Beyond apoptosis …

The Potential Non-apoptotic Functions of Canonical Apoptotic Proteins

Caspases
- **Spermatogenesis in flies**
- **Erythrocyte differentiation and maturation**
- **Capase-8 and wound healing**
- **Caspase-6 in neuronal axonal degeneration**

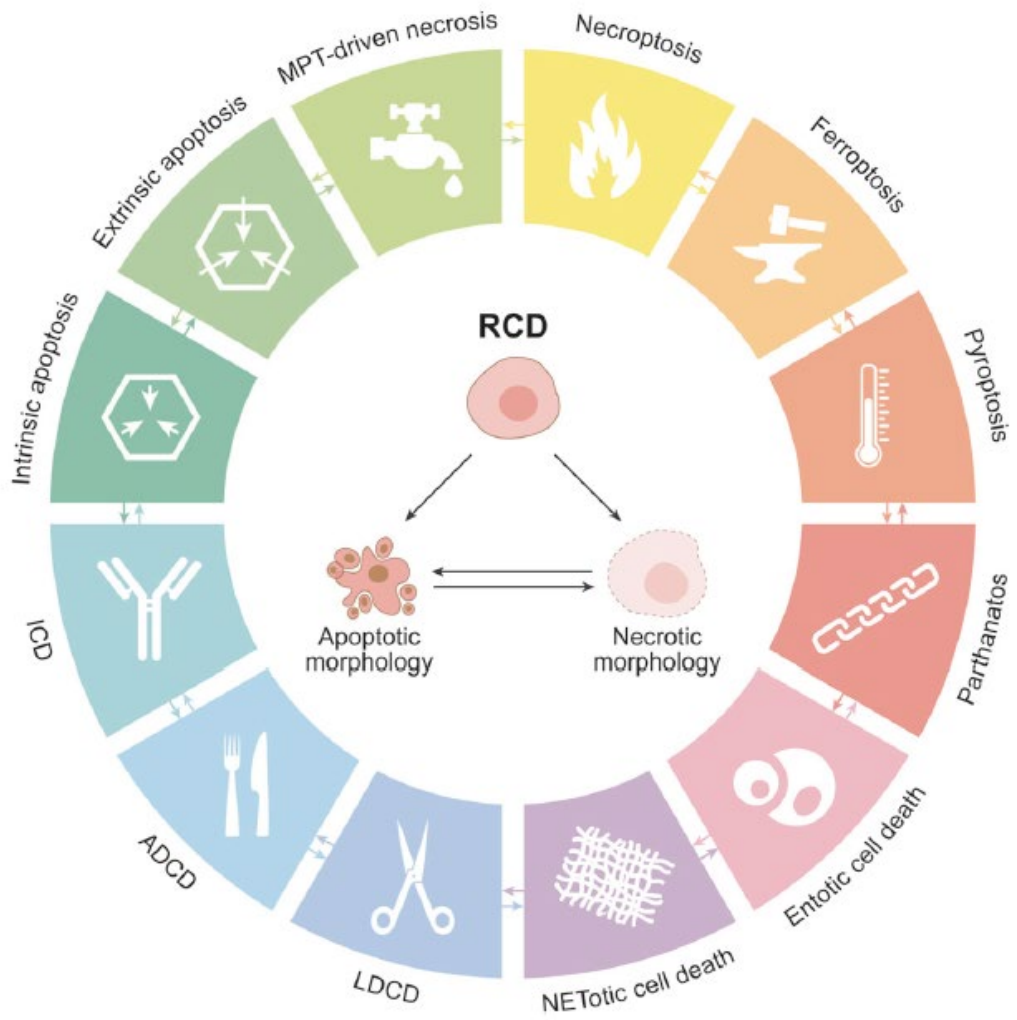
(Partial death?)
- **Cytochrome c pathway in long-term neuronal depression**
- **Casp-8 and -3/7 in microglia activation and neurotoxicity**

•(Life-and-death determination)

Bcl-2 members
- **Cellular calcium homeostasis**
- **Glucose metabolism**
- **Mitochondrial dynamics (Bak/Bax, fusion)**

IAP proteins
- **Cell cycle (survivin for kinetochore function)**
- **NF-κB signaling**

Various forms of programmed/regulated cell death



Galluzzi et al, Cell Death Diff (2018) 25:486

Everyday, an average adult eliminates **50-70 billion** cells of different tissue origins through cell death (not including RBC). Over the course of a year, each of us produces and destroys a mass of cells almost equal to our entire body weight.

$20\sim30 \times 10^{12}$ red blood cells; lifespan ~120 days, thus 2~3 million RBC deceased and generated per second

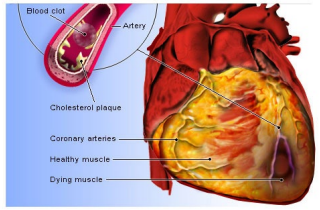
Gastrointestinal epithelial cells (lifespan 3~5 days)

Immune cells (made and eliminated as needed) ……

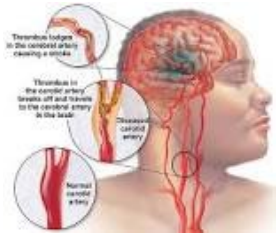
Clinical relevance of cell death

- **Cancer**
- **Ischemic organ damage**

1. **Ischemia and ischemia/reperfusion (I/R) are pathological conditions that can cause human organ damage.**



2. **Ischemic heart disease (aka coronary heart disease) = No. 1 deadly disease in the world, killing ~ 9 million people in 2017 [a].**



3. **Annual medical and indirect cost in US = $188 billion in 2015 [b]**

4. **Preventing and intervention treatments for these diseases are limited**

Reference:

a. *https://academic.oup.com/ehjqcco/article/8/1/50/5918025*

b. *https://www.heart.org/-/media/Files/About-Us/Policy-Research/Fact-Sheets/Public-Health-Advocacy-and-Research/CVD-A-Costly-Burden-for-America-Projections-Through-2035.pdf*

Cells Die by Different Mechanisms

- **Necrotic cell death (pathological death)**
  - **Cell swelling and breakage**
- **Apoptotic cell death (programmed type I)**
  - **Caspase activation**
  - **Nuclear condensation and DNA fragmentation**
- **Autophagic cell death (programmed type II)**
  - **Autophagy morphology**

Non-apoptotic, programmed cell death

- **Caspase-independent**
- **Distinctive morphology**
- **Programmed (what does that mean?)**

TNF receptor pathway:

From necrosis to apoptosis and back to necrosis

What is necrosis?

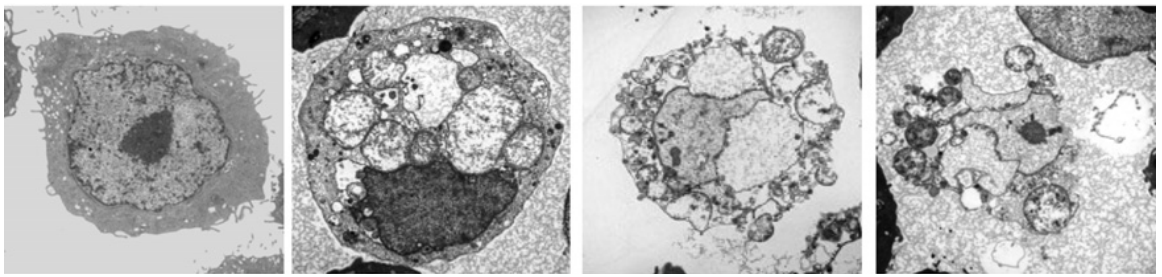
Necrosis, originally a morphological term

Apoptosis

- Chromatin condensation
- Cell shrinkage
- Preservation of cell membranes
- Rapid engulfment of apoptotic bodies by neighboring cells to prevent inflammation

Necrosis

- Nuclear Swelling
- Cell Swelling
- Disruption of organelles
- Rupture of cells and release of cellular contents
- Inflammatory response
- Thought to be always passive and harmful



He et al, 2009, Cell 137:1100

Can necrosis ever be programmed?

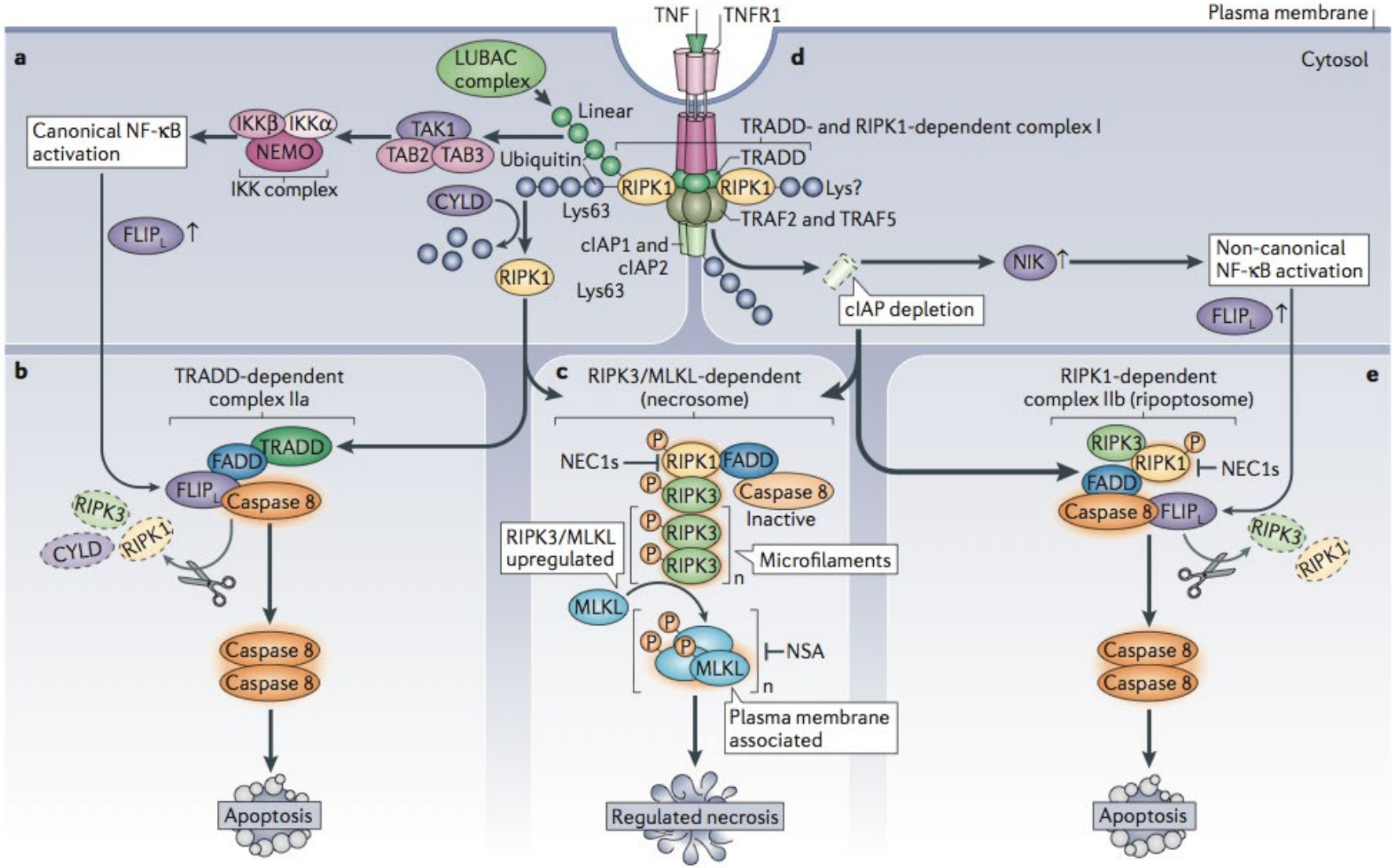
Definition of "programmed"

