Autophagy

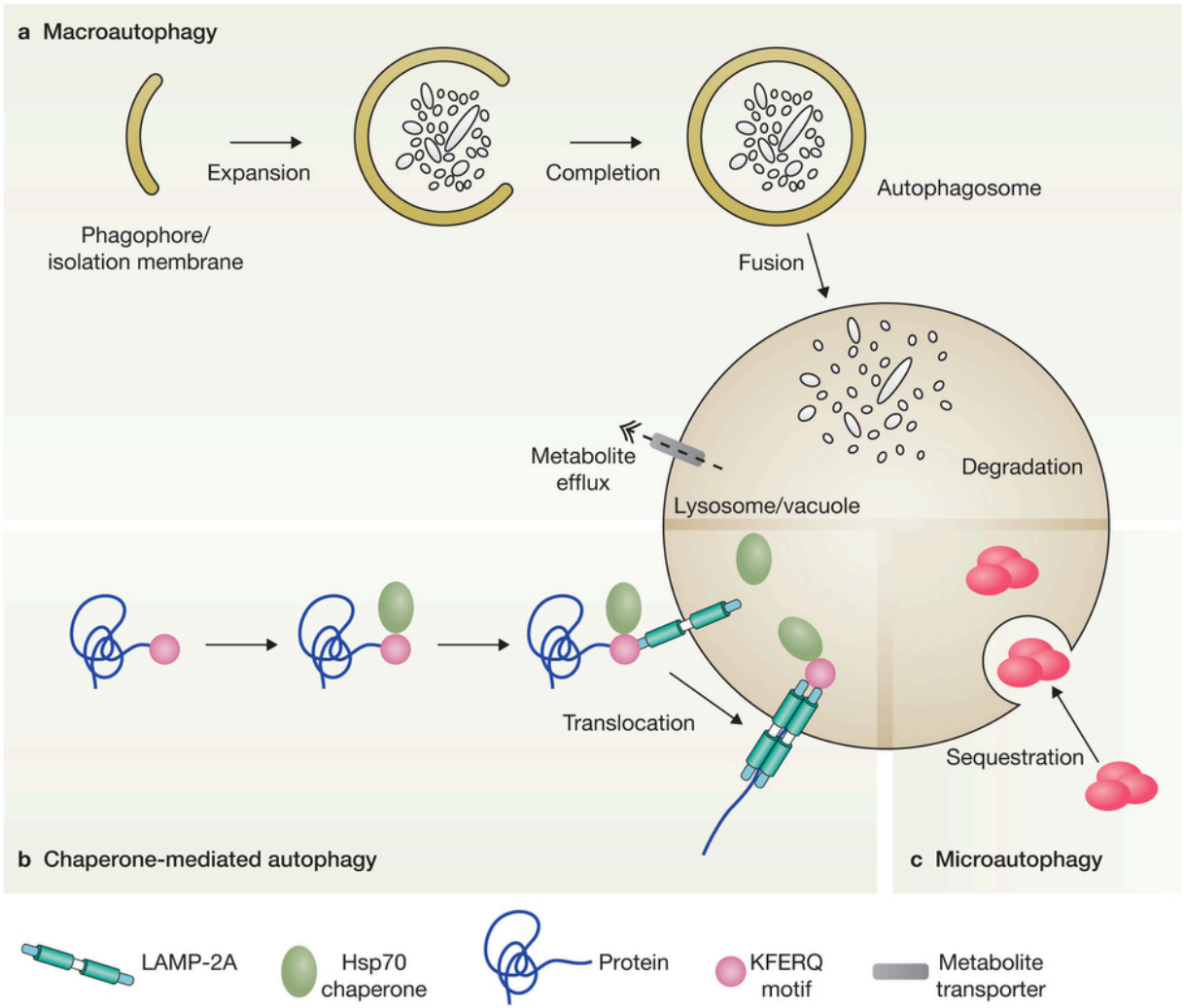
Macroautophagy
Microautophagy
Chaperone mediated autophagy (CMA),

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

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,

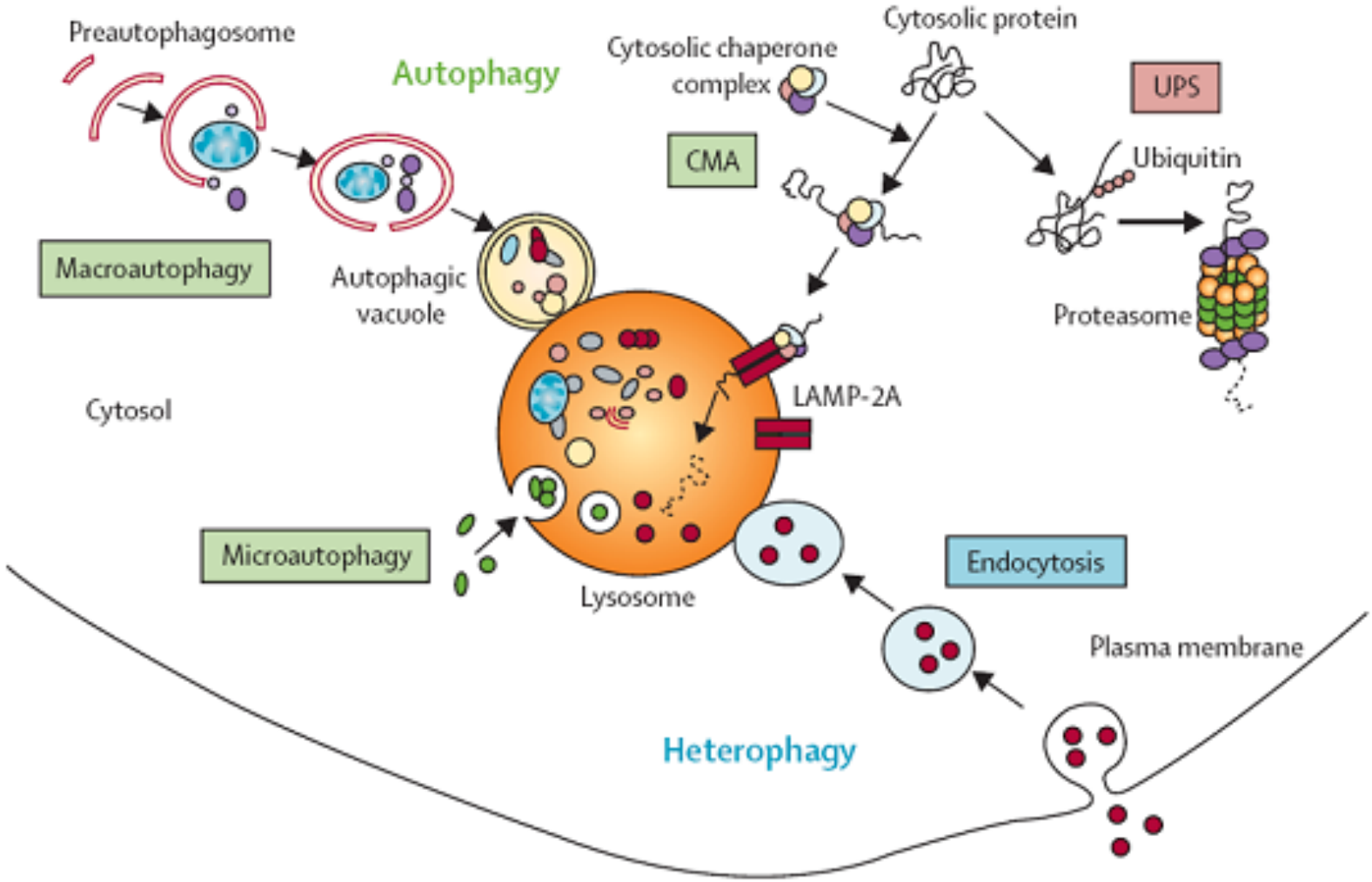
Chaperione Mediated Autophagy: translocates unfolded, soluble proteins directly across the limiting membrane of the lysosome.



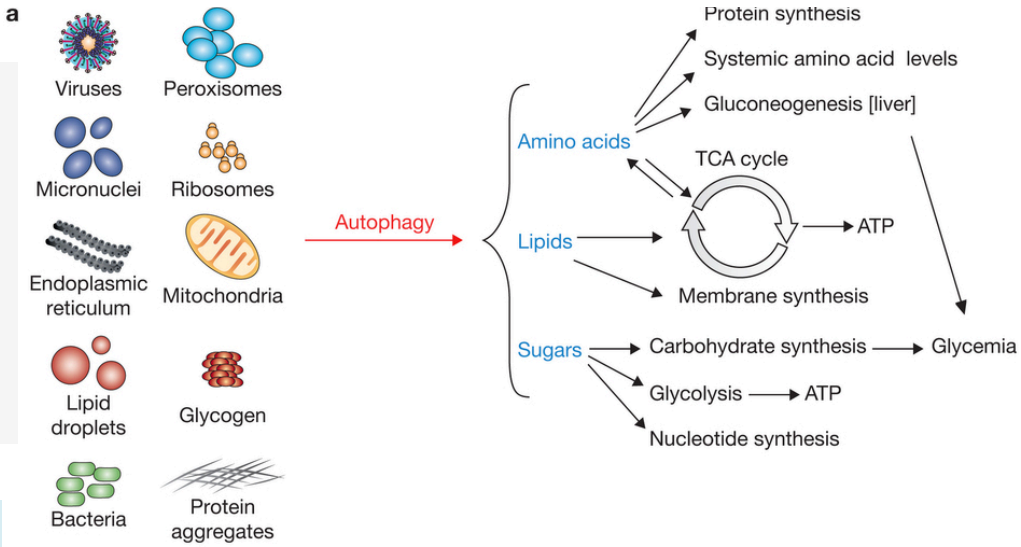
(a) Macroautophagy is characterized by the sequestration of structures targeted for destruction into double-membrane vesicles called autophagosomes. Complete autophagosomes first fuse with endosomes before finally exposing their content to the hydrolytic interior of lysosomes. The resulting metabolites are transported into the cytoplasm and used either for the synthesis of new macromolecules or as a source of energy.

(b) During chaperone-mediated autophagy, proteins carrying the pentapeptide KFERQ-like sequence are recognized by the Hsc70 chaperone, which then associates with the integral lysosome membrane protein LAMP-2A, triggering its oligomerization. This event leads the translocation of the bound protein into the lysosome interior through a process that requires Hsc70.

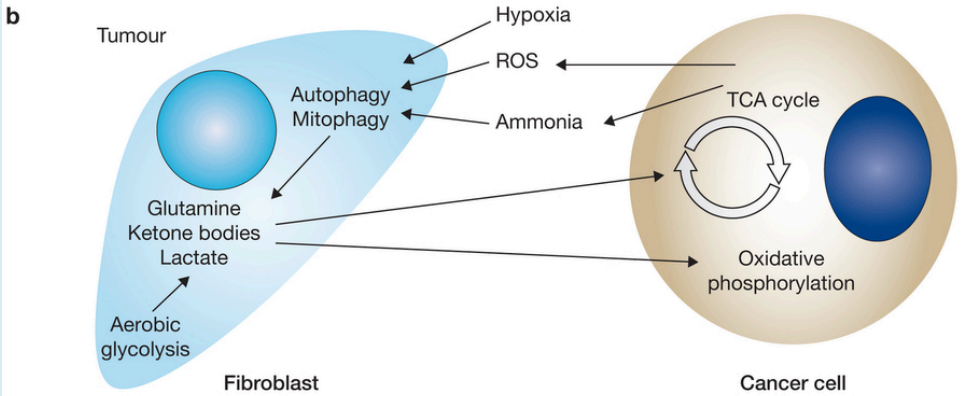
(c) Microautophagy entails the recruitment of targeted components in proximity with the lysosomal membrane, which subsequently invaginates and pinches off.


Preautophagosome
Autophagy
Cytosolic chaperone complex
Cytosolic protein
UPS
CMA
Ubiquitin
Macroautophagy
Autophagic vacuole
Proteasome
Cytosol
LAMP-2A
Microautophagy
Lysosome
Endocytosis
Plasma membrane
Heterophagy

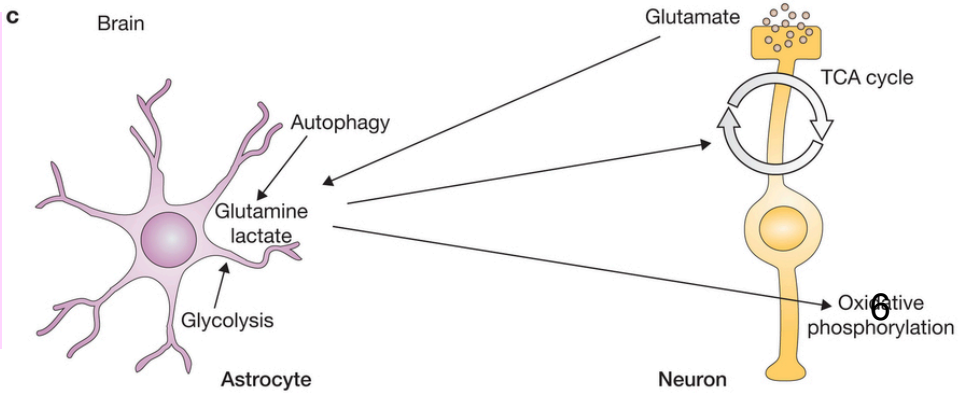
(a) The **catabolic products** of the intracellular structures that are targeted by autophagosomes, such as amino acids, lipids and sugars, are used for **anabolic reactions** to generate new proteins, glycans, oligonucleotides and membranes to sustain cell functions. Lipids, amino acids abd sugars generate energy in the form of **ATP**. Sugars can also be metabolized to generate ATP



(b) Inside tumours, hypoxia and oxidative stress trigger autophagy and mitophagy in the stromal fibroblasts. This induces a metabolic switch towards aerobic glycolysis (known as the Warburg effect), leading to the production of lactate and other metabolites that are liberated into the intracellular space and reabsorbed by tumour cells. A more oxidative metabolism in these cells generates oxidative stress and ammonia (from glutaminolysis), which signals back to fibroblasts to further stimulate autophagy.



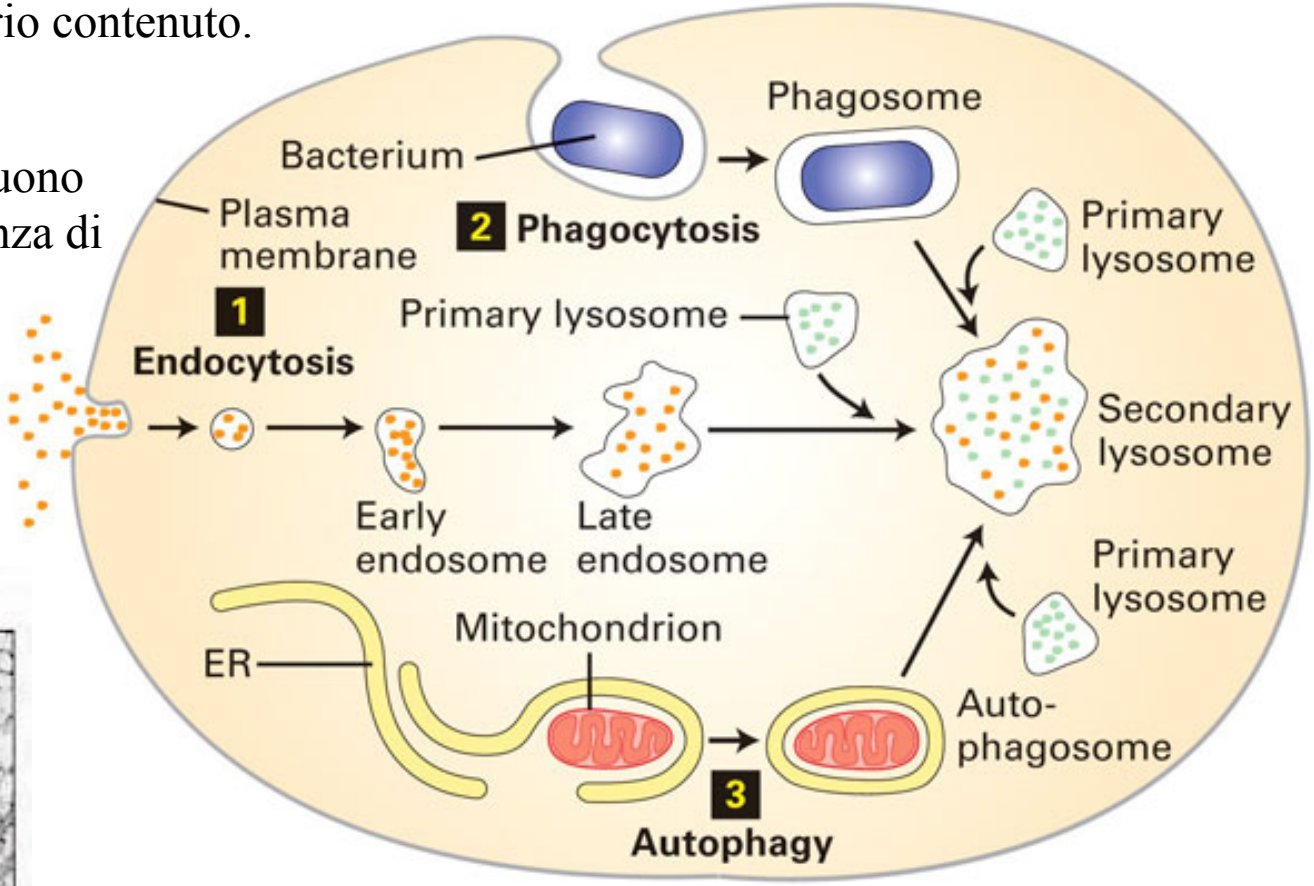
(c) In brain tissue, astrocytes produce lactate from glucose through glycolysis and glutamine through autophagy. These metabolites are taken up by neurons and oxidized to generate ATP. Moreover, the neurotransmitter glutamate, released by neurons, can be retransformed into glutamine by astrocytes.

