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Research Focus

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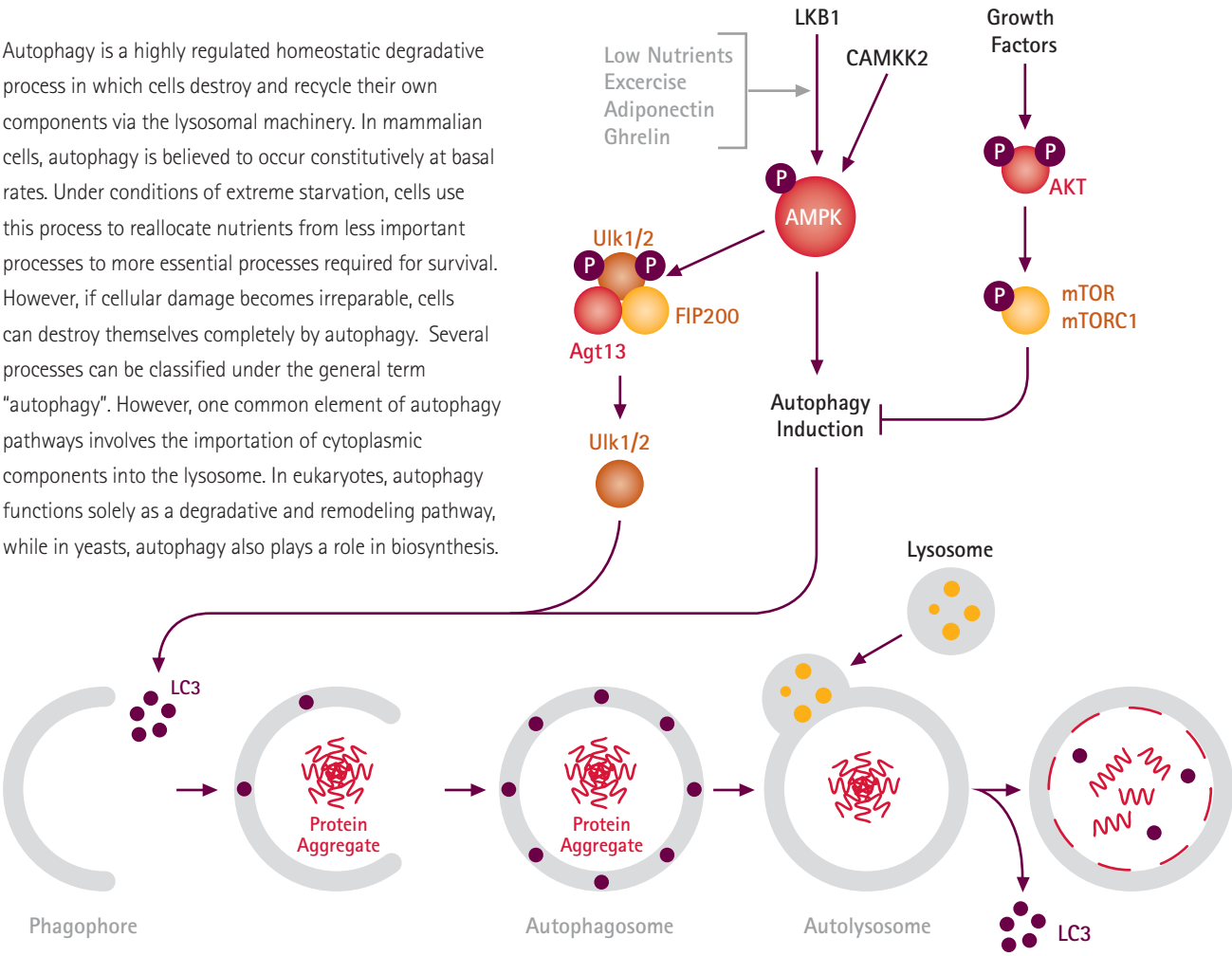
Autophagy:

Mechanisms and Connections to Apoptosis

Autophagy is a highly regulated homeostatic degradative process in which cells destroy and recycle their own components via the lysosomal machinery. In mammalian cells, autophagy is believed to occur constitutively at basal rates. Under conditions of extreme starvation, cells use this process to reallocate nutrients from less important processes to more essential processes required for survival. However, if cellular damage becomes irreparable, cells can destroy themselves completely by autophagy. Several processes can be classified under the general term "autophagy". However, one common element of autophagy pathways involves the importation of cytoplasmic components into the lysosome. In eukaryotes, autophagy functions solely as a degradative and remodeling pathway, while in yeasts, autophagy also plays a role in biosynthesis.



