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MOLECULAR CELL BIOLOGY

Autophagy: molecular mechanisms and disease outcomes

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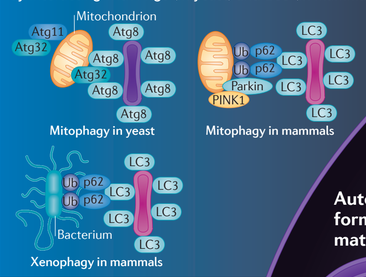
Autophagy is a cytoplasmic, homeostatic process by which cells degrade their interior components, including targets that are too large for other degradative systems, in response to external and internal triggers. Of the different types of autophagy, macroautophagy is the best characterized. The morphological hallmark of this process is the sequestration of a portion of the cytoplasm within double-membrane vesicles called autophagosomes. These fuse with the lysosome in mammalian cells (or the vacuole in yeast) to allow degradation of the cargo. Autophagy can be nonspecific in terms of its cytoplasmic targets in certain situations, for example in response to starvation. However, depending on the signal, highly specific autophagy can target superfluous or damaged organelles, protein aggregates or invasive microorganisms. When properly regulated, autophagy supports normal cellular and developmental processes, whereas autophagic dysfunction is associated with various human diseases. Our molecular understanding of autophagy has increased exponentially in recent years and is depicted here for both yeast and mammals. This knowledge holds the promise of allowing us to target this pathway for therapeutic purposes.

Cargo selection

Autophagy can selectively target superfluous or damaged organelles, specific proteins or invasive microorganisms. Some targets come with a built-in tag, as with prApe1 (propeptide) and mitochondria (Atg32 in yeast and NIX in mammals), or a ubiquitin modification is added to the cargo by a ubiquitin ligase such as parkin. Tags are recognized by receptors (for example, Atg19, p62, NBR1 and NDP52) and/or adaptors (for example, Atg11 in yeast and ALFY in mammals) linking the cargo with nascent autophagosomes by interacting with Atg8 (in yeast) and LC3 (in mammals).



Phagophore expansion

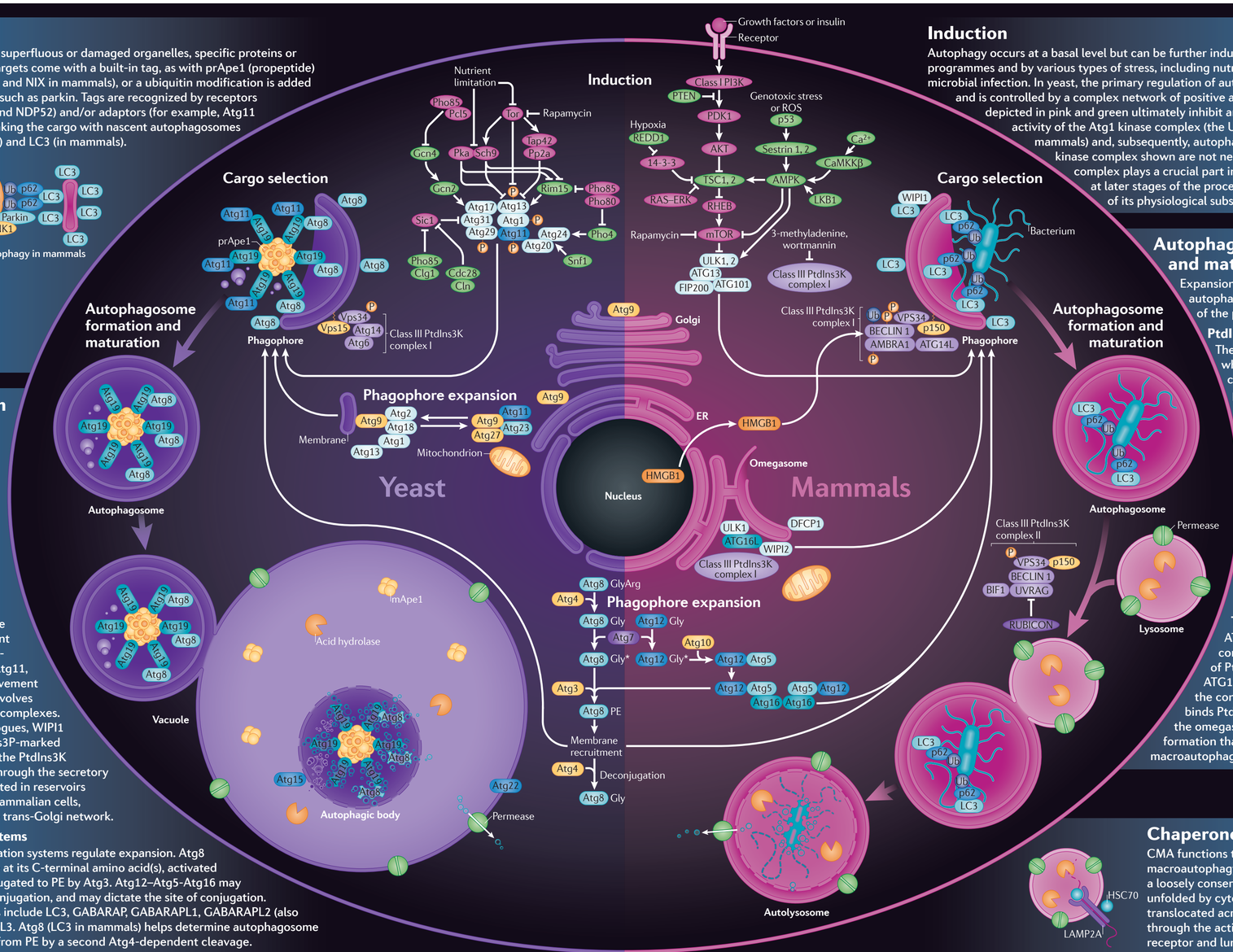
The initial sequestering compartment, the phagophore, expands to form the autophagosome, probably by incorporating membrane from the ER, Golgi complex, mitochondria and/or plasma membrane.

