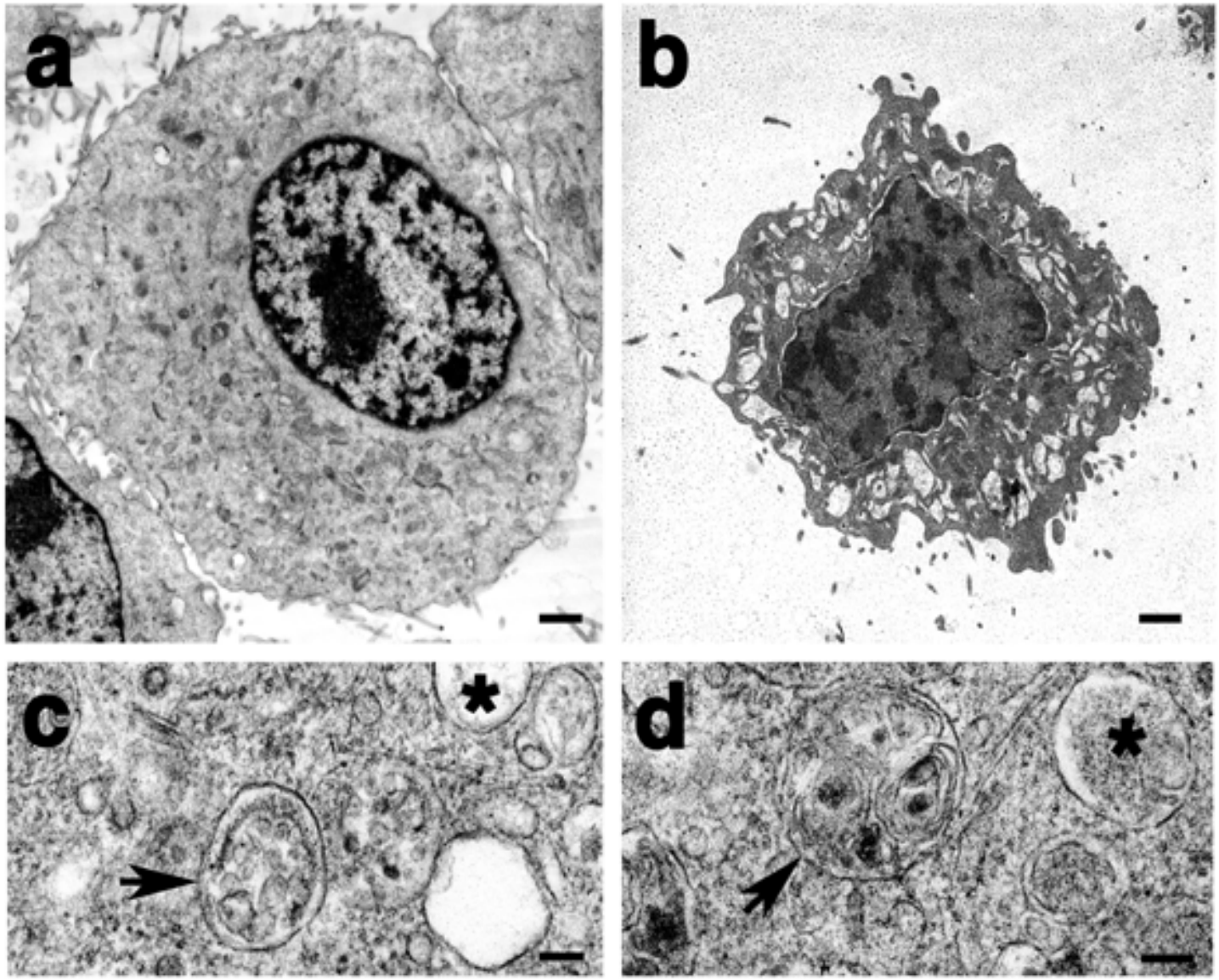
zVAD Induces L929 Cell Death





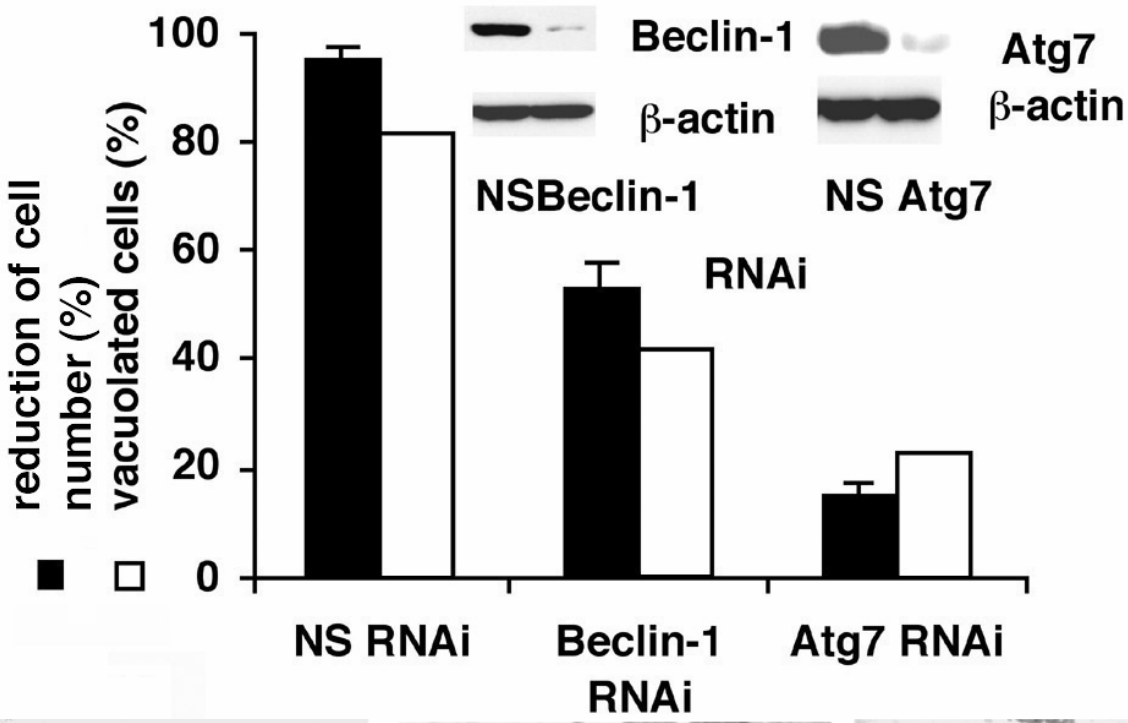
Yu et al., Science 2004.