Molecular basis
(What is the molecular executioner?)
Biological function

Necroptosis, or programmed necrosis
Degterev et al, 2005 Nat. Chem. Biol. 1:112

(TNF pathway, requirement of RIPK1 and RIPK3)

Overview of TNF-induced apoptosis and necroptosis



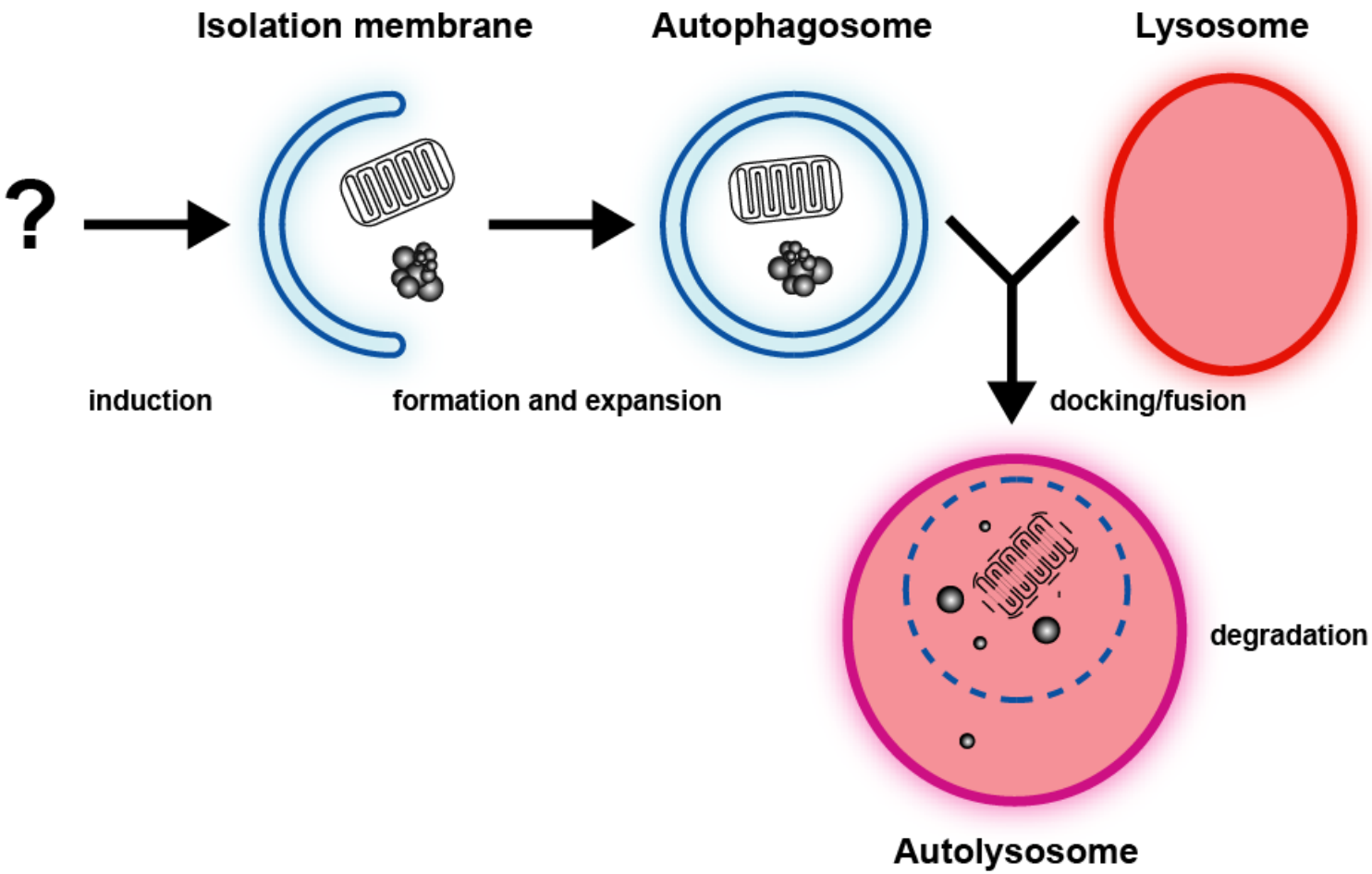
Berghe et al. , *Nat Rev Mol Biol Cell* (2014)

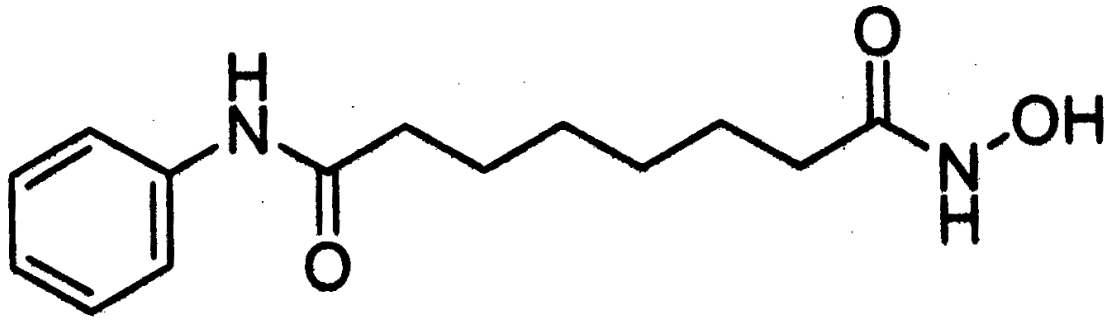
Biological contexts…

Cells Die by Different Mechanisms

- **Necrotic cell death (pathological or not)**
  - **Cell swelling and breakage**
- **Apoptotic cell death (programmed type I)**
  - **Caspase activation**
  - **Nuclear condensation and DNA fragmentation**
- **Autophagic cell death (programmed type II)**
  - **Autophagy morphology**

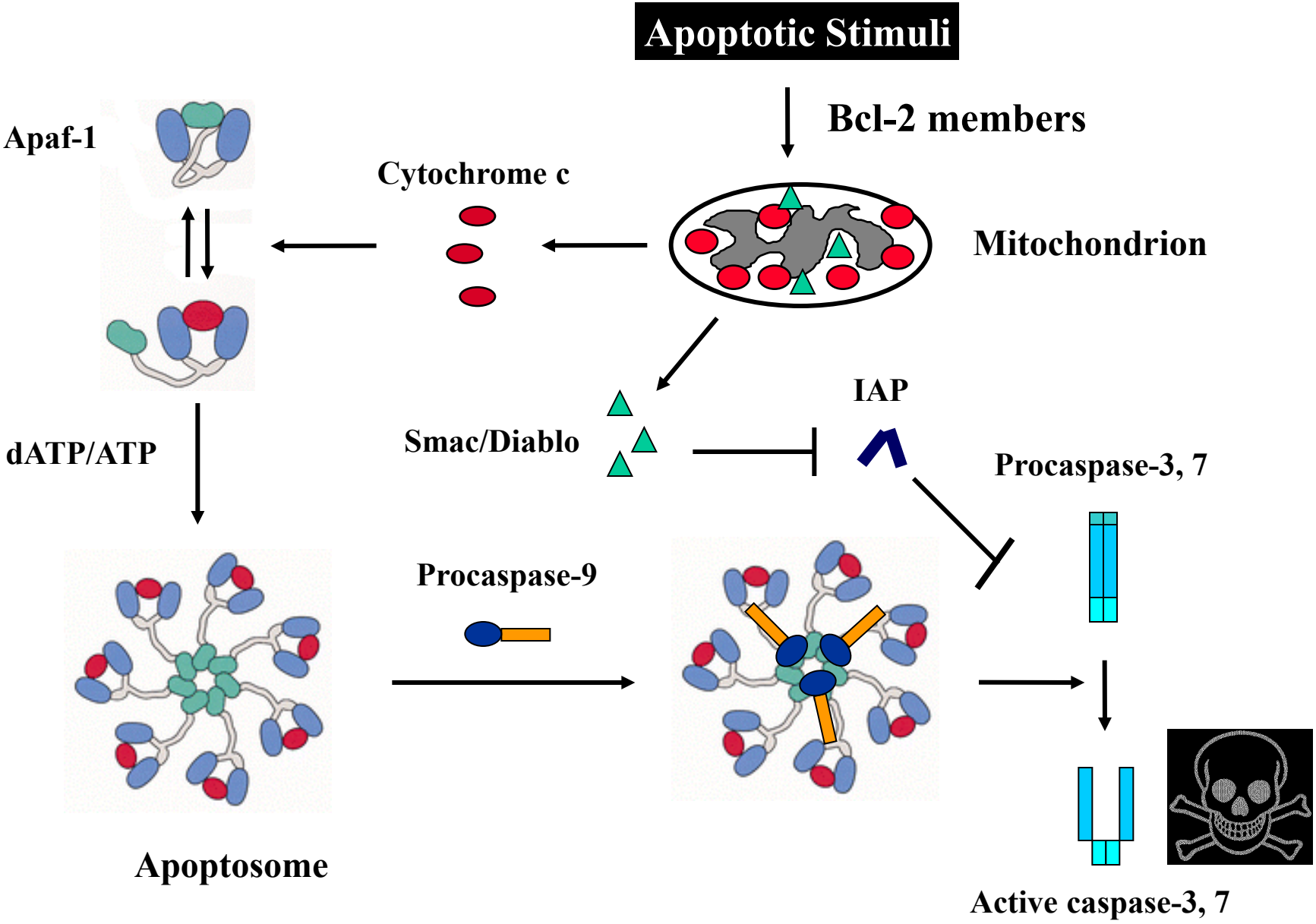
Autophagy (Self-eating)



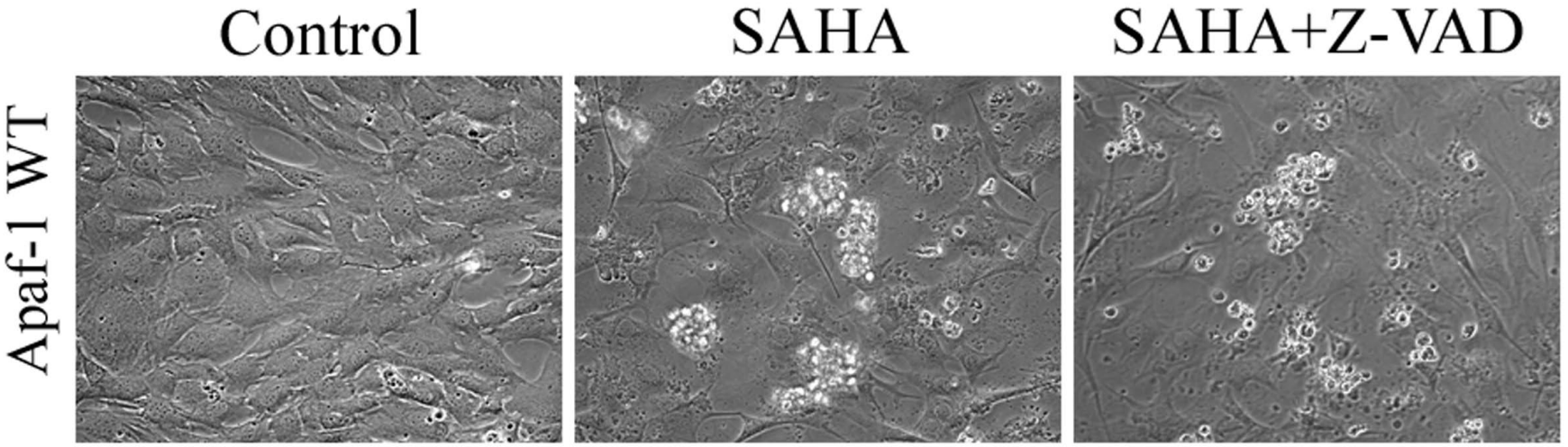


Suberoylanilide Hydroxamic Acid

(SAHA)