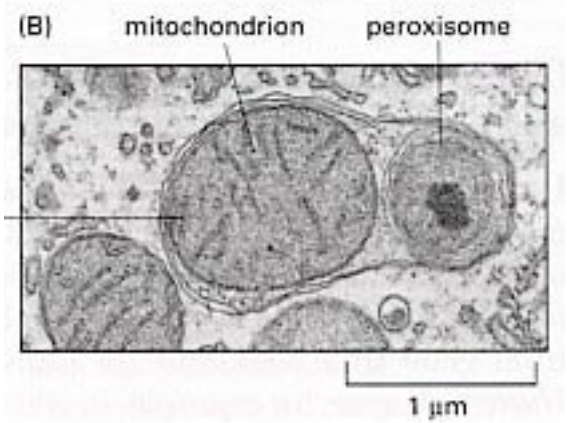


•Funzioni del REL: macroautofagia

Per macroautofagia si intende la degradazione di componenti della cellula stessa, siano essi macromolecole o organelli. In questo caso questi vengono circondati da vescicole derivanti dalla membrana del reticolo endoplasmatico, portando alla formazione del cosiddetto autofagosoma. Successivamente questo si fonde col lisosoma che è quindi libero di riversarvi il proprio contenuto.

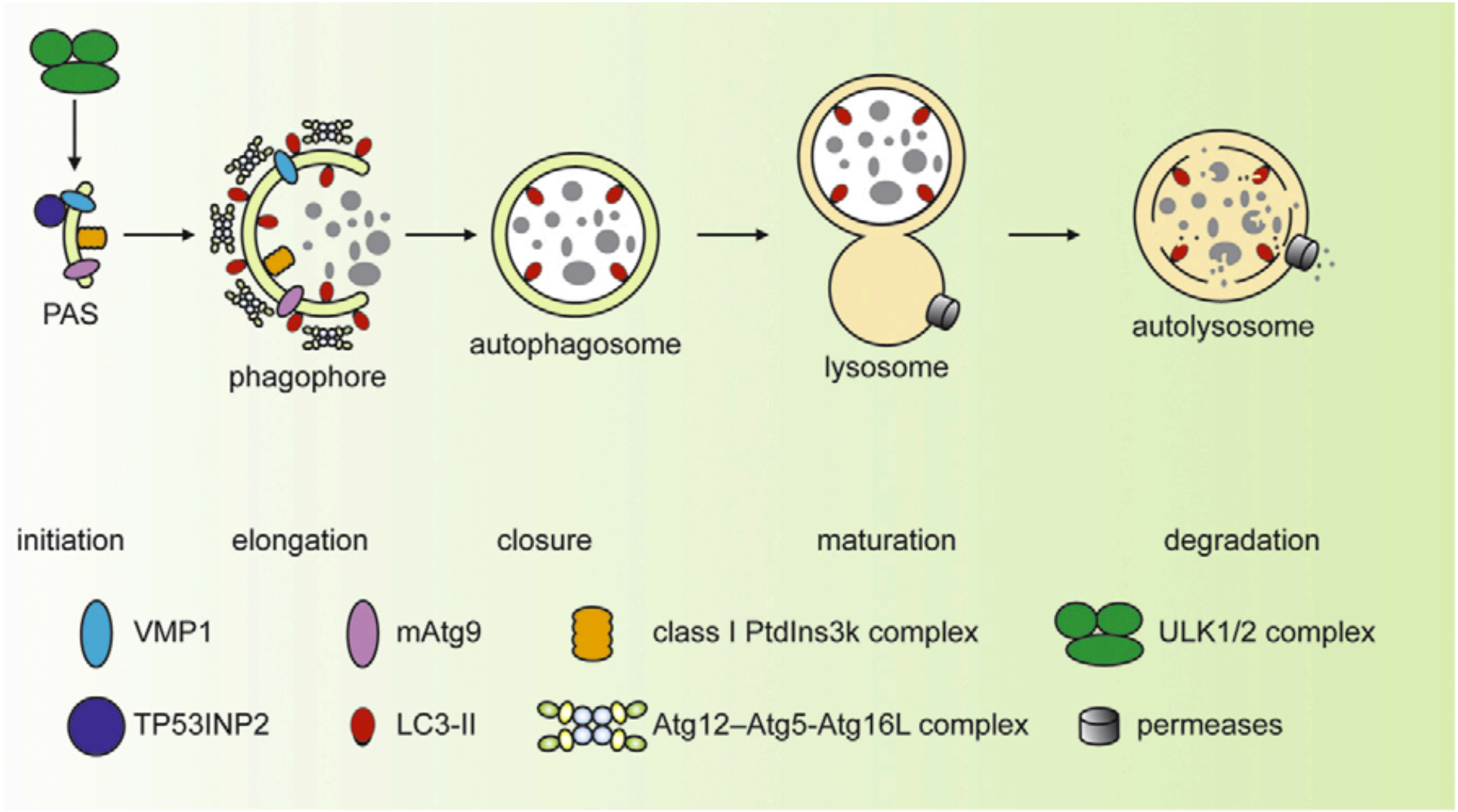
Gli autofagosoma si distinguono dagli fagosoma per la presenza di una doppia membrana



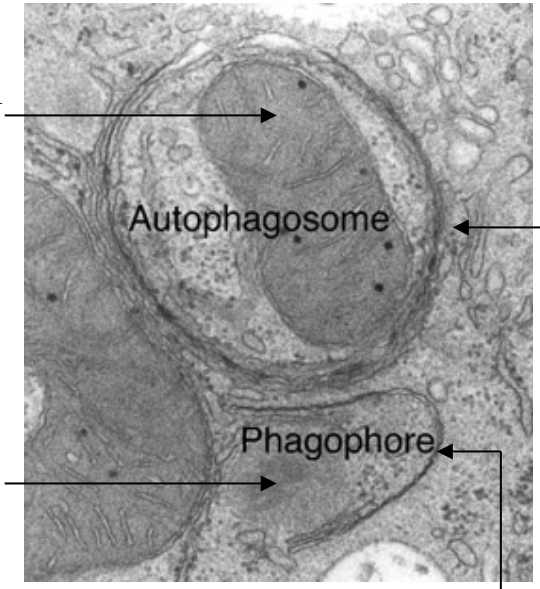


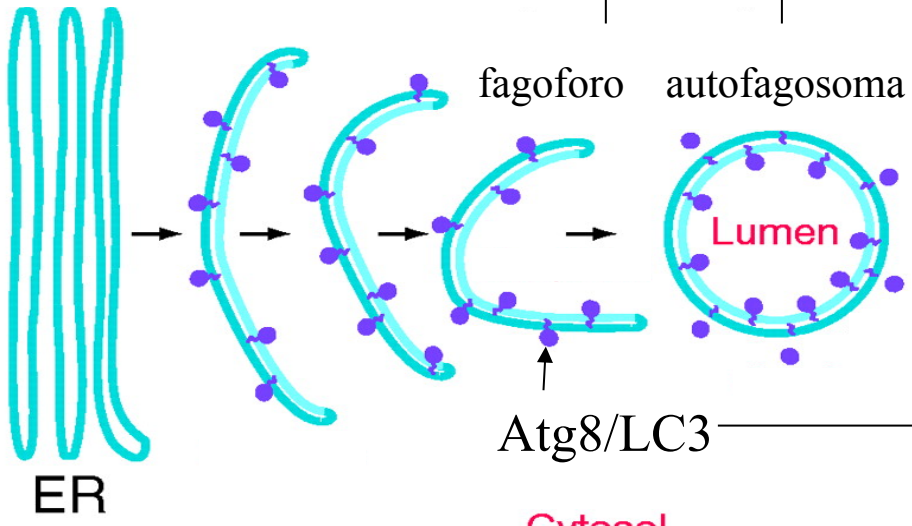
Autofagosoma circondato da una doppia membrana e contenente un mitocondrio e un perossisoma.

autophagy-related (ATG) genes

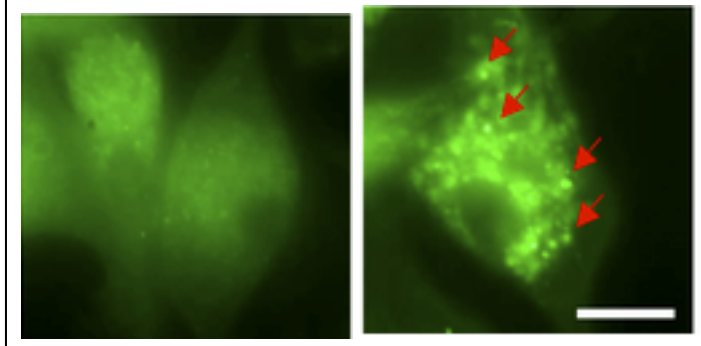


Formazione dell'autofagosoma: Emissione di un prolungamento della membrana del RE e avvolgimento di materiale intracellulare da eliminare (organelli oppure materiale citoplasmatico compresi i ribosomi ad esempio).



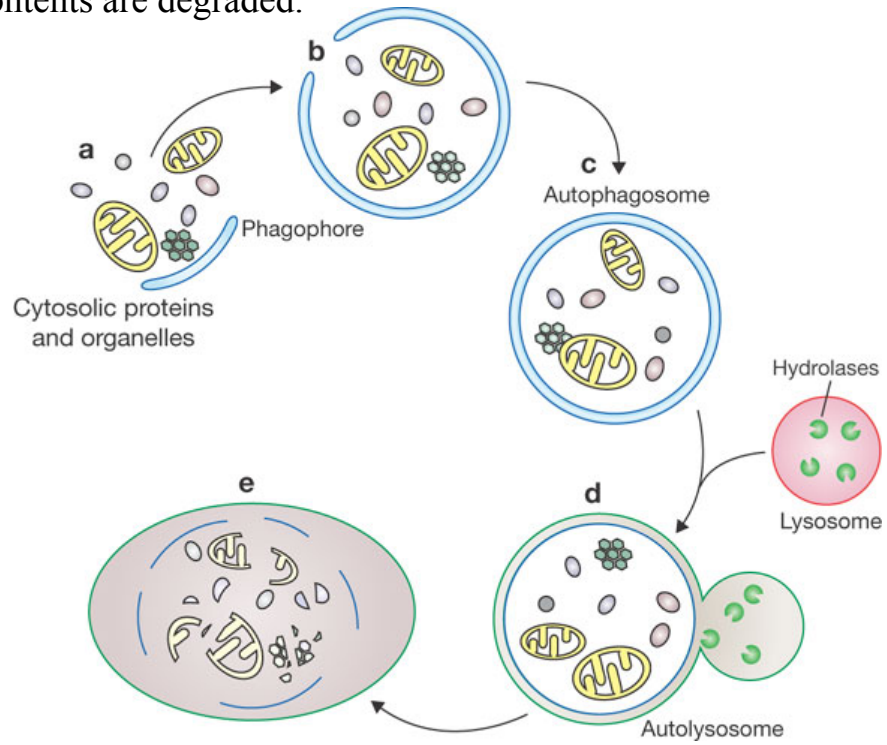


Cellule trasfettate con GFP-LC3



Fusione dell'autofagosoma con endosomi e/o lisosomi: formazione di anfisoma/autolisosoma: degradazione della membrana interna dell'autofagosoma e del contenuto

Schematic depiction of autophagy.
(a, b) Cytosolic material is sequestered by an expanding membrane sac, the phagophore, (c) resulting in the formation of a double-membrane vesicle, an autophagosome; (d) the outer membrane of the autophagosome subsequently fuses with a lysosome, exposing the inner single membrane of the autophagosome to lysosomal hydrolases; (d) the cargo-containing membrane compartment is then lysed, and the contents are degraded.



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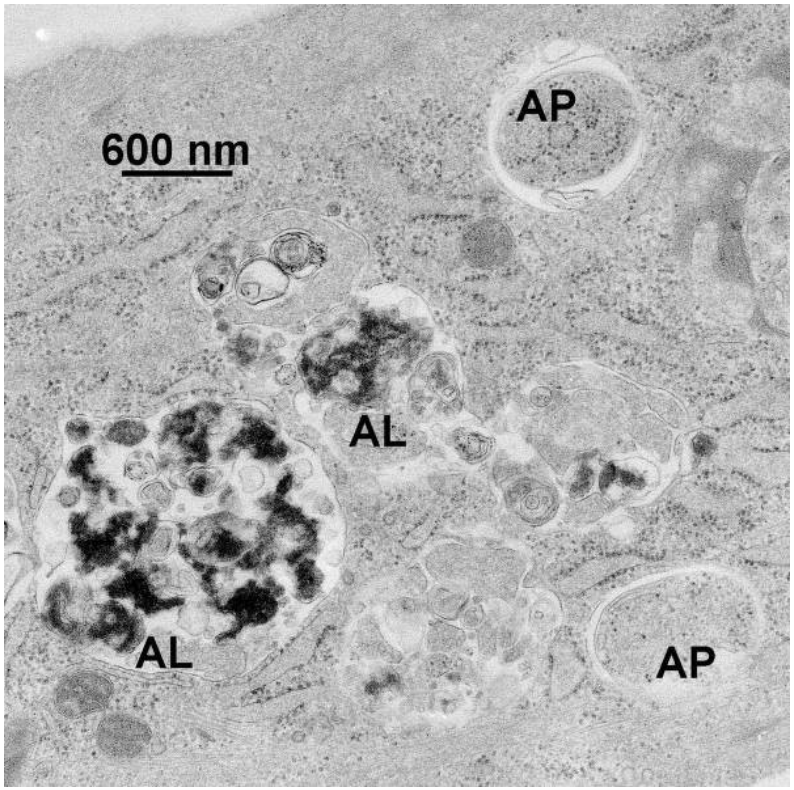


TEM demonstrating the ultrastructure of autophagosomes and amphisomes/autolysosomes in a mouse fibroblast. Early autophagosomes (AP) contain morphologically intact cytoplasm. Degradative amphisomes/autolysosomes (AL) contain partially degraded cytoplasmic material, above all remnants of ribosomes, which form electron dense amorphous aggregates.

Cell Death and Differentiation (2009) 16, 3–11

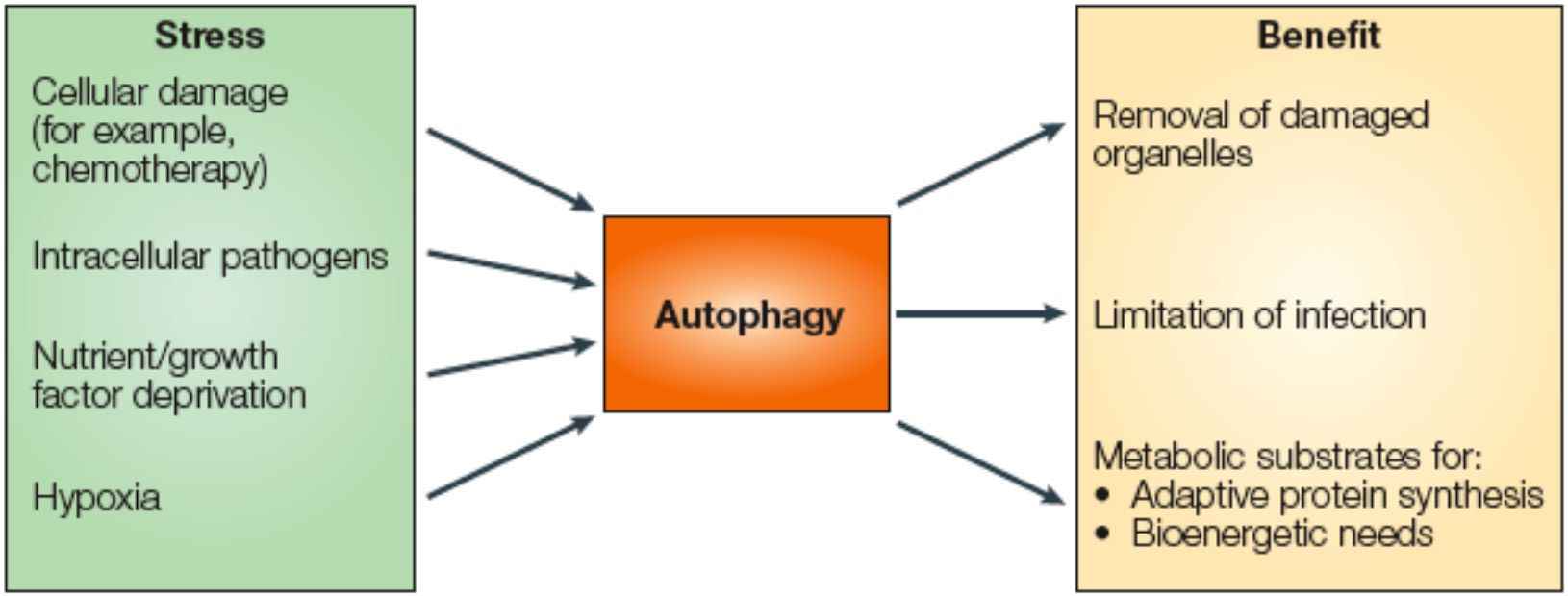
Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009

G Kroemer*[,1,2,3], L Galluzzi[1,2,3], P Vandenabeele[4,5], J Abrams[6], ES Alnemri[7], EH Baehrecke[8], MV Blagosklonny[9], WS El-Deiry[10], P Golstein[11,12,13], DR Green[14], M Hengartner[15], RA Knight[16], S Kumar[17], SA Lipton[18,19,20], W Malorni[21], G Nuñez[22], ME Peter[23], J Tschopp[24], J Yuan[25], M Piacentini[26,27], B Zhivotovsky[28] and G Melino[29,30]

Different types of cell death are often defined by morphological criteria, without a clear reference to precise biochemical mechanisms. The Nomenclature Committee on Cell Death (NCCD) proposes unified criteria for the definition of cell death and of its different morphologies, while formulating several caveats against the misuse of words and concepts that slow down progress in the area of cell death research. Authors, reviewers and editors of scientific periodicals are invited to abandon expressions like 'percentage apoptosis' and to replace them with more accurate descriptions of the biochemical and cellular parameters that are actually measured. Moreover, at the present stage, it should be accepted that caspase-independent mechanisms can cooperate with (or substitute for) caspases in the execution of lethal signaling pathways and that 'autophagic cell death' is a type of cell death occurring together with (but not necessarily by) autophagic vacuolization. This study details the 2009 recommendations of the NCCD on the use of cell death-related terminology including 'entosis', 'mitotic catastrophe', 'necrosis', 'necroptosis' and 'pyroptosis'.

