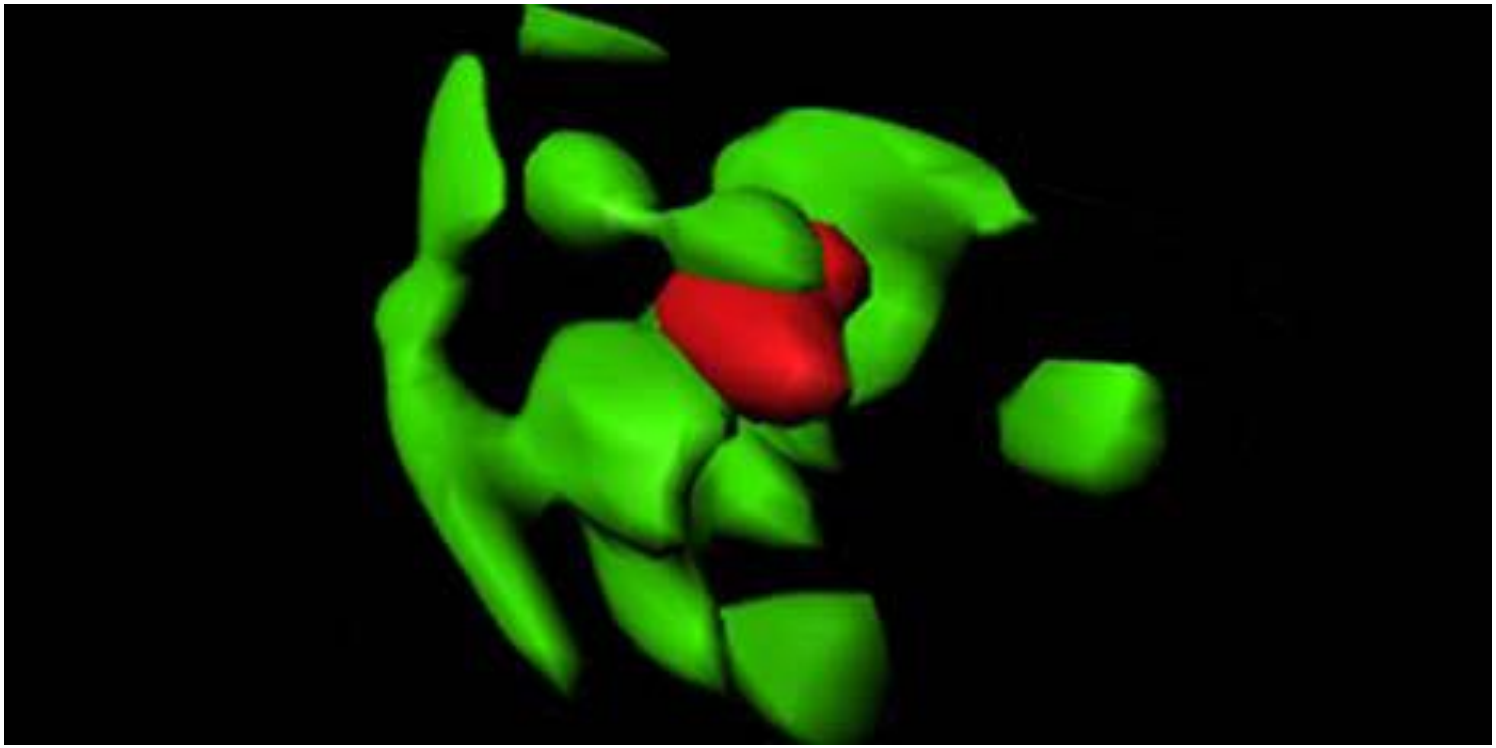
Concept course „Cell Biology“:
551-0326-00L
Spring semester 2017

Autophagy



Dr. Werner Kovacs

ETH Zürich, Institute of Molecular Health Sciences, HPL H16

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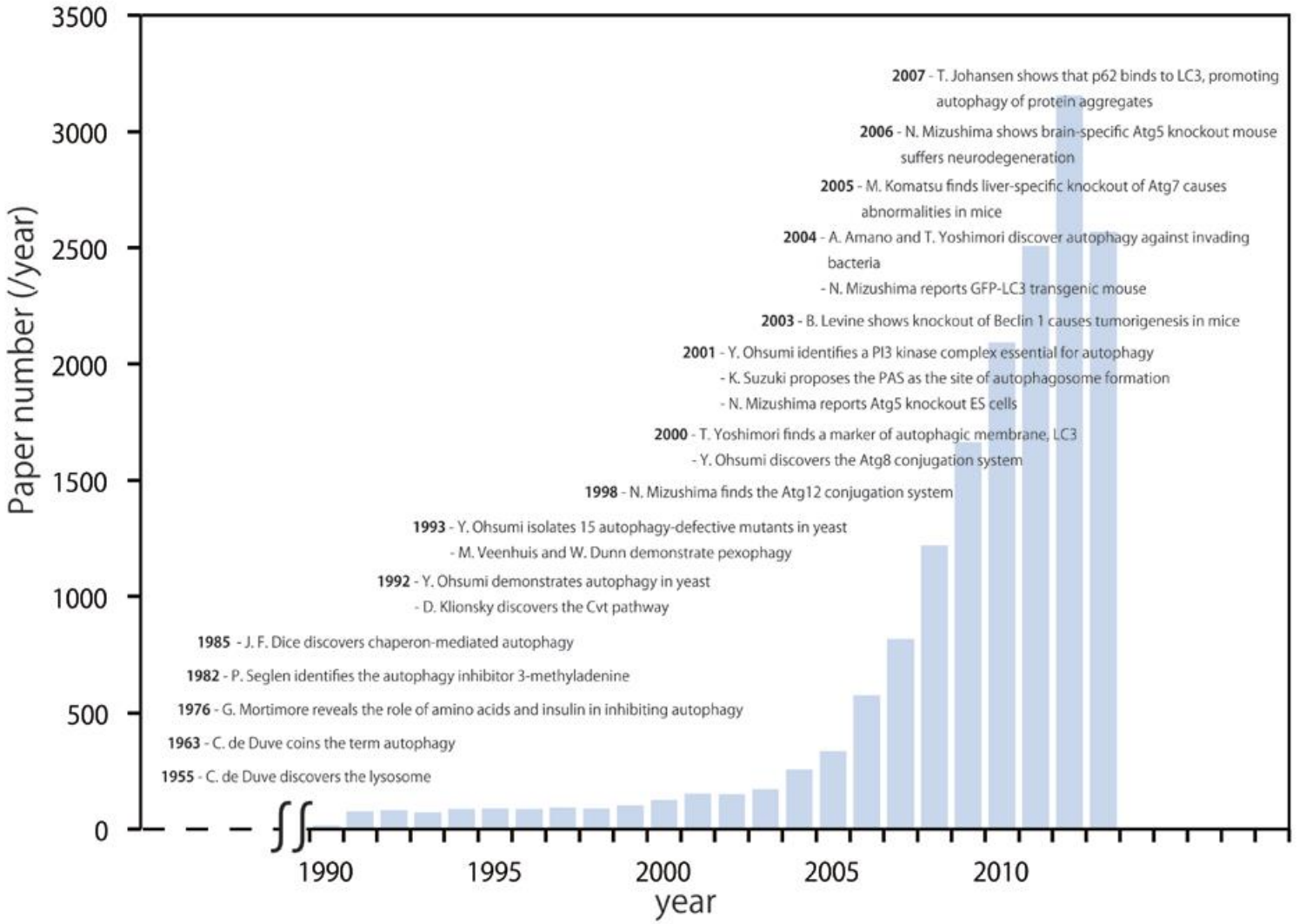
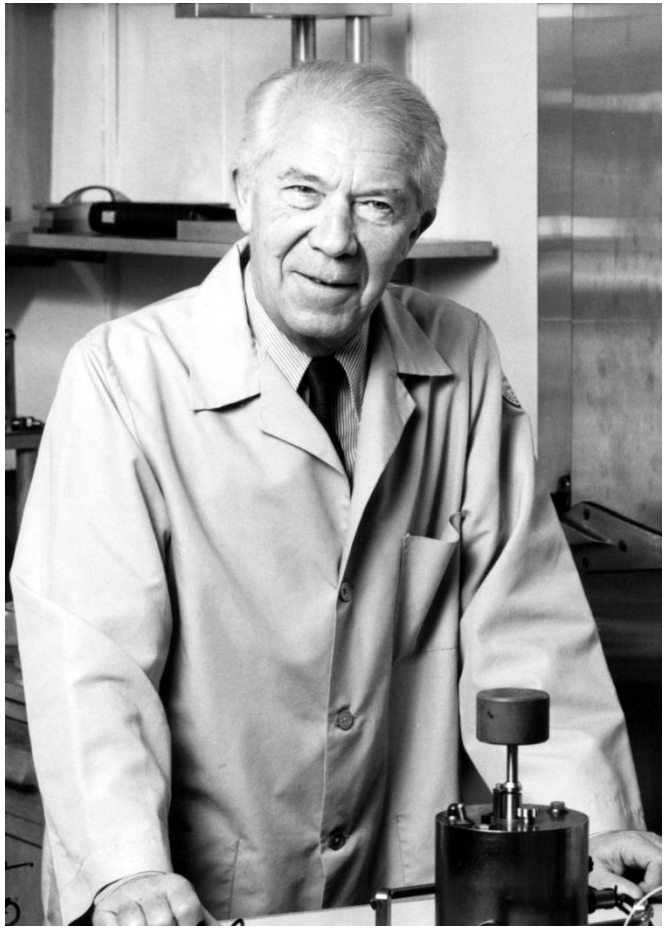
The growth of autophagy research and historical landmarks



Ohsumi, Cell Res. (2014)



Christian de Duve

Nobel Prize (1974)

Discovery of the lysosome, peroxisome, and autophagy

Yoshinori Ohsumi

Nobel Prize (2016)

Discovery of mechanisms underlying autophagy: **A**u**T**opha**G**y-related genes (ATGs)

Schematic depiction of autophagy in yeast and mammalian cells

Cvt: cytoplasm to vacuole targeting

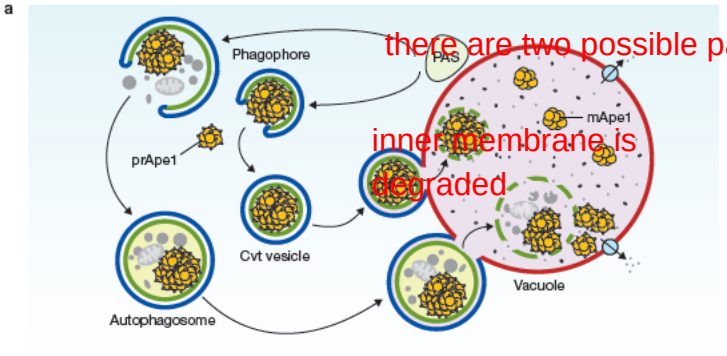
PAS: Phagophore assembly site

yeast

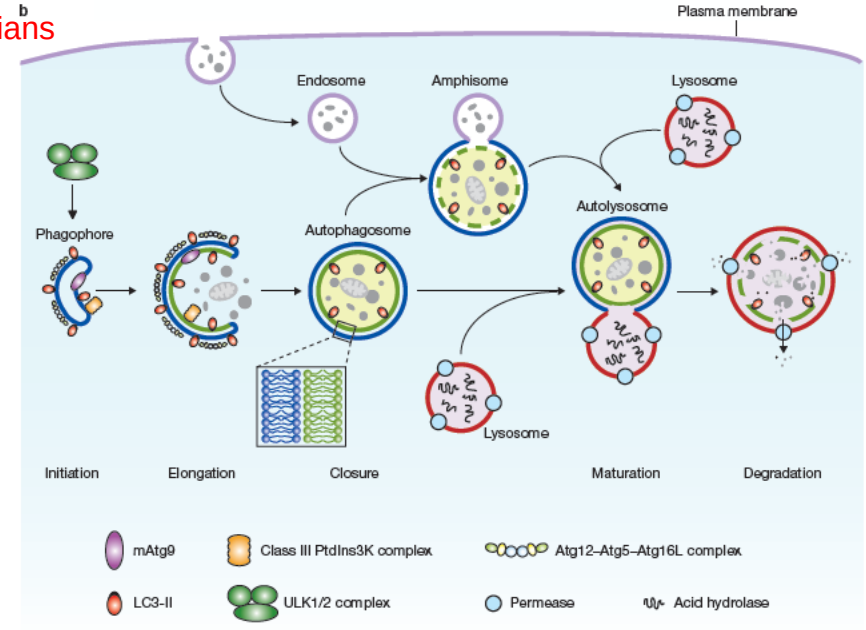
there are two possible pathways

inner membrane is degraded

cvt pathway is a biosynthetic way for ... (what exactly?)



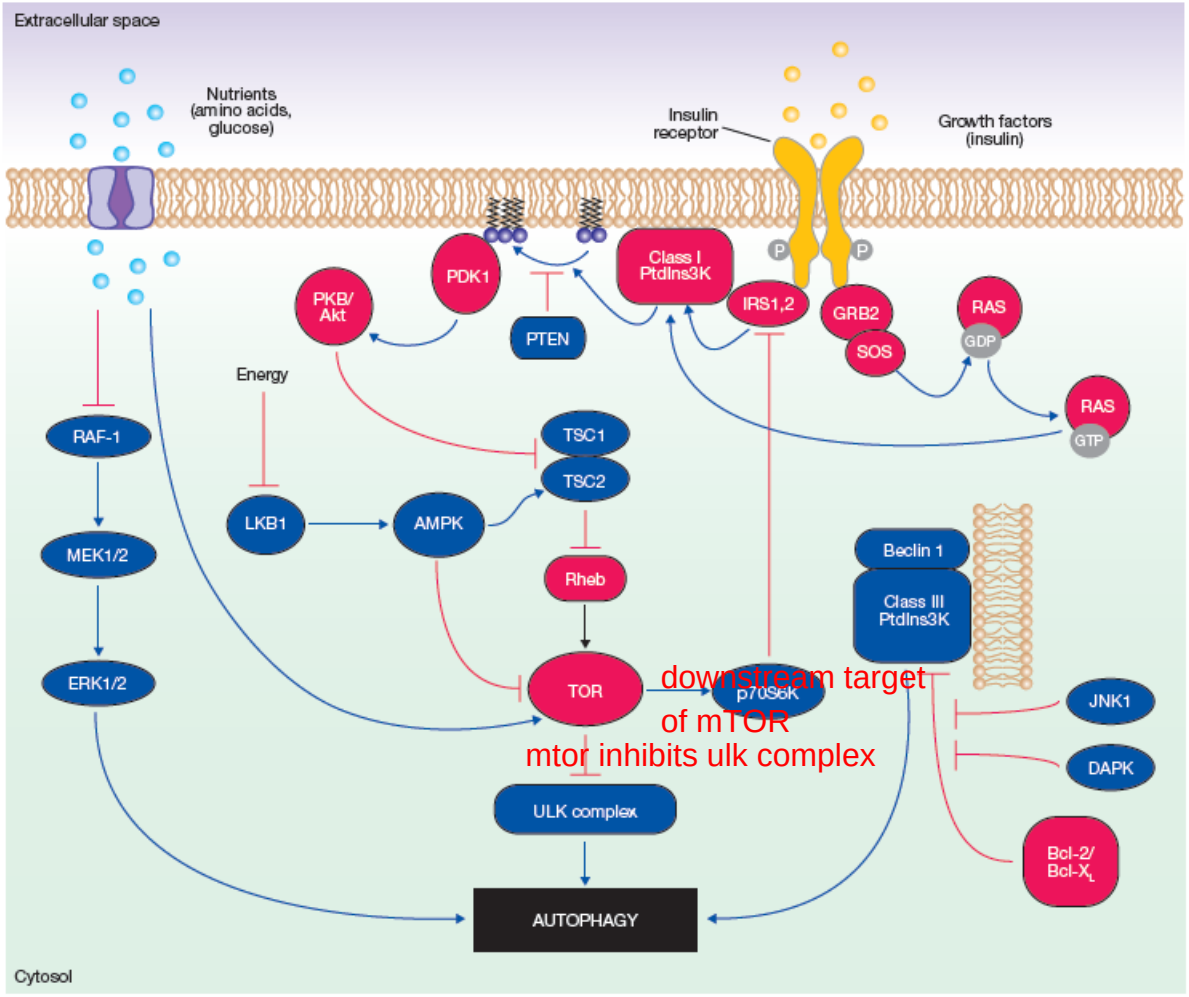
mammalians



Yang and Klionsky, Nat. Cell Biol. (2010)