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Three types of autophagy

Generally, three types of autophagy have been recognized. They are chaperone-mediated autophagy, microautophagy, and macroautophagy. Chaperone-mediated autophagy involves the direct translocation of cytosolic proteins across the lysosomal membrane. It requires cytosolic and lysosomal chaperones to unfold substrates. During microautophagy, cytoplasm is sequestered directly at the lysosomal surface by separation and/or invagination of the lysosomal membrane. In macroautophagy the sequestering of membrane is distinct from lysosome and it involves the formation of autophagosome that fuses with lysosome, which provides the hydrolytic enzyme machinery. The fused structure is termed as autophagolysosome. The maturation of autophagolysosome requires acidification by H1-ATPase. Hence, several inhibitors of H1-ATPase, such as Bafilomycin A1, are shown to diminish autophagy. Following their breakdown macromolecules are released back into the cytoplasm for reuse in the metabolic processes.

Steps of macroautophagy

The process of autophagy can be divided into several phases. In the initial phase, the cell senses signals released in response to lack of nutrients, hypoxia, hormones or other sources of cell stress. These signals can induce macroautophagy, either via inhibition of mTOR or by AMPK activation of the ULK1/2 kinase complex (Atg1 in yeast). mTOR is essential to nutrient-sensing signal transduction and regulation of translation and cell cycle progression. mTOR activity negatively regulates autophagy, which may involve AMP kinase induced phosphorylation of mTOR on specific sites. Autophagy can also be induced in an mTOR-independent manner, such as by lowering the levels of myo-inositol-1,4,5-triphosphate in cells by using lithium, carbamezapine, or sodium valproate.

The second phase of macroautophagy involves the formation of the phagophore, a subcellular structure. The origin of the phagophore membrane is still unclear – the endoplasmic reticulum, Golgi, mitochondria, and endosomes may contribute. The ULK1,2/Atg13 complex, by mechanisms still being characterized, catalyzes the scaffolding of Atg protein complexes onto the phagophore membrane. The ATG14L/Vps34 protein complex generates phosphatidylinositol triphosphate, which recruits still more Atg complexes to the membrane.

In the third phase of macroautophagy, elongation of the phagophore to form the autophagosome is mediated by two protein conjugation systems that are reminiscent of the ubiquitin conjugation pathway. Atg7 is an E1-like activating enzyme that, along with Atg10 (E2-like conjugating enzyme), links Atg12 to Atg5 and Atg16L. This latter complex has an E3-like ligase activity that conjugates PE to LC3 (Atg8), the small protein which mediates elongation. Atg9 is a transmembrane protein that cycles between the autophagosome membrane and other intracellular membranes, regulating membrane addition to the growing autophagosome.

During autophagosome maturation, the protease ATG4 cleaves LC3-II (LC3-PE) from the lipid bilayer of the autophagosome, in preparation for fusion with the lysosome. Finally, the autophagosome fuses with the lysosome, whose contents enable recycling of macromolecules and amino acids for reuse by the cell.

Relationship to apoptosis

Correlation between autophagy and apoptotic cell death has become an emerging topic of great scientific interest, especially in the field of tumor biology. On one hand, autophagy may induce cell death by degrading essential components, but on the other, it may facilitate survival of cancer cells under unfavorable metabolic conditions. Hence, cancer cells, with mutated Bcl-2, may survive chemotherapy by employing a protective autophagic process.