Table 2 Distinct modalities of cell death

Cell death mode	Morphological features	Notes
Apoptosis	Rounding-up of the cell Retraction of pseudopodes Reduction of cellular and nuclear volume (pyknosis) Nuclear fragmentation (karyorrhexis) Minor modification of cytoplasmic organelles Plasma membrane blebbing Engulfment by resident phagocytes, *in vivo*	'Apoptosis' is the original term introduced by Kerr *et al.*[14] to define a type of cell death with specific morphological features. Apoptosis is NOT a synonym of programmed cell death or caspase activation.
Autophagy	Lack of chromatin condensation Massive vacuolization of the cytoplasm Accumulation of (double-membraned) autophagic vacuoles Little or no uptake by phagocytic cells, *in vivo*	'Autophagic cell death' defines cell death occurring with autophagy, though it may misleadingly suggest a form of death occurring by autophagy as this process often promotes cell survival.[15,16]
Cornification	Elimination of cytosolic organelles Modifications of plasma membrane Accumulation of lipids in F and L granules Extrusion of lipids in the extracellular space Desquamation (loss of corneocytes) by protease activation	'Cornified envelope' formation or 'keratinization' is specific of the skin to create a barrier function. Although apoptosis can be induced by injury in the basal epidermal layer (e.g., UV irradiation), cornification is exclusive of the upper layers (granular layer and stratum corneum).[17,18]
Necrosis	Cytoplasmic swelling (oncosis) Rupture of plasma membrane Swelling of cytoplasmic organelles Moderate chromatin condensation	'Necrosis' identifies, in a negative fashion, cell death lacking the features of apoptosis or autophagy.[4] Note that necrosis can occur in a regulated fashion, involving a precise sequence of signals.


Stress
Cellular damage (for example, chemotherapy)
Intracellular pathogens
Nutrient/growth factor deprivation
Hypoxia
Autophagy
Benefit
Removal of damaged organelles
Limitation of infection
Metabolic substrates for:
• Adaptive protein synthesis
• Bioenergetic needs

Science. 2004 Nov 5;306, 990-995

Autophagy in Health and Disease: A Double-Edged Sword

Takahiro Shintani and Daniel J. Klionsky*

Autophagy, the process by which cells recycle cytoplasm and dispose of excess or defective organelles, has entered the research spotlight largely owing to the discovery of the protein components that drive this process. Identifying the autophagy genes in yeast and finding orthologs in other organisms reveals the conservation of the mechanism of autophagy in eukaryotes and allows the use of molecular genetics and biology in different model systems to study this process. By mostly morphological studies, autophagy has been linked to disease processes. Whether autophagy protects from or causes disease is unclear. Here, we summarize current knowledge about the role of autophagy in disease and health.

Table 1. Possible roles of autophagy in health and disease.

Disease state	Beneficial effects of autophagy	Negative effects of autophagy
Cancer	Acts as a tumor suppressor; may be involved in type II PCD in cancer cells, could limit cell size or may remove damaged organelles that could generate free radicals and increase mutations.	May allow survival of cancer cells within the nutrient-poor environment of a tumor, could prevent cell death, and may protect against some cancer treatments.
Liver disease	Allows removal of nonfunctional endoplasmic reticulum resulting from accumulation of aggregated α_1-antitrypsin Z protein.	Increased mortality due to excessive mitochondrial autophagy.
Muscular disorder	Increased autophagy may compensate for defects in lysosome function.	Increased autophagy or defects in completing autophagy result in the accumulation of autophagosomes that may impair cell function.
Neurodegeneration	Allows the removal of protein aggregates before they become toxic.	May induce cell death in neurons that accumulate aggregated proteins.
Pathogen infection	Cellular defense against invasion by bacteria and viruses.	Subversion of the autophagic pathway allows pathogens to establish a replicative niche and supplies nutrients for growth.

Macroautophagy

Macroautophagy is a **lysosomal degradation pathway for cytoplasmic material**. In mammalian cells macroautophagy is an important survival mechanism during short-term starvation. By degrading some non-essential components cells get nutrients for energy production and vital biosynthetic reactions. Autophagy also contributes to growth regulation and longevity. In addition, autophagy plays a role in innate immunity against viral infection and intracellular bacteria, as well as in the processing of viral antigens. Defective autophagy has been connected to many human diseases including cancer, myopathies, Alzheimer's disease, and Huntington's disease.

Cell, Vol. 120, 237–248, January 28, 2005, Copyright ©2005 by Elsevier Inc.

Growth Factor Regulation of Autophagy and Cell Survival in the Absence of Apoptosis

Julian J. Lum,[1] Daniel E. Bauer,[1] Mei Kong,[1]
Marian H. Harris,[1] Chi Li,[1] Tullia Lindsten,[1,2]
and Craig B. Thompson[1,*]

Following growth factor withdrawal, $Bax^{-/-}$ $Bak^{-/-}$ cells activate autophagy, undergo progressive atrophy, and ultimately succumb to cell death.

Cells from Bax$^{-/-}$ Bak$^{-/-}$ animals fail to undergo apoptosis in response to serum deprivation, loss of attachment, and growth factor withdrawal. Thus, Bax and Bak are essential and redundant regulators of apoptosis and extracellular signals.

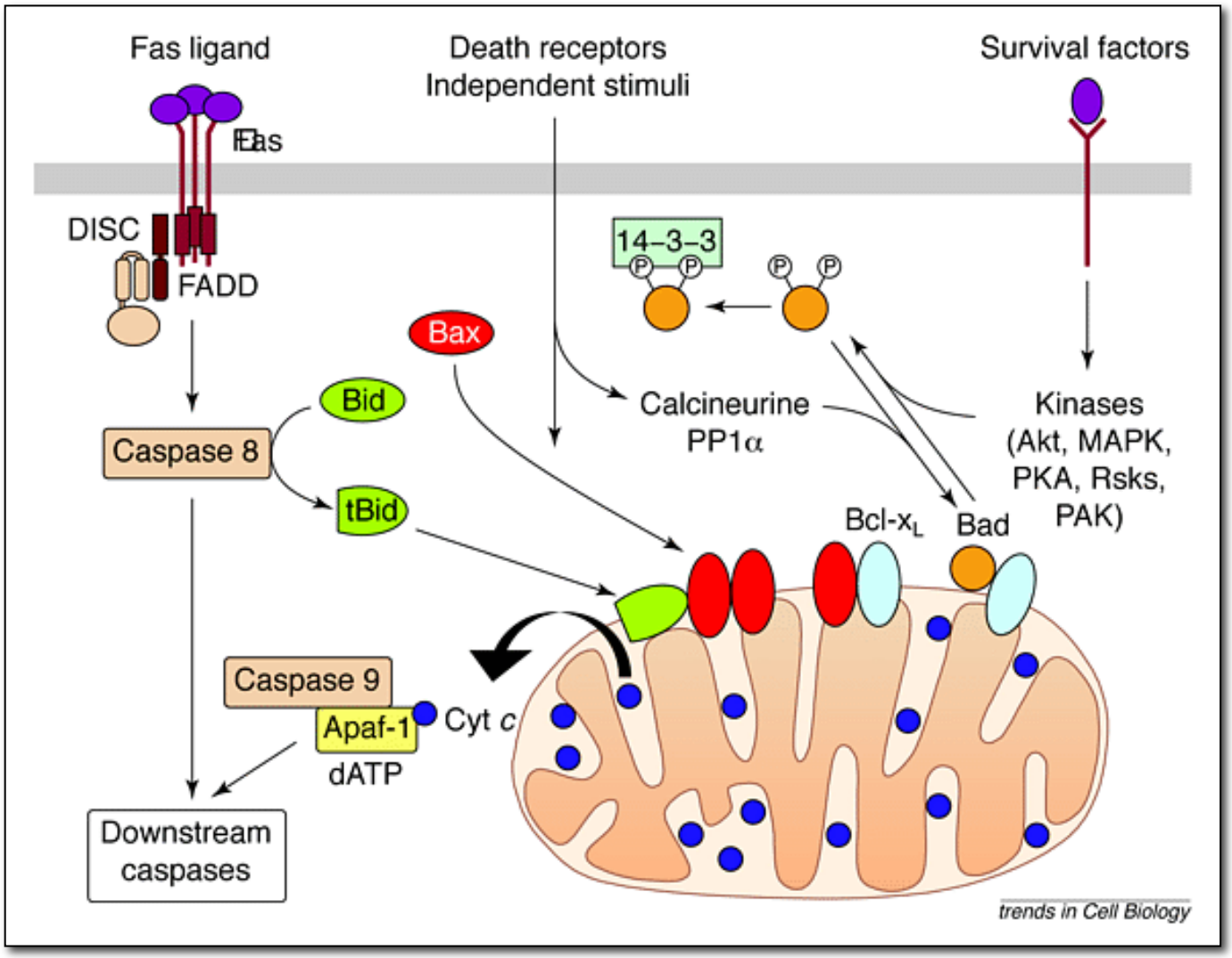
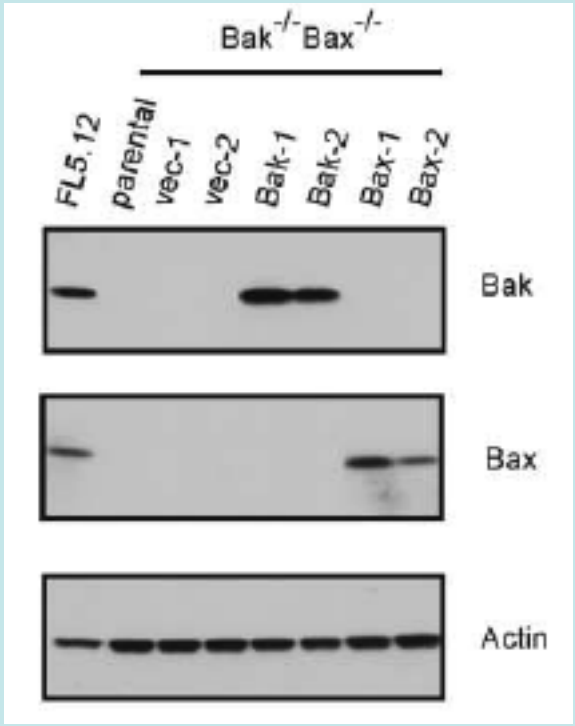
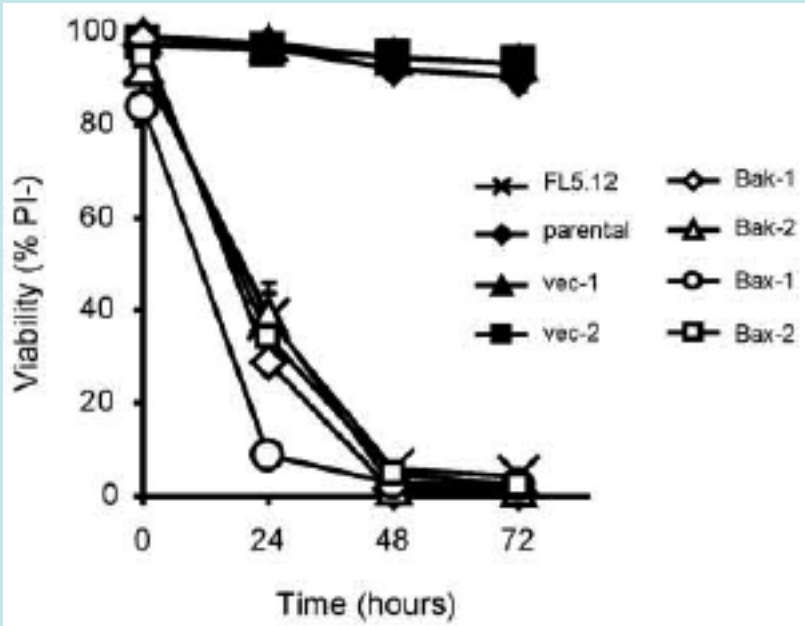


Figure 1. Bax -/- Bak -/- Cells Undergo Atrophy and Maintain Prolonged Survival Following Withdrawal of Growth Factor

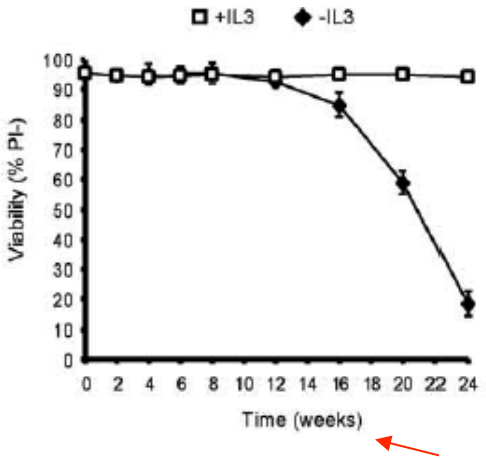


(A) Two independent clones of Bax -/- Bak -/- IL-3-dependent cells (parental) stably transfected with either Bax, Bak, or empty vector (vec) were generated, and expression levels were assessed by Western blot. The IL-3-dependent Bax / Bak / cell line FL5.12 is shown for comparison.



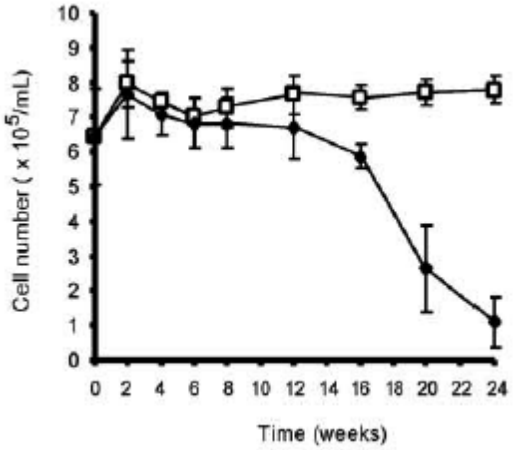
(B) Kinetics of cell death in Bax- or Bak-reconstituted cells following IL-3 withdrawal. Viability was measured by propidium iodide exclusion. Data are averages of three experiments standard deviation (SD).

(C) **Cell viability** of Bax -/- Bak -/- cells in the presence or absence of IL-3. Cells were washed and cultured in the presence (open squares) or absence (closed diamonds) of IL-3. At the indicated time points, cells were collected and viability was assessed. Cells grown in the presence of IL-3 were passaged every 2–3 days to restore a cell concentration of 7.5 x10^5 cells/ml. The medium in IL-3-deprived cultures was replaced with an identical volume of fresh complete medium without IL-3 every 10 days. Data are averages of three independent experiments SD.



+IL-3

-IL-3

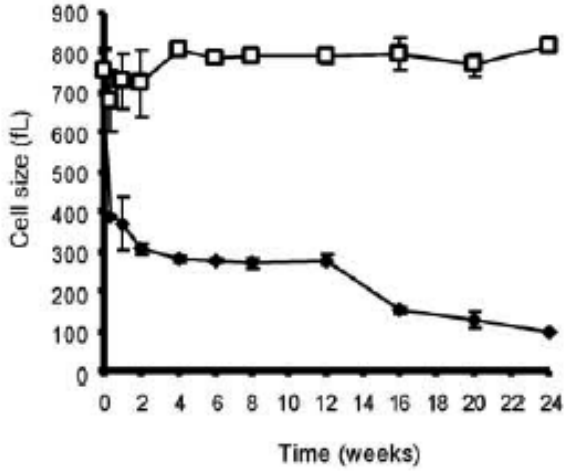


+IL-3

-IL-3

(D) **Cell numbers** of cultures that were grown in the presence or absence of IL-3 and were cultured as in (C). Data are averages of three independent experiments SD.

