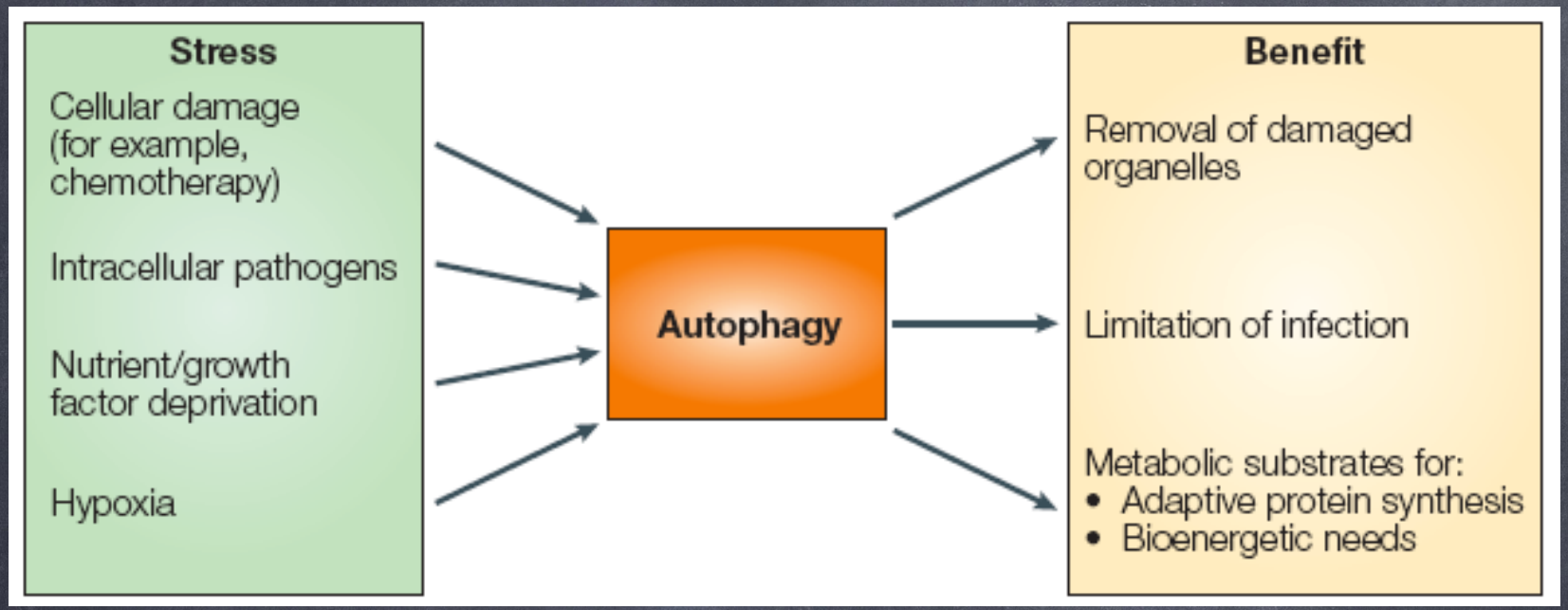
Autophagy and stem cells



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"Autophagy promotes life"

Autophagy ("self eating") is an old, evolutionarily conserved stress response that is present in all living cells. Like apoptosis, autophagy is a programmed response and has several sub-pathways. Unlike apoptosis, autophagy promotes life rather than death.

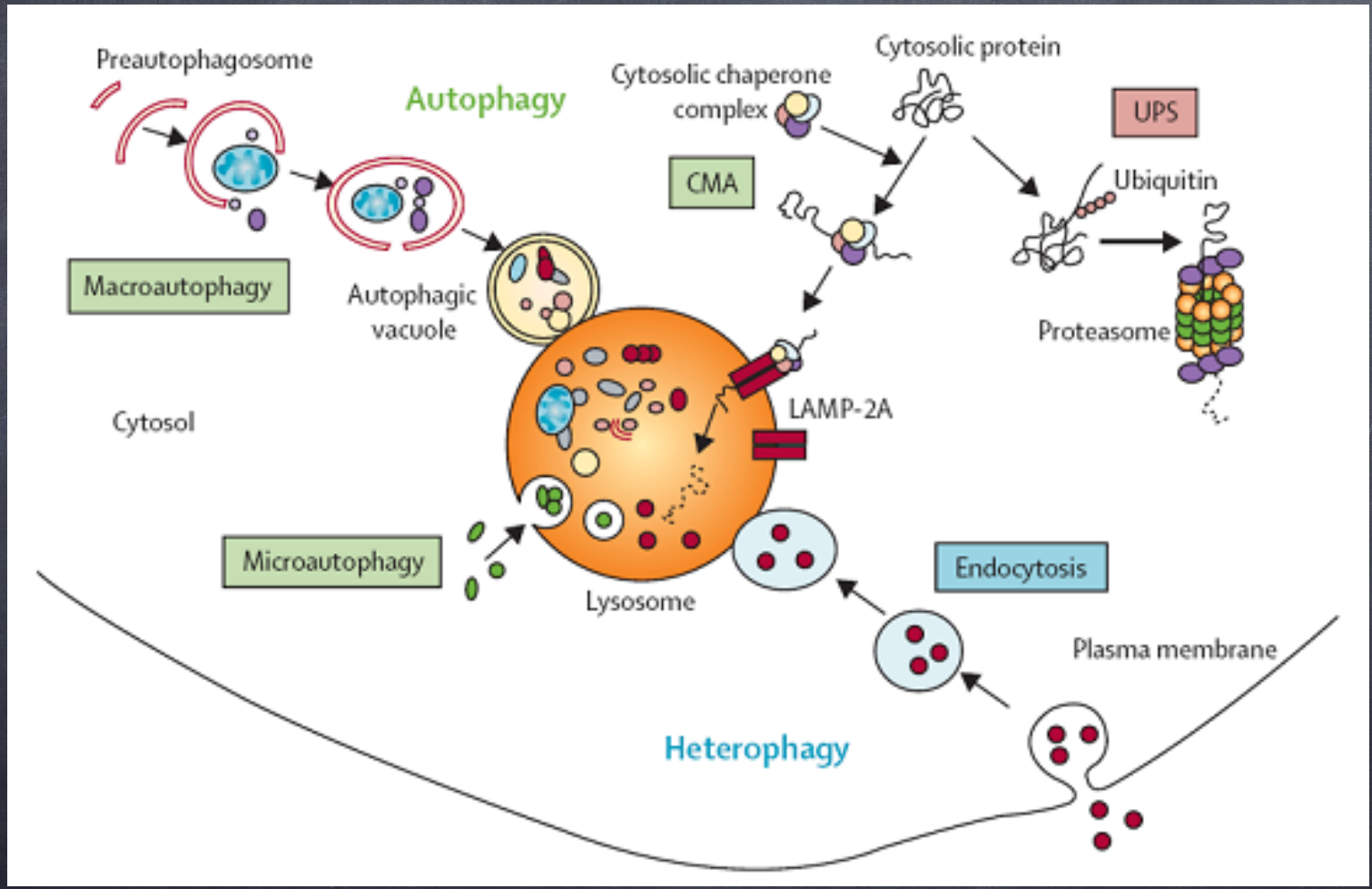
Recent discoveries have shown that almost every genetic, dietary, and pharmacologic manipulation proven to extend lifespan activates autophagy as part of its mechanism of action.

Three different types of Autophagy

- Macroautophagy
- Microautophagy
- Chaperone mediated autophagy (CMA)

Share a common destiny of lysosomal degradation, but are mechanistically different from one another.

