Nobel Lecture
in Physiology or Medicine
Karolinska Institute
2016. 12. 7

AUTOPHAGY
an intracellular recycling system

Yoshinori OHSUMI
Institute of Innovative Research
Tokyo Institute of Technology

Personal History

1945 Born in Fukuoka, half a year before the end of World War II
sickly child, mother's TB, for long time bedbound
Childhood spent among nature, collecting insects, watching stars

1960 High school, chemistry club

1963 Undergraduate School, The Univ. of Tokyo
establishment of the central dogma, molecular biology

1967 Graduate Student, The Univ of Tokyo, Dr. Kazutomo Imahori
ribosome, protein synthesis, mechanism of action of colicin E3

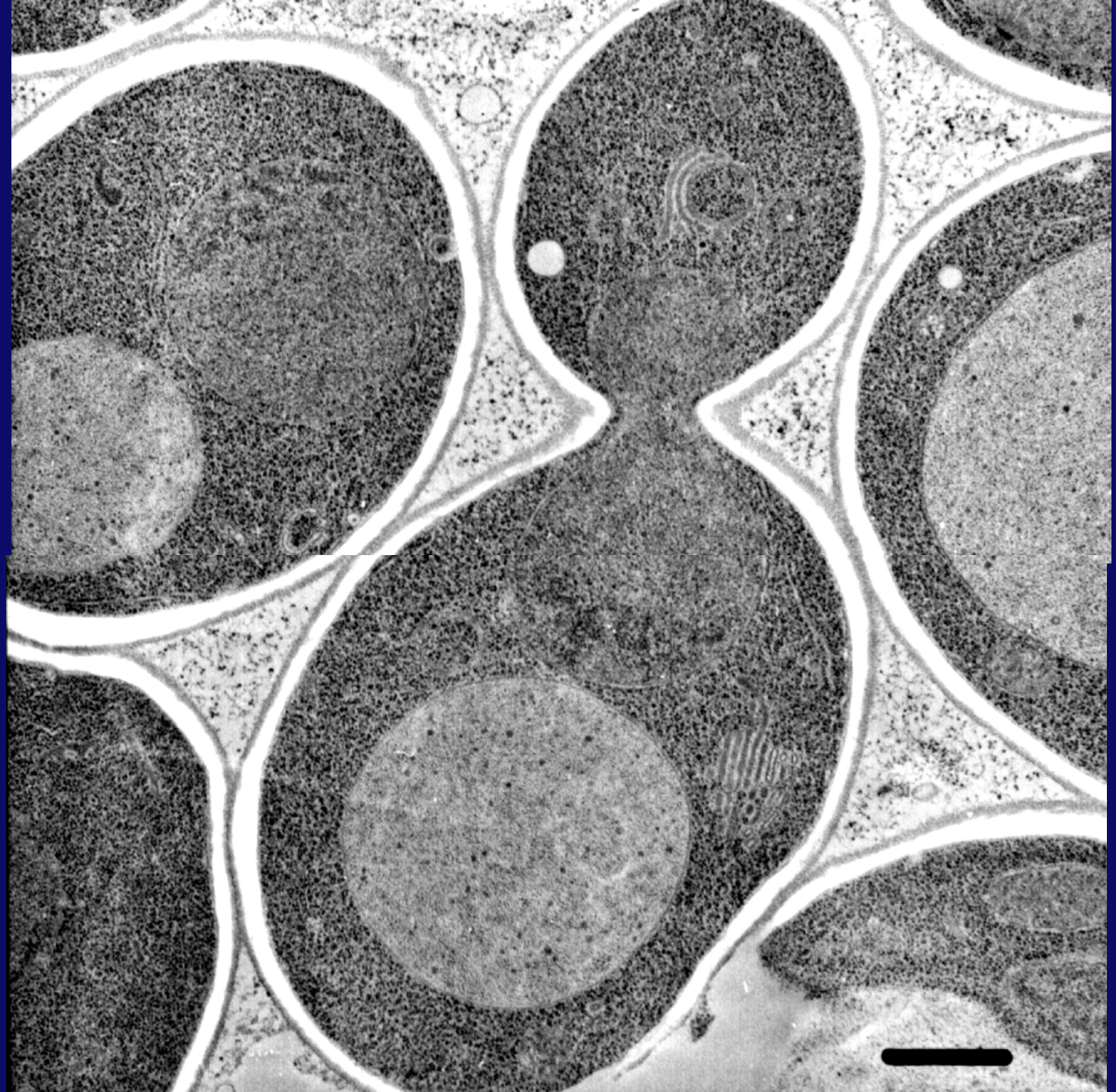
1974 Research Fellow, Rockefeller Univ., Dr. G. M. Edelman (Nobel Prize in 1972)
initiation of DNA replication in yeast with Dr. M. S. Jazwinski

1977 Assistant professor, The Univ. of Tokyo, Dr. Yasuhiro Anraku
vacuole, amino acid transport, V-type ATPase

1988 Associate Professor, Department of Biology, College of Arts and Sciences, The Univ. of Tokyo
lytic function of the yeast vacuole

1996 Professor, National Institute for Basic Biology, Okazaki

2009- Professor, Tokyo Institute of Technology



The yeast vacuole : just a cellular garbage dump?

Why is the vacuole so large?

in plants, the vacuole occupies more than 90% of cell volume

many physiological functions

storage compartment: sugar, acids, ions, protein

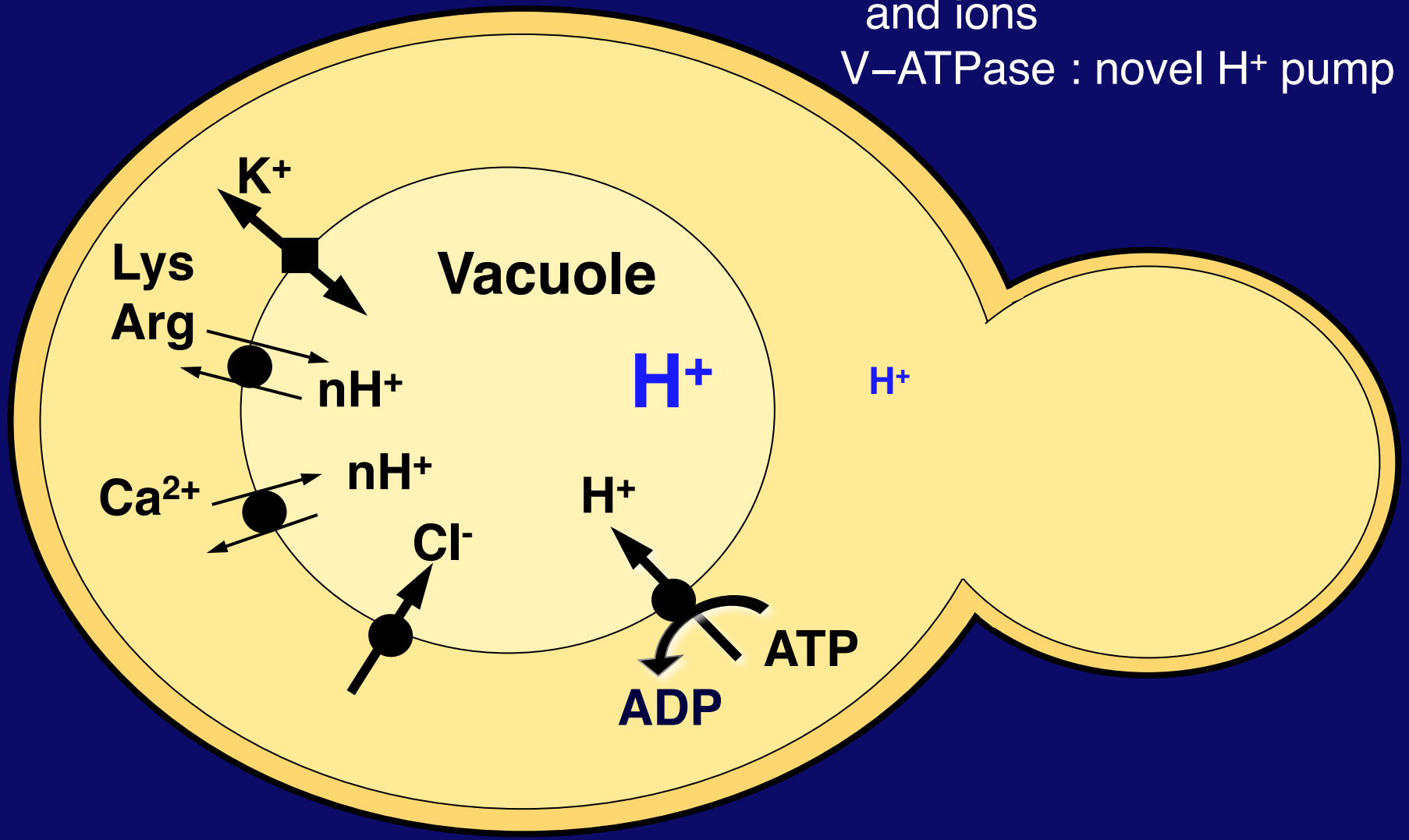
defense: secondary metabolites, pigments, alkaloids

inhibitors

gravitropism etc

Transport Systems of Vacuolar Membrane

Active transport :
homeostasis of amino acids and ions
V–ATPase : novel H^+ pump



In 1988 lab at the College of Arts and Sciences, The University of Tokyo

Lytic function of the yeast vacuole

Acidic compartment

Various hydrolytic enzymes

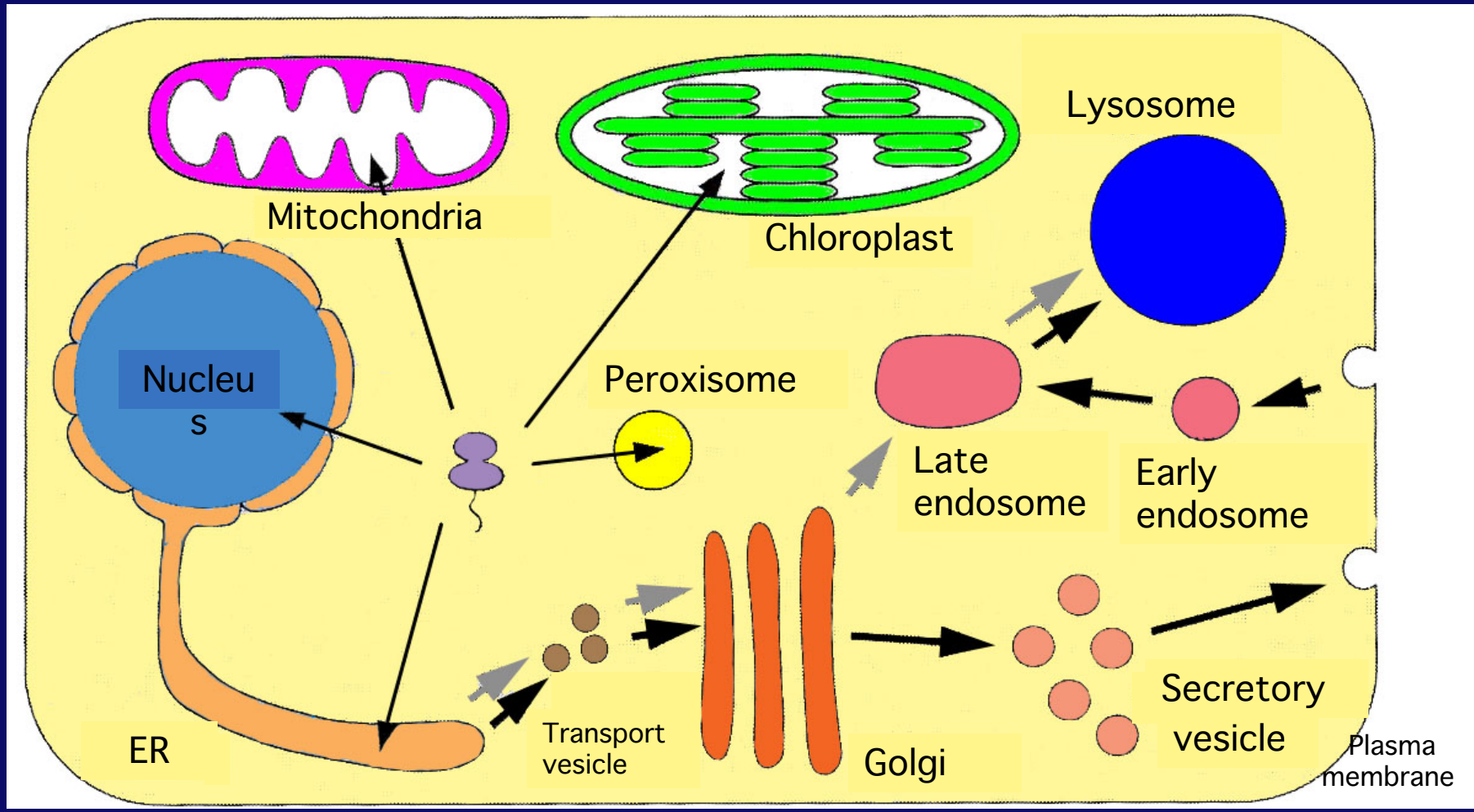
proteinase, peptidase, nucleotidase

mannosidase, phosphatase etc.

homologous to the lysosome in mammals?

The Central dogma

DNA ⇒ RNA ⇒ Protein { Folding, Trafficking



A question
a biology class for first-year undergraduate students at of The University of Tokyo

How many red blood cells are made per second in an average human body?

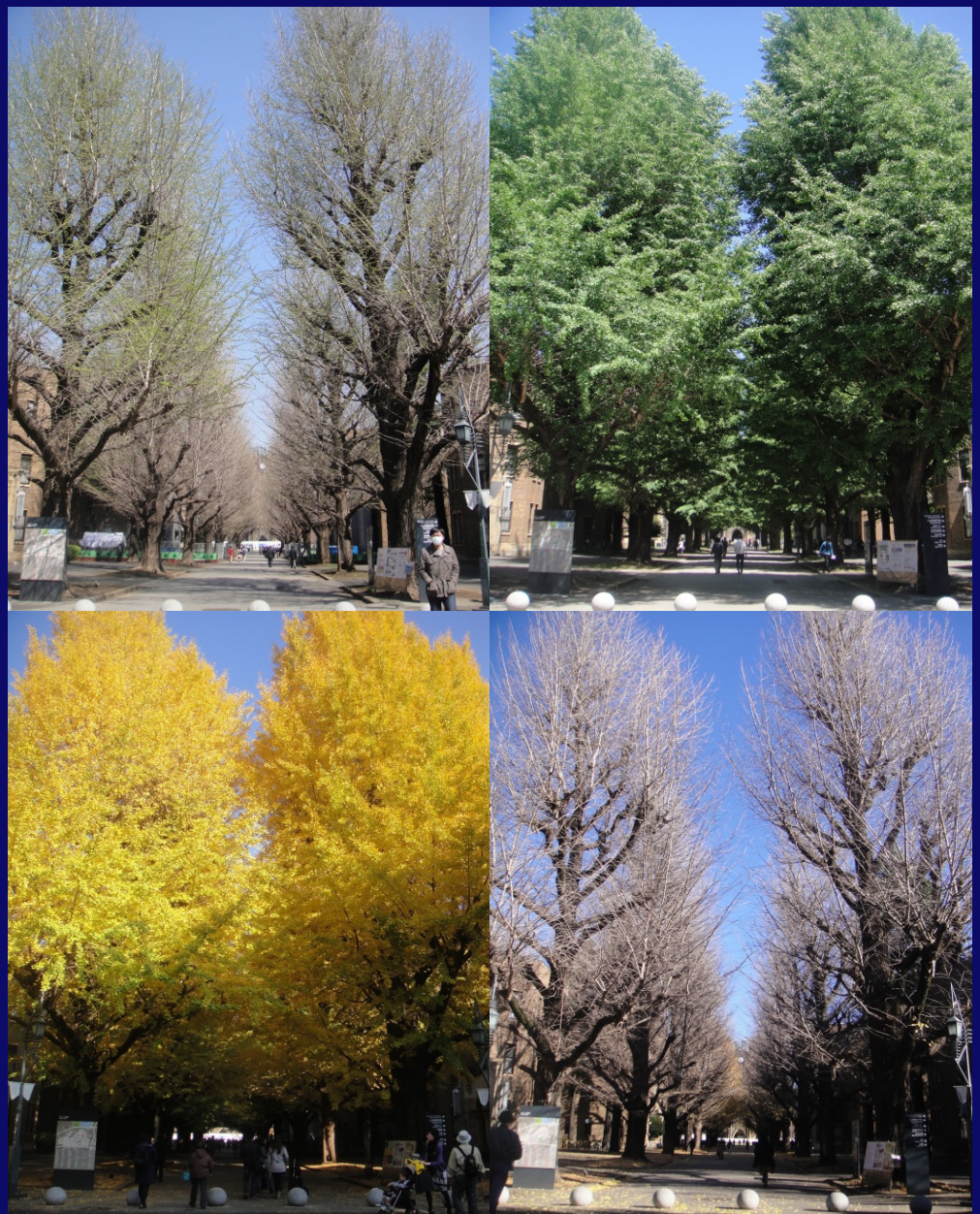
Red blood cell :

3×10^{6} cells/sec

Hemoglobin :

1×10^{15} molecules/sec

Four seasons in Japan

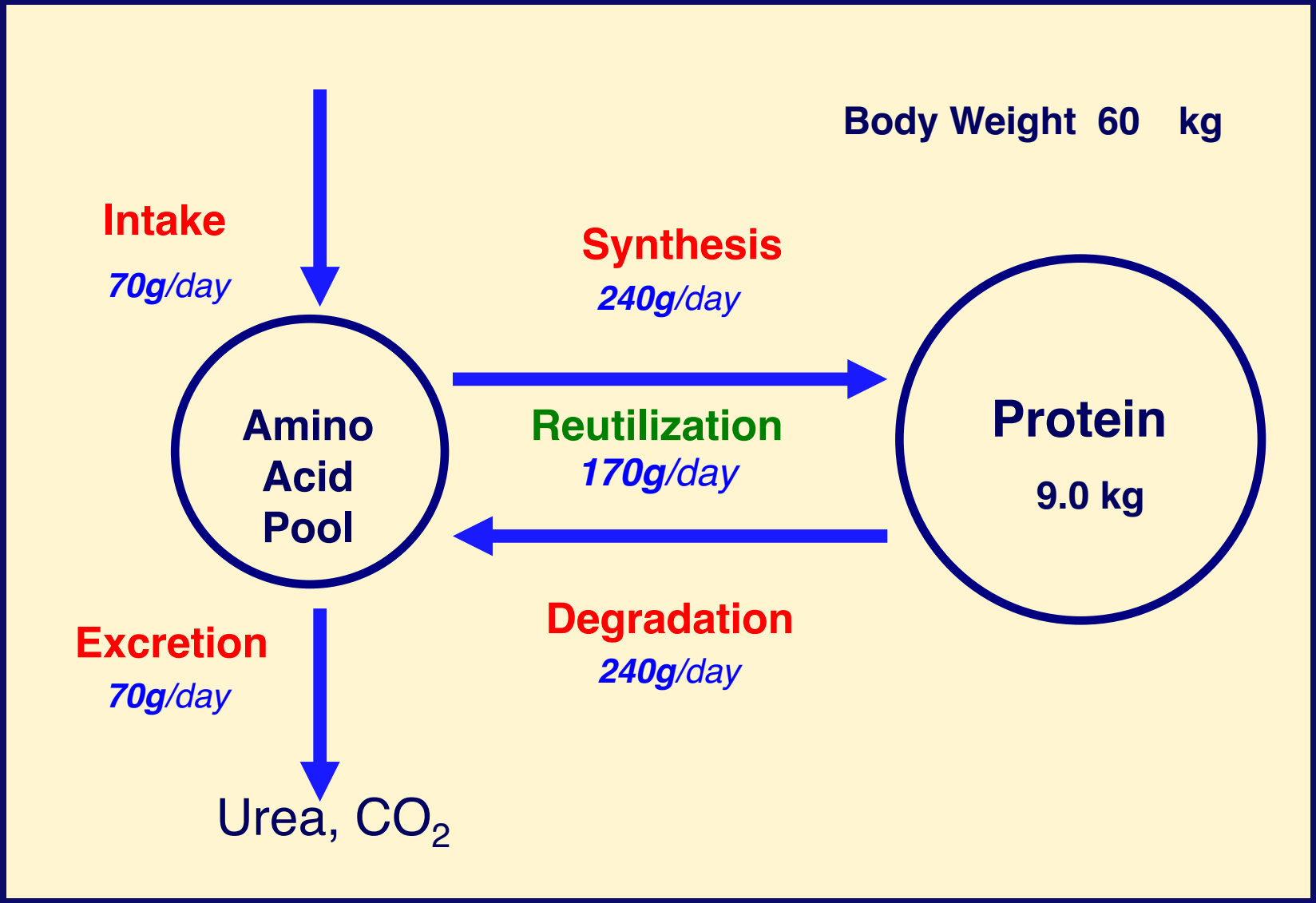


生々流転
諸行無常

All things are in a state of relentless and ephemeral flux

Rice

Protein Dynamics in Body

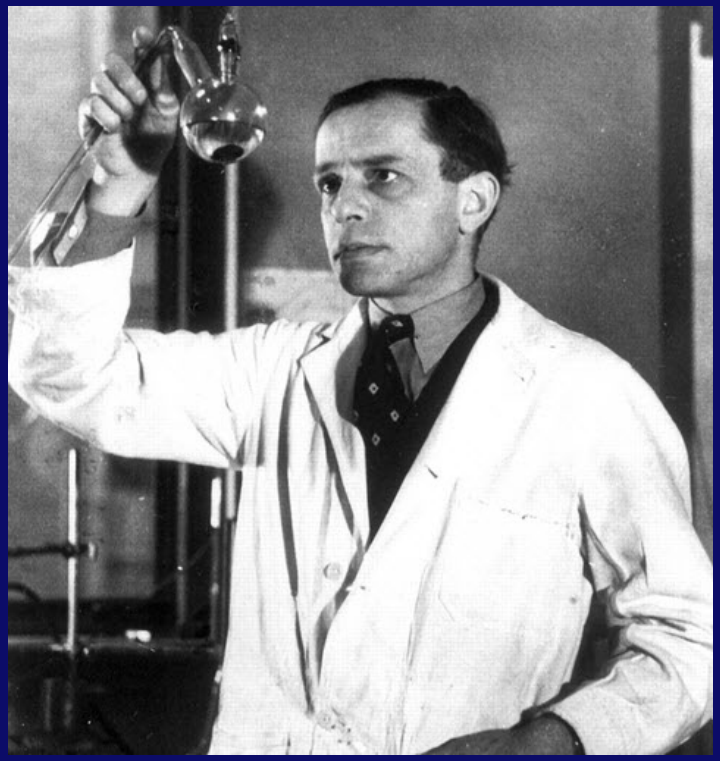


Life is in an equilibrium state between synthesis and degradation of proteins.

