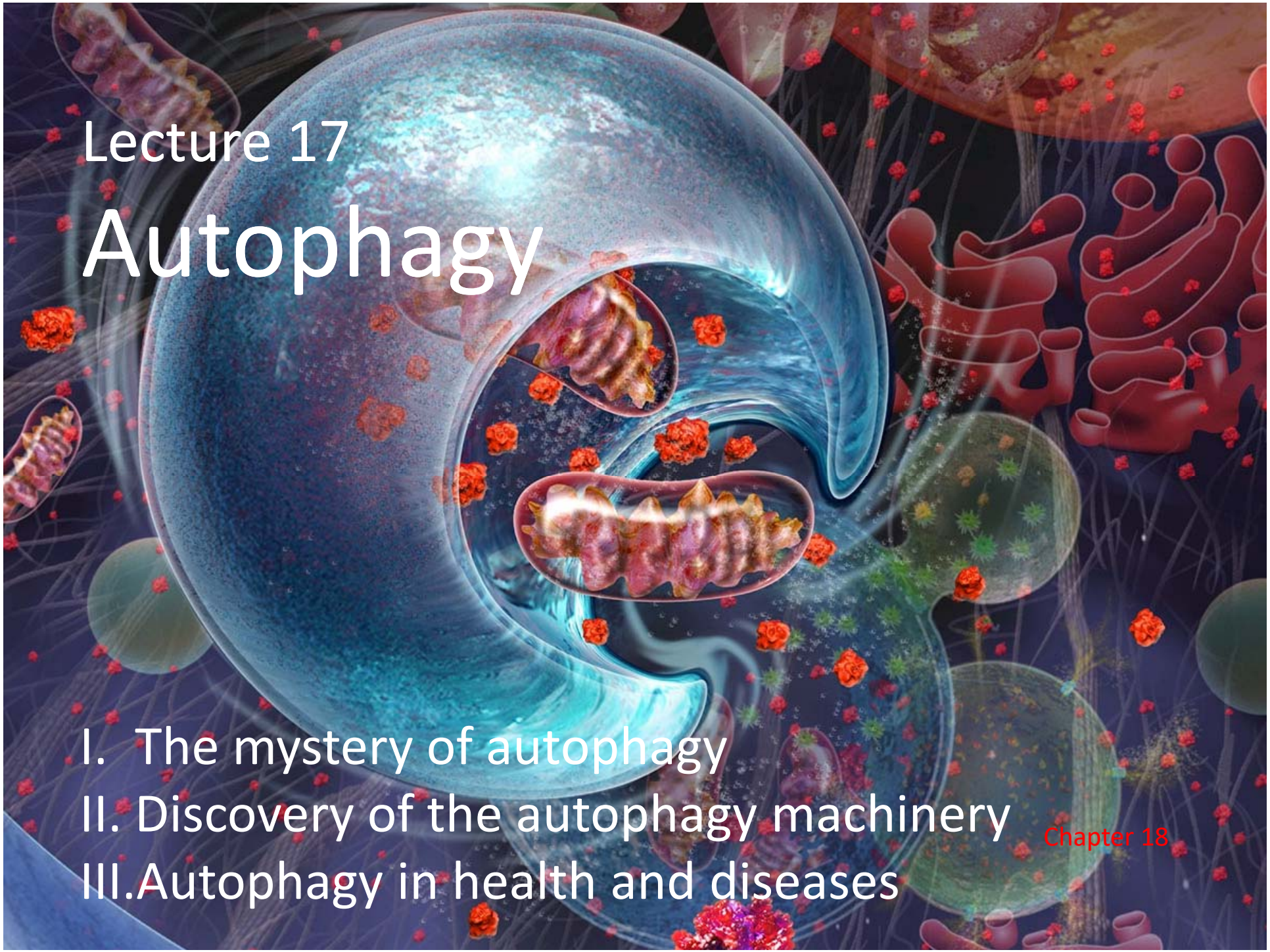


Lecture 17

Autophagy

I. The mystery of autophagy
II. Discovery of the autophagy machinery
III. Autophagy in health and diseases

Chapter 18

Autophagy and homoeostasis

Different degradation mechanisms for different targets:

proteins & protein **complexes** → 26S proteasome → degradation

Larger portions of cytosol, organelles (ER, ribosomes, mitochondria & plastids → lysosomes → degradation

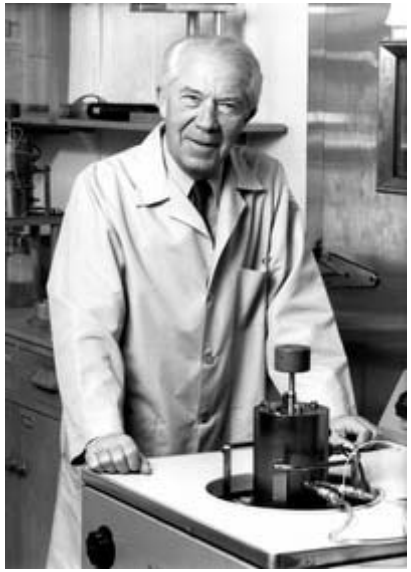
Ubiquitin/ proteasome system:

specific target recognition (regulation); short-lived proteins

Lysosome/ vacuolar system:

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

Discovery of the lysosome by Christian de Duve, 1955



1917-2013

Milestones:

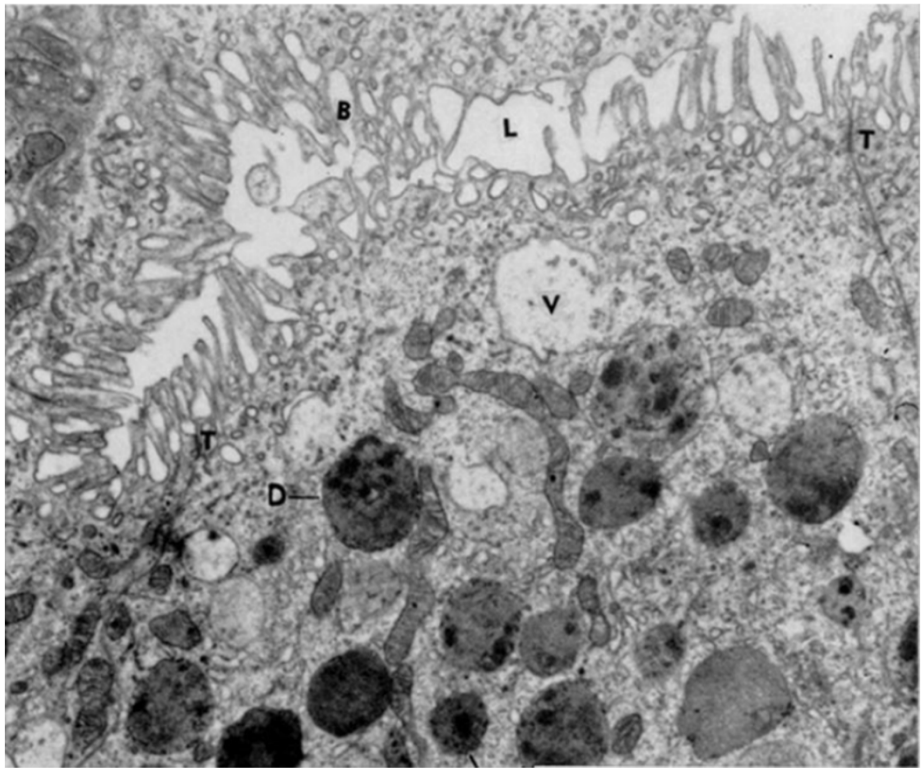
- Discovery of the lysosome (1955)
- Discovery of the autophagosome (1962-1963), *auto* (self) – *phagy* (eating)
- Nobel laureate jointly with Albert Claude & George E. Palade for their discoveries concerning "**the structural & functional organization of the cell**", 1974