Autophagy versus heterophagy



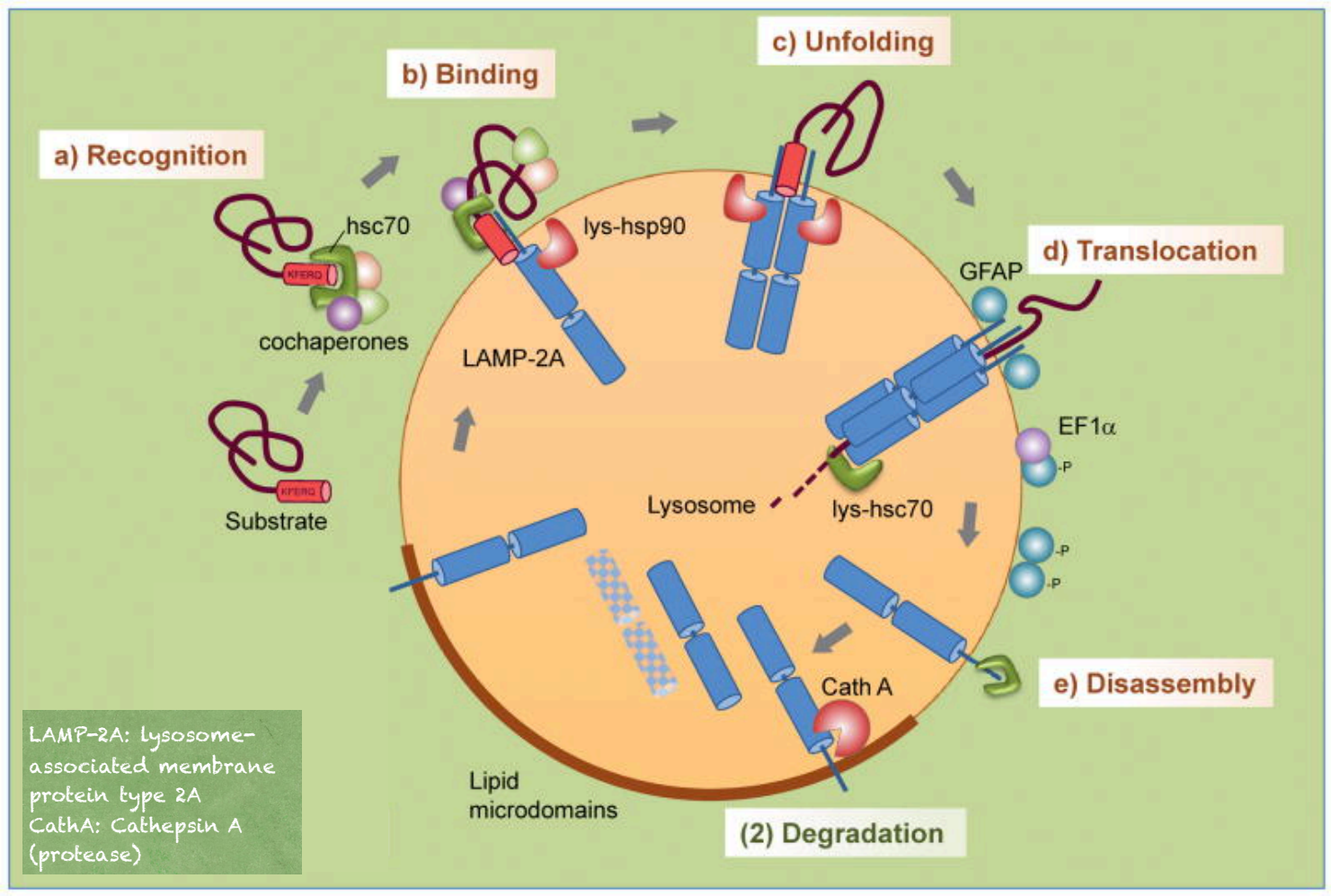
CMA

Chaperon-mediated autophagy

All cellular proteins undergo continuous synthesis and degradation. This permanent renewal is necessary to maintain a functional proteome and to allow for rapid changes in levels of specific proteins with regulatory purposes.

Although for a long time lysosomes were considered unable to contribute to the selective degradation of individual proteins, the discovery of chaperone-mediated autophagy (CMA) changed this notion.

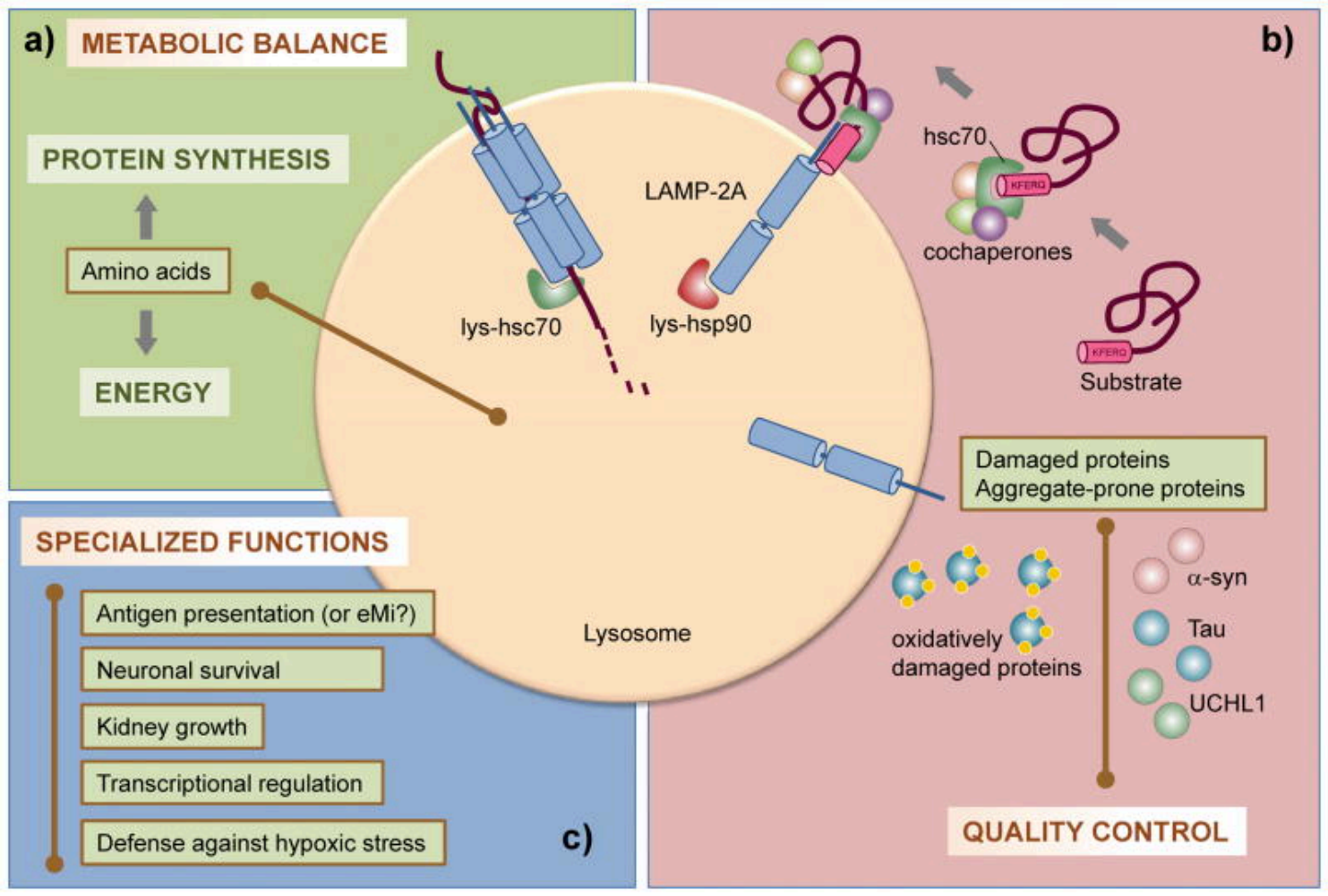
Steps and regulation of CMA: Cytosolic proteins rerouted for lysosomal degradation



Properties of the CMA-targeting motif

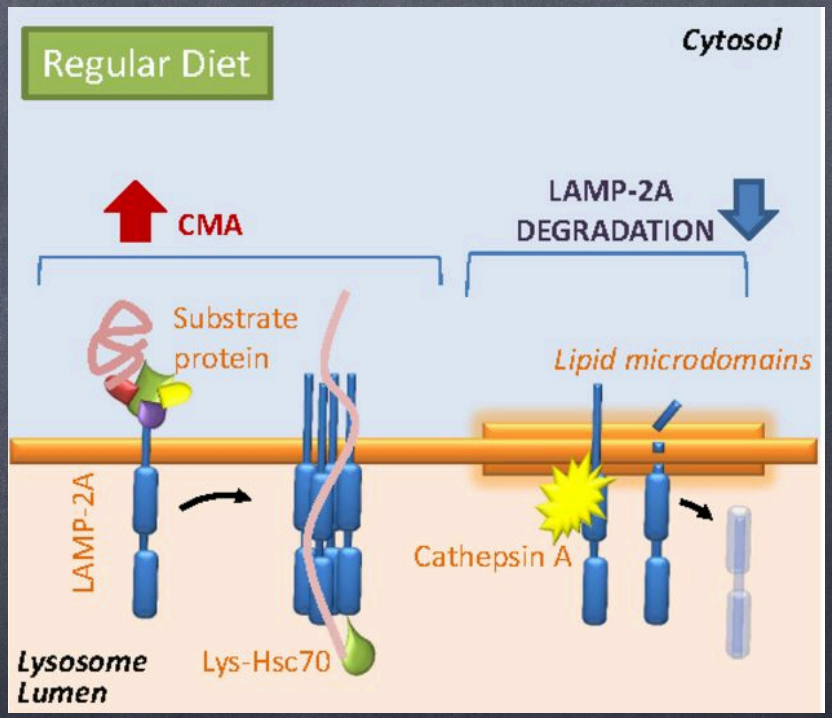
- Composition: a pentapeptide motif (ex: KFERQ) that is necessary and sufficient for their targeting to lysosomes
- Location: only requirement being that it becomes exposed or accessible for chaperone binding. Mb protein: no CMA-targeting motif except those in which a cytosolic fragment can be released.
- Abundance: Sequence analysis reveals that about 30% of soluble cytosolic proteins contain a putative CMA-targeting motif
- Selectivity: This motif has recently been shown to be also utilized for targeting of proteins to late endosomes in order to undergo microautophagy. In addition, it is possible that some KFERQ-like motifs are utilized for targeting of proteins that exert a function at the lysosomal membrane and do not necessarily undergo degradation in this compartment.