- replacement of most proteins every 3 months
- “difference between organisms and machine”

Recycling is essential for life

- important ability for survival against starvation
- critical selection factor in evolution



Rudolf Schoenheimer
(1898-1941)
Biochemist

STUDIES IN PROTEIN METABOLISM

X. THE METABOLIC ACTIVITY OF BODY PROTEINS INVESTIGATED WITH *l*(−)-LEUCINE CONTAINING TWO ISOTOPES*

By RUDOLF SCHOENHEIMER, S. RATNER, AND D. RITTENBERG

(From the Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York)

(Received for publication, July 28, 1939)

Adult animals on an adequate diet, in nitrogen equilibrium, excrete an amount of nitrogen equivalent to that in the diet. Increase of dietary amino acid or protein results in an immediate or somewhat retarded excess excretion of nitrogen corresponding to the additional intake. It has usually been assumed that the urinary nitrogen is mainly of dietary origin. Almost all investigators, however, have postulated the occurrence of at least some replacement of body proteins necessary for repair of losses due to wear and tear (maintenance quota). The classical "balance" experimentation has been unable to measure the extent of this normal replacement.

J. Biol. Chem., 1939

"Protein Turnover"

using isotope as a tracer

1955

Discovery of the Lysosome

EM analysis by Rockefeller group

1962

Auto (Self) - Phagy (Eating)

"Autophagy"

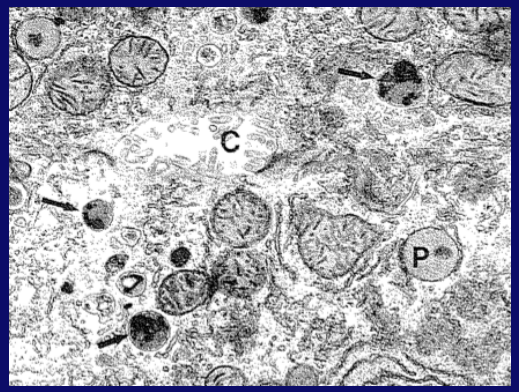
Christian de Duve

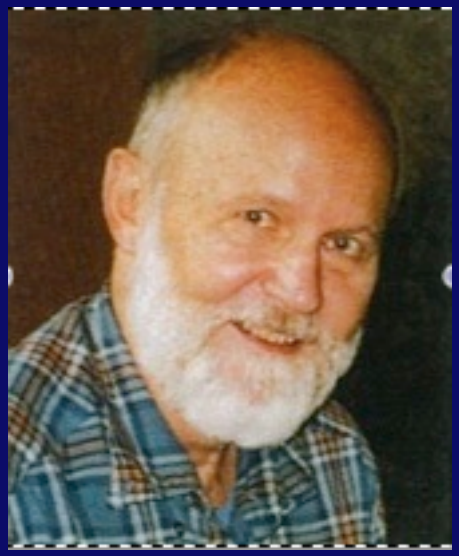
Rockefeller University

Nobel Prize (1974)

Glenn E. Mortimore

Liver perfusion





Per O Seglen (1943 -)

Cultured liver cell

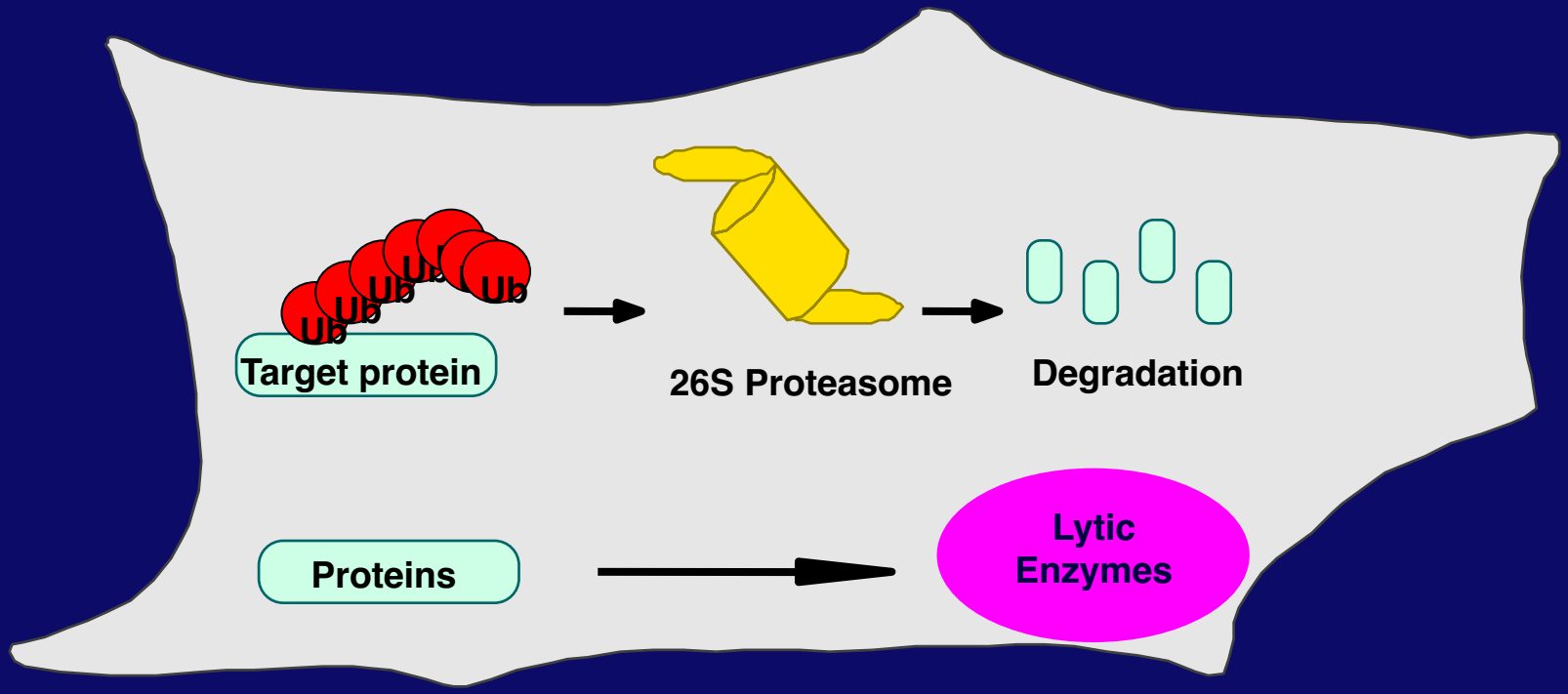
Cell Biologist

Non-selective proteolysis

Physiological Regulation

by amino acids, hormone

Two major intracellular protein degradation systems

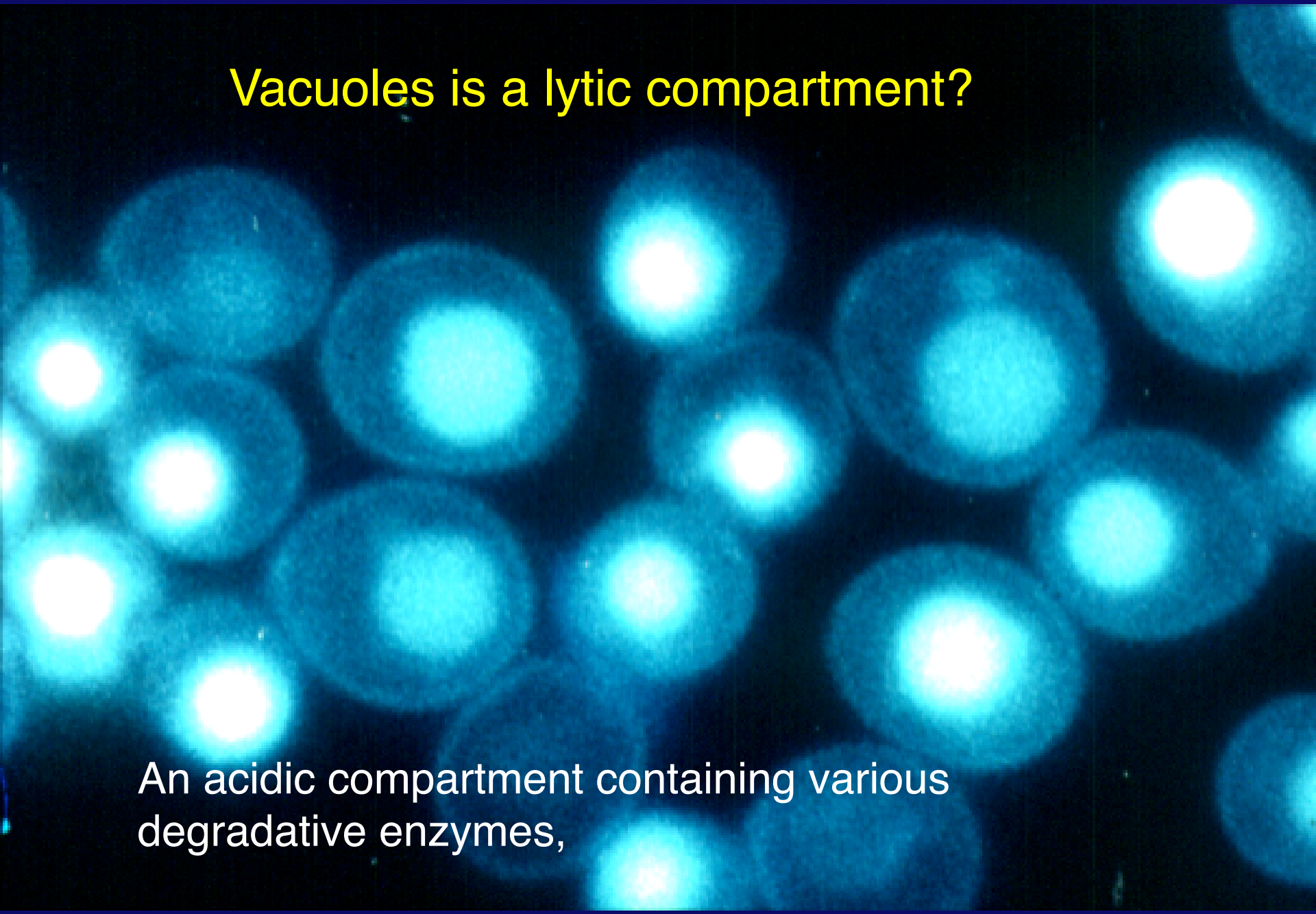


Ubiquitin/Proteasome System
- Specific target recognition
- Short-lived Proteins

Nobel prize in Chemistry 2004
- Aaron Ciechanover
- Avram Hershko
- Irwin Rose

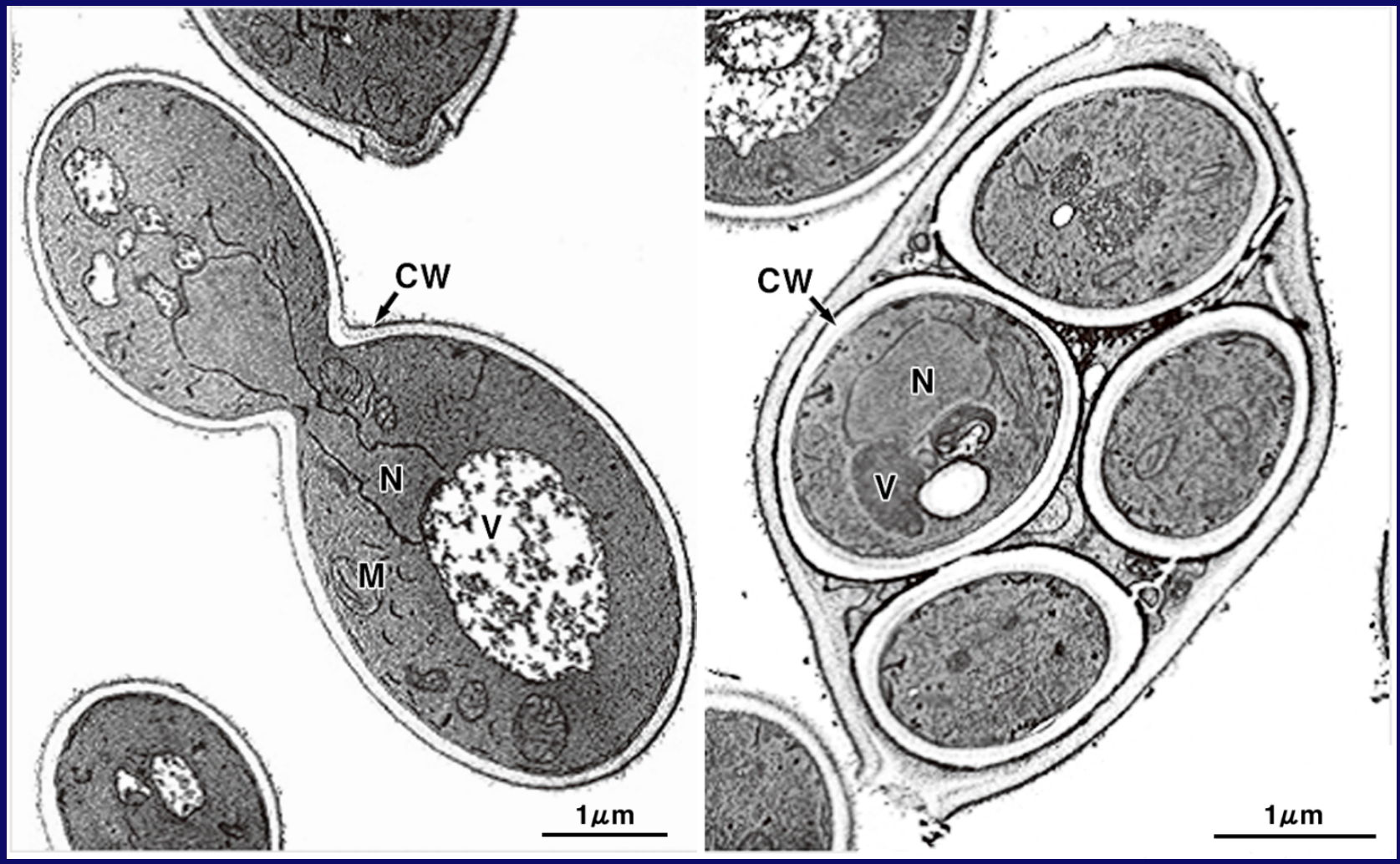
Lysosome/Vacuolar System
- Bulk and Non-selective
- Long-lived Proteins

Vacuoles is a lytic compartment?



An acidic compartment containing various degradative enzymes,

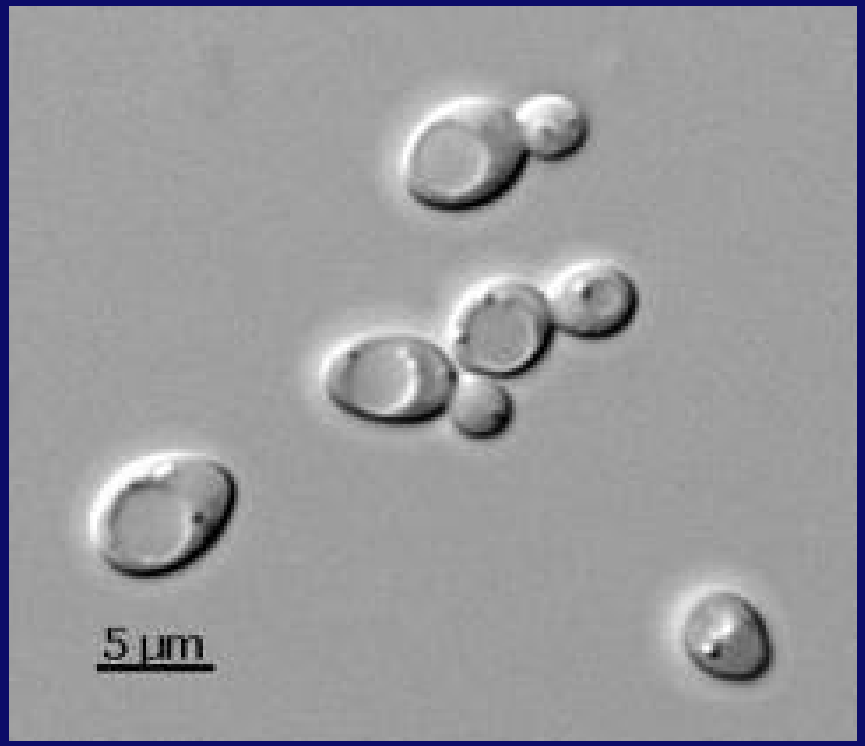
Growing cell → (-N starvation; cellular remodeling) → Spores



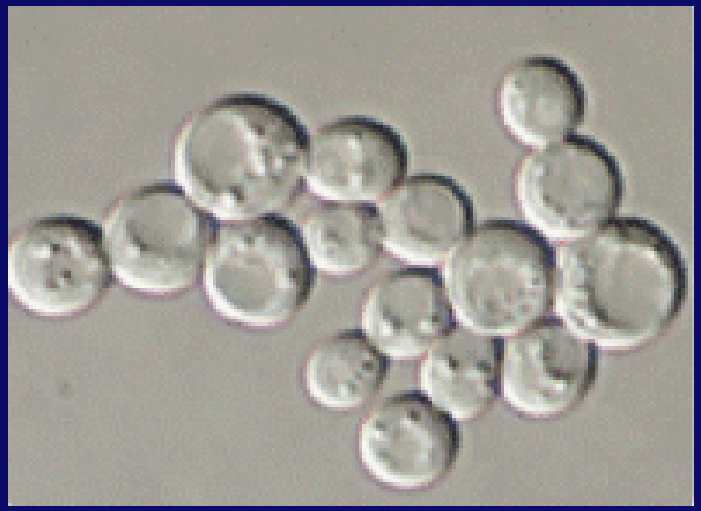
by Masako Osumi

Yeast cells under a light microscope

Vacuole: sole organelle visible under a light microscope
relatively large size, no structure inside
low protein concentration, low viscosity



Phase Contrast



Nomarski

Starvation of vacuolar proteinase-deficient mutants

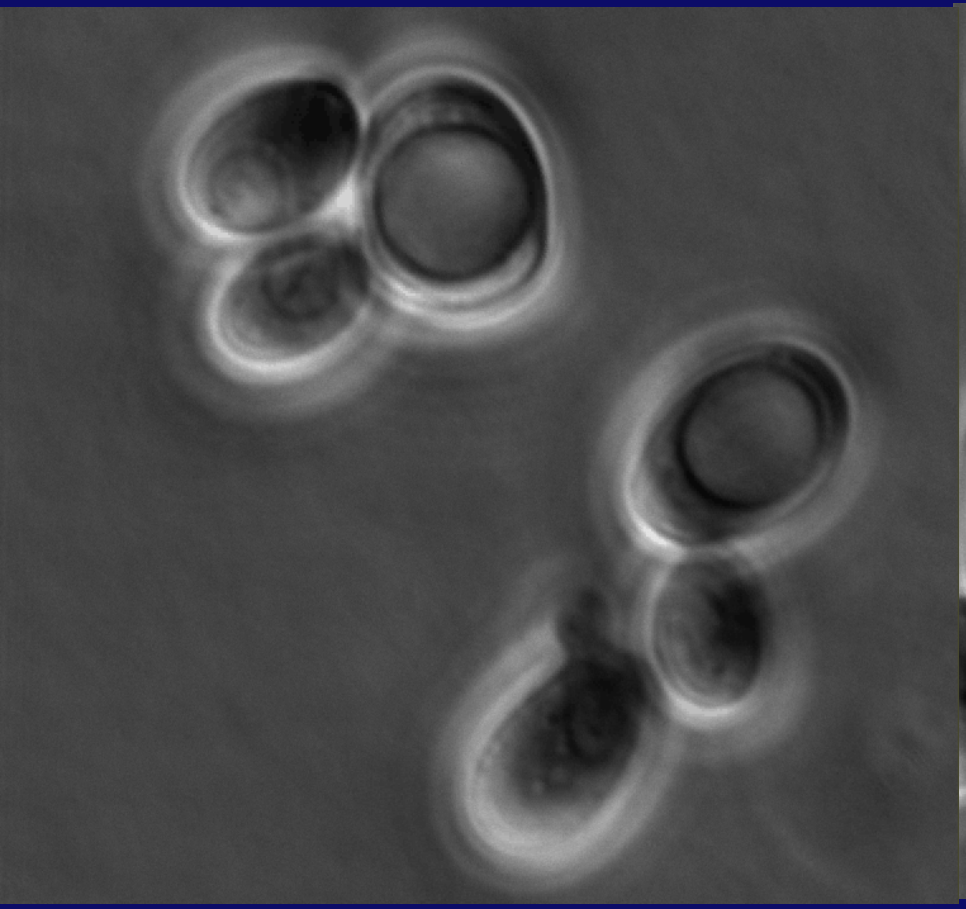
BJ-926 : ***a prb1-1122 pre1-407 pep4-3 leu2 trp1 ura3-52***
α prb1-1122 pre1-407 pep4-3 leu2 trp1 ura3-52