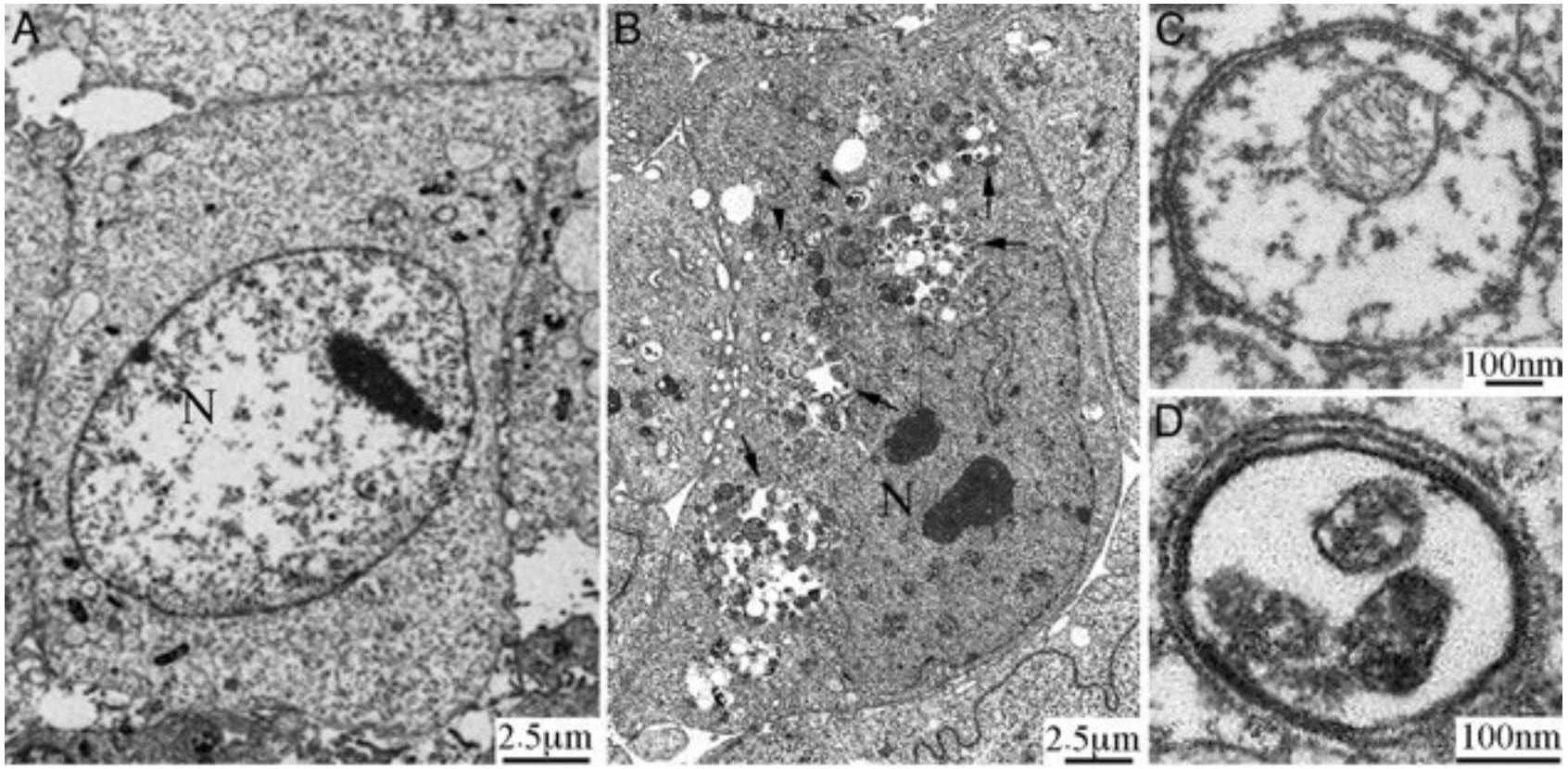

Apoptotic Stimuli
Bcl-2 members
Apaf-1
Cytochrome c
Mitochondrion
IAP
Smac/Diablo
dATP/ATP
Procaspase-3, 7
Procaspase-9
Apoptosome
Active caspase-3, 7

SAHA Can Induce Caspase-Independent Cell Death



Autophagy Detected by Electron Microscopy

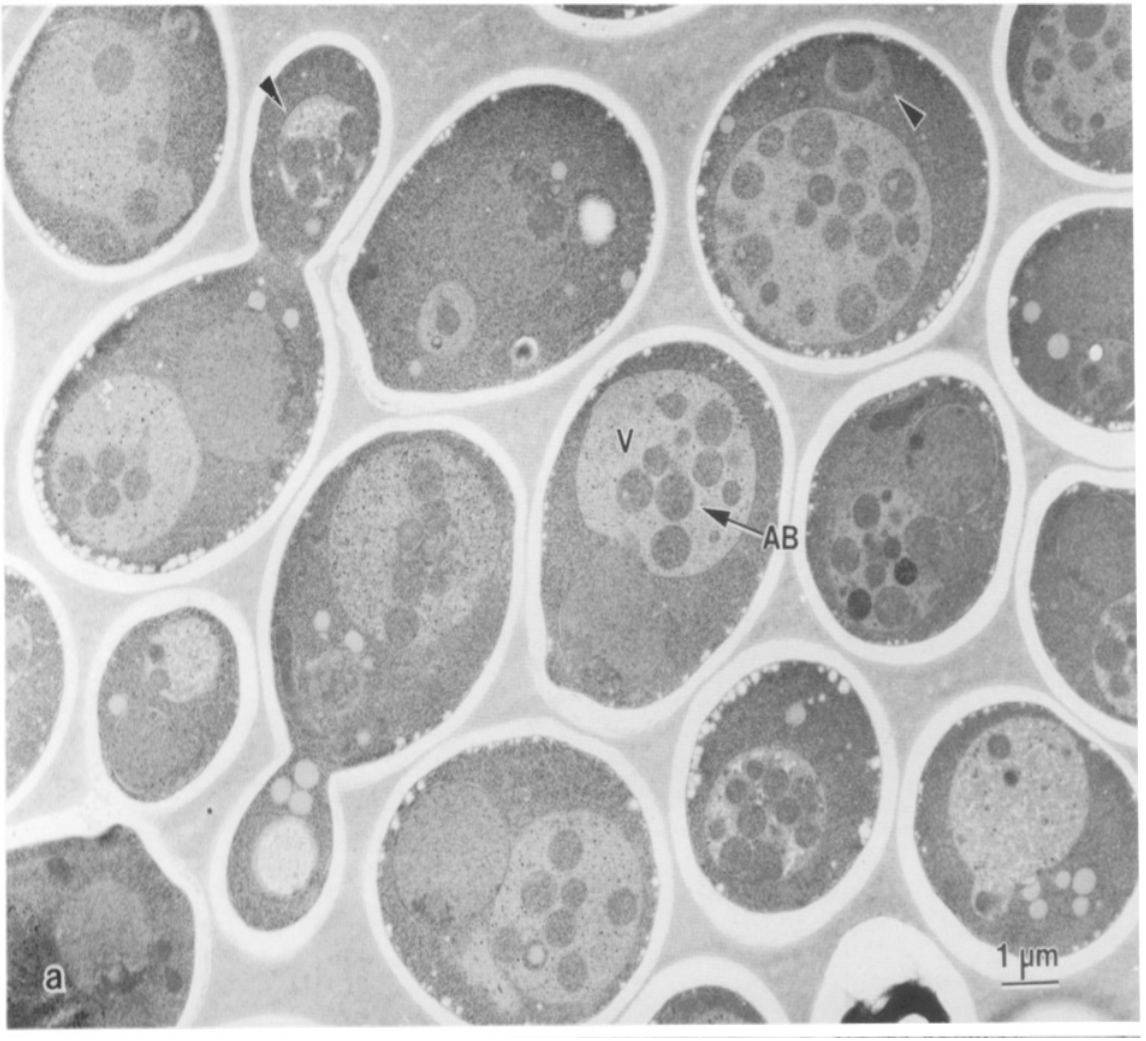
Control **SAHA treatment**



Yufang Shao

Correlation, consequence, versus causality

Basis of Yoshinori Ohsumi's yeast genetic screening



Takeshige et al, JCB 1992 (119) 301

Autophagy, A Highly Conserved Cellular Process

Table 3. Orthologs of Autophagy-Related Genes in Higher Eukaryotes[a]

Gene Designation								
ATG	At[b]	Ce	Dd	Dm	Hs	Mm	Rn	Reference
1	—	*unc-51*	*DdAPG1*	—	—	—	—	Meléndez et al., 2003; G.P. Otto and R.H. Kessin, personal communication
3	—	—	—	*DrAUT1*	*hAPG3*	—	—	Juhasz et al., 2003; Tanida et al., 2002b
4	—	—	—	*APG4/AUT2*	—	—	—	Thumm and Kadowaki, 2001
5	—	—	*DdAPG5*	—	*hAPG5*	*APG5*	—	Mizushima et al., 2001; Otto et al., 2003; Mizushima et al., 1998b
6	—	*bec-1*	*DdAPG6*	—	*beclin 1*	—	—	Liang et al., 1999; Meléndez et al., 2003; G.P. Otto and R.H. Kessin, personal communication
7	*AtAPG7*	*M7.5*	*DdAPG7*	—	*HsGSA7* *hAP G7*	*mAPG7*	—	Doelling et al., 2002; Otto et al., 2003; Tanida et al., 2001; Yuan et al., 1999; Meléndez et al., 2003
8	—	*lgg-1*	*DdAPG8*	—	*MAP1LC3*[c]	*mAPG8*	*LC3*	He et al., 2003; Tanida et al., 2002c; Otto et al., 2003; Meléndez et al., 2003
9	*AtAPG9*	—	—	—	—	—	—	Hanaoka et al., 2002
10	—	—	—	—	—	*mAPG10*	—	Mizushima et al., 2002
12	—	—	*DdAPG12*	—	*hAPG12*	*APG12* *mAPG12*	—	Mizushima et al., 2001; Tanida et al., 2002a; Mizushima et al., 1998b; Otto et al., 2003
16	—	—	*TipD*	—	—	*APG16L*	—	Mizushima et al., 2003; G.P. Otto and R.H. Kessin, personal communication
18	—	*F41E6.13*	—	—	—	—	—	Meléndez et al., 2003

[a] Only genes that have been mutated and shown to function in autophagy or that have been shown to interact with other autophagy-related proteins in published papers have been included in this table.

[b] Abbreviations: *At*, *Arabidopsis thaliana*; *Ce*, *Caenorhabditis elegans*; *Dd*, *Dictyostelium discoideum*; *Dm*, *Drosophila melanogaster*; *Hs*, *Homo sapiens*; *Mm*, *Mus musculus*; *Rn*, *Rattus norvegicus*.

[c] There are three homologs of human MAP1LC3, designated A, B, and C.

