Lecture 10 Autophagy

I. The mystery of autophagy

II. Discovery of the autophagy machinery

III. Autophagy in health and diseases

Autophagy is an intracellular recycling system

♥**Life is in an equilibrium state between synthesis and degradation of proteins.**

Every 3 months, most proteins in the body will be replaced.

♥**Recycling is essential for life**

Important ability for survival against starvation, it serves as a critical selection factor during evolution

Autophagy and homoestasis

Target protein → 26S proteosome → degradation

Proteins/organelles → lysosomes

Ubiquitin/ proteosome system:

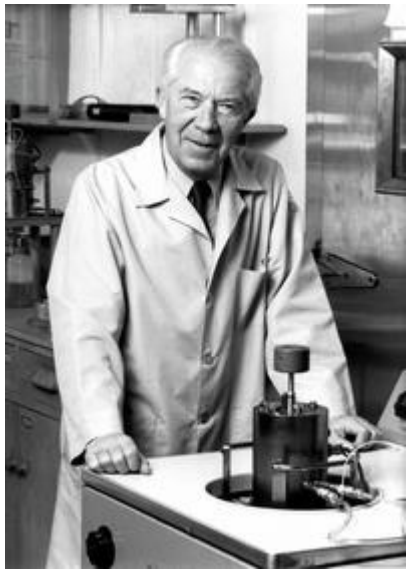
specific target recognition; short-lived proteins

Lysosome/ vacuolar system:

obsolete /damaged organelle, bulk and non-selective; long-lived proteins

Discovery of lysosome

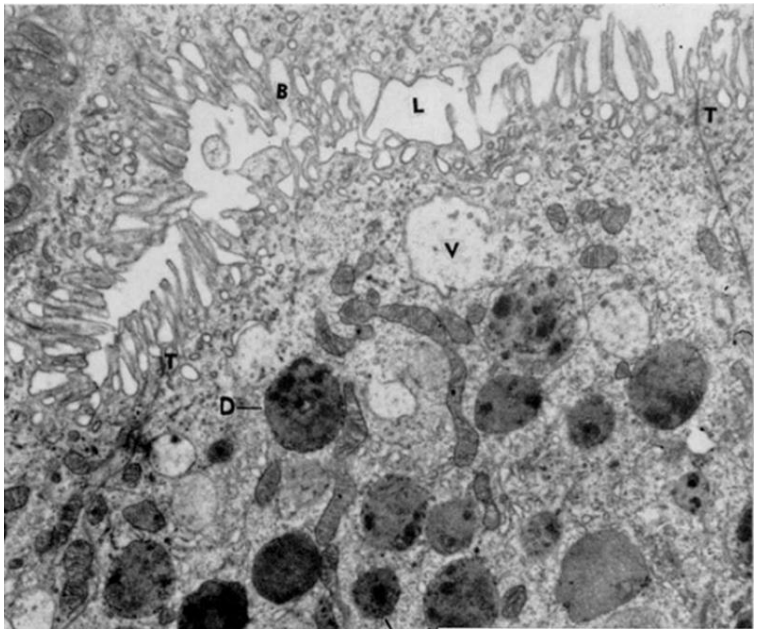
- 1955, by Christian de Duve
- 1962-1963, auto (self) – phagy(eating)
- 1974, Nobel laureate

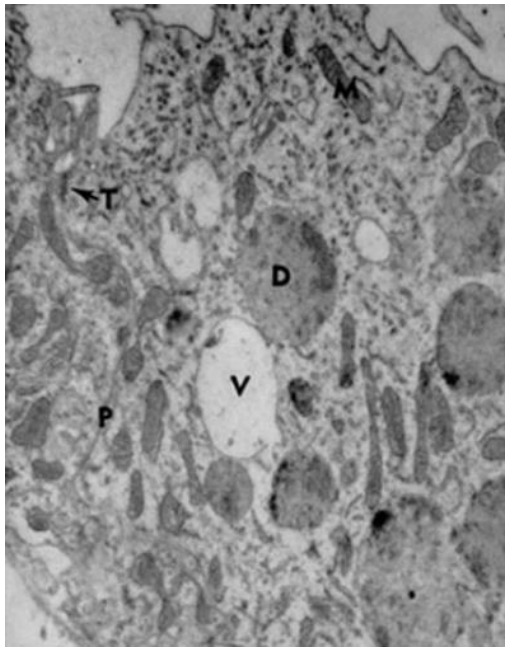


1917-2013

His interest in glucose-6-phosphotase leaded him to study an acid phosphotase, which usually Interfere with his results. When trying to identify the nature of this acid phosphatase, he found Acid phosphatase is latent and particle bound, subsequently he found this particle to be Lysosome. He also discovered peroxisomes. All these discoveries were through differential centrifugation.

Early indication of autophagy in normal kidney development

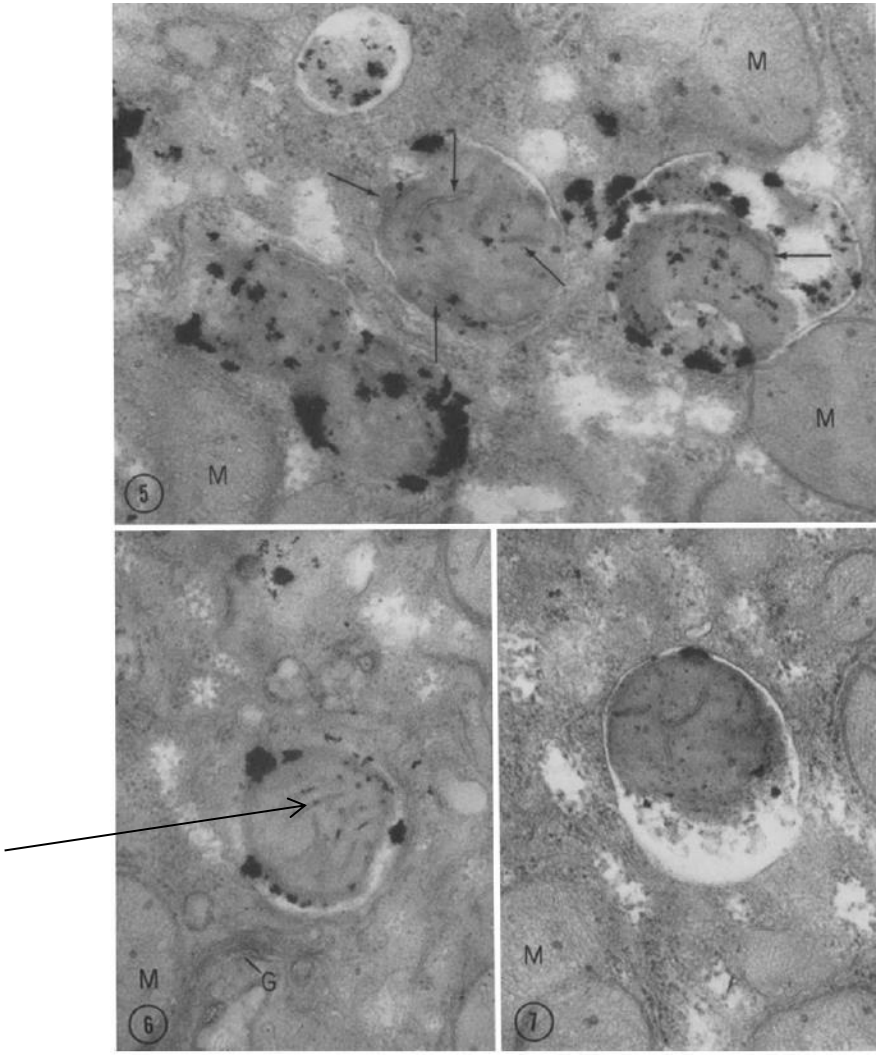




Part of cellular content will be encircled into vacuoles, and then fuse with lysosoms

EM images , 1956

Early hint for autophagy



Acid phosphatase-containing granules occurred during liver degeneration, dissolved mitochondria is visible inside.