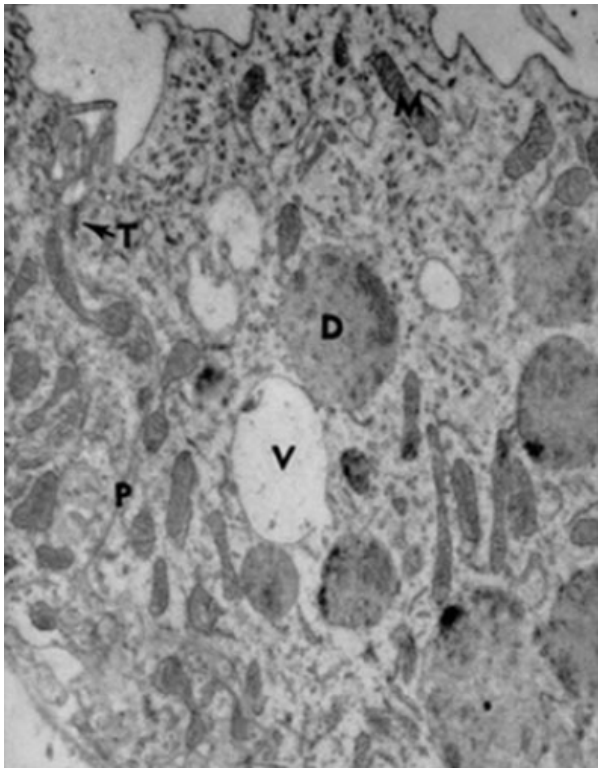
- His interest in **glucose-6-phosphatase** leaded him to study an **acid phosphatase**, which usually interfere with his results.
- When trying to identify the nature of this acid phosphatase, he found that the **acid phosphatase is latent and particle bound**, subsequently he found that this particle is the **lysosome**.
- He **also discovered peroxisomes**.
- All discoveries were possible due to the **combination** of **biochemistry**, **ultrastructural analysis** (**electron microscopy**) and **subcellular fractionation** via **differential centrifugation**.

Early indication of autophagy in normal kidney development

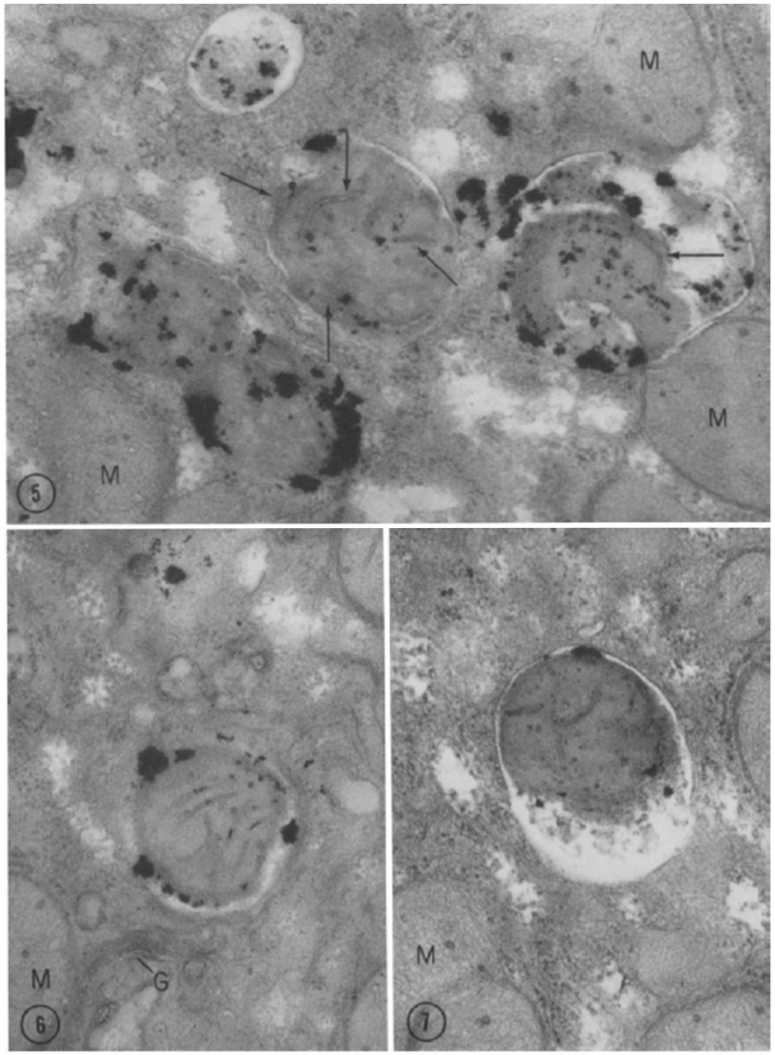
"CELLULAR DIFFERENTIATION IN THE KIDNEYS OF NEWBORN MICE STUDIED WITH THE ELECTRON MICROSCOPE"

Clark, S., J. Biophysic. and Biochem. Cytology 3, 1956





Early hint for autophagy: degeneration of mitochondria



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

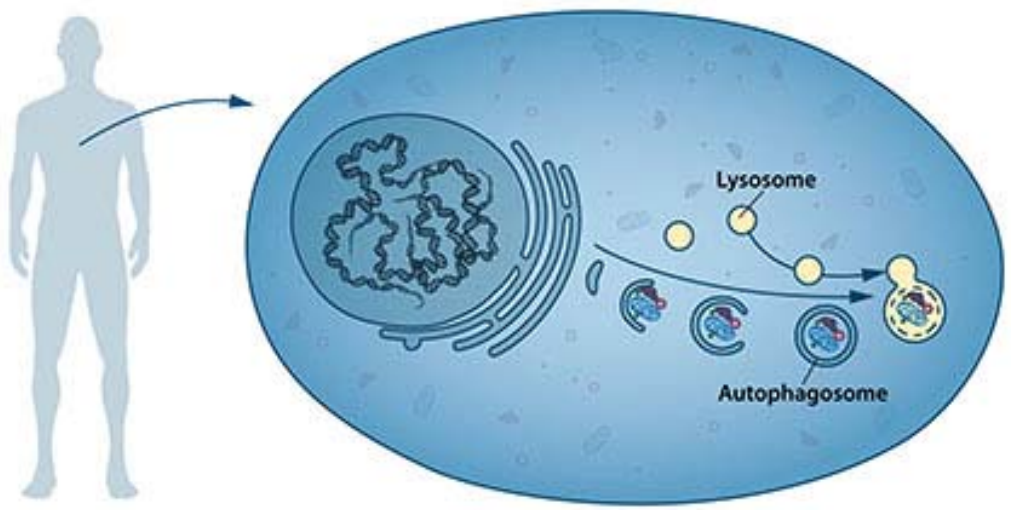
Novikoff & Essner (1962)
Cytolysomes and mitochondrial degeneration, JCB ***15***, 140.

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 remained a mystery for ~ 30 years…

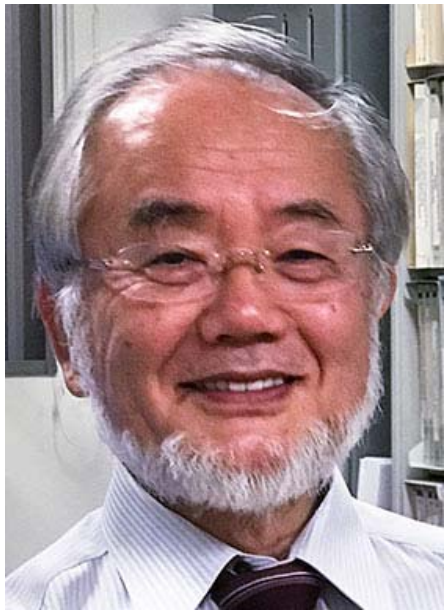
What they knew was:
A type of vesicle called **autophagosome** was observed within the cell.
As the autophagosome forms, **it engulfs cellular contents, such as damaged proteins and organelles**. Finally, **it fuses with the lysosome,** where the contents are degraded into smaller constituents.