Klionsky et al, Developmental Cell, 2003 (5) 539–545

The role of autophagy during the early neonatal starvation period

Akiko Kuma[1,2,5,7], Masahiko Hatano[2,4], Makoto Matsui[5,6,7], Akitsugu Yamamoto[8], Haruaki Nakaya[3], Tamotsu Yoshimori[9], Yoshinori Ohsumi[5,6], Takeshi Tokuhisa[2] & Noboru Mizushima[1,5,7]

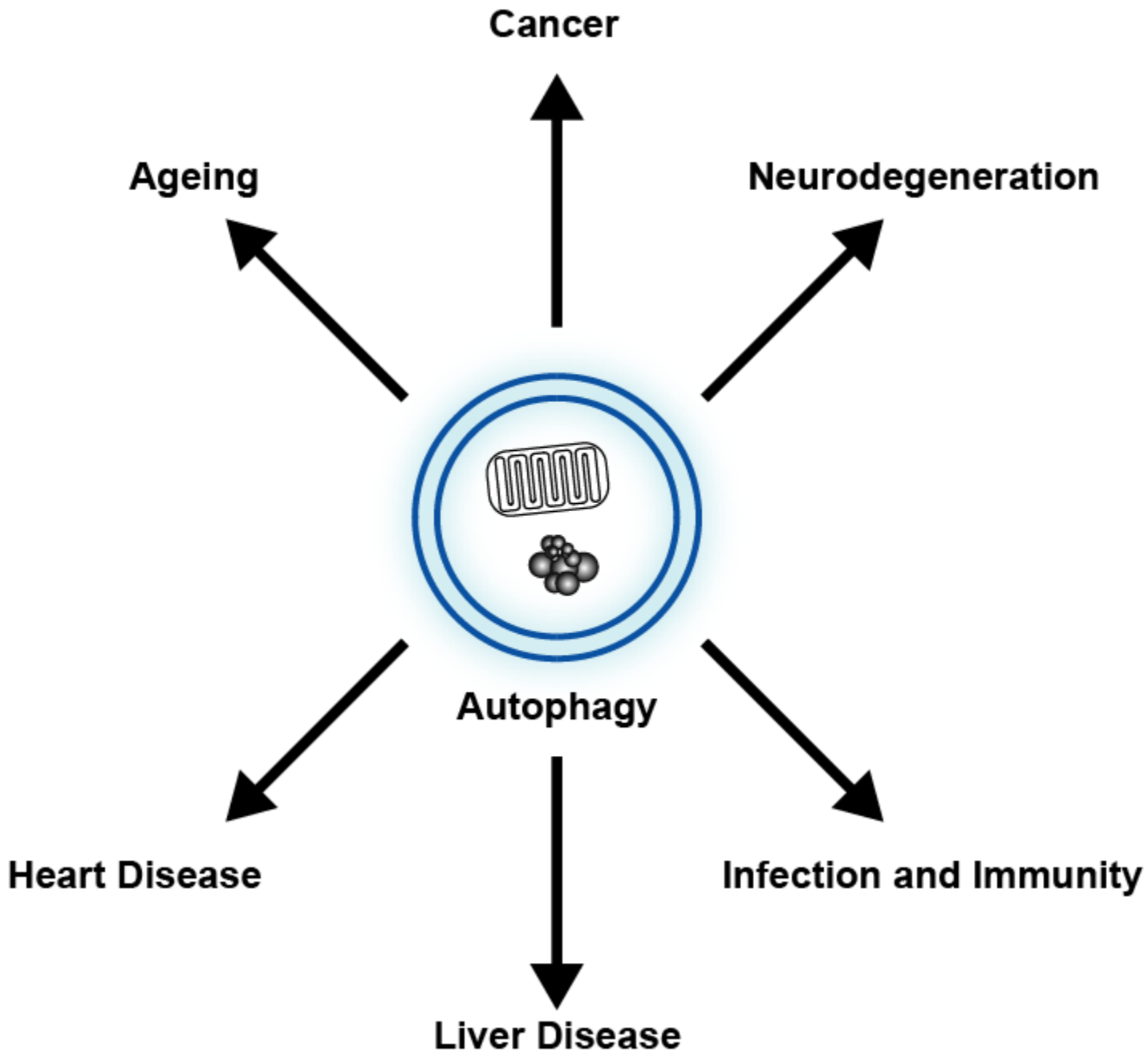
At birth the trans-placental nutrient supply is suddenly interrupted, and neonates face severe starvation until supply can be restored through milk nutrients[1]. Here, we show that neonates adapt to this adverse circumstance by inducing autophagy. Autophagy is the primary means for the degradation of cytoplasmic constituents within lysosomes[2–4]. The level of autophagy in mice remains low during embryogenesis; however, autophagy is immediately upregulated in various tissues after birth and is maintained at high levels for 3–12 h before returning to basal levels within 1–2 days. Mice deficient for *Atg5*, which is essential for autophagosome formation, appear almost normal at birth but die within 1 day of delivery. The survival time of starved *Atg5*-deficient neonates (~12 h) is much shorter than that of wild-type mice (~21 h) but can be prolonged by forced milk feeding. *Atg5*-deficient neonates exhibit reduced amino acid concentrations in plasma and tissues, and display signs of energy depletion. These results suggest that the production of amino acids by autophagic degradation of 'self' proteins, which allows for the maintenance of energy homeostasis, is important for survival during neonatal starvation.

www.sciencemag.org SCIENCE VOL 321 4 JULY 2008

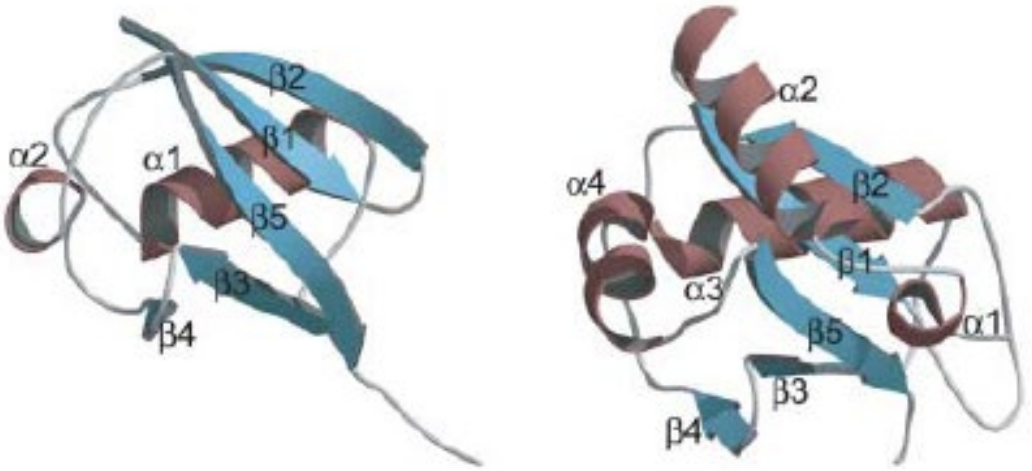
Autophagy Is Essential for Preimplantation Development of Mouse Embryos

Satoshi Tsukamoto,[1]* Akiko Kuma,[1,2]† Mirei Murakami,[1] Chieko Kishi,[1] Akitsugu Yamamoto,[3] Noboru Mizushima[1,2]‡

After fertilization, maternal proteins in oocytes are degraded and new proteins encoded by the zygotic genome are synthesized. We found that autophagy, a process for the degradation of cytoplasmic constituents in the lysosome, plays a critical role during this period. Autophagy was triggered by fertilization and up-regulated in early mouse embryos. Autophagy-defective oocytes derived from oocyte-specific Atg5 (autophagy-related 5) knockout mice failed to develop beyond the four- and eight-cell stages if they were fertilized by Atg5-null sperm, but could develop if they were fertilized by wild-type sperm. Protein synthesis rates were reduced in the autophagy-null embryos. Thus, autophagic degradation within early embryos is essential for preimplantation development in mammals.

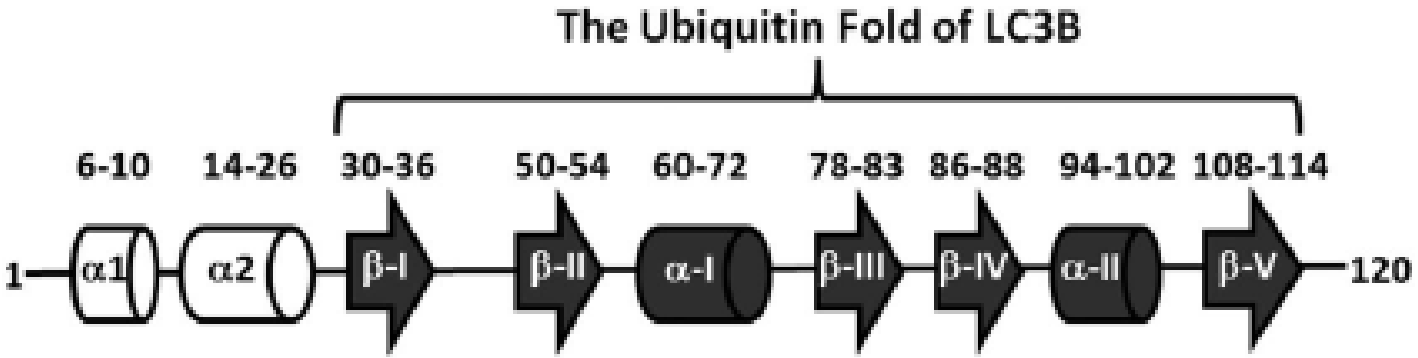

Cancer
Ageing
Neurodegeneration
Autophagy
Heart Disease
Infection and Immunity
Liver Disease