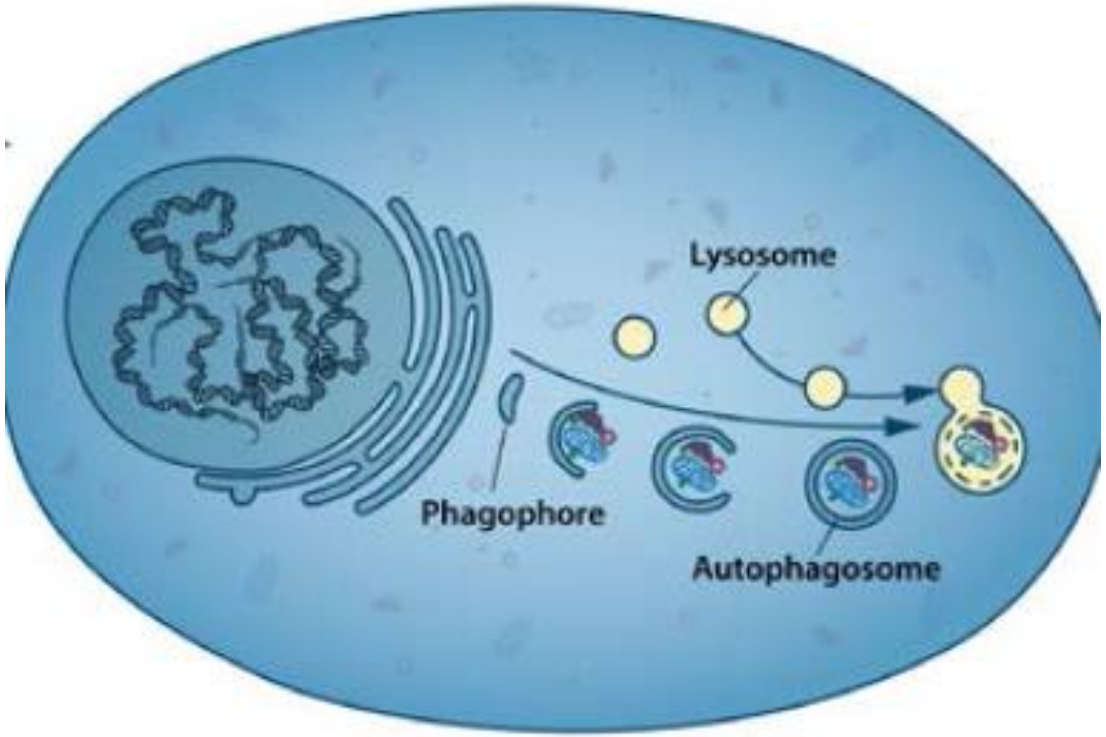
(1962, JCB)

Autophagy concept in 1963

- Autophagy occur at a low basal level in normal condition
- It can be induced by stress such as starvation
- It may have roles in pathogenesis or disease
- It occurs in a wide range of cells including amoeba, tetrahymena, insect, frogs , etc

Autophagy remains a mystery for ~ 30 years…

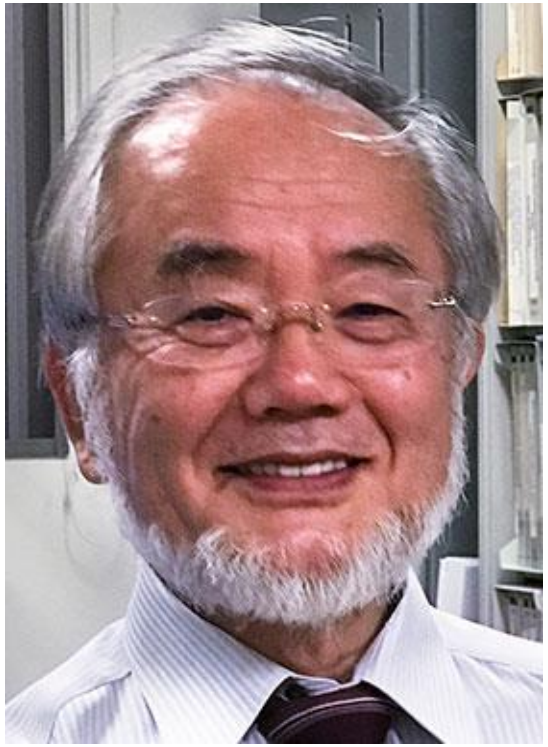
- What they know was:



To study autophagy was difficult at that time, autophagosome is transient, only exist for ~20-30 minutes before fusing with lysosome… until ….

In early 1990's, Yoshinori Ohsumi

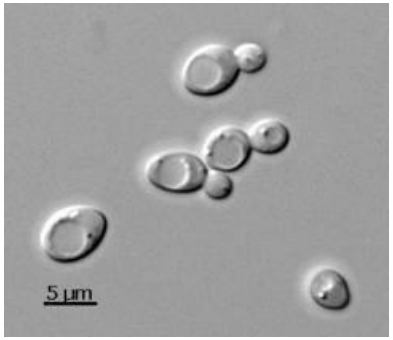
- Made seminar discoveries about autophagy using yeast model system
- He was awarded 2016 Nobel prize in Physiology/Medicine
- He identified multiple key genes involved in autophagy and revealed the molecular mechanism for autophagy initiation, formation, regulation, and link to human diseases, etc.



1945 - Japan

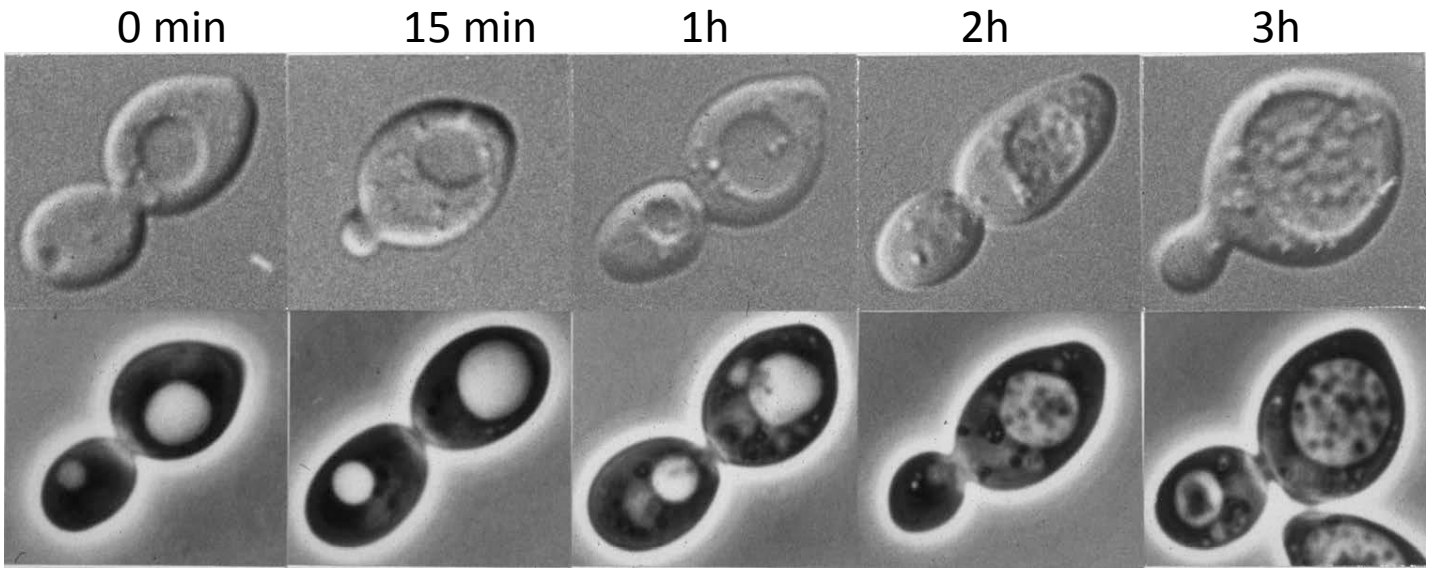
He initially set up an efficient autophagy analytical platform