Functions of CMA



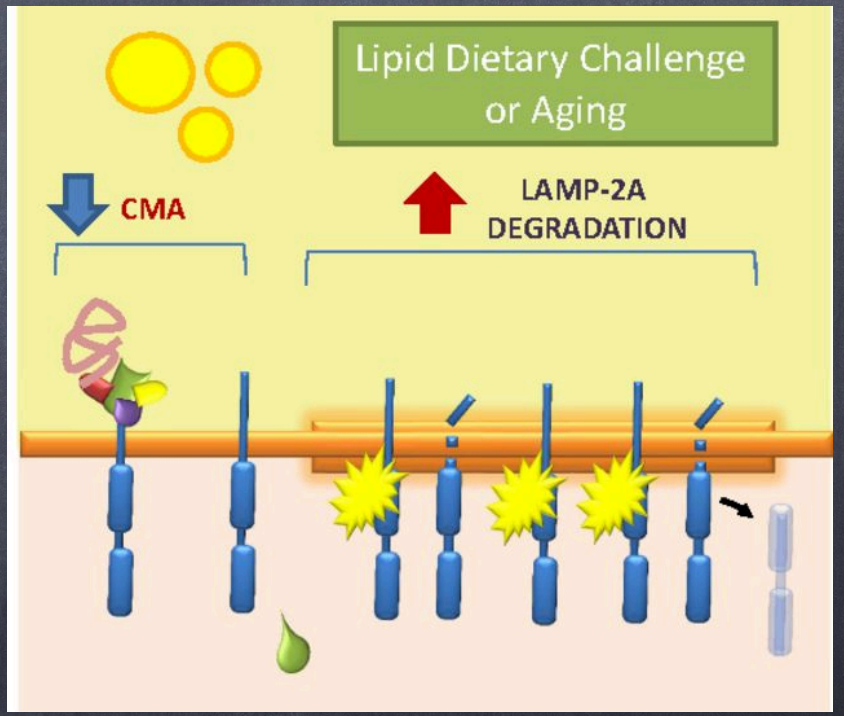
CMA is inhibited on lipid dietary challenge and aging

Under normal dietary conditions, the membrane protein LAMP-2A facilitates binding and translocation of proteins in the cellular cytosol across the lysosomal membrane, where they are degraded. A fraction of LAMP-2A is normally turned over in microdomains of discrete lipid composition at the lysosomal membrane.



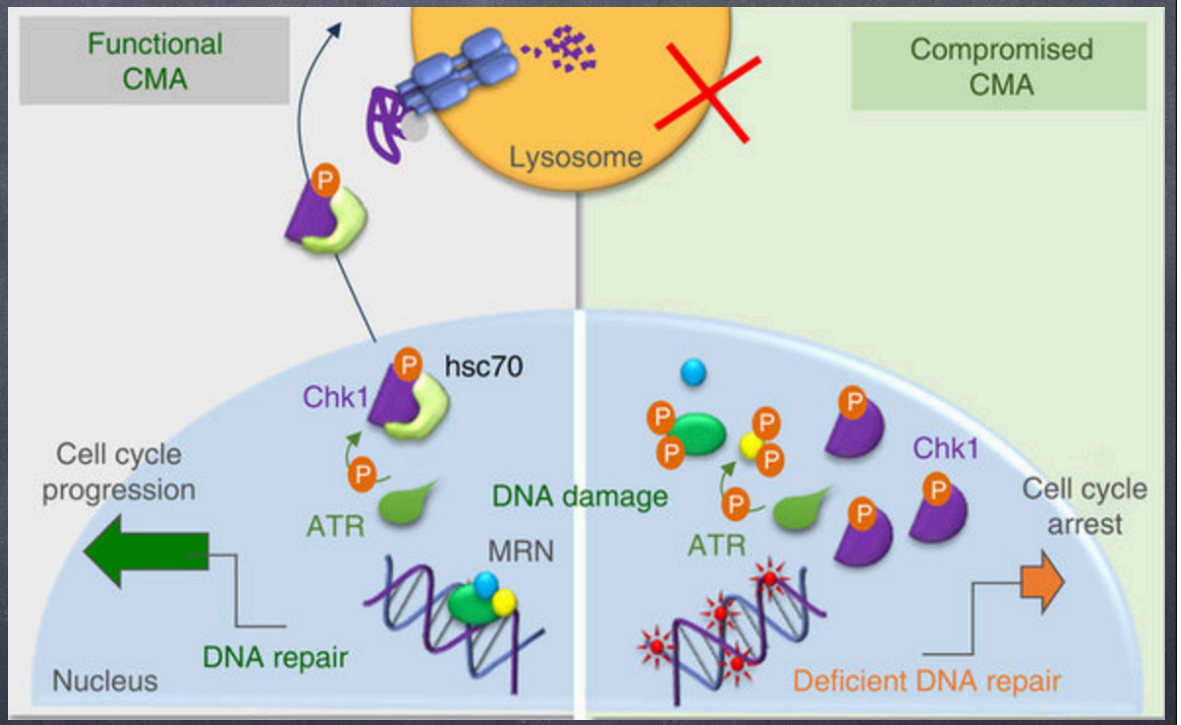
CMA is inhibited on lipid dietary challenge and aging

High-lipid diets and aging induce quantitative and qualitative changes in the lipid composition of the lysosomal membrane, favoring accelerated degradation of LAMP-2A in this compartment. The reduced levels of LAMP-2A under these conditions limit the CMA capacity of lysosomes.



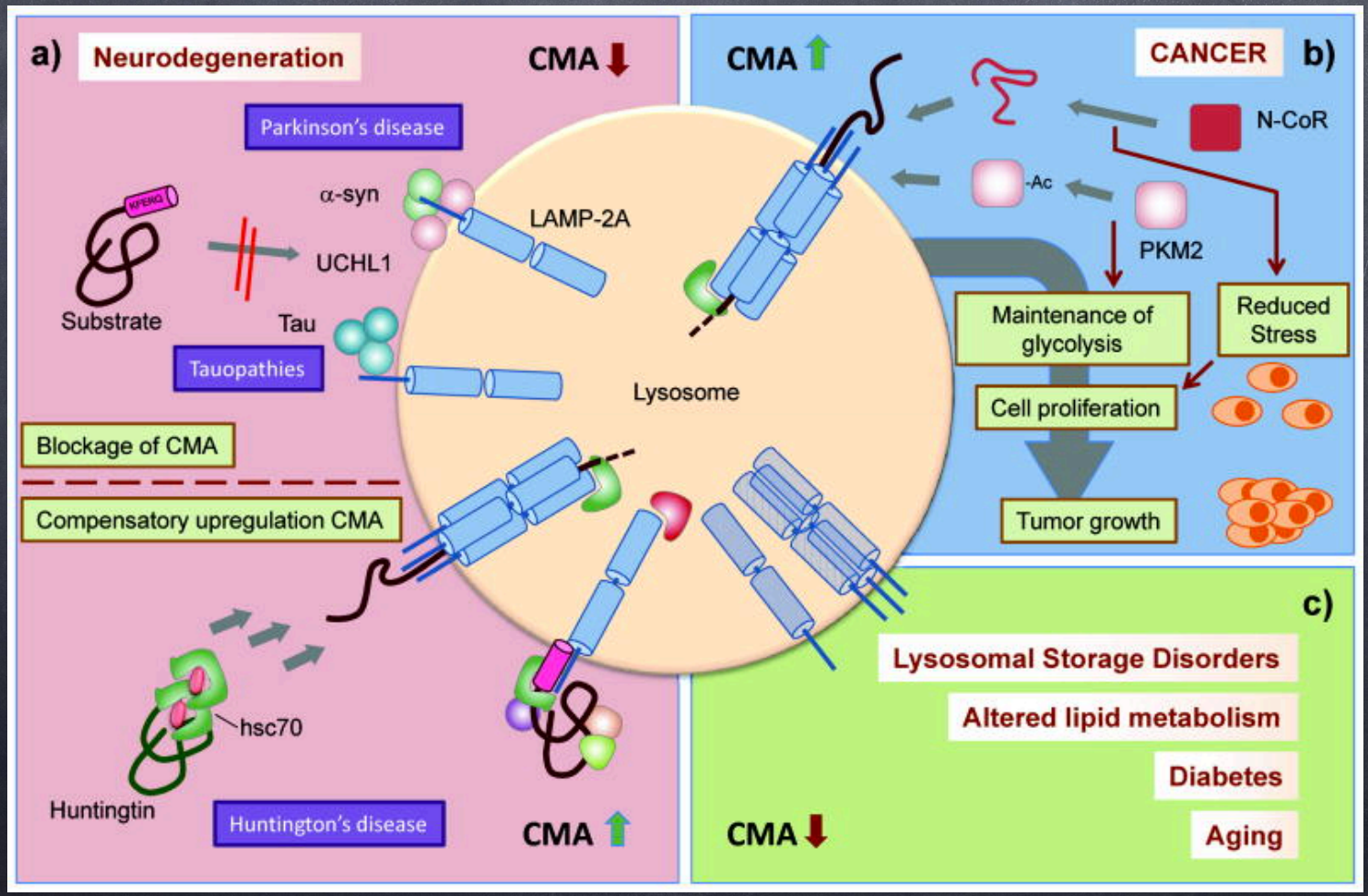
CMA contributes to maintain genome stability by assuring nuclear proteostasis