To study autophagy was difficult at that time:

- autophagosomes are transient and only exist
  for about 20-30 minutes before fusing with lysosome… until ….

In early 1990's, Yoshinori Ohsumi

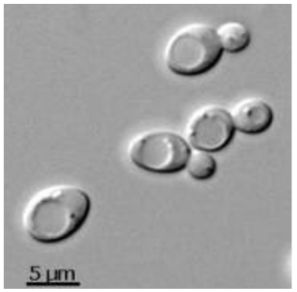


- Made seminar discoveries about autophagy using yeast model system
- He was awarded 2016 Nobel prize in Physiology/Medicine
- He **identified** multiple **key genes involved in autophagy** and revealed the molecular mechanism for autophagy initiation, formation, regulation, **and link to human diseases,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

1945 - Japan

He initially set up an efficient autophagy analytical platform

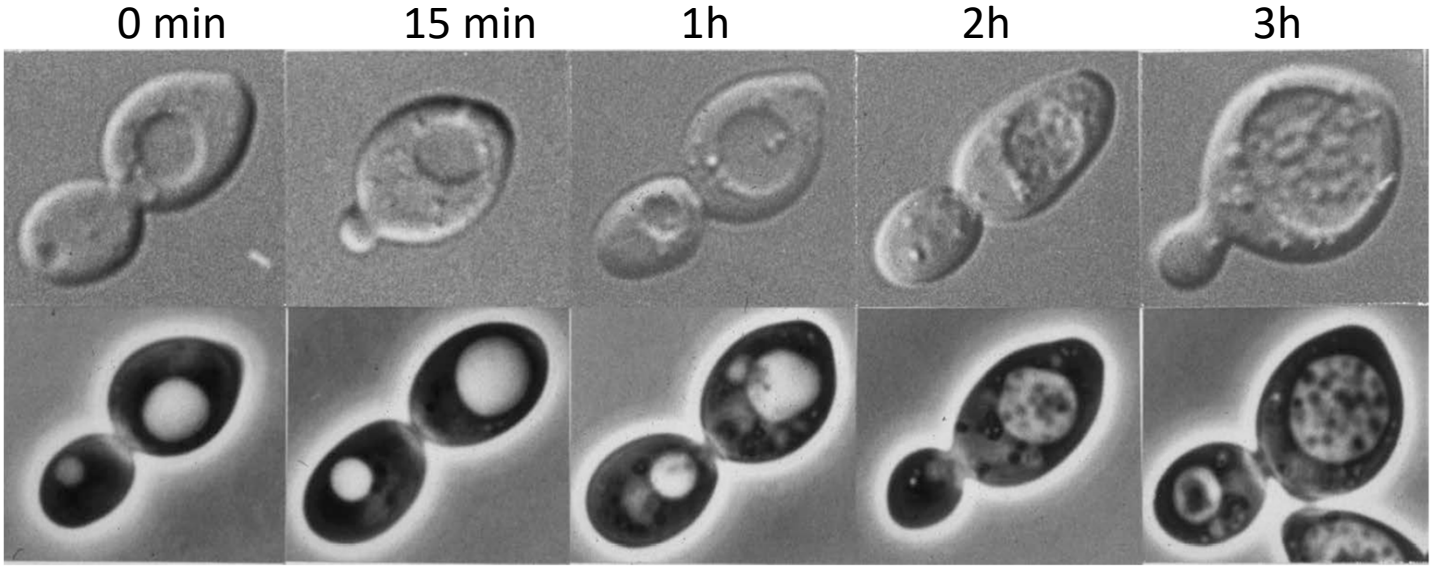
Regular yeast cells are small



Choose a vacuolar **proteinase deficient** mutant

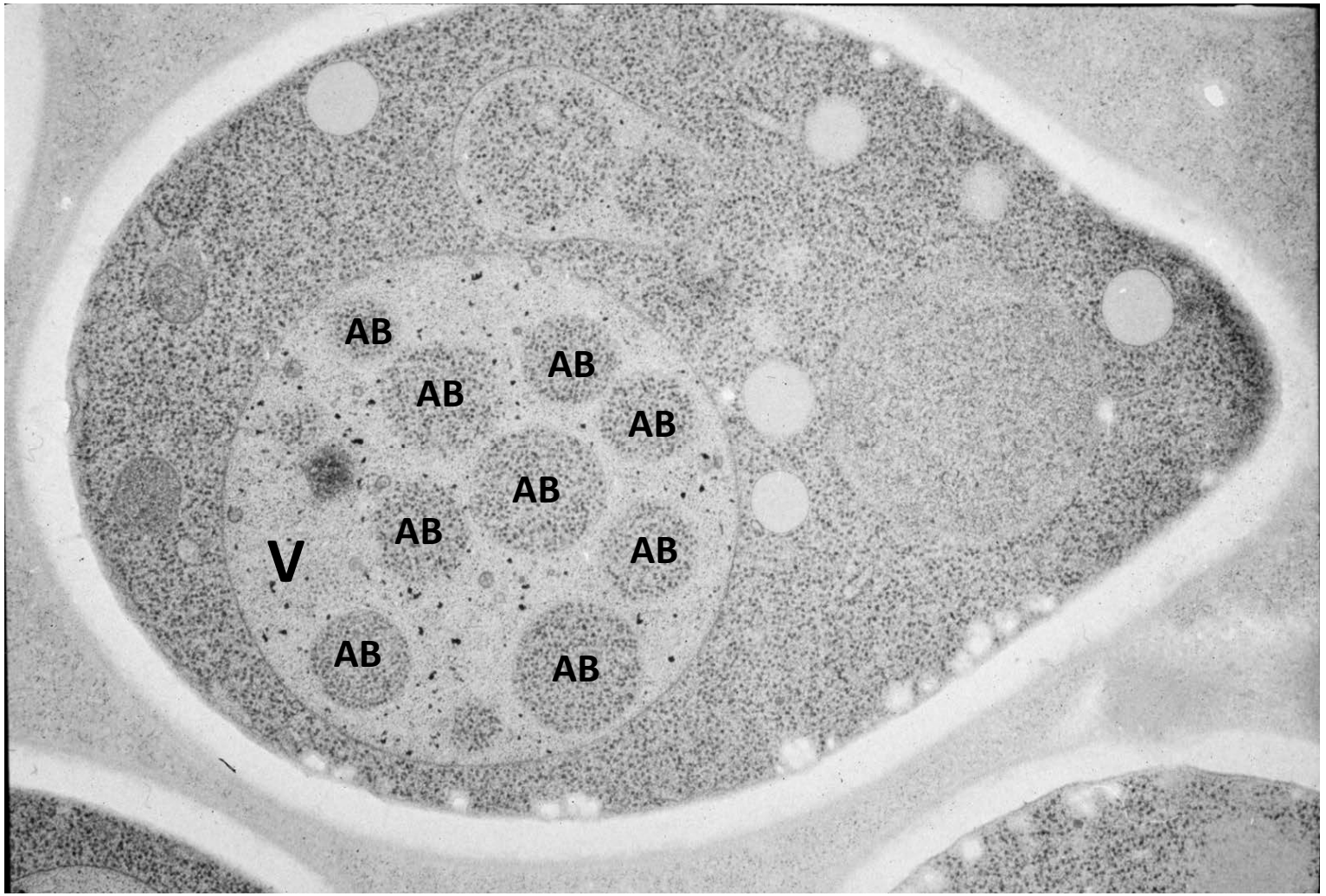
Switch into nitrogen **starvation medium**

