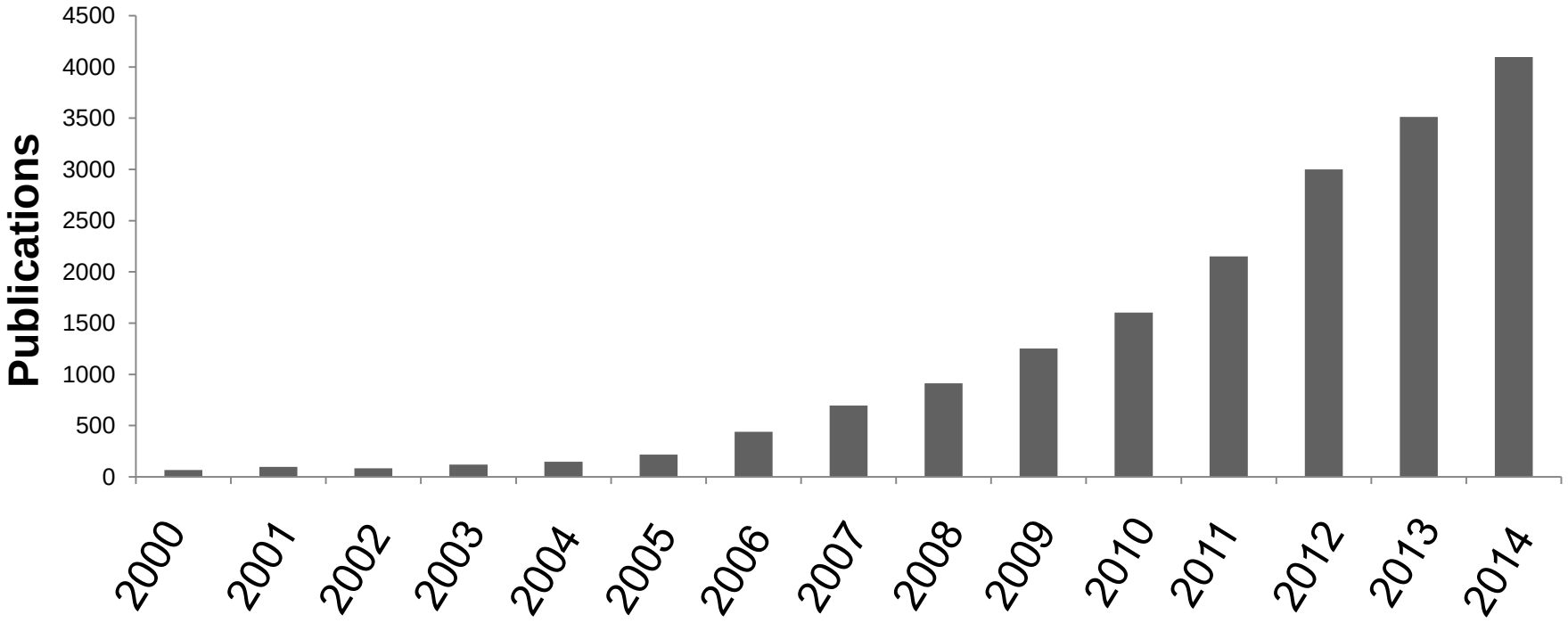
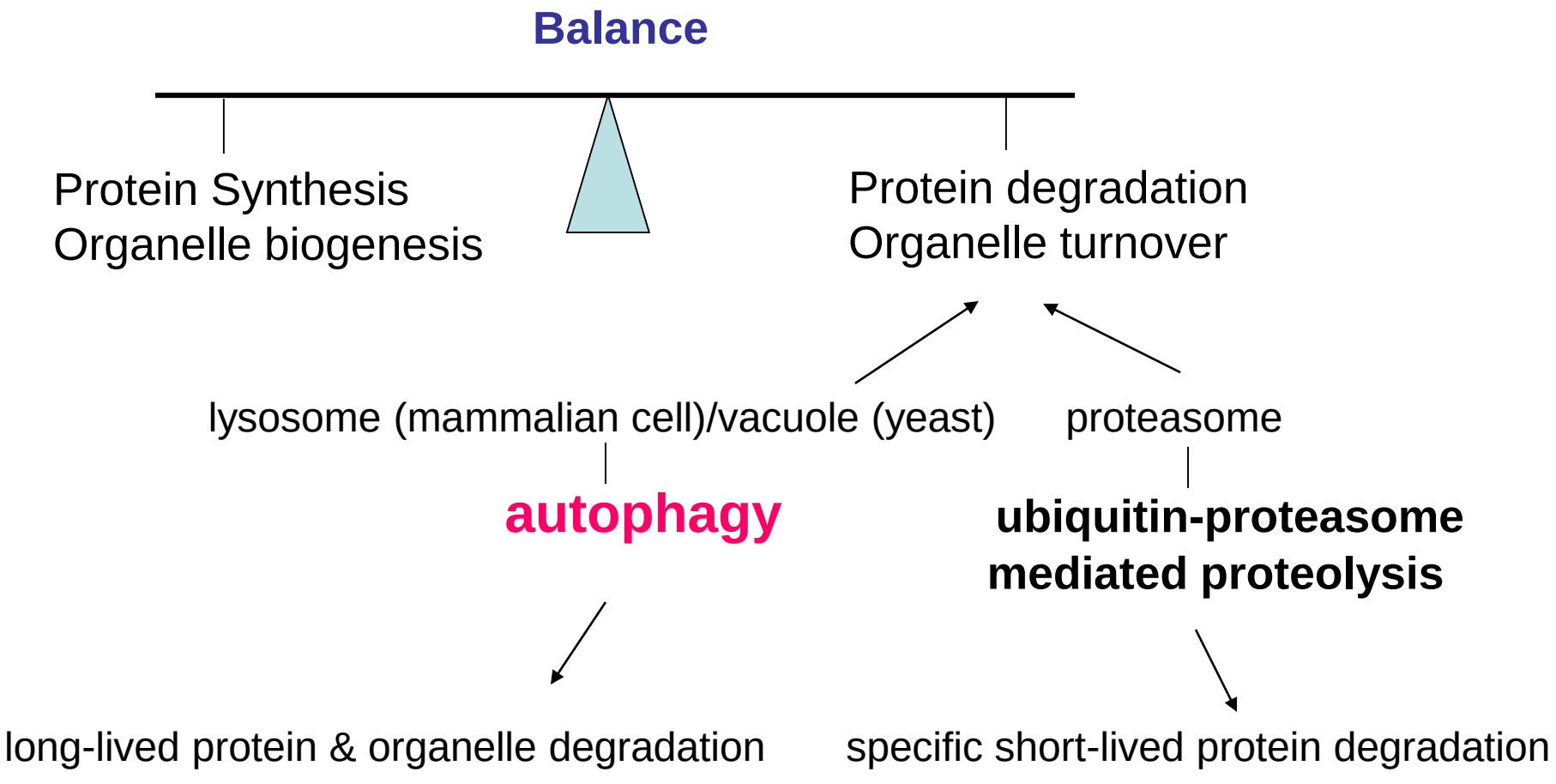
Autophagy and cancer: from yeast to humans

Lin Yan, PhD

Autophagy Research




Balance
Protein Synthesis
Organelle biogenesis
Protein degradation
Organelle turnover
lysosome (mammalian cell)/vacuole (yeast)
proteasome
autophagy
ubiquitin-proteasome
mediated proteolysis
long-lived protein & organelle degradation
specific short-lived protein degradation

What is autophagy

- Autophagy ("self-eating”) is a regulated lysosomal degradation pathway responsible for the turnover of unnecessary or dysfunctional organelles and cytoplasmic proteins.

- Autophagy is evolutionarily conserved in all eukaryotic cells from yeast to mammal. It is maintained at a basal level under normal cell growth conditions but is rapidly upregulated when cells need to
  1) generate intracellular nutrients and energy (e.g., during starvation or trophic factor withdrawal);
  2) undergo cell remodeling (e.g., during developmental transitions);
  3) rid themselves of damaging cytoplasmic components (e.g., during oxidative stress, infection, hypoxia/ischemia, and accumulation of protein aggregates)

- Under stress conditions, cells utilize their autophagic mechanism to survive by degrading non-essential and dysfunctional organelles and proteins to generate amino acids to synthesize new proteins that are needed for functional recovery and the continued cell survival.