ATG8/LC3, a ubiquitin-like protein essential for autophagy

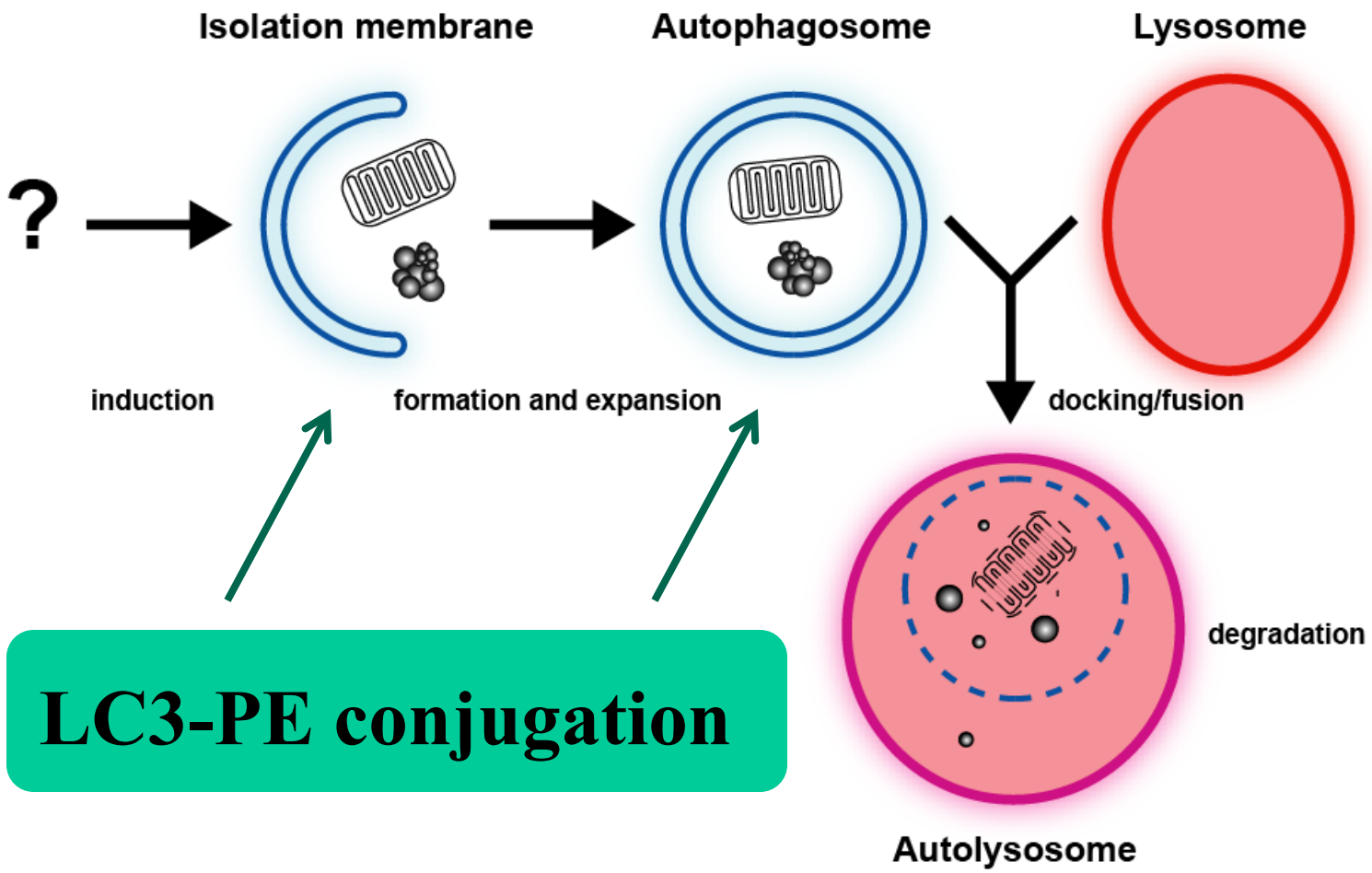


Ubiquitin **LC3**

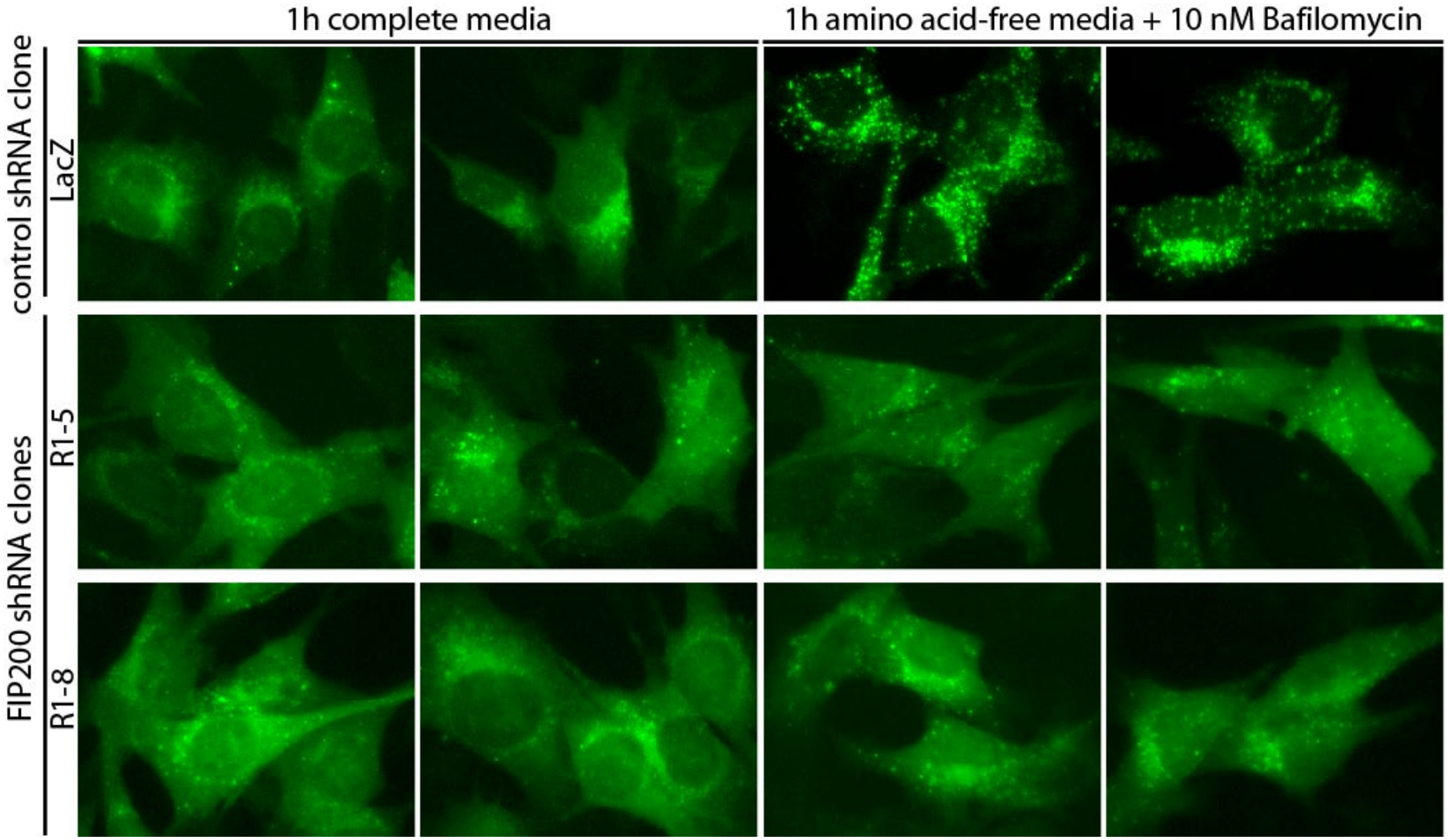
Sugawara et al, Genes to Cells, 2004, 9:611



Autophagy (self-eating)

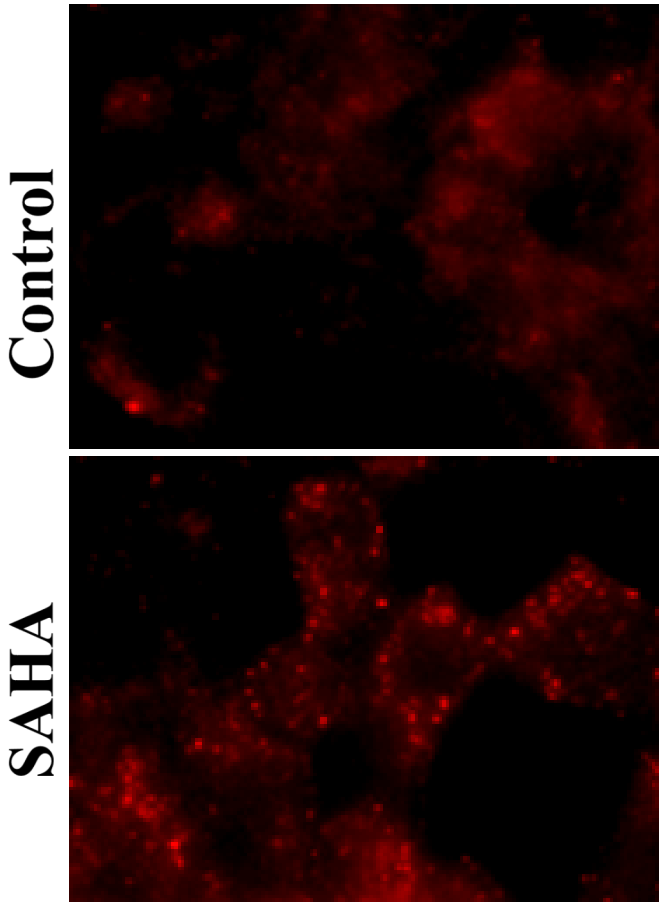


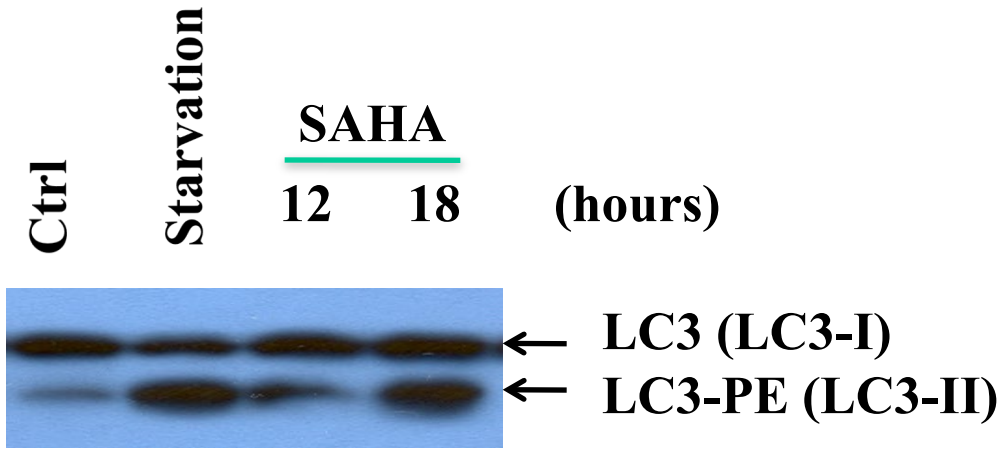
GFP-LC3/ATG8 as a cellular marker for autophagy