accumulation of autophagic bodies in the vacuole



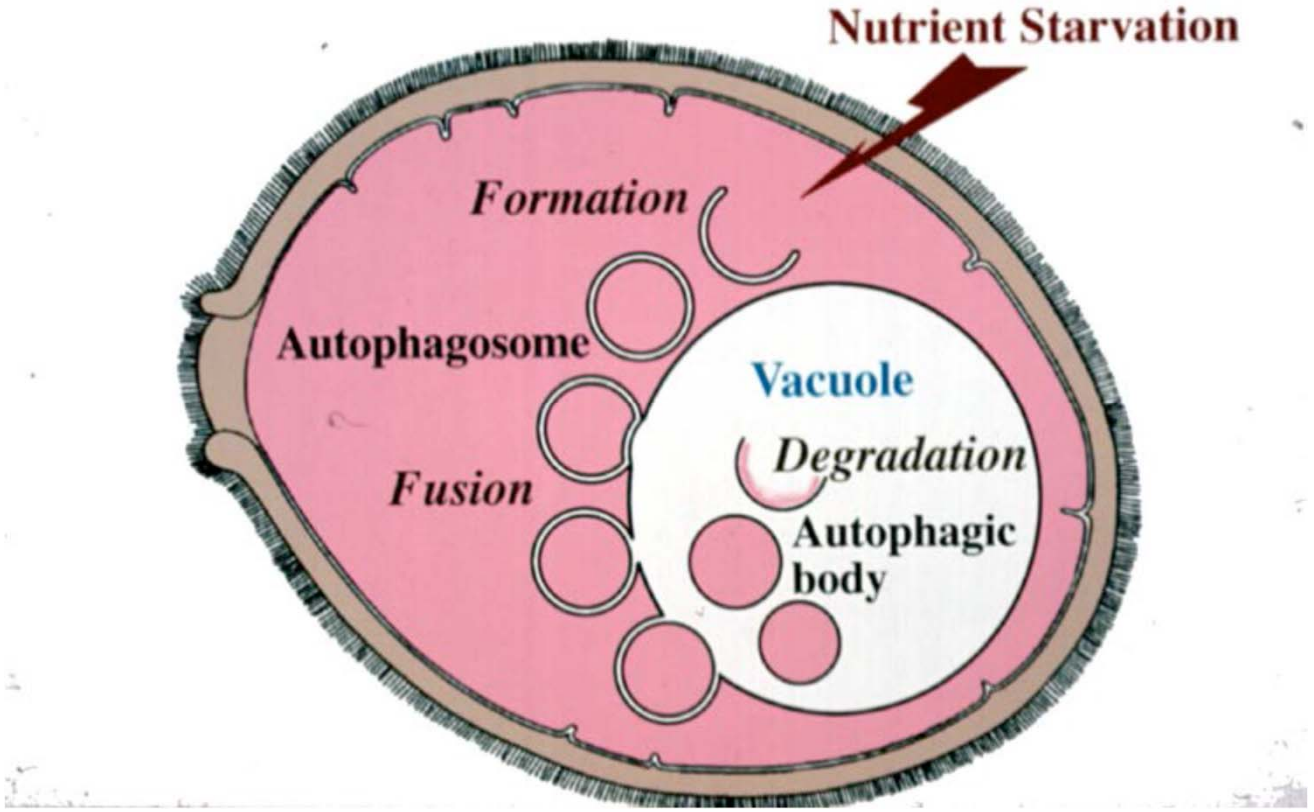
Takeshige, K.*, et al.* (1992) Autophagy in yeast demonstrated with proteinase-deficient mutants and conditions for its induction. *J. Cell Biol.* 119, 301-311

Accumulation of autophagic bodies (AB) in vacuoles of protease-deficient yeast mutants at starvation conditions



Takeshige, K.*, et al.* (1992) Autophagy in yeast demonstrated with proteinase-deficient mutants and conditions for its induction. *J. Cell Biol.* 119, 301-311

The autophagic process in yeast



- Autophagosomes consists of **two membranes**.
- Autophagosomes **fuse** with the vacuole, thereby **releasing a single membrane limiting vesicle** into the lumen of the vacuole.
- Upon release, vesicle and its content is subsequently degraded in the vacuole.

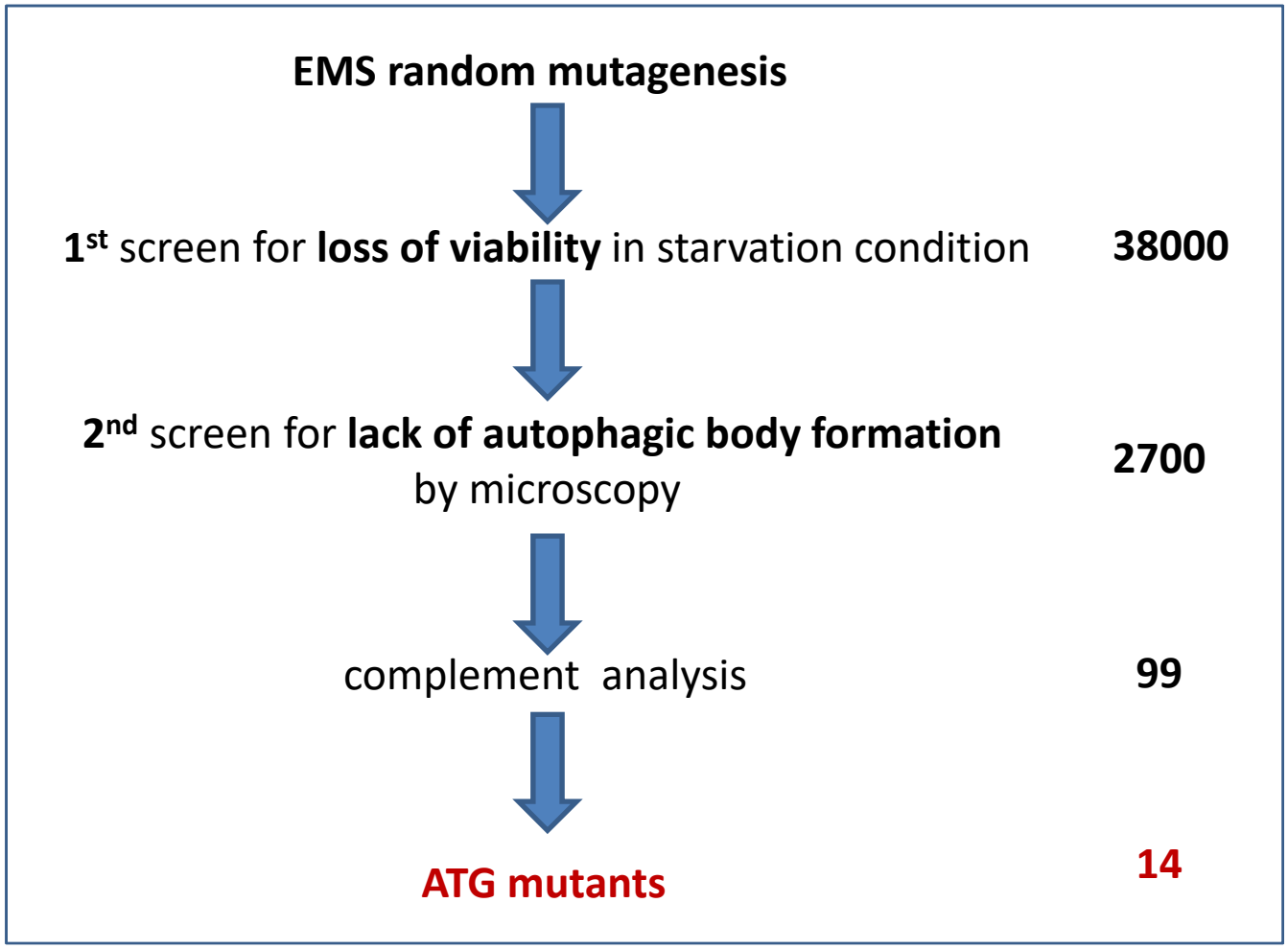
Screening of autophagy defective mutants with the vacuolar proteinase null cells to identify “autophagy related genes”

- Vacuolar proteinase **null yeast cells** allow the analysis of autophagy by judging by the analysis of accumulating autophagic bodies in the vacuole
  - Random mutagenesis of vacuolar proteinase null yeast cells allow identification of genes that are **required for the formation of autophagic bodies**
  - Phenotypic screen via microscopy for mutants, **lacking the accumulation of autophagic bodies** in the vacuole

- **Screen resulted in the identification** of the first mutant gene: **APG1** (**a**uto**p**ha**g**y **1**) → newer nomenclature ATG1 (autophagy gene 1)

- **ATG1 mutants die soon** at starvation conditions (Loss of viability).