(E) **Cell size** of cultures that were grown in the presence or absence of IL-3 and were cultured as in (C). Data are averages of three independent experiments SD.



+IL-3

-IL-3

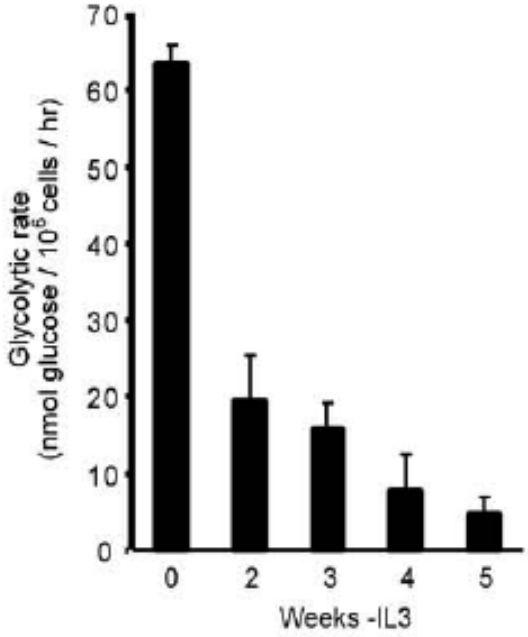
An additional consequence of growth factor limitation is a rapid decline in the surface expression of nutrient transporters including the major glucose transporter GLUT1, the LDL receptor, amino acid transporters and receptors for iron uptake

This decrease in nutrient transporter expression has been proposed to perturb mitochondrial physiology resulting in the induction of apoptotic cell death.

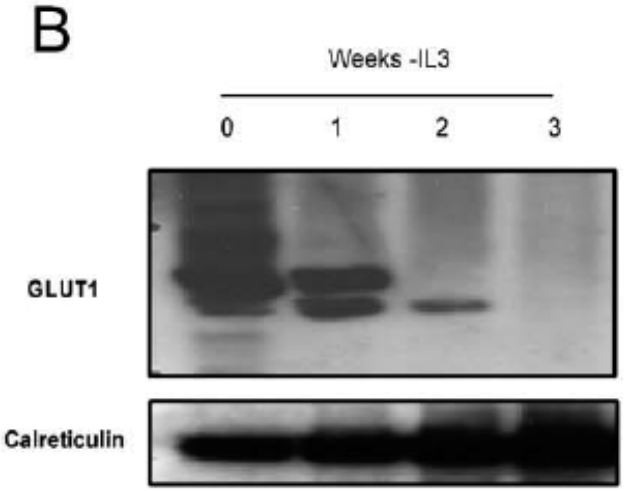
An alternative explanation is that the decline in surface expression of nutrient transporters simply reflects a secondary response to the decreased metabolic demand on the cell following the cessation of growth and the withdrawal from the cell cycle.

Figure 2. Metabolic Effects of IL-3 Withdrawal on Bax-/- Bak -/- Cells

(A) Glycolytic rate of cells grown in the absence of IL-3 as measured by the conversion of 5-H^3-glucose to ^3H2O at the indicated time points. The data presented at week 0 represent values of control cells growing in IL-3 throughout the time course of the experiment. Data are averages of three experiments SD.



time-dependent loss of GLUT1, the major glucose transporter expressed on these cells



(B) Western blot analysis of GLUT1 expression in cells cultured in the absence of IL-3. The GLUT1 expression at week 0 is representative of GLUT1 expression of cells grown in IL-3.

Figure 2. Metabolic Effects of IL-3 Withdrawal on Bax-/- Bak -/- Cells

Coincident with the decline in glycolysis, there was a decline in mitochondrial membrane potential

(C) Mitochondrial membrane potential as measured by TMRE staining in cells grown without IL-3 (solid histogram) at the indicated time point. Baseline TMRE was determined by using cells treated with the uncoupler CCCP (dotted histogram). The numbers in the top right corner indicate the averge mean fluorescence intensity of three independent experiments. The week 0 time point indicates the mean fluorescence intensity of cells growing in IL-3 and is representative of the values obtained for such cells over the time course of the experiment.

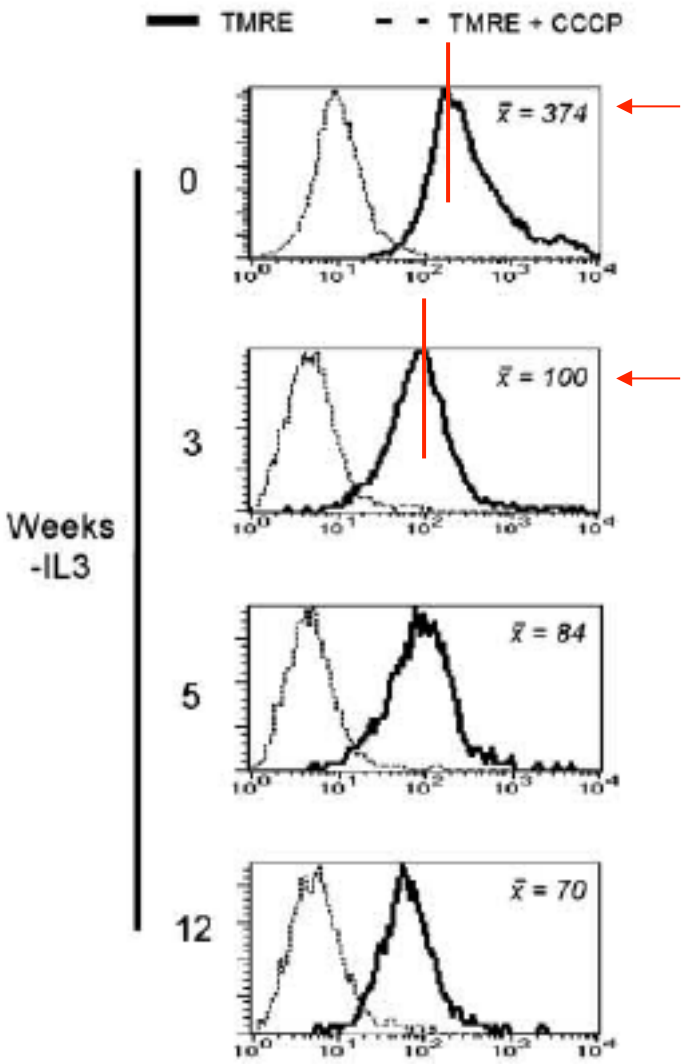
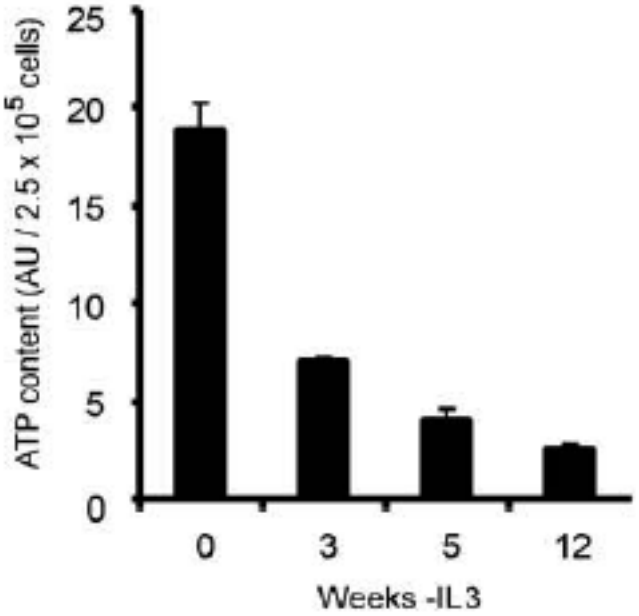
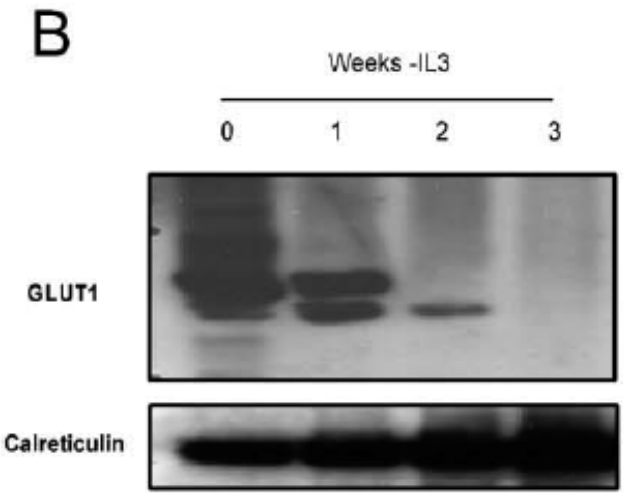


Figure 2. Metabolic Effects of IL-3 Withdrawal on Bax-/- Bak -/- Cells

(D) ATP levels in cells grown without IL-3 and expressed as arbitrary units (AU). ATP levels for IL-3-grown cells did not decline significantly over the time course of the experiment (data not shown). Data are averages of three independent experiments SD.



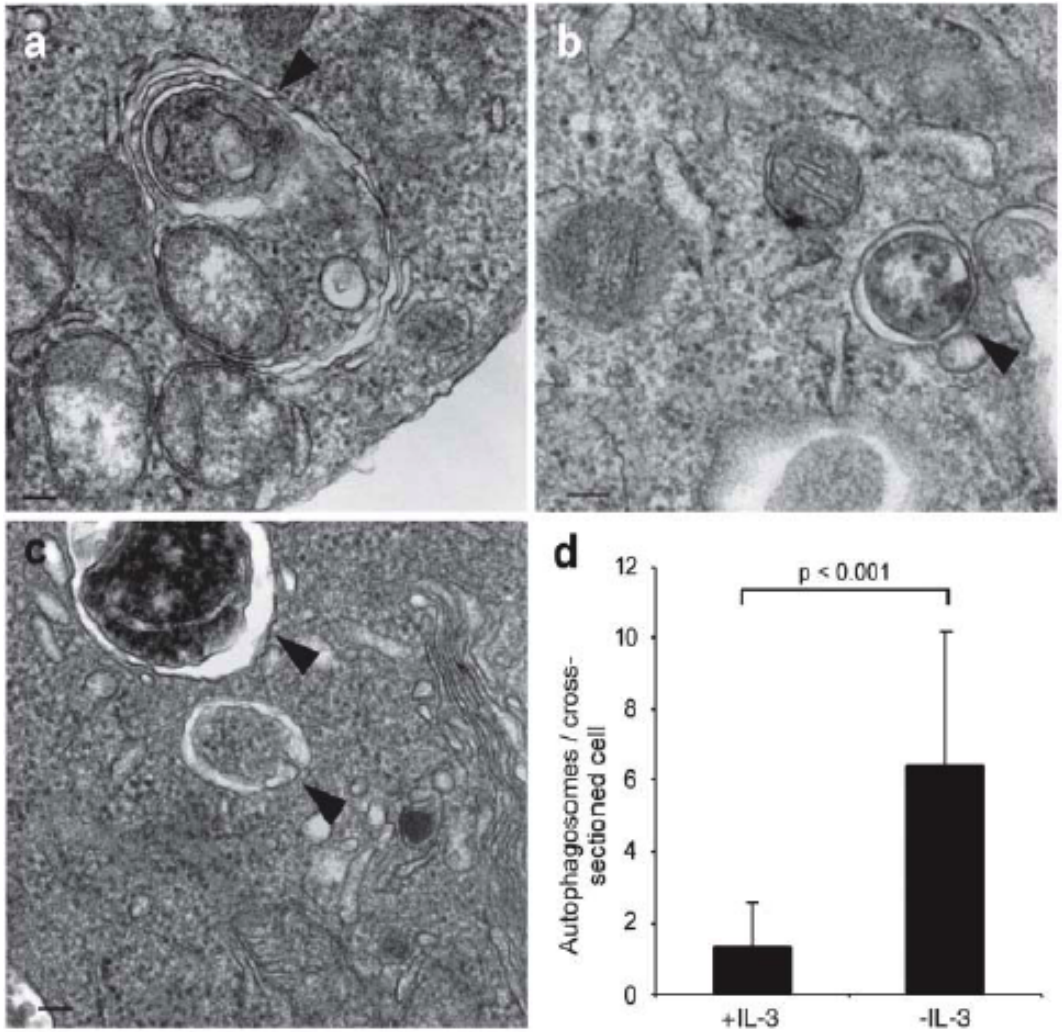
Cellular ATP levels also fell, but the decline in glucose transporter expression was greater than that expected based on the ATP decline, suggesting that cells were utilizing alternative substrates to maintain their bioenergetics.



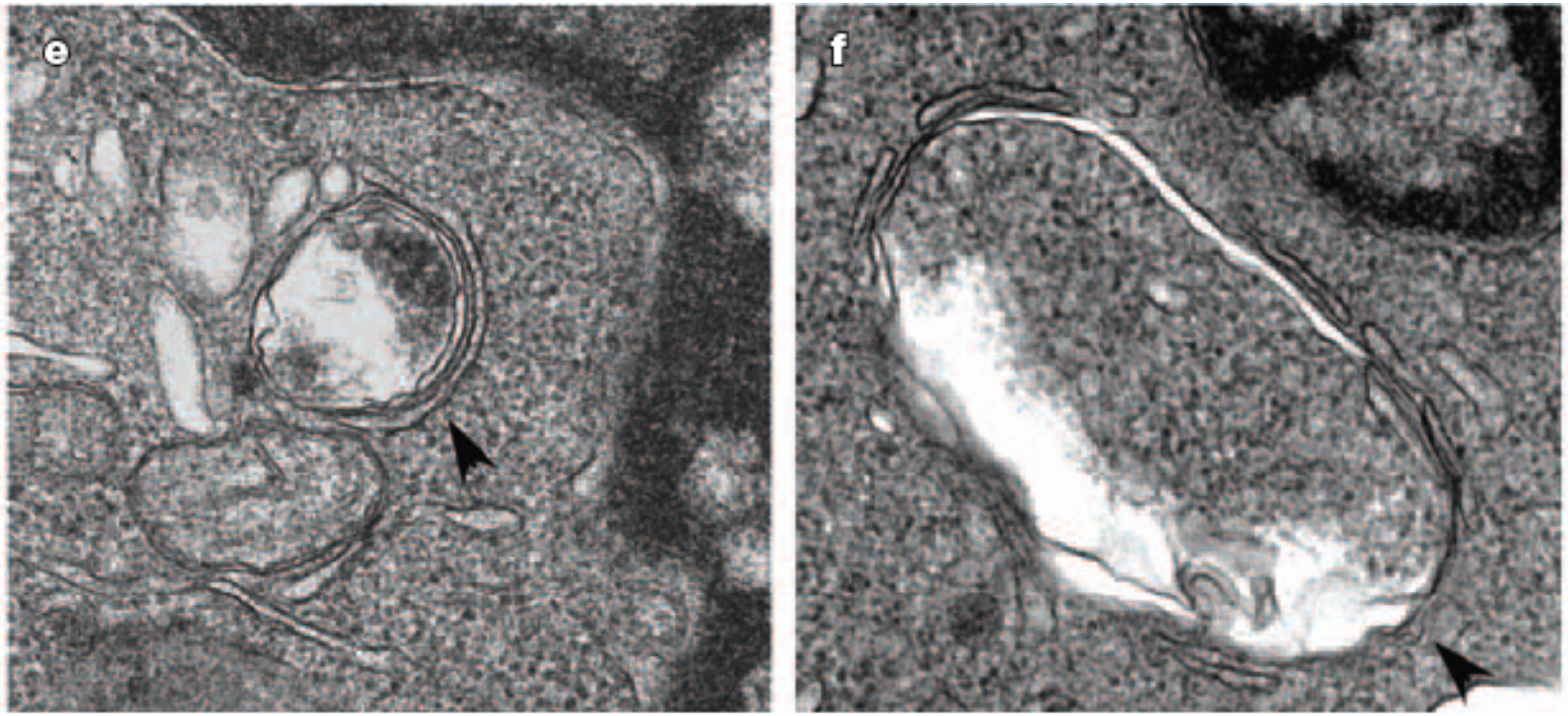
The continued decline in cell size of the G0/G1 arrested cells following growth factor withdrawal suggested the possibility that cells were utilizing **macroautophagy to catabolize intracellular substrates to maintain their survival**.