Signaling regulation of mammalian autophagy

blue: stimulating factors
red: inhibiting factors
for autophagy



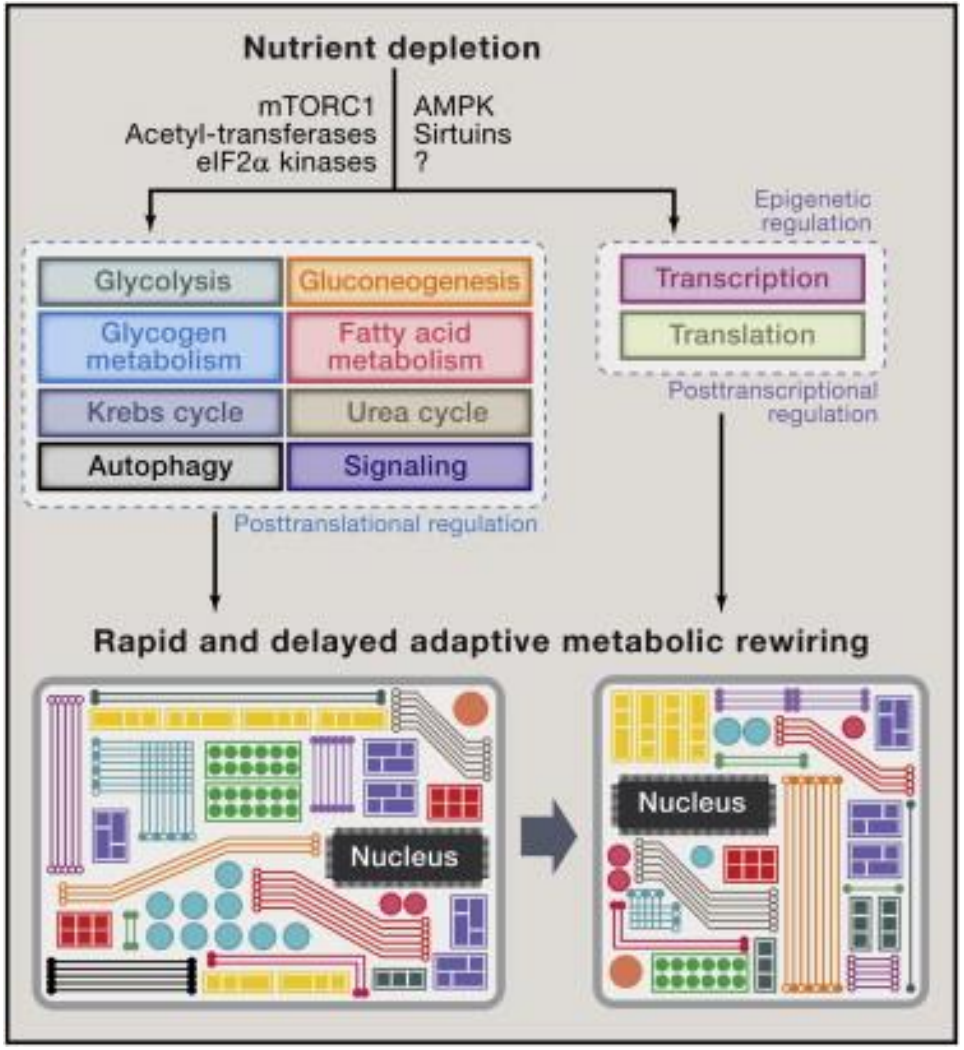
downstream target of mTOR
mtor inhibits ulk complex

some reasonalble level of autophagy is needed to maintain homeostasis

Yang and Klionsky, Nat. Cell Biol. (2010)

Cell-wide metabolic rewiring associated with the activation of autophagy

not soo important

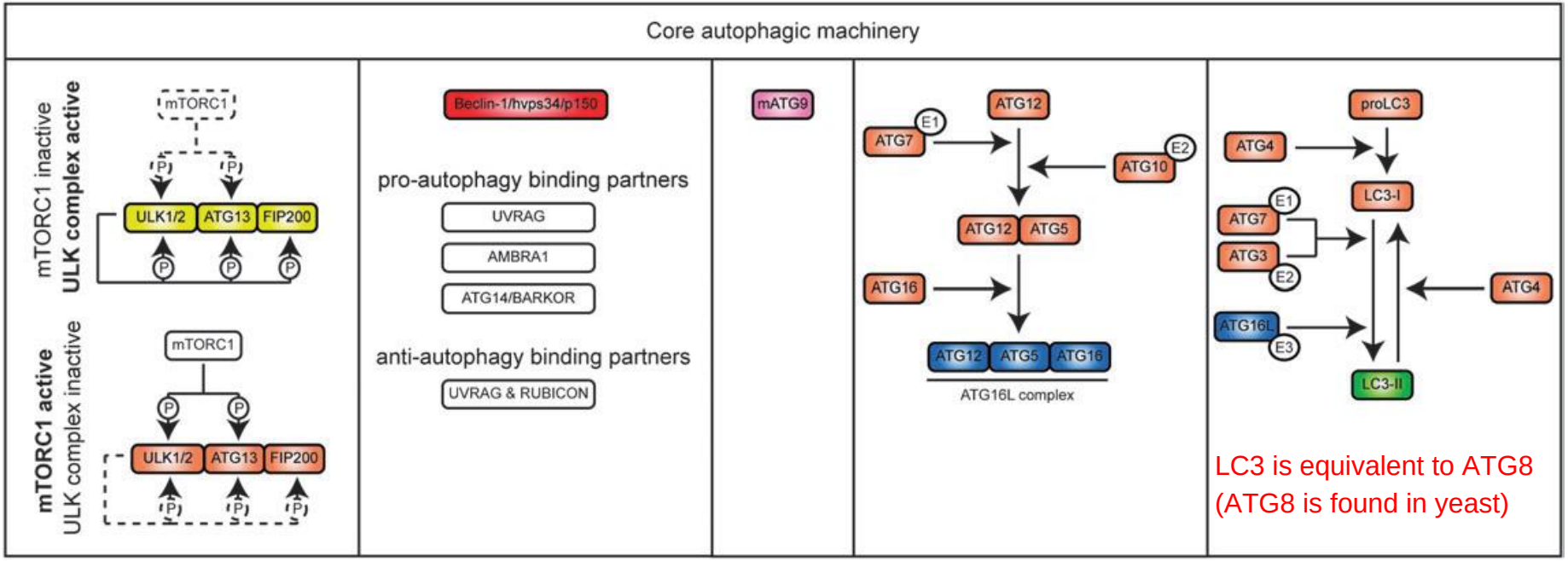


Galluzzi et al., Cell (2014)

very important slide

Autophagic core machinery

ATG101 is a new component of the dashed mTORC1 process



ULK1/2: orthologues of yeast Atg1
BARKOR: Beclin-1-associated autophagy-related key regulator
UVRAG: protein product of the ultraviolet radiation resistance gene
AMBRA1: activating molecule in Beclin-1-regulated autophagy
RUBICON: RUN domain and cysteine-rich domain

2 ubiquitin-like conjugation systems

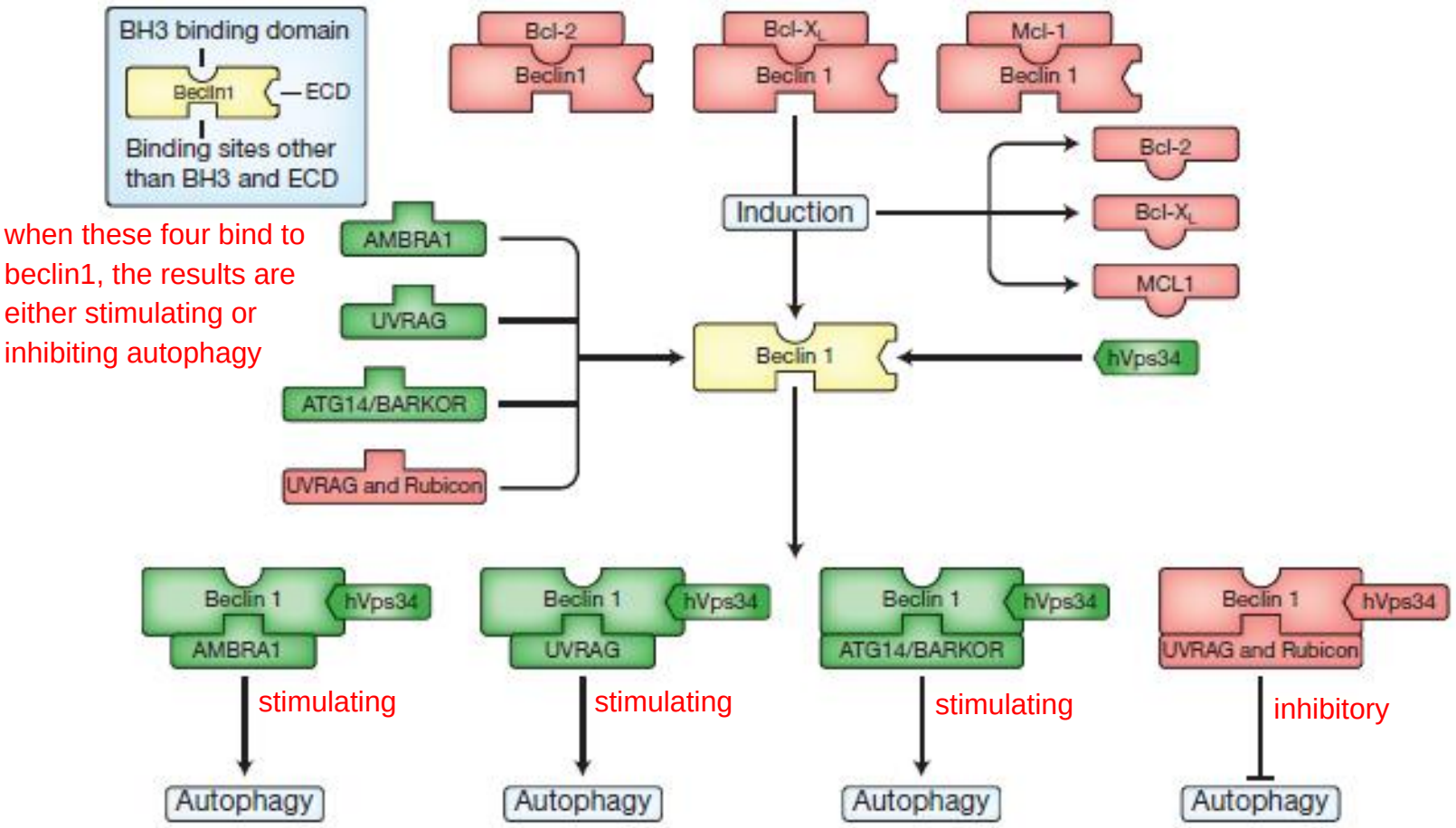
mTORC1: mTOR+ RAPTOR
mTORC2: mTOR + RICTOR
RAPTOR: reuglatory associated protein of mTOR
RICTOR: rapamycin insensitive companion of mTOR

mTORC1/2 are simply complexes

beclin-1 initially identified as a tumorsuppressor

Rosenfeldt and Ryan, Carcinogenesis (2011)

Regulation of autophagy by Beclin 1 complexes



when these four bind to beclin1, the results are either stimulating or inhibiting autophagy

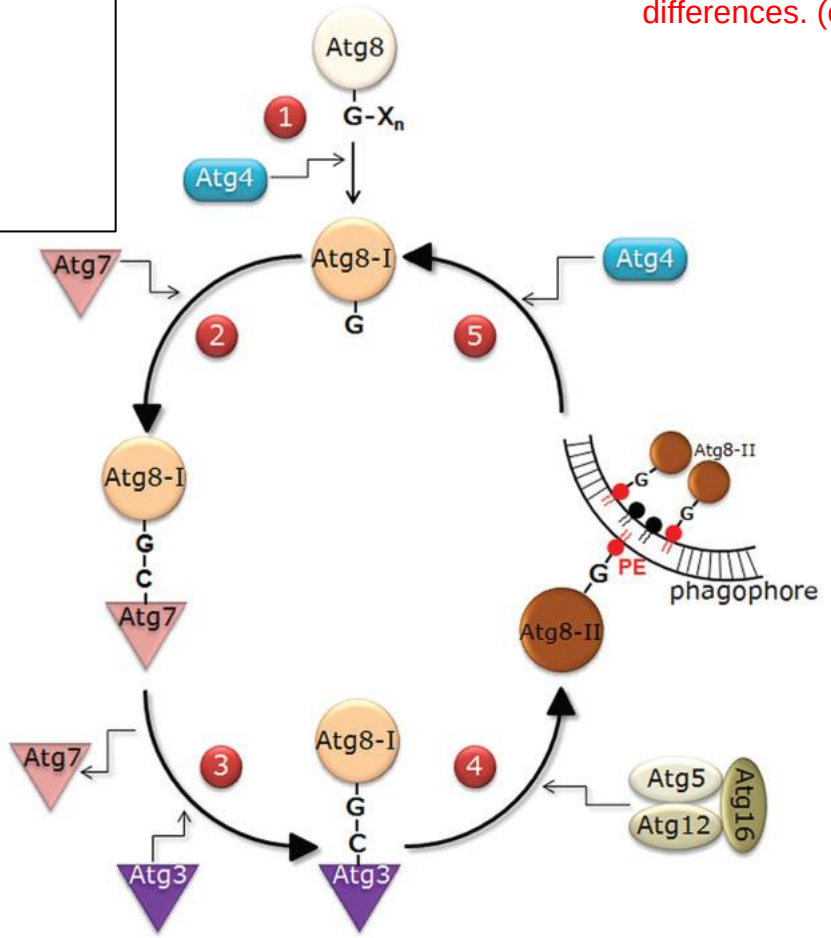
Beclin was found to have tumor suppressor functions.
Antiapoptotic members of the Bcl-2 family: Bcl-2, Bcl-Xl, Mcl-1

Rosenfeldt and Ryan, Exp. Rev. Mol. Med. 2009

Processing of Atg8s

Mammalian Atg8 orthologues:
MAP1LC3A (LC3A)
MAP1LC3B (LC3B)
MAP1LC3C (LC3C)
GABARAP
GABARAPL1
GABARAPL2 (GATE-16)

Atg8 is in yeast, in mammalians ther eare three; work very similiarly, but there are tissue distribution differences. (can you think of a reason?)