Large scale screening to identify other autophagy related genes

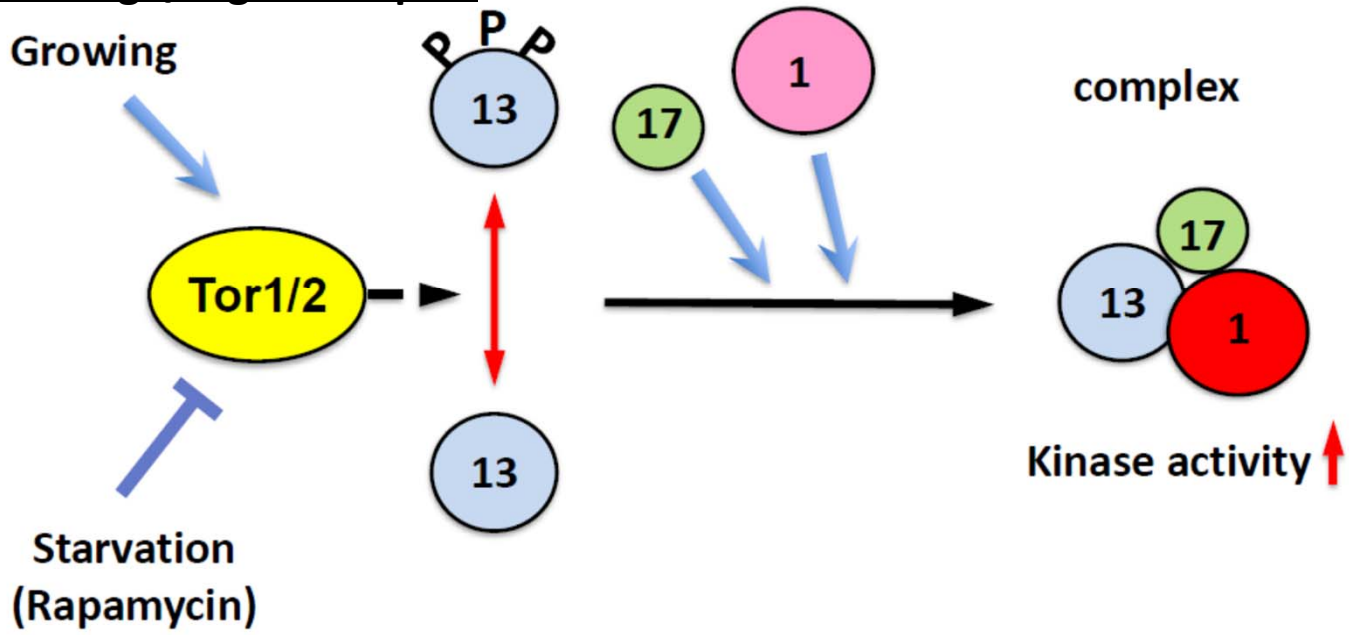


All of these genes are elusive as they were only triggered by starvation, while regular analysis was carried out in nutrient rich condition….

What are the functions for these genes?

- Atg**1** is a **serine/threonine kinase**
- Atg**1** forms a complex with Atg**13**
- Interaction between Atg**1**/Atg**13** is regulated by **TOR** (**t**arget **o**f **r**apamycin) **kinase**
- Atg**1**/Atg**13**/Atg**17**/Atg**29**/Atg**31** form a **pentameric complex** which marks the initial step of autophagosome formation.

TOR regulates Atg1/Atg13 complex

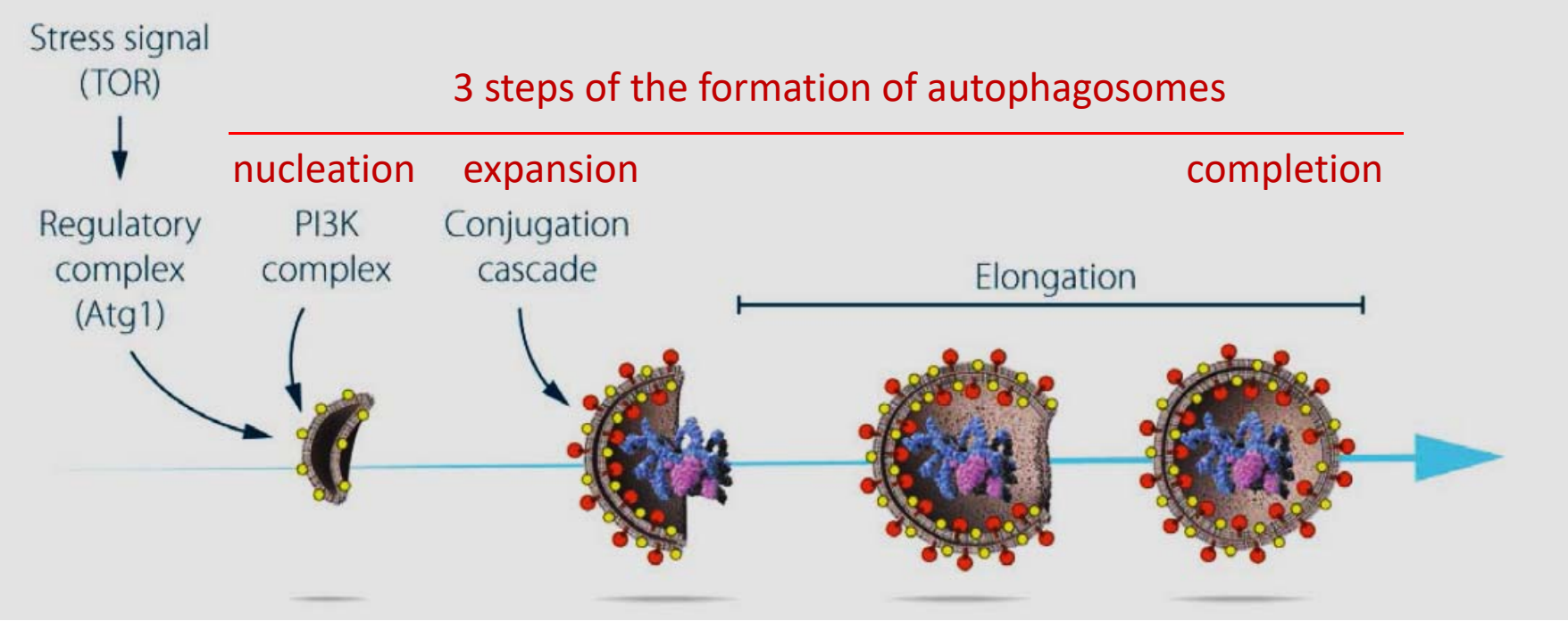


Noda and Ohsumi et al. 1998

Kamada et al. 2000

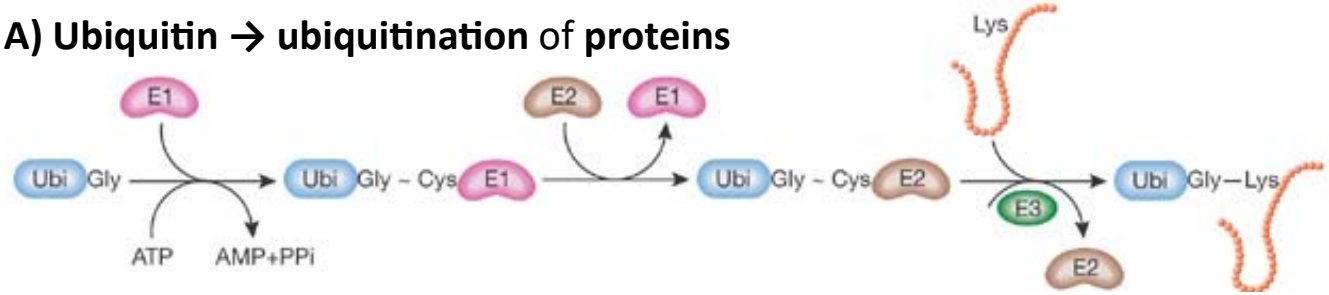
What are the functions for these genes?

