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# Autophagy A Cellular Mechanism: A Review

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## ABSTRACT

Autophagy is a basically survival mechanism of body in which cell digests its own content to maintain cellular homeostasis in multiple stress conditions and starvation. The term 'autophagy' was first described in 1963 by Christian de Duve, a Japanese cell biologist to describe presence of double-membrane vesicles containing cytoplasmic constituents within the cell. These vesicles that encapsulate cytoplasm, organelles and proteins, are known as autophagosomes. After formation of autophagosome it fuses with endosomes and travels *via* cytoplasm to fuse with lysosomes for degradation. In lysosomes internal content material of autophagosome is degraded with the action of acid hydrolases. Autophagy is very important for regulation of diverse cellular functions *i.e*., growth, differentiation, response to nutrient deficit and oxidative stress, cell death and macromolecule and organelle turnover. Nutrient starvation is most typical trigger of autophagy. Depending on the mechanism by which intracellular materials are delivered into lysosome for degradation, there are four types- Macroautophagy, Microautophagy, Chaperon mediated autophagy and Crinophagy. Autophagy is important in normal development and it responds to changing environmental stimuli. It plays role in cancer and numerous important diseases such as bacterial and viral infections, liver and kidney diseases, Diabetes mellitus, inflammatory bowel diseases, neurodegenerative disorders, several myopathies and cardiovascular diseases.


**Key words:** Acid hydrolases, Autophagy, Cellular-molecular mechanism, Stress/starvation.

Autophagy is a major intracellular pathway for the degradation and recycling of long-lived proteins and cytoplasmic organelles. In yeast, nitrogen starvation is most potent stimulus, but withdrawal of other essential factors such as carbon, auxotrophic amino acids and nucleic acids and even sulfate can induce autophagy but less efficiently (Takeshige *et al*., 1992). In plant cells, nitrogen or carbon starvation triggers the autophagy (Moriyasu and Ohsumi, 1996; Yoshimoto *et al*., 2004; Kelekar, 2006). Most typical trigger for autophagy is nutrient starvation (Mizushima, 2007).

It is a self-digesting mechanism responsible for removal of damaged organelles, malformed proteins during biosynthesis and nonfunctional long-lived proteins by lysosome. It is for regulating diverse cellular functions *i.e*., growth, differentiation, response to nutrient deficit and oxidative stress, cell death and macromolecule and organelle turnover (Badadani, 2012).

## History of autophagy

Autophagy becomes first discovered by Keith R.Porter and his student Thomas Ashford at Rockefeller Institute. The term was emerged during 1960s and was given by Christian de Duve in 1963 to describe the presence of double-membraned vesicles containing cytoplasmic materials within the cellular. Christian de Duve with Russell Deter, established lysosomes are responsible for glucagon-induced autophagy.Yoshinori Ohsumi and Michael Thumm examined starvation-induced non-selective autophagy. Daniel J. Klionsky discovered the cytoplasm-to-vacuole targeting (CVT) pathway, which is a form of selective autophagy. In 1999, a landmark discovery connecting autophagy with cancer by Beth Levine's group. The 2016 Nobel Prize in Physiology or Medicine was awarded to Yoshinori Ohsumi for his discovery of the mechanisms underlying autophagy (Table 1).

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## Key discoveries in the autophagy field

There are several types of such self-degradative and recycling pathways identified, out of this, autophagy is most basic and important process occurring at cellular level. It occurs in following conditions (Fig 1).

## Process of macroautophagy in mammalian cells

A phagophore or isolation membrane, which is primarily formed from the endoplasmic reticulum, plasma membrane, or mitochondria to form an autophagosome, encloses a section of the cytoplasm, including organelles. Outer membrane of autophagosome subsequently fuses with endosome and then to lysosome and the internal material is degraded. In yeast, autophagosomes generated from pre-autophagosomal structure (PAS), which has not yet been identified in mammalian cells. The control of autophagy in mammals seems to be very intricate. In many different types

of cultivated cells, a reduction in total amino acids dramatically induces autophagy, but the effects of particular amino acids vary. Leucine, Tyrosine, Phenylalanine, Glycine, Proline, Histidine, Tryptophan, Methionine and Alanine suppress autophagy in *ex vivo* perfused liver. Only leucine has a predominate impact on the heart and skeletal muscle. Liver autophagy is suppressed by insulin and enhanced by glucagon (Mortimore *et al*., 1987).