The lipidated Atg8 form of MAP1LC3B is termed "LC3B-II".

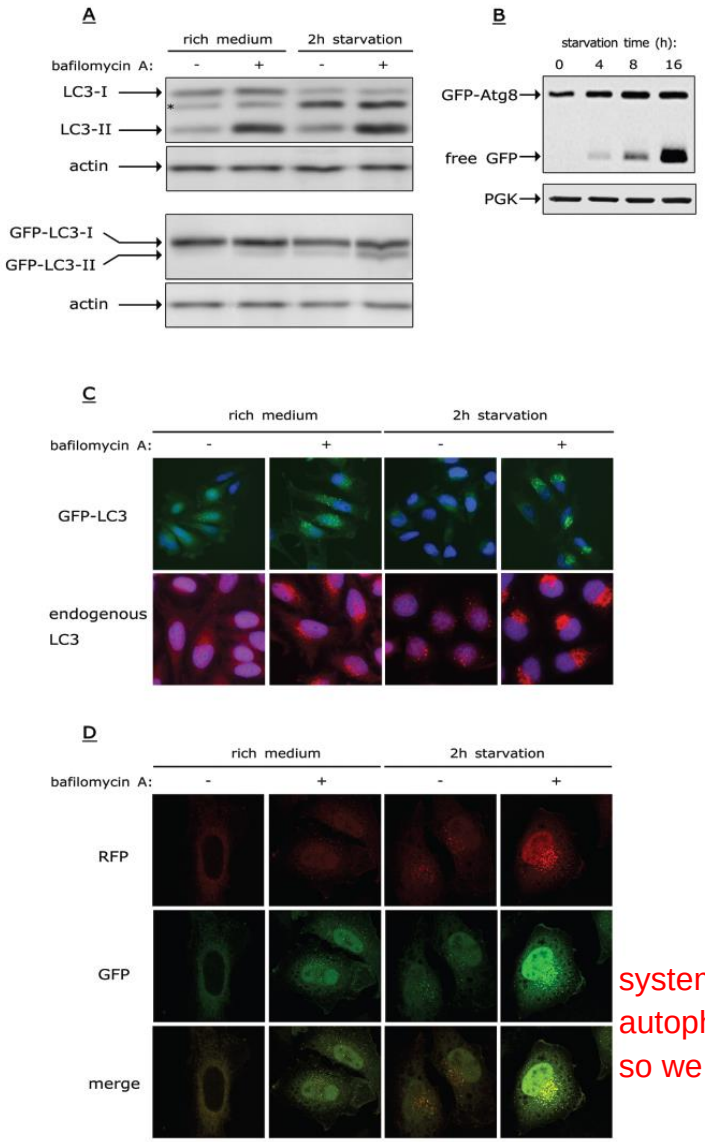
The mammalian Atg8-I homolog, MAP1LC3B, is dubbed the "LC3B-I form".

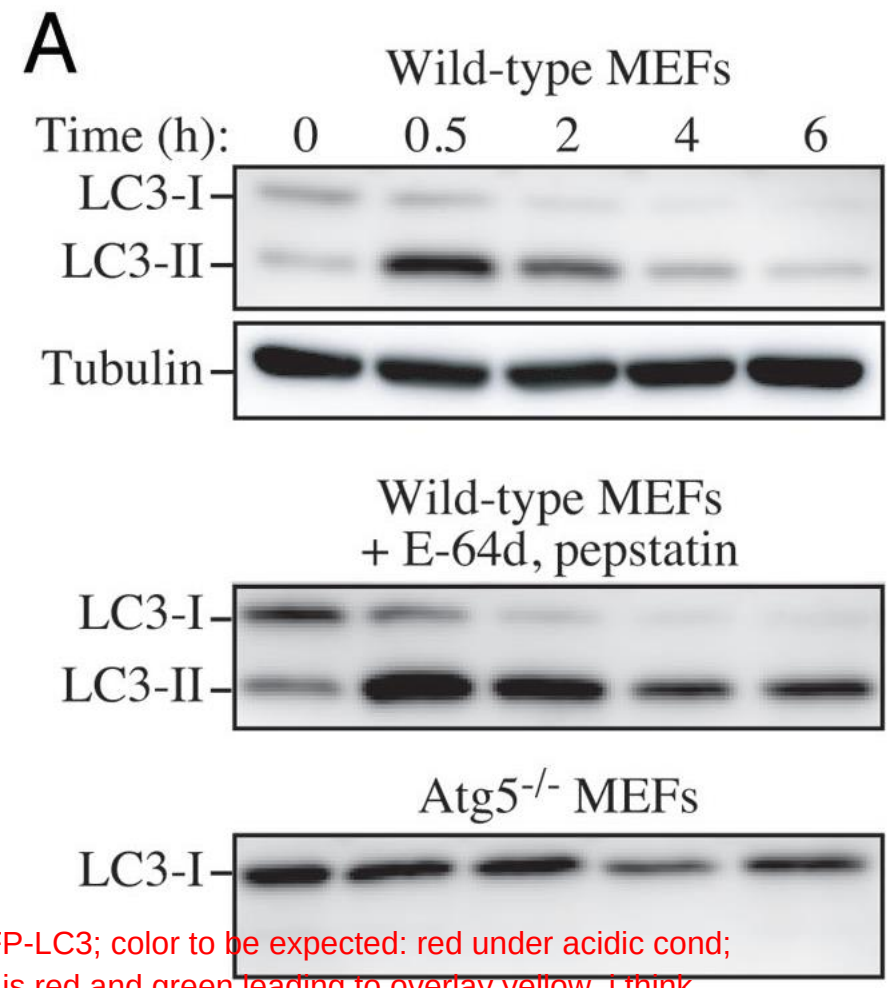
The ATG16L1-ATG12-ATG5 complex is localized to autophagosomal membranes by WIPI2.

Slobodkin and Elazar, Essays Biochem. (2013)

one can increase ph value to
inhibit degradation of proteins,
I think-

LC3-processing to monitor autophagy

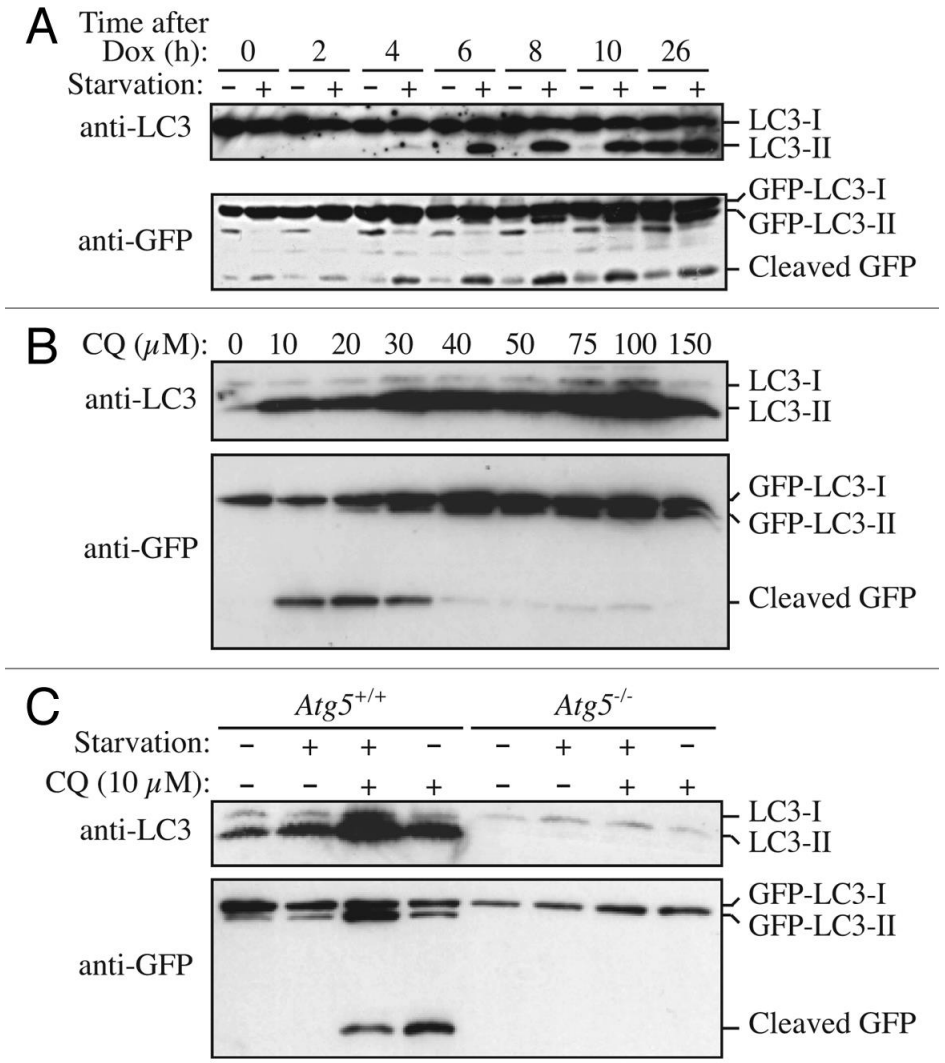




system: RFP-GFP-LC3; color to be expected: red under acidic cond;
autophagasome is red and green leading to overlay yellow, I think
so we can distinguish autophagasomes and autolysosomes

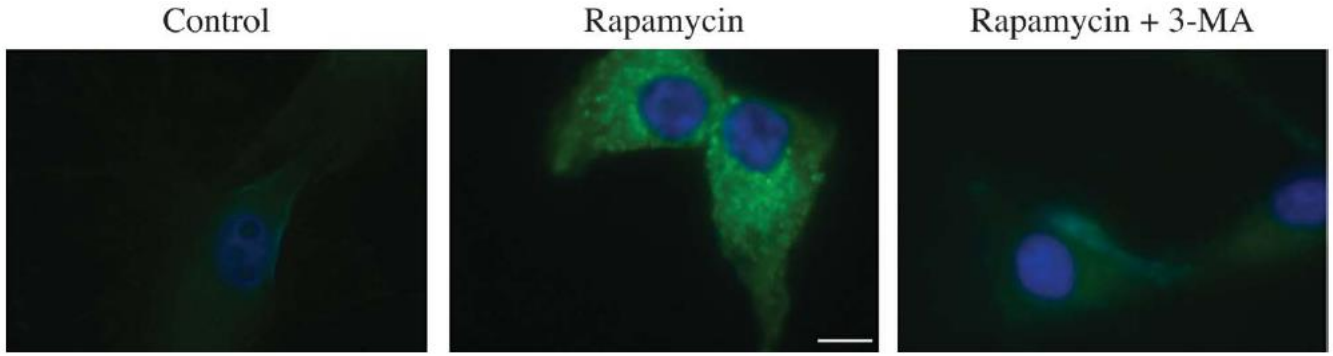
Slobodkin and Elazar, Essays Biochem. (2013)

GFP-LC3 processing to monitor autophagy

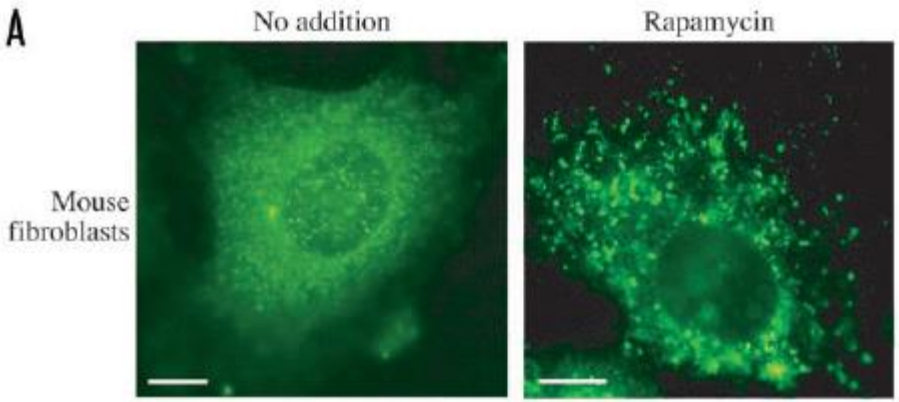


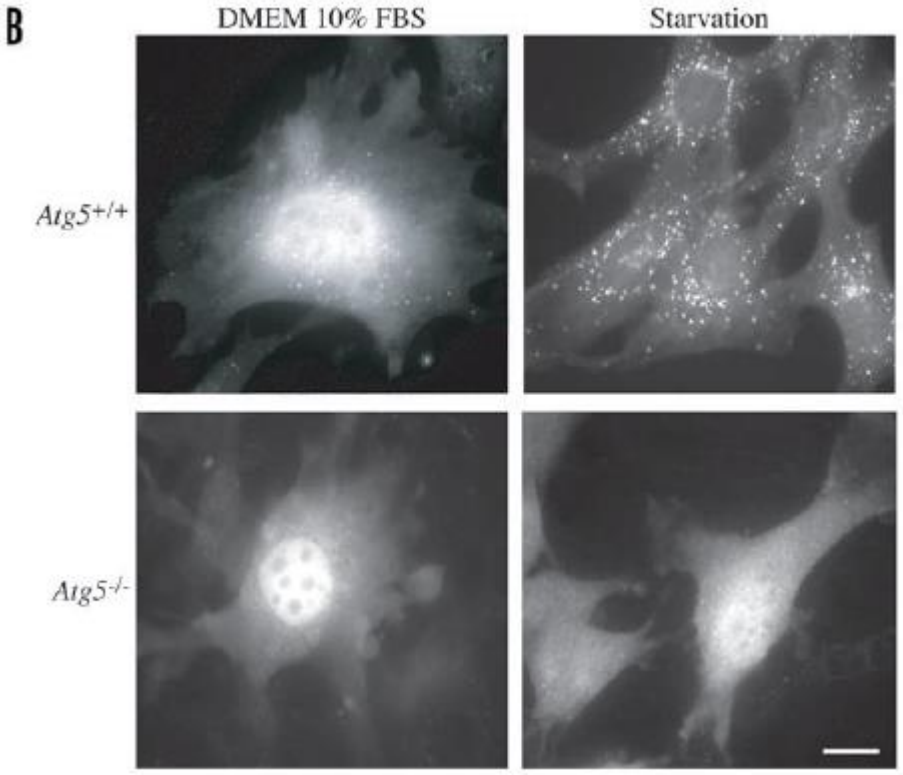
Klionsky et al., Autophagy (2012)