Regular yeast cells are small



Choose a vacuolar proteinase deficient mutant

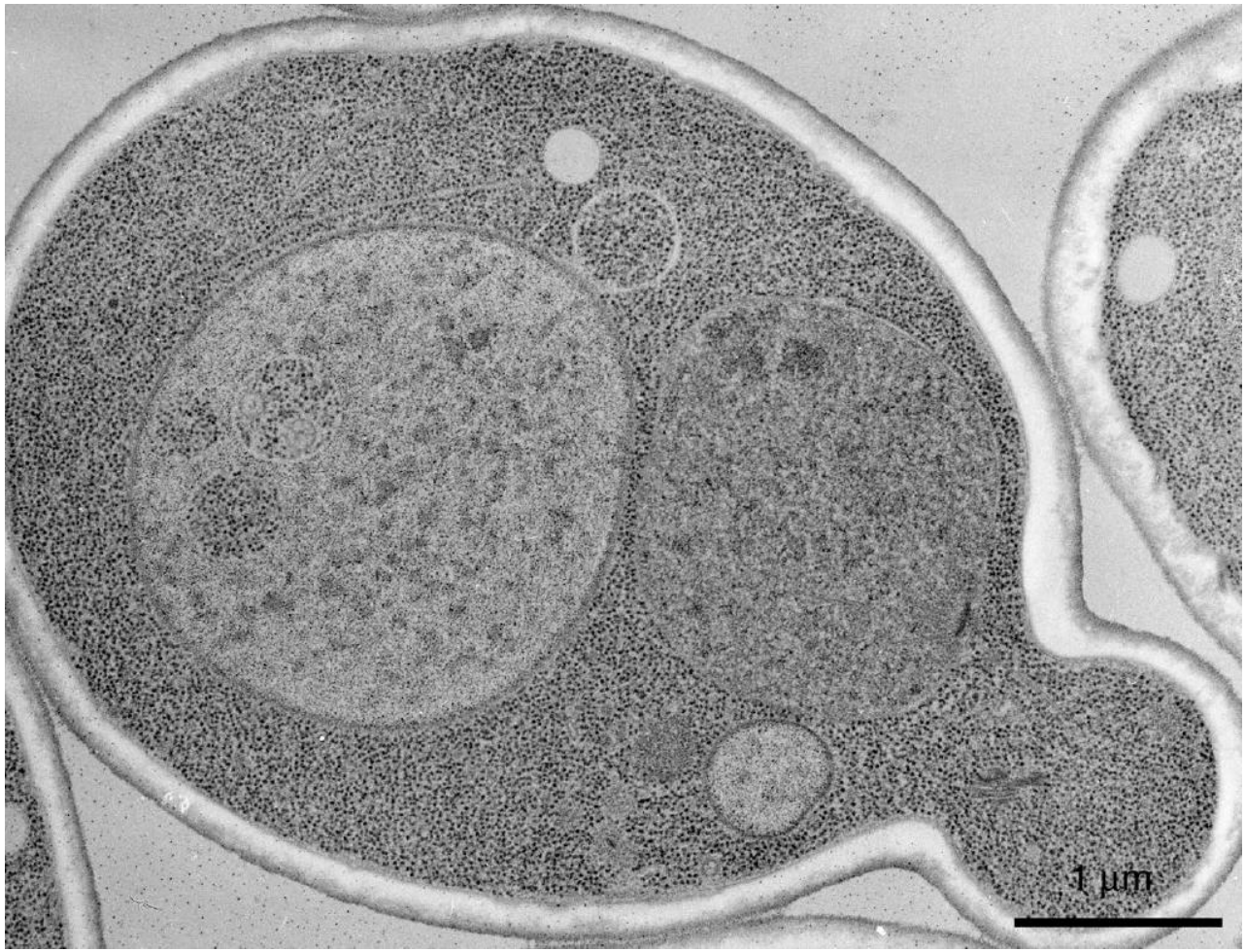
Switch into nitrogen Starvation medium



Large vacuole inside the yeast cell

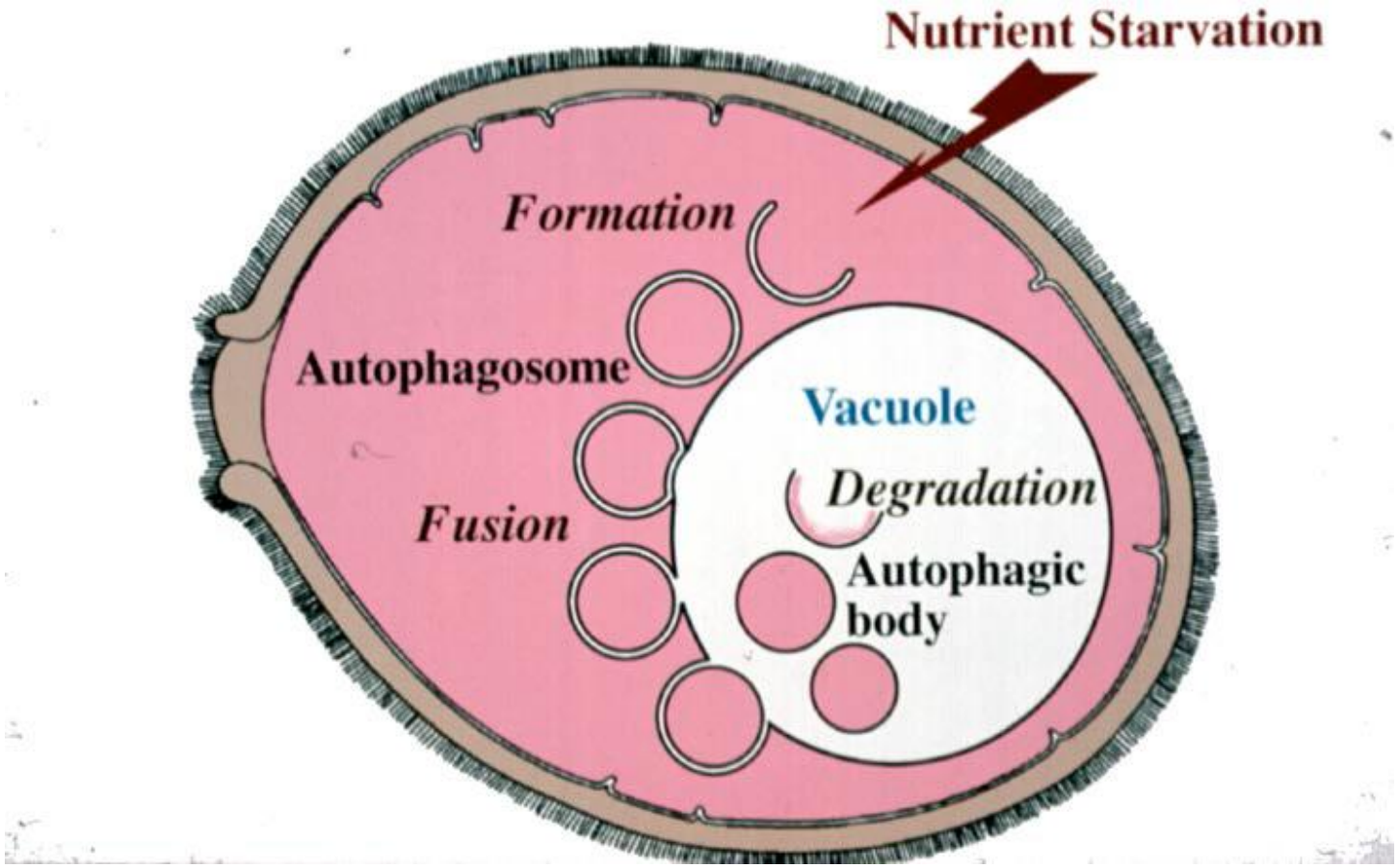
Baba, et al, 1994

Large vacuole inside yeast cells



Adapted from Ohsumi lecture

Autophagic process in yeast



Screening of autophagy defective mutants with the vacuolar proteinase null cells

- Random mutagenize vacuolar proteinase null yeast cells
- Phenotypic screen under microscope: no accumulation of autophagic bodies in the vacuole
- Identify the first mutant gene: ATG1 (autophagy 1)
- ATG1 mutant soon die in starvation condition

  (Loss of viability)

Large scale screening to identify other autophage related genes

EMS random mutagenesis

1st screen for loss of viability in starvation condition 38000

2nd screen for lack of autophagic body by microscope 2700