Autophagy pathways as therapeutic targets

A better understanding of autophagy will allow us to develop therapeutic agents to either increase or decrease the extent of this process. A number of disease states, including those where mutant proteins cause pathological changes, could become target of autophagy inducing agents. Some of the examples include Parkinson's disease, Huntington's disease, and Alzheimer's disease where undesirable aggregates of proteins are causative factors of disease. Armed with newly developed immunoreagents, small molecule inhibitors and autophagy assays, researchers in diverse fields are poised to shed light on the potential effects of perturbing autophagy pathways on the progression of these diseases.

NEW! AUTOPHAGY RESEARCH TOOLS

Antibodies

Product	Catalogue No.
Anti-Abl (c-, v-, Bcr-), clone 24-21	MABT203
Anti-APG10	AB15408
Anti-APG12	AB15410
Anti-APG5	AB15404
Anti-APG5	AB15404P
Anti-APG9	AB15407
Anti-ATG12, clone EPR4800, rabbit	MABC135
Anti-ATG13, clone 2H4.2	MABC46
Anti-ATG16L1	ABC25
Anti-ATG18 (WIPI-2), clone 2A2	MABC91
Anti-ATG3	AB2953
Anti-ATG3, clone 1F7	ST1526-100UG
Anti-ATG4A	ABC29
Anti-ATG4A, clone EPR4122, rabbit	MABC136
Anti-ATG4B	ABC32
Anti-ATG4C	ABC21
Anti-ATG4C	ST1538-50UG
Anti-ATG4D	ABC22
Anti-ATG5	ABC14
Anti-ATG5, clone 177.19	MAB2605
Anti-ATG5, clone EPR1755(2), rabbit	MABC137
Anti-ATG7	AB10511
Anti-Atg7, clone EP1759Y, rabbit	04-1055
Anti-ATG9	ABC23
Anti-Beclin-1 (C-term), clone EPR1733Y, rabbit	MABN16
Anti-Beclin-1	AB15417
Anti-Beclin-1, Rabbit	AP1055-50UG
Anti-Beclin-1, clone 9A1.1	MABC34
Anti-DRAM1	MABC62
Anti-DRAM2	MABC64
Anti-GABARAP	AB15415
Anti-GABARAPL1	AB15278
Anti-GATE-16	ABC24
Anti-LC3A (N-term), clone EP1528Y, rabbit monoclonal	MABC177
Anti-LC3A, clone EPR1754, rabbit monoclonal	MABC175
Anti-LC3A/B (N-term), clone EP1983Y, rabbit monoclonal	MABC176
Anti-phospho-ULK1 (Ser555)	ABC124
Anti-ULK1, clone 2H8	ST1521-100UG
Anti-phospho-ULK1/ATG1 (Ser758)	ABC112

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Kits & Assays

Product	Catalogue No.
Akt/mTOR/S6K Pathway Explorer Antibody MiniPack	15-104
FlowCellect™ Autophagy LC3 Antibody-based Assay Kit (100 tests)	FCCH100171
FlowCellect™ Autophagy Reagent Pack	FCCF200097
FlowCellect™ LC3-GFP Reporter Autophagy Assay Kit (CHO)	FCCH100170
FlowCellect™ LC3-GFP Reporter Autophagy Assay Kit (U2OS)	FCCH100181
Autophagy Detection Reagent Pack	CF200097
Autophagy Detection Reagent Pack	CF200097
LC3-II Enrichment Kit (Western Blot)	17-10232
LentiBrite™ GFP-LC3-II Enrichment Kit (Flow Cytometry)	17-10230
LentiBrite ™ GFP-LC3 Lentiviral Biosensor	17-10193
LentiBrite ™ GFP-LC3 Control Mutant Lentiviral Biosensor	17-10189
LentiBrite ™ RFP-LC3 Lentiviral Biosensor	17-10143
LentiBrite ™ RFP-LC3 Control Mutant Lentiviral Biosensor	17-10188
mTOR phosphorylation Pathway Explorer Antibody MiniPack	15-105