Figure 3. Growth Factor Withdrawal Induced Autophagosome Formation Is Required for Survival

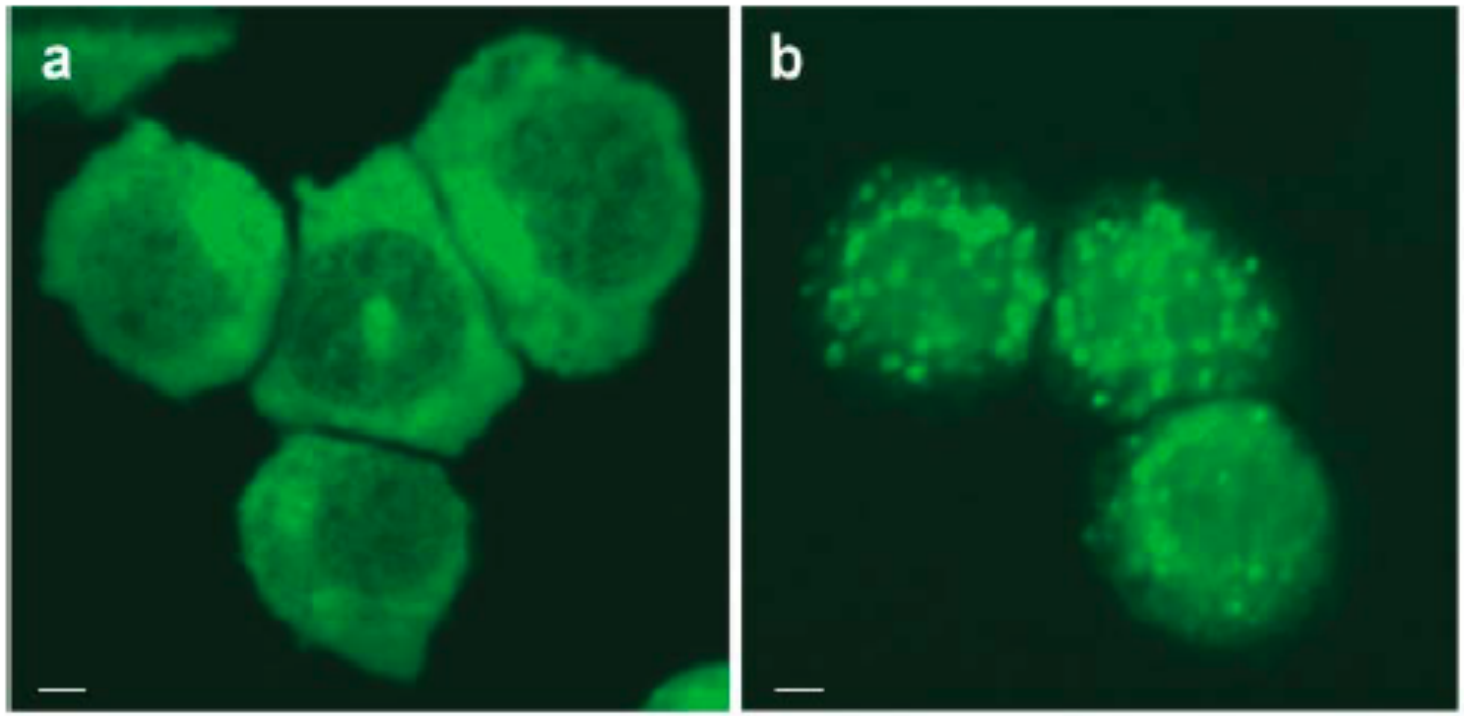
(A) Electron microscopy of cells grown in the absence of IL-3 for 48 hr (a–c) showing the presence of autophagosomes. Arrowheads depict representative autophagosomes quantitated in (d). Scale bar, 100 nm. (d) Quantitation of the number of autophagosomes per cross-sectioned cell cultured in the presence or absence of IL-3 for 48 hr. Error bar represents SD. Statistical significance determined by Student's t test.



Higher-power magnification photomicrographs of IL3-dependent cells deprived of IL3 show autophagosomes that contain intracellular contents. Autophagosomes are indicated by arrowheads.

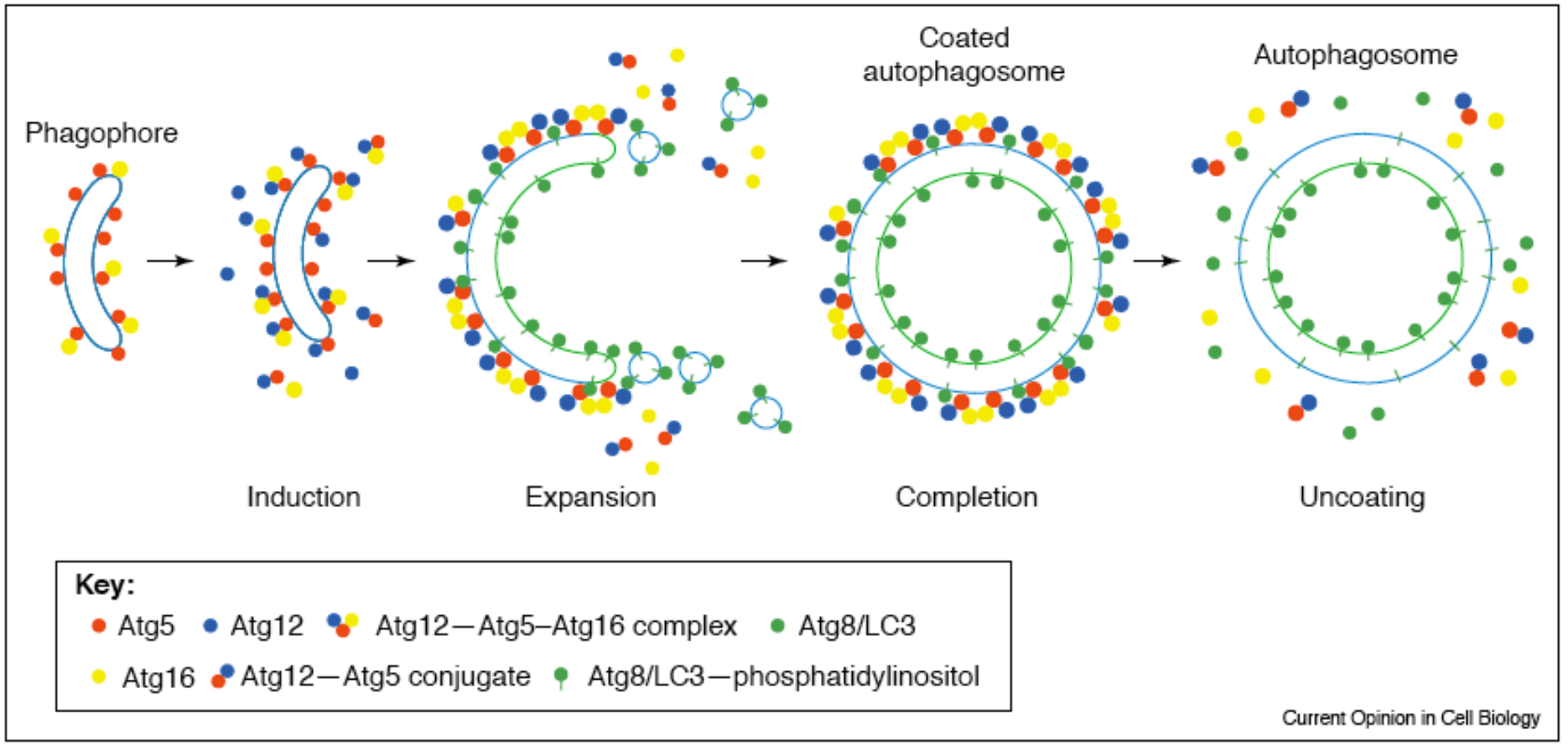


Immunofluoresence with anti-LC3* antibody on cells grown in the presence (a) or absence (b) of IL-3 for 48 hr.



(*) antibody specific for the mammalian homolog of the yeast Atg8 protein, microtubule-associated protein-1 light chain-3 (LC3).

Atg: autophagocytosis associated molecules in yeast

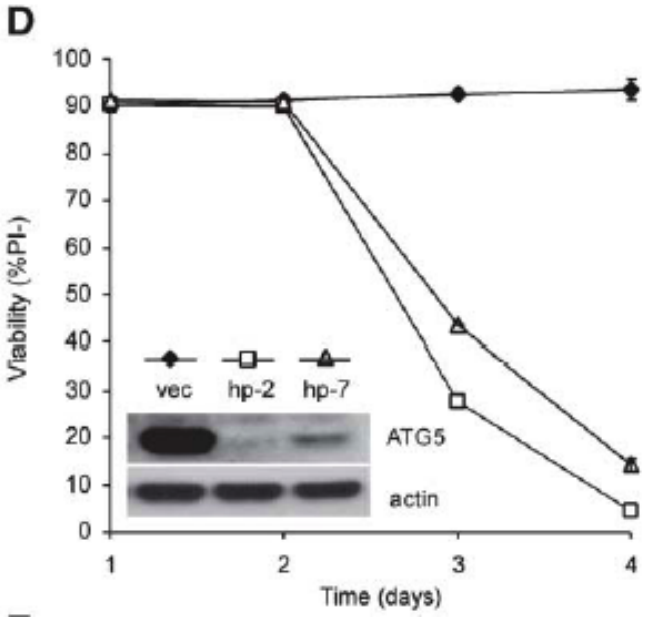


Current Opinion in Cell Biology 2005, **17**:415–422

Inhibition of Autophagy Leads to Cell Death

absence of IL-3 for 48 hr

(D) Time course of cell viability following IL-3 withdrawal in cells with inactivation of ATG5. Data are averages of three experiments SD.
Western blot analysis of ATG5 protein expression in cells transfected with vector control, hp-2, or hp-7 shRNA is shown as a representative experiment. Actin was used as loading control.



(E) Time course of cell viability following IL-3 withdrawal in cells transfected with FITC tagged-siRNA for ATG7 (Yu et al., 2004) or a control siRNA. Cells which had incorporated the siRNA for ATG7 or control were purified by FACS sorting based on FITC-positive cells, and viability was assessed at the indicated time points. Data are averages of three experiments SD.

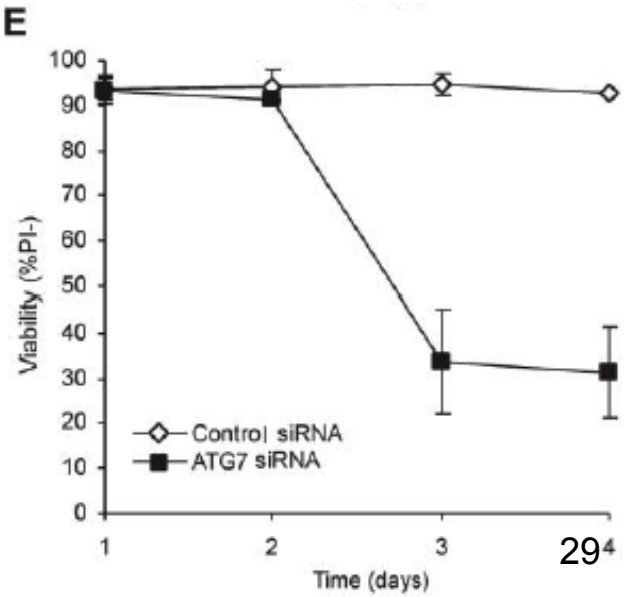
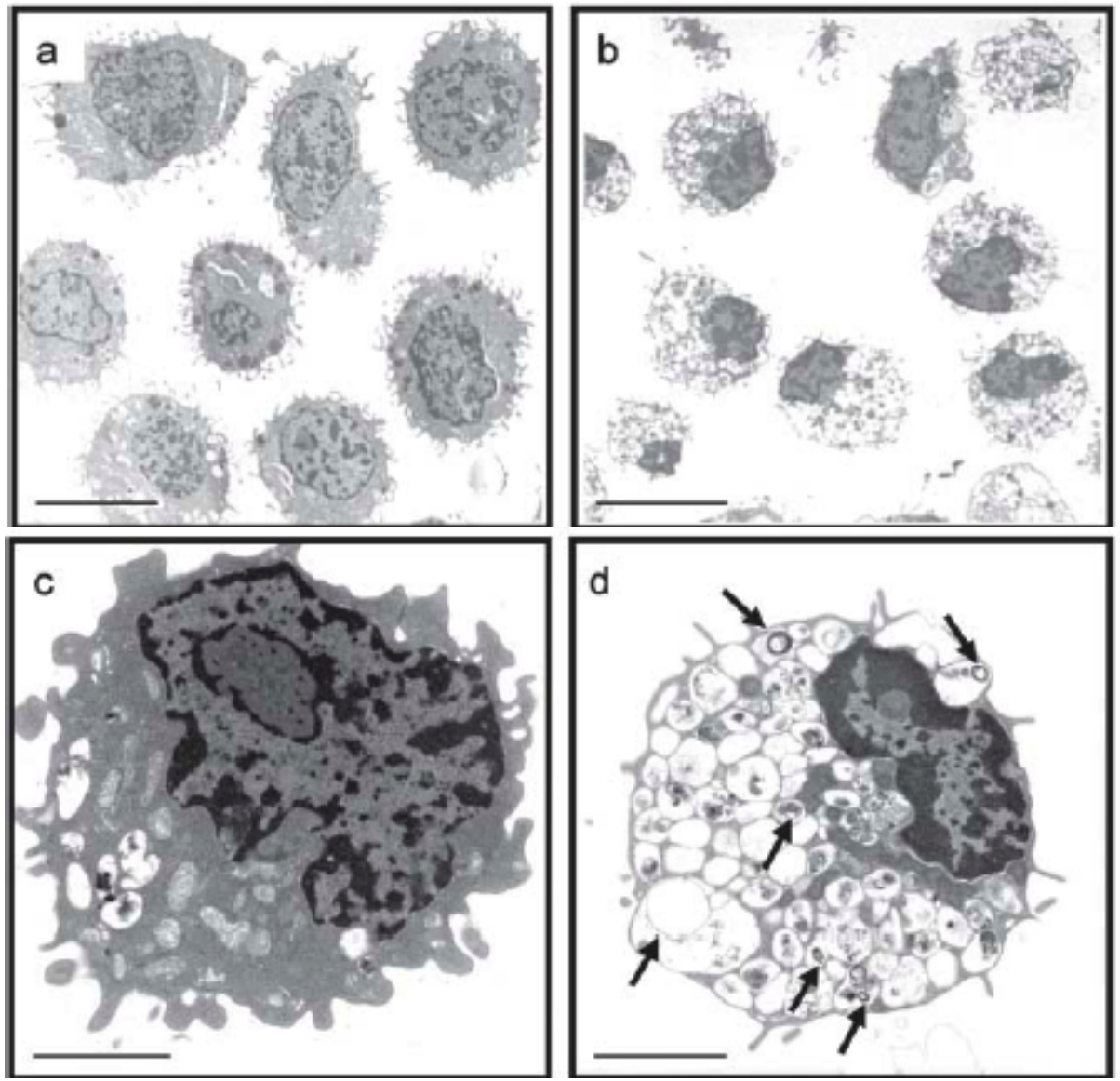


Figure 4. Persistent Autophagy in Long-Term (6 weeks) Growth Factor-Withdrawn Cells