complement analysis 99

ATG mutants 14

All of these genes are elusive as they were only triggered by starvation, while regular analysis was carried out in nutrient rich condition….

What do ATG genes encode?

Cloning of ATG gene

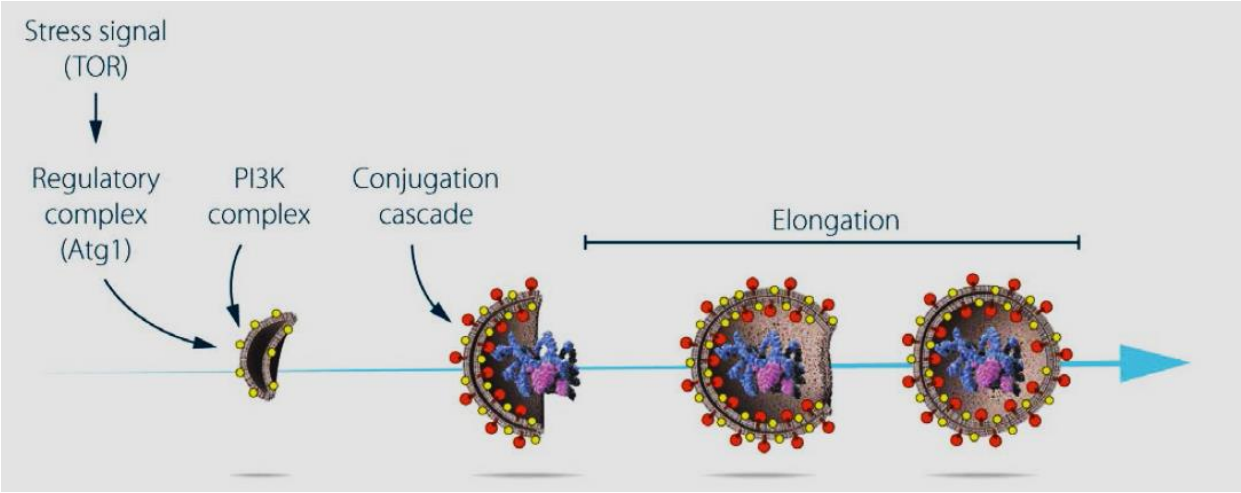
Sequencing of ATG gene

Sequence alignment to known genes

They found no hints about the functions for these proteins

What are the functions for these genes?

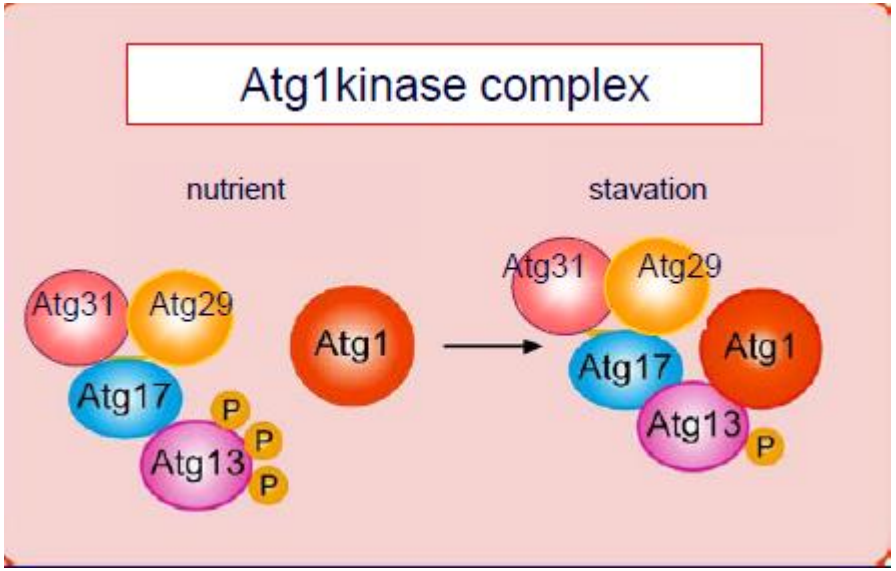
- Atg1 is serine/threonine kinase
- Atg1 forms a complex with atg 13
- Interaction between atg1/atg13 is regulated by TOR kinase
- Atg1/atg13/atg17/atg29/atg31 form a pentameric complex which marks the initial step of autophagosome formation.
- This complex interacts with PI3K complex to form phagophore
- Extention of phagophore involves two ubiquitin-like protein conjugation cascades to form the mature autophagosome.