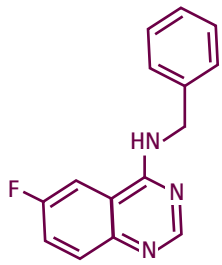
Small Molecules & Inhibitors

Product	Catalogue No.
Akt Inhibitor X	124020
AMPK Activator VI, RSVA314	171272
AMPK Inhibitor, Compound C	171260
Autophagy Inducer, STF-62247	189497
Autophagy Inhibitor, 3-MA	189490
Autophagy Regulators Panel	189488-1EA
Bafilomycin A1, Streptomyces griseus	196000
bpV(phen)	203695
Cathepsin L Inhibitor, CAA0225	219502
DAPK Inhibitor	324788
Dynamin Inhibitor I, Dynasore	324410
ICMT Inhibitor	420350
Leupeptin, Hemisulfate, Synthetic	108976
mTOR Inhibitor III, PP242	475988
Necrostatin-1	480065
Niclosamide	481909
Nigericin, Sodium Salt, *Streptomyces hygroscopicus*	481990
Nocodazole	487928
PI-103	528100
Pifithrin-μ	506155
PIKfyve Inhibitor	524611
Rapamycin	553210
SMER28	573121
Spautin-1	567569
Thapsigargin	586005
Wiskostatin	681525

FEATURED INHIBITORS

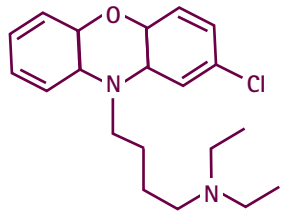
Spautin-1

This specific, potent autophagy inhibitor stops autophagosome formation by promoting degradation of the Vps34 complex by blocking the activity of USP 10 (IC_{50} = 580 nM) and USP 13 (IC_{50} = 690 nM) deubiquitinating enzymes.

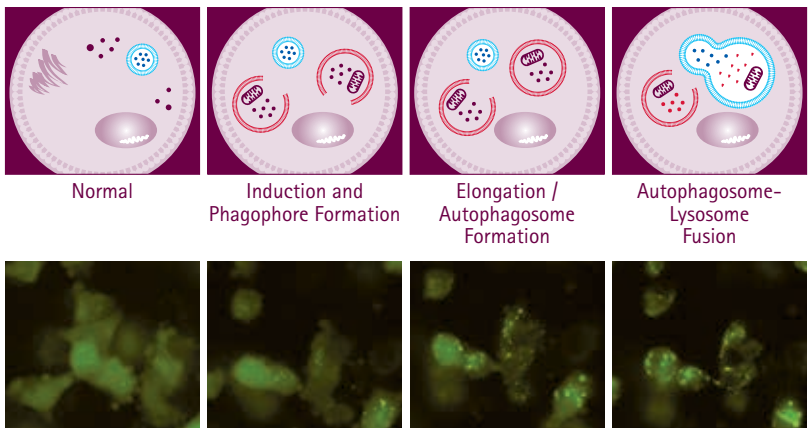


Akt Inhibitor X

Induce autophagy with this cell-permeable, reversible, and selective Akt inhibitor (complete inhibition <5 µM). Shown to induce neuronal autophagy, this compound enhances the clearance of misfolded protein.



FEATURED AUTOPHAGY ASSAYS AND ASSAY KITS



Timecourse imaging of HT-1080 cells after transduction with LentiBrite™ GFP-LC3 (Catalogue No. 17-10193). GFP-LC3 displays a diffuse nuclear and cytosolic distribution in normal cells, and a punctate distribution in starved, autophagic cells.

*To see the full video, scan with your mobile device:

NEW! LentiBrite™ Lentiviral Biosensors

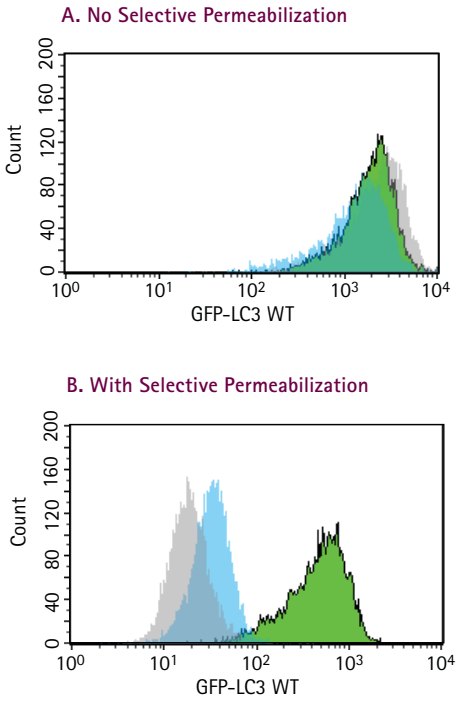
Visualize autophagy in real time, even in difficult-totransfect cell types, using LentiBrite™ GFP- and RFP-LC3 lentiviral particles, which reveal precise localization of LC3 during autophagosome formation.