Localization of LC3 upon induction of autophagy

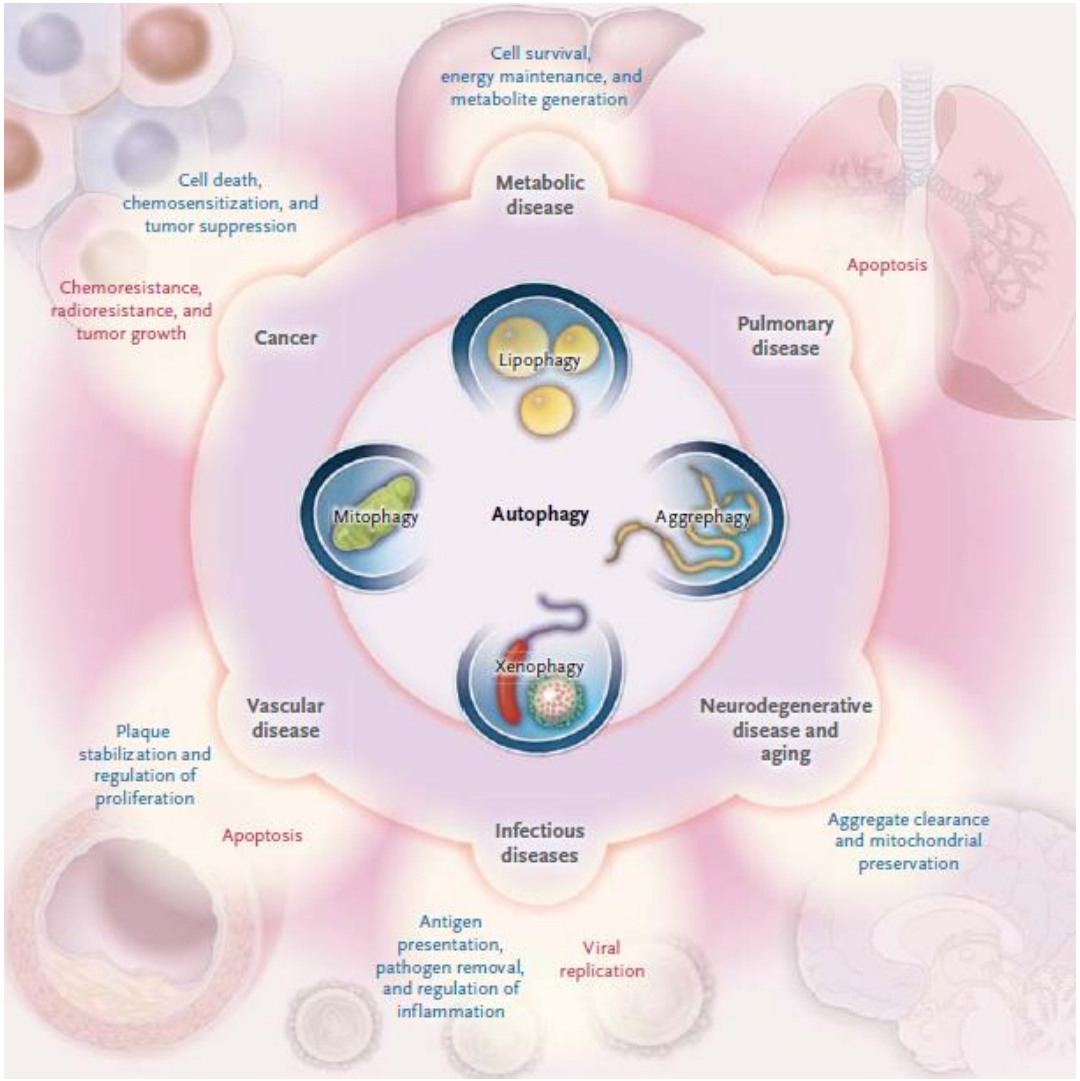


3-MA := 3-methyl adenine





Effects of autophagy on disease progression



Choi et al., N. Engl. J. Med. (2013)

Principles of selective autophagy

The 4 key steps of selective autophagy

Induction

↓

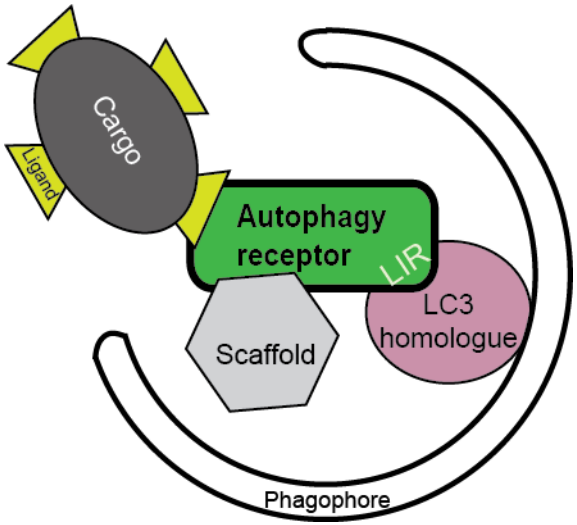
Cargo tagging

↓

Sequestration

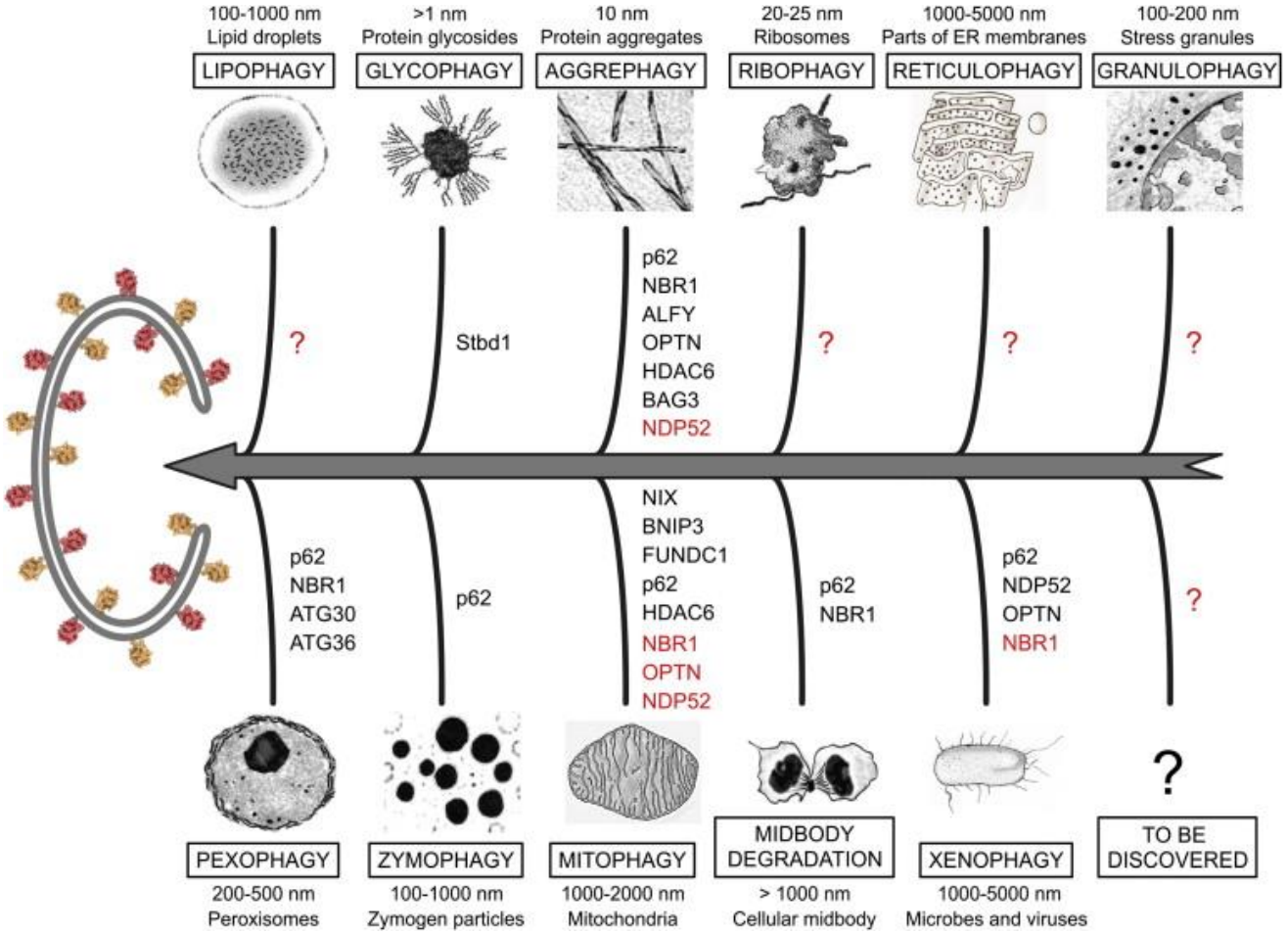
↓

Degradation of cargo and receptor



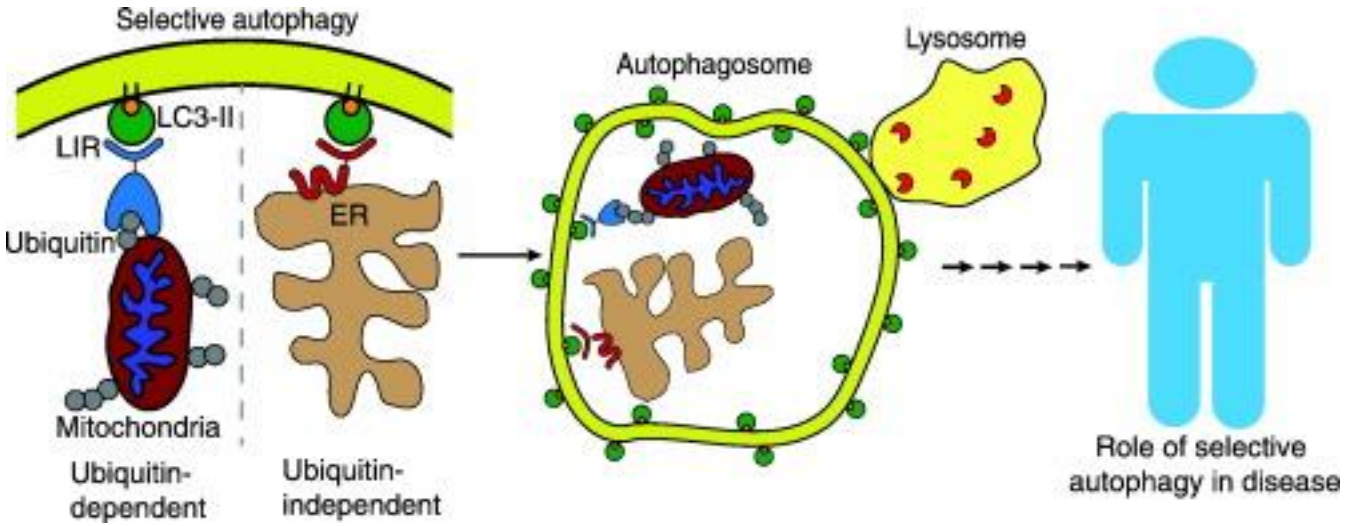
At the heart of this selectivity lies the LC3-interacting region (LIR) motif, which ensures the targeting of autophagy receptors to LC3 (or other ATG8 family proteins) anchored in the phagophore membrane.

Types of selective autophagy in mammalian cells



Rogov et al., Mol. Cell (2014)

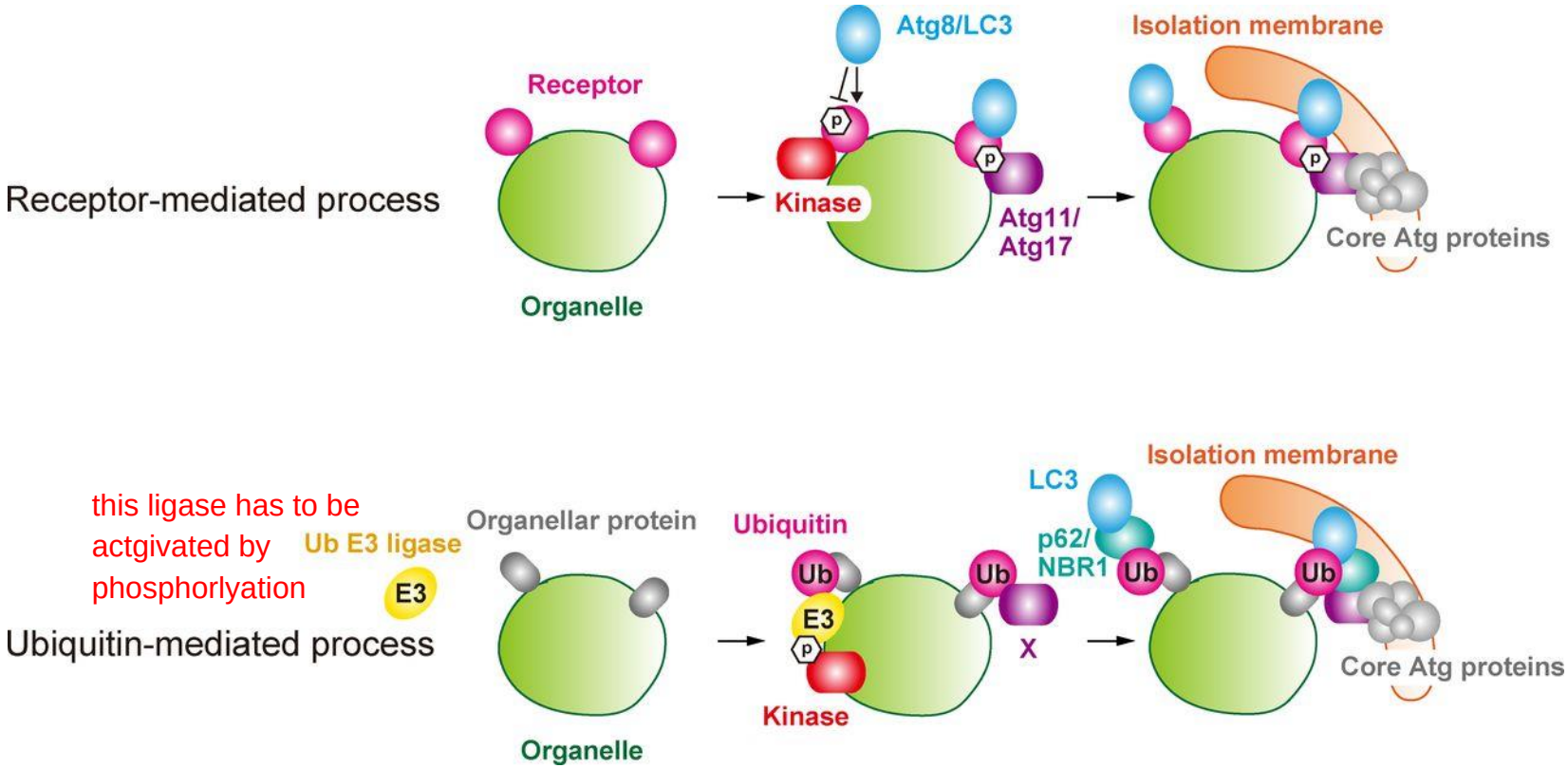
The process and regulation of selective autophagy



Mancias and Kimmelman, J. Mol. Biol. (2016)