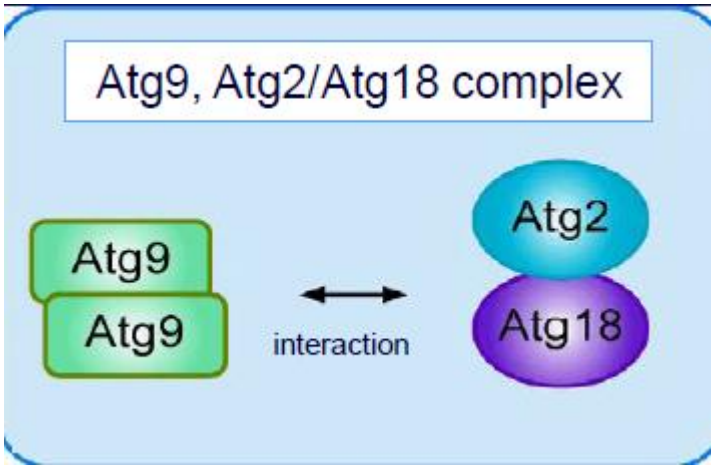


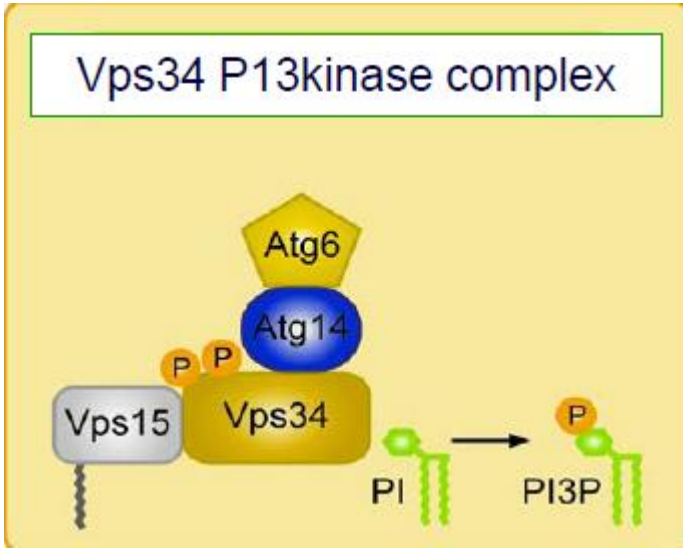
Totally 18 Atg proteins are required for autophagosome formation

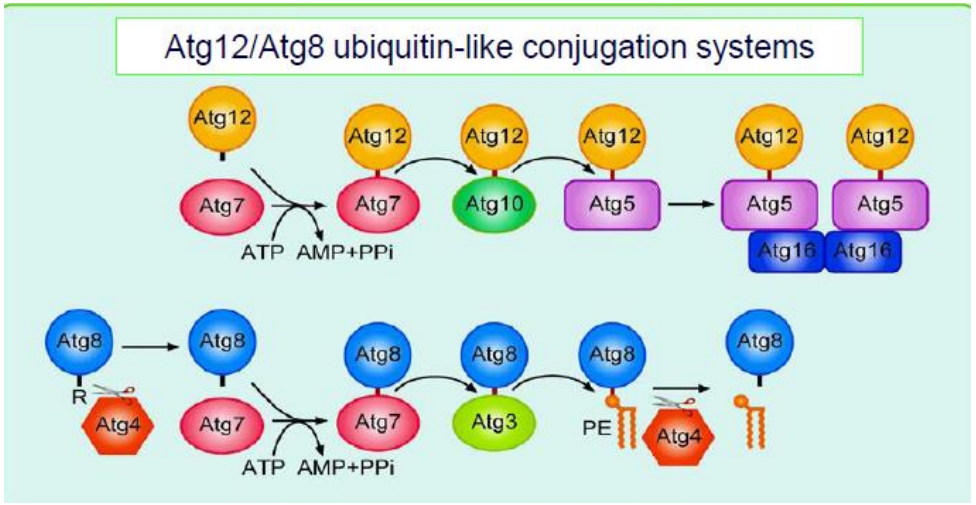
- ♥ Atg1 kinase complex
- ♥ Atg9, Atg2, Atg18 complex
- ♥ Vps34 PI3K complex
- ♥ Atg12/Atg 8 ubiquitin-like conjugation system