IL-3

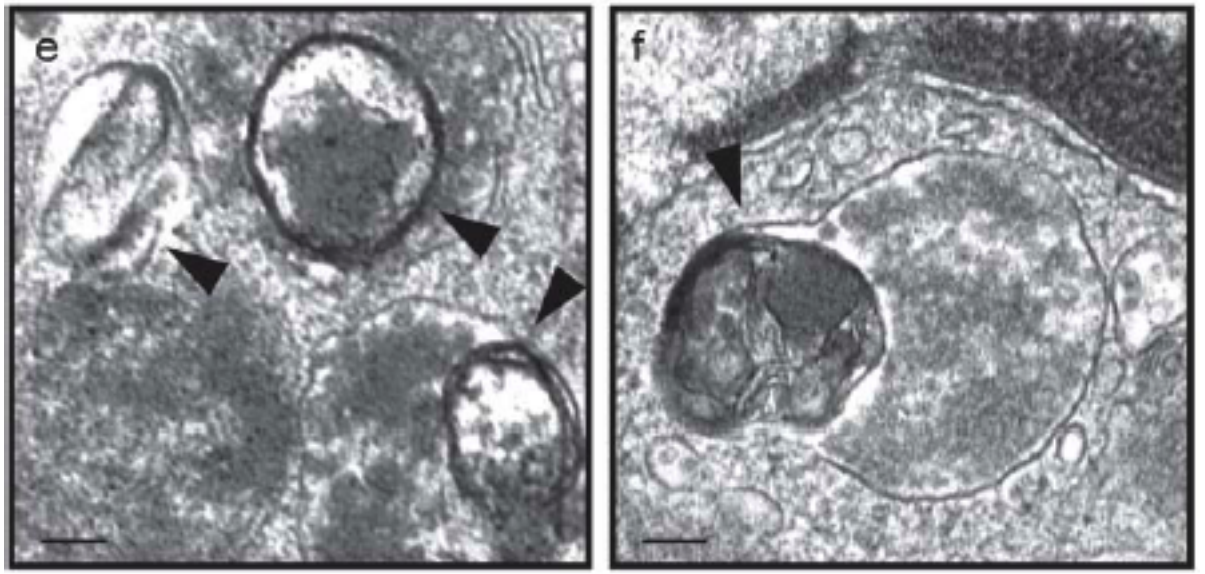


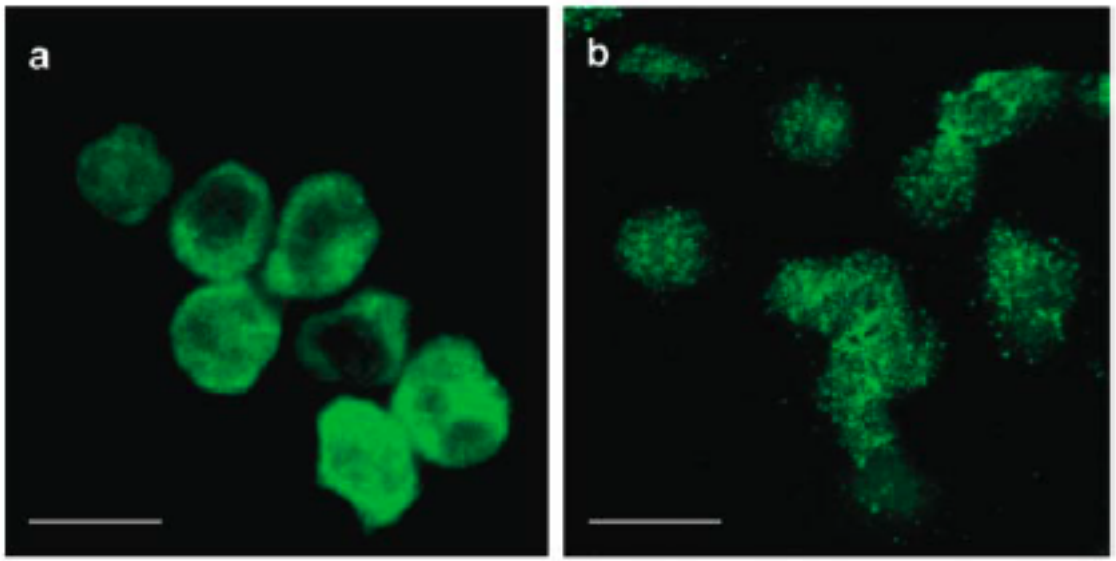
-IL-3

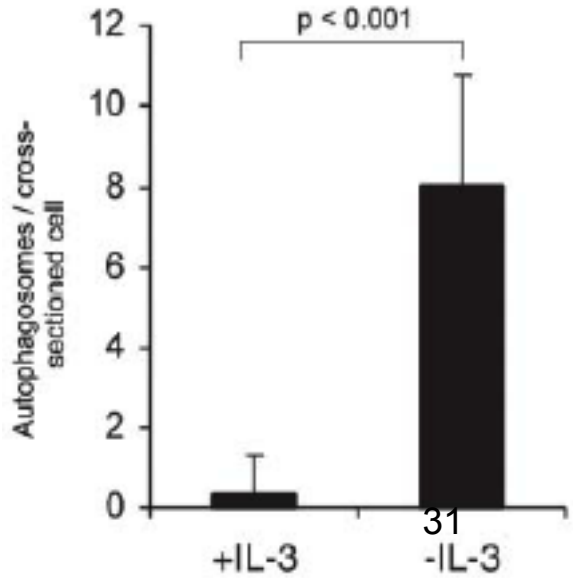
(A) Electron microscopy of cells grown in the presence (a) or absence (b) of IL-3 for 6 weeks. Scale bar, 8.5 m. Magnification image of a cell grown in the presence (c) or absence (d) of IL-3 showing 30 autophagosomes (arrows). Scale bar, 2.3 um.

Higher magnification of cells grown in the absence of IL-3 (e and f). Arrowheads depict autophagosomes in cells containing recognizable cellular material (e) or a late autophagosome fusing with a lysosome (f). Arrowheads depict representative autophagosomes quantitated in (B).

Long term deprivation (6 weeks)





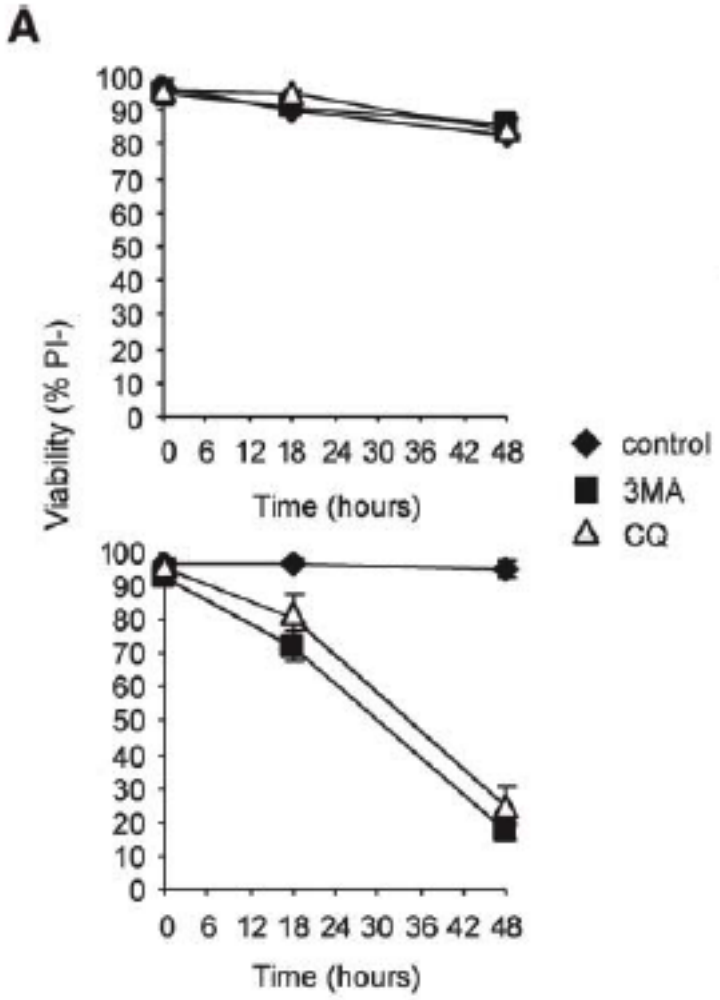


While macroautophagy in yeast and plant cells is required to promote cell survival in the absence of nutrients, the macroautophagy observed following IL-3 deprivation occurred in the presence of abundant extracellular nutrients.

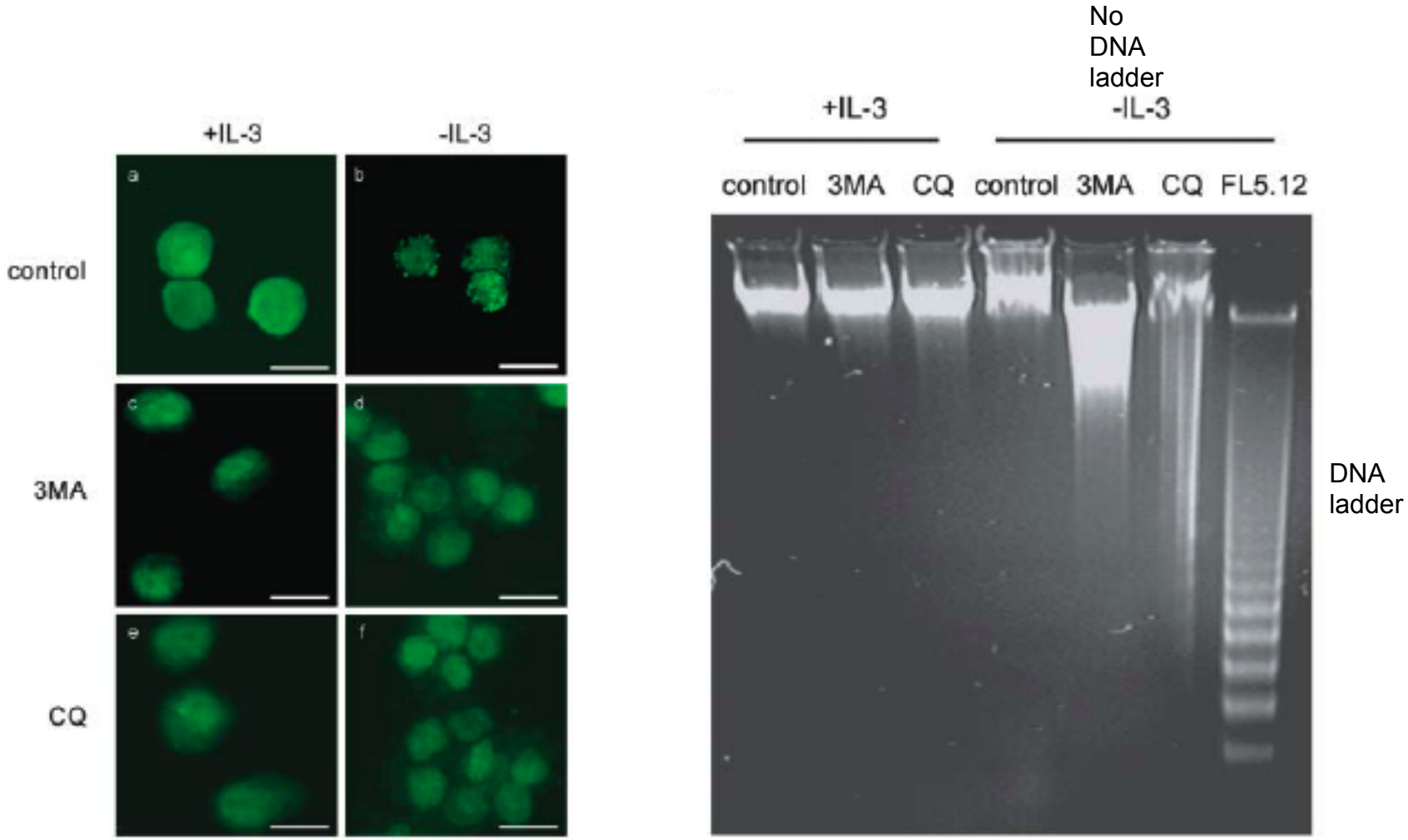
The IL-3-deprived cells were maintained in complete RPMI medium supplemented with 10% serum, and the medium was replaced every 10 days. The medium removed from these cultures was not nutrient deficient since it supported proliferative expansion of the parental $Bax^{-/-}$ $Bak^{-/-}$ cells when supplemented with IL-3 (data not shown). Therefore, macroautophagy in $Bax^{-/-}$ $Bak^{-/-}$ cells was induced by growth factor withdrawal and not by a lack of nutrients in the extracellular environment.

Figure 5. Cell Death Following Inhibition of Autophagy

(A) Viability of cells grown in the presence (top panel) or absence (bottom panel) of IL-3 for 6 weeks treated with 5 mM 3-MA (closed squares) or 10 M CQ (open triangles). PBS was used as a vehicle control (closed diamonds).



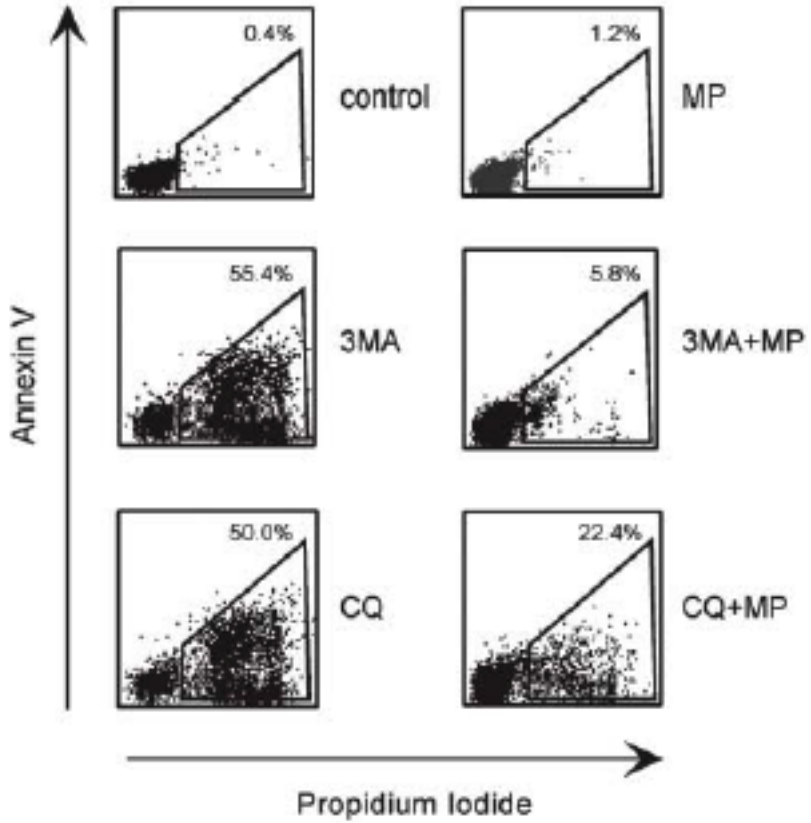
Existing shRNA transfection methods proved ineffective in cells that had undergone prolonged growth factor withdrawal, therefore we used two independent and widely used inhibitors of macroautophagy, 3-methyladenine (3-MA) and chloroquine (CQ) to block autophagy.

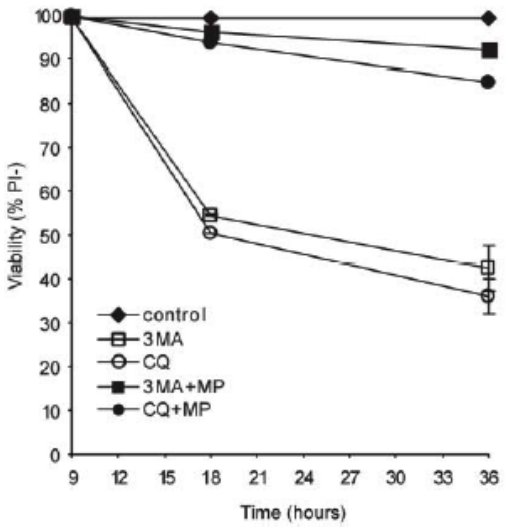


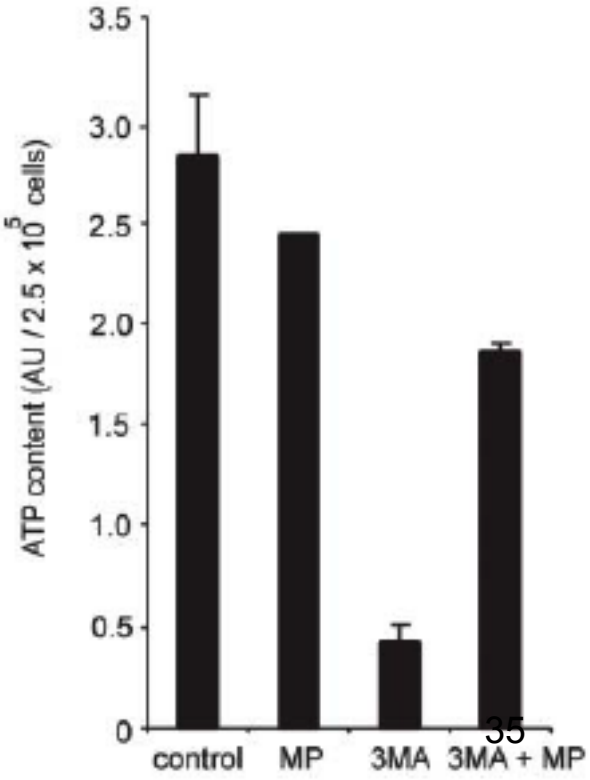
(B) Immunofluorescence staining of LC3 in cells grown in the presence (a) or absence (b) of IL-3 for 6 weeks. Cells grown in the presence or absence of IL-3 were treated for 18 hr with 5 mM 3-MA (c and d) or 10 M CQ (e and f) followed by LC3 staining. PBS was used as a vehicle control.

(C) DNA fragmentation assay was performed on Bax / Bak / cells grown in the presence or absence of IL-3 for 6 weeks and treated for 36 hr with 5 mM 3-MA, 10 M CQ, or PBS as a vehicle control. IL-3-dependent Bax -/- Bak -/- FL5.12 cells grown in the absence of IL-3 for 36 hr were used as a positive control for DNA laddering.

Cell Death Following Inhibition of Autophagy Is Reversed by Methylpyruvate





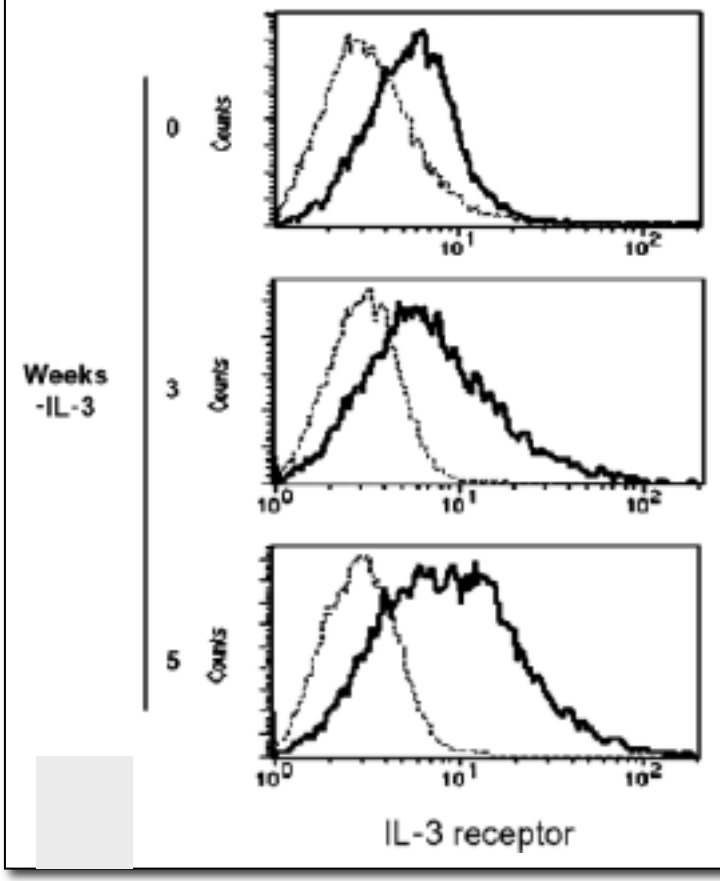


A cell-permeable form of pyruvate, methylpyruvate (MP), was added to the cultures at the time 3-MA or CQ treatment. Once internalized, this substrate can be oxidized in the tricarboxylic acid cycle to produce NADH to fuel electron transport and ATP production.