## Process of autophagy

Autophagy is a multi-step procedure of sequential activities such as induction, nucleation of a phagophore structure,

**Table 1:** Timeline selection of important discoveries in autophagy.

| Year | Invention |
|---|---|
| 1963 | The term 'autophagy' coined by de Duve |
| 1977 | Inhibition of autophagy by amino acids |
| 1978 | Inhibition of autophagy byinsulin |
| 1982 | Autophagy inhibitor 3-methyladeine |
| 1992 | Autophagy in yeast, The CVT pathway |
| 1993 | Isolation of apg mutants |
| 1994 | Isolation of aut mutant |
| 1995 | Isolation of cvt nutants, Induction of autophagy by rapamycin |
| 1998 | The Atg 12 conjugation system |
| 1999 | Bectin 1, role of autophagy in cancer |
| 2000 | The Atg12 conjugation system The autophagosomal marker |
| 2001 | The Atg 14-PI3K complex |
| 2002 | Neuroprotective role of autophagy |
| 2003 | Antiaging effect of autophagy |
| 2004 | Xenophagy, Role of autophagy in neonates |
| 2005 | Slective degradation of p62/SQSTM1 |
| 2006 | Role of autophagy in neurons |
| 2007 | ATG16L 1 mutation in Crohn's disease |
| 2008 | Parkin in mitophagy induction |
| 2011 | TFEB in ATG gene regulation |
| 2012 | WDR45 mutation in SENDA/BPAN disease |
| 2013 | EPG5 mutations in Vici syndrome |
| 2014 | LIR cooperativity, ferritinophagy SAR - NcoA4; 1st ubiquitin binding SAR in yeast - Cue5 |
| 2015 | Ephagy SARs - Atg39, Atg40, abd FAM134B, mitophagy SARs - NDP52 and OPTN |
| 2016 | LIR/UFIM in UBA5; LIR;ATG5 interaction; pexophagy E3 ligase (PEX2) lipophagy SARs - ATGL/HSL |
| 2017 | LIR-based LC3/GABARAP sensors; ARG7-independent degradation of SL Rs; IMM SAR in mitophagy - PHB2 |
| 2018 | FIP2000 binding ER-phagy SAR - CCPG1; ribophagy SAR - NUFIPa; LIR peptides with nanomolar binding affinity to LC3/GABARAPs |

Source: (Mizushima, 2018 and Vladimir, 2020).

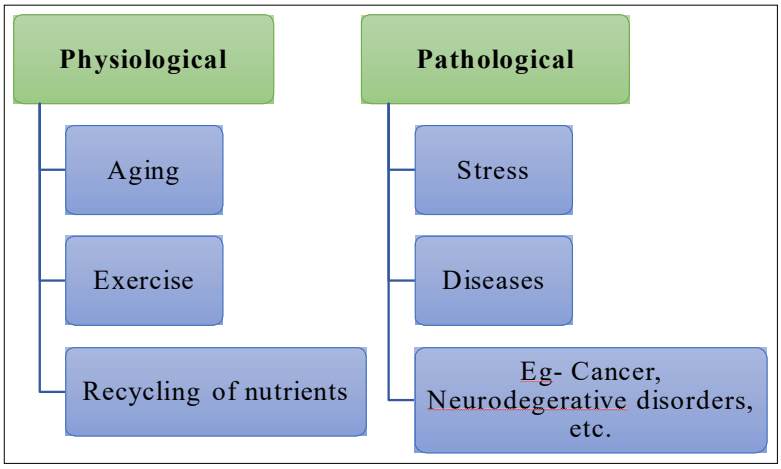


**Fig 1:** Depicts various etiological agents for the autophagy.

maturation of the autophagosome, autophagosome fusion with the lysosome and the degradation and recycling of nutrients. The term "autophagy" usually indicates macroautophagy unless otherwise specified (Fig 2).

## Ultrastructural mechanism of autophagy

It involes the following steps:

### Phagophore formation

Autophagy starts with formation of double membrane structure are called as phagophore. It is assumed that it should be originates from cellular components such as golgi apparatus, smooth and rough rough endoplasmic reticulum.