18 Atg proteins required for autophagosome formation



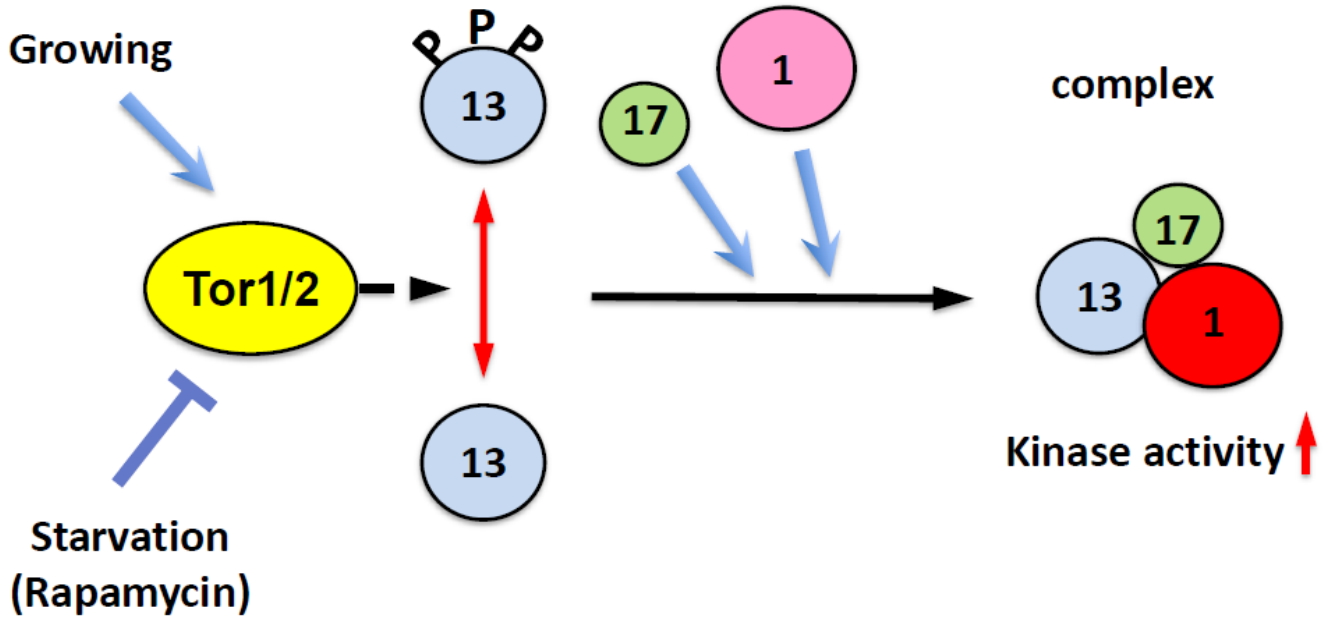






Adapted from Ohsumi lecture

TOR regulates atg1/atg13 complex



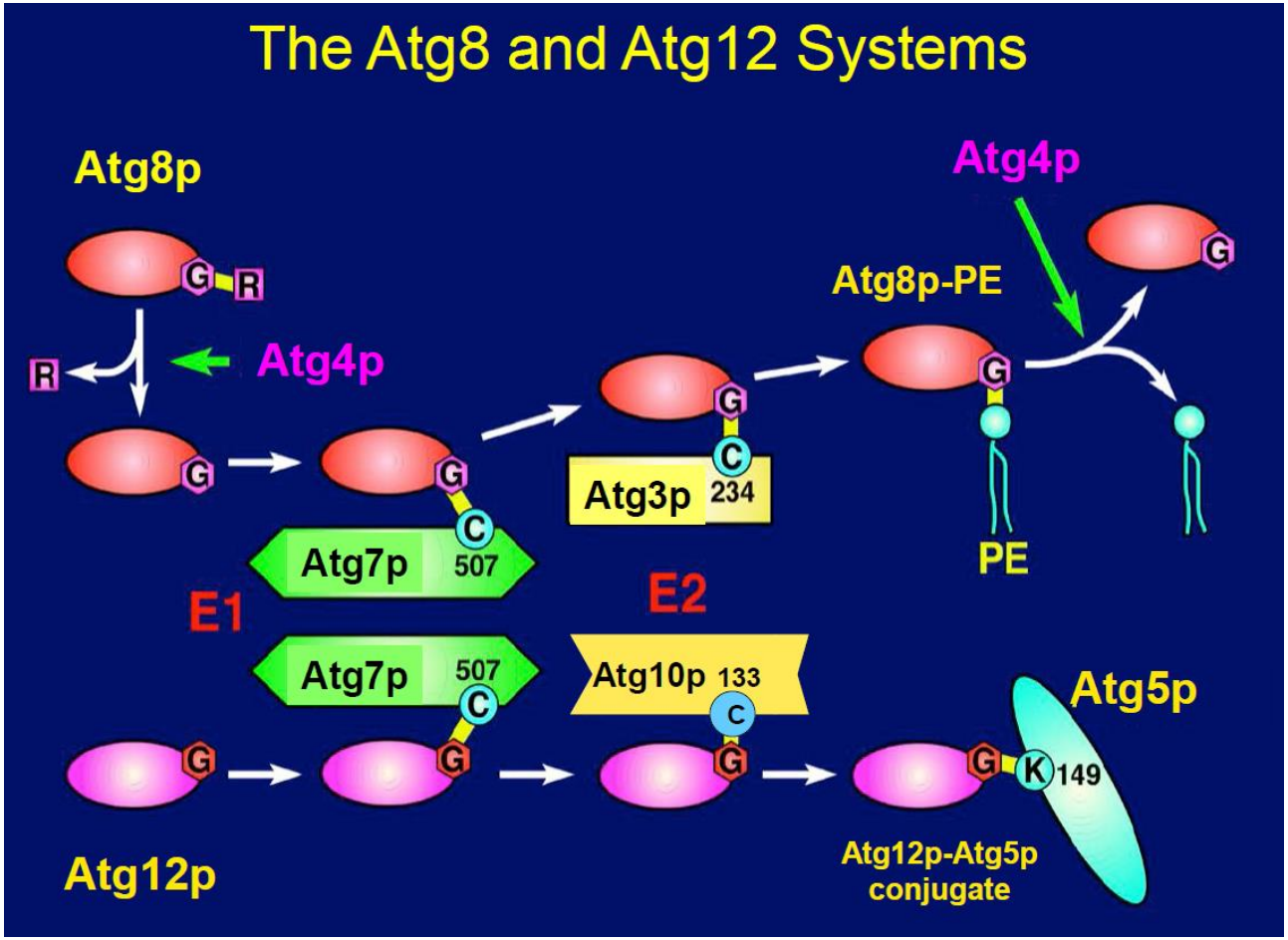
Noda and Ohsumi et al. 1998

Kamada et al. 2000

Atg8 conjugation to PE(phosphotidylethanolamine) is a key driver for autophagosome elongation

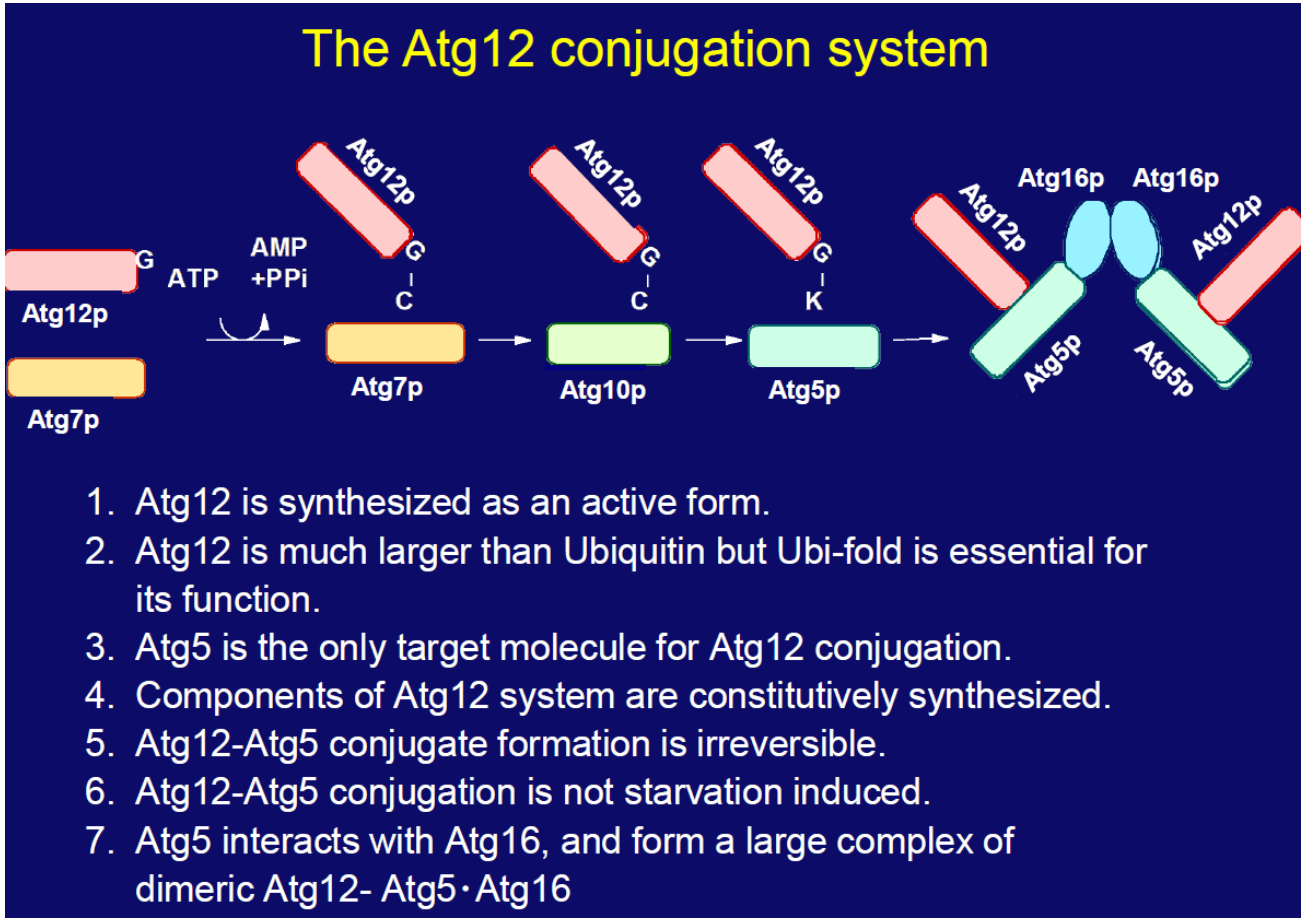
- Two ubiquitation –like systems are important to catalyze the conjugation