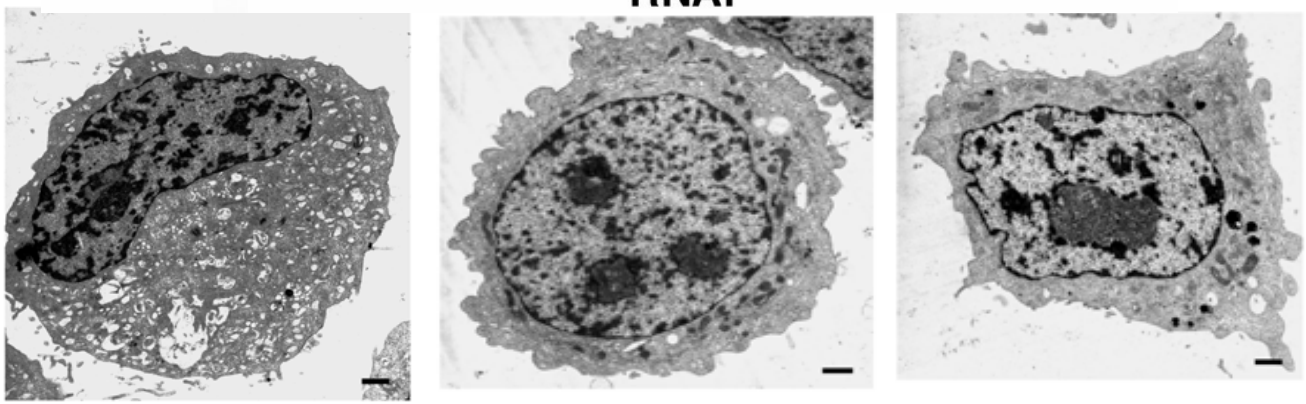
zVAD Induces Autophagy in L929 Cells



Yu et al., Science 2004.

zVAD Induced Cell Death and Autophagy Requires Atg7 and Beclin-1 (Atg6)