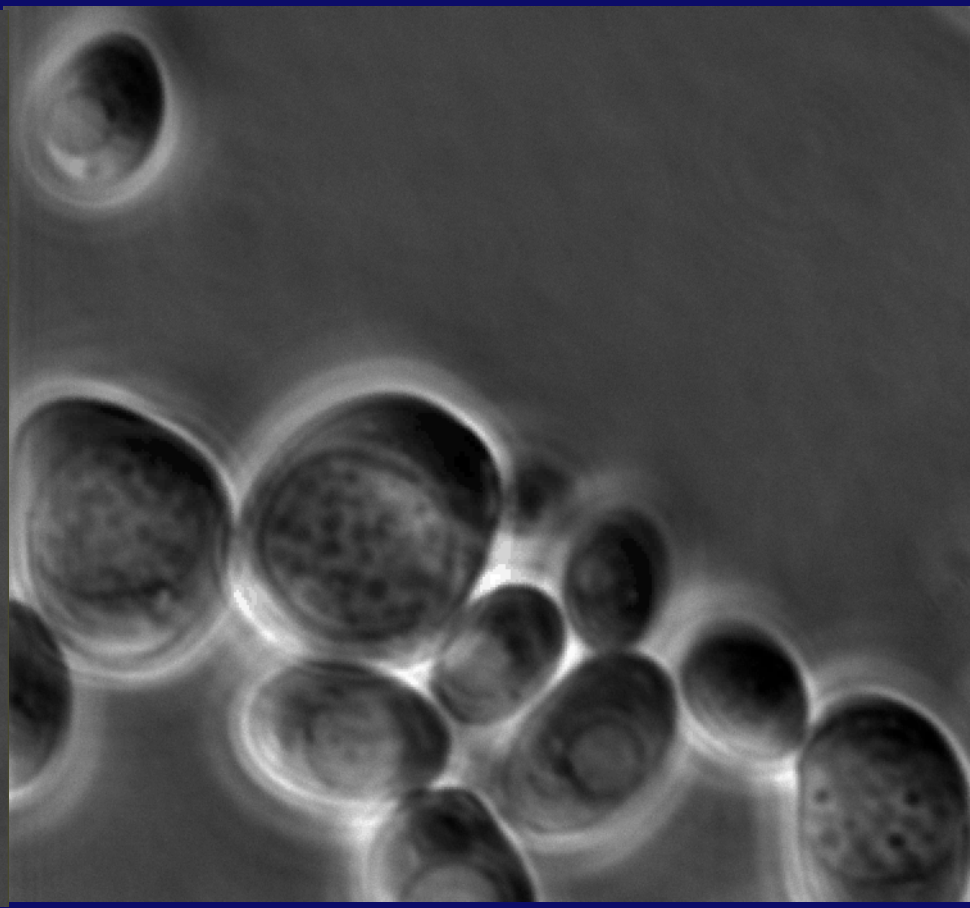
Strains constructed by Elizabeth W. Jones

Cells grown in nutrient rich medium (YEPD)

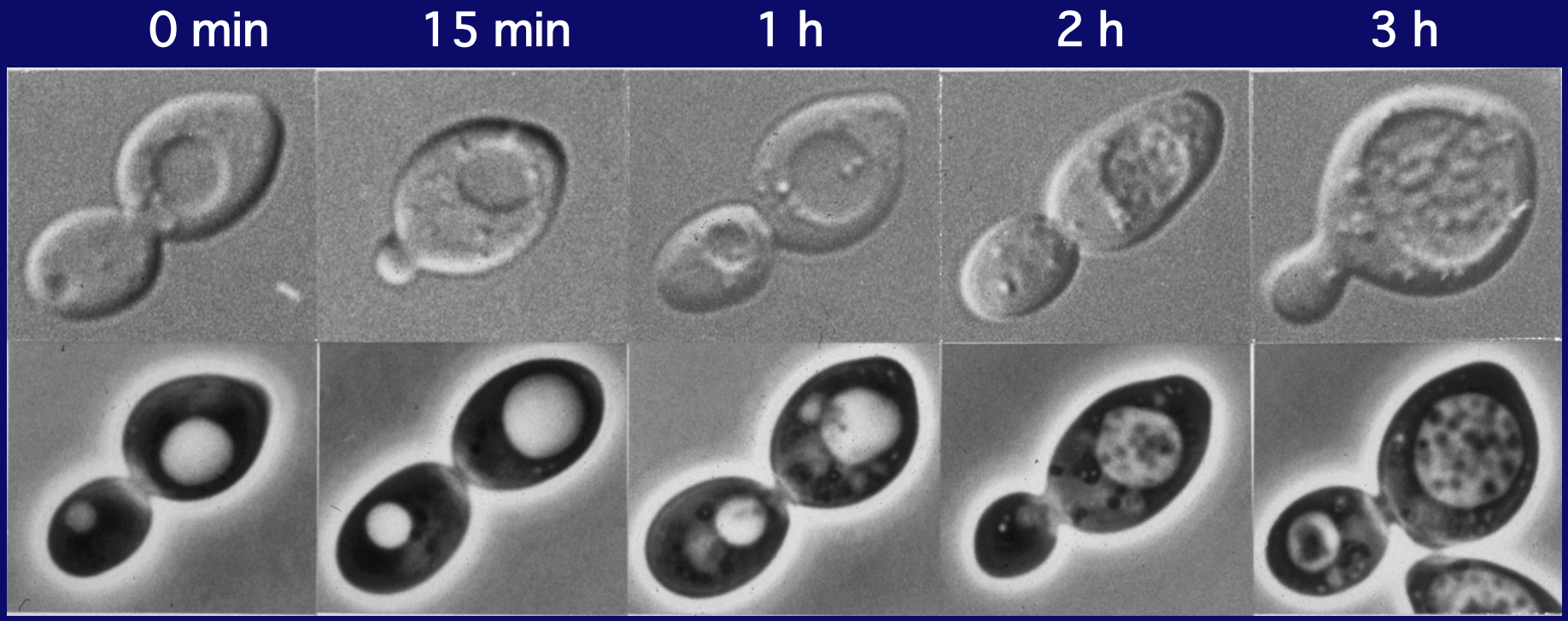
Nitrogen-Starvation medium (SD(-N))

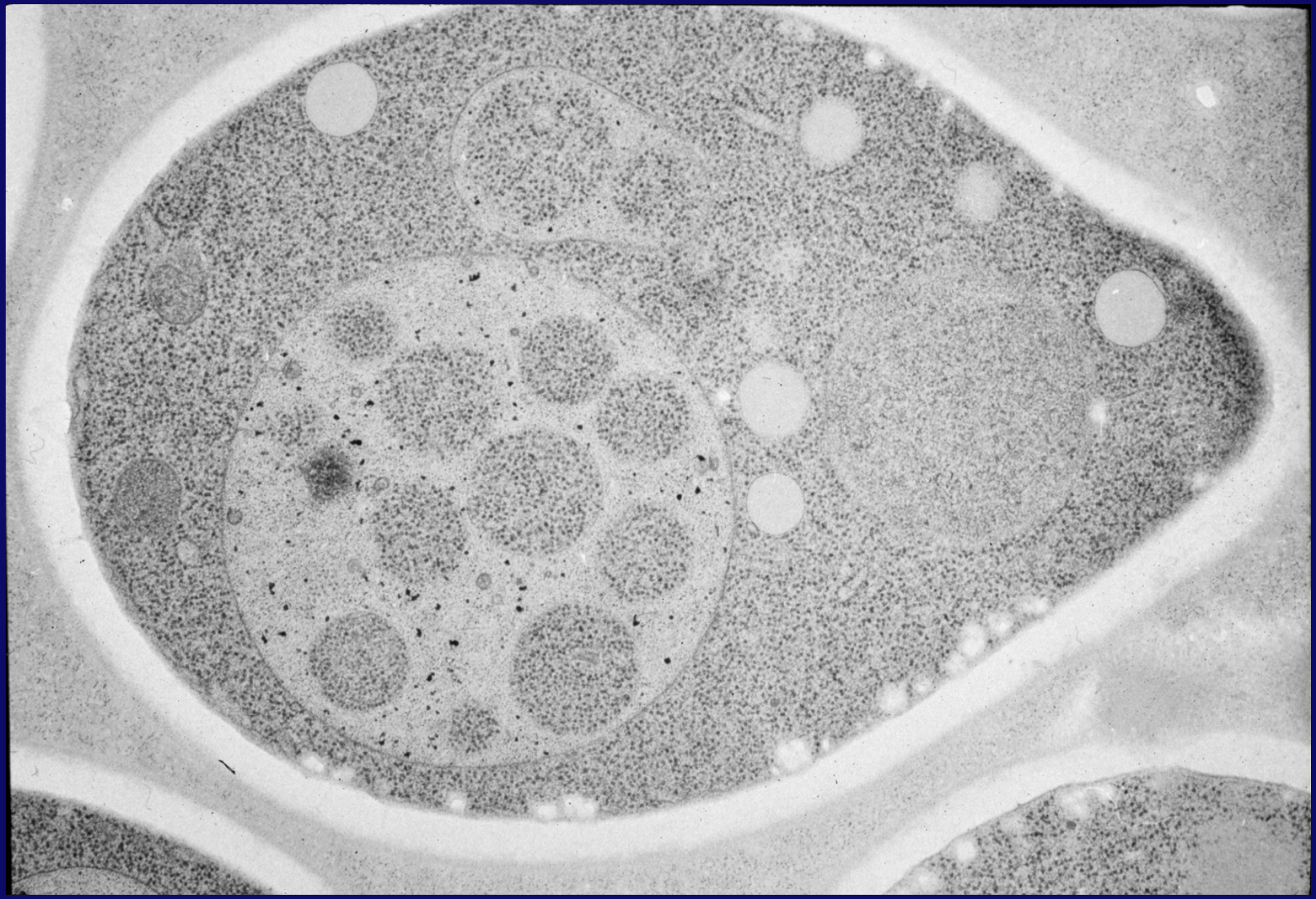
Morphology of the vacuole of proteinase-deficient mutant strains subjected to nitrogen starvation



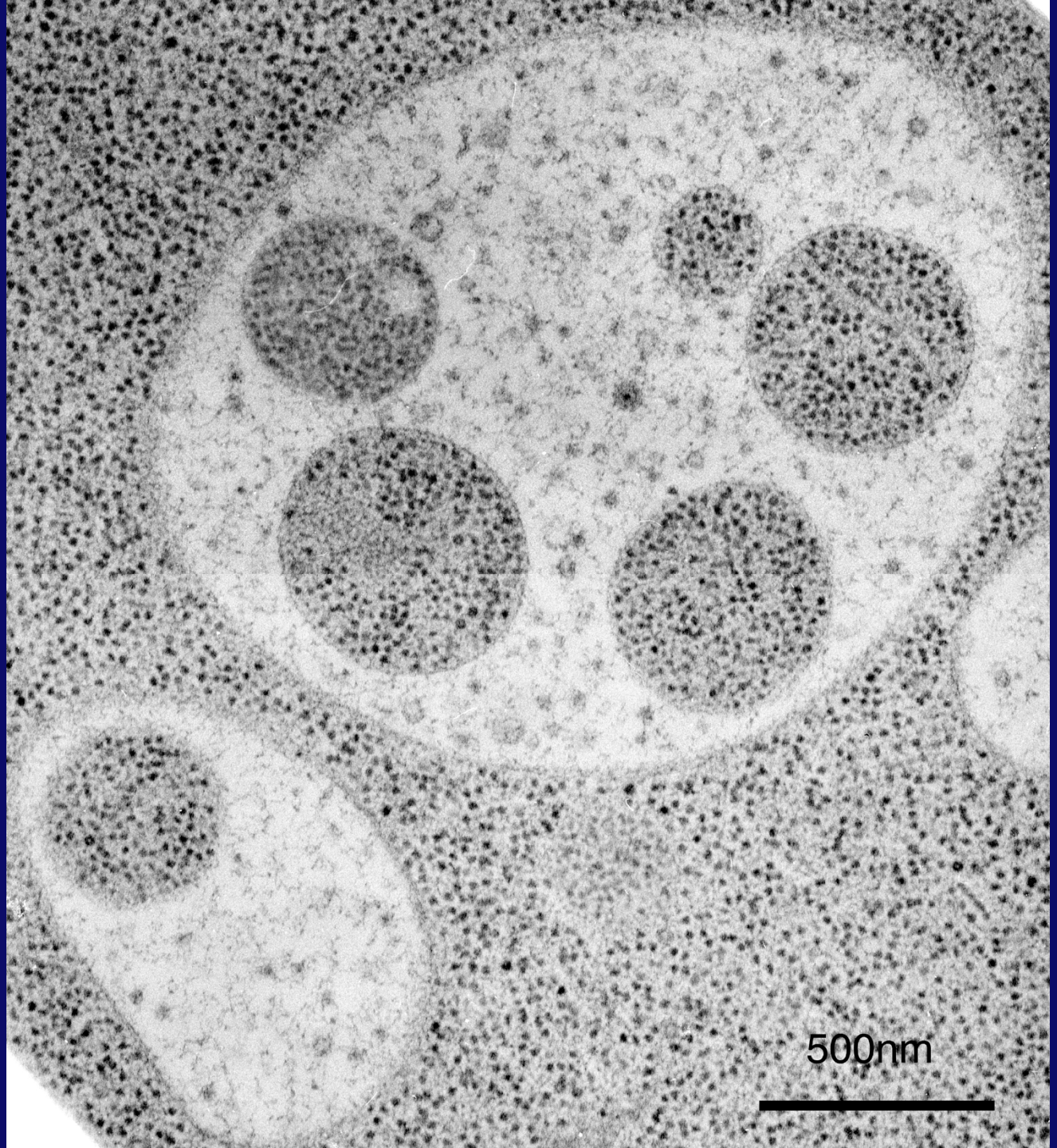


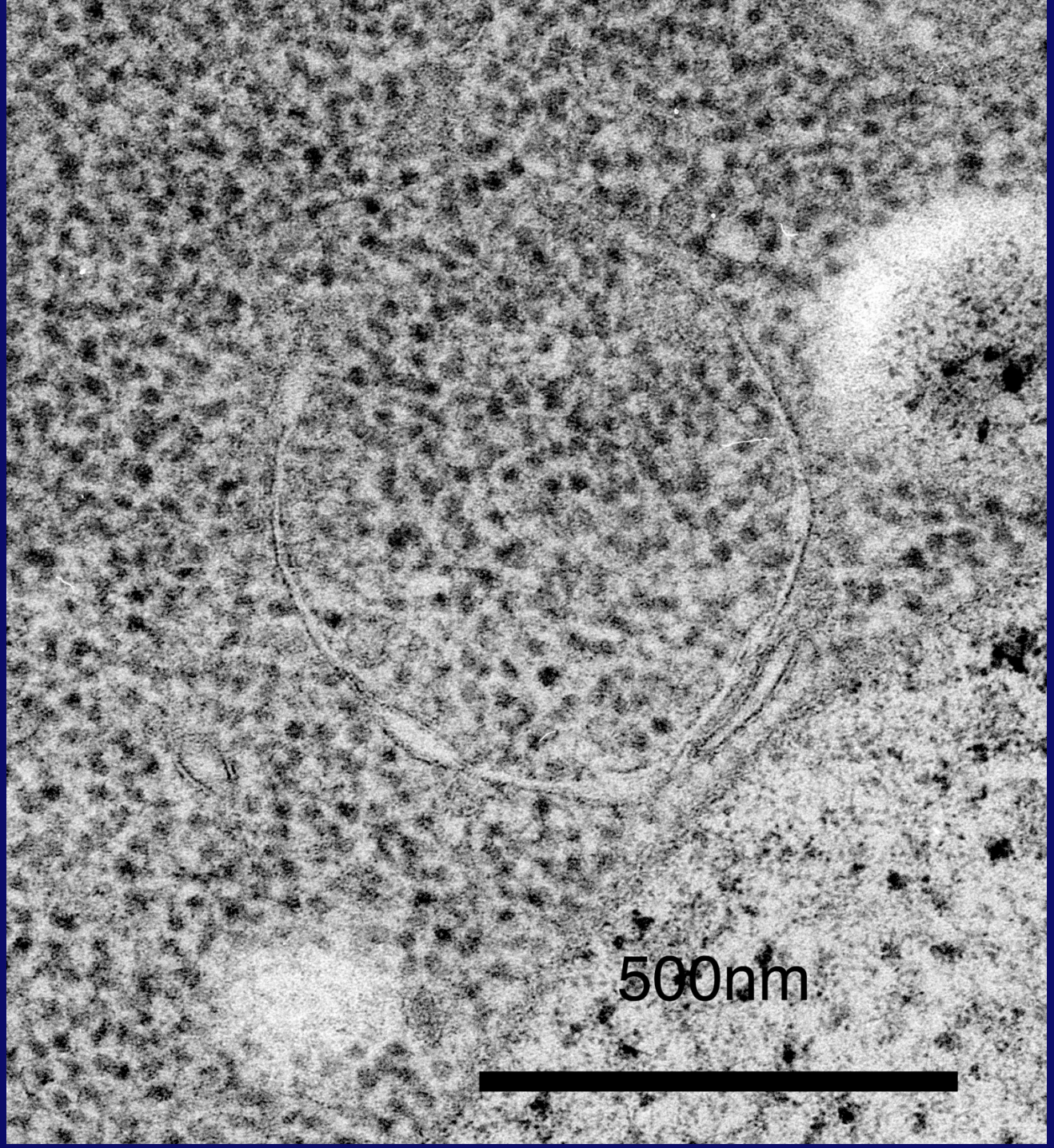
Time course of accumulation of autophagic bodies