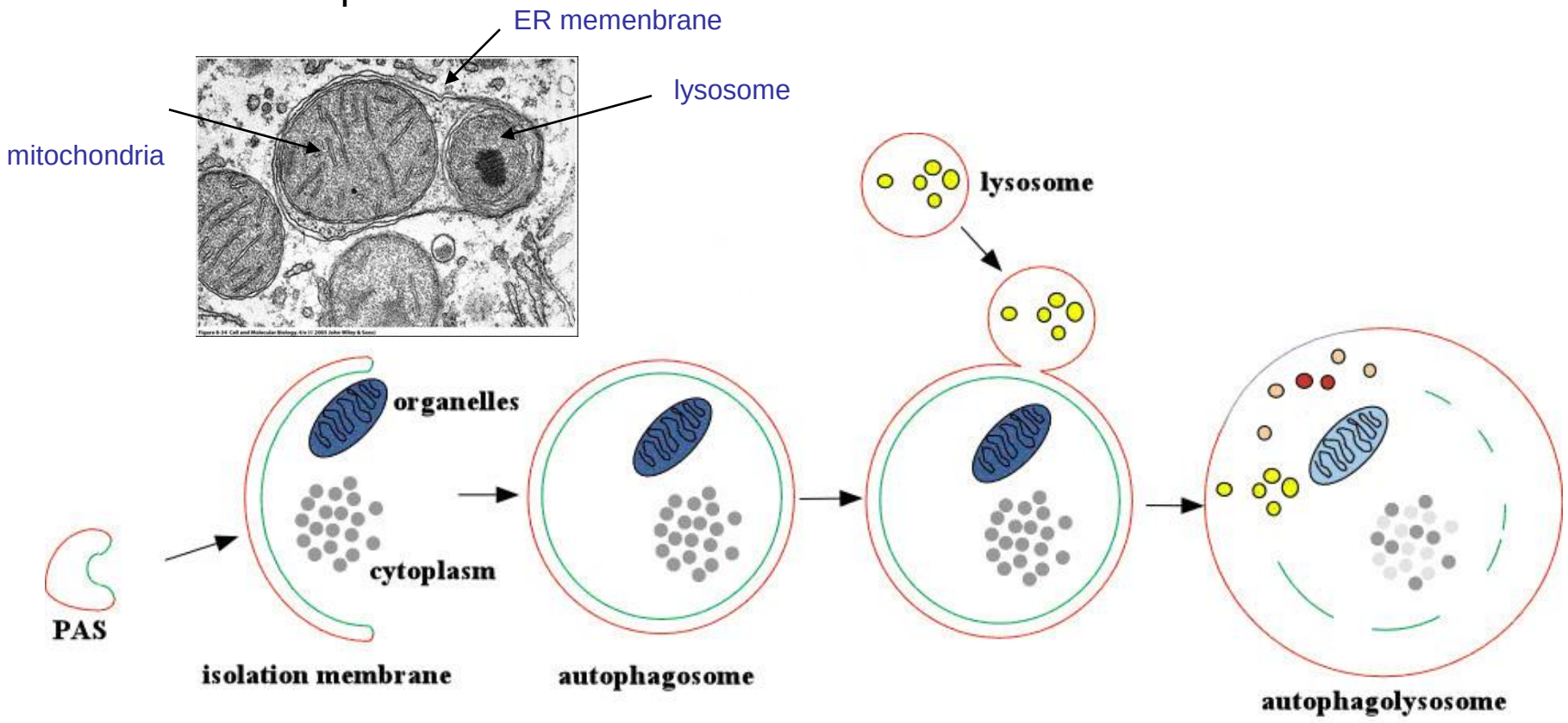
The control of autophagy

- Nutritional status
- growth factors
- Temperature
- Oxygen concentration
- Cell density

Major autophagy mechanisms

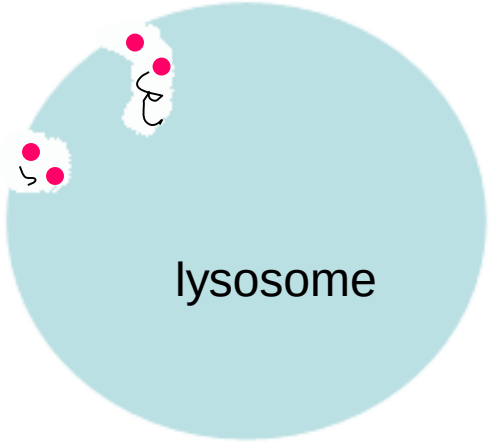
- Macroautophagy
- Microautophagy
- Chaperone-mediated autophagy

Macroautophagy ---- a dynamic process for the bulk degradation of proteins, in which cytoplasmic proteins as well as entire organelles are sequestered in a double-membrane-bound vesicle, termed autophagosome, delivered to the lysosome (mammalian cell) or vacuole (yeast) by fusion for degradation and recycle. This process is mainly activated under conditions of nutrient deprivation.

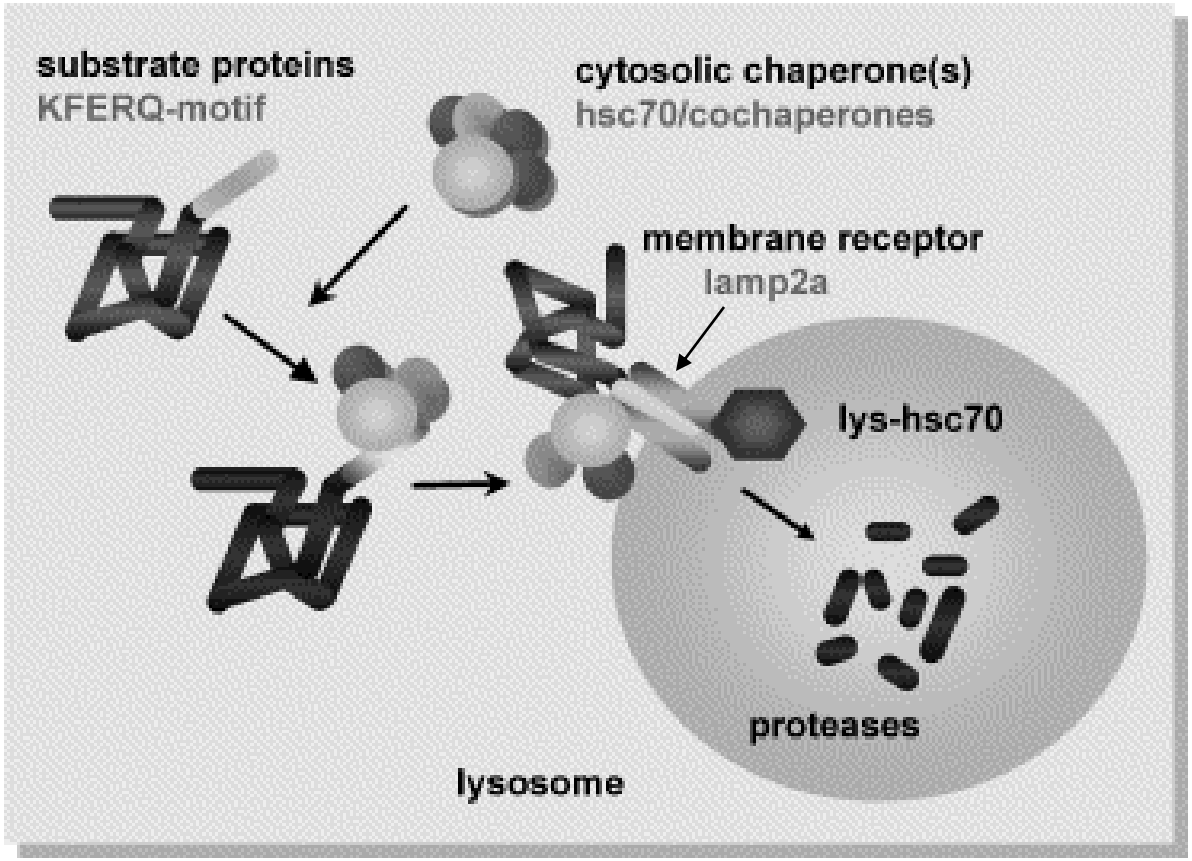


4 steps: Signaling → sequestration of cytoplasm → completion of vesicle formation → targeting of the completed vesicle to the lysosome/vacuole followed by docking and fusion, and breakdown

Microautophagy ---- only small portions of cytoplasm are engulfed by small invaginations in the surface of lysosomes. After internalization of those vesicles and breakage of their surrounding membrane, the cytoplasmic proteins are degraded inside lysosomes. This process is mainly responsible for the continuous basal degradation of long lived proteins. It has not been well characterized in mammalian cell.



Chaperone-mediated autophagy --- a selective degradation pathway, in which proteins containing a pentapeptide KFERQ motif are bound by Hsc70, a cytosolic chaperone and targeted to the lysosomal membrane. The substrate complexes bind to lamp2a at the lysosomal membrane, are unfolded and get translocated into the lysosomal lumen assisted by the lysosomal chaperone lys-hsc70. Once in the lysosomal matrix, substrates are rapidly degraded by the lysosomal proteases.



Comparison of the three main forms of autophagy

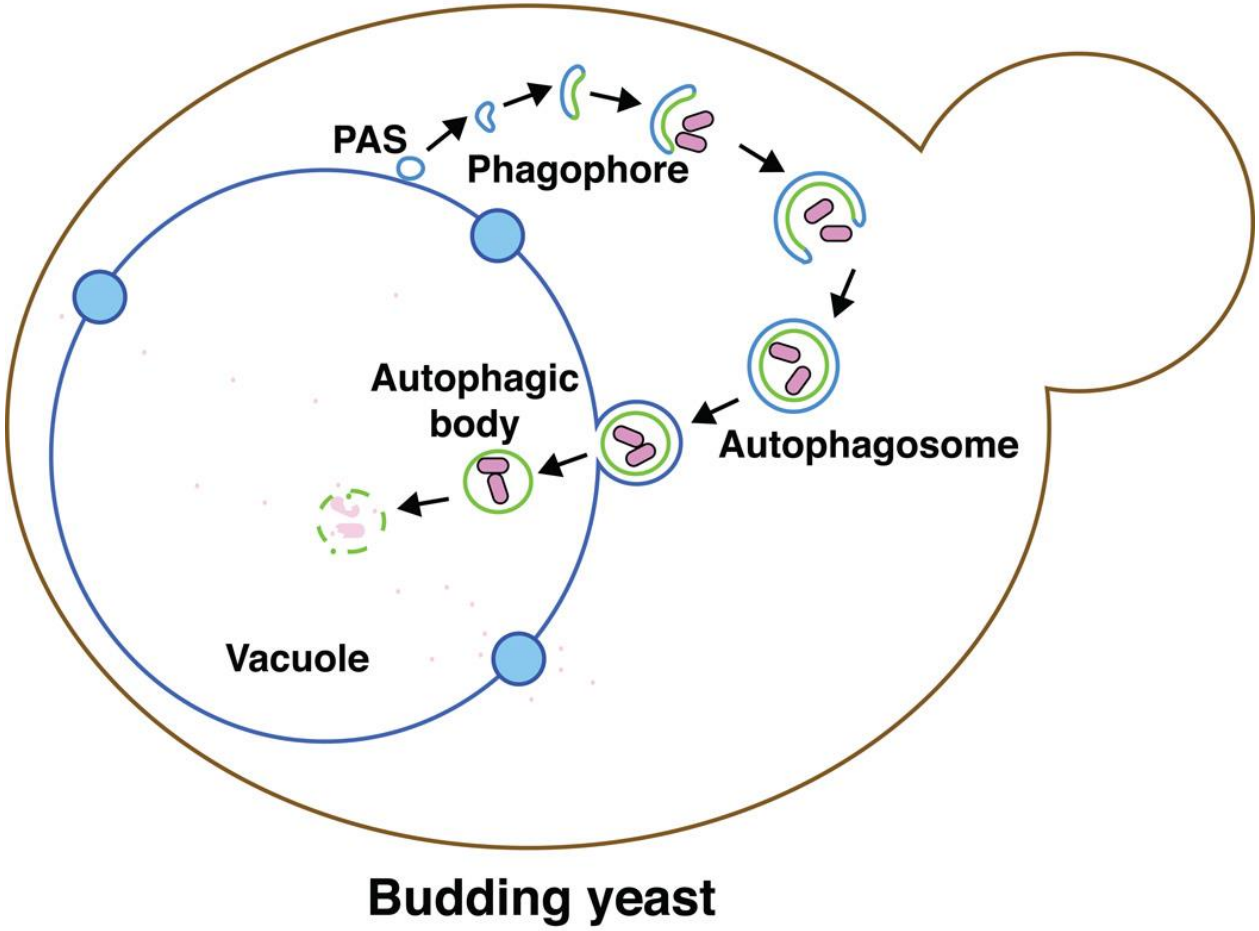
Characteristic	Macroautophagy	Microautophagy	Chaperone-mediated autophagy
Activation	Stress	Constitutive	Stress
Species			
Mammals	Yes	Yes	Yes
Nonmammals	Yes	Yes	?[b]
Mechanism			
Engulfment	Yes	Yes	No
Sequestering membrane	Nonlysosomal	Lysosomal	–
Receptor mediated	No	No	Yes
Requirements[c]			
ATP	Yes	Yes	Yes
GTP and GTPases	Yes	Yes	No
Cytoskeleton	Yes	No	?[b]
Chaperones	?[b]	?[b]	Yes
Acidic vacuolar pH	Yes	Yes	No
Membrane potential	?[b]	Yes	Yes
PI3 kinases	Yes	?[b]	No
Substrates			
Organelles	All types	All types	No
Soluble cytosolic proteins	All types	All types	KFERQ[d]-tagged
Targeting signals	Ubiquitination?[e] Removal of glucose?[e]	?[b]	KFERQ[d]-like motif
Unfolding	No	No	Yes
Selectivity	Some forms[f]	Some forms[f]	Always