- **Th pentameric** Atg**1**/Atg**13**/Atg**17**/Atg**29**/Atg**31 complex interacts with PI3K complex** to form the initial **phagophore** (**p**re**a**utophagosomal **s**tructure (**PAS**)
- **Extension of the phagophore** involves **two ubiquitin-like protein** conjugation cascades to form the mature autophagosome:
  - **Atg8** (**LC3**)system
  - **Atg12** system



Conjugation processes in yeast

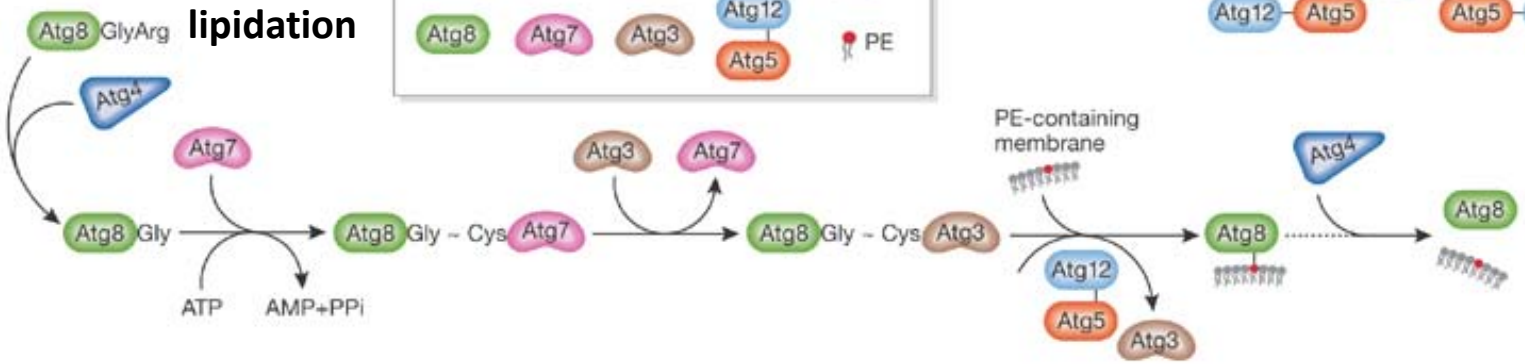
A) Ubiquitin → ubiquitination of **proteins**



B) Atg12 → Atg12 conjugation of **Atg5: Formation of tetrameric Atg12–Atg5-Atg16 complex**

Ubl	E1	E2	E3	Substrate
Atg12	Atg7	Atg10	?	Atg5
Atg8	Atg7	Atg3	Atg12–Atg5	PE

C) Atg8 → Atg8 lipidation

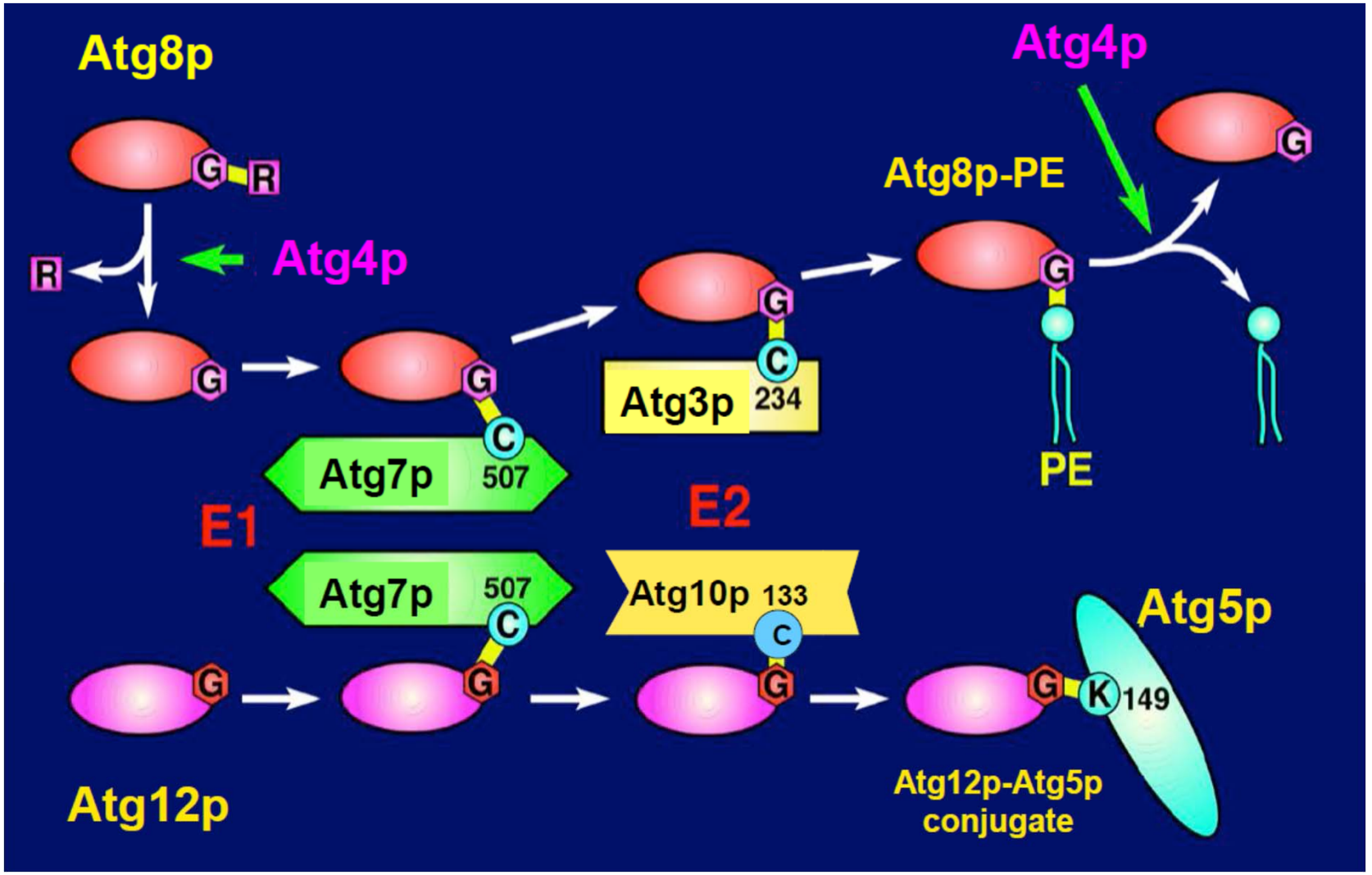


Atg8 conjugated to **p**hosphatidyl**e**thanolamine (**PE**), **Atg8-PE,** is a key driver for autophagosome elongation