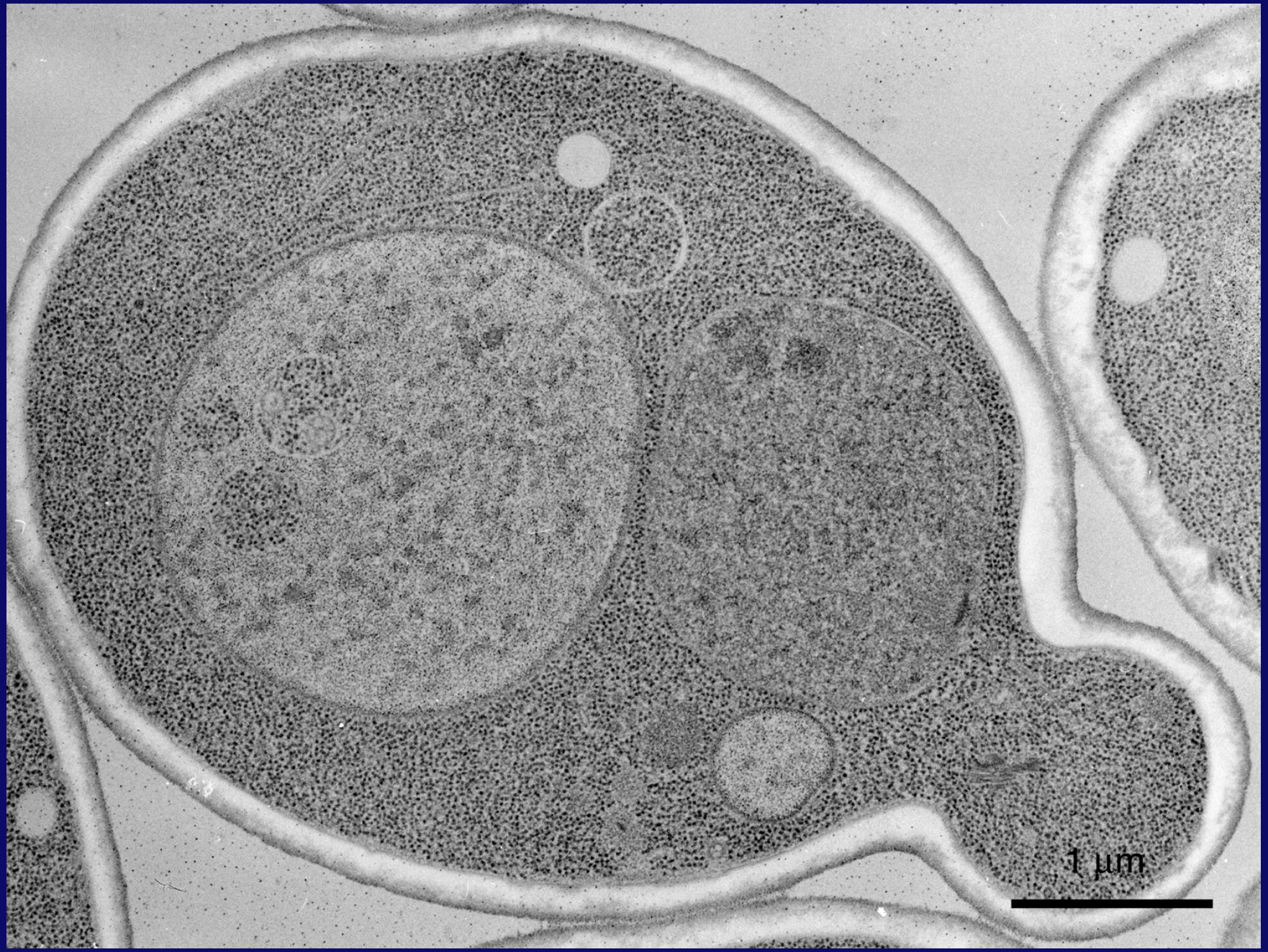




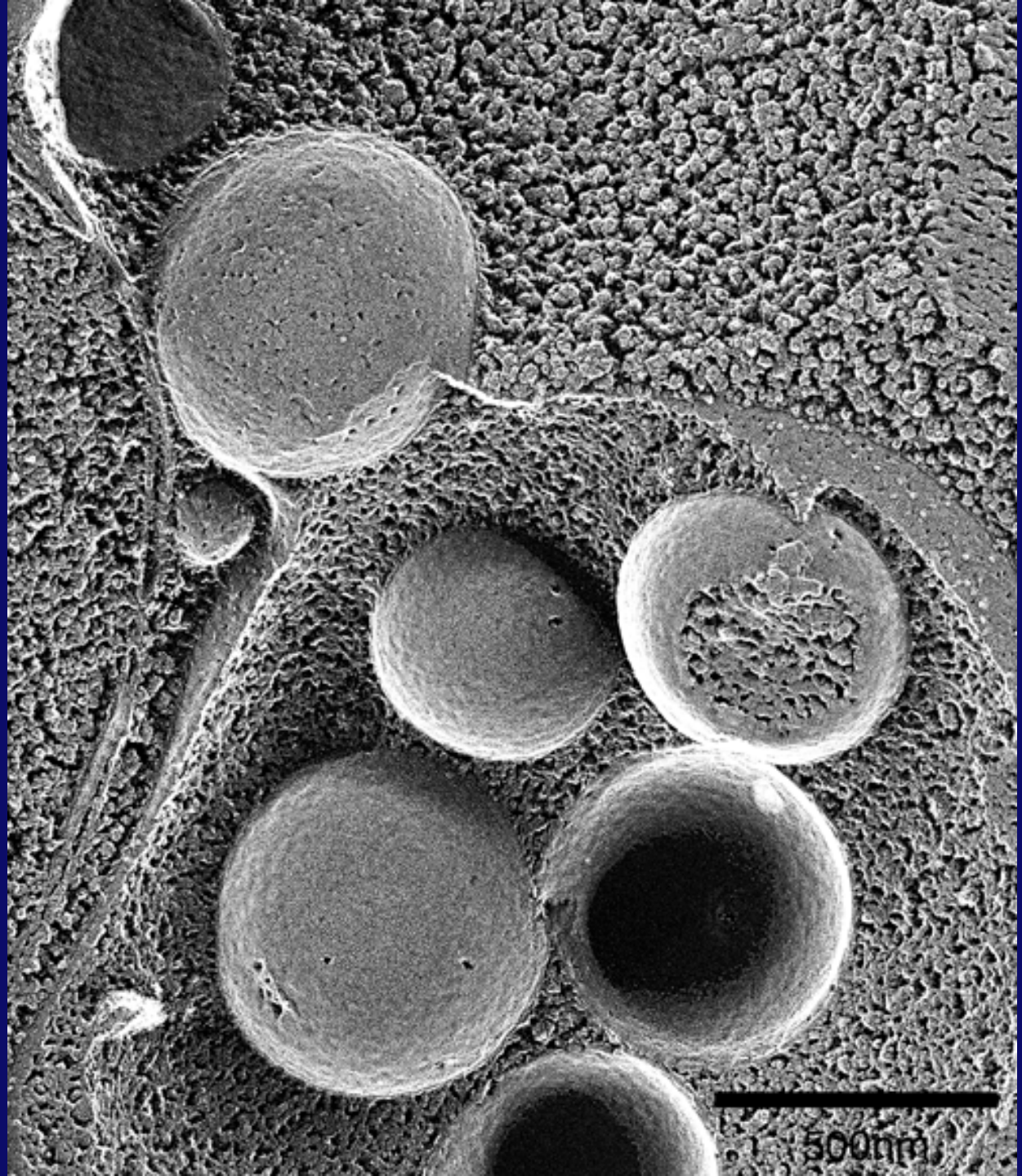
Takeshige et al. 1992

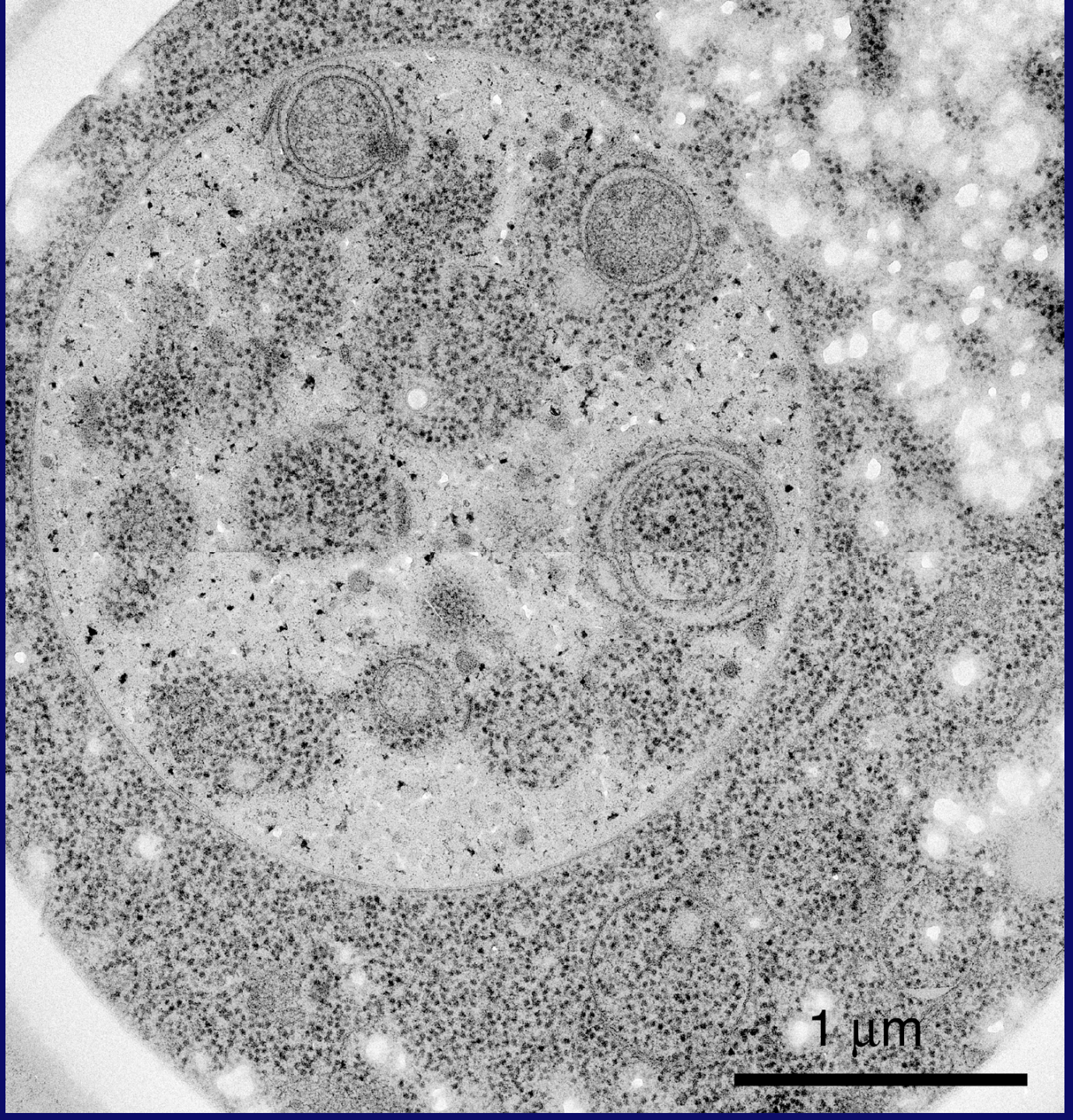

500nm


500nm

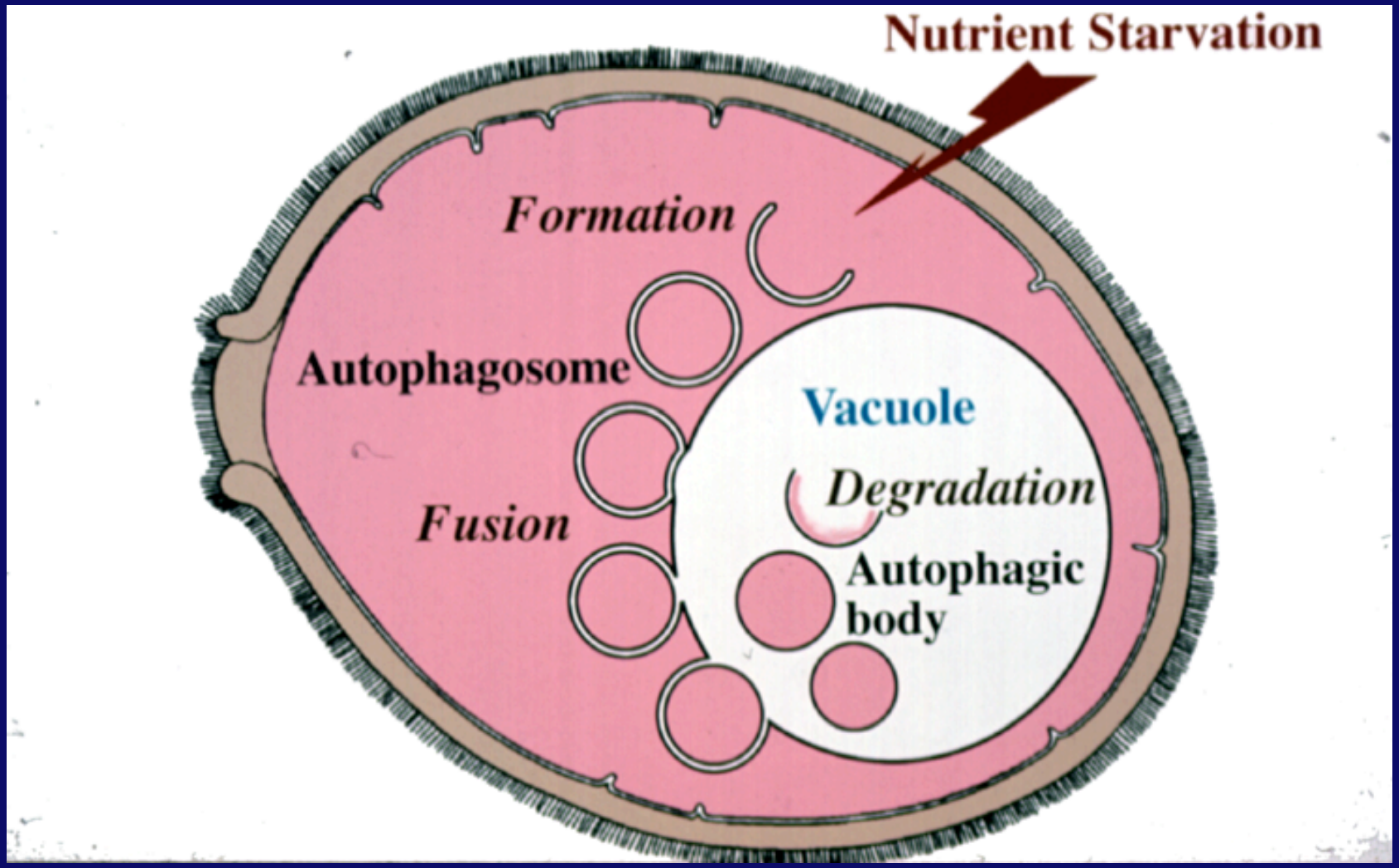


Baba et al. 1994


500nm


1 µm

The autophagic process in yeast



By M. Baba

Using genetics to understand autophagy

cdc mutants : cell cycle

By Lee H. Hartwell, Nobel Prize 2004

sec mutants: secretory pathway

By Randy W. Schekman, Nobel Prize 2013

Isolation of autophagy-defective mutants

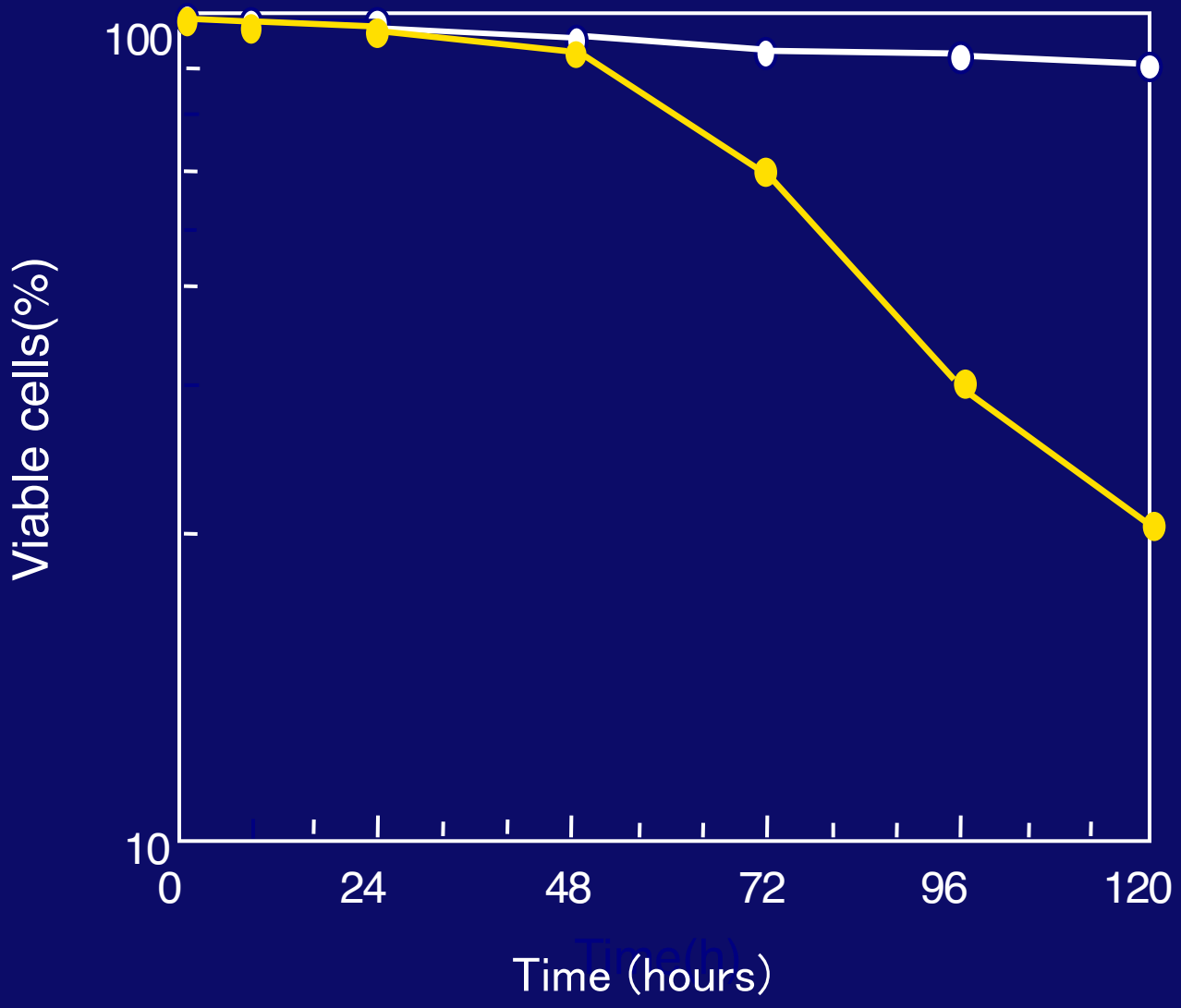
What is the phenotype of the mutants?

Microscopic screen : no accumulation of autophagic bodies in the vacuole

Only one mutant, *apg1*-1 (*atg1*)

Miki Tsukada

Loss of viability of *atg1* mutant cell under N-starvation



Isolation of *apg* (*atg*) mutants

EMS treated cells

↓

first screen by plate assay for the loss viability in SD(-N) medium — 38000

↓

second screen for lack of autphagic body accumulation by light microscopy under starvation — 2700

↓

complementation analysis — 99

↓

apg (atg) mutants — 14

Tsukada and Ohsumi 1993

ATG genes

A set of genes encoding machinery essential for the unique membrane dynamics of autophagosome formation

Why are so many genes are yet to be unidentified?

Most researchers interested in "essential genes" in extremely rich medium, such as YEPD.

atg mutants can grow normally and show little phenotype under growing conditions.

What is encoded by the *ATG* genes?