Cuervo AM, TRENDS in Cell Biology, 2004: 14(2): 70-77

Selective autophagic pathways

- Mitophagy (mitochondria degradation)
- Pexophagy (peroxisomes degradation)
- Ribophagy (ribosomes degradation)
- ERphagy (endoplasmic reticulum degradation)
- Lipophagy (lipid droplets degradation)
- Aggrephagy (protein aggregates degradation)
- Xenophagy (invasive microbes degradation)

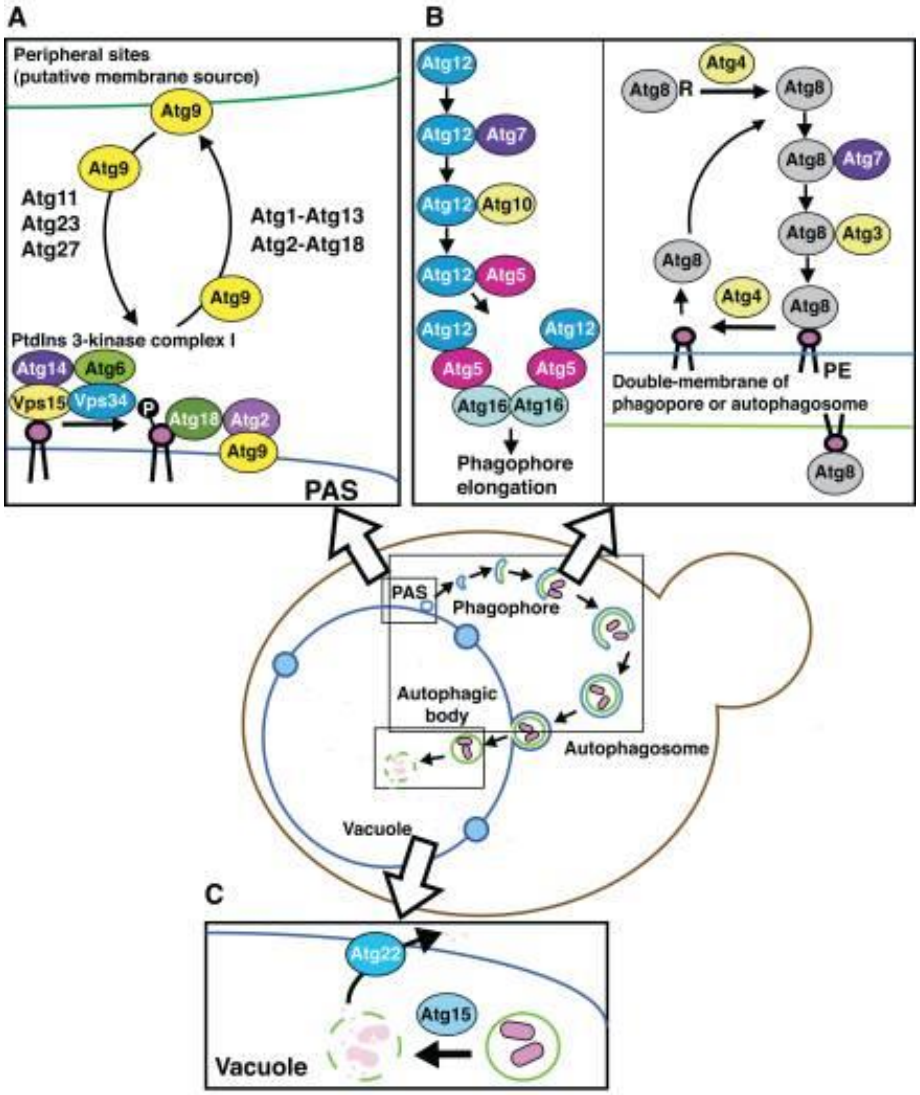
Autophagy in yeast



Inoue Y, Klionsky J., Semin Cell Dev Biol. 2010 Sep; 21(7): 664–670.

The molecular mechanisms of autophagy

-more than 30 autophagy-related (*ATG*) genes have been identified in yeast.



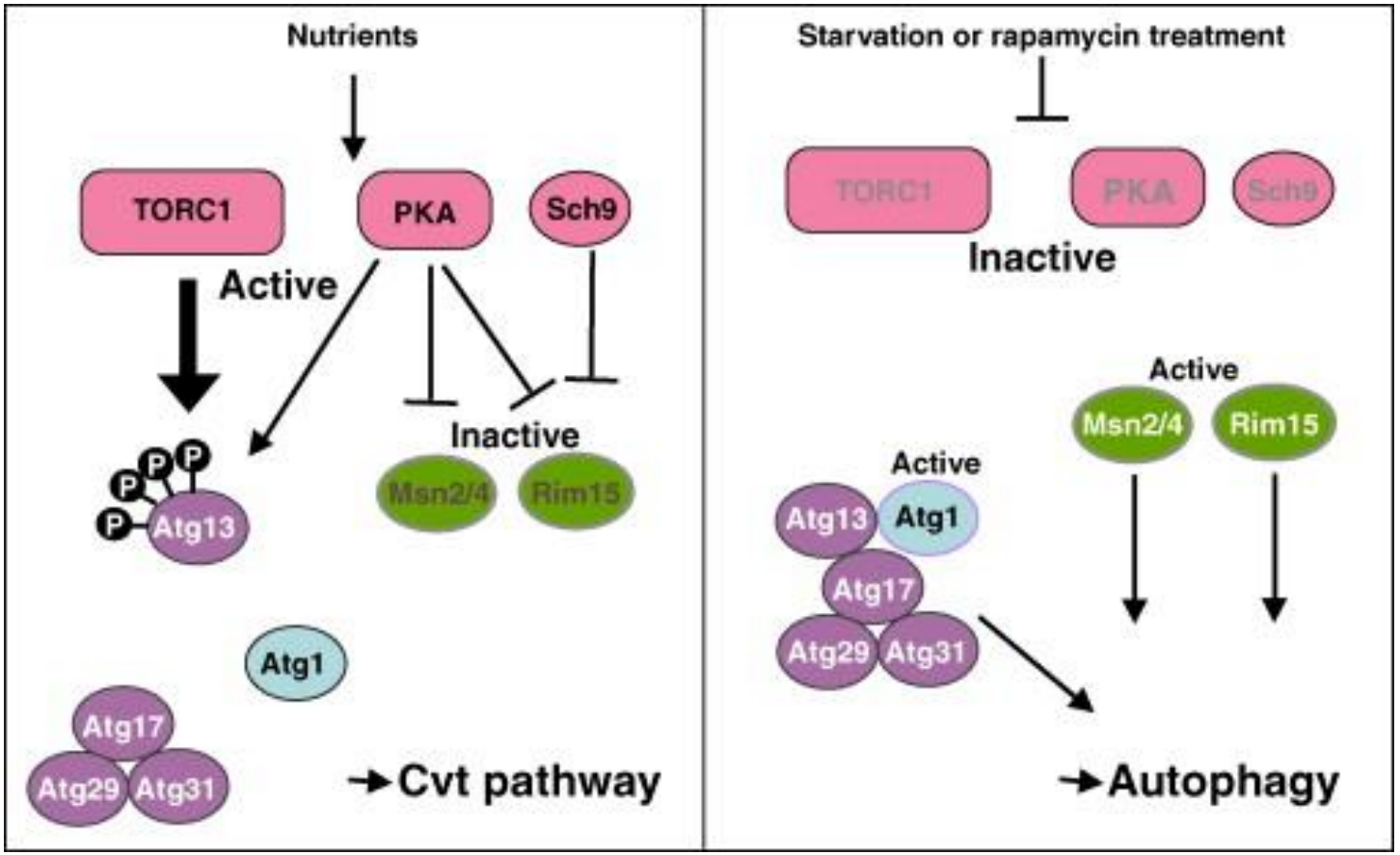
Inoue Y, Klionsky DJ., Semin Cell Dev Biol. 2010 Sep; 21(7): 664–670.