Chaperone-mediated autophagy (CMA) is activated in response to cellular stressors to prevent cellular proteotoxicity through selective degradation of altered proteins in lysosomes.

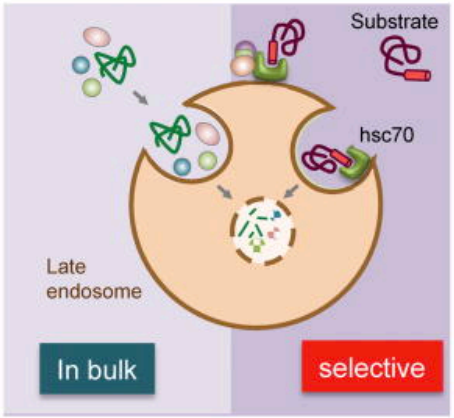


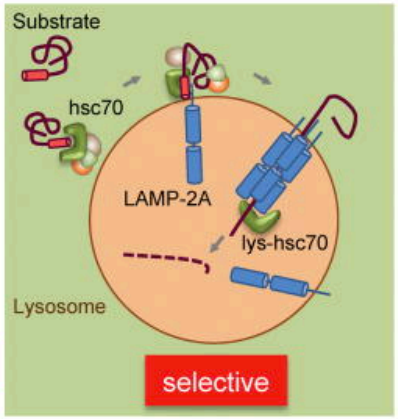
Reduced CMA activity contributes to the decrease in proteome quality in disease and ageing.

Pathology of CMA



CMA and microautophagy - how are they different?

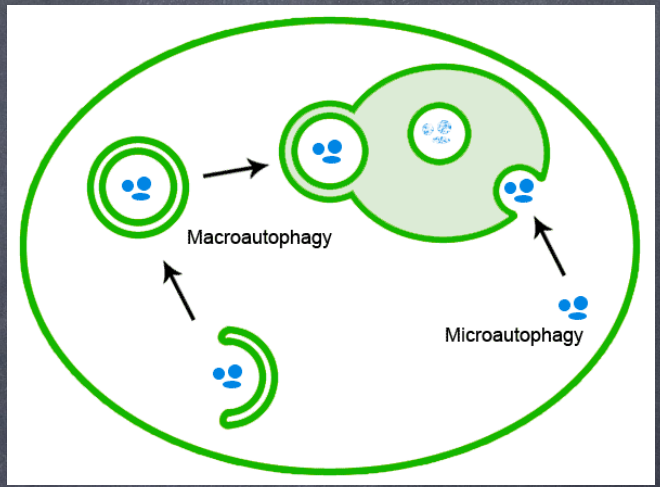




		e-Microautophagy		CMA
		In bulk	selective	
COMPARTMENT		Endosomes		Lysosomes
DELIVERY		Vesicles (MVB)		Transporter
TARGETING	Motif	None	KFERQ	KFERQ
	Chaperone	None	hsc70	hsc70
CARGO	Organelles	YES	NO	NO
	Proteins	YES		YES
	Unfolding	NO		YES
HSC70 BINDING		NO	Lipids	LAMP-2A
SELECTIVITY		NO	YES	Selective

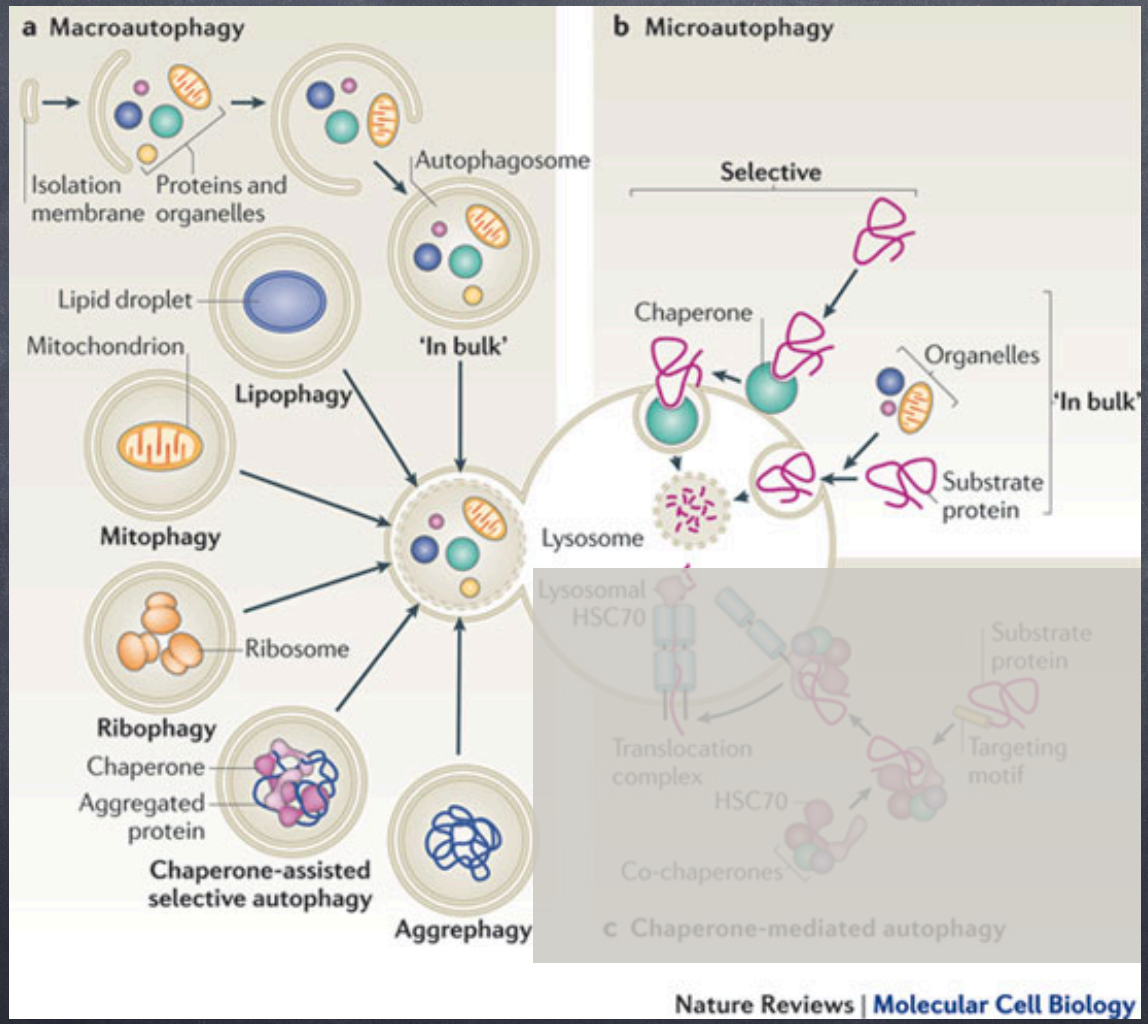
Macroautophagy vs microautophagy

MACROAUTOPHAGY: During macroautophagy, intact organelles (such as mitochondria) and portions of the cytosol are sequestered into a double-membrane vesicle, termed an autophagosome. Subsequently, the completed autophagosome matures by fusing with an endosome and/or lysosome, thereby forming an autolysosome. This latter step exposes the cargo to lysosomal hydrolases to allow its breakdown, and the resulting macromolecules are transported back into the cytosol through membrane permeases for reuse.