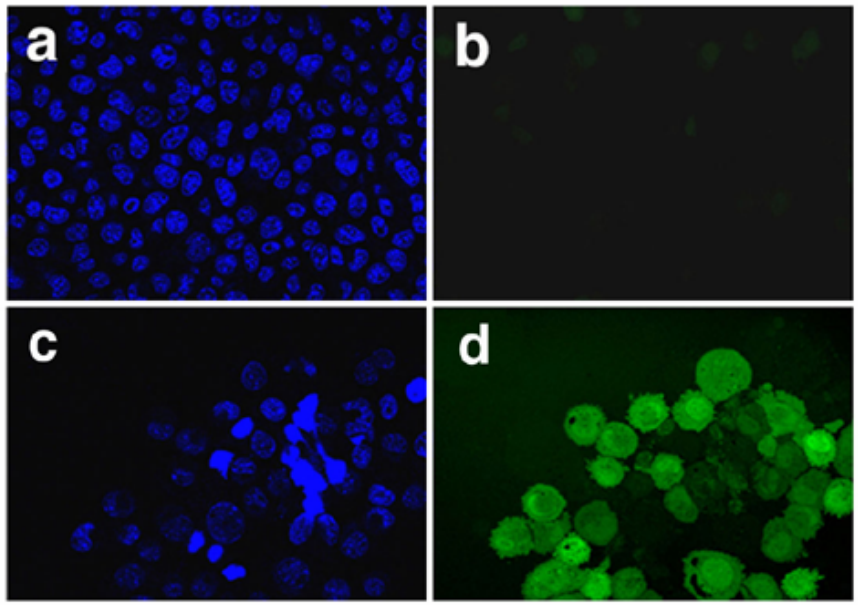




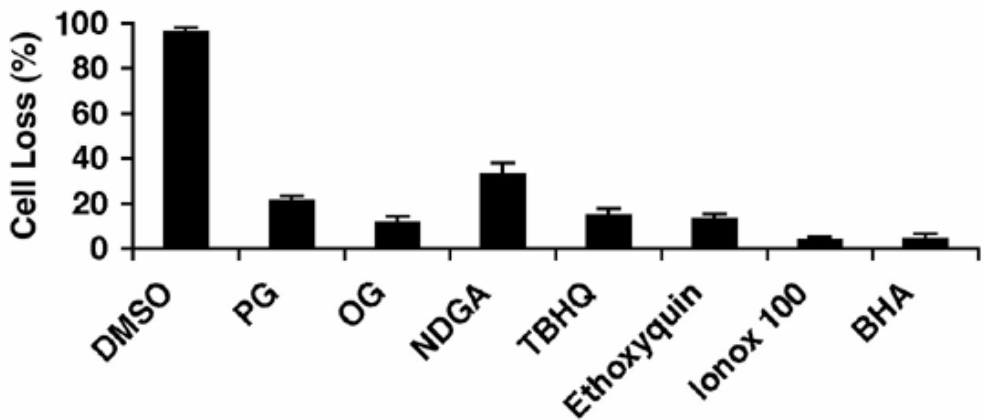
NS RNAi Beclin-1 RNAi Atg7 RNAi Yu et al., 2004