Orthologs of Yeast Autophagy-Related Genes in Mammals

	Yeast	Mammals	Characteristics and functions
Atg1/ULK complex	Atg1	ULK1/2	Ser/Thr protein kinase; phosphorylated by M/TORC1; recruitment of Atg proteins to the PAS
	Atg13	ATG13	Regulatory subunit through phosphorylation by M/TORC1 and/or PKA, linker between Atg1 and Atg17
	Atg17	RB1CC1/FIP200 (functional homolog)	Scaffold protein, ternary complex with Atg29 and Atg31. Phosphorylated by ULK1; scaffold for ULK1/2 and ATG13
	Atg29		Ternary complex with Atg17 and Atg31
	Atg31		Ternary complex with Atg17 and Atg29
	Atg11		Scaffold protein in selective autophagy for PAS organization
		C12orf44/Atg101	Component of the complex with ATG13 and RB1CC1
Atg9 and its cycling system	Atg2	ATG2	Interacts with Atg18
	Atg9	ATG9A/B	Transmembrane protein, directs membrane to the phagophore
	Atg18	WIPI1/2	PtdIns3P-binding protein
PtdIns3K complex	Vps34	PIK3C3/VPS34	PtdIns 3-kinase
	Vps15	PIK3R4/VPS15	Ser/Thr protein kinase
	Vps30/Atg6	BECN1	Component of PtdIns3K complex I and II
	Atg14	ATG14	Component of PtdIns3K complex I
Atg8 Ubl conjugation system	Atg8	LC3A/B/C, GABARAP, GABARAPL1/2	Ubl, conjugated to PE
	Atg7	ATG7	E1-like enzyme
	Atg3	ATG3	E2-like enzyme
	Atg4	ATG4A/B/C/D	Deconjugating enzyme, cysteine proteinase
Atg12 Ubl conjugation system	Atg12	ATG12	Ubl
	Atg7	ATG7	E1-like enzyme
	Atg10	ATG10	E2-like enzyme
	Atg16	ATG16L1	Interacts with Atg5 and Atg12
	Atg5	ATG5	Conjugated by Atg12

Feng Y. et al., *Cell Res*. 2014 Jan; 24(1): 24–41.

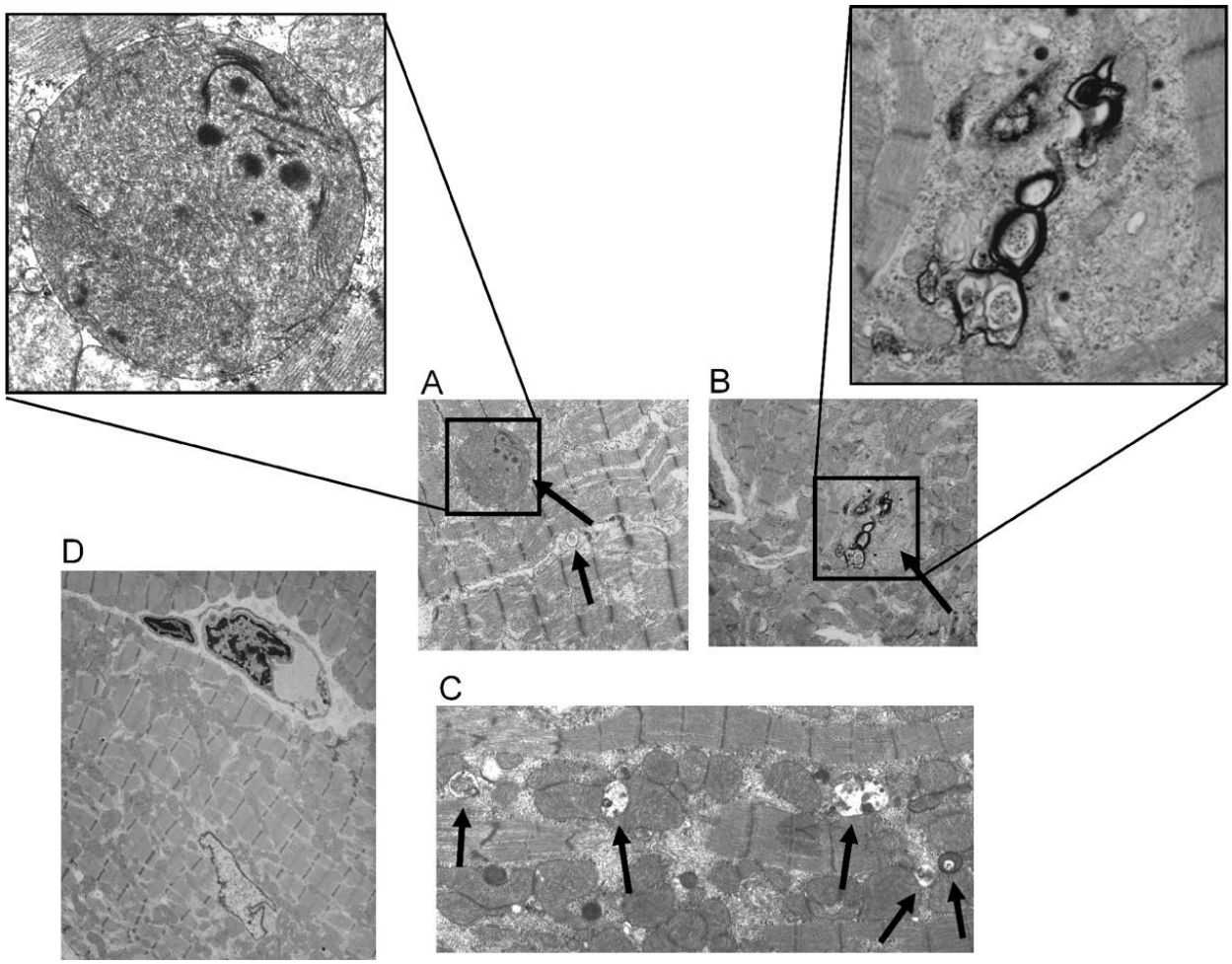
Induction of autophagy in response to starvation



Inoue Y, Klionsky DJ., Semin Cell Dev Biol. 2010 Sep; 21(7): 664–670.

Methods for monitoring autophagy

- **Morphological method**
  - Electron microscopy
- **Biochemical methods**
  - Bulk degradation of long-lived proteins
  - Delivery of cytoplasmic components to lysosome
- **Specific markers for autophagy**
  - GFP-LC3 localization
  - Conversion of LC3-I to LC3-II
  - LysoTracker



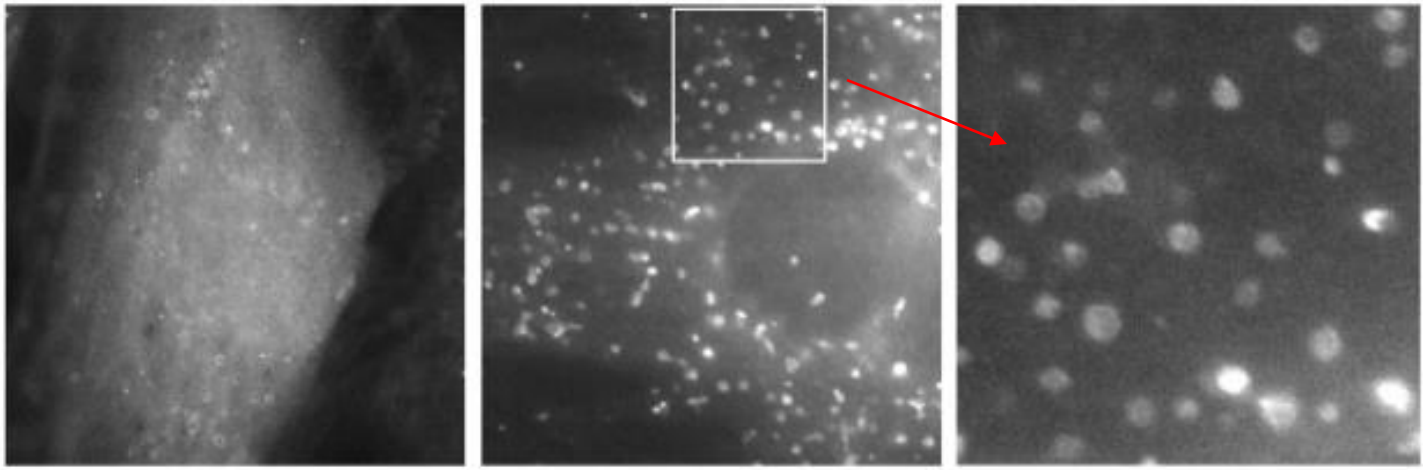
Electron micrographs of different types of autophagic vacuoles (AVs) observed in chronically ischemic region (*A*–*C*) and non-ischemic region (*D*). (*A*) AVs containing remnants of mitochondria are demonstrated. (*B*) Double-membrane AVs containing recognizable cytoplasmic contents are displayed. (*C*) AVs containing multivesicular bodies surrounded by a sequestering membrane are demonstrated. (*D*) These AVs were not observed in the six-episode NI. Arrows indicate AVs. (Magnifications: x1,400–2,000, *A*–*D*; x5,000, *A* and *B Insets*.)

L. Yan et al. ***PNAS****, 2005; 102 (39):13807-12*

- The rat microtubule-associated protein LC3, a mammalian homolog of yeast Atg8 (essential for yeast autophagy), was identified as the first mammalian protein localized in the autophagosome membrane and therefore has been suggested as an excellent marker for the detection of autophagosomes.