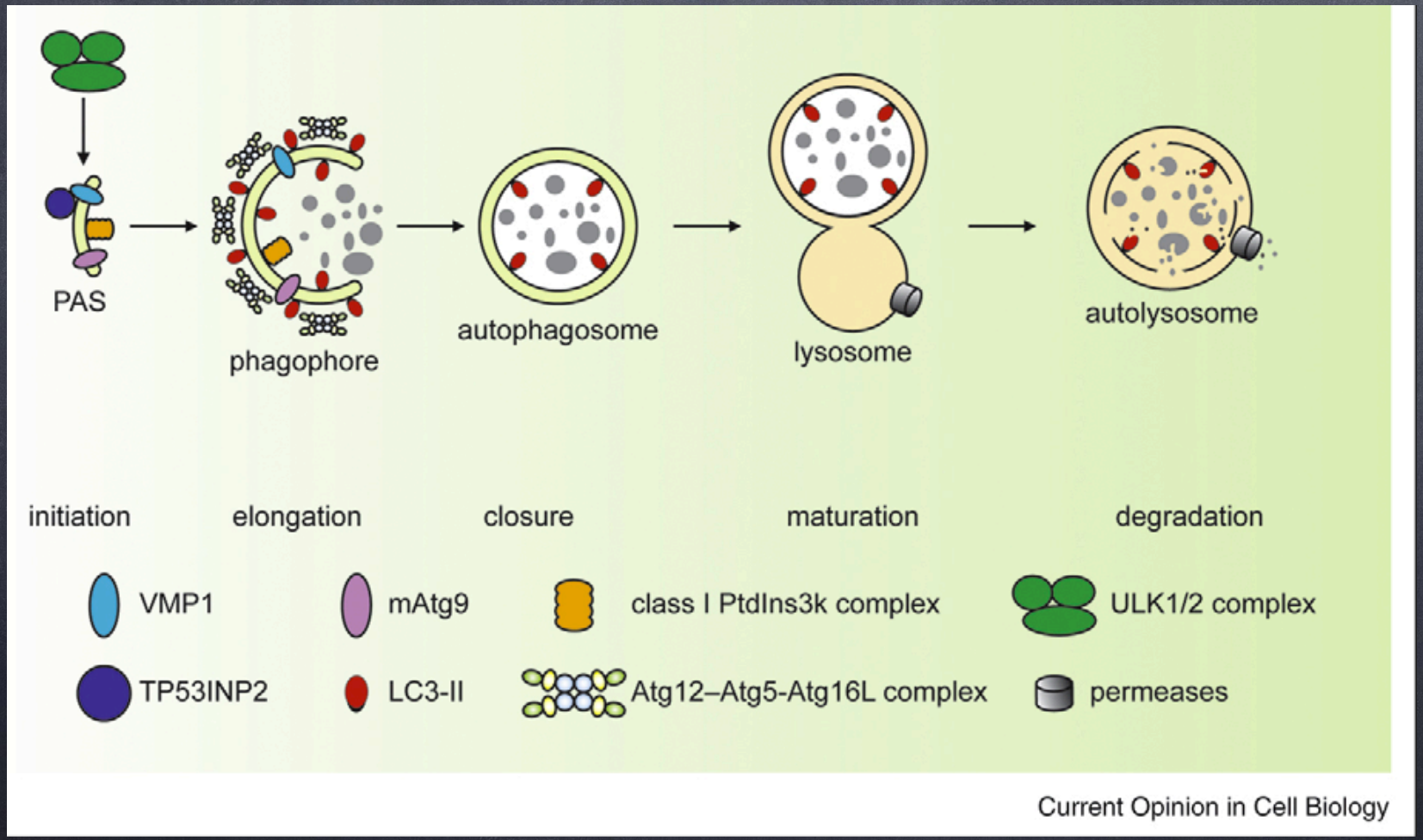
MICROAUTOPHAGY: direct engulfment of cytoplasm at the lysosome surface

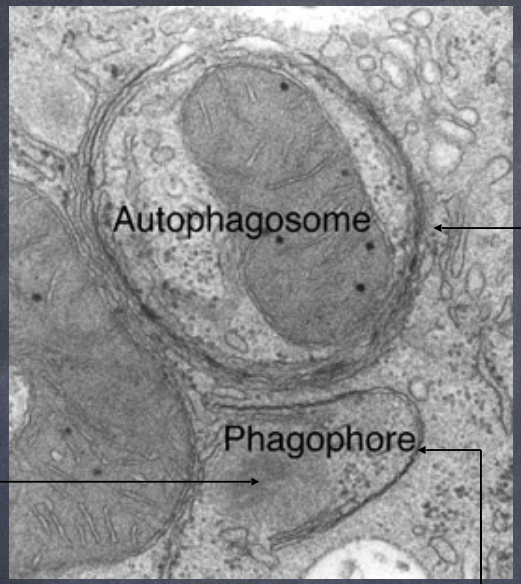
Autophagy versus heterophagy

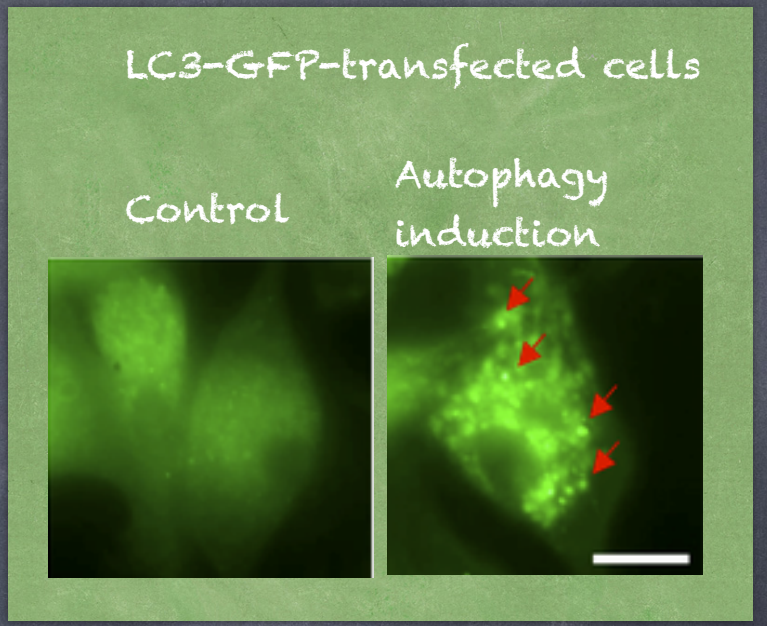


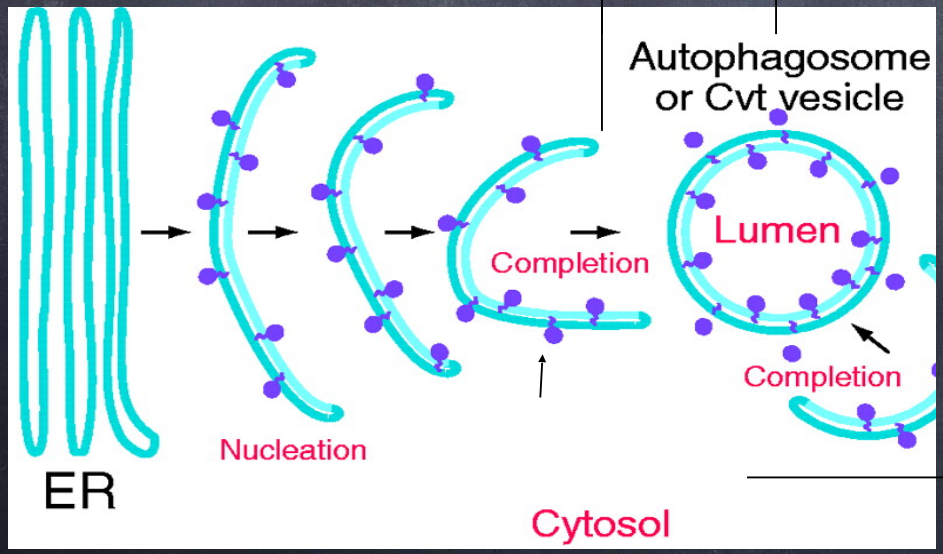
Nature Reviews Molecular Cell Biology 12, 535-541, 2011