ROS Accumulates in zVAD Treated Cells, and ROS Inhibitors Prevent Cell Death

A

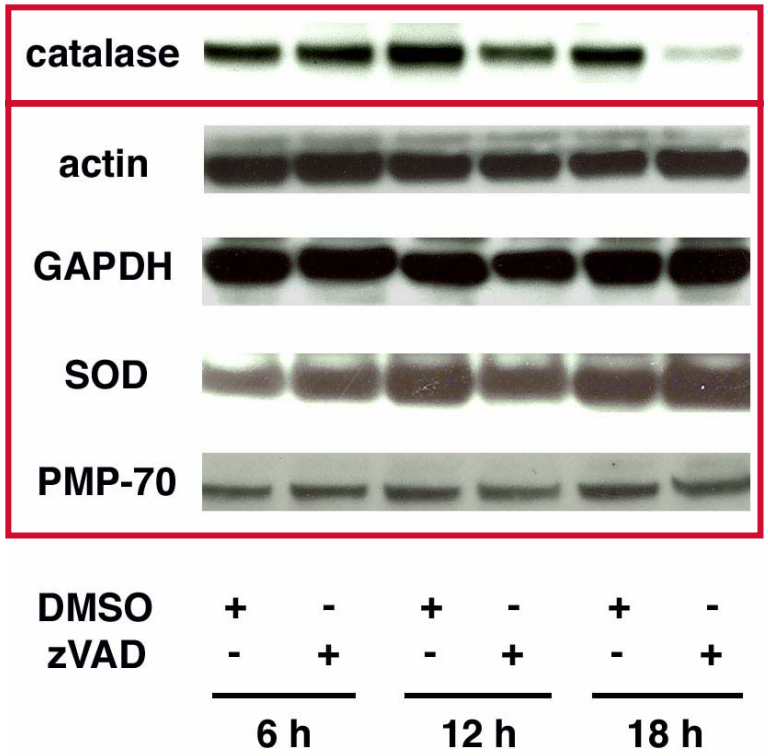


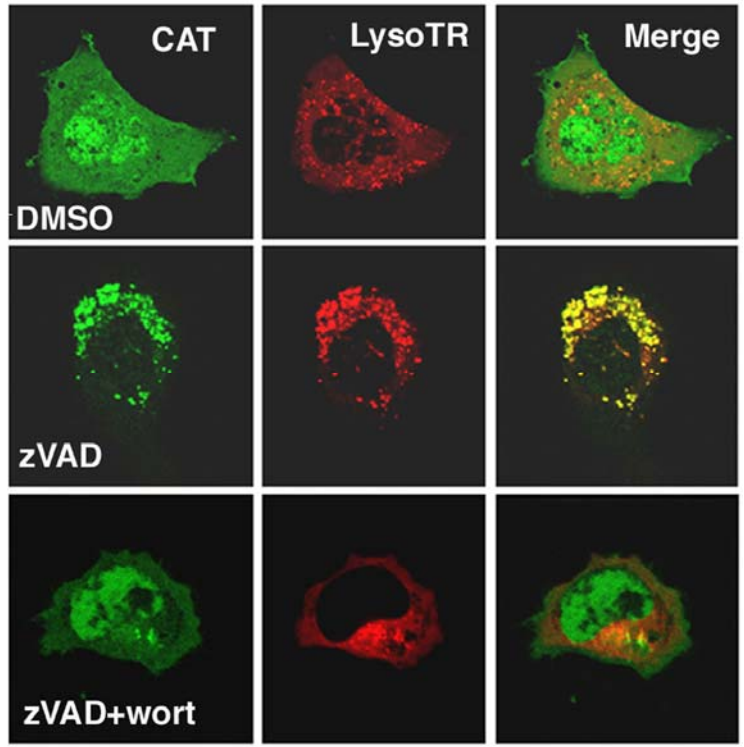
B

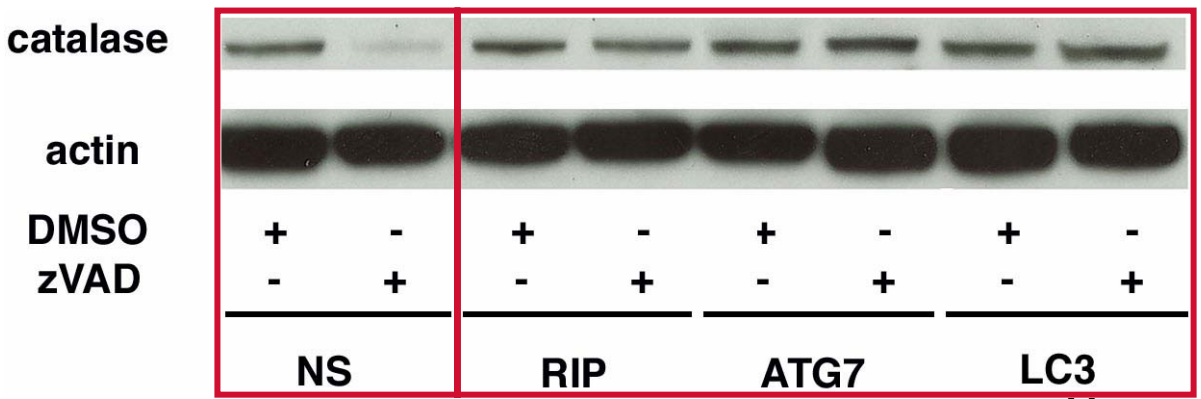


Yu et al., PNAS 2006.