Cloning of *ATG* genes

Sequencing of *ATG* genes

Identification of Atg proteins

→ A group of novel uncharacterized genes

no hint about protein function

1988-

College of Arts and Sciences, The University of Tokyo

Small lab, but foundation of autophagy research

1996-

National Institute of Basic Bilogy

Tamotsu Yoshimori

Takeshi Noda & Yoshiaki Kamada

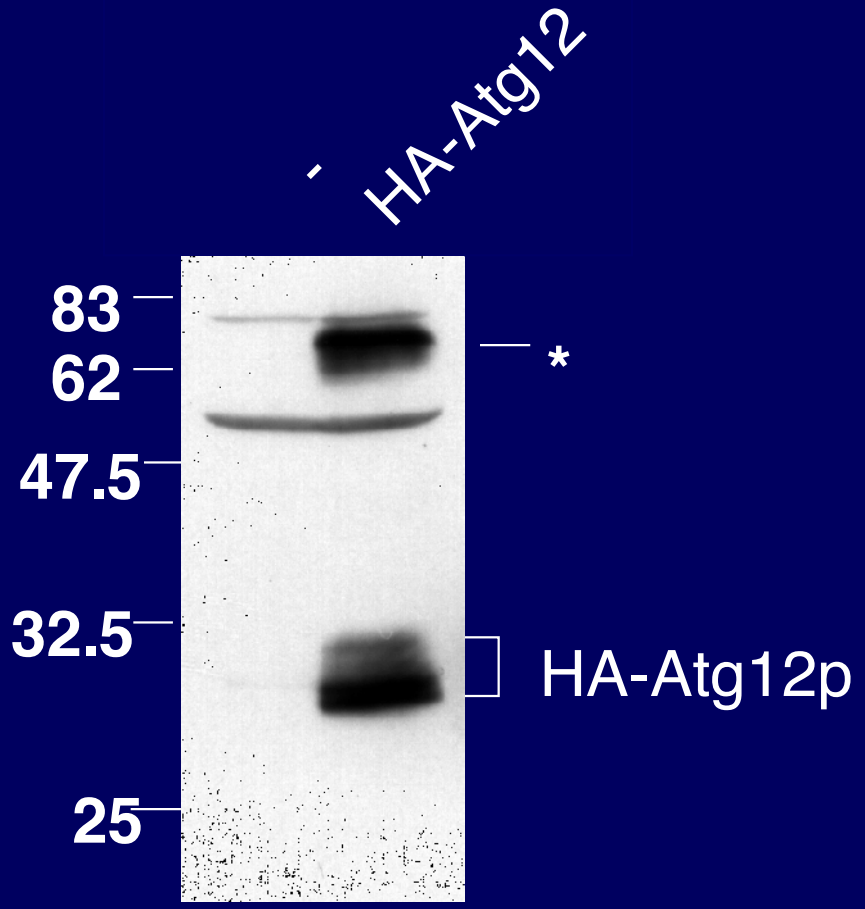
Noboru Mizushima,

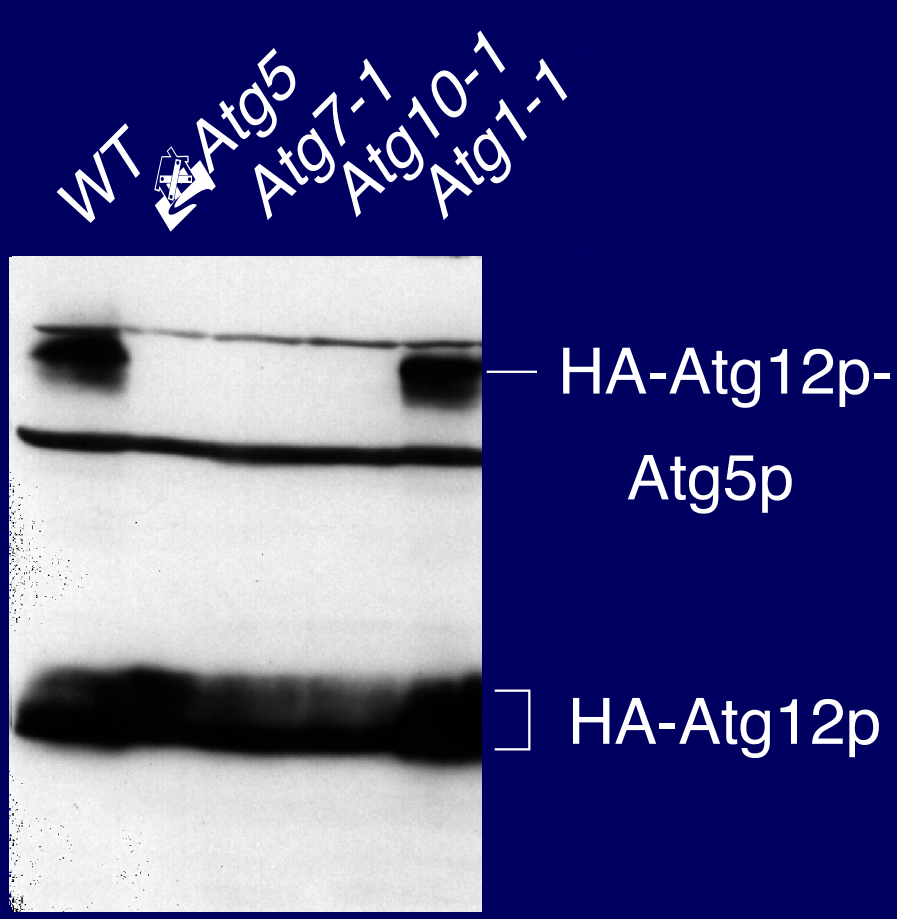
then Post Docs, Graduate Students

Cloning of ATG genes, collaboration with Mariko Ohsumi

Autophagy in Yeast, Mammals, and Plant

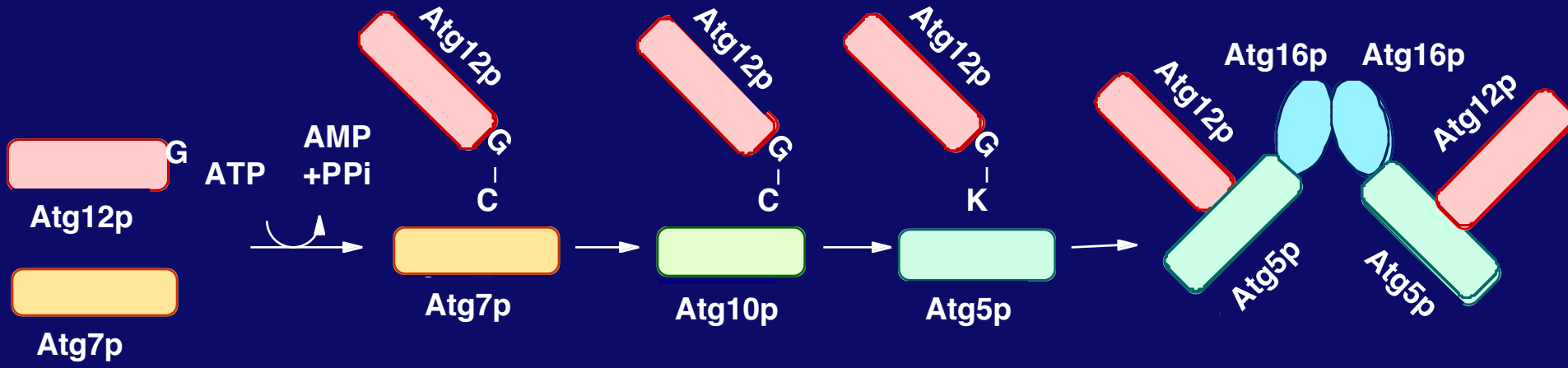
Atg12 is covalently attached to Atg5



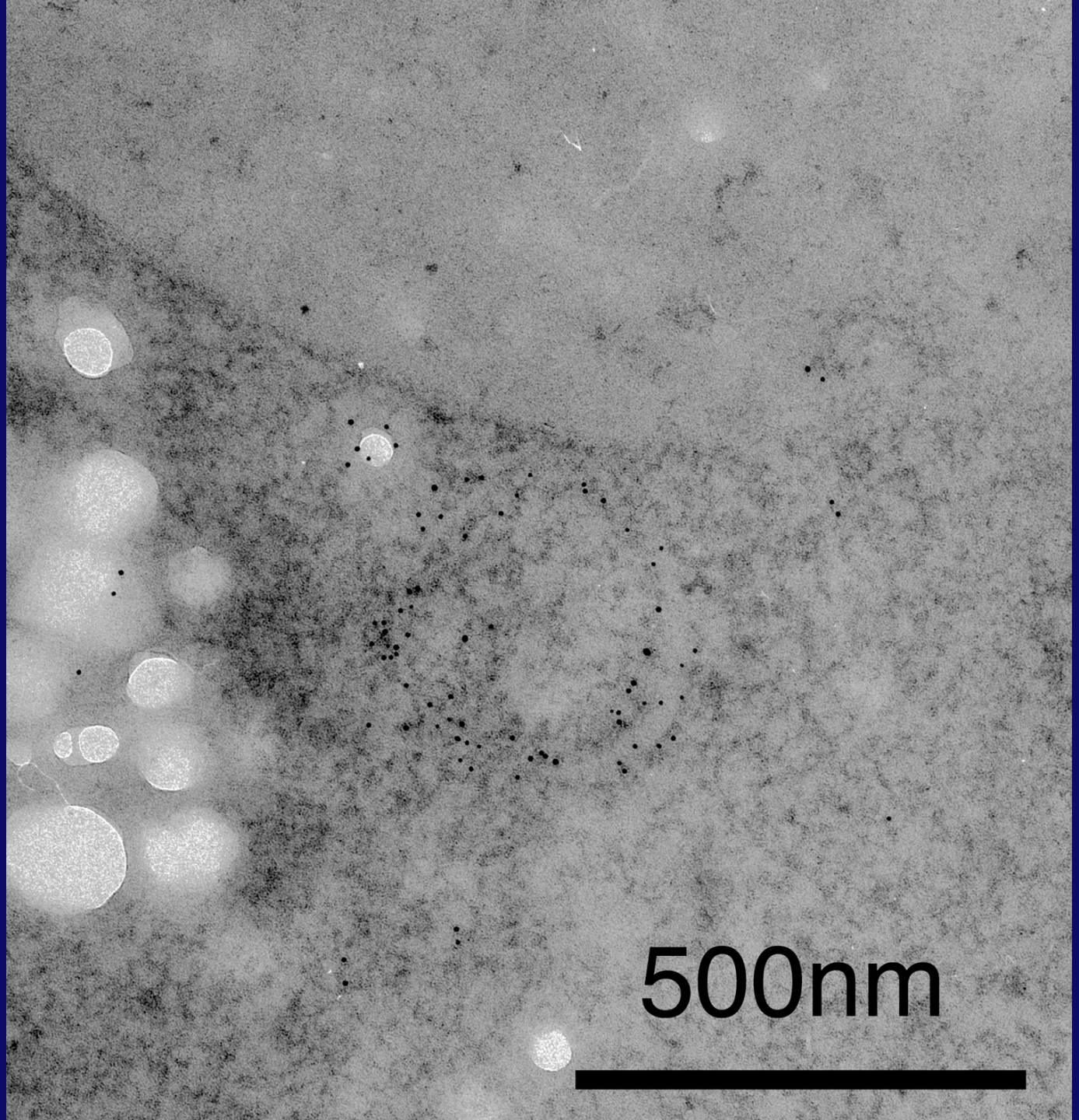


Mizushima et al. 1998

The Atg12 conjugation system

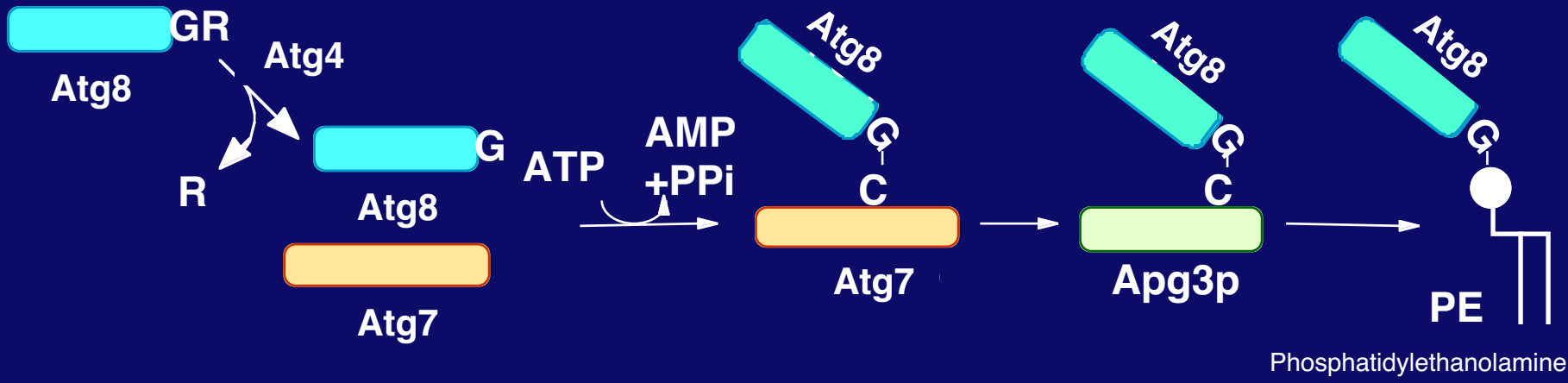


1. Atg12 is synthesized as an active form.
2. Atg12 is much larger than Ubiquitin but Ubi-fold is essential for its function.
3. Atg5 is the only target molecule for Atg12 conjugation.
4. Components of Atg12 system are constitutively synthesized.
5. Atg12-Atg5 conjugate formation is irreversible.
6.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



M. Baba

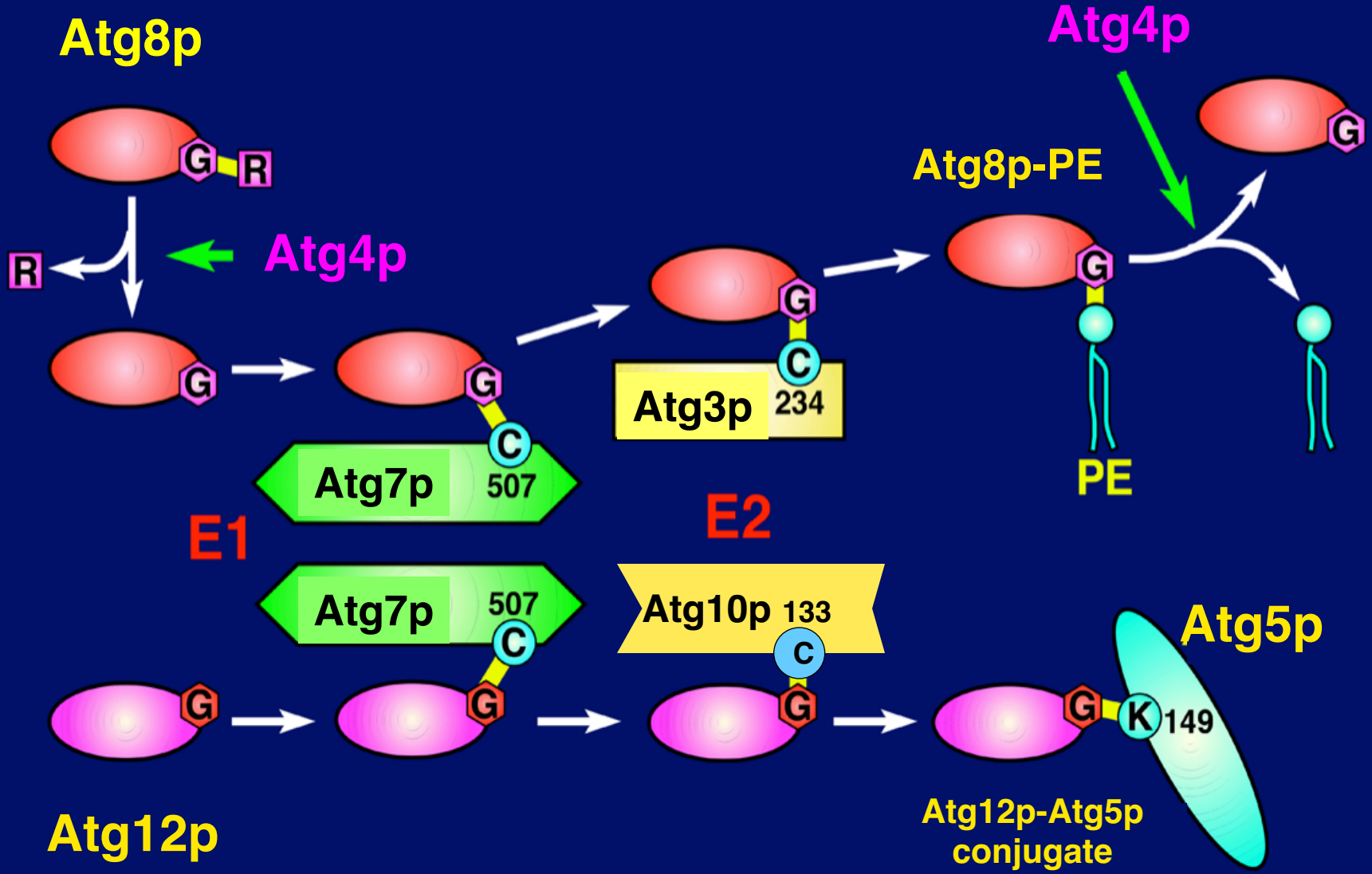
Atg8 conjugation system



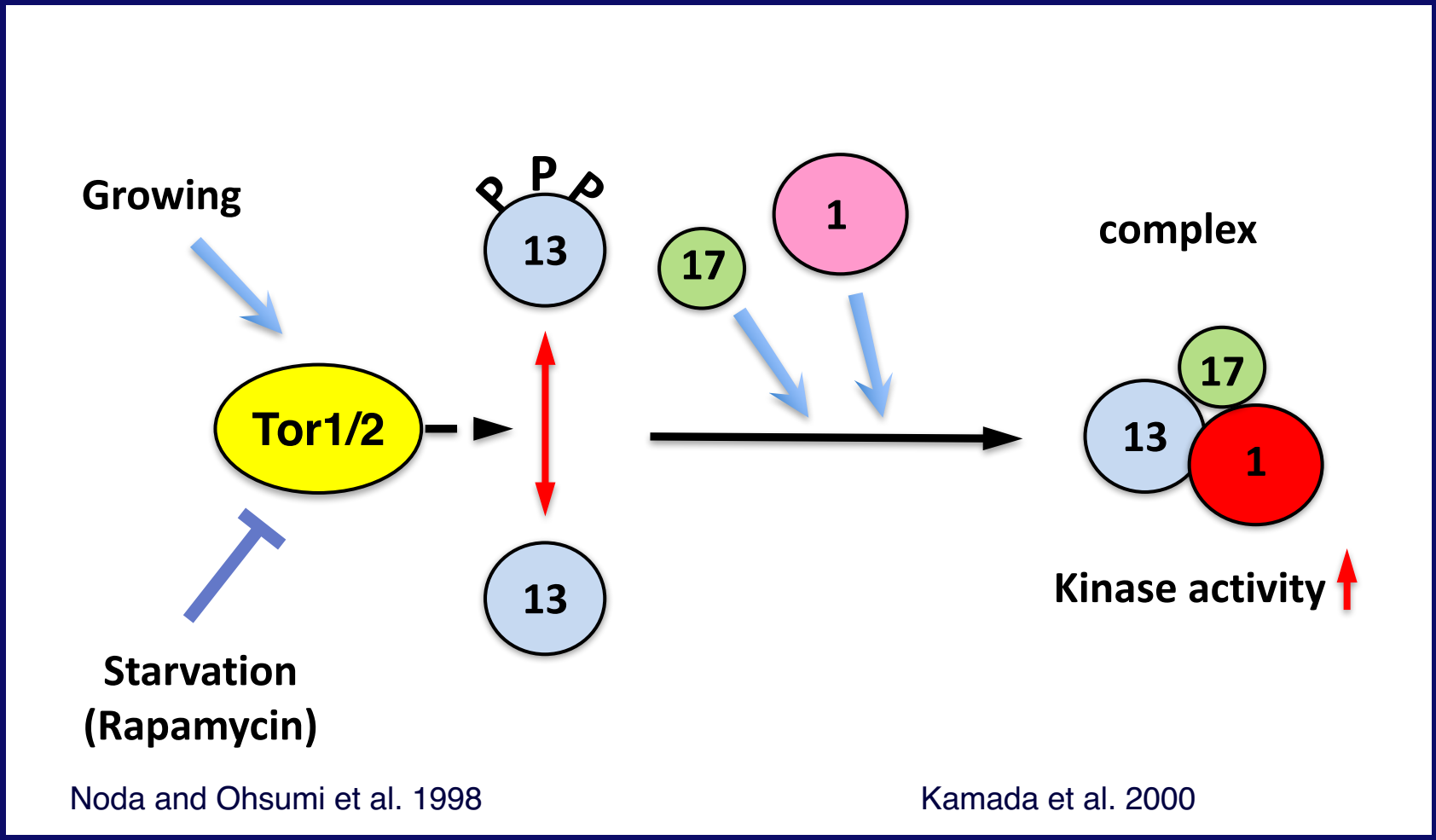
1. Atg8 forms a large protein family in eukaryotes.
2. Nascent Atg8 is processed by cysteine proteinase Atg4 to C-terminal Gly exposed form.
3. Atg8 is also activated by Atg7 E1 enzyme.
4. Deconjugation of Atg8-PE by Atg4p is necessary for normal progression of autophagy.

Kirisako et al. 2000, Ichimura et al. 2000

The Atg8 and Atg12 Systems



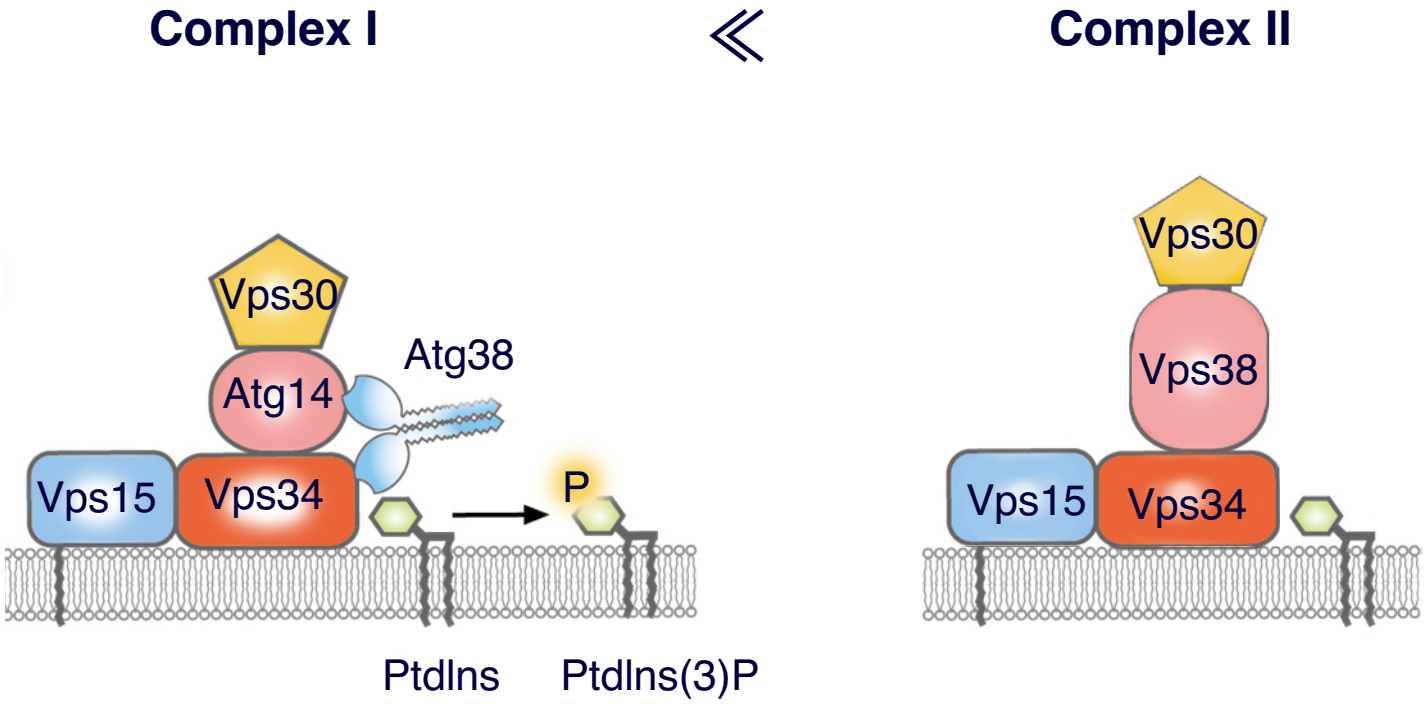
Dephosphorylated Atg13 strongly binds to Atg17 and Atg1, and enhance its kinase activity



Noda and Ohsumi et al. 1998

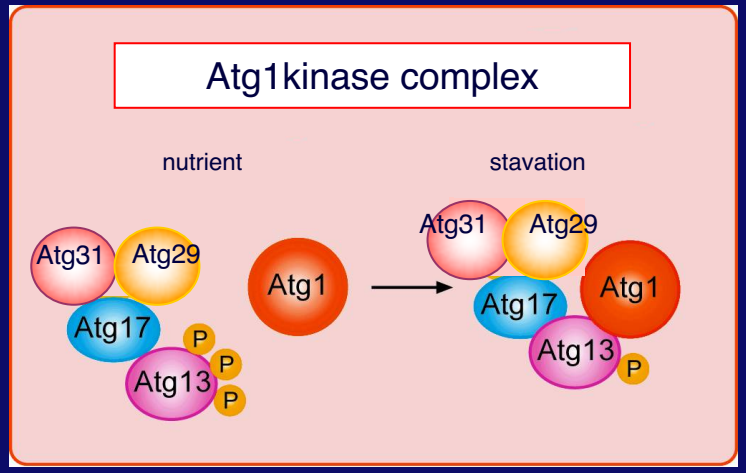
Kamada et al. 2000

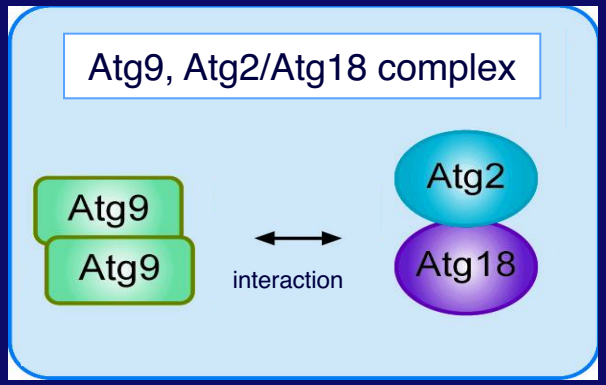
PI3 kinase essential for autophagy

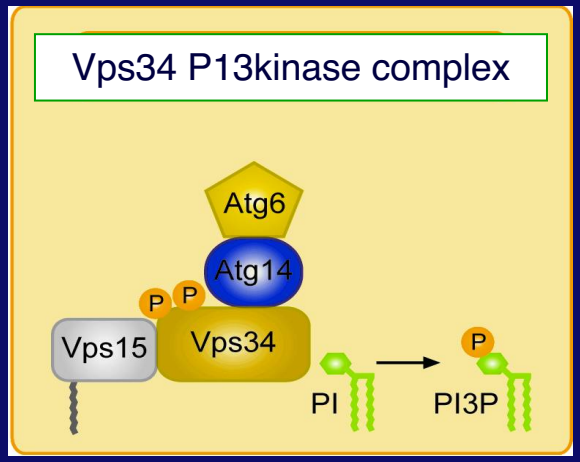


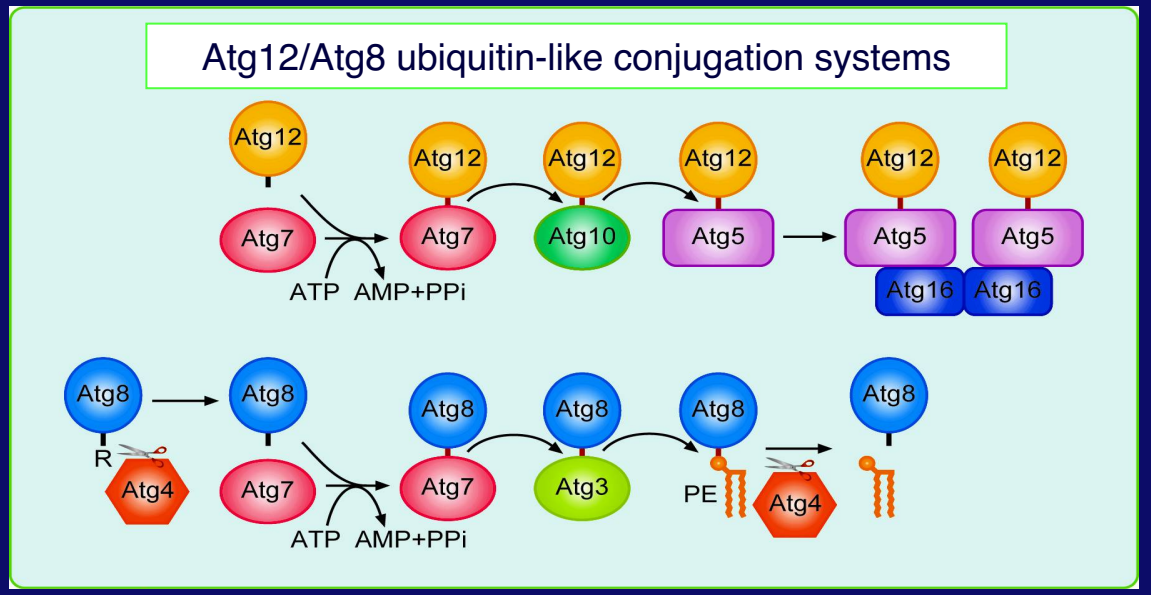
Kihara et al. 2001

18 Atg proteins required for autophagosome formation



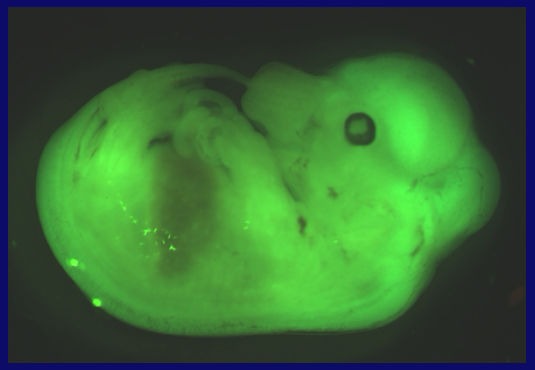




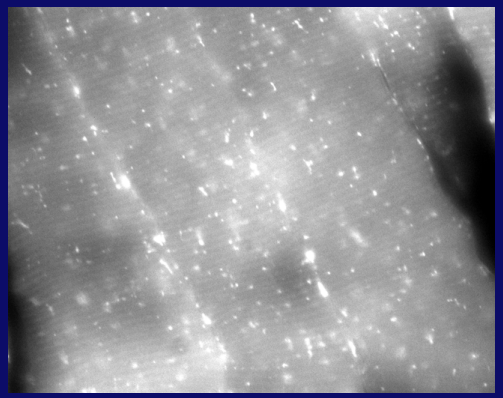


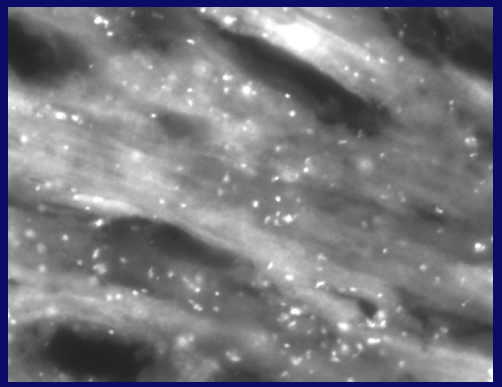
	Yeast	Mammalian	Plant(Arab.)
Atg1 kinase and its regulators	Atg1	ULK1/2	AtATG1a-1c,1t
	Atg13	Atg13	AtATG13a,13b
	Atg17	FIP200	-
	Atg29	Atg101	-
	Atg31		-
PtdIns 3-kinase complex	Atg6/Vps30	Beclin-1	AtATG6
	Atg14	Atg14	-
	Vps34	Vps34	AtVps34
	Vps15	p150	AtVps15
Atg2-Atg18 complex and Atg9	Atg2	Atg2s	AtATG2
	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