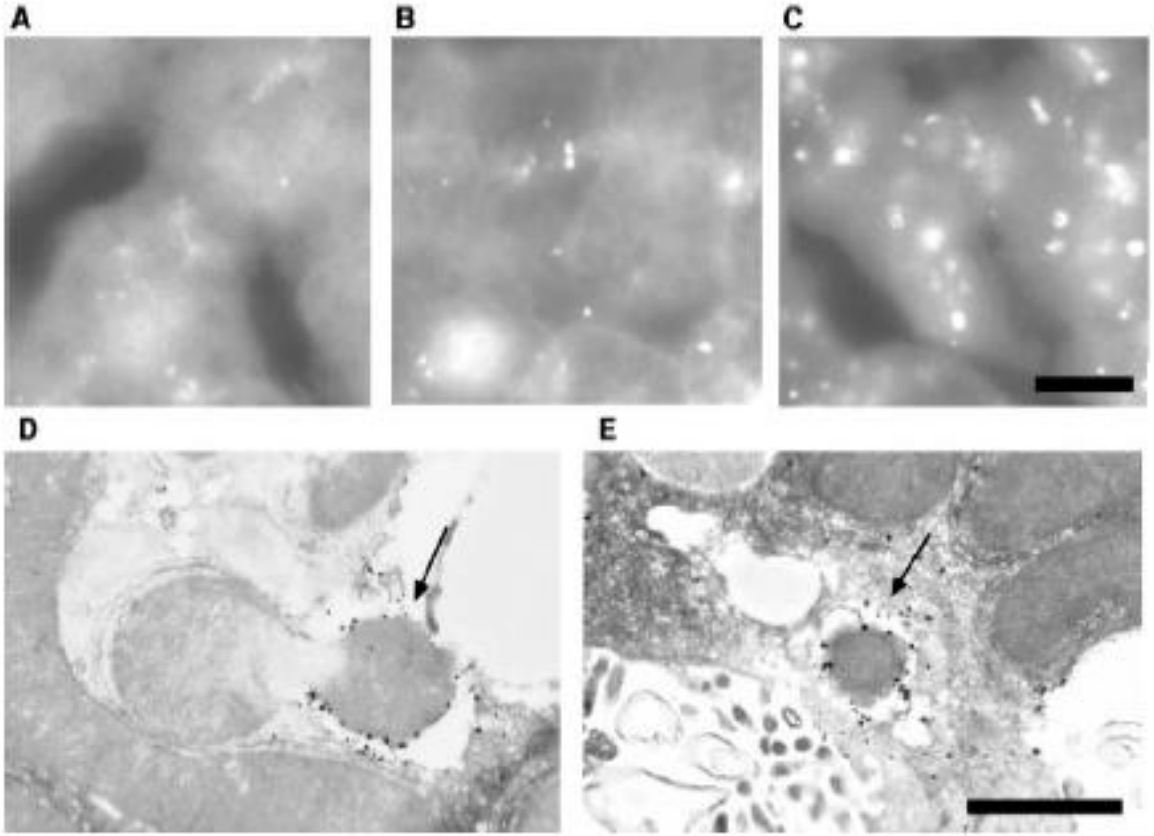
- LC3 localizations can be examined by generating chimeric proteins fused with green fluorescent protein (GFP)

Before stavation After 2h starvation



Embryonic fibroblasts expressing GFP-LC3 before and after 2h starvation

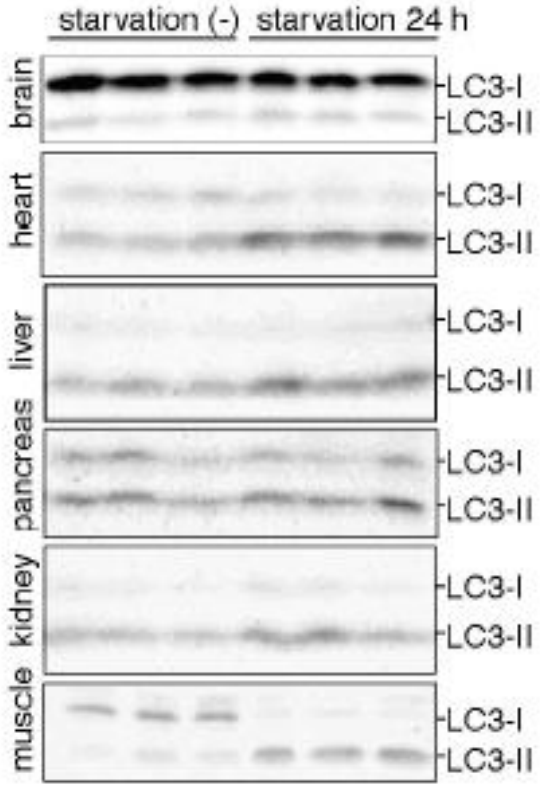
N. Mizushima. Int. J of Biochem cell biol. 2004;36(12):2491-502 .



Liver autophagy in response to starvation. (A–C) Liver samples were prepared from GFP-LC3 transgenic mice before (A) or after 24-h starvation (B and C) and fixed with 4% paraformaldehyde. Cryosections were analyzed by fluorescence microscopy. Panel C demonstrates the most highly induced case. Bar, 10 μm. (D and E) Localization of GFP-LC3 in hepatocytes from 24-h starved GFP-LC3 mice. Liver samples were prepared from GFP-LC3 transgenic mice after 24-h starvation and fixed with 4% paraformaldehyde. The localization of GFP-LC3 was examined by silver-enhanced immunogold electron microscopy using an anti-GFP antibody. A cup-shaped isolation membrane (arrow in D) and double-membrane autophagosome (arrow in E) were shown. Bar, 1 μm.

N. Mizushima et al. Mol Biol Cell. 2004;15(3):1101-11.

The ratio of LC3-II/LC3-I is correlated with the extent of autophagosome formation



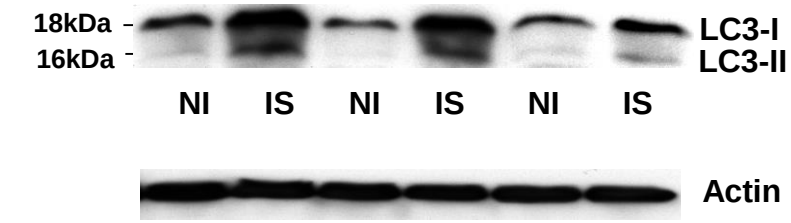
cytosolic form

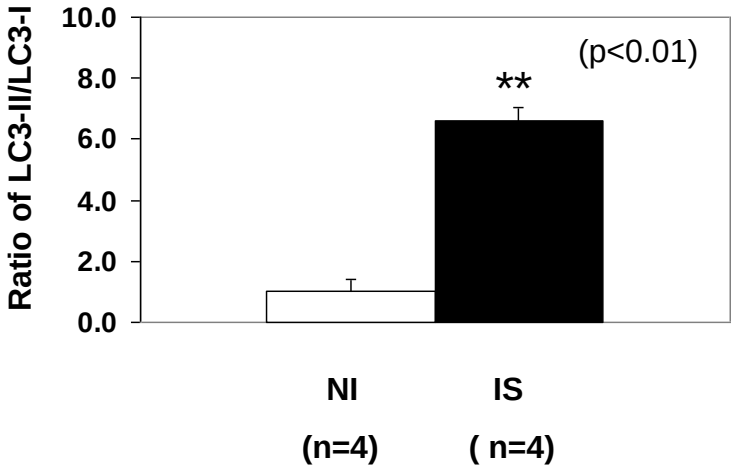
processed form associated tightly with the autophagosomal membrane

Conversion of LC3-I to LC3-II in mouse tissues during 24-h starvation. Tissue homogenates were prepared from three fed mice and three 24-h starved mice and subjected to immunoblot analysis using anti-LC3 antibody. The positions of LC3-I and LC3-II are indicated.

N. Mizushima et al. Mol Biol Cell. 2004;15(3):1101-11.

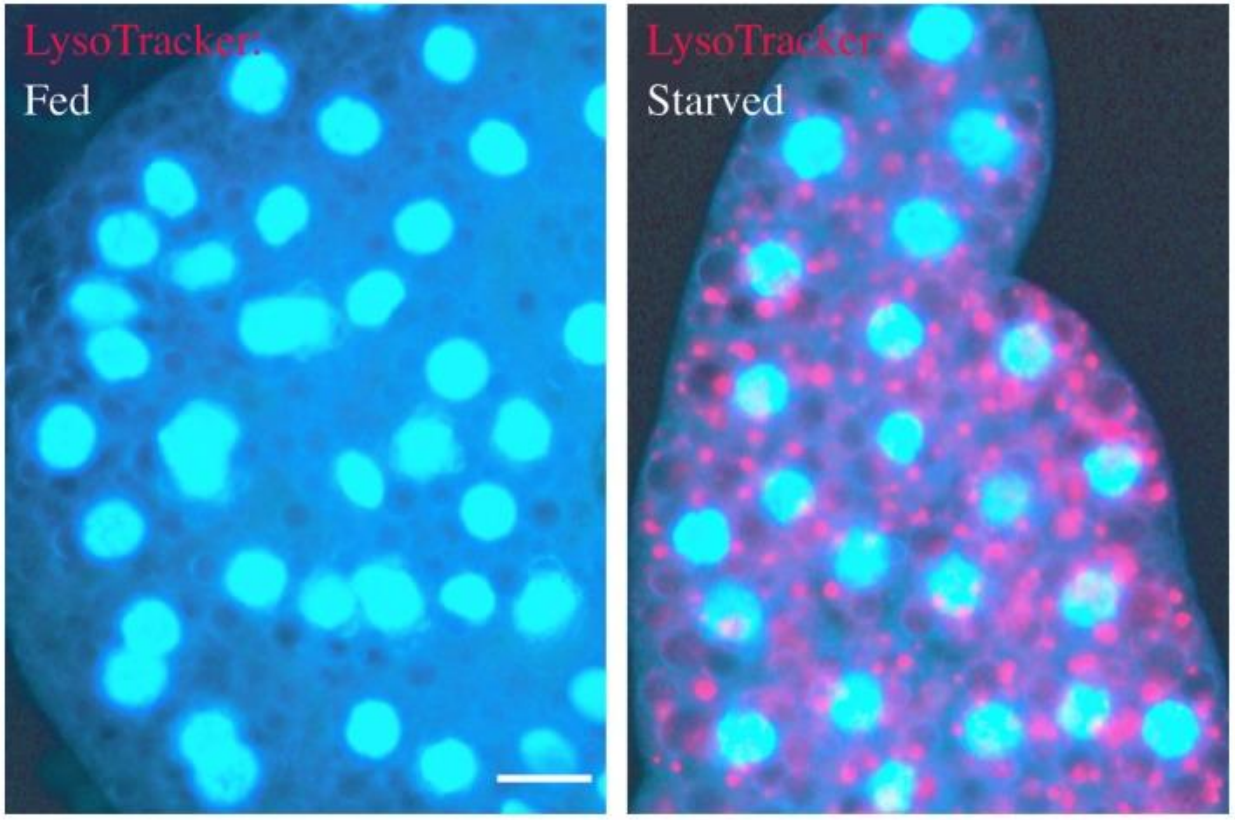
LC3-II/LC3-I ratio was significantly increased in the chronically ischemic myocardium





L. Yan et al. PNAS, 2005; 102 (39):13807-12

LysoTracker



Guidelines for the use and interpretation of assays for monitoring autophagy, *Autophagy.* 2012 Apr 1; 8(4): 445–544.