### Autophagosome formation

The phagophore ultimately swallow the target objectives *i.e*., protein aggregates or injured cellular organelles to develop vesicle sealed doulble membrane call it as autophagosome. It should be came out from cytoplasm and helps to protect from breakdown until fuse with lysosome.

### Maturation and cargo recognition

For the maturation of Autophagosomes they need source of protein and lipids for their maintaince. For the maturation they need autophagy related protein and cargo recognition receptors *i.e*., p62/SQSTM1 for the conjugation with cargo and fuse with autophagosome membrane results in breakdown.

### Fusion with lysomes

Lysosome fuses with the autophagosomes leads to autolysosome. Due to the action of lysosomes they helps to breakdown the autolysosome contain cargo.

### Cargo degradation

After the release of lysosome enzyme leads to rupture of nucleic acid, protein, lipids and cellular components. These ruptured products accumulates in the cytoplasm of the cell where they were utilised for the source of energy for the further process.

### Autophagosome recycling

In this process we can observe separation of membrane between internal and external membrane of autolysosome. This helps lysosomal enzymes to fuse with the inner membrane of autolysosomes for the further breakdown whereas outer membrane gets reuse for autophagososme formation.

### Release of degradation products

End product results after the rupture of autophagy *i.e*., fatty acid and amino acids leaks into cytoplasm and were used for the source of energy for the various cellular components.

The ultra structural physiology involves arrangement and synchronization of protein complex for the sequestration and degradation of cellular cargo. This is important step for the maintaince of the healthy cell and to protect cell from the unavoidable stress and vital nutrients. Due to the affection of autophagy results in various changes in the body such as cancer and neurodegenerative conditions (Dikic and Elazar, 2018).

## Mechanism of autophagy

Molecular circuitry and signaling pathways regulateprocess of autophagy. It is a complex self-degradative process that involves the following key steps: (a) Control of phagophore formation by Beclin-1/VPS34 at the ER and other membranes in response to stress; (b) Atg5–Atg12 conjugation, interaction with Atg16L and multimerization at the phagophore; (c) LC3 processing and insertion into the extending phagophore membrane; (d) Capture of random or selective targets for degradation, completion of the autophagosome accompanied by recycling of some LC3-II/ ATG8 by ATG4, followed by; (e) Fusion of the autophagosome with the lysosome and proteolytic degradation by lysosomal proteases of engulfed molecules (Tadashi *et al*., 2020).

Autophagy is negatively regulated by the nutrient-sensing mammalian target of rapamycin (mTOR) kinase, a master regulator of cellular growth and metabolism. Under nutrient-rich conditions, mTOR inhibits autophagy by preventing the formation of the autophagy initiation ULK1 Ser/Thr kinase protein complex. mTOR represses autophagy by direct phosphorylation of ULK1 at serine residue. Phosphorylation of ULK1 by mTOR at this site prevents ULK1 from interacting with AMPK. AMPK induces autophagy

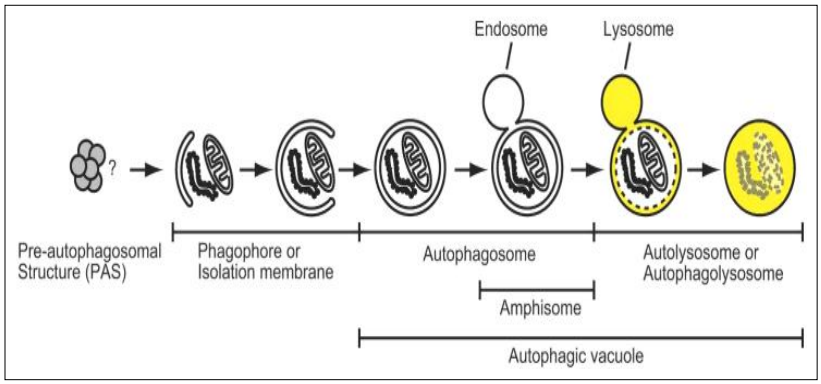


**Fig 2:** Process of autophagy (Mizushima, 2007).

by alleviating the negative regulation by mTOR through the phosphorylation and activation of TSC2 and *via* direct phosphorylation of Raptor. AMPK induces the formation of the ULK1 complex through direct phosphorylation of ULK1 at Ser 317 and Ser 777, which result in the activation of ULK1 kinase activity (Tadashi *et al*., 2020).