Two common mechanisms of organellophagy

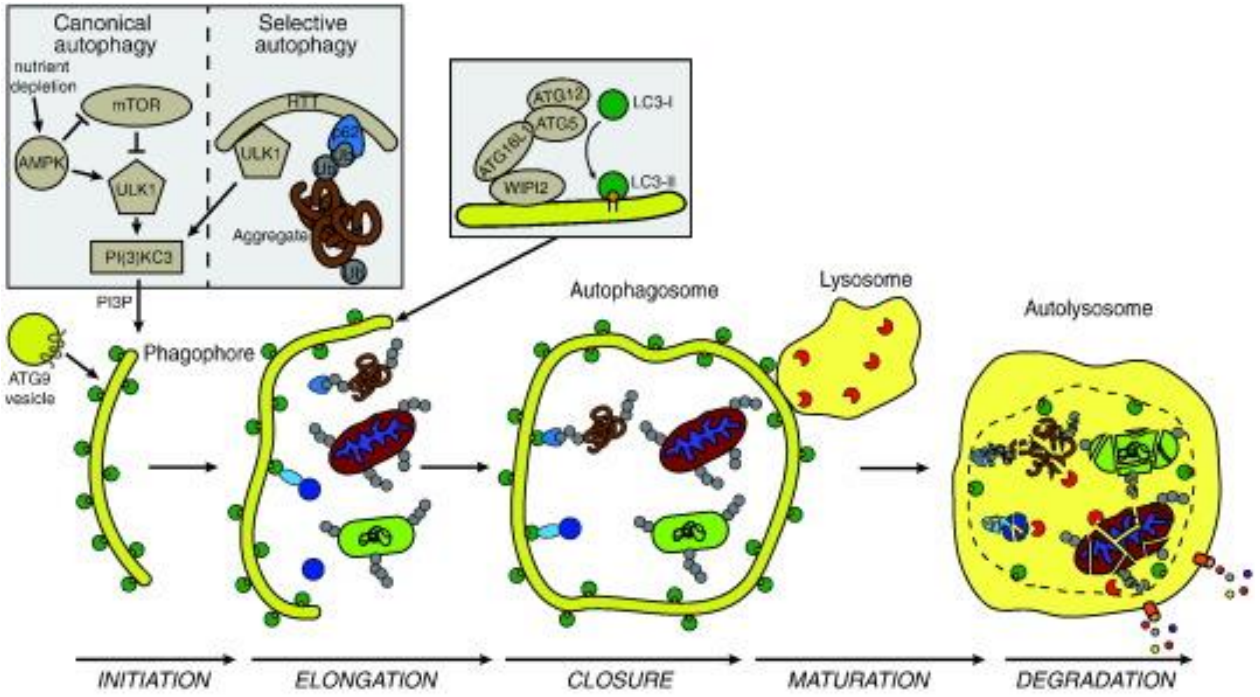
phosphylrations can be inhibitory or activating



Okamoto et al., J. Cell Biol. (2014)

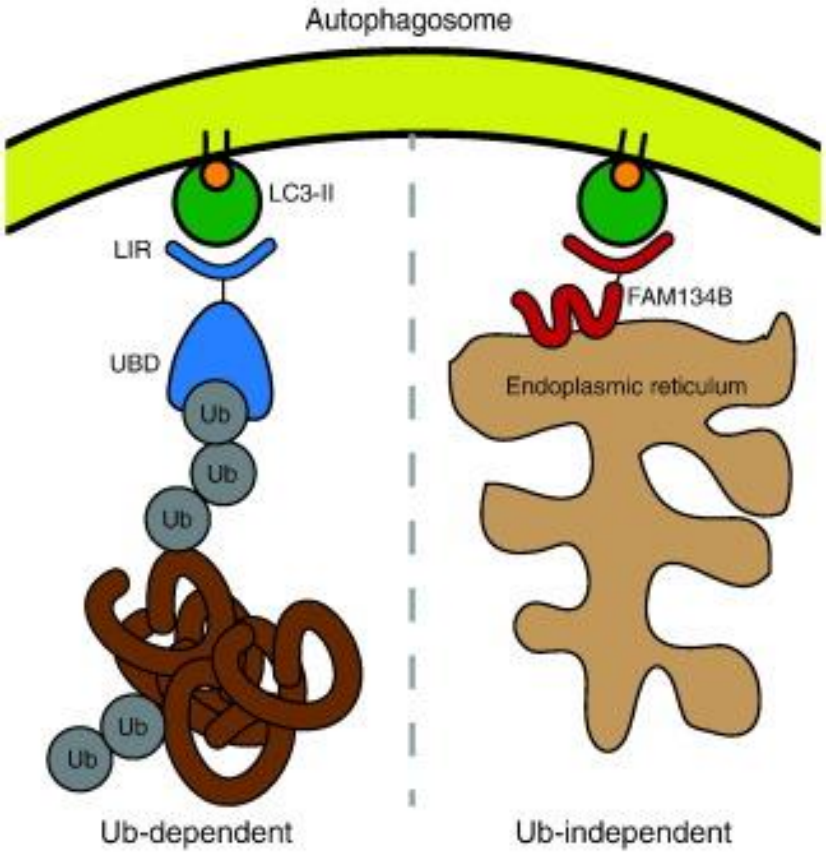
The process and regulation of selective autophagy

a summary



Mancias and Kimmelman, J. Mol. Biol. (2016)

Ubiquitin-dependent and -independent selective autophagy

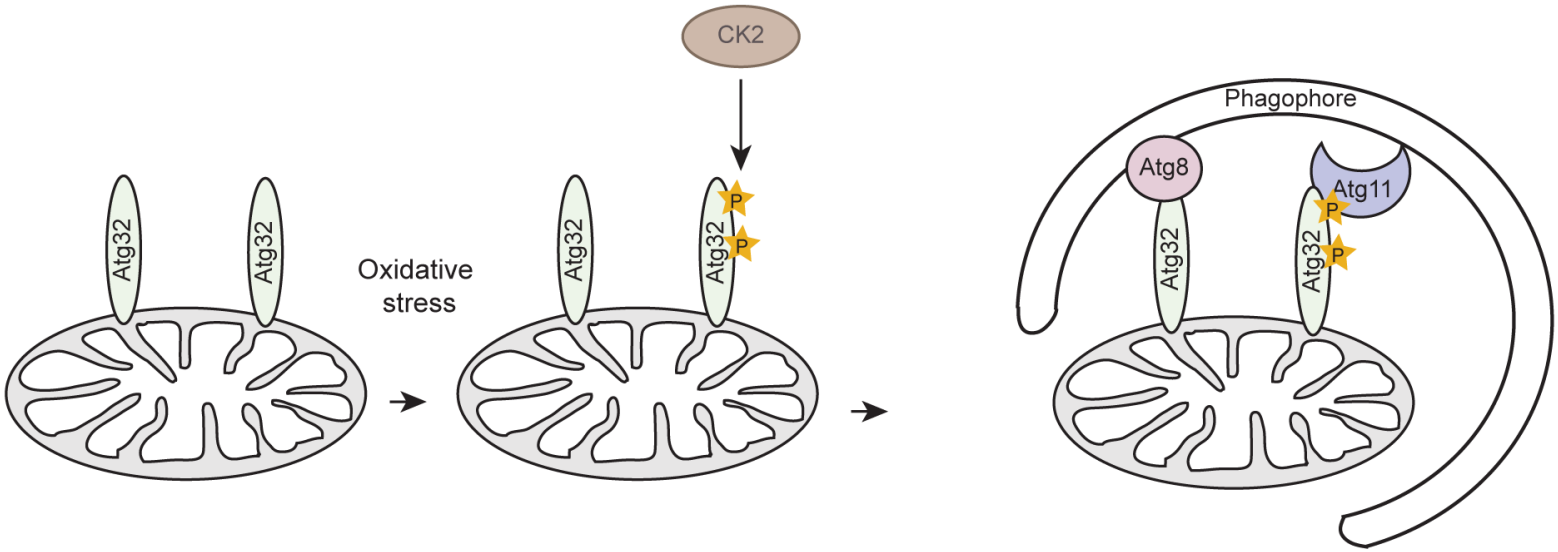


Mancias and Kimmelman, J. Mol. Biol. (2016)

ubiqutitin independent degradation

Receptor-mediated mitophagy in yeast

our focus of today: mitophagy (degradation of mitochondria)

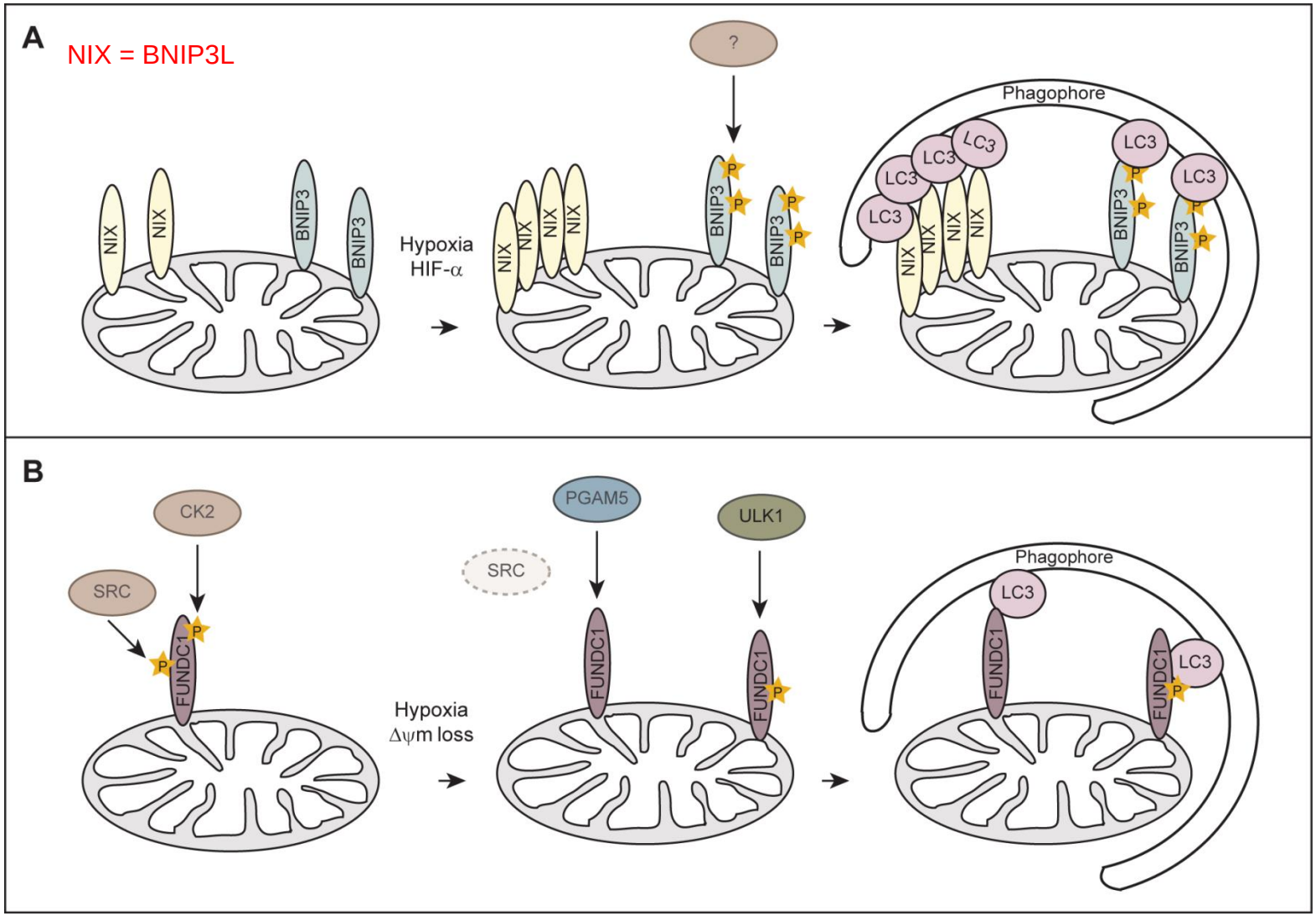


CK2 := casein kinase 2
involved in many selective autophagy processes, both in mammals and yeast, is found not only in mitochondra

Schönenberger and Kovacs, Front. Cell Dev. Biol. (2015)

HIF-dependent regulation of mitophagy

exam question; how does HIF downregulate mitochondrial metabolism?



Schönenberger and Kovacs, Front. Cell Dev. Biol. (2015)

Ubiquitin-mediated cargo recognition

There is a cooperative function of the autophagy-lysosome system with the ubiquitin-proteasome system to manage the turnover of damaged proteins to maintain the proteome.

The ubiquitin-proteasome system requires unfolding of substrates for degradation via the proteasome core.

The autophagy-lysosome system is capable of handling much larger protein aggregates or tightly folded proteins without a requisite unfolding step.

There is some overlap in specificity for ubiquitylated cargo among selective autophagy receptors. In some cases this overlap is cooperative to mediate delivery to autophagosomes (e.g., mitophagy). In other cases multiple different autophagy receptors appear capable of mediating the process individually (e.g., xenophagy).

Post-translational modifications of both the selective autophagy receptors as well as the cargo (and in some cases ubiquitin itself on the cargo) are integral to regulating autophagy receptor function.

Additional complexity given that many of the selective autophagy receptors have non-autophagy functions.

Receptors and substrates in selective autophagy pathways

Pathway	Receptor	Substrate	Refs
Ub-dependent			
Aggrephagy	p62, NBR1, OPTN, Cue5, TOLLIP	Protein aggregates	[32–36]
Mitophagy	OPTN, NDP52, Tax1BP1	Mitochondria	[41–43]
Xenophagy	p62, NDP52, OPTN	Bacteria	[37–39]
Pexophagy	NBR1	Peroxisomes	[40]
Zymophagy	p62	Zymogen	[16]
Proteaphagy	RPN10	Proteasomes	[24]
Midbody disposal	p62, NBR1	Midbody	[15,44]
Nucleic acid disposal	p62, NDP52	Nucleic acids	[18,45]
Ub-independent			
Mitophagy	NIX, BNIP3, FUNDC1, Atg32	Mitochondria	[84–89]
ER-phagy	FAM134B, Atg40	ER	[93,95]
Nucleophagy	Atg39	Nuclear envelope	[95]
Ferritinophagy	NCOA4	Ferritin	[12,13]
Pexophagy	NBR1, Atg30, Atg36	Peroxisomes	[40,90,91]
Glycophagy	Stbd1	Glycogen	[92]
Signalophagy	c-Cbl	Src	[19]
Cvt targeting	Atg19, Atg34	Ape1, Ams1	[82,83]
Lysophagy	Galectin-8	Lysosomes	[97]
Xenophagy	Galectin-8	Bacteria	[97]
Virophagy	TRIM5α, SMURF1	Viral components	[17,20]
Fatty acid synthase (FAS) disposal	FAS	FAS	[21]

Khaminets et al., Trends Cell Biol. (2016)

Mitochondrial stress

Various insults can cause damage:

- Environmental (radiation, toxic chemicals)
- Genetic (mutations in genes for metabolic processes or repair pathways)
- Spontaneous (ROS generated as byproduct of electron transport)

Types of damage:

- DNA
- Proteins
- Lipids

Problems caused by damage:

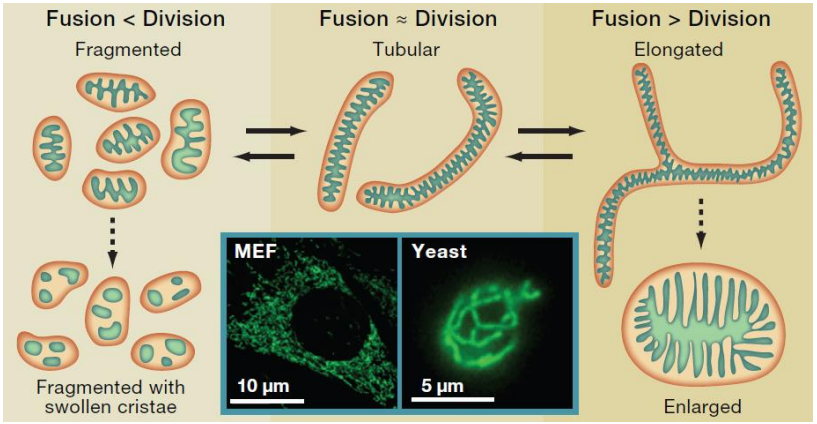
- Loss of metabolic functions (ATP synthesis, etc.)
- More ROS made by defective mitochondria
- F_1F_0-ATPase may, instead of making ATP, consume ATP to generate membrane potential