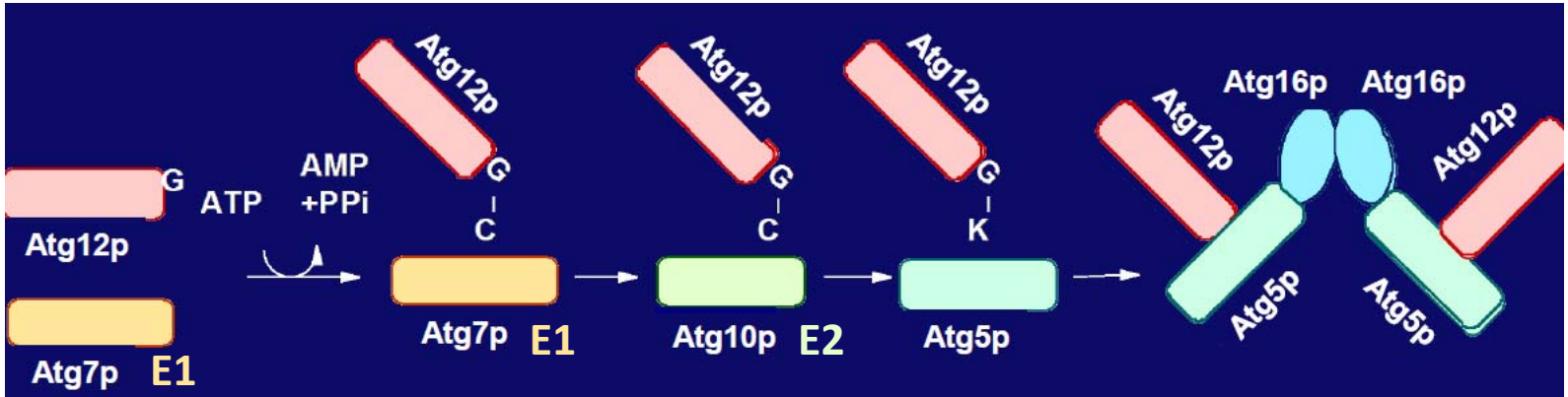
Abbreviations: Atg, autophagy-related; PE, phosphatidylethanolamine; PPi, pyrophosphate; Ubi, ubiquitin; Ubl, ubiquitin-like

Jiefei Geng, and Daniel J. Klionsky EMBO Rep. 2008;9:859-864

Side -by side comparison of the Atg8 and the Atg12 systems

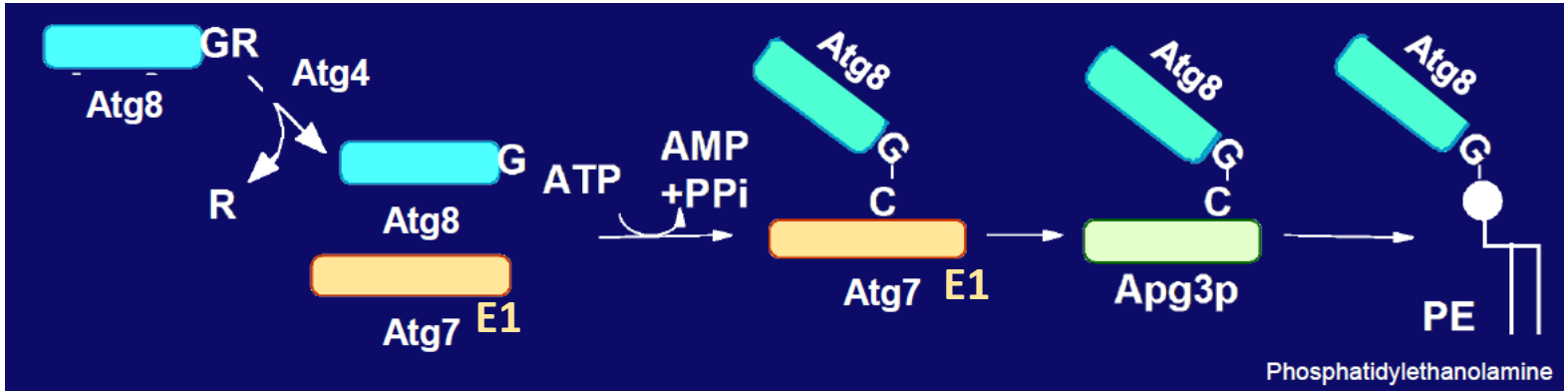


ATG12 conjugation system



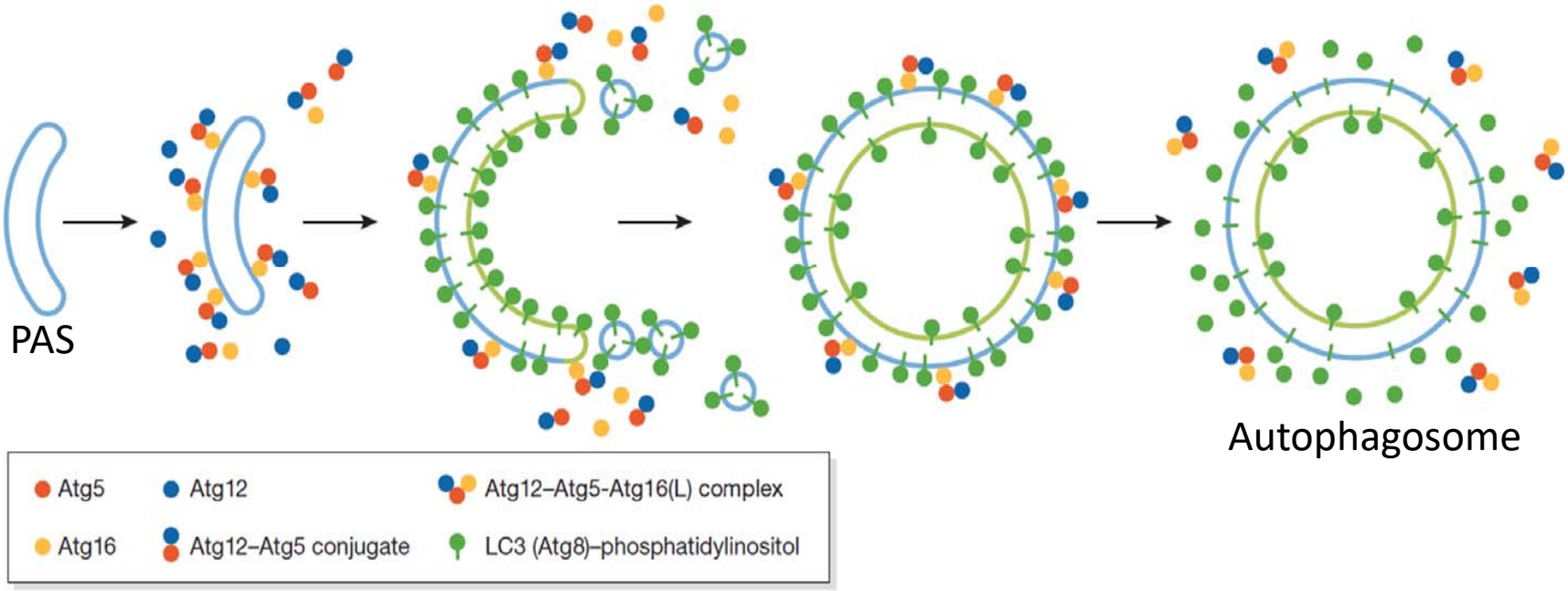
1. Atg12 is synthesized as an active form.
2. Atg12 is much larger than Ubiquitin but Ubi-fold is essential for its function.
3. Atg5 is the only target molecule for Atg12 conjugation.
4. Components of Atg12 system are constitutively synthesized.
5. Atg12-Atg5 conjugate formation is irreversible.
6. Atg12-Atg5 conjugation is not starvation induced.
7. Atg5 interacts with Atg16, and form a large complex of dimeric Atg12- Atg5·Atg16

The Atg8 conjugation system



1. Atg8 forms a large protein family in eukaryotes.
2. Nascent Atg8 is processed by cysteine proteinase Atg4 to C-terminal Gly exposed form.
3. Atg8 is also activated by Atg7 E1 enzyme.
4. Deconjugation of Atg8-PE by Atg4p is necessary for normal progression of autophagy.