Cells cultured in the absence of IL-3 had higher levels of surface IL-3 receptor than cells grown in the presence of IL-3.

Despite the loss of cell surface nutrient transporters, the absence of an observable Golgi/ER, and a profound decline in total protein content, the cells cultured in the absence of IL-3 had higher levels of surface IL-3 receptor than cells grown in the presence of IL-3.

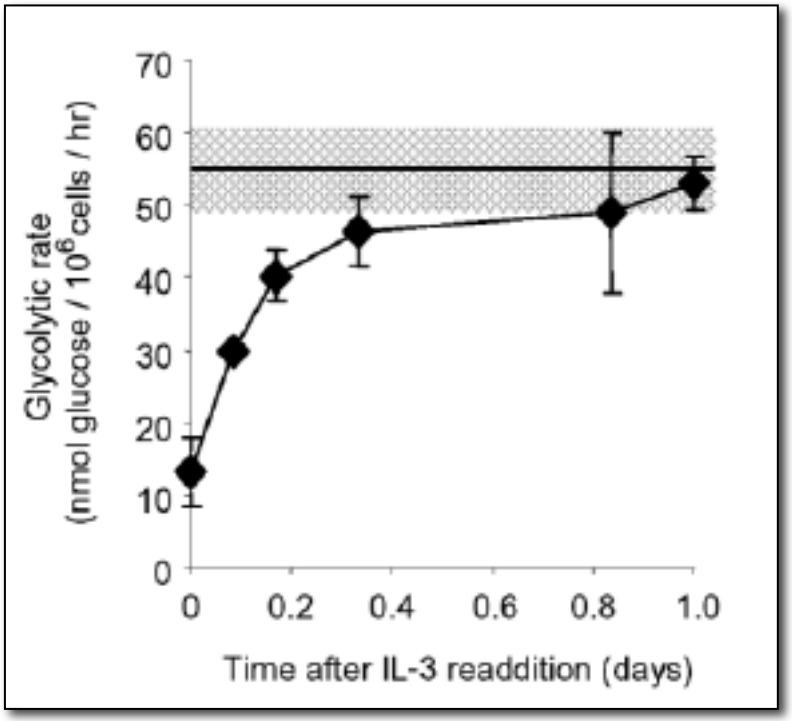
Cell surface staining of IL-3 receptor chain. Dotted histogram represents isotype control and solid histogram represents IL-3 receptor expression.



IL-3 Restimulates Glycolysis and Growth/Proliferation in Growth Factor-Deprived Cells

IL-3 was readded to cells that were cultured in the absence of IL-3 for 4 weeks and collected at the indicated time points for measurement of glycolytic rate. Solid line indicates average glycolytic rate of cells grown in the presence of IL-3 over the time course of the experiment.

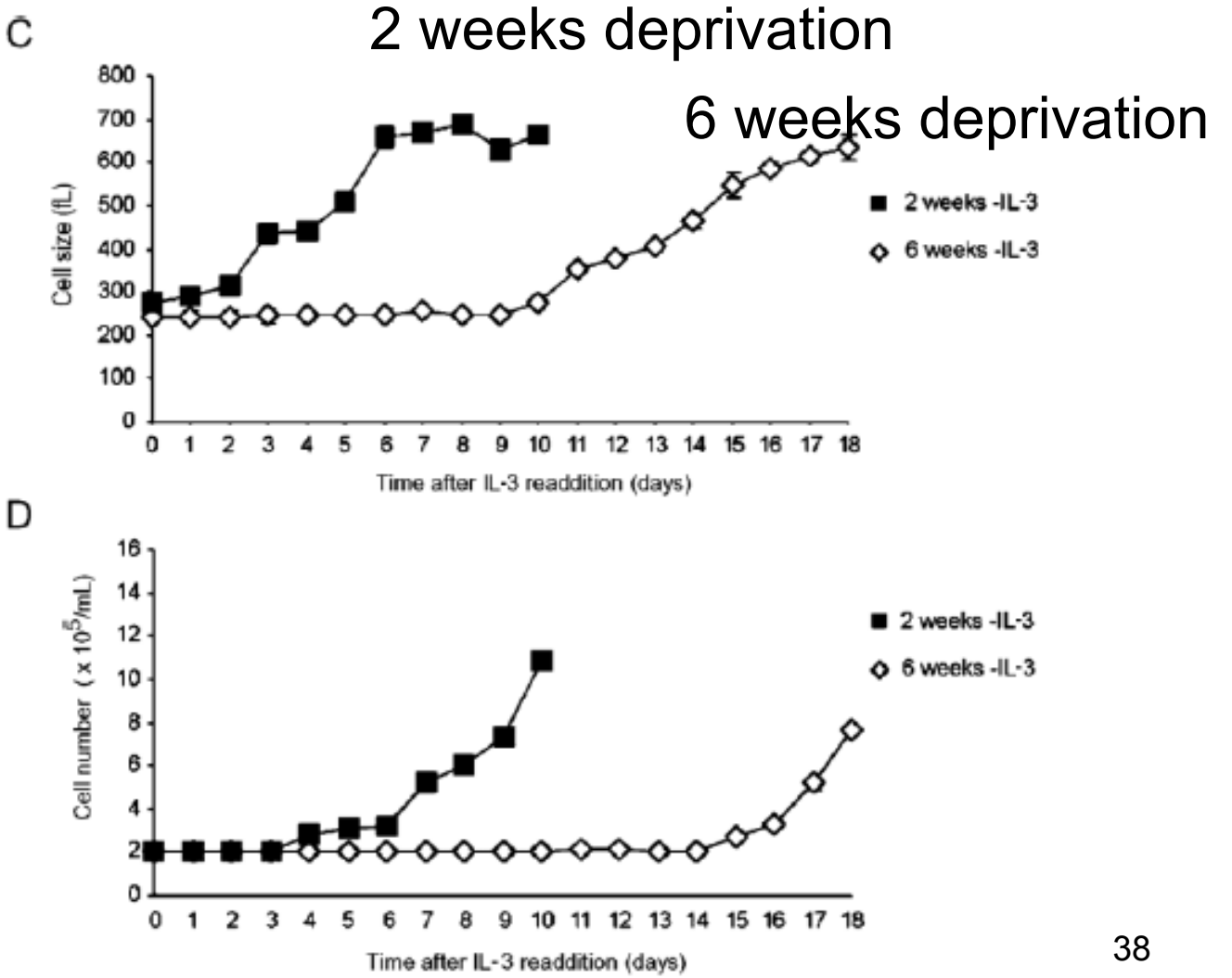
Glycolytic rate of cells following readdition of IL-3.



Cell size and cell number of cultures cultured without IL-3 for 2 (closed squares) or 6 (open triangles) weeks followed by readdition of IL-3.

Cell size

Cell number



Macroautophagy Is a Conserved but Self-Limited Survival Mechanism

Based on the results, macroautophagy appears to be an evolutionarily conserved survival strategy. Macroautophagy can support growth factor-independent cell survival of hematopoietic cells for several weeks.

Thus, it appears eukaryotic cells share a common survival pathway that promotes cell-autonomous survival in the face of starvation and/or neglect. Animal cells may have evolved an apoptotic response in part to limit this form of cell-autonomous survival. Nevertheless, as previously demonstrated in unicellular organisms, macroautophagy is a self limited survival strategy and ultimately will result in cell death if not reversed.

The best-characterized regulator of autophagy is mTOR* complex 1 (mTORC1)

mTOR is a kinase that negatively regulates autophagy by inhibiting the activity of the Atg1 (ULK1) complex through direct phosphorylation. The activity of mTORC1 is stimulated by a variety of anabolic inputs, which include the energy and nutrient status of the cell as well as the presence of amino acids and growth factors. Conversely, mTORC1 is inhibited when amino acids are scarce, growth factor signalling is reduced and/or ATP concentrations fall, and this results in a de-repression of autophagy

Nutrients → mTOR active → No autophagy

Reduced nutrients → mTOR inhibited → Autophagy

(*) mTOR: the mammalian target of rapamycin. Rapamycin is a macrolide used to immunosuppressant functions in humans and is used to prevent rejection in organ transplantation; it is especially useful in kidney transplants. It prevents activation of T cells and B cells by inhibiting their response to interleukin-2.

AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1

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Affiliations | **Contributions** | **Corresponding author**

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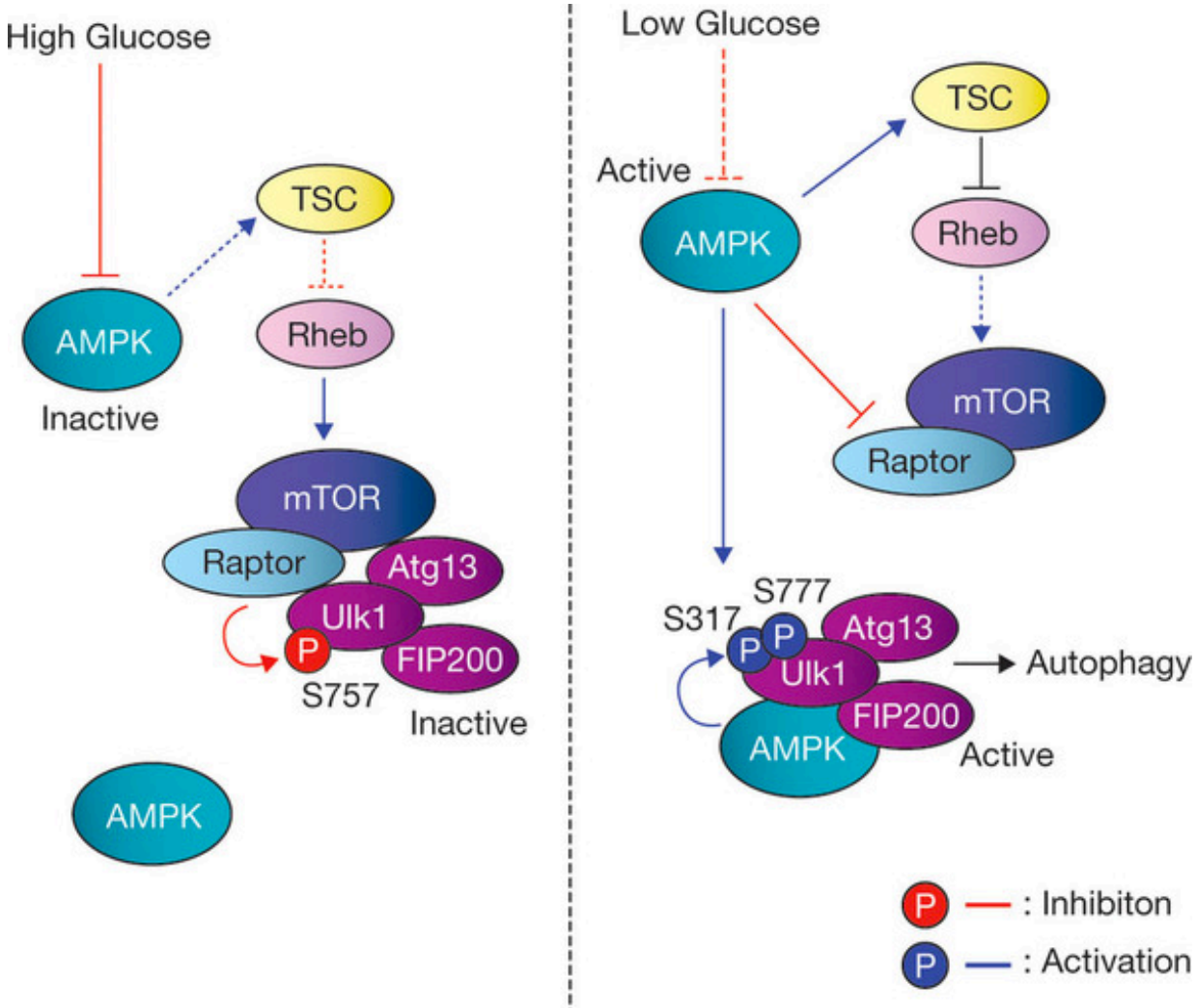
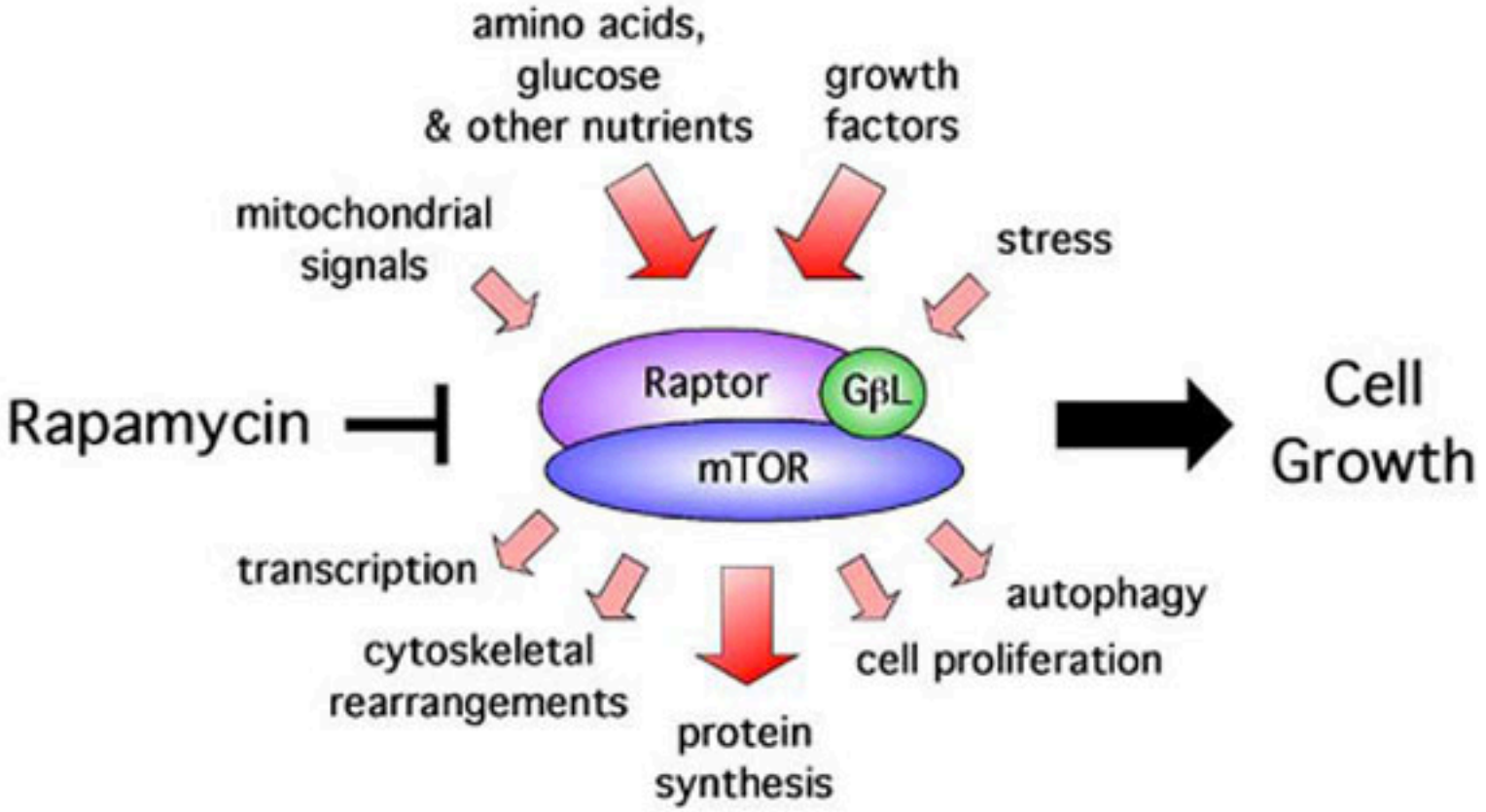
Left: when glucose is sufficient, AMPK is inactive and mTORC1 is active. The active mTORC1 phosphorylates Ulk1 on Ser 757 to prevent Ulk1 interaction with and activation by AMPK. Right: when cellular energy level is limited, AMPK is activated and mTORC1 is inhibited by AMPK through the phosphorylation of TSC2 and Raptor. Phosphorylation of Ser 757 is decreased, and subsequently Ulk1 can interact with and be phosphorylated by AMPK on Ser 317 and Ser 777. The AMPK-phosphorylated Ulk1 is active and then initiates autophagy.