Pathogenomonic features of autophagy

- Increased autophagic vacuole and autolysosomes.
- Increased lysosomal activity.
- Increased an expression of genes and proteins known to be involved in autophagy.

  cathepsins - *lysosomal proteins*

  Hsc70 - *a key protein marker for chaperone-mediated autophagy*

  Beclin1 – *a mammalian autophagy gene*

  LC3-II – *a maker for autophagosomes*

The Regulation of Autophagy

Induction

- Starvation
- Growth factor deprivation
- Energy depletion (AMPK)
- ER stress (PERK, ATF1)
- p53 (nuclear)
- Sirtuins
- Immune signals

  PAMPs: bacterial PGN, MDP, endotoxin (LPS), Pam_3Cys_4

  DAMPs: ATP, ROS, misfolded proteins

  PRRs: PRGP-LE, TLRs (3, 4, 7, others), NOD-like receptors (NOD1, NOD2), PKR

  Other: TLR adaptors (MyD88, TRIF), cytokines (IFN-γ, TNF-α), DAP kinase, JNK, IKK, NF-κB, immunity-related GTPases, HMGB1, vitamin D–cathelicidin
- Cell-surface receptors (Fcγ, CD46, CD40)
- Infection (viruses, intracellular bacteria)
- Bacterial toxins
- HIV envelope fusion activity

Suppression

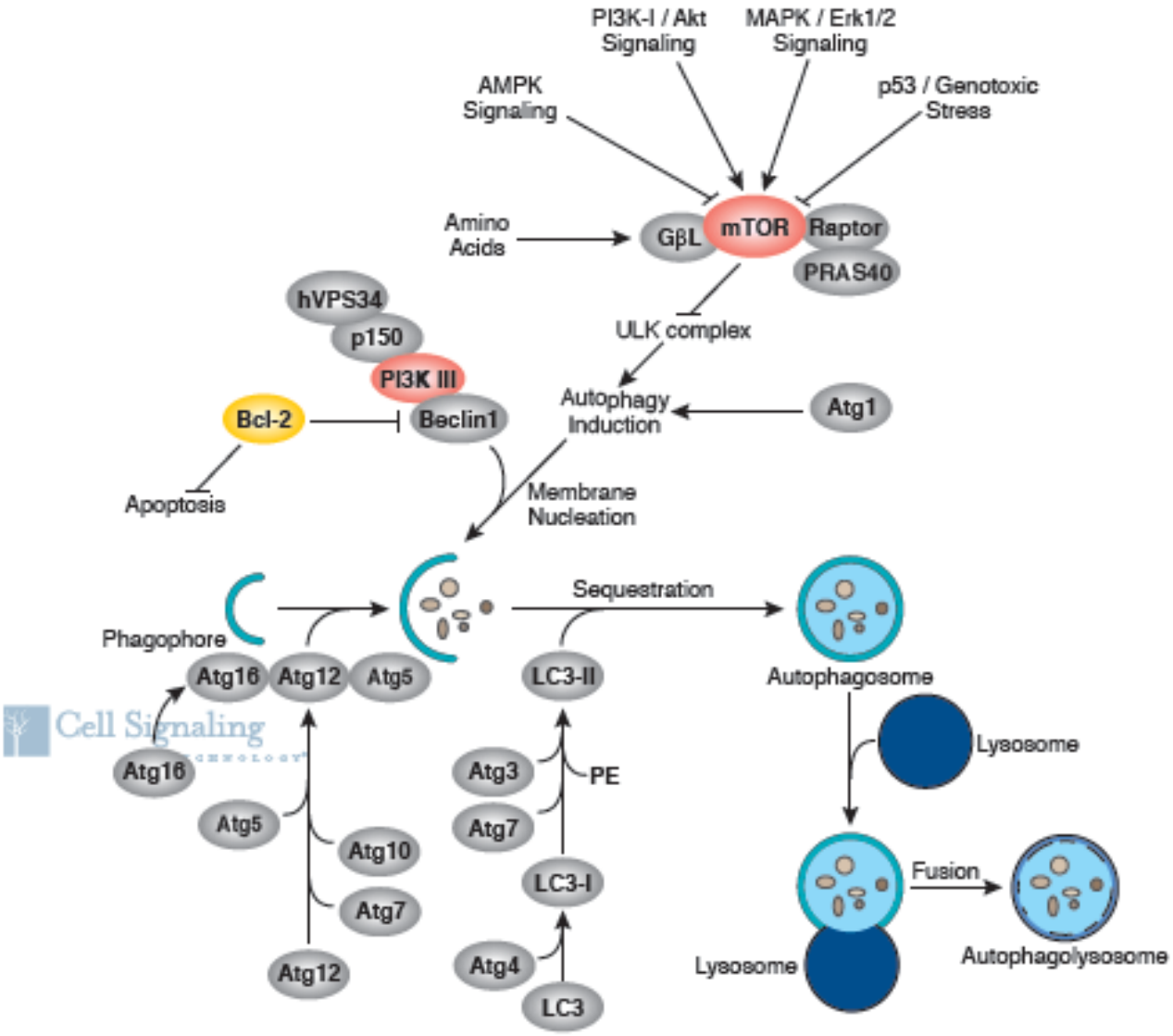
- Nutrient abundance
- Growth factor/insulin–AKT–TOR signalling
- p53 (cytoplasmic)
- Immune signals

  Cytokines (IL-4, IL-13, CLCF1, LIF, IGF1, FGF2, SDF1/CXCL12), STAT3, NF-κB, BCL2
- Microbes and microbial virulence factors

  mTOR activators (CMV, EBV, KSHV, HBV, HIV envelope protein), beclin 1 inhibitors (HSV-1 ICP34.5, viral BCL2s), ATG3 inhibitors (KSHV v-FLICE)

B. Levine, N. Mizushima, HW Virgin. ***Nature.*** 2011 Jan 20;469(7330):323-35

Autophagy signaling



The role of autophagy

- **Generate amino acids to synthesize new proteins when nutrients are scarce.**

  - starvation (serum or amino acids withdrawal) or growth factor deprivation

  - protect early postnatal life during the transition from the trans-placental nutrient supply to milk supply (early neonatal starvation period) *Kuma A, et al.* ***Nature****, 2004; 432:1032-36*

- **Involved in remodeling during development and differentiation**

  - dauer formation and life-span extension in C. elegans *Melendez A, et al.* ***Science****, 2003;301:1387-91*

  - regulatory of self-eating of the fat during the larval period in Drosophila *RC. Scott, et al.* ***Dev Cell.*** *2004;7(2):167-78.*

- **Remove damaged organelles and molecules**

  - regulate lifespan *VD Longo et al..* ***Science****, 2003;299:1342,* *A. Terman et al. Exp. Gerontol. 2004; 39:701*

  - prevent accumulation of misfolded and aggregated proteins in Parkinson's, Huntington's and Alzheimer's disease *Shintani, T & Klionsky DJ.* ***Science****, 2004;306:990-995*

  - protect against apoptosis in chronic ischemia *L. Yan et al.* ***PNAS****, 2005; 102 (39):13807-12*

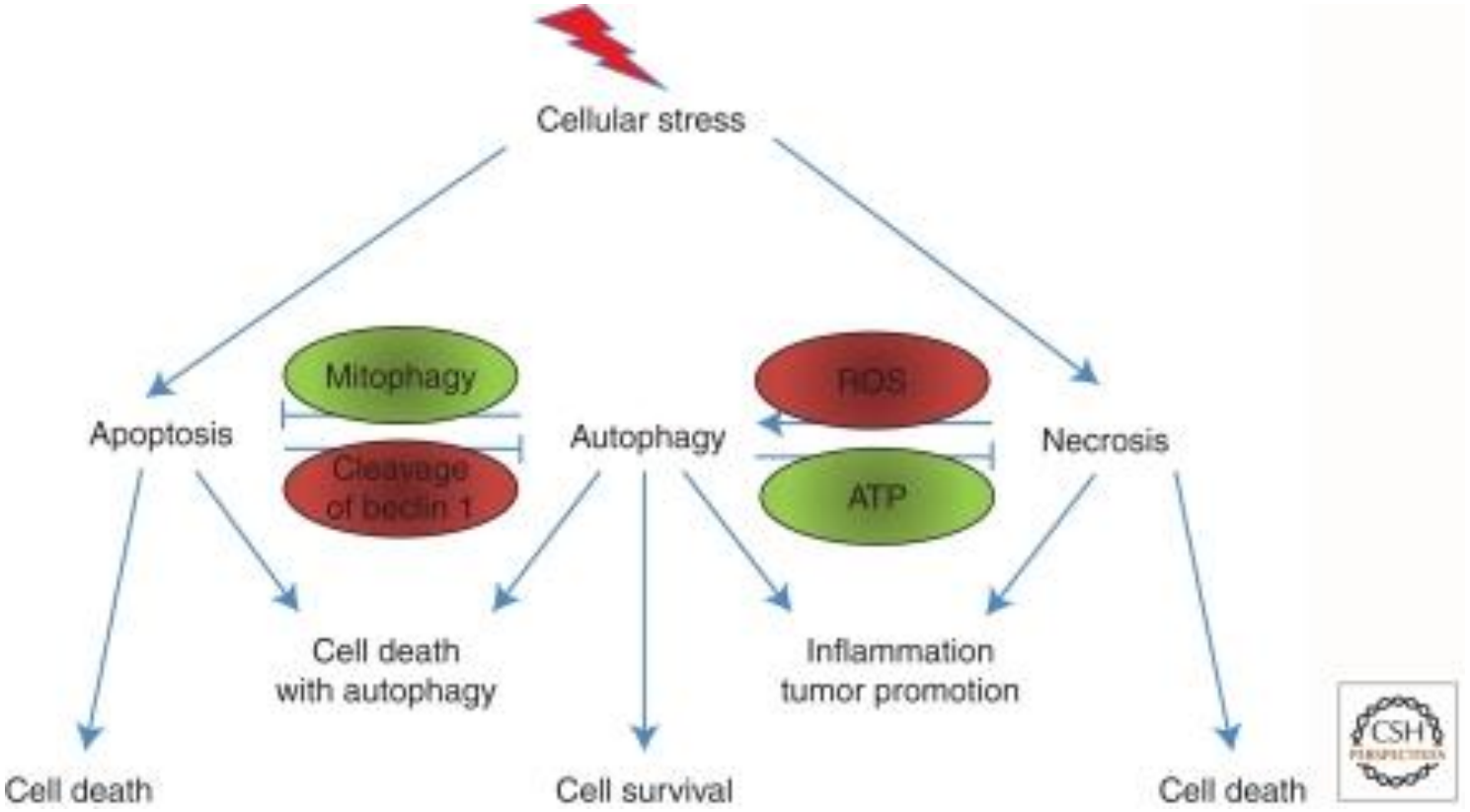
  - tumor suppression *Y. Kondo et al. Nature Reviews Cancer* ***9****, 726-734 (2005)*

Autophagy: a survival or death pathway?