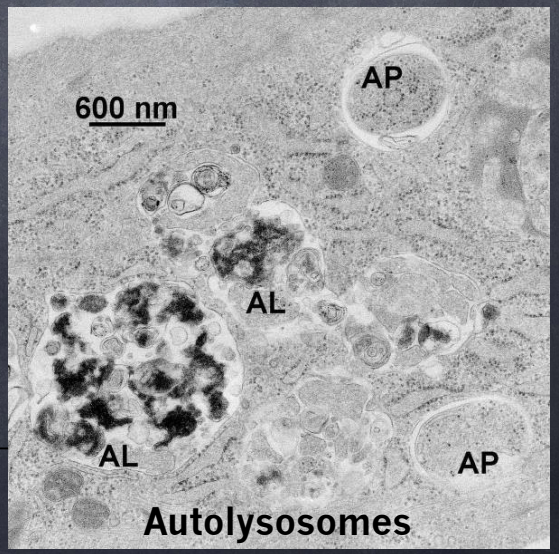
Autophagy-related (ATG) genes



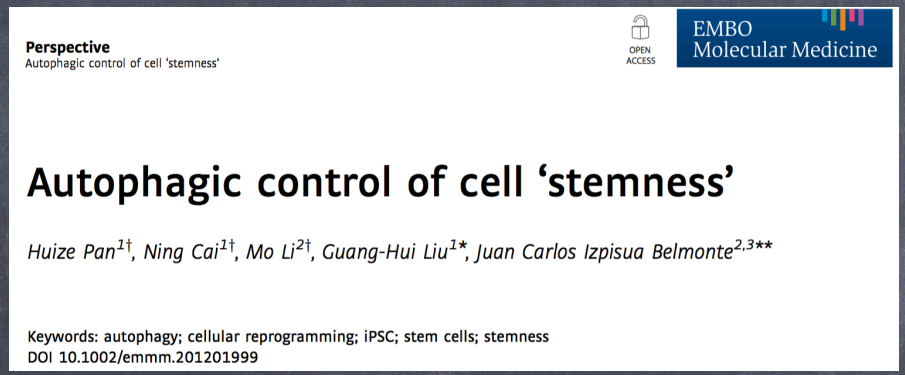

Autophagosome
Phagophore


LC3-GFP-transfected cells
Control
Autophagy induction


Autophagosome or Cvt vesicle
Lumen
Completion
Completion
Nucleation
ER
Cytosol


600 nm
AP
AL
AL
AP
Autolysosomes

Autophagic control of cell 'stemness'



Perspective
Autophagic control of cell 'stemness'

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EMBO Molecular Medicine

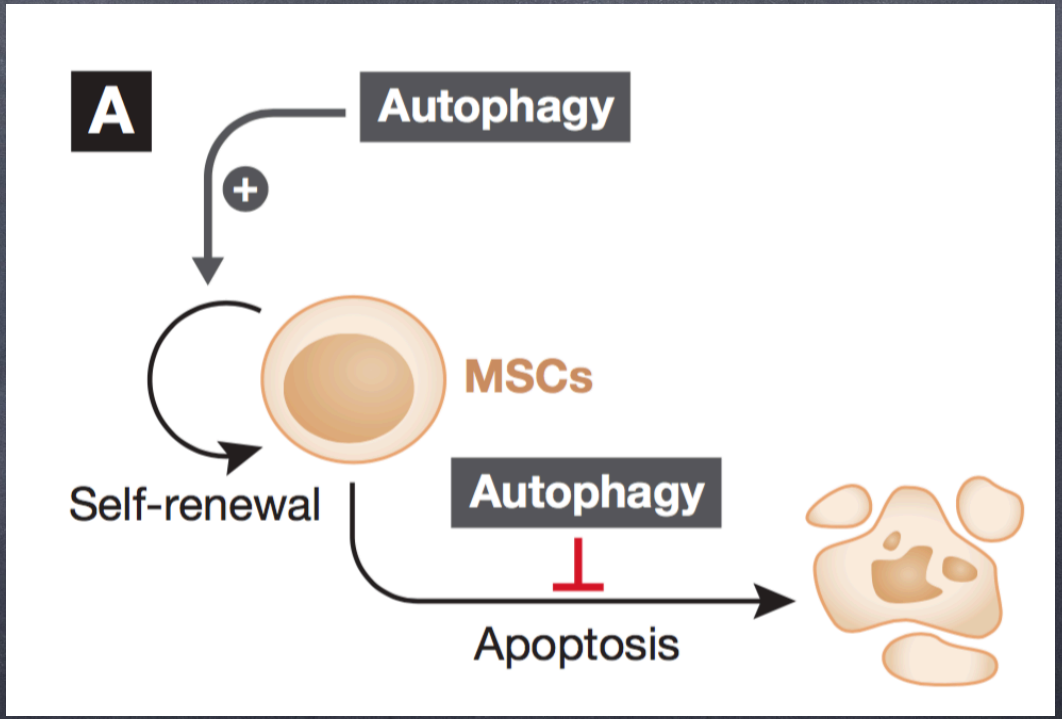
Autophagic control of cell 'stemness'

Huize Pan[1†], Ning Cai[1†], Mo Li[2†], Guang-Hui Liu[1], Juan Carlos Izpisua Belmonte[2,3**]*

Keywords: autophagy; cellular reprogramming; iPSC; stem cells; stemness
DOI 10.1002/emmm.201201999

Autophagy is required for the maintenance of MSCs and inhibits their death

- The level of constitutive autophagy in human mesenchymal stem cells (hMSC) is high. Once hMSCs are differentiated into osteoblasts, however, basal autophagy becomes undetectable.
- autophagy induced by hypoxia promotes the maintenance and self-renewal of MSC

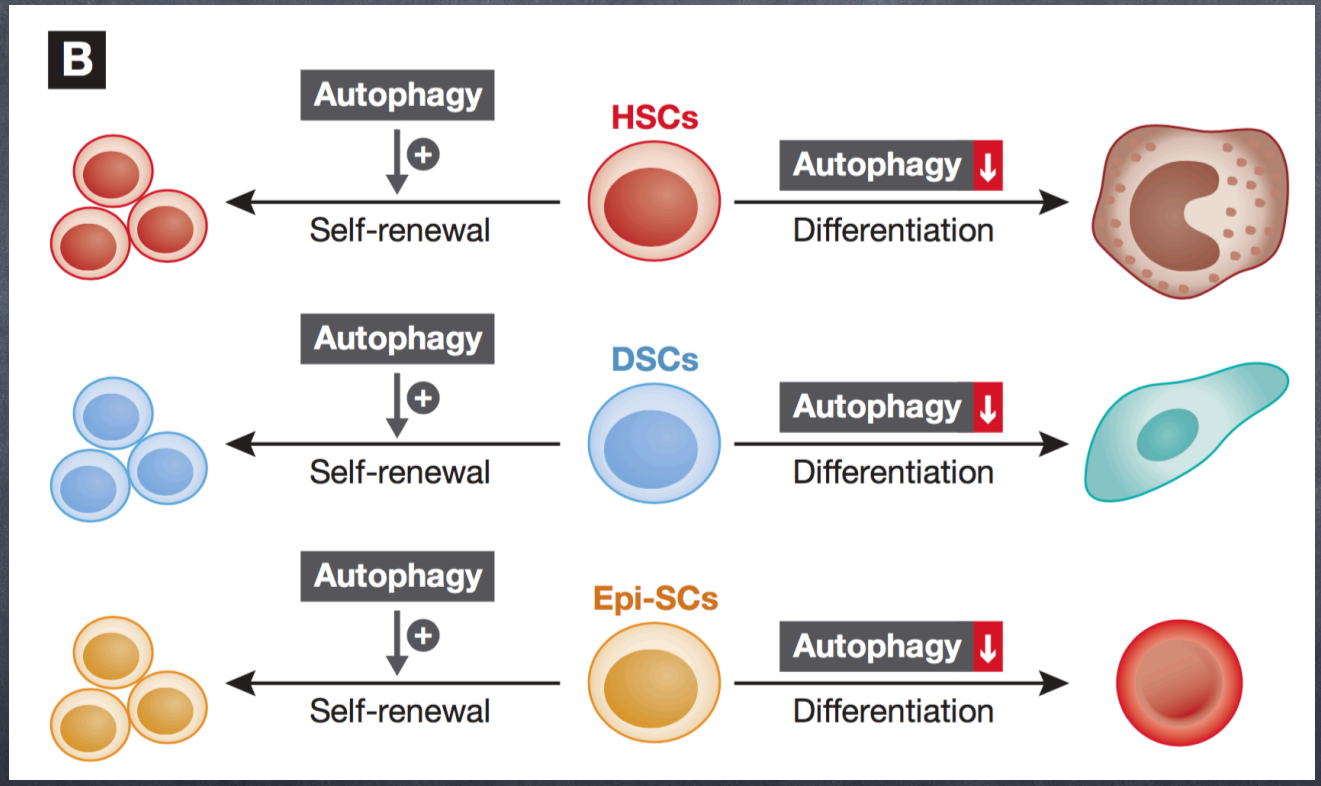


- activation of autophagy antagonized, while inhibition of autophagy promoted MSC apoptosis during hypoxia/serum deprivation

Autophagy in SCs maintenance and differentiation

- HSC: Hematopoietic stem cells,
- DSC Dermal stem cells
- Epi-SCs Epidermal stem cells

Autophagy remains at high levels in SCs and promotes their maintenance; after induced differentiation, autophagic activity is down regulated.