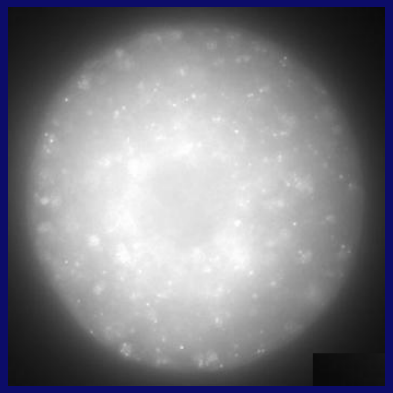
Autophagy in whole organisms:

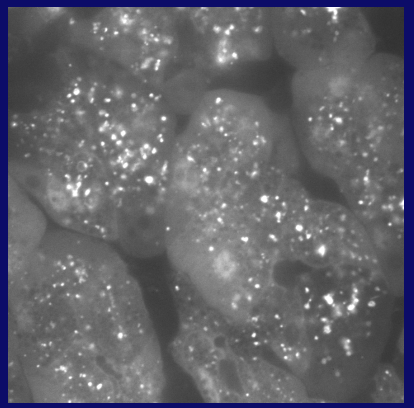


GFP-LC3
Transgenic mouse



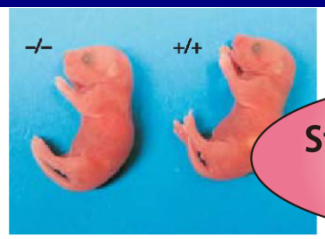






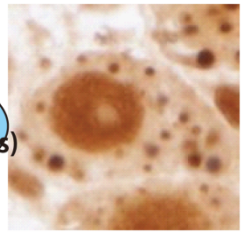
N. Mizushima

Expanding autophagy research
Various physiological function of autophagy

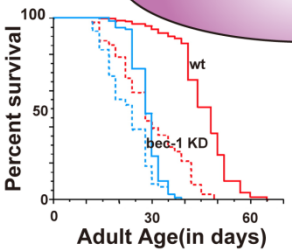


Starvation adoptation
(amino acid production)

Intracellular clearance
(suppression of degenerative diseases)

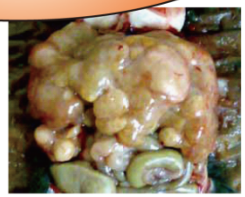


Longevity

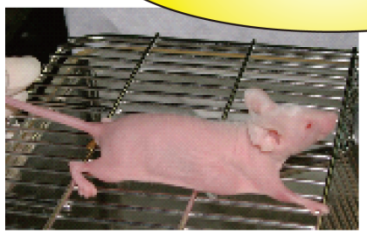


Autophagy

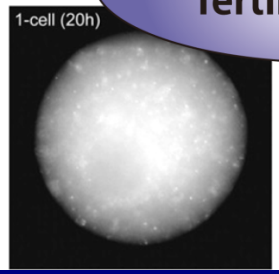
Tumor suppression

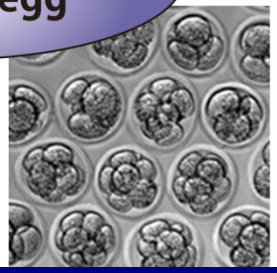


Antigen presentation

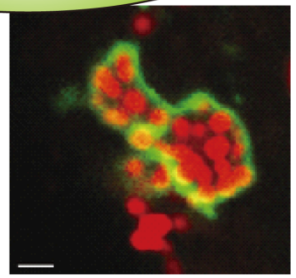


Development of
fertilized egg





Elimination of
invasive bacteria



Two major roles of autophagy

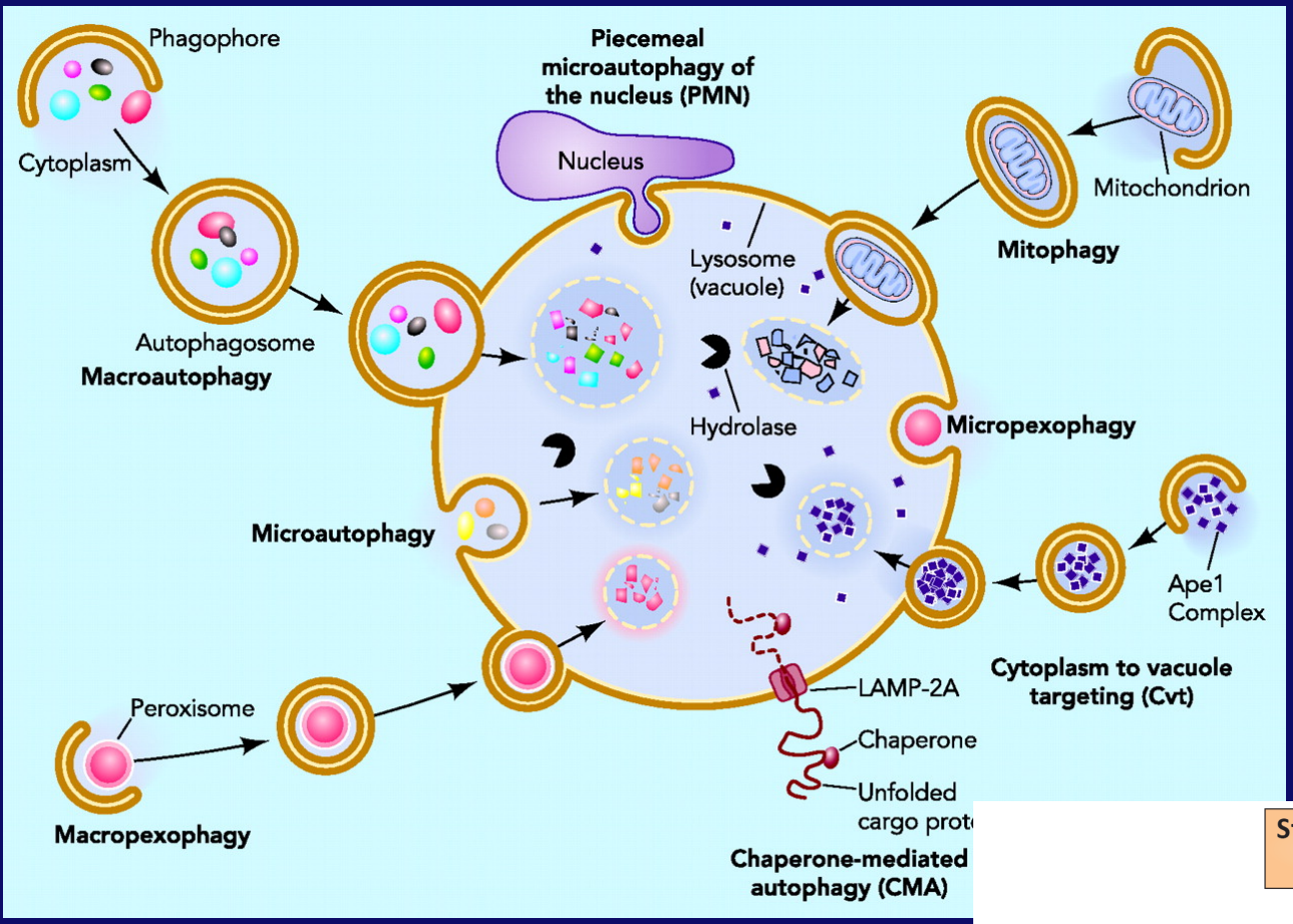
Nutrient Recycling:

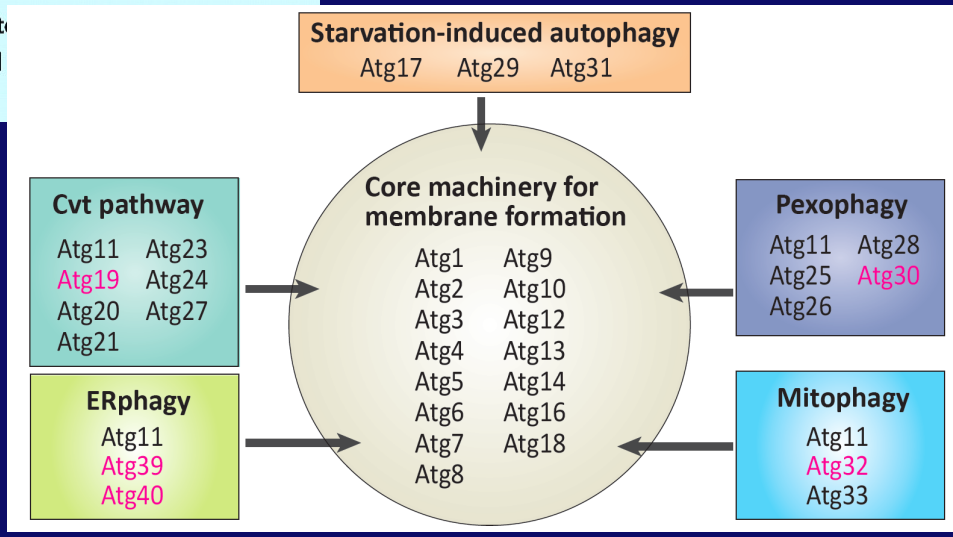
- essential for survival under starvation
- bulk, non-selective degradation
- amino acids for protein synthesis, energy source

Elimination of excessive or harmful materials:

- essential for clearance of cytoplasm
- selective degradation
  - specific protein, protein aggregates
  - supramolecular structures : ribosome……
  - organelles : mitochondria, peroxisomes, lysosomes, ER, nucleus….
  - Invasive bacteria, Virus particles

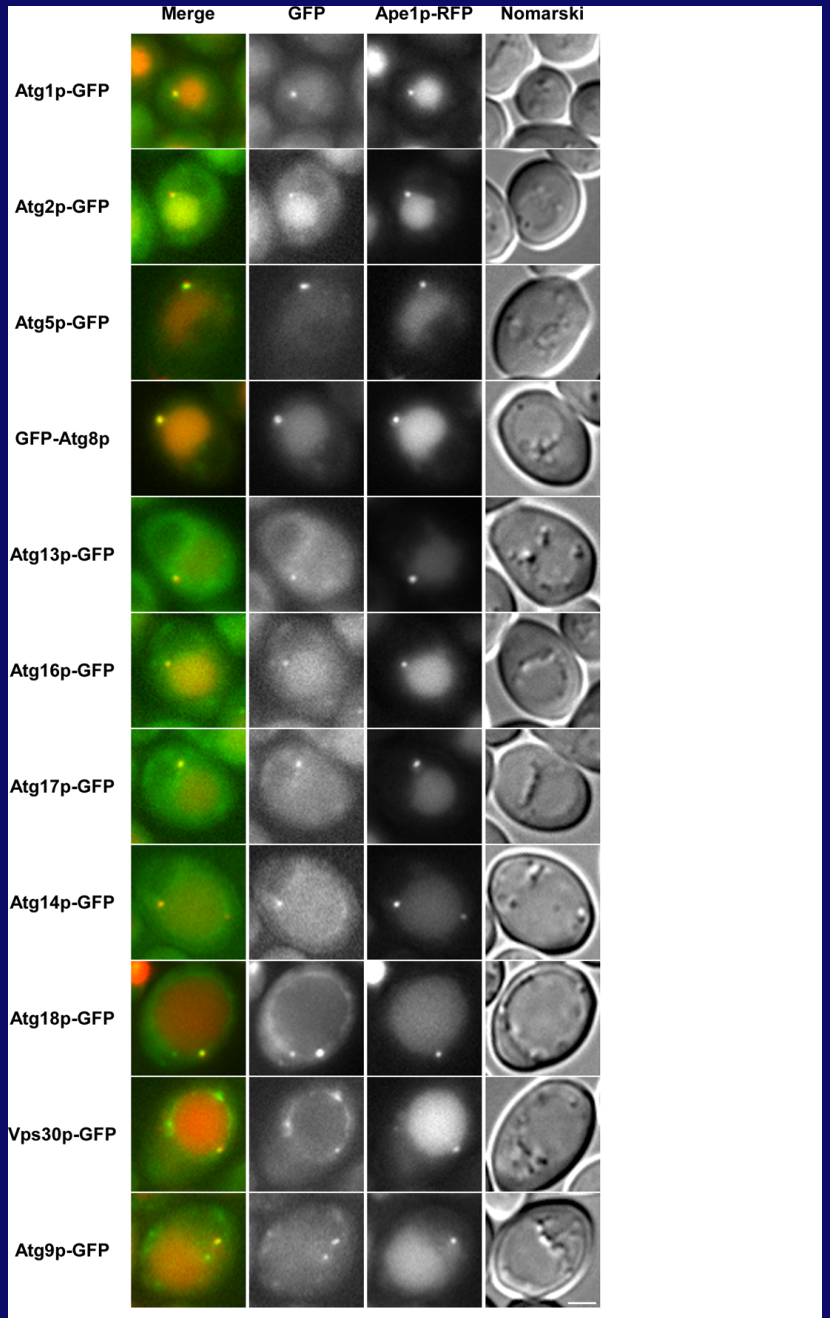
Diverse modes of autophagy





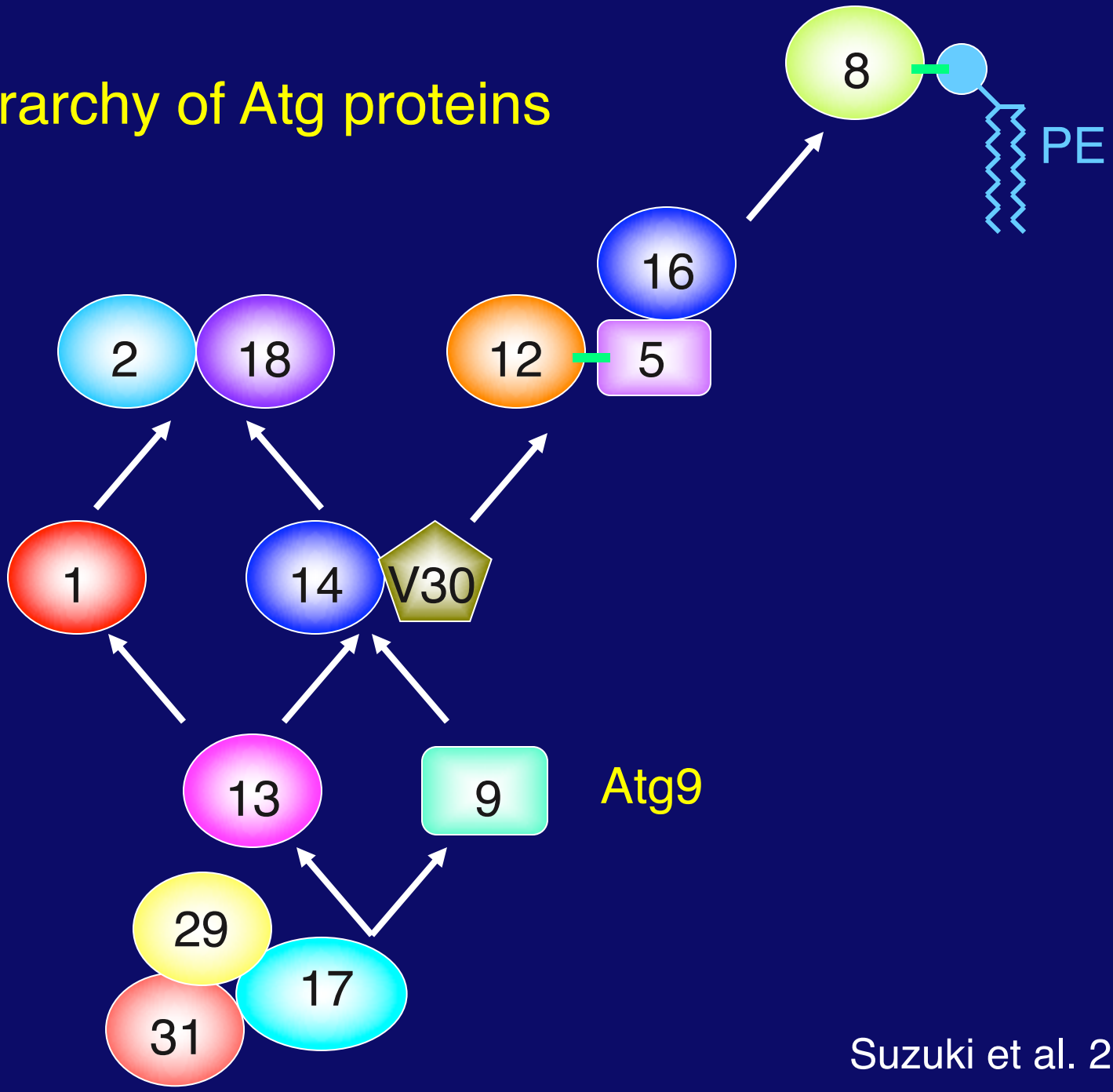
Studies of Atg proteins function during autophagosome formation

Localization of Atg proteins to the PAS



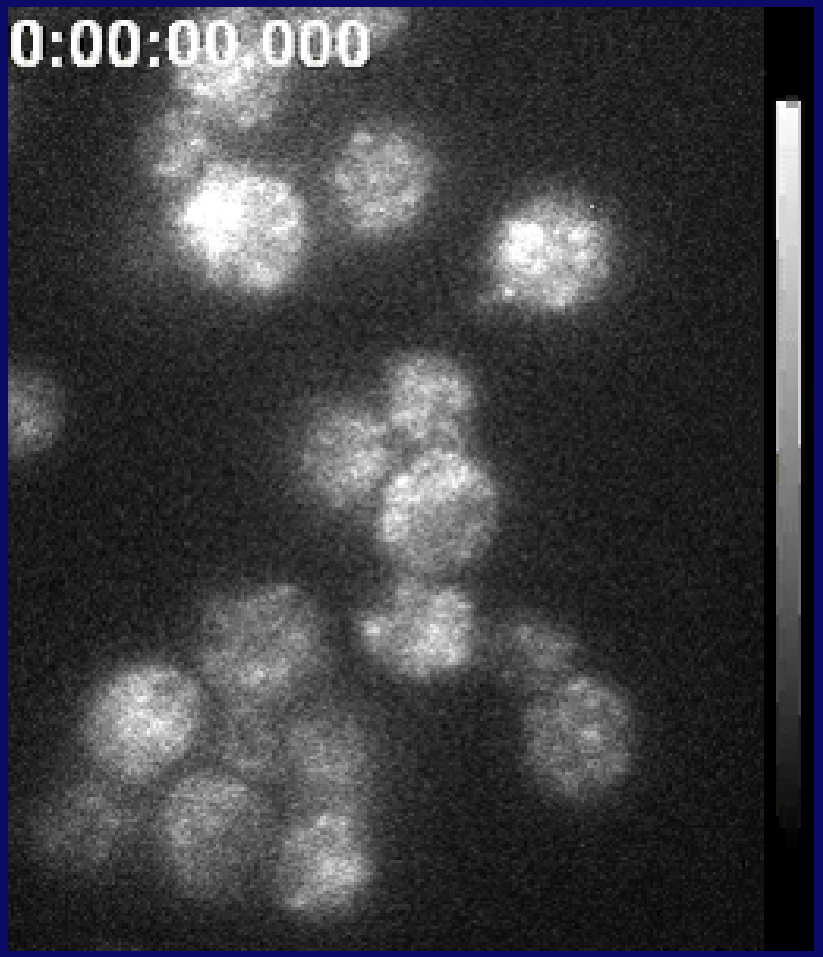
Suzuki et al. 2001

Hierarchy of Atg proteins

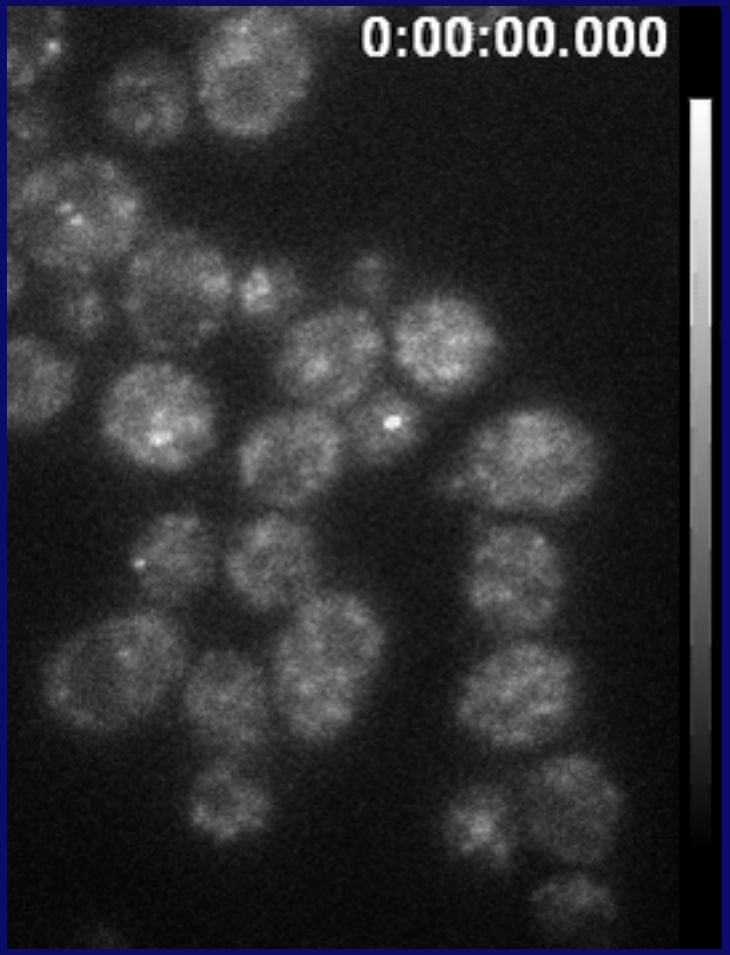


Suzuki et al. 2007

Atg17-2xGFP (dimer form of Atg17-Atg29-Atg31)