- Evidence as a death pathway
  - Type II programmed cell death
  - Presence of autophagic vesicles in dying cells
- Evidence as a survival pathway
  - An adaptive response to maintain continual cell survival under stress conditions
- Act as both protector and killer of the cell

Our viewpoint: The major role of autophagy is to prolong the life of a cell against environmental stresses. Clearly, the primary function of autophagy is to degrade nonessential and dysfunctional organelles and proteins to produce amino acids to be used during functional recovery and help the continual survival of the cell when nutrients are scarce. Thus, autophagy acts to rescue in the early phase when cells are threatened from stress. However, when the insult is overwhelming or sustained, it can no longer reverse cell disintegration and imposes on cells “point of no return”, which develops into a type II programmed cell death.

Autophagy and the control of cell death



Li Yen Mah, Kevin M. Rya Cold Spring Harb Perspect Biol. 2012 January; 4(1): a008821.

Comparison of two types of programmed cell death

	Type I apoptotic	*Type II autophagic*
Nucleus	Chromatin condensation Pyknosis of nucleus DNA laddering and nuclear fragmentation	Partial chromatin condensation Sometimes pyknosis of nucleus Nucleus intact until late stages No DNA laddering
Cytoplasm	Cytoplasmic condensation Ribosome loss from RER Fragmentation to apoptotic bodies Lysosomal protease release to cytosol may be involved Mitochondrial permeability transition is often involved Caspases are active	Increased autophagic vesicle number Increased autolysosome number Increased lysosomal activity Enlarged Golgi, sometimes dilatation of ER Mitochondrial permeability transition may be involved Caspase-independent
Cell membrane	Blebbing	Blebbing
Corps clearence	Heterophagy by other cells	Late and occasional heterophagy by other cells
Detection methods	Electron microscopy Nuclear/cellular fragmenation detection Caspase activation tests Caspase substrate cleavage tests DNA laddering detection TUNEL staining Increase in sub G1 cell population assessed by FACS analysis Annexin V staining	Electron microscopy Test of increased long-lived protein degradation Tests of increased lysosomal activity (MDC, acridine orange or lysotracker staining, etc) Test of increased cytoplasmic sequestration (LDH or sucrose sequestration tests) Detection of LC3 recruitment to autophagic membranes (protein band shift or change in intracellular localization)

D. Gozuacik and A. Kimchi Oncogene. 2004;23(16):2891-906

Autophagy in health and disease

- Shintani, T & Klionsky DJ. " Autophagy in Health and Disease: A Double-Edged Sword" ***Science****,* 2004;306:990-995
- Cuervo AM, "Autophagy: in sickness and in health" ***TRENDS in Cell Biology***, 2004: 14(2): 70-77.
- B. Levine and J. Yuan, "Autophagy in cell dealth: an inncent convict" ***J. Clin. Invest****.* 2005; 115:2679-2688.
- Morselli E, Galluzzi L, Kepp O, Vicencio JM, Criollo A, Maiuri MC, Kroemer G. "Anti- and pro-tumor functions of autophagy" ***Biochim Biophys Acta.*** 2009 Sep;1793(9):1524-32.
- B. Levine, N. Mizushima, HW Virgin. "Autophagy in immunity and inflammation" ***Nature.*** 2011 Jan 20;469(7330):323-35
- Lavandero S, Chiong M, Rothermel, RA, Hill JA, Autophagy in cardiovascular biology. ***J Clin Invest****.* 2015;125(1):55–64.
- Gatica D, Chiong M, Lavandero S, Klionsky DJ, Molecular Mechanisms of Autophagy in the Cardiovascular System. ***Circ Res*** 2015;116:456-467

  - cancer

  - Neurodegeneration

  - Pathogen Infection and inflammation

  - Muscular Disorder

  - Aging

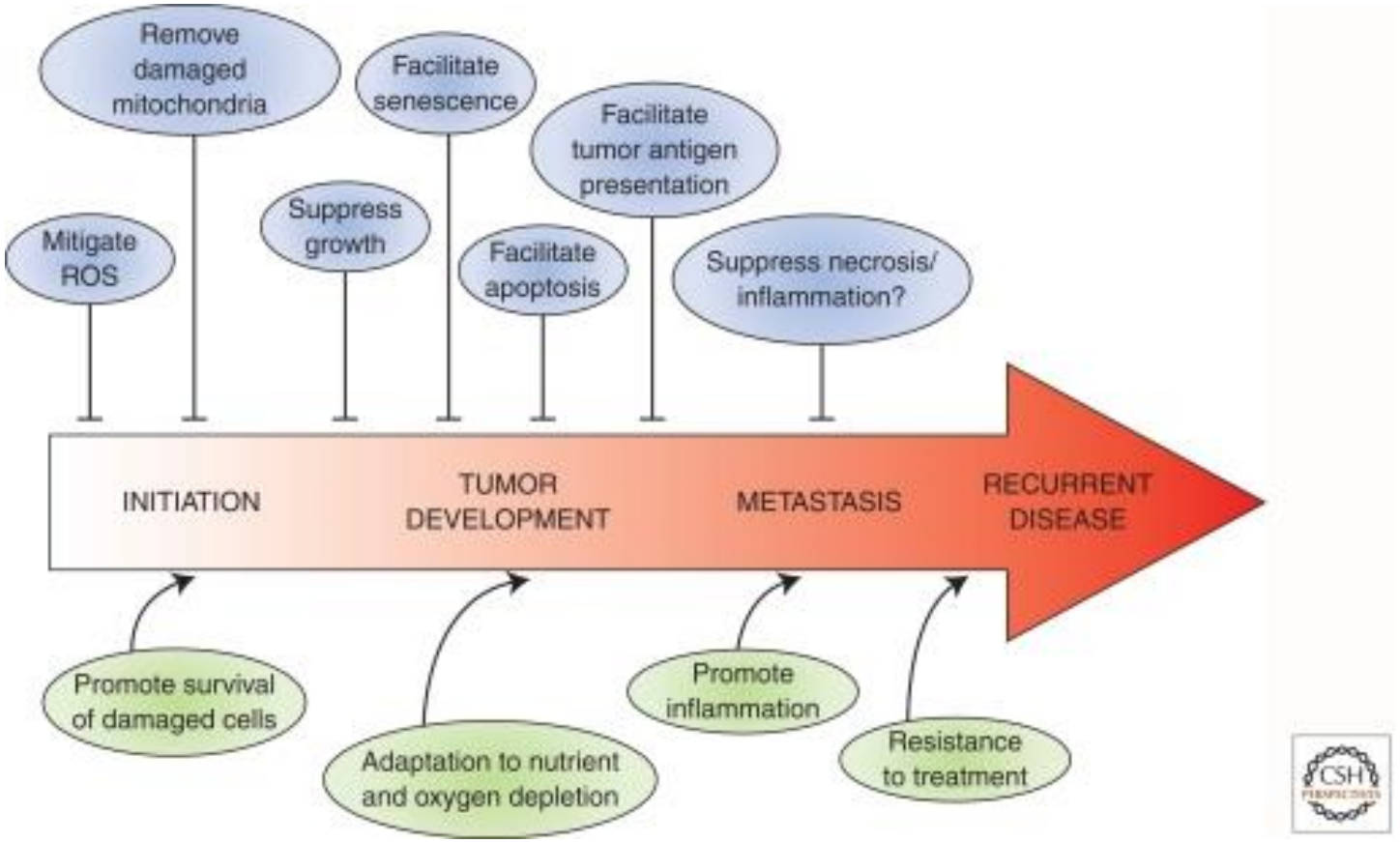
  - Cardiovascular disease

Possible roles of autophagy in health and disease

Disease state	Beneficial effects of autophagy	Negative effects of autophagy
Cancer	Acts as a tumor suppressor; may be involved in type II PCD in cancer cells, could limit cell size or may remove damaged organelles that could generate free radicals and increase mutations.	May allow survival of cancer cells within the nutrient-poor environment of a tumor, could prevent cell death, and may protect against some cancer treatments.
Liver disease	Allows removal of nonfunctional endoplasmic reticulum resulting from accumulation of aggregated α_1-antitrypsin Z protein.	Increased mortality due to excessive mitochondrial autophagy.
Muscular disorder	Increased autophagy may compensate for defects in lysosome function.	Increased autophagy or defects in completing autophagy result in the accumulation of autophagosomes that may impair cell function.
Neurodegeneration	Allows the removal of protein aggregates before they become toxic.	May induce cell death in neurons that accumulate aggregated proteins.
Pathogen infection	Cellular defense against invasion by bacteria and viruses.	Subversion of the autophagic pathway allows pathogens to establish a replicative niche and supplies nutrients for growth.