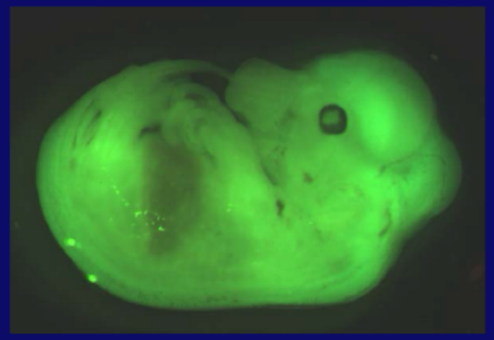
Formation of the autophagosome: the role of Atg8-PE and Atg12-Atg5



- **Recruitment** of the **Atg12–Atg5-Atg16 complex** to the **PAS**
- Atg12–Atg5-Atg16 redistributes mostly to the **outer surface** of the phagophore
- Atg12–Atg5-Atg16 **directs** additional **Atg8** to this site.
- **Presence** of **Atg8** on the phagophore **supports its expansion**
- On completion of the autophagosome, the **Atg12–Atg5-Atg16 complex dissociates** from the vesicle, and **Atg4** proteolytically **releases** the **Atg8** from the outer surface.

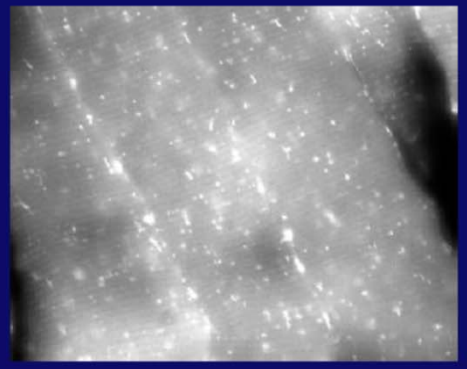
Autophagy naturally occurs in mouse development

Autophagy in whole organisms:

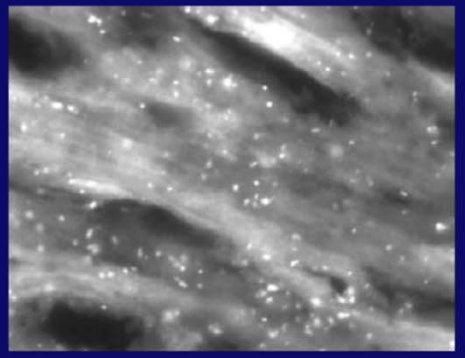


GFP-LC3
Transgenic mouse

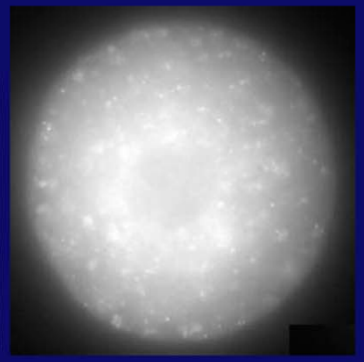
starvation (muscle)

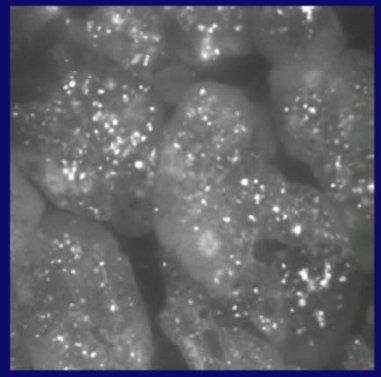


birth (heart)

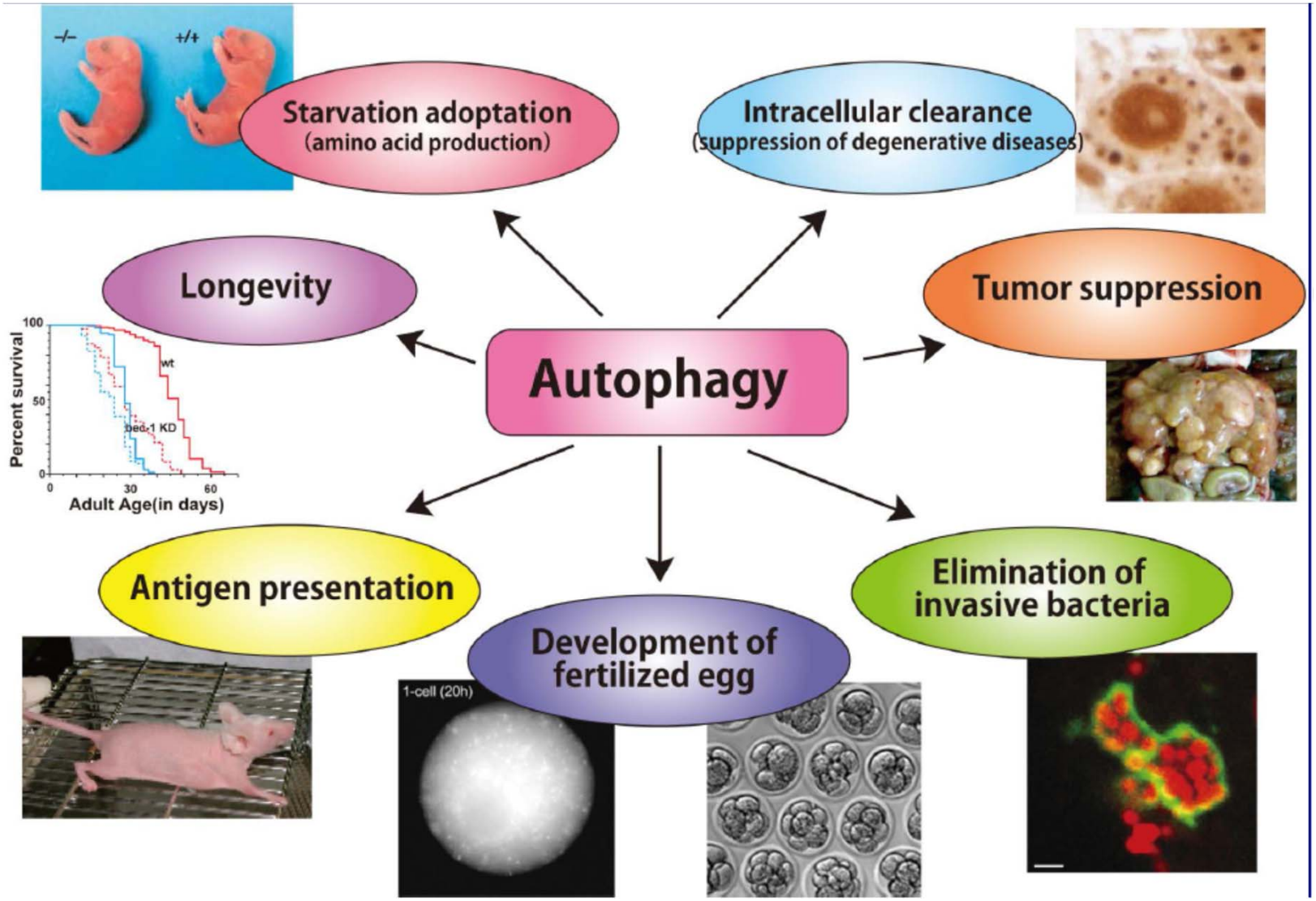


fertilization (egg)





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

Autophagy in plants