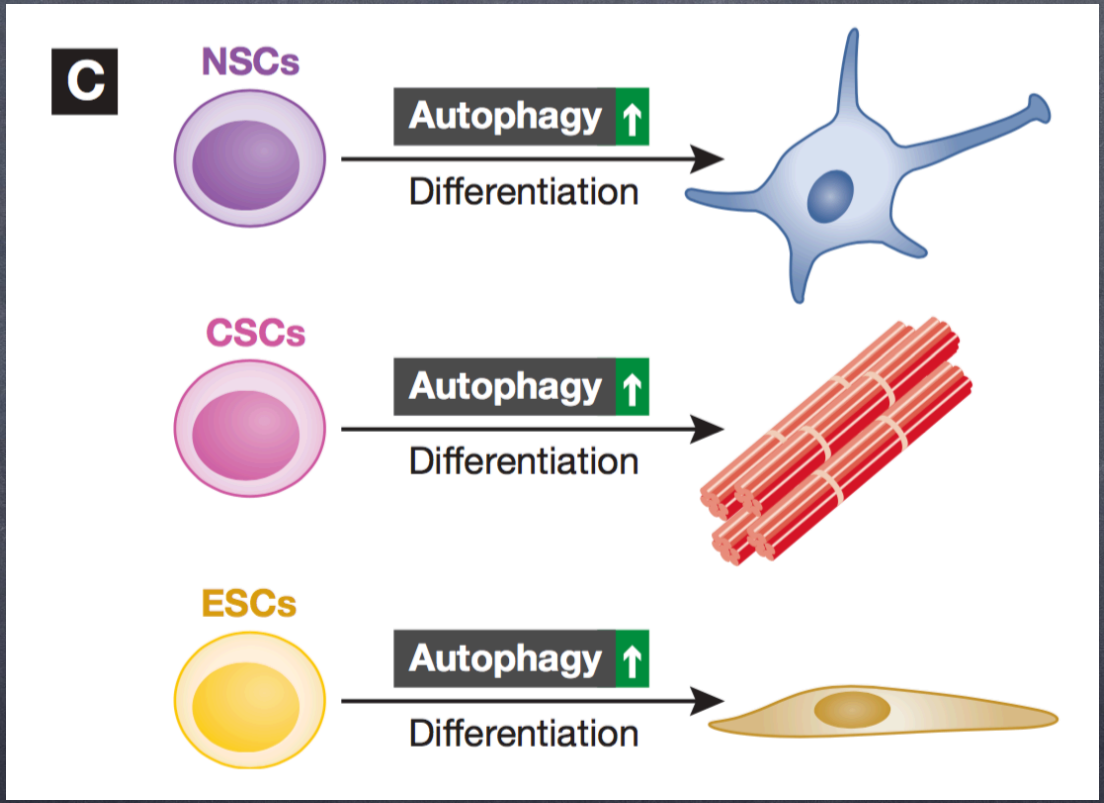
HSC: Hematopoietic stem cells,
DSC Dermal stem cells
Epi-SCs Epidermal stem cells

After induced differentiation, autophagic activity in immature keratinocytes, fibroblasts and neutrophils is down regulated to a basal level similar to that observed in most cell types

Autophagy in SCs maintenance and differentiation

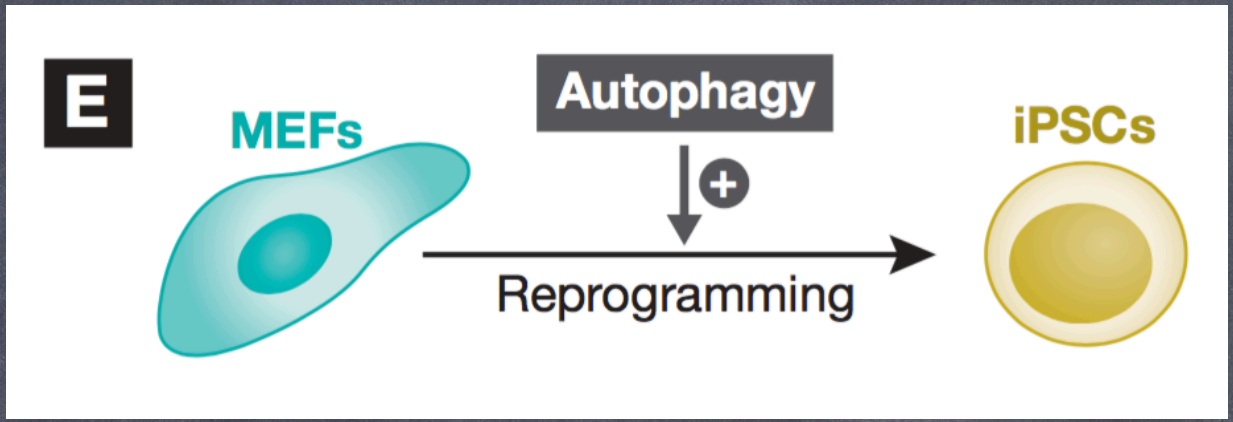
- NSCs neuronal stem cells,
- CSCs cardiac stem cells
- ESCs embryonic stem cells
- iPSCs induced pluripotent stem cells

Autophagy in NSCs, CSCs and ESCs is up regulated during their differentiation.



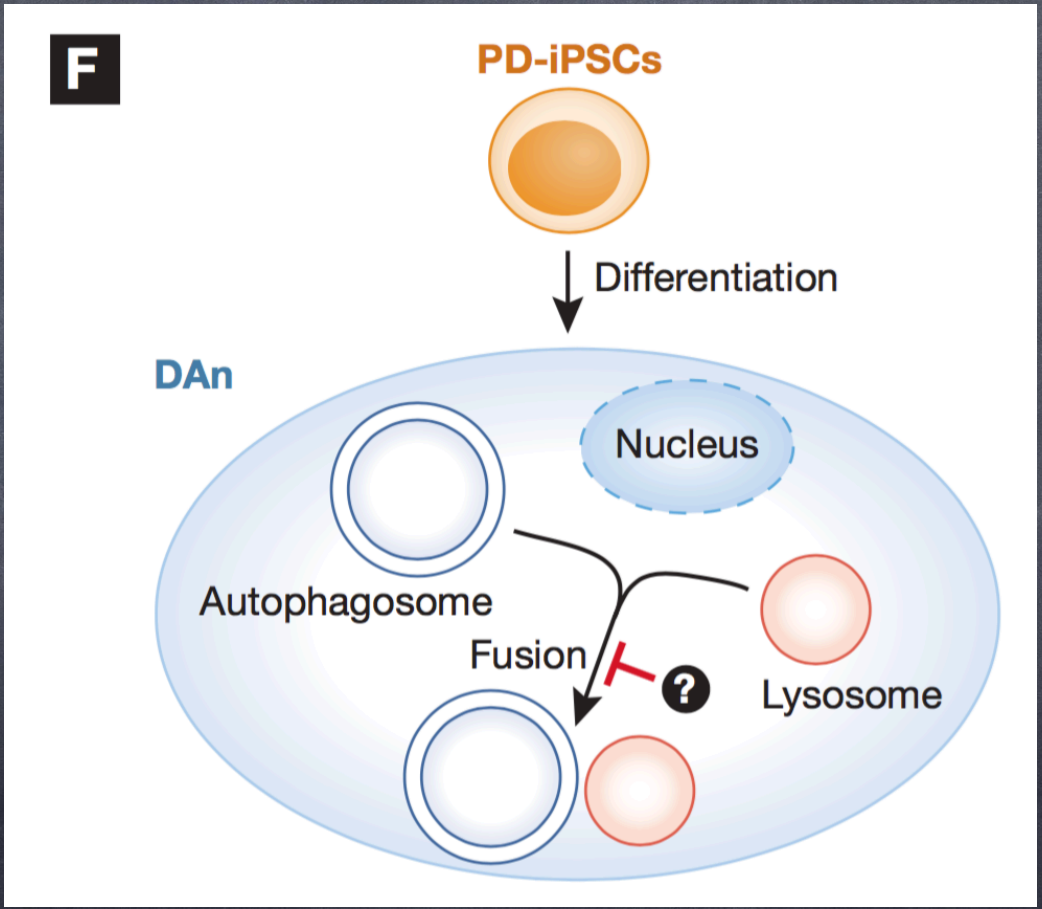
Autophagy is up regulated during differentiation of neural and cardiac stem cells

Autophagy increases the reprogramming efficiency, and promotes the generation of iPSC.