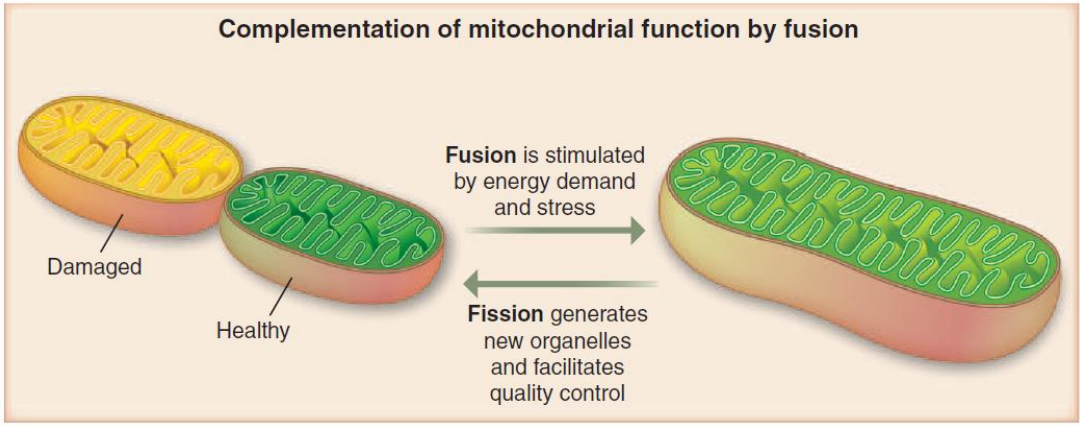
Cellular responses to damage:

- DNA repair
- Proteases
- Lipases
- Mitochondrial unfolded protein response
- Mitophagy
- Apoptosis

Youle and van der Bliek, Science (2012)

Mitochondrial fission and fusion



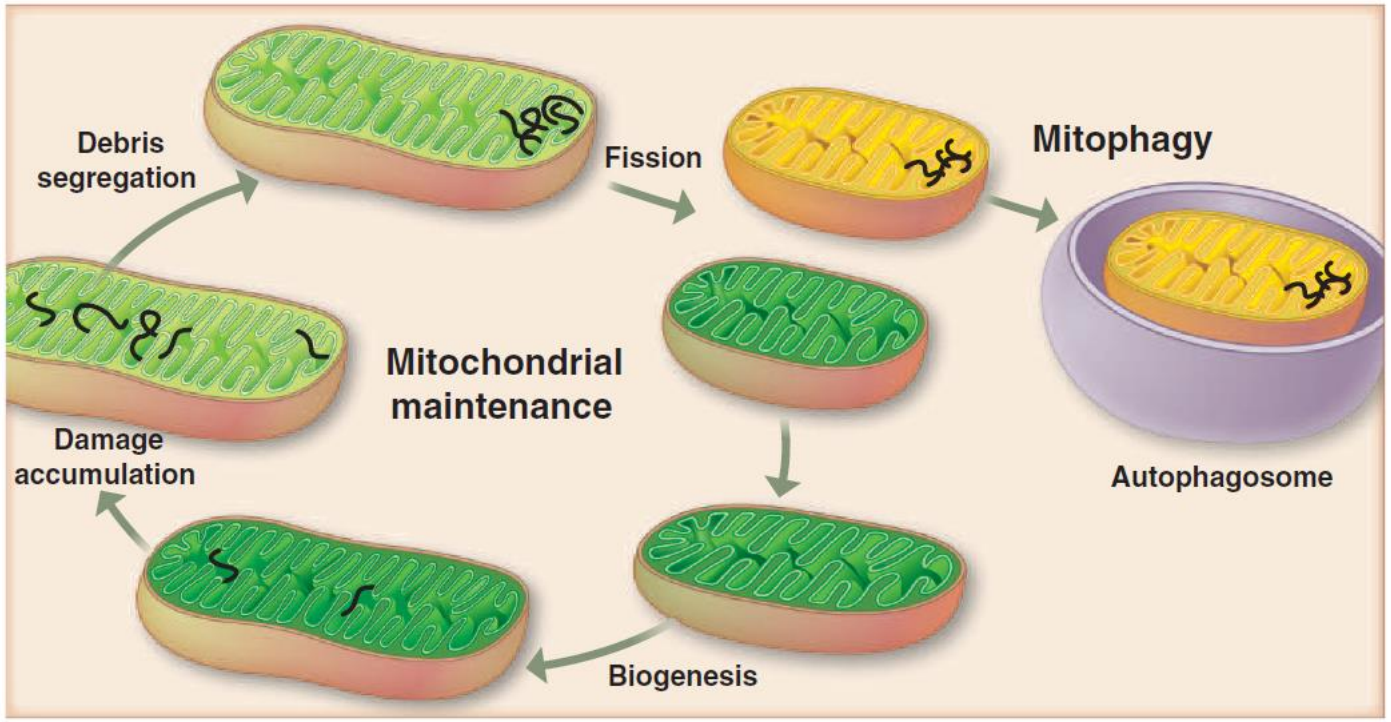


Fission proteins:
Dynamin-related GTPase (Drp1/Dlp1)
Mitochondrial fission factor (Mff)
Fission 1 (Fis1)
GDAP1

Fusion proteins:
Optic atrophy 1(Opa1)
Mitofusin 1 (Mfn1)
Mitofusin 2 (Mfn2)

Youle and van der Bliek, Science (2012)

Segregation of damaged parts of mitochondria by fission



Youle and van der Bliek, Science (2012)

Parkinson's disease

- The term parkinsonism is used for a motor syndrome whose main symptoms are tremor at rest, stiffness, slowing of movement and postural instability.
- 1817 first described by James Parkinson.
- The second most common age-related neurodegenerative disease.
- The central pathological feature is the loss of neurons in the substantia nigra pars compacta (SNpc).
- 1997: discovery that mutations in the gene for a-synuclein cause an inherited form of PD.

Illustration of Parkinson's disease by William Richard Gowers from *A Manual of Diseases of the Nervous System in 1886*

Parkinson's disease

Identifizierte Gene familiären Parkinsonismus

Gen	Lokus	Alter	Mutationen	Klinik	Pathologie	Bemerkung
LRRK2	Park8 (12cen)	50–70a	Dominant, über 20 verschiedene missense Mutationen (G2019S, R1441C/G, Y1699C)	wie sporadischer M.P., Demenz Amytrophie	überwiegend Lewy Bodies, Neurofibrilläre Tangles (selten) und/oder nigrale Degeneration	etwa 1–5% der sporadischen, 10–20% der dominanten Fälle, 20–40% der Ashkenazi Juden bzw. der nordafrikanischen Bevölkerung
α-Synuklein	Park1 und Park4 (4q21)	38–65a Duplikation 24–48y Triplikation	Dominant A30P, E46K, A53T, genomische Multiplikationen			Allelvariationen prädisponieren für sporadischen M. P.
UCHL1	Park5 (4p14)	55–58 a	Dominant (I93M)	sporadisch	n.b	Allelvariationen prädisponieren für sporadischen M.P
Parkin	Park2 (6q25–q27)	~30 a (20–70 a)	Rezessiv, Missense, Deletionen, Duplikationen, Rearrangements	Beginn oft mit Dystonie, gutes Ansprechen auf L-Dopa	Nigrale Degeneration	50% aller früh beginnenden familiären Fälle (~20a); 20% aller frühen sporadischen Fälle (<50 a)
PINK1	Park6 (1p35–p36)	20–40 a	Rezessiv, Missense, Deletionen	Langsam progredient gutes Ansprechen auf L-Dopa	n.b	Selten, 1–2% der früh beginnenden Fälle (~50a), Haploinsuffizienz prädisponiert möglicherweise für späten M. P
DJ1	Park7 (1p36)	20–40 a	Rezessiv, Missense, Deletionen	Langsam progredient eventuell psychiatrisch Symptome	n.b	Selten, < 1% der früh beginnenden Fälle (~50a)
ATP13A2	Park9	~20 a	Rezessiv, splice site, Frame shift Mutation	Degeneration pyramidaler Zellen, Demenz	n.b	

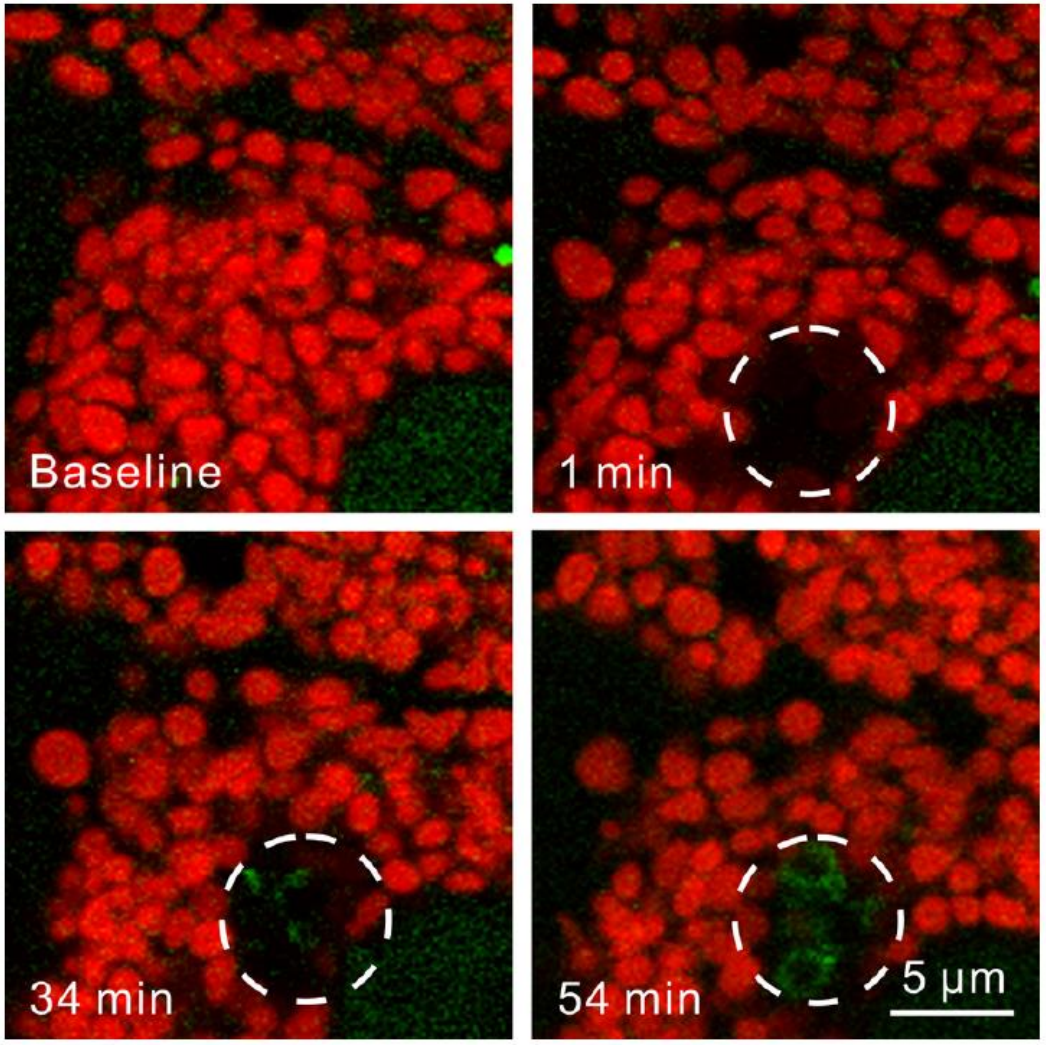
n.b.: nicht bestimmt; M.P.: Morbus Parkinson

Tab.: Auf Basis von Kopplungsanalysen in großen monogenen Parkinson-Familien gelang in den letzten 10 Jahren die Identifikation chromosomaler Loci für familiären Parkinsonismus, für 7 der 10 Genorte konnten die entsprechenden Gene gefunden werden

Parkin is an E3 ubiquitin ligase
PINK1: PTEN-induced kinase 1

Genetik des Morbus Parkinson: Zimprich A, Jatros, 2007

Photodamage-induced mitophagy



Mitochondria (TMRM)

GFP-LC3

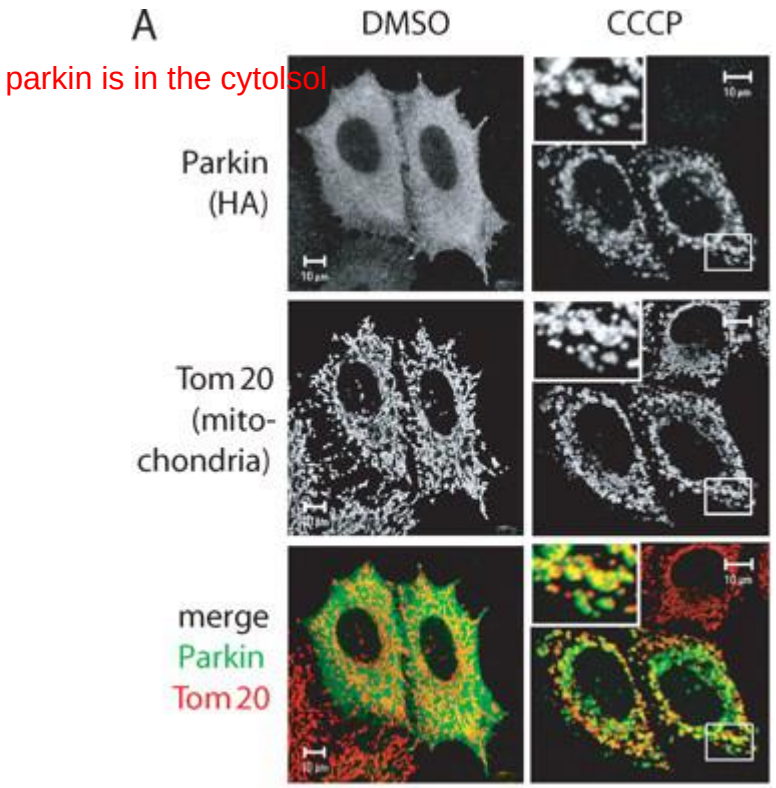
laser shot on mitochondria

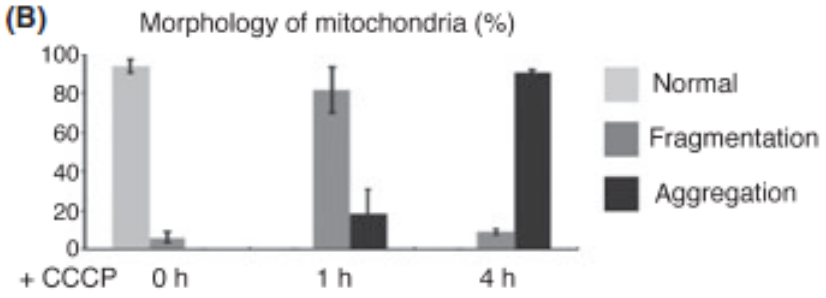
after around 1h, the damaged mitochondria were removed; green are autophagosomes containing damages mitochondria