•*Shintani, T & Klionsky DJ. Science, 2004;306:990-995*

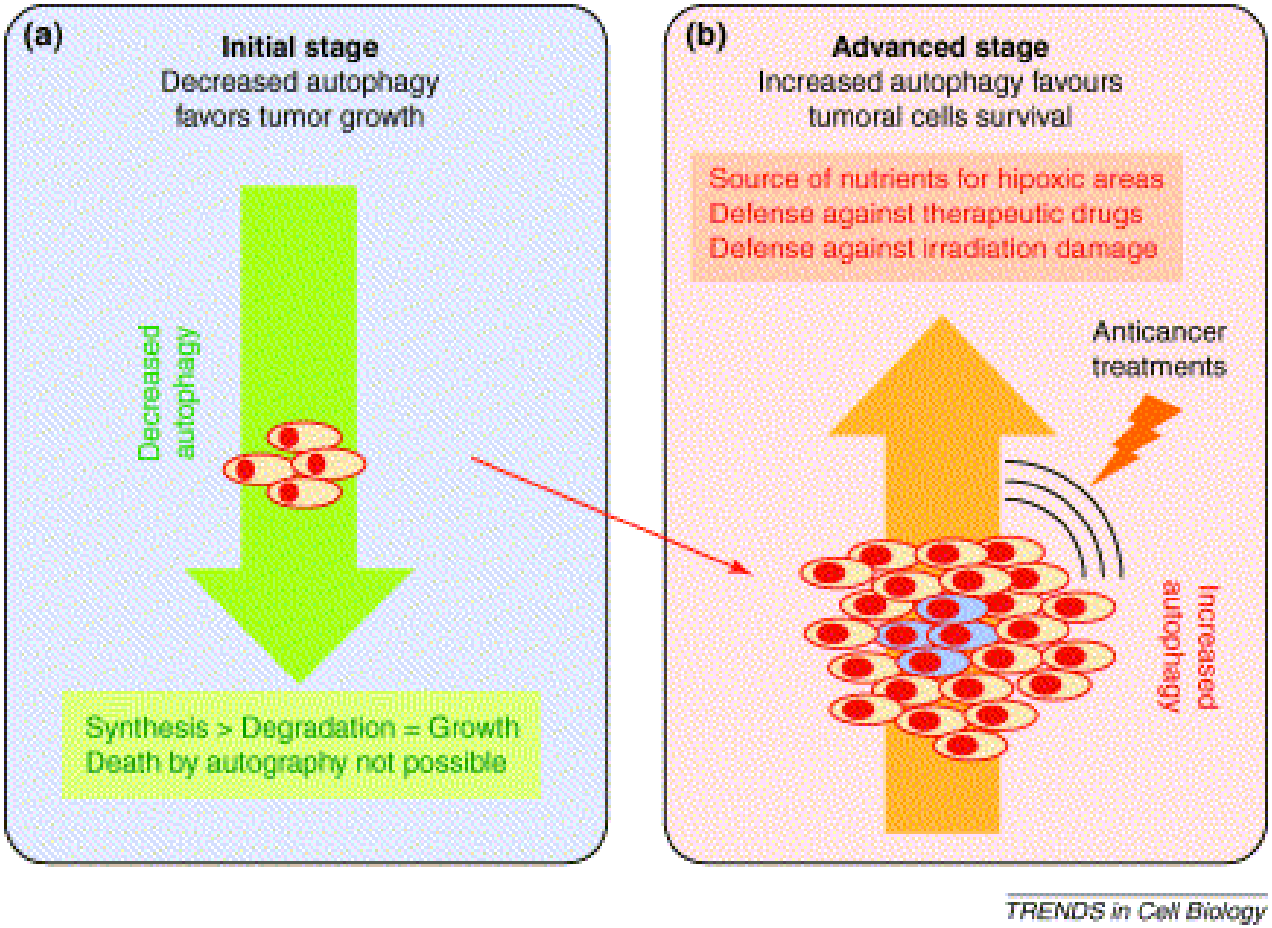
The contrasting roles of autophagy in cancer



Li Yen Mah, Kevin M. Rya Cold Spring Harb Perspect Biol. 2012 January; 4(1): a008821.

Autophagy in Cancer

Hypothetical model for the double role of macroautophagy in cancer



At early stage of tumor development, autophagy functions as a tumor suppressor

At advanced stage of tumor development, autophagy promotes tumor progression. The tumor cells that are located in the central area of the tumor mass undergo autophagy to survive low-oxygen and low-nutrient conditions.

•*Cuervo AM, TRENDS in Cell Biology, 2004: 14(2): 70-77*

Autophagy in Cancer

- Expression of **beclin 1**, a mammalian orthologue of the yeast autophagy-related gene *Atg6,* reduces tumorigenesis through induction of autophagy. Heterozygous mice (*beclin1*$^{+/-}$*)* display a remarkable increase in the incidence of lung cancer, hepatocellular carcinoma and lymphoma.

XH Liang, et al. "Induction of autophagy and inhibition of tumorigenesis by beclin 1, ***Nature****, 1999, 402:672-676*

XP Qu, et al. "Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene"
J. Clin. Invest*., 2003;* ***112****:1809-1820.*

Table 1 | **Therapies that induce autophagy in cancer cells**

Treatment	Proposed target	Cancer type	References
Tamoxifen	Oestrogen receptor	Breast cancer	28,29
Temozolomide	DNA	Malignant glioma	30
γ-Irradiation	DNA	Breast cancer, prostate cancer, colon cancer, malignant glioma	24,31,32
Sodium butyrate and SAHA	HDAC	Cervical cancer that overexpresses BCL-X_L	33
Hyperthermia	Unknown	Malignant glioma	34
Arsenic trioxide	Multiple targets (for example, mitochondria)	Malignant glioma	35,36
Resveratrol	Multiple targets (for example, oestrogen receptor and mitochondria)	Ovarian cancer	37
Soybean B-group triterpenoid saponins	Unknown	Colon cancer	38
Rapamycin	mTOR	Malignant glioma	39

HDAC, histone deacetylase; mTOR, mammalian target of rapamycin; SAHA, suberoylanilide hydroxamic acid.

*Y. Kondo et al. Nature Reviews Cancer 2005; **9**:726-734*

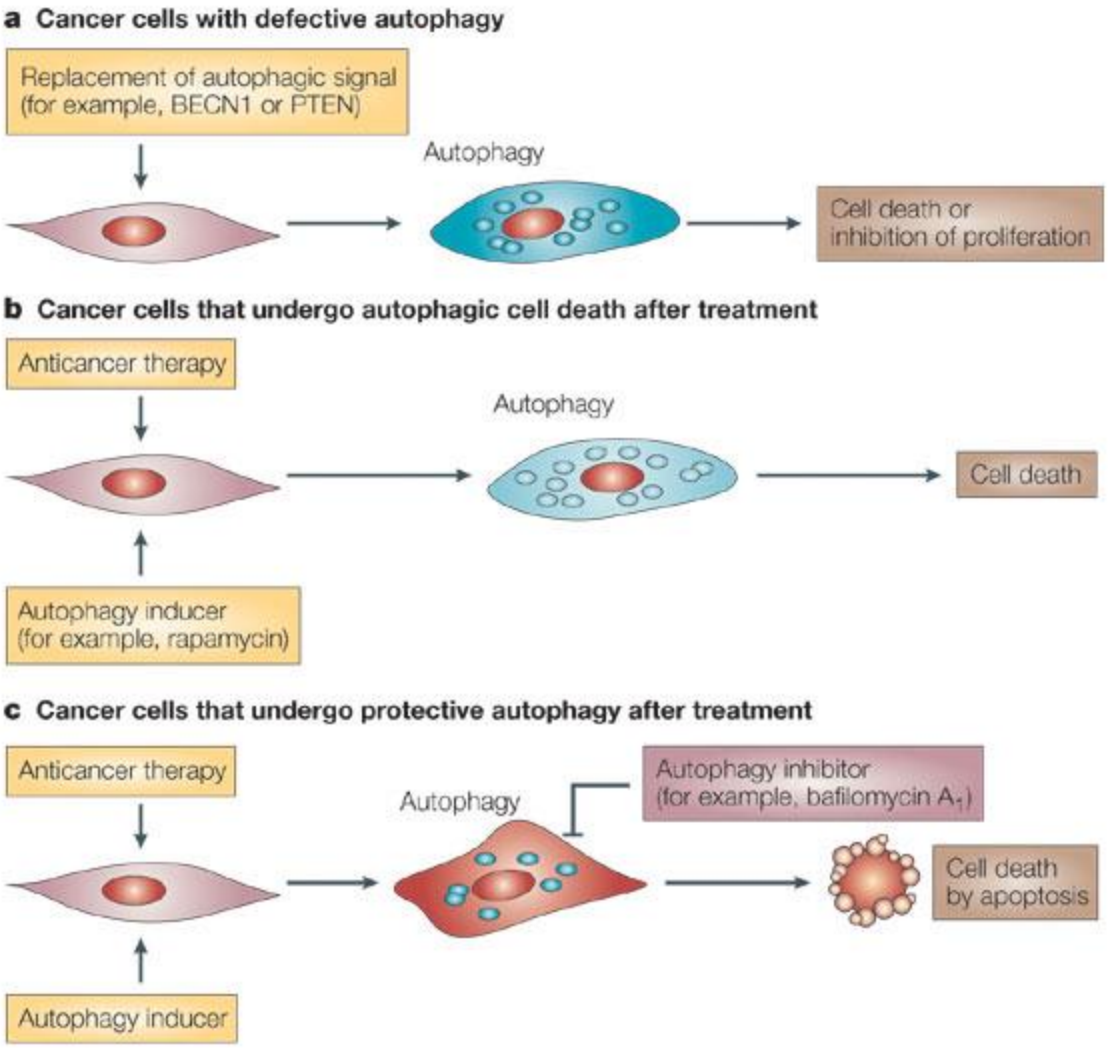
Table 2 | **Inhibitors of autophagy**

Compound	Modification of cellular component	Cancer type	References
3-MA	PI3K inhibitor. Inhibits the formation of pre-autophagosomal structure	Breast cancer, prostate cancer, colon cancer, malignant glioma and cervical cancer	28,30,31,46, 64,67
Bafilomycin A_1	H^+-ATPase inhibitor. Blocks the fusion of the autophagosome and lysosome	Breast cancer, prostate cancer, colon cancer, malignant glioma, cervical cancer	30,31,34, 35,67
HCQ	A lysosomotropic agent. Blocks the fusion of the autophagosome and lysosome	Cervical cancer	67
Monensin	Proton exchange for potassium or sodium. Blocks the fusion of the autophagosome and lysosome	Cervical cancer	67
siRNA against ATG5, BECN1, ATG10, ATG12	Blocks translation of these proteins	Cervical cancer	67

3-MA, 3-methyladenine; HCQ, hydroxychloroquine; PI3K, phosphatidylinositol 3-phosphate kinase; siRNA, small interfering RNA.

*Y. Kondo et al. Nature Reviews Cancer **9**, 726-734 (2005)*

Potential strategies for treating cancer by manipulating the autophagic process



Y. Kondo et al. Nature Reviews Cancer ***9****, 726-734 (2005)*

Summary

- What is autophagy
- Three major autophagy mechanisms
- Methods for monitoring autophagy
- The general roles of autophagy
- Autophagy signaling
- Dual functions of autophagy in health and disease
- The role of autophagy in Cancer
- Potential strategies for treating cancer by manipulating the autophagic process