Atg9 transport

Atg9 is the primary transmembrane Atg protein required for autophagosome formation. It is detected in multiple discrete puncta, which probably mark the donor membranes for phagophore expansion. Anterograde movement of Atg9 to the site of vesicle formation, the PAS in yeast, requires Atg11, Atg23 and Atg27. Retrograde movement of Atg9 back to the donor sites involves the Atg1–Atg13 and Atg2–Atg18 complexes. Atg18 and its mammalian homologues, WIPI1 and WIPI2, are recruited to PtdIns3P-marked membranes (the phagophore) by the PtdIns3K complex. In yeast, Atg9 transits through the secretory pathway, and a population is located in reservoirs juxtaposed to mitochondria; in mammalian cells, mATG9 is initially localized to the trans-Golgi network.

Ubiquitin-like conjugation systems

Two ubiquitin-like protein conjugation systems regulate expansion. Atg8 is processed by the Atg4 protease at its C-terminal amino acid(s), activated by Atg7 (shown as Gly*) and conjugated to PE by Atg3. Atg12–Atg5-Atg16 may act as an E3 ligase for Atg8–PE conjugation, and may dictate the site of conjugation. Atg8 family members in mammals include LC3, GABARAP, GABARAPL1, GABARAPL2 (also known as GATE16) and GABARAPL3. Atg8 (LC3 in mammals) helps determine autophagosome size, and it can be deconjugated from PE by a second Atg4-dependent cleavage.



Induction

Autophagy occurs at a basal level but can be further induced during developmental programmes and by various types of stress, including nutrient limitation, hypoxia and microbial infection. In yeast, the primary regulation of autophagy is nutrient dependent and is controlled by a complex network of positive and negative pathways. Proteins depicted in pink and green ultimately inhibit and stimulate, respectively, the activity of the Atg1 kinase complex (the ULK1 and ULK2 complex in mammals) and, subsequently, autophagy. The interactions with the Atg1 kinase complex shown are not necessarily direct. The Atg1 kinase complex plays a crucial part in autophagy induction and also acts at later stages of the process; however, it is likely that not all of its physiological substrates have been identified.

Autophagosome formation and maturation

Expansion of the phagophore into an autophagosome requires the generation of the phospholipid PtdIns3P.

PtdIns3K complex

The Vps34 complex generates PtdIns3P, which may allow recruitment of certain Atg components to the phagophore. There are multiple Vps34 complexes, each of which contains the Vps34 lipid kinase, the Vps15 regulatory kinase (p150 in mammals) and Atg6 (BECLIN 1 in mammals). During phagophore formation, the Vps34 complex contains Atg14 (ATG14L in mammals), whereas during maturation into an autolysosome in mammals the complex includes UVRAG. Additional modulating proteins in mammalian cells include AMBRA1, BIF1, RUBICON and HMGB1.

The omegasome

ATG14L targets the PtdIns3K complex to the ER. The generation of PtdIns3P recruits WIPI2, along with ATG16L and ULK1, which in part direct the conjugation of LC3 to PE. DFCP1 also binds PtdIns3P and directs the formation of the omegasome, a scaffold for phagophore formation that is involved in some types of macroautophagy.

Chaperone-mediated autophagy

CMA functions temporally after the induction of macroautophagy. Individual proteins that contain a loosely conserved KFERQ consensus motif are unfolded by cytosolic HSC70 chaperones and translocated across the lysosome membrane, through the action of the LAMP2A membrane receptor and lumenal HSC70, for degradation.