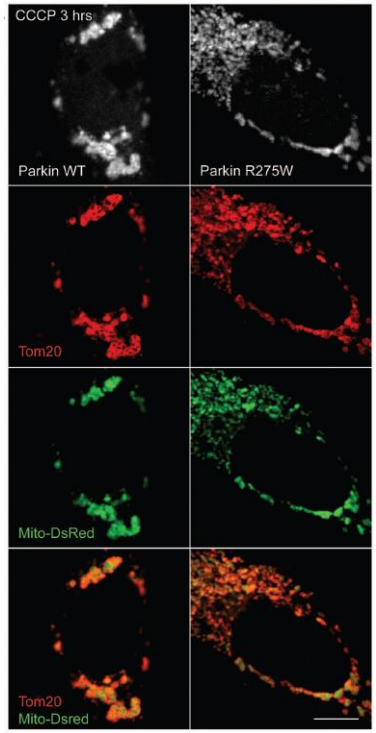
Kim et al., Arch. Biochem. Biophys. (2007)

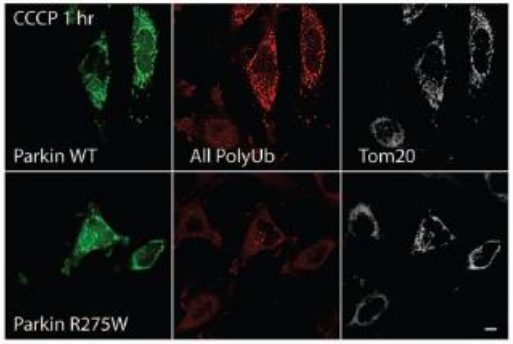
Recruitment of Parkin to damaged mitochondria

CCCP is a drug that can depolairze mitochondira, simulating mitochondrial damage



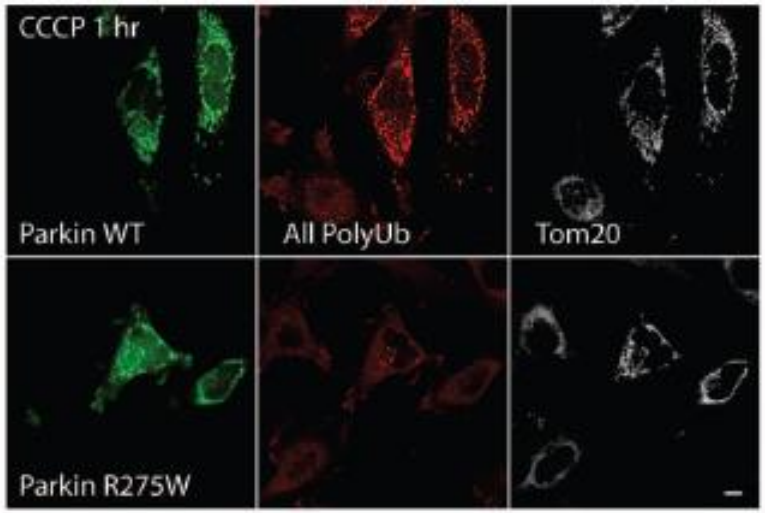


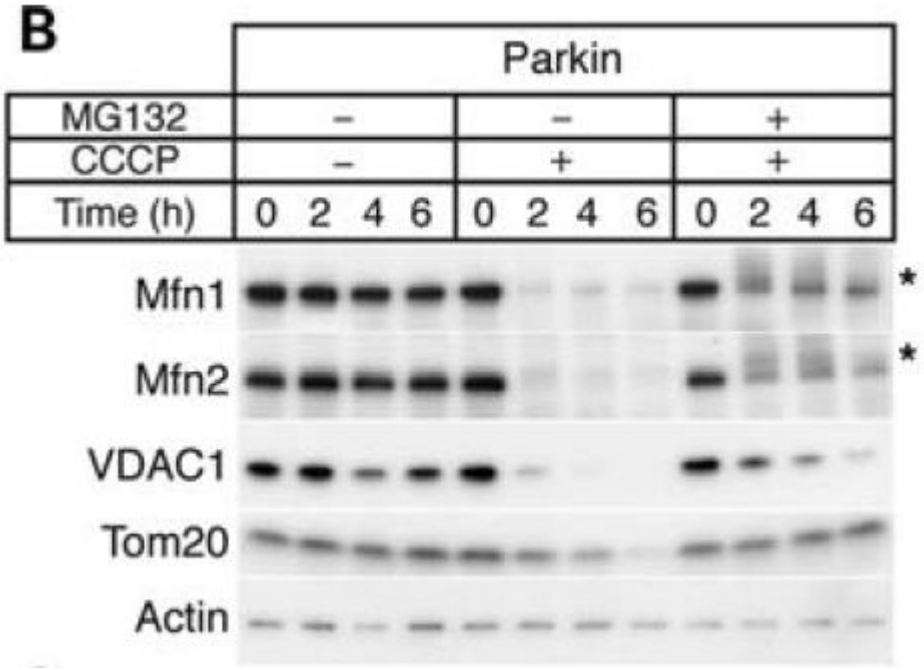




Matsuda et al., J. Cell Biol. 2010
Okatsu et al., Genes Cells 2010
Narendra et al., Autophagy 2010

Parkin mediates extensive proteolysis of outer mitochondrial membrane proteins via the ubiquitin proteasome system (UPS)





Narendra et al., Autophagy 2010

Youle and van der Bliek, Science (2012)

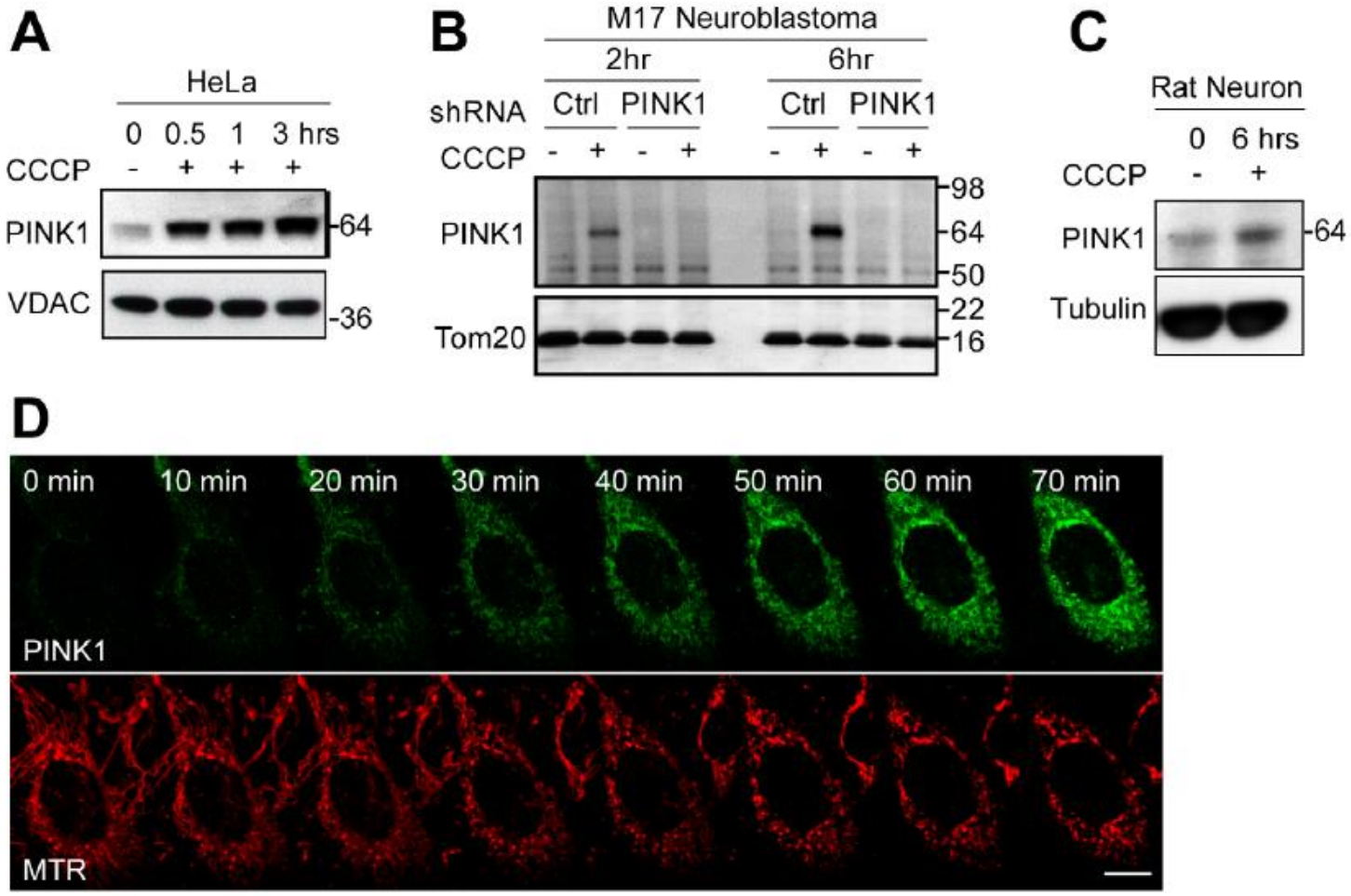
1. Summary

1. Mitochondria are depolarized (with CCCP or due to damage)
2. Parkin translocates to damaged mitochondria.
3. Parkin ubiquitylates mitochondrial surface proteins (e.g., MFN1, MFN2, VDAC).
4. Proteasome translocates to damaged mitochondria.
5. Surface proteins are degraded.

How does Parkin detect damaged mitochondria?

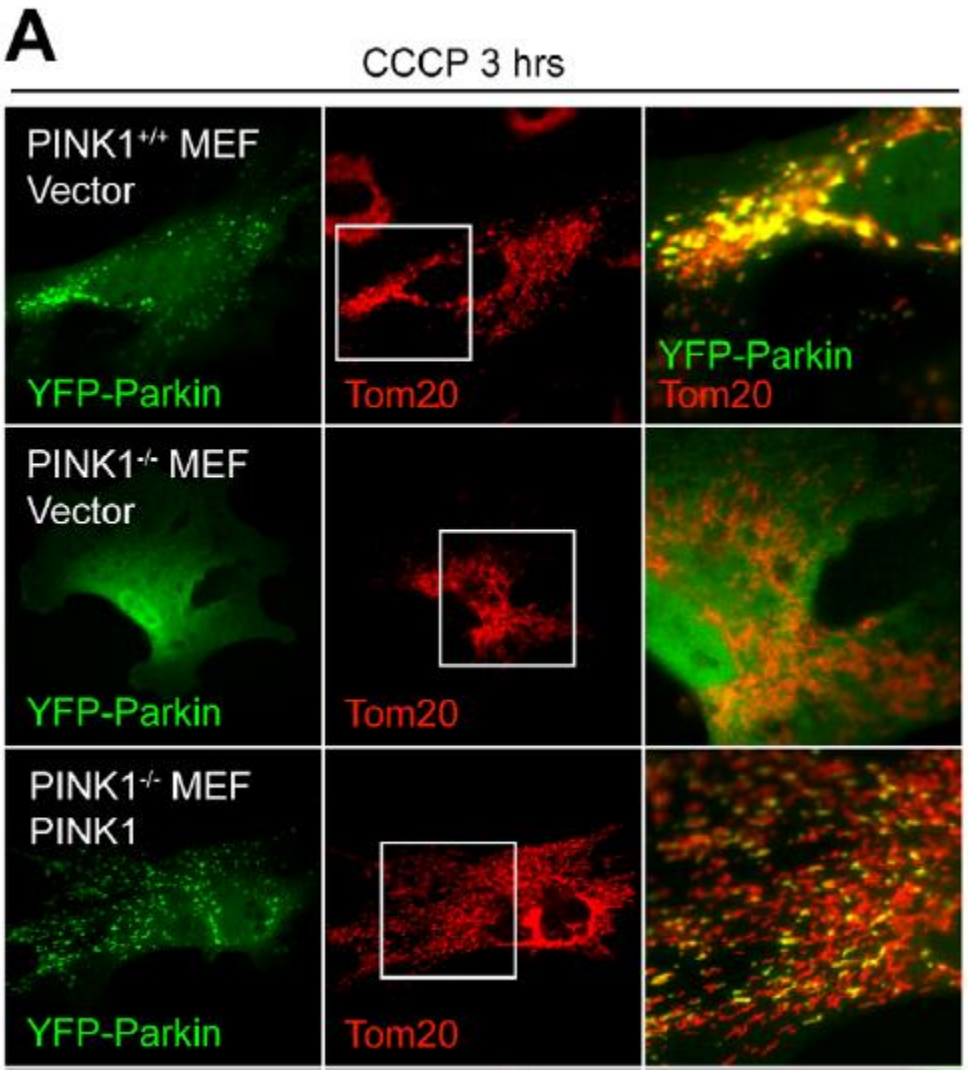
How does the autophagic machinery detect these mitochondria?

PINK1 gets stabilized and accumulates on depolarized mitochondria



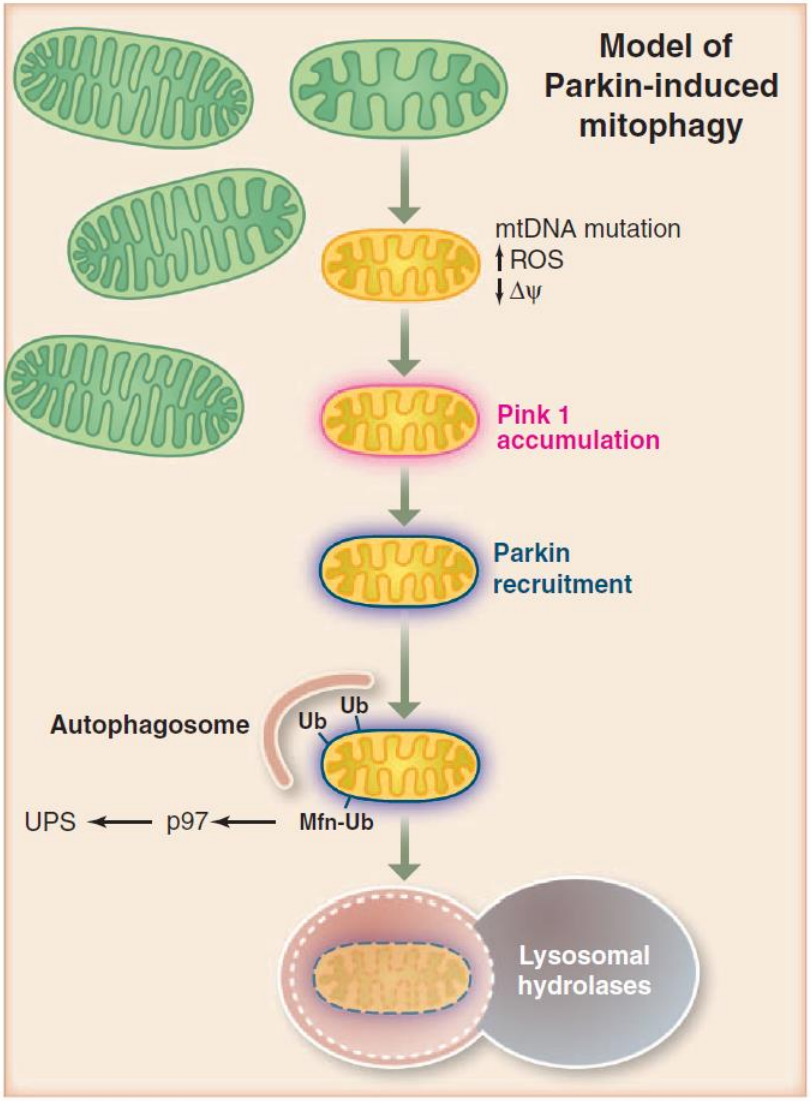
Narendra et al., PLOS (2010)