Following activation, ULK1 phosphorylates several downstream targets to initiate the autophagic process. Formation of the ULK1 multi-protein complex requires ULK1-mediated phosphorylation of ATG13 and the scaffold protein FIP200. Resulting in the assembly of the ULK1-ATG13-FIP200-ATG101 complex. ULK1 complex translocates to sites of autophagosome initiation, where it is responsible for the activation of a second essential autophagy effector protein complex, the phosphatidylinositol 3-kinase (PI3K) complex. VPS34, a class III PI3K, is responsible for producing the phosphatidylinositol 3-phosphate- required for autophagosome formation. Enrichment in PI (3) P at specialized sites form the initiating autophagosome structure, known as the Omegasome. Omegasome serves as a membrane platform, that remains in contact with the endoplasmic reticulum and with vesicles containing the transmembrane autophagy protein ATG9, to recruit the necessary subsequent core autophagy machinery that drive elongation of the autophagosome membrane (Tadashi *et al*., 2020).

Haplo insufficient tumor suppressor and critical autophagy effector protein Beclin 1- key member of the PI3K complex- can regulate autophagy through its ability to interact with members of the anti-apoptotic BCL-2 family. BCL-2 directly interacts with Beclin 1 to negatively regulate autophagy, a process that is mediated by the BCL-2 adapter protein CISD2/NAF-1 at the endoplasmic reticulum. Elongation of the autophagosome membrane is dependent on two ubiquitin-like conjugation steps. First involves the conjugation ATG12 to ATG5-is catalyzed by ATG7 and ATG10. ATG12-ATG5 conjugate forms a multi-protein complex with ATG16L1 and functions as an E3-like ligase.Second conjugation step is mediated by ATG7 and ATG3 which together with the ATG5-ATG12:ATG16L1 complex responsible for conjugating phosphatidyl-ethanolamine to microtubule-associated protein 1 light chain 3 beta- MAP1LC3B (LC3B), which has been proteolytically cleaved by ATG4 (LC3-I). Lipidated LC3B (referred to as LC3-II) is incorporated into the autophagosome membrane during elongation and is commonly used as an experimental marker to detect and quantify autophagosomes within. Mature autophagosomes carrying cytoplasmic cargo are trafficked to lysosomes and subsequent autophagosome-lysosome fusion is mediated by the Rab family of small GTPases, SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) proteins and membrane tethering proteins (reviewed by Nakamura and Yoshimori, 2017). The contents of the autophagolysosome are degraded by lysosomal acidic hydrolases and are exported back to the cytoplasm to be reused for metabolic processes.

Conversely, autophagy is inhibited by increased growth factor signaling through the insulin receptor and its adaptor, IRS1, as well as other growth factor receptors that activate the Class I group of PI3-kinases and Akt, to promote mTOR activity through inhibition of TSC1/TSC2 and increased Rheb GTPase activity (Tadashi *et al*., 2020).

## Types of autophagy

Depending on the mechanism by which intracellular materials are delivered into lysosome for degradation.

- Macroautophagy.
- Microautophagy.
- Chaperon mediated autophagy.
- Crinophagy.

## Macroautophagy

It is a major catabolic pathway for energy generation and for breakdown of macromolecules and damaged organelles into their essential constituents during periods of stress or nutrient deprivation. In this pathway phagophore engulfs the material and forms autophagosome around the organelle. Autophagosome then travels through cytoplasm to a lysosome in mammals, or vacuoles in yeast and plants, then two organelles fuse. Within lysosome/vacuole, contents of autophagosome degraded *via* acidic lysosomal hydrolase (Fig 3).

Macroautophagy is again divided into bulk and selective macroautophagy. Bulk type is non-selective toward its substrates, whereas Selective type is autophagy of selective organelles (Table 2).

Subclassification of macroautophagy into “induced autophagy” and “basal autophagy” can also be done, where induced autophagy is used to produce amino acids following starvation, while basal autophagy is important for constitutive turnover of cytosolic components (Mizushima, 2018).

## Microautophagy

In this type there is direct engulfment of cytoplasmic material into lysosome which occurs by invagination or cellular protrusion. It is mediated by lysosomal action in mammals and by vacuolar action in plants and fungi. Cytoplasmic