Catalase is Degraded in zVAD Treated Cells

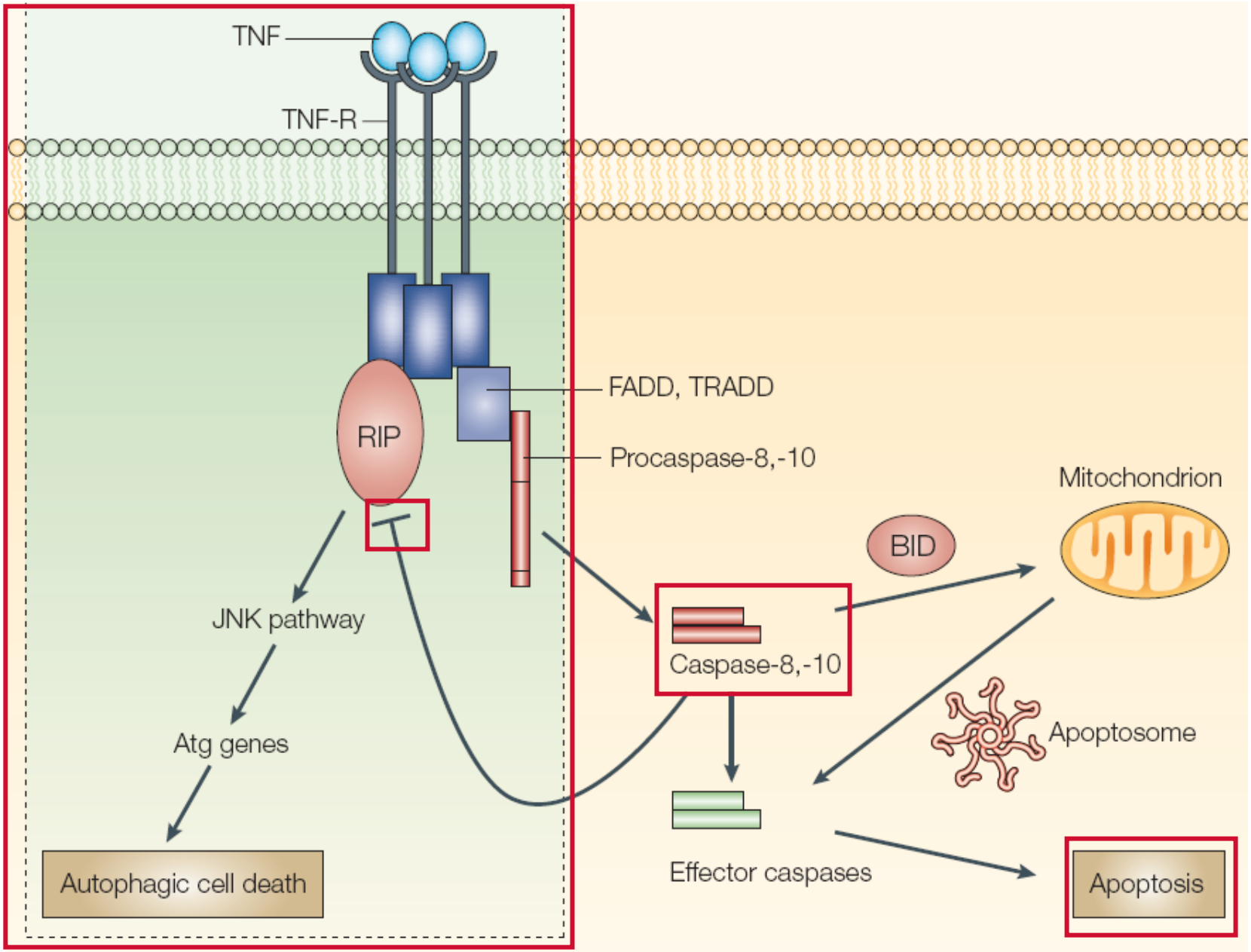






Yu et al., PNAS 2006.

Autophagic Cell Death in L929 Cells



Baehrecke, Nat. Rev. Mol. Cell Biol. (2005)

Conclusions

Autophagic cell death occurs in many cell types in animals from diverse taxa.

Autophagy may function to either promote cell survival, or to kill cells.

Autophagy may regulate cell death by depleting nutrients and/or cell survival factors.

Studies in physiologically relevant systems are needed to clarify the role of autophagy in cell survival and cell death.

Some future questions:

- What distinguishes use of autophagy for cell survival and death?
- What is the relationship between caspases and autophagy?
- What role does nutrition and growth play in cell death?
- How does autophagy kill cells?
- Why does disruption of autophagy result in pathological conditions?
- Why less *Atg* genes in animals than yeast?

Thanks!

Past and present members of the Baehrecke laboratory.
Especially Deborah Berry for figures used in this lecture!

Collaborators and colleagues

Center for Biosystems Research
University of Maryland Biotechnology Institute

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for supporting our studies of autophagy