LentiBrite™ Biosensor Advantages:

- **Reproducible & convenient** - pre-packaged, GFP- & RFP-tagged protein constructs
- **Robust** - Long-term, stable fluorescent expression
- **Efficient** - High transduction efficiency, nondisruptive to cell function, low immunogenicity
- **Easy interpretation** - LC3 Control Mutant lentiviral particle contains the translocation-defective protein LC3-G120A for comparison studies.

FlowCellect™ GFP-LC3 Reporter Cell Line Autophagy Assay Kits

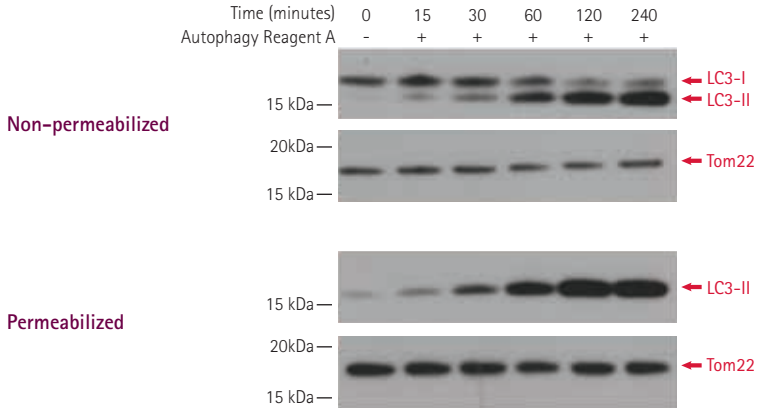
Merck Millipore's optimized FlowCellect™ kits take the guesswork out of assay development so you can focus on your research. Our newest kits provide a quantitative solution for studying autophagy and measuring the potency of autophagy inducers using flow cytometry. Like all FlowCellect™ assays, these are validated for easyCyte™ benchtop flow cytometers as well as other flow cytometry instruments.



Graphs (right): Flow cytometry detection of LC3 translocation to autophagosomes by addition of a lysosome inhibitor. The FlowCellect™ LC3-GFP Reporter Autophagy Kit was used (A), without selective permeabilization to show high levels of LC3-GFP before and after starvation (induction of autophagy). With selective permeabilization (B), LC3-GFP level remains high in autophagosomes when starved in the presence of lysosome inhibitor (green); even without the inhibitor, a slight shift is observed when starved (blue). All cytosolic LC3-GFP is washed away if no autophagy is induced by starvation (gray).

LC3-II Enrichment Kits (WB & Flow Cytometry)

Merck Millipore's LC3-II Enrichment Kit (Western Blot) & LentiBrite™ LC3-II Enrichment Kit (Flow Cytometry) enables sensitive and accurate quantification of autophagosome density by utilizing a selective permeabilization procedure that removes cytosolic LC3-I and retains autophagosome-bound LC3-II. This procedure reveals quantities of LC3-II, without interference from LC3-I, by Western blotting analysis and Flow Cytometry.



Western Blot Detection of LC3-II Before and After Enrichment. Western Blot of non-enriched lysate and LC3-II-enriched protein fraction from HeLa cells prepared with the LC3-II Enrichment Kit (Western Blot) (Catalogue No. 17-10232). The PVDF membrane was probed with primary antibodies against LC3 and TOMM22 (mitochondria organelle marker), followed by secondary antibodies. Immunoblotting results of non-enriched lysates indicate the LC3-I signal decreases over time after induced autophagy, as the LC3-II signal increases. After enrichment, the LC3-I signal is no longer detectable and the LC3-II signal is retained.

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