Ian Ganley

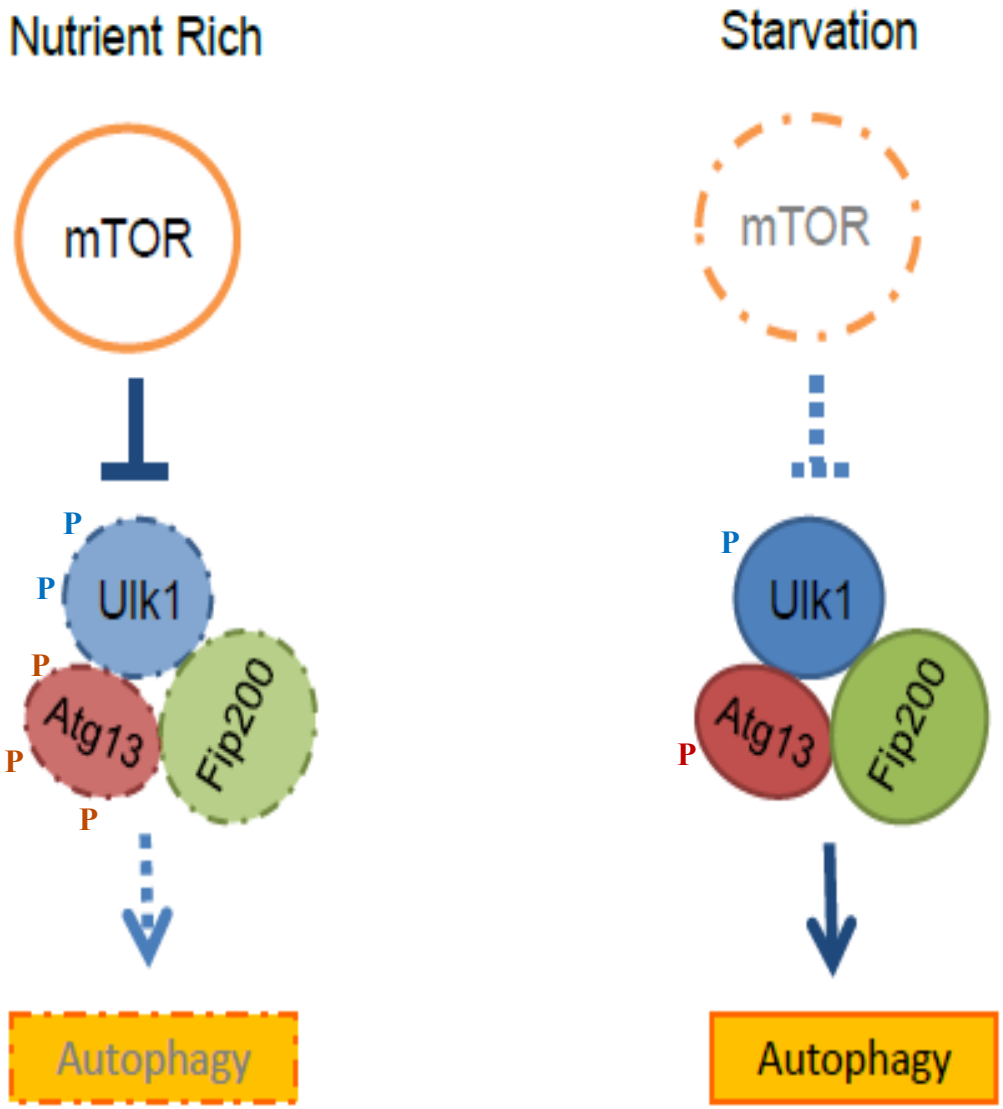
LC3/ATG8-PE conjugation Induced by SAHA



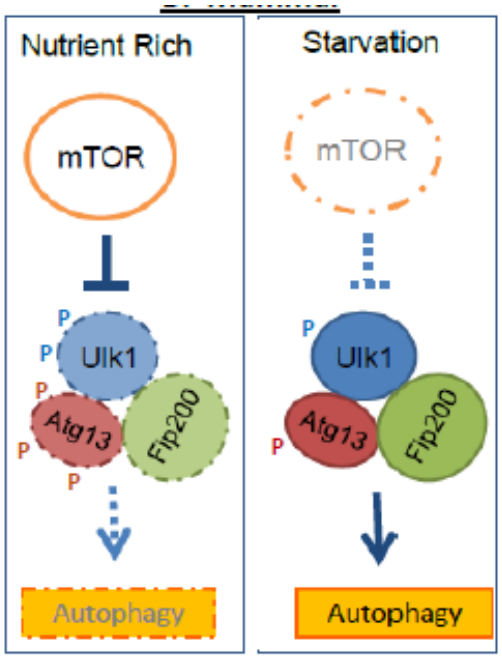


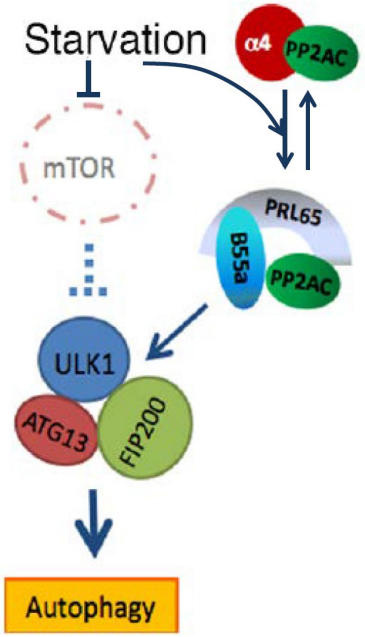
Du Lam

Mammalian ULK1 complex initiates autophagy



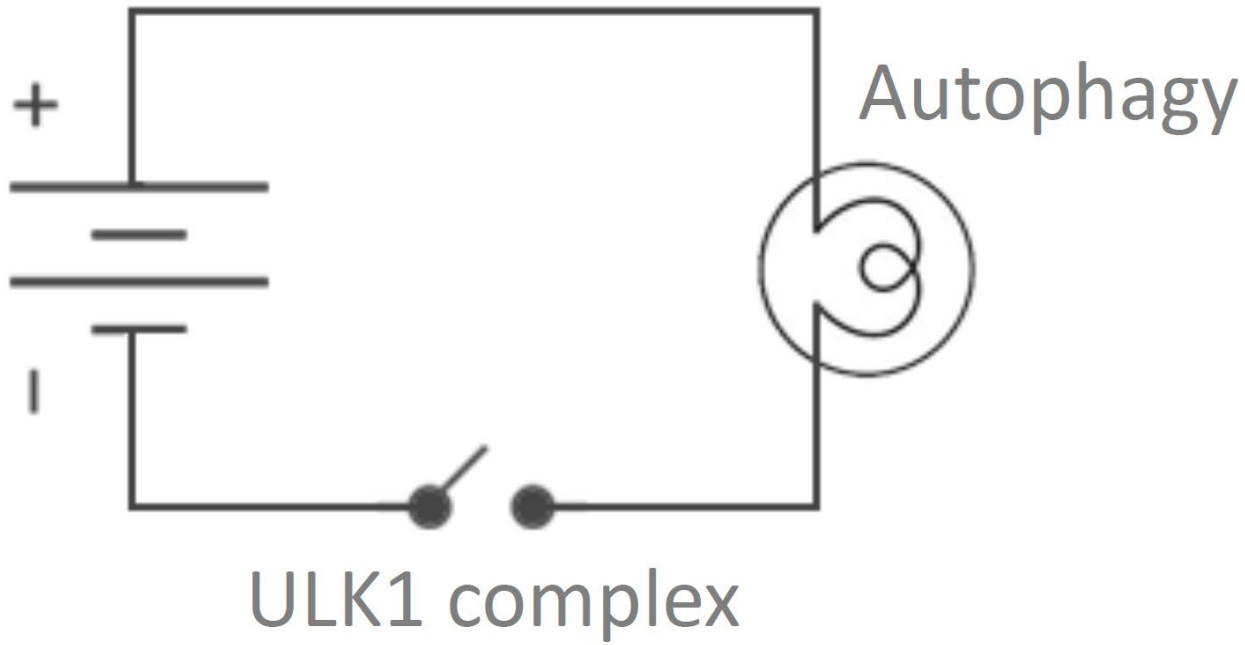
Paradox of mTORC1 and ULK1 in Autophagy-Addicted Cancer Cells





Wong P *et al*, *Nature Communications* 2015 Aug 27; 6:8048.

Autophagy addiction in cancer cells



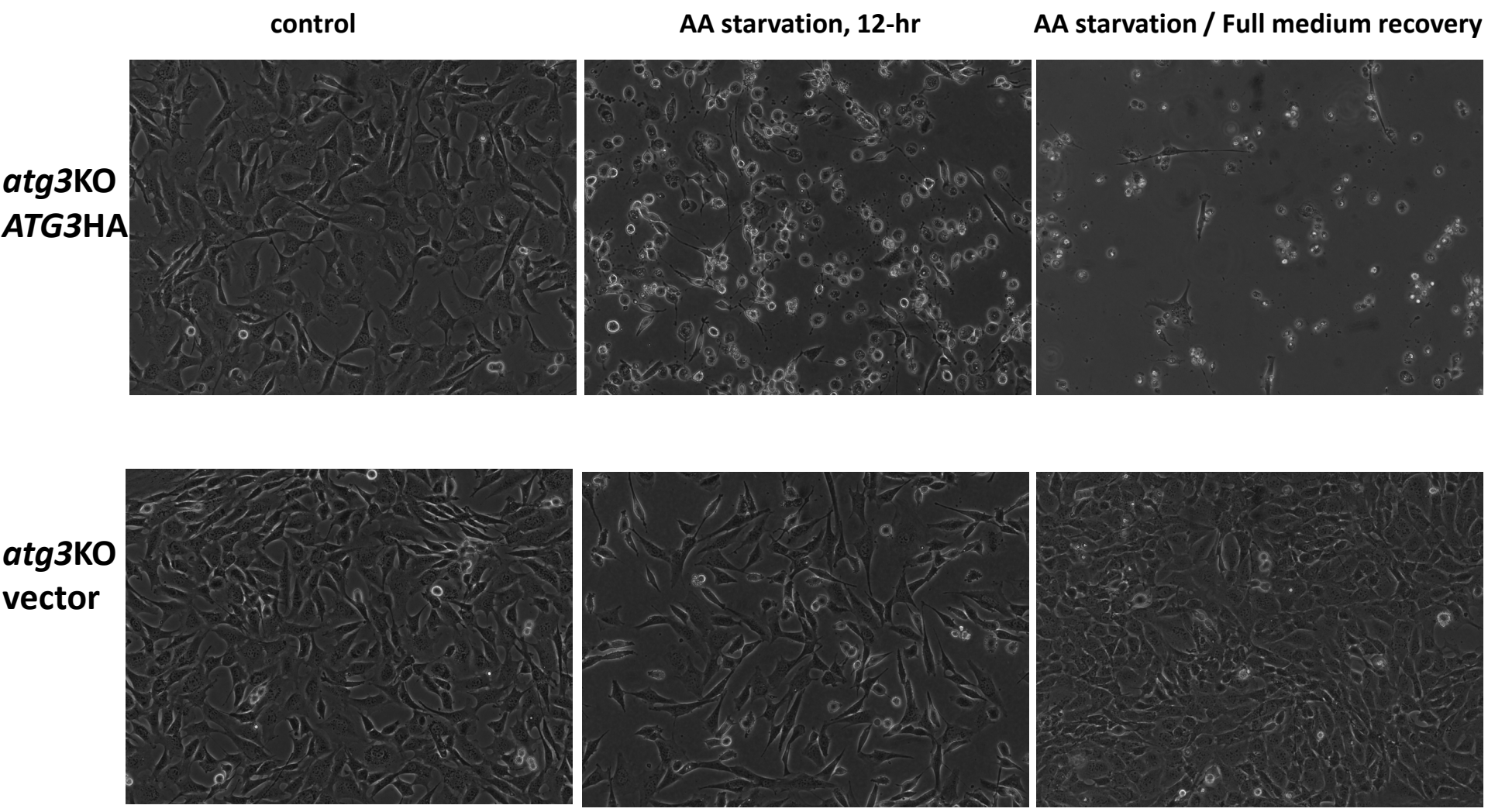
Autophagy, ever a cause of cell death?

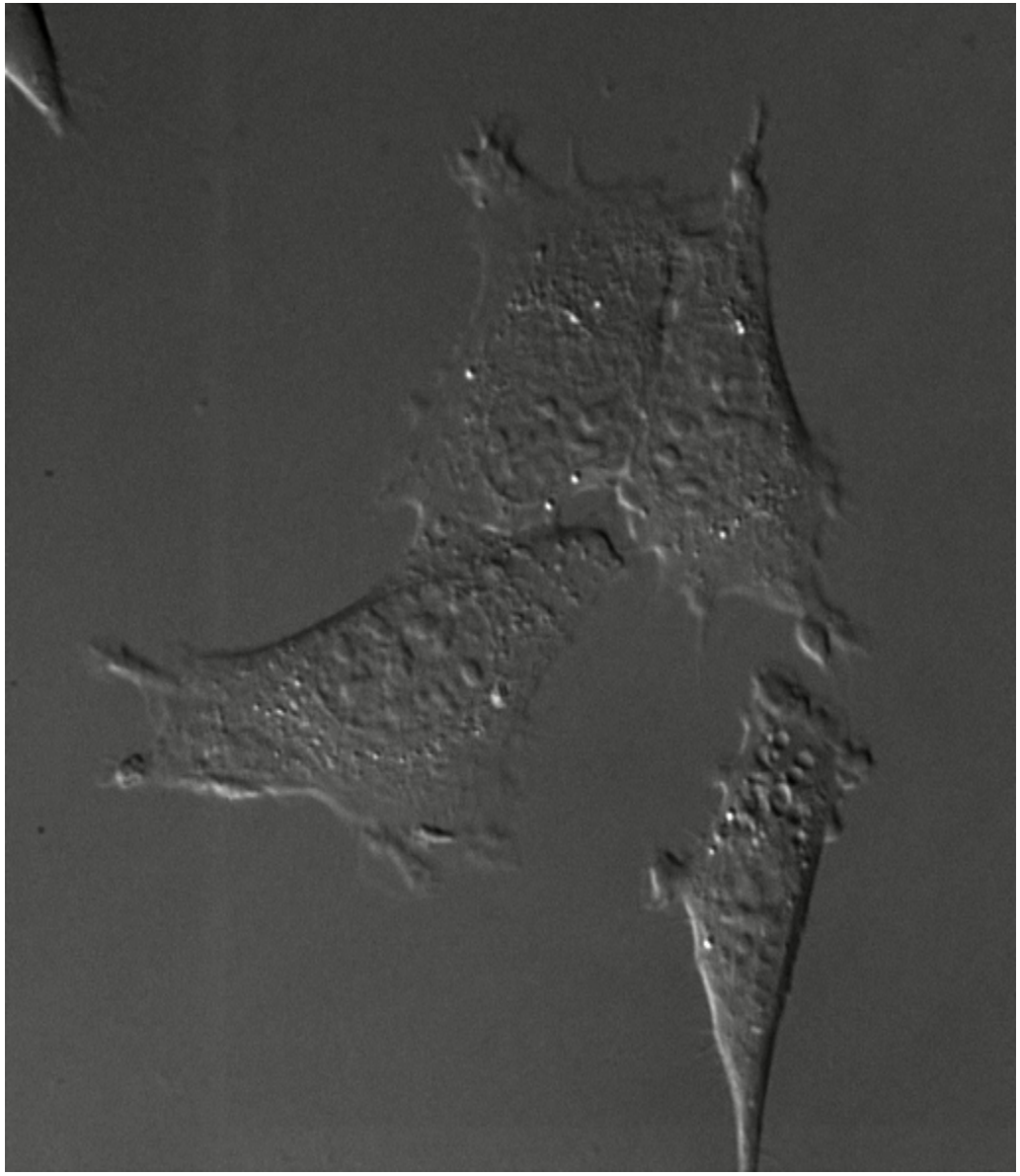
- **Survival argument**
- **Developmental observation**
- **Therapeutic implication**