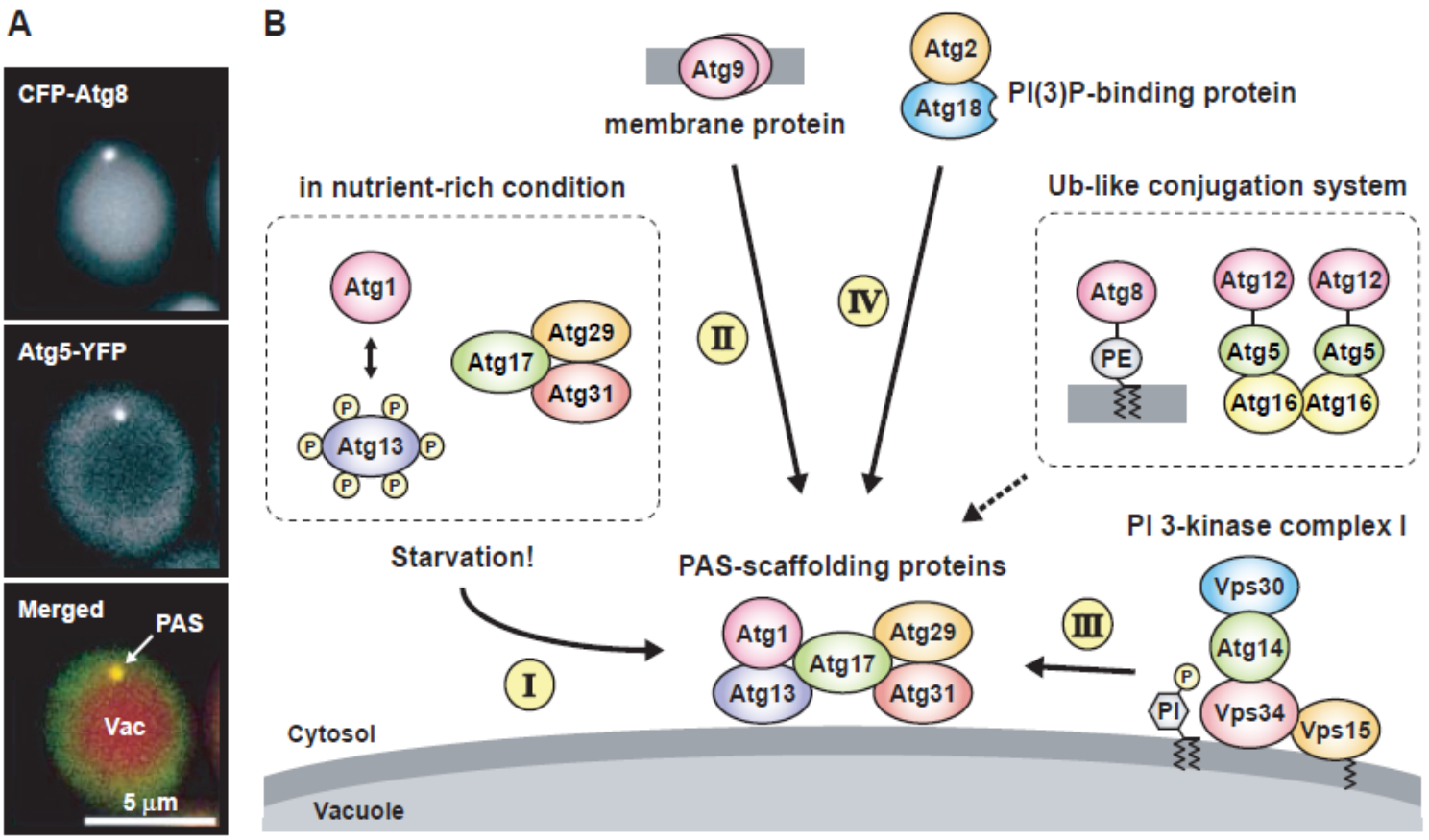


Nutrient rich condition
[in YPD medium]

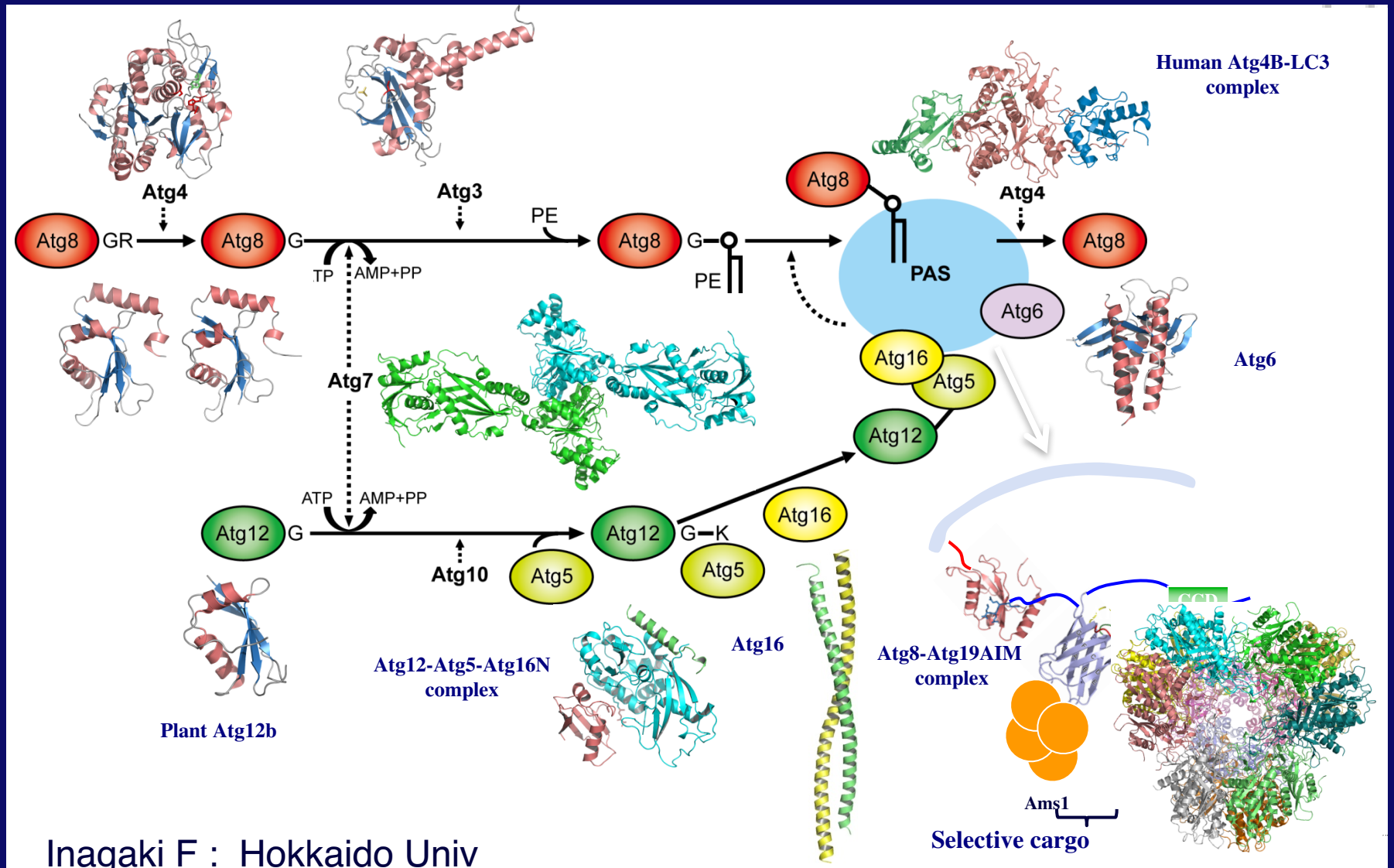


Nitrogen starvation for 2 h
[in SD(-N) medium]