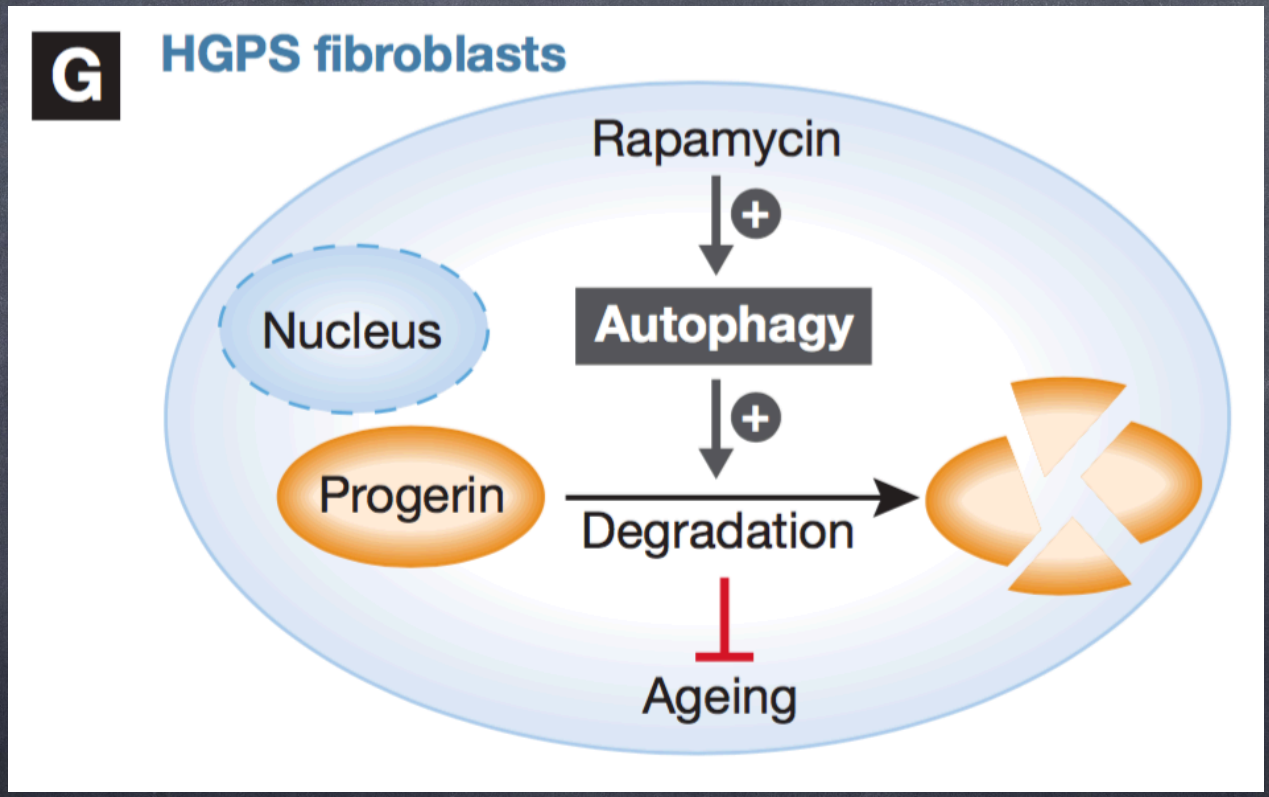
human iPSCs generated by inducing expression of the four reprogramming factors (OCT-4, SOX-2, KLF-4 and c-MYC)

Autophagosome clearance is inhibited in PD-iPSC-derived dopaminergic neurons (DAn)



PD-iPSC: Parkinson's disease

Rapamycin, an autophagy inducer, can effectively facilitate the degradation of progerin and thus prevent progeria-associated ageing phenotypes in Hutchinson-Gilford progeria syndrome (HGPS) fibroblasts.

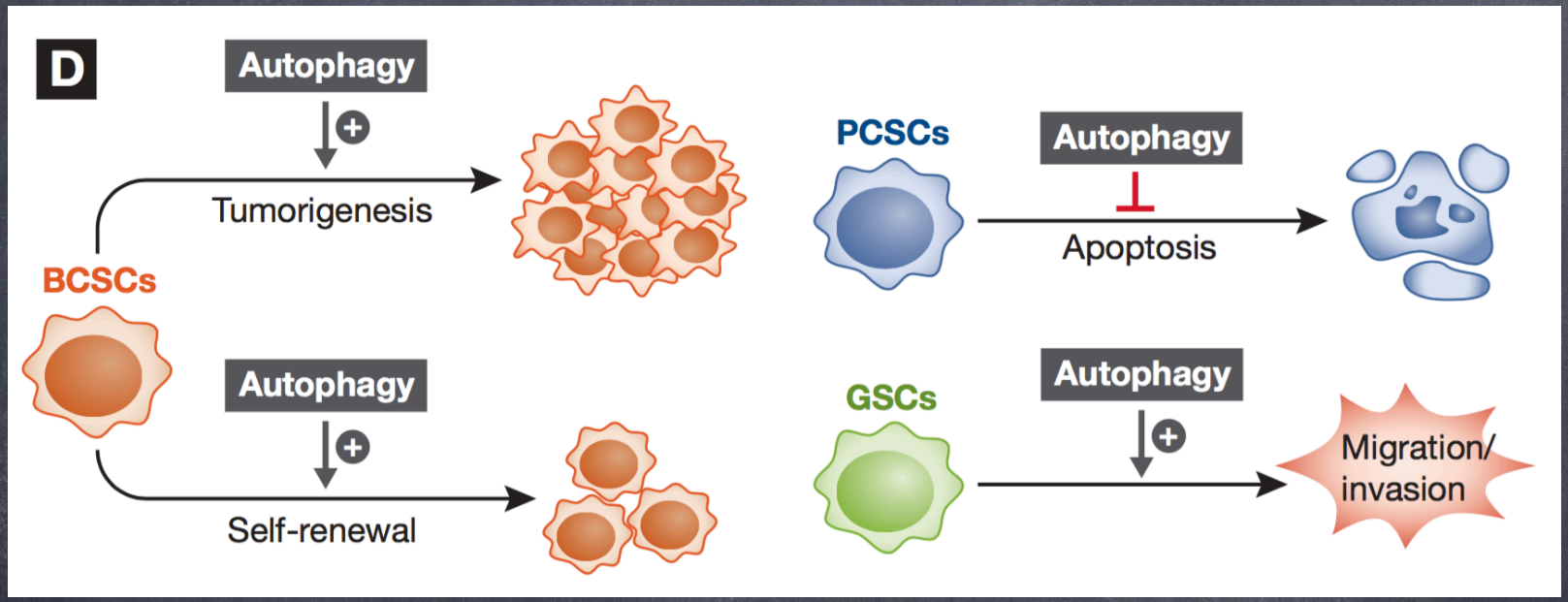


HGPS: Hutchinson-Gilford progeria syndrome

Autophagy in cancer SCs

- BCSC: breast cancer stem cells
- PCSC: pancreatic cancer stem cells
- GSC: glioblastoma stem cell

Autophagy is required for the maintenance and the tumourigenic potential of BCSCs, enhances the survival of PCSCs, and plays an important role in GSC migration.



BCSC: breast cancer stem cells
PCSC: pancreatic cancer stem cells
GSC: glioblastoma stem cell

assignment

Autophagy and stem cells

- group work (4 students/group)
- select an experimental article of interest
- Present the aim of the study and the main experimental approach using no more than 4 slides and 10 min