Atg8 and Atg12 systems in driving autophagosome formation

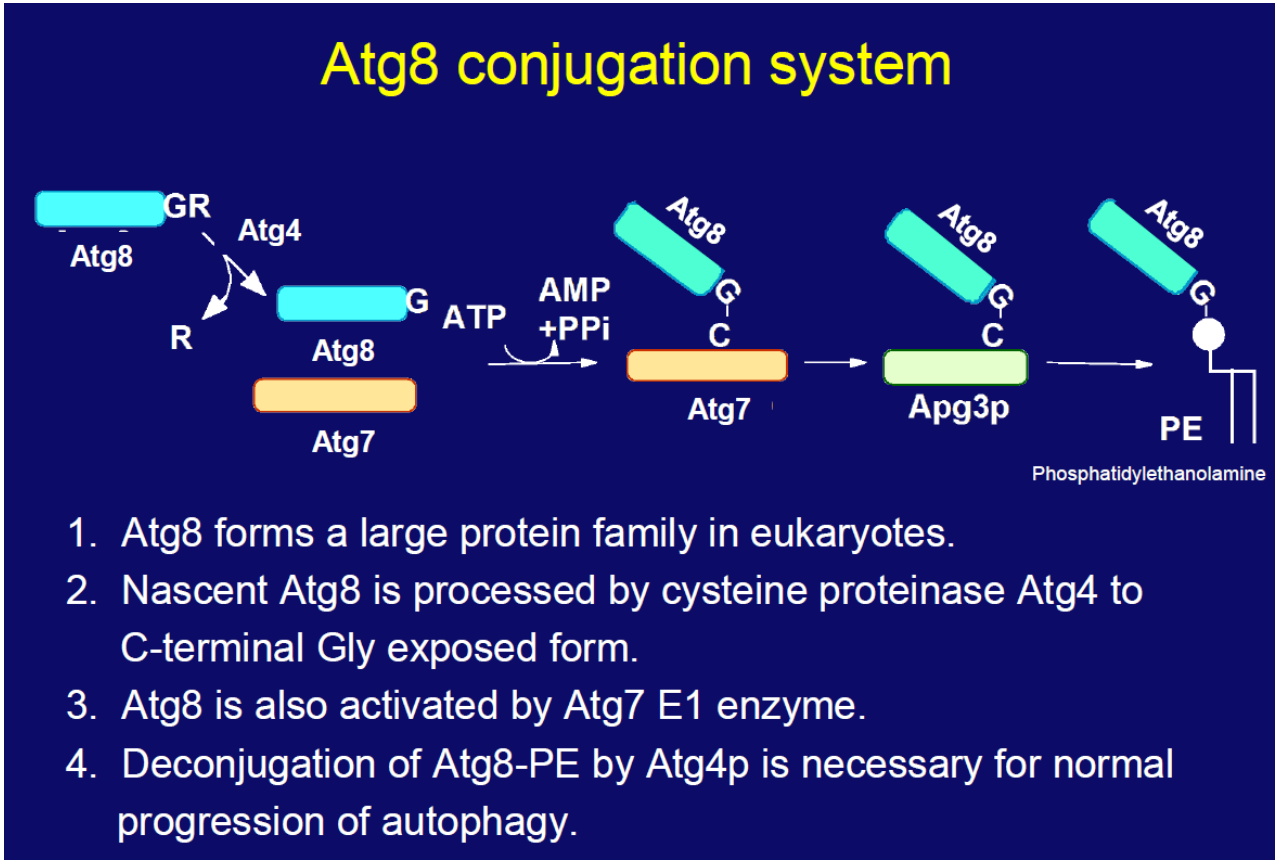


---Adapted from Ohsumi lecture

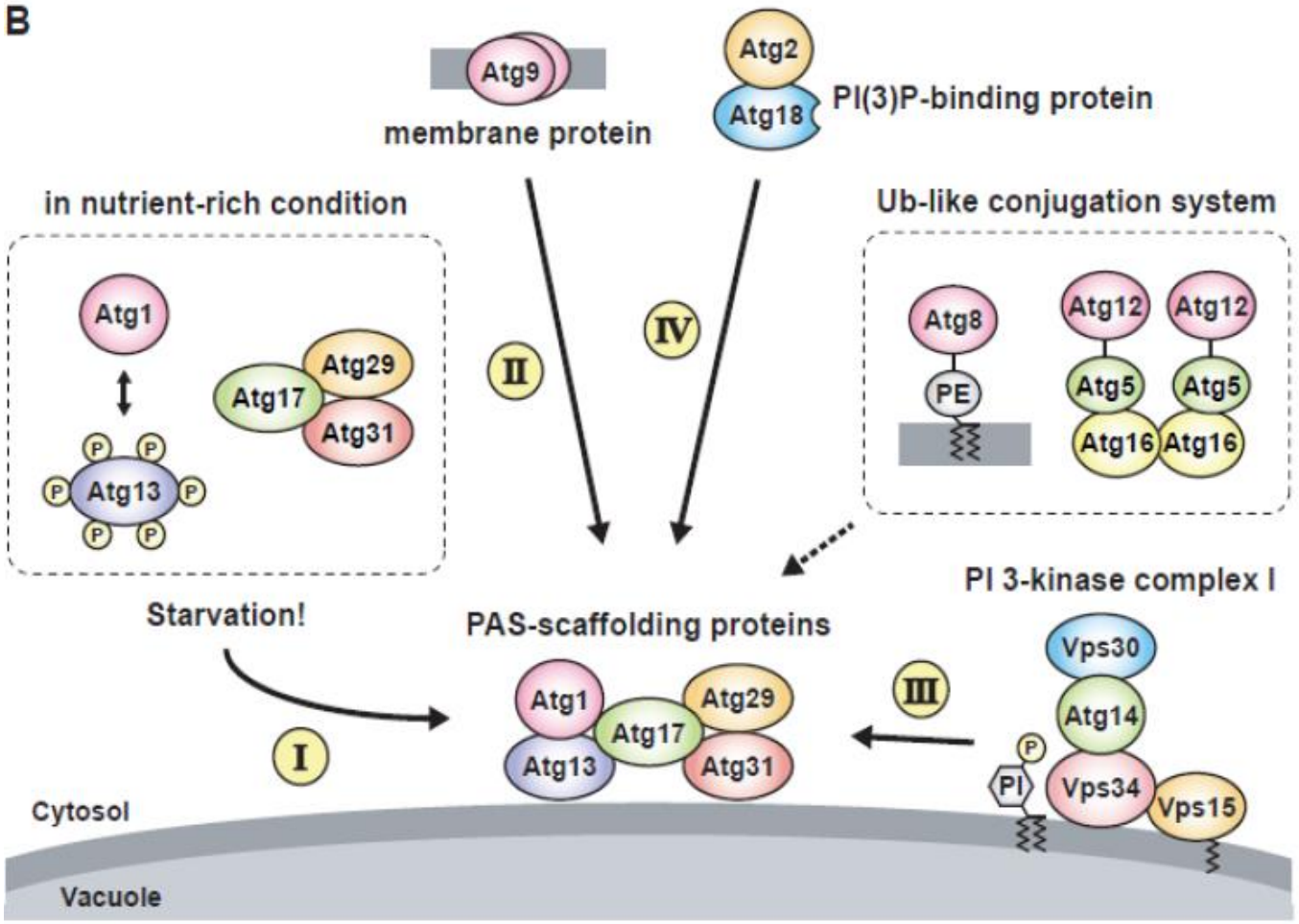
ATG12 conjugation system



Atg8 conjugation system



Overview of sequential events in autophagy



Adapted from Ohsumi lecture

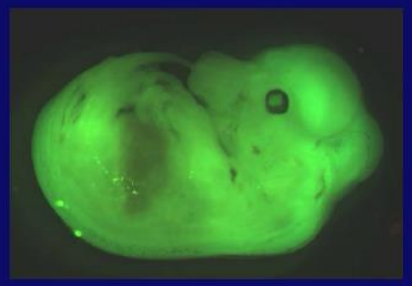
These yeast Atg genes have homolog in mammalian and plant cells