Current overview of sequential events at the PAS



Structural Analysis of Atg proteins

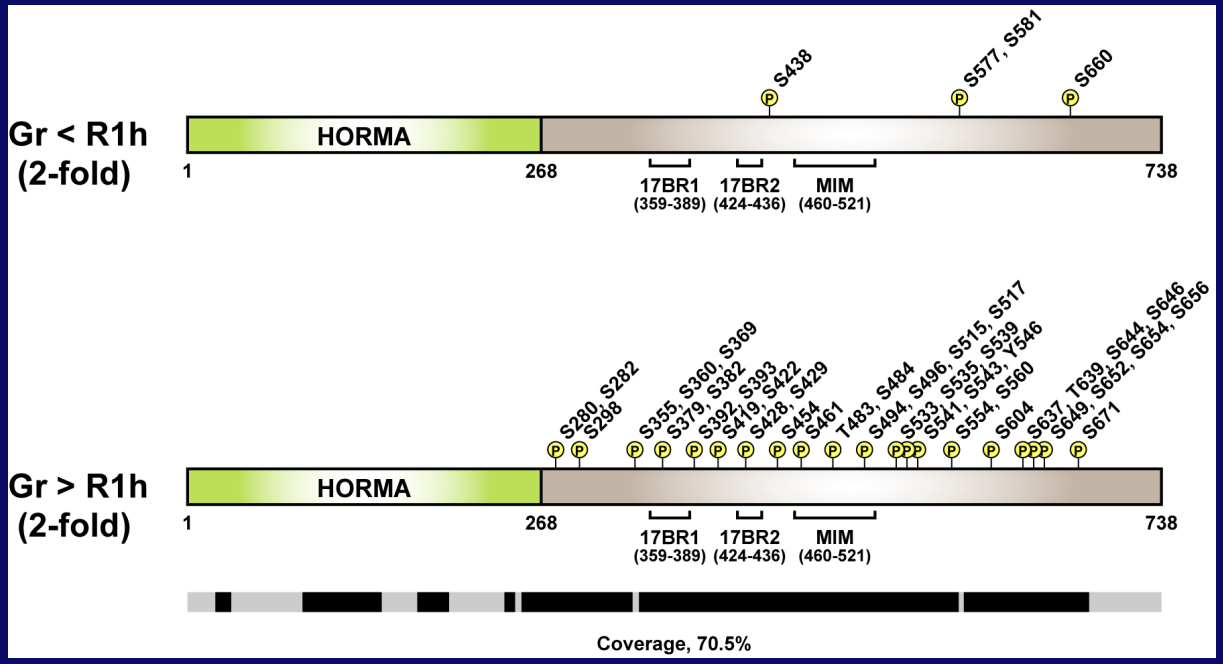


Inagaki F : Hokkaido Univ
Noda N : Inst of Microbial Chem

Early events of autophagosome formation

Dynamics of the Atg1 complex

Atg13 as a key regulator




```
MVAEEDIEKQ VLQLIDSFFL KTTLLICSTE SSRYQSSTEN IFLFDDTWFE     50
DHSELVSELP EIISKWSHYD GRKELPPLVV ETYLDLRQLN SSHLVRLKDH    100
EGHLWNVCKG TKKQEIVMER WLIELDNSSP TFKSYSEDET DVNELSKQLV    150
LLFRYLLTLI QLLPTTELYQ LLIKSYNGPQ NEGSSNPITS TGPLVSIRTC    200
VLDGSKPILS KGRIGLSKPI INTYSNALNE SNLPAHLDQK KITPVWTKFG    250
LLRVSVSYRR DWKFEINNTN DELFSARHAS VSHNSQGPQN QPEQEGQSDQ    300
DIGKRQPQFQ QQQQPQQQQQ QQQQQQRQHQ VQTQQQRQIP DRRSLSLSPC    350
TRANSFEPQS WQKKVYPISR PVQPFKVGSI GSQSASRNPS NSSFFNQPPV    400
HRPSMSSNYG PQMNIEGTSV GSTSKYSSSF GNIRRHSSVK TTENAEKVSK    450
AVKSPLQPQE SQEDLMDFVK LLEEKPDLTI KKTSGNNPPN INISDSLIRY    500
QNLKPSNDLL SEDLSVSLSM DPNHTYHRGR SDSHSPLPSI SPSMHYGSLN    550
SRMSQGANAS HLIARGGGNS STSALNSRRN SLDKSSNKQG MSGLPPIFGG    600
ESTSYHHDNK IQKYNQLGVE EDDDDENDRL LNQMGNSATK FKSSISPRSI    650
DSISSSFIKS RIPIRQPYHY SQPTTAPFQA QAKFHKPANK LIDNGNRSNS    700
NNNNHNGNDA VGVMHNDEDD QDDDLVFFMS DMNLSKEG                 738
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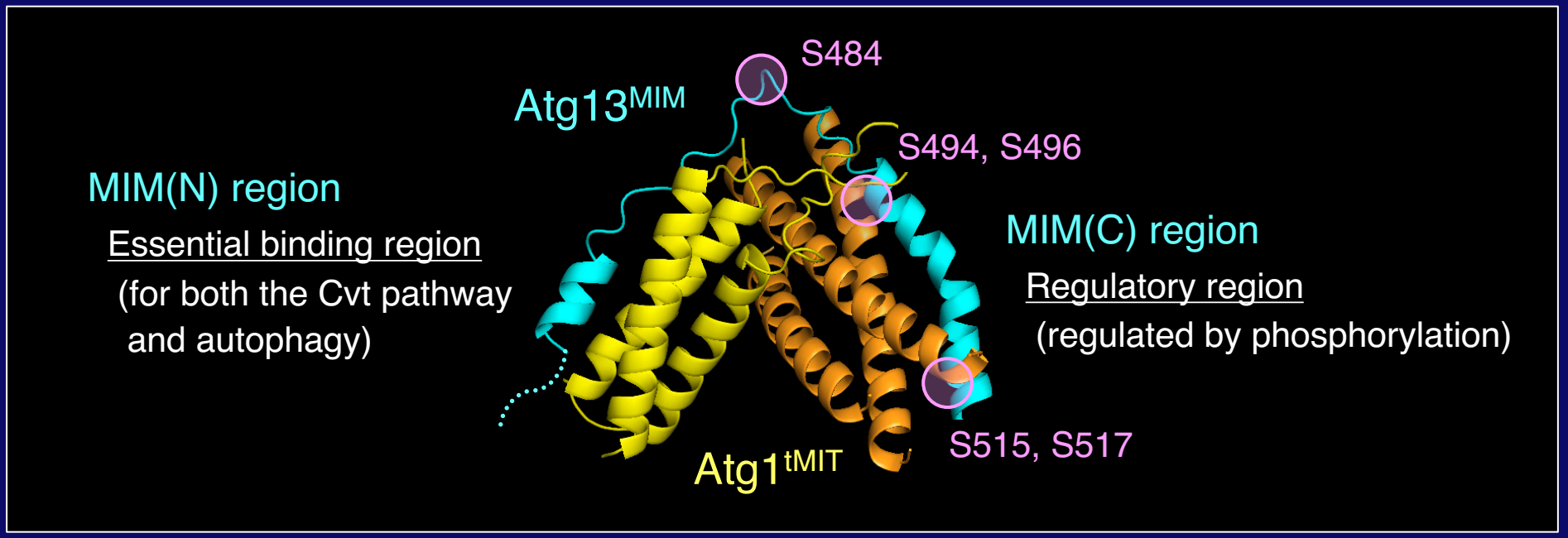
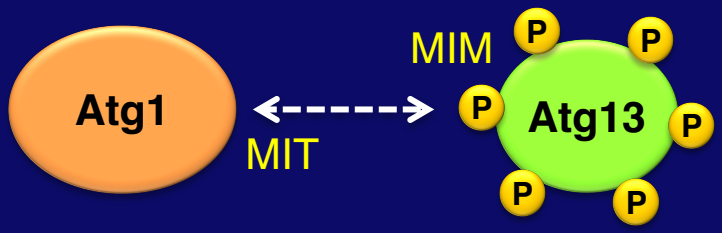
The Atg13^{MIM} region has two roles in recognition of Atg1^{tMIT}

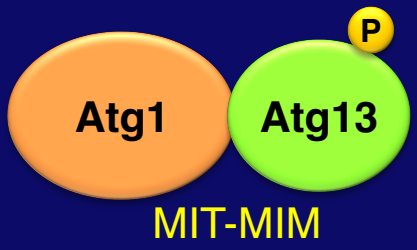


Nutrient rich



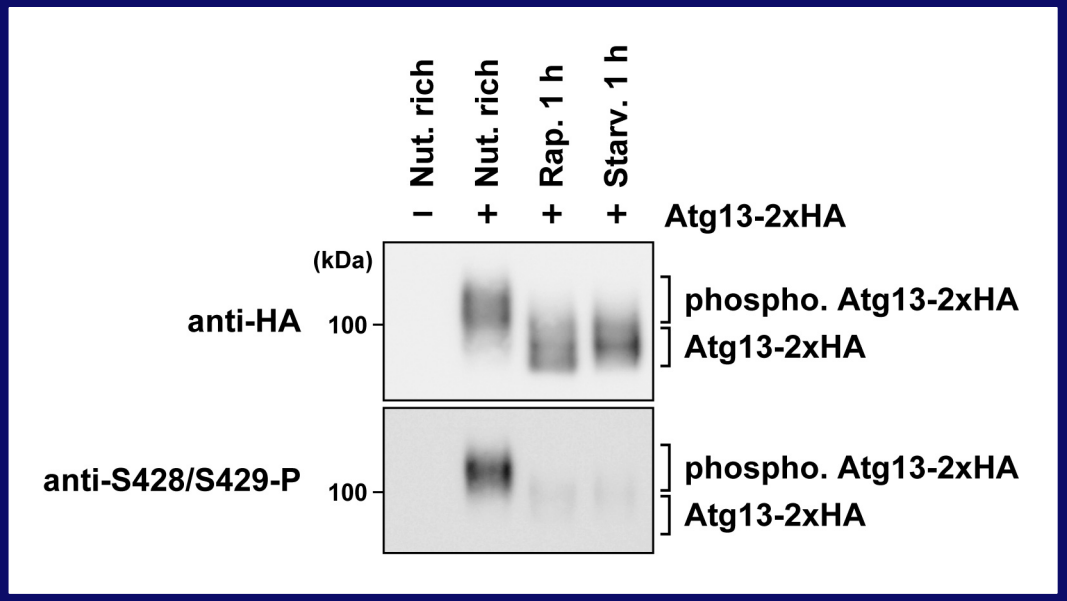
"Low affinity"
for the Cvt pathway

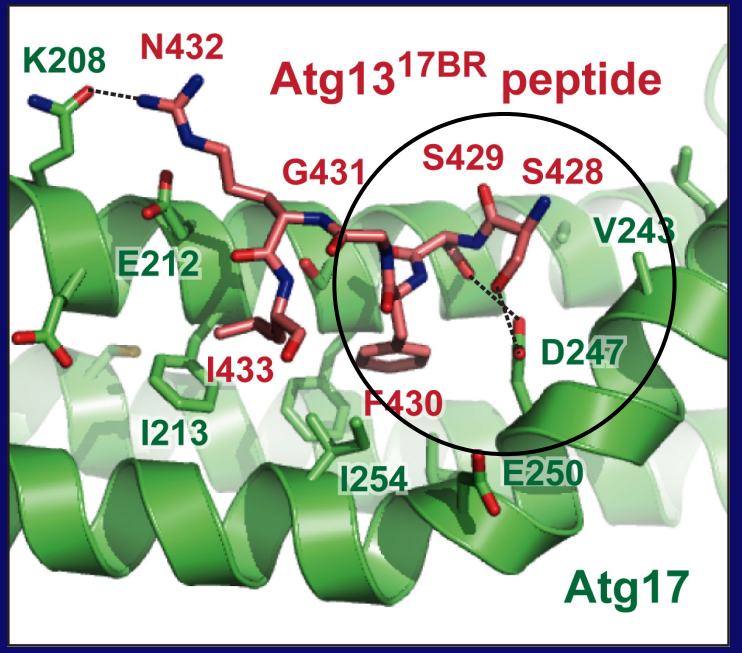
Starvation

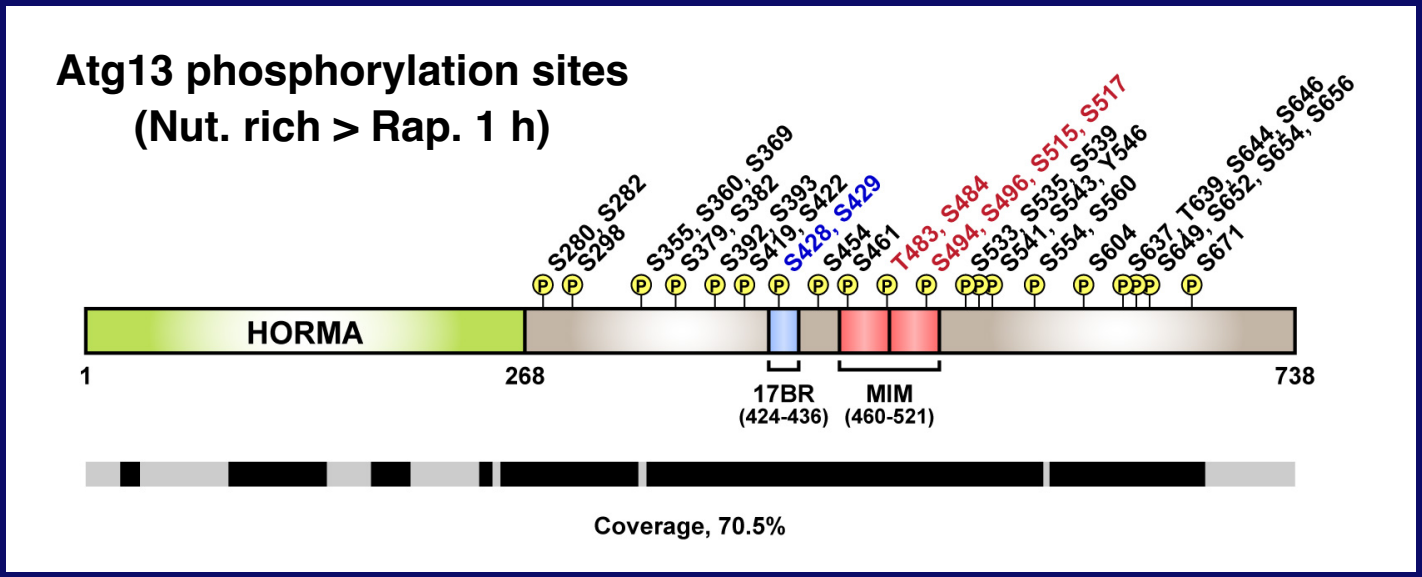


"High affinity"
for Autophagy

S428/S429 are phosphorylated in nutrient-rich conditions

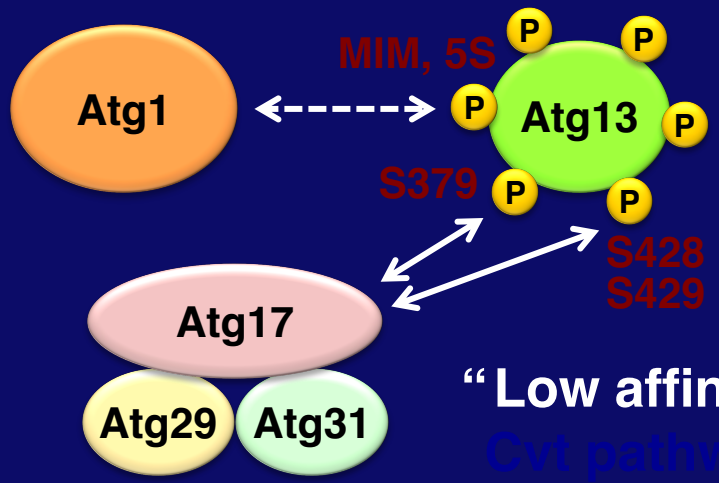






Formation of Atg1 complex

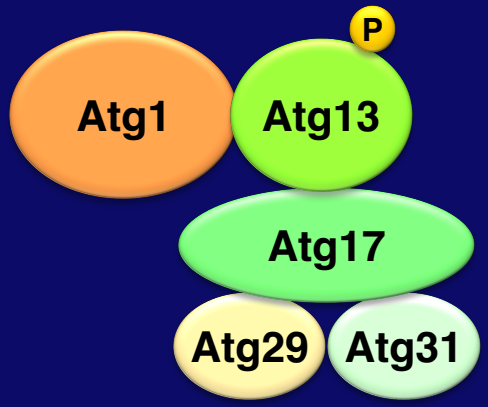
Nutrient rich



"Low affinity"

Cvt pathway

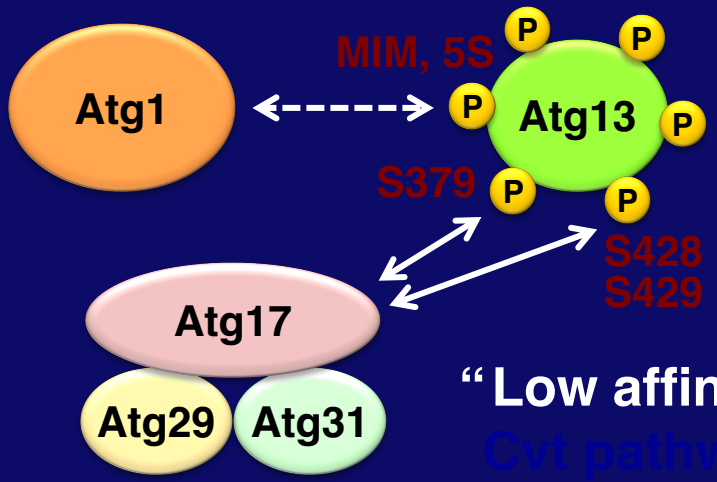
Starvation



"High affinity" Autophagy

Atg1 complex at the PAS

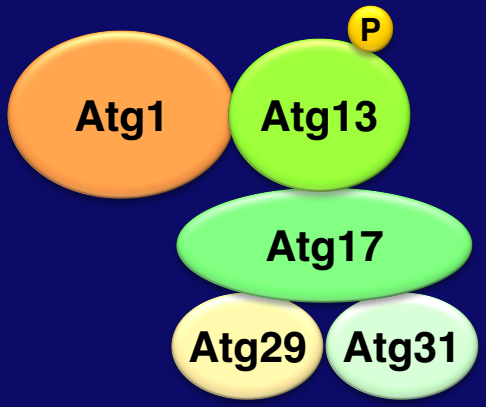
Nutrient rich



"Low affinity"

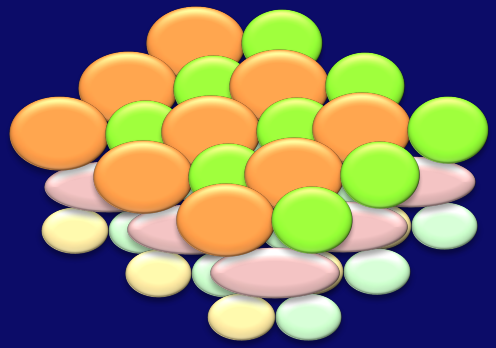
Cvt pathway

Starvation

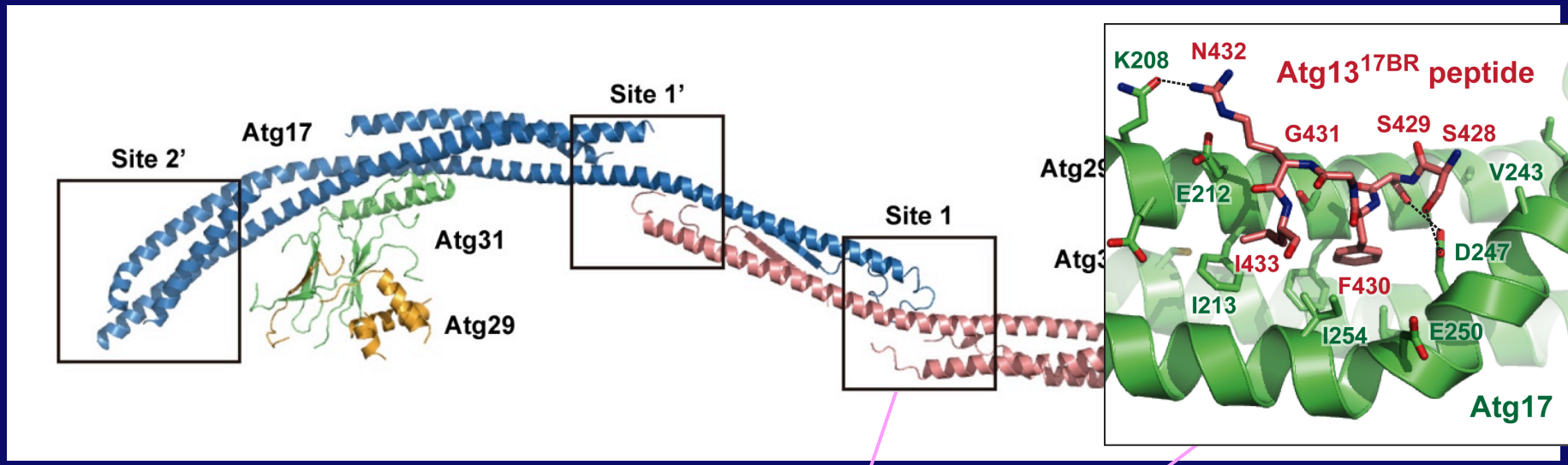


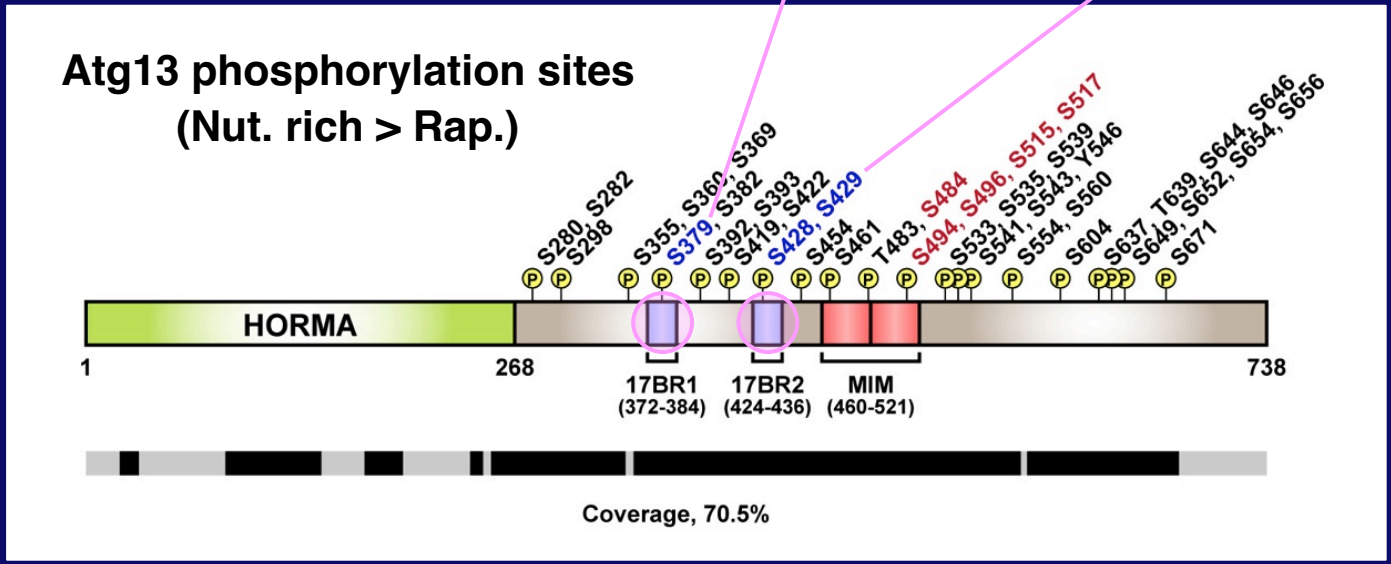
"High affinity" Autophagy

?

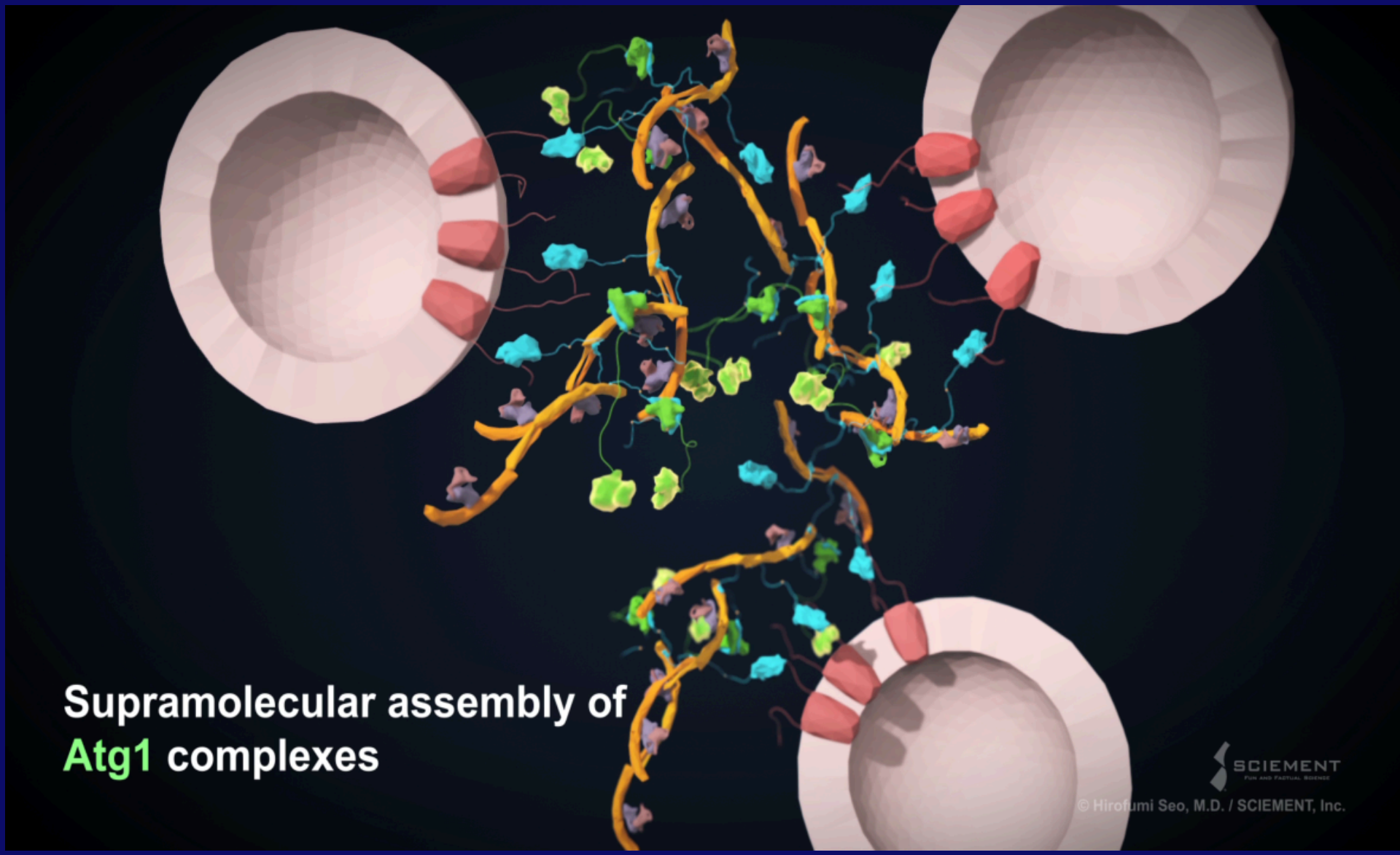


Atg13-Atg17 interaction may be regulated by phosphorylation





Working Model of early step of autophagy induction



The PAS

Flexible supramolecular-assembly made up multimeric complexes of Atg proteins and membrane structure

The PAS assembly is highly regulated by modification and transient interactions of Atg proteins at each stage of membrane formation

Fundamental questions to be answered

Autophagic degradation of cytoplasmic components: when, What and How?

Induction conditions, what kind of nutrient limitation?

- signal transduction for induction

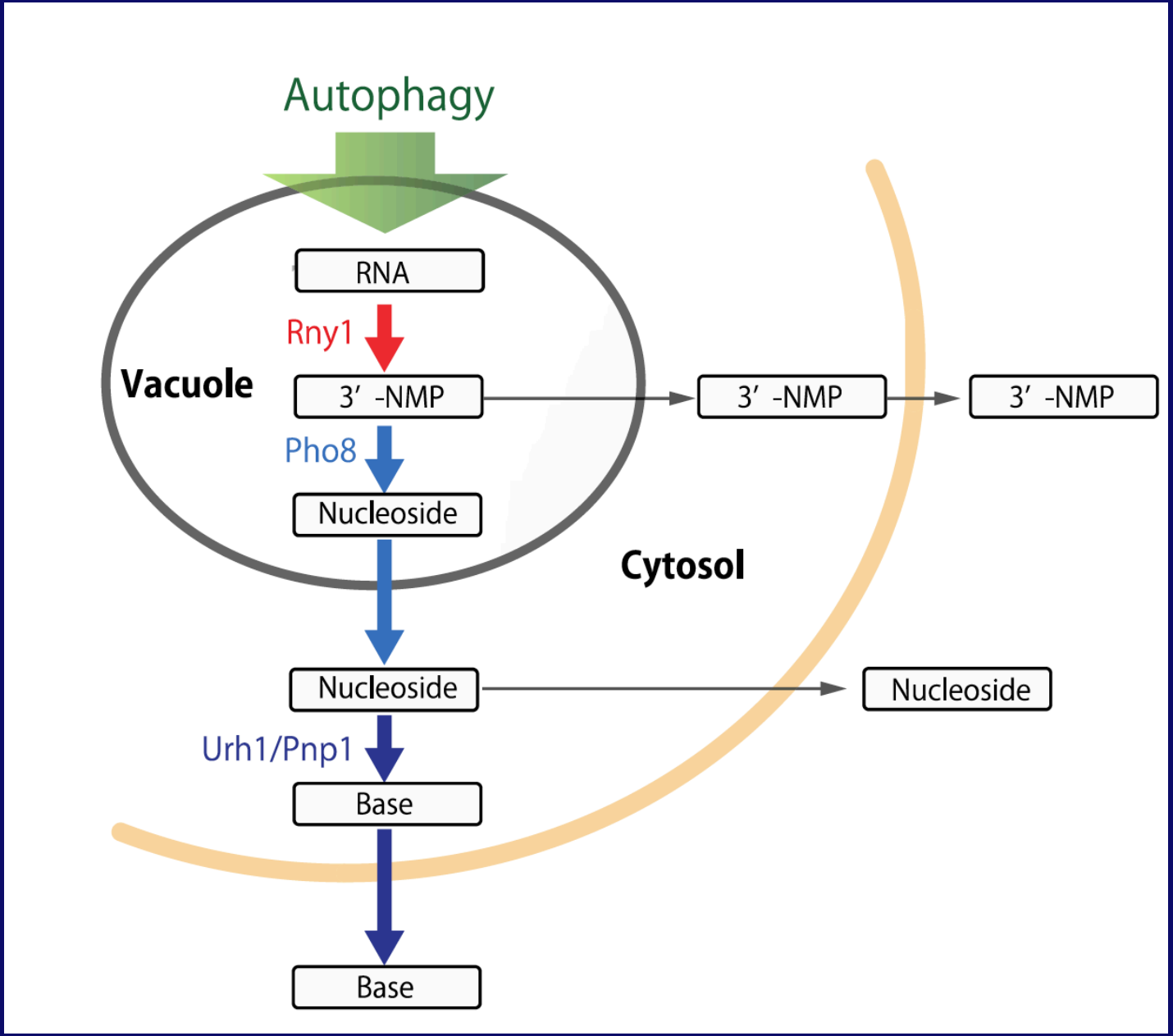
Identification of targets of autophagy

Various modes of autophagy, distinct molecular machinery

- constitutive autophagy
- selectivity autophagy
- micro- and macro-autophagy

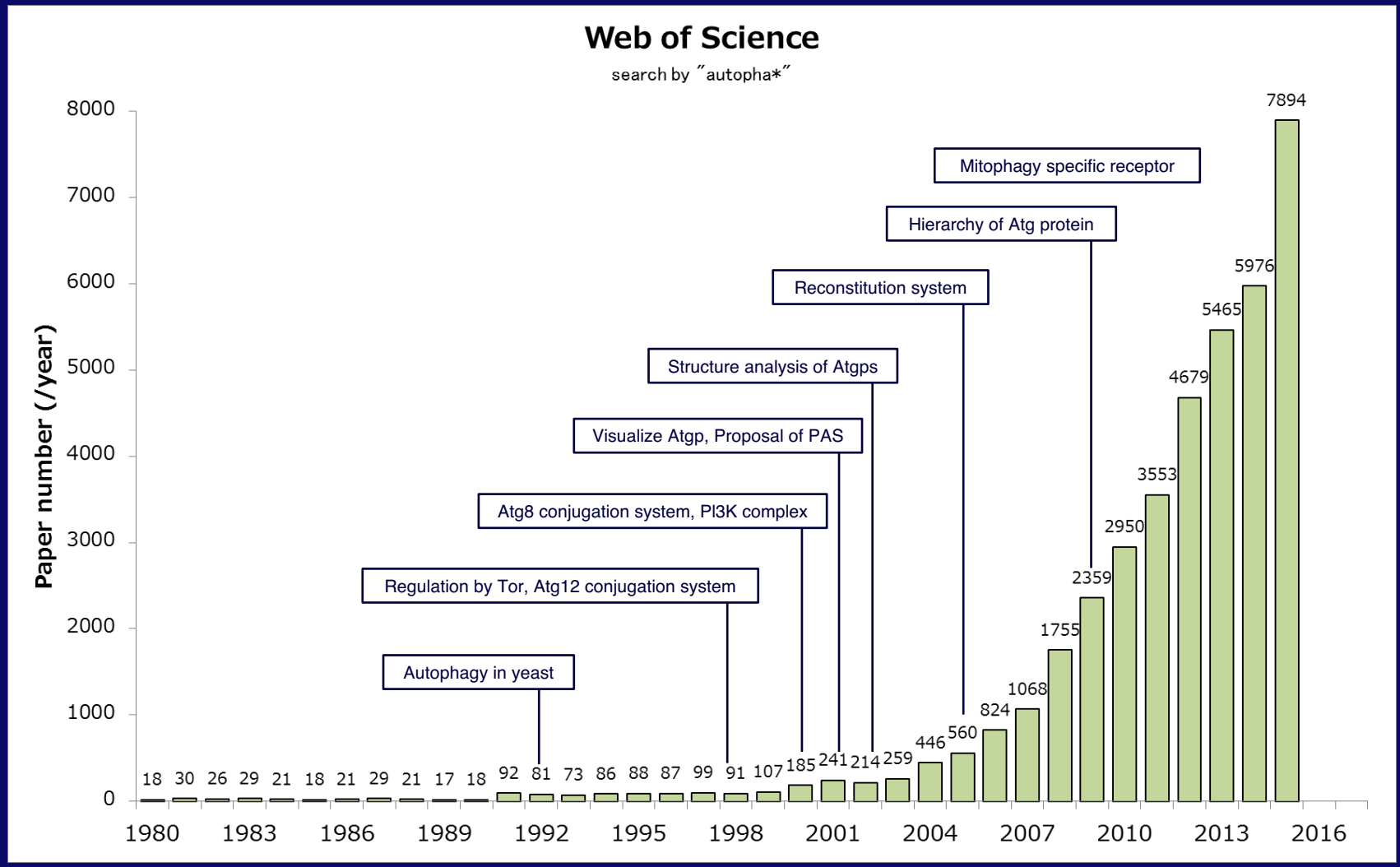
Identification and fates of degradation products

- their effects on cellular metabolism



Huang and Kawamata et al. 2015

The explosion of autophagy research



Looking back on 27 years of autophagy research

A long and winding path

- many accidents and wonderful encounters

Intellectual curiosity driven research

Fortune

- profound subjects, autophagy
- excellent colleagues, nice collaborators
- indispensable grant support
- supportive family

- Thank You for Your Attention!