Cancer Therapeutic Implication

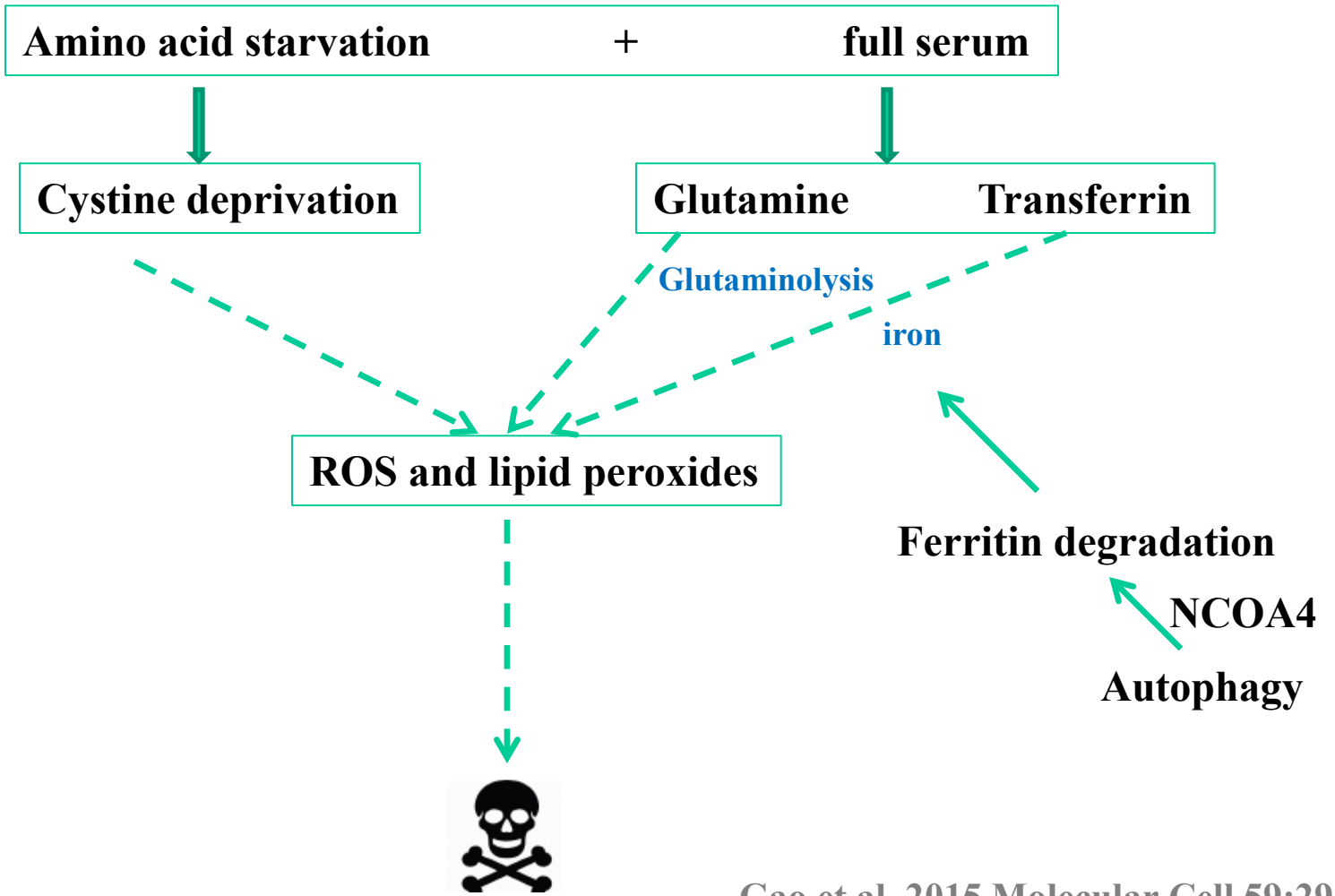
- **Autophagy is frequently activated during cancer development, so shall we inhibit it?**
- **Autophagy can be activated by many cancer therapeutic treatments (mTOR as an example), so shall we inhibit it (combination therapy)?**
- **Autophagy and immunity**
- **Currently, there is not autophagy-specific inhibitor available**

Autophagy Machines Regulate Starvation-Induced, Serum-Dependent Cell Death



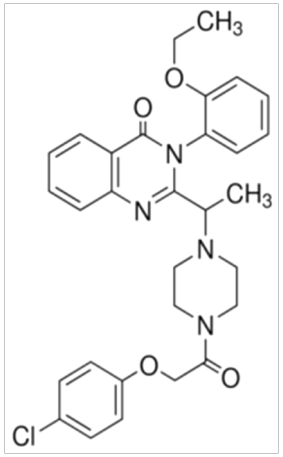


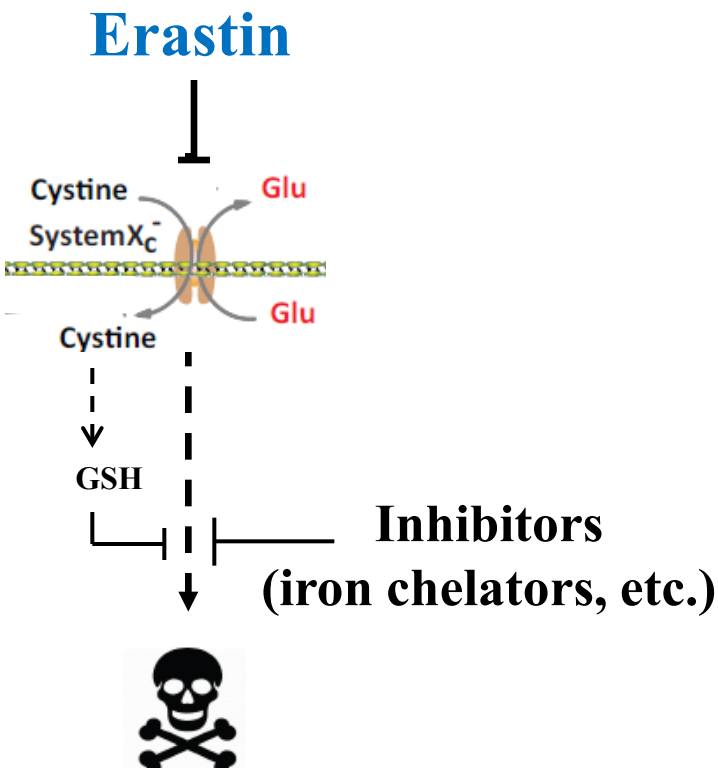
Metabolic Cell death



Gao et al, 2015 Molecular Cell 59:298
Gao et al, 2016 Cell Research 26:1021

Brent Stockwell





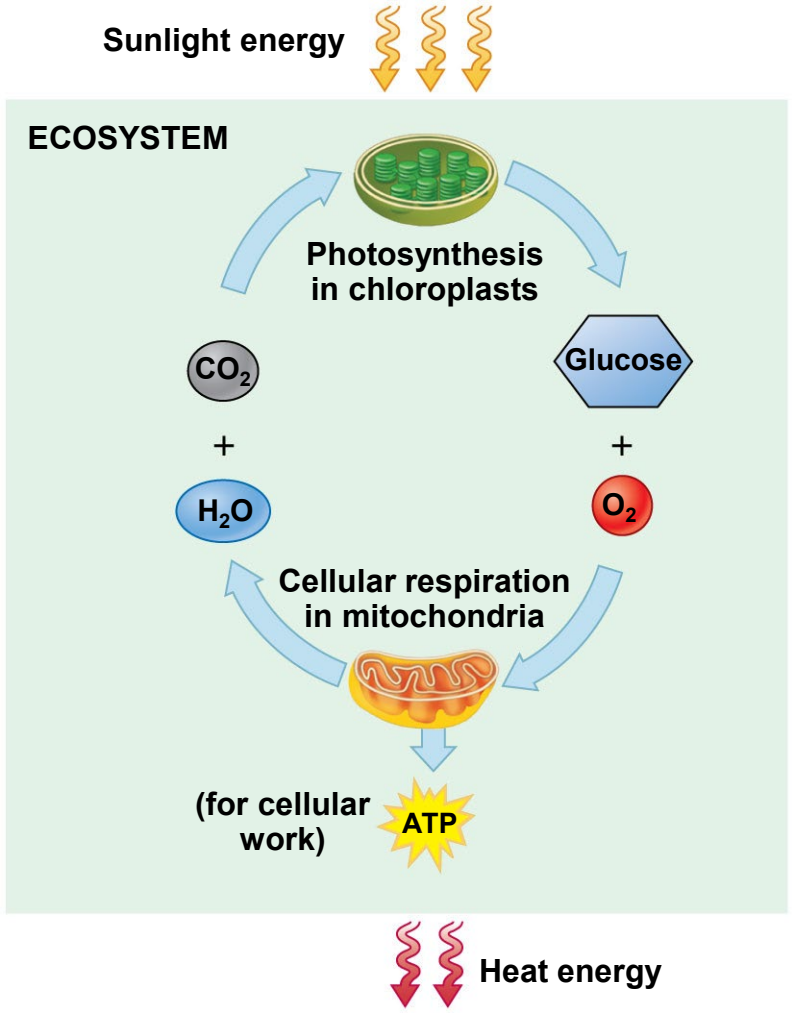
Unique mode of death

Ferroptosis, 2012

Dixon, S.J., Lemberg, K.M., Lamprecht, M.R., Skouta, R., Zaitsev, E.M., Gleason, C.E., Patel, D.N., Bauer, A.J., Cantley, A.M., Yang, W.S., *et al.* (2012). Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell *149*, 1060-1072.