	Yeast	Mammalian	Plant(Arab.)
Atg1 kinase and its regulators	Atg1	ULK1/2	AtATG1a-1c,1t
	Atg13	Atg13	AtATG13a,13b
	Atg17	FIP200	-
	Atg29	Atg101	-
	Atg31		-
PtdIns 3-kinase complex	Atg6/Vps30	Beclin-1	AtATG6
	Atg14	Atg14	-
	Vps34	Vps34	AtVps34
	Vps15	p150	AtVps15
Atg2-Atg18 complex and Atg9	Atg2	Atg2s	AtATG2
	Atg9	Atg9Ls	AtATG9
	Atg18	WIPIs	AtATG18a-18h
Atg12 conjugation system	Atg12	Atg12 DFCP1	AtATG12a,12b
	Atg7	Atg7	AtATG7
	Atg10	Atg10	AtATG10
	Atg5	Atg5	AtATG5
	Atg16	Atg16Ls	AtATG16L
Atg8 conjugation system	Atg4	Atg4s	AtATG4a,4b
	Atg8	LC3/Atg8s	AtATG8a-8i
	Atg3	Atg3	AtATG3

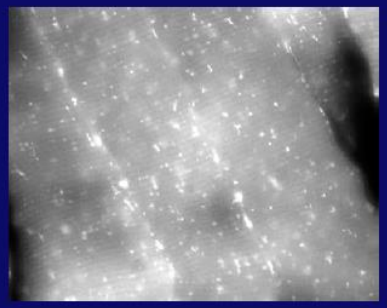
Adapted from Ohsumi lecture

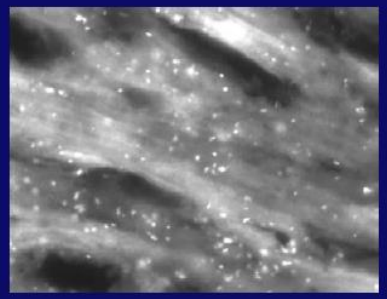
Autophagy naturally occurs in mouse development

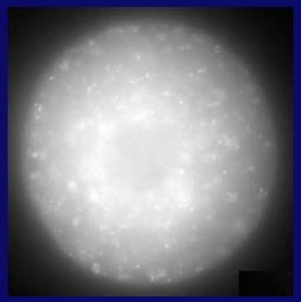
Autophagy in whole organisms:

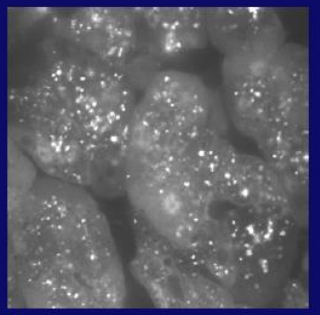


GFP-LC3
Transgenic mouse

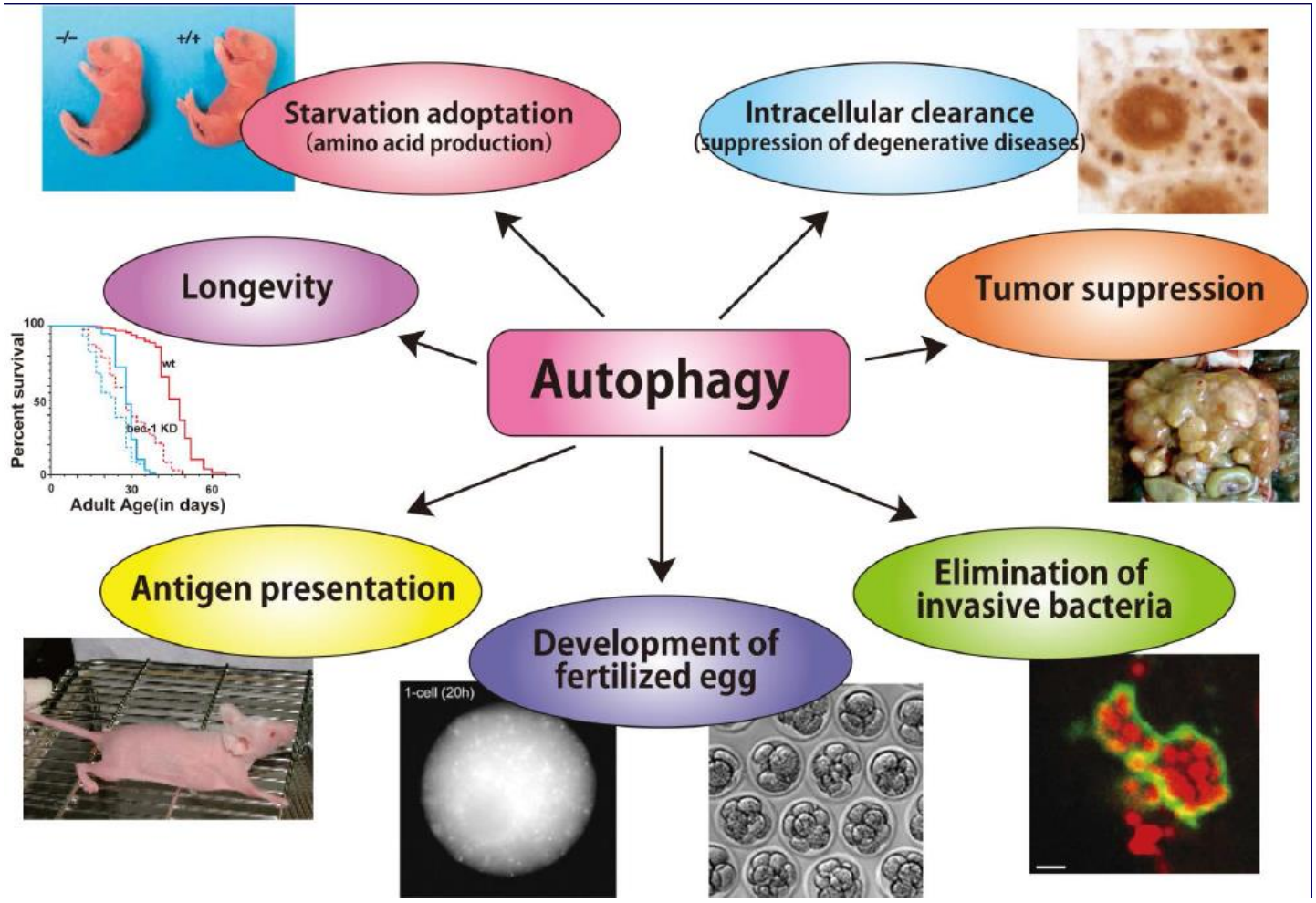








Various physiological functions of autophagy



Two major roles of autophagy

- **Nutrient Recycling:**

  1).essential for survival under starvation

  2).recycling for amino acids for protein synthesis, energy source

- **Elimination of excessive or harmful materials:**

  1).essential for clearance of cytoplasm

  2).specific protein, protein aggregates

  3).organelles : mitochondria, peroxisomes, lysosomes, ER, nucleus….

  4).Invasive bacteria, Virus particles