Parkin recruitment to depolarized mitochondria requires PINK1



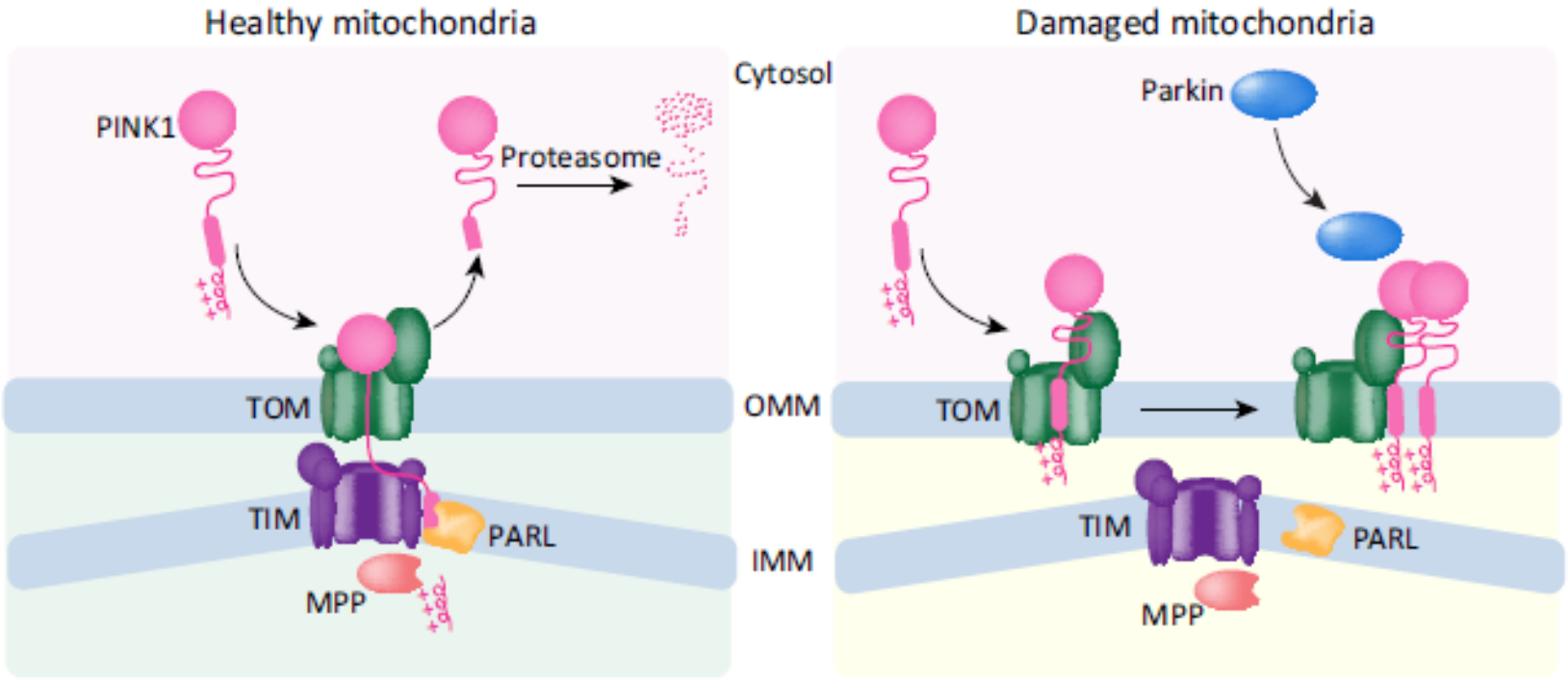
Narendra et al., PLOS (2010)

Model of Parkin-induced mitophagy



Youle and van der Bliek, Science (2012)

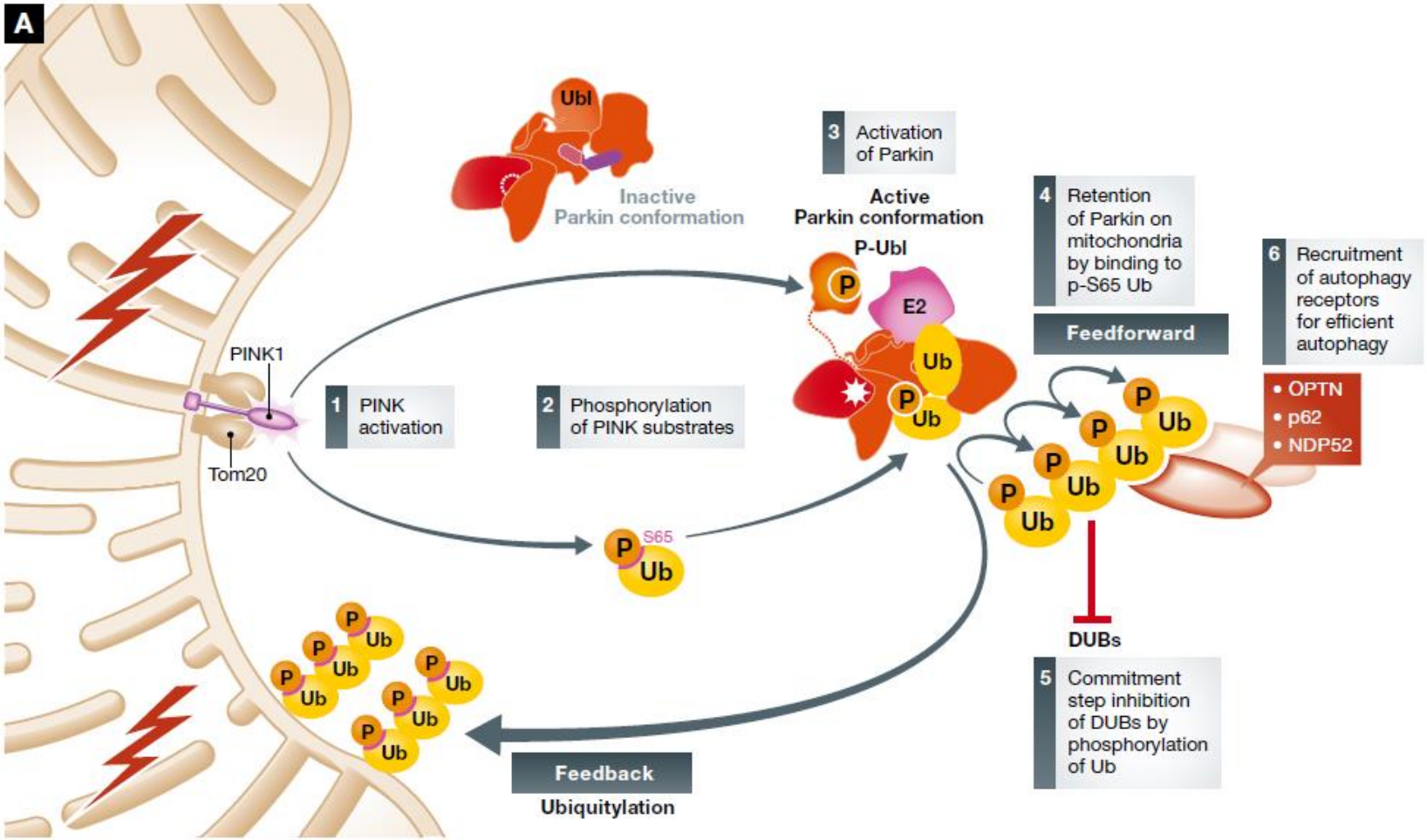
PINK1/Parkin-mediated mitophagy



MPP: matrix processing peptidase, removes PINK1's N-terminal mitochondrial targeting signal
PARL: rhomboid presenilin-associated rhomboidlike

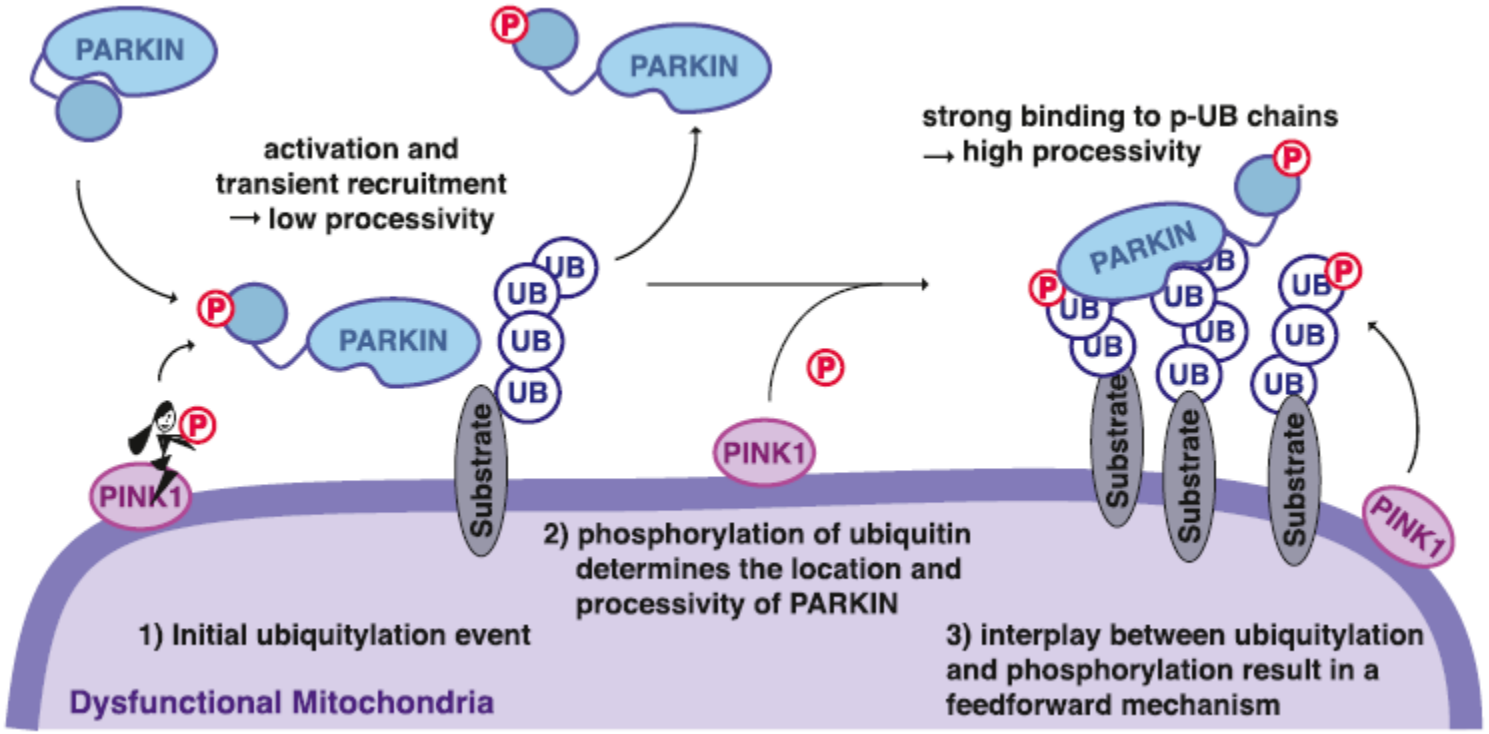
Nguyen et al., Trends Cell Biol. (2016)

PINK1-mediated phosphorylation of ubiquitin and Parkin



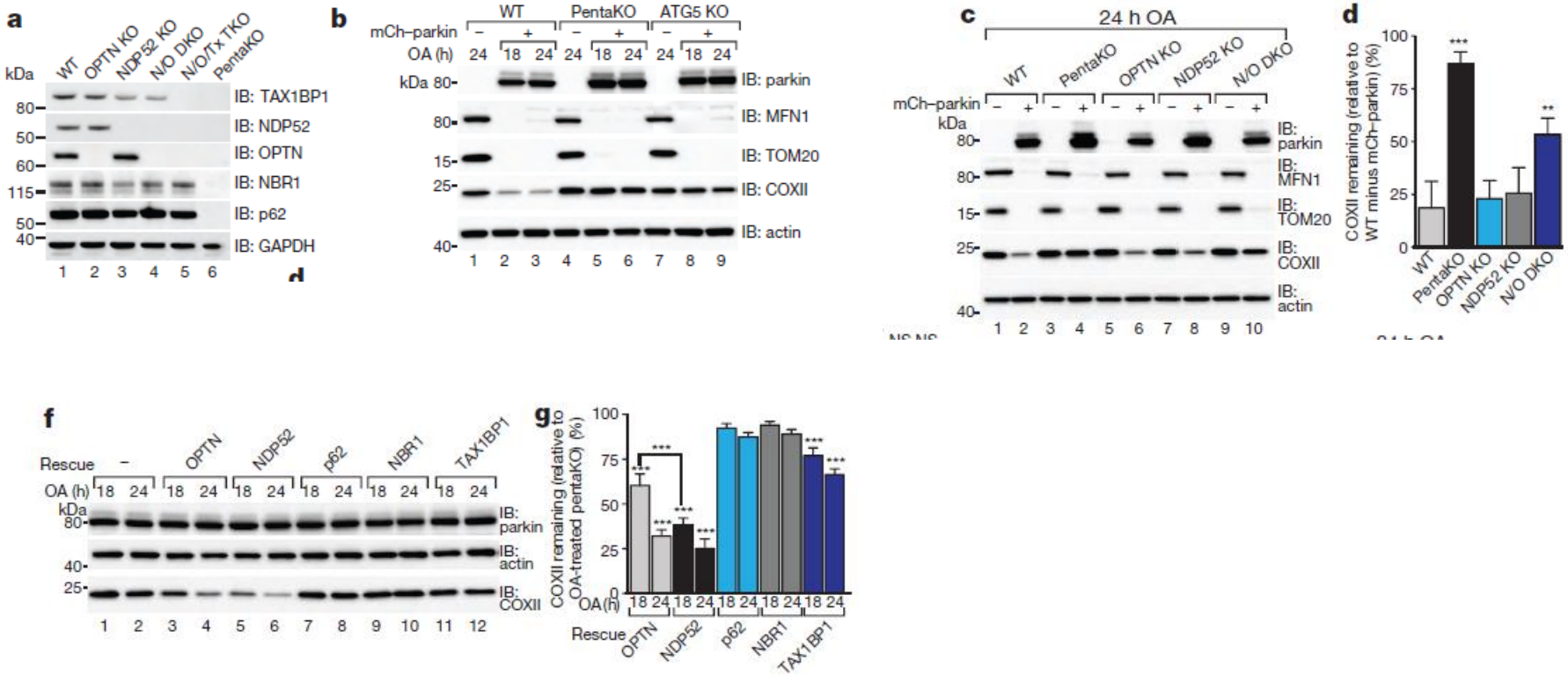
Herhaus and Dikic, EMBO Rep. (2015)

Phosphorylation of ubiquitin during PINK1/Parkin-mediated mitophagy



Stolz and Dikic, Mol. Cell (2014)

NDP52 and optineurin are the primary receptors for PINK1- and parkin-mediated mitophagy



OA: Oligomycin and antimycin A treatment