Autophagy in health and disease

Disease or process	Role of autophagy*
Cancer[1,2]	✓ Functions in tumour suppression
	✗ Used by cancer cells for cytoprotection
Neurodegenerative diseases[3,4]	✓ Basal levels clear toxic protein aggregates in neurons; selectively removes damaged mitochondria by mitophagy
	✗ Amyloid precursor protein in autophagosomes can generate pathology-associated peptides
Myopathies; lysosomal storage diseases[5]	✓ Removes proteins and organelles to prevent the accumulation of protein aggregates or dysfunctional organelles and maintain cellular homeostasis
	✗ Accumulation of autophagosomes when maturation is impeded can compromise cellular physiology; excessive levels cause muscle wasting
Microbial infection[6,7]	✓ Helps eliminate invasive microorganisms and regulates innate immunity and the protective inflammatory response to microbial products
	✗ Some pathogens have adaptations that counter autophagy or use it to promote their own growth
Immune response; inflammatory bowel disease[8]	✓ Processes endogenous antigens for MHCII presentation; regulates naive T cell repertoires, T cell maturation and B cell and T cell homeostasis; counters damaging inflammation
	✗ May promote excess inflammatory cytokines when defective
Liver disease[9]	✓ Role in organelle homeostasis allows portions of the ER to be removed when protein misfolding overloads the UPR and ERAD
	✗ Excessive autophagic removal of the ER can cause liver damage
Heart, vascular and renal diseases[10–12]	✓ Its homeostatic properties are essential to cardiomyocytes and podocytes; protective during ischaemia and pressure overload; may protect against apoptosis in plaques; prevents glomerular disease
	✗ Can be harmful during reperfusion
Diabetes[13,14]	✓ Basal levels maintain normal islet structure and function; involved in the response of β-cells to a high-fat diet; may affect neutral lipid metabolism
	✗ Exposure to free fatty acids can lead to excessive autophagy and pancreatic β-cell death
Development[15–19]	✓ Removal of mitochondria by mitophagy in reticulocytes is key to erythrocyte differentiation
	✗ Unknown
Embryogenesis[20,21]	✓ Required for embryo implantation; allows neonates to survive after termination of the transplacental supply of nutrients; involved in the removal of dead cells during programmed cell death
	✗ Unknown
Ageing[22]	✓ Removes damaged organelles and oxidized or aggregated macromolecules to increase health and prolong life
	✗ Increased levels may lead to muscle and organ wasting in progeria

*Beneficial (✓) or harmful (✗) roles of autophagy in health and disease.
For the reference list see: http://www.nature.com/nrm/posters/autophagy

Abbreviations

ALFY, autophagy-linked FYVE (also known as WDFY3); AMBRA1, activating molecule in BECLIN 1-regulated autophagy 1; Atg, autophagy-related; ATG14L, yeast Atg14-like (also known as BARKOR); ATG16L, yeast Atg16-like; BECLIN 1, BCL-2 interacting myosin/moesin-like coiled-coil protein 1; BIF1, BAX-interacting factor 1; C-terminal, carboxy-terminal; CMA, chaperone-mediated autophagy; DFCP1, double FYVE domain-containing protein 1 (also known as ZFYVE1); ER, endoplasmic reticulum; ERAD, ER-associated degradation; GABARAP, γ-aminobutyric acid receptor-associated protein; GABARAPL, GABARAP-like; HMGB1, high-mobility group B1; HSC70, heat shock cognate 70; LAMP2A, lysosome-associated membrane glycoprotein 2; LC3, microtubule-associated protein 1 light chain 3 (also known as MAP1LC3); mApe1, mature aminopeptidase I; mATG, mammalian autophagy-related; MHCII, major histocompatibility complex II; NBR1, next to BRCA1 gene 1; NDP52, nuclear dot protein 52 kDa (also known as CALCOCO2); NIX, NIP3-like X (also known as BNIP3L); PAS, phagophore assembly site; PE, phosphatidylethanolamine; prApe1, precursor aminopeptidase I; PtdIns, phosphatidylinositol; PtdIns3K, PtdIns 3-kinase; PtdIns3P, PtdIns 3-phosphate; ROS, reactive oxygen species; RUBICON, RUN domain BECLIN 1-interacting and cysteine-rich containing; Ub, ubiquitin; ULK, UNC-51-like kinase; UPR, unfolded protein response; UVRAG, UV radiation resistance-associated gene; Vps, vacuolar protein sorting; WIPI, WD repeat domain phosphoinositide-interacting.

Further Reading

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http://www.nature.com/nrm/posters/autophagy

SUPPLEMENTARY INFORMATION

Supplementary information to poster | **Autophagy: molecular mechanisms and disease outcomes.**
Daniel J. Klionsky and Vojo Deretic
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