Essential factors for life can also function as killers

- **Transferrin/iron**
- **Glutamine**
- **Mitochondria (different from apoptosis)**

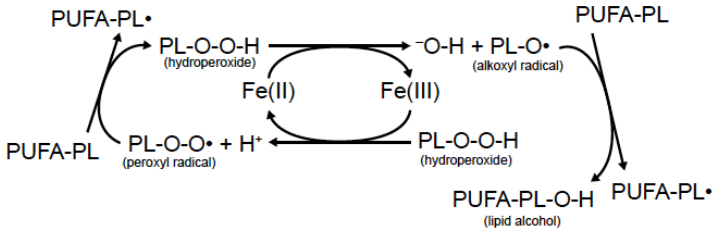


$Fe^{3+} + e^- \longleftrightarrow Fe^{2+}$

Iron/oxygen-driven, redox-based metabolism

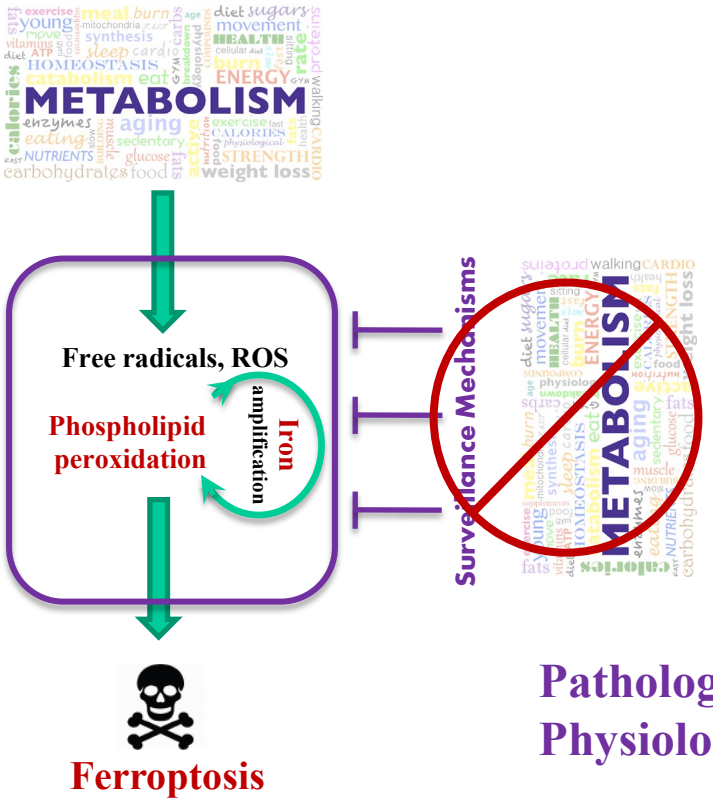
Reactive oxygen species (ROS)

Phospholipid peroxides (PLOOH)



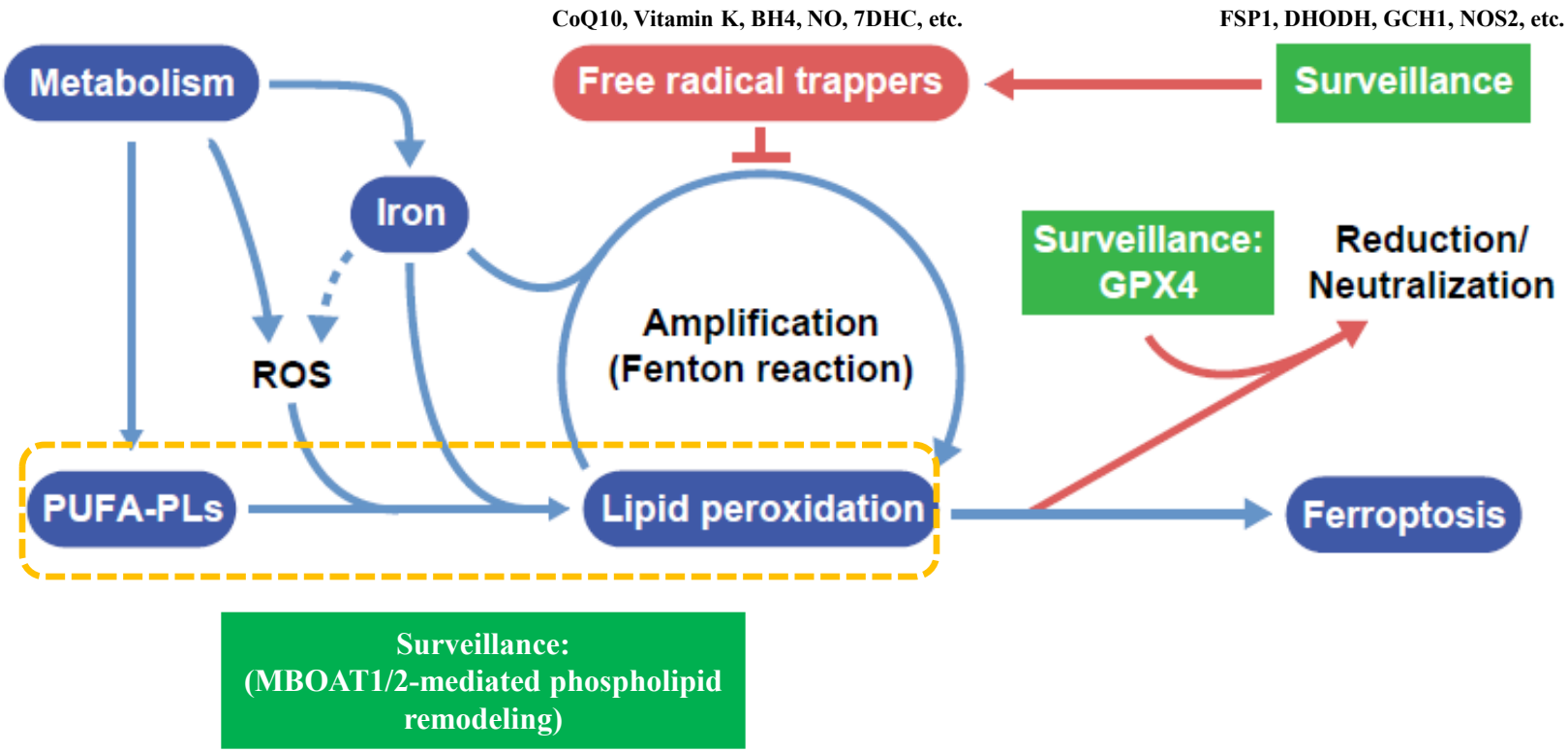
Ferroptosis

The Metabolic Nature of Ferroptosis



Pathological disruption
Physiological suppression?

Ferroptosis Surveillance

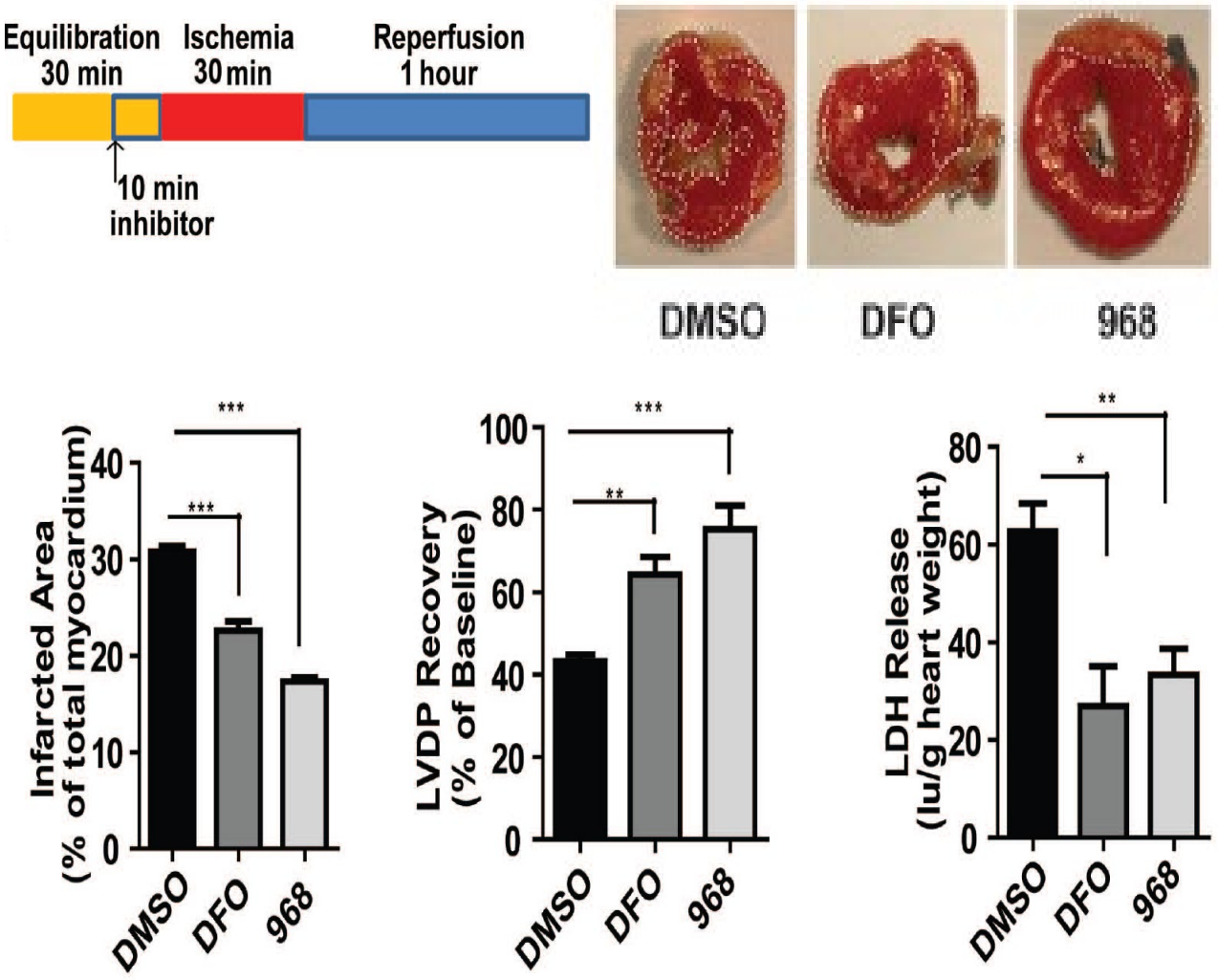


Physiological and Pathological Relevance of Ferroptosis?

Is tumor suppression a physiological function of ferroptosis?

- p53 (Nature 2017. 520:57)
- BAP1 (NCB 2018. 20:1181)
- Fumarase (Molecular Cell 2019. 73:354)

Ferroptosis Inhibitors Attenuate Ischemia/Reperfusion-Induced Heart Damage (ischemic heart diseases)



Gao et al, 2015 Molecular Cell 59:298

Brief summary of several non-apoptotic cell death modalities

- **Necroptosis**
  - Mechanism: caspase-independent; requires RIP3 and MLKL
  - Biology: involved in inflammation and likely immune defense
  - Role in disease: inflammation; defective in most cancer cells due to RIP3 silence
- **Pyroptosis**
  - Mechanism: caspase/inflammasome-dependent; gasdermin-mediated membrane permeabilization
  - Biology: immunity
  - Role in disease: immune diseases; implicated in cancer cell death and cancer immunity
- **Ferroptosis**
  - Mechanism: caused by iron-dependent phospholipid peroxidation
  - Biology: likely involved in immunity and tumor suppression
  - Role in disease: ferroptosis modulation is a promising approach for treating multiple diseases, including ischemic organ injuries and cancer

General Questions

- **Why so many cell death pathways**
- **Point of no-return**
- **Biomarkers**
- **Crosstalk**
- **Physiological functions**
- **Disease implication**
- **… …**