AMPK: AMP activated kinase
Ulk1= mammalian Atg analog

- mTOR **regulates** many fundamental biological processes, such as cell growth and survival.
- mTOR **integrates** both intracellular and extracellular signals, including growth factors, nutrients, energy levels, and cellular stress.
- When bound to different proteins, mTOR forms **distinctive complexes** with very **different physiological functions**.



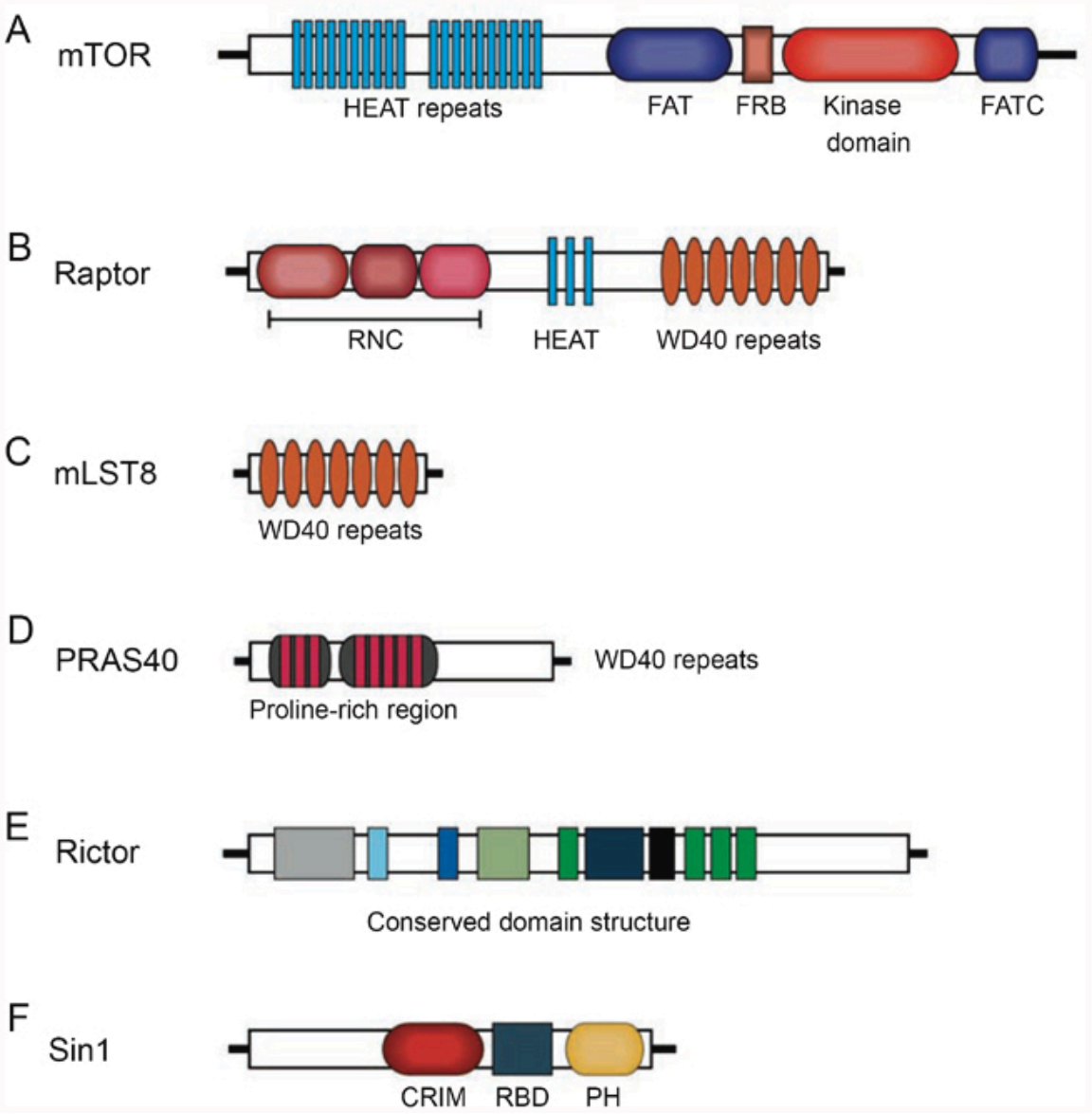
Schematic of mTOR complex components

HEAT: a protein-protein interaction structure of two tandem anti-parallel a-helices found in huntingtin, elongation factor 3, PR65/A and TOR

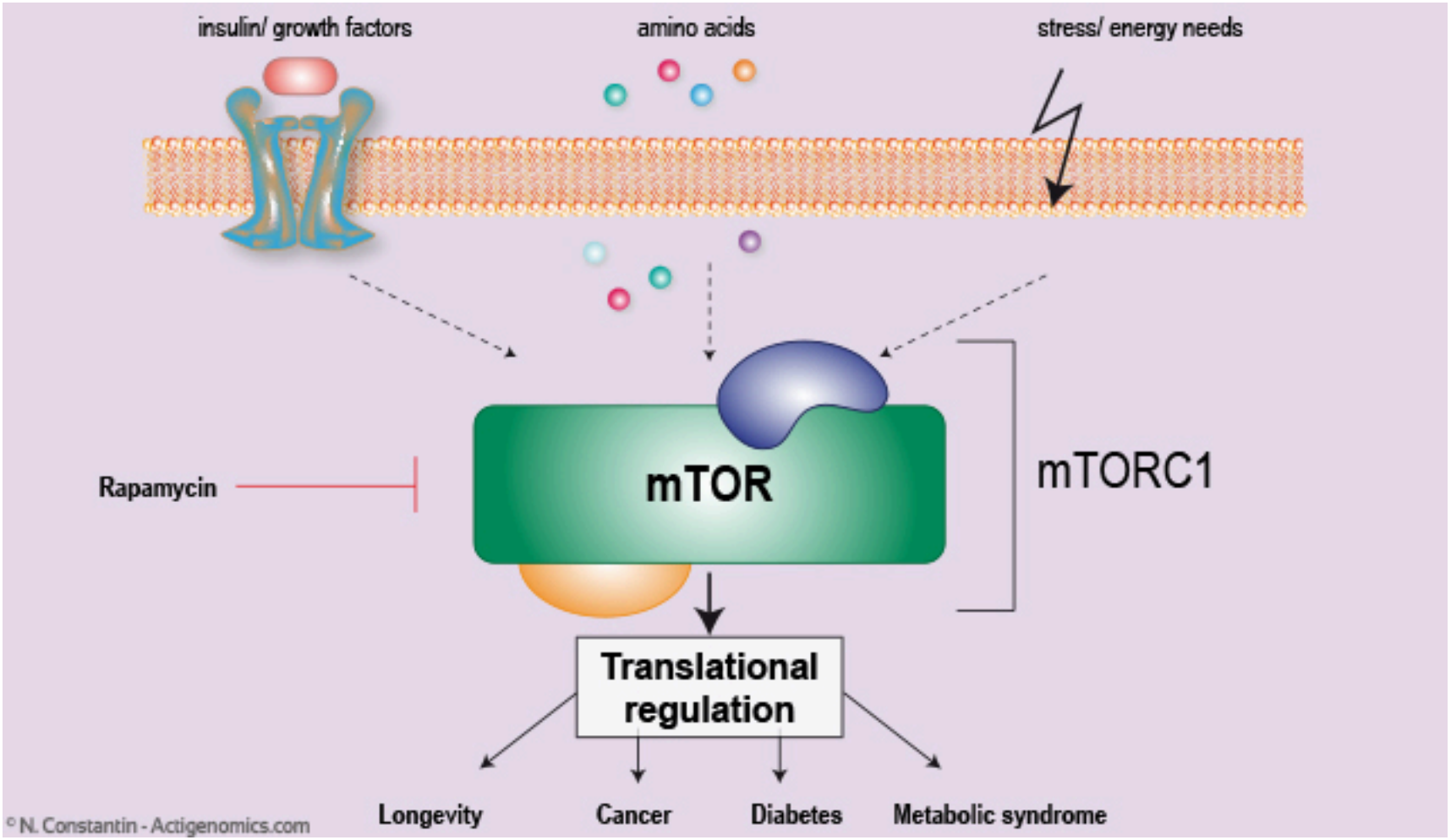
FAT: a domain structure shared by FRAP, ATM and TRRAP, all of which are PIKK family members (atypic PI3K involved also in DNA damage/repair)

FRB: FKBP12/rapamycin binding domain; FATC:

FATC: FAT C-terminus;



RNC: Raptor N-terminal conserved domain; WD40: about 40 amino acids with conserved W and D forming four anti-parallel beta strands; CRIM: conserved region in the middle; RBD: Ras binding domain.


insulin/ growth factors
amino acids
stress/ energy needs
Rapamycin
mTOR
mTORC1
Translational regulation
Longevity
Cancer
Diabetes
Metabolic syndrome
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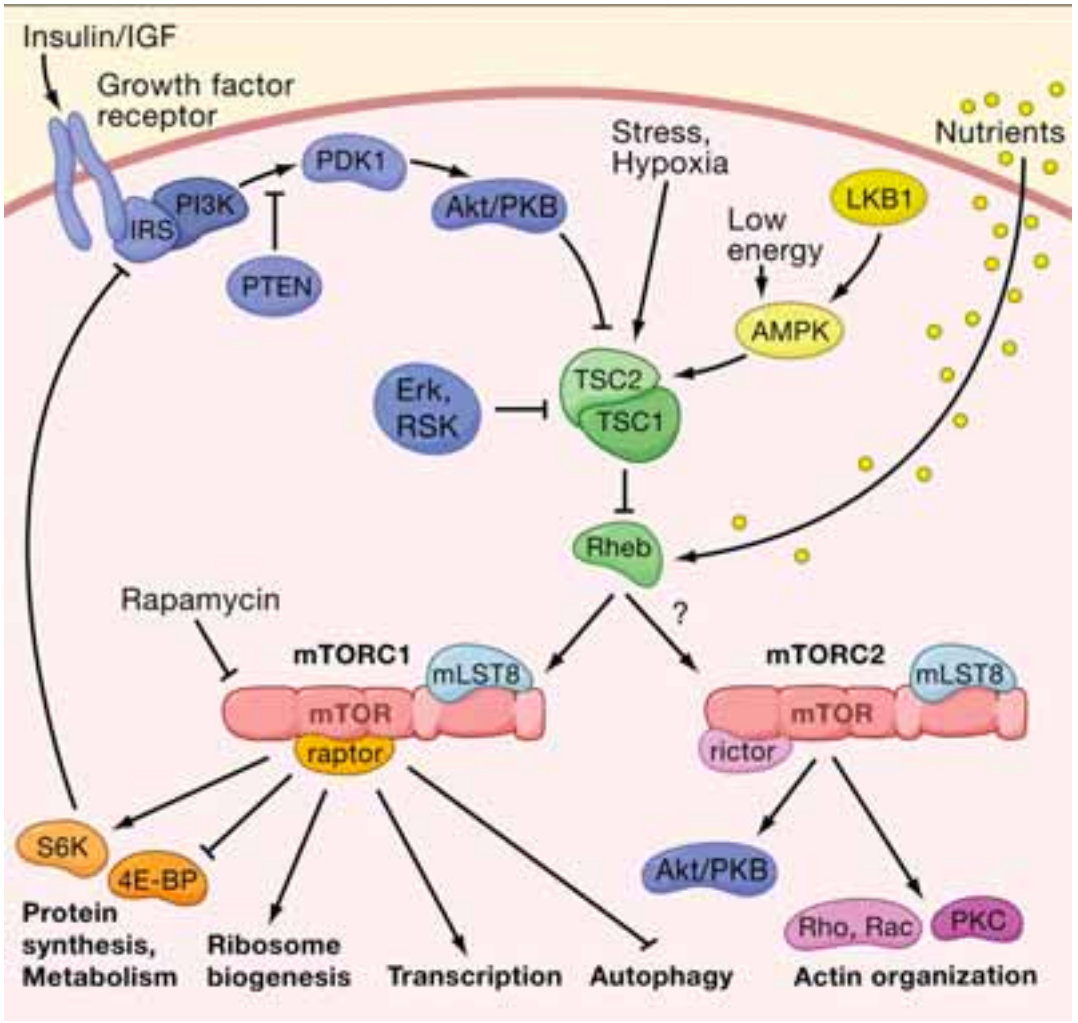
mTOR signaling network in mammalian cells.

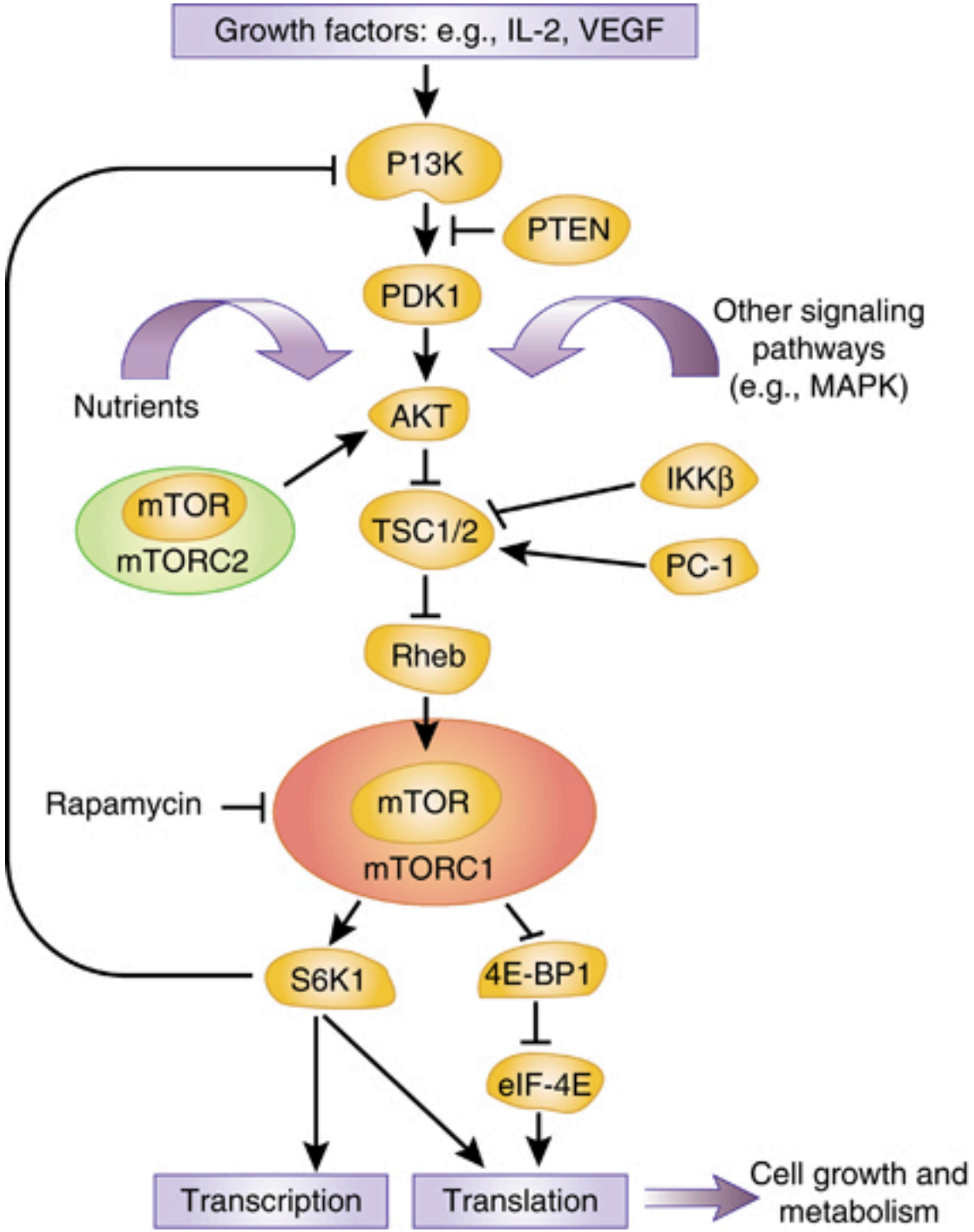
mTORC1= mTOR, Raptor, mLST8, and PRAS40

TSC1/2-Rheb is the major upstream regulator of mTORC1.
Through the TSC1/2-Rheb axis, mTORC1 integrates cellular energy levels, growth factors, and Wnt signals to regulate protein translation by phosphorylating S6K1 and 4EBP1. Phosphorylated S6K1 (active) inhibits IRS1 function and thus attenuates insulin/PI3K signaling.

The mTORC2 subunits include mTOR, Rictor, Sin1, and mLST8. mTORC2 controls cell structure and survival by regulating PKCalpha and Akt. The upstream regulation of mTORC2 remains unknown.

Arrows represent activation, bars represent inhibition.




Growth factors: e.g., IL-2, VEGF
P13K
PTEN
PDK1
Other signaling pathways (e.g., MAPK)
Nutrients
AKT
IKKβ
mTOR
mTORC2
TSC1/2
PC-1
Rheb
Rapamycin
mTOR
mTORC1
S6K1
4E-BP1
eIF-4E
Transcription
Translation
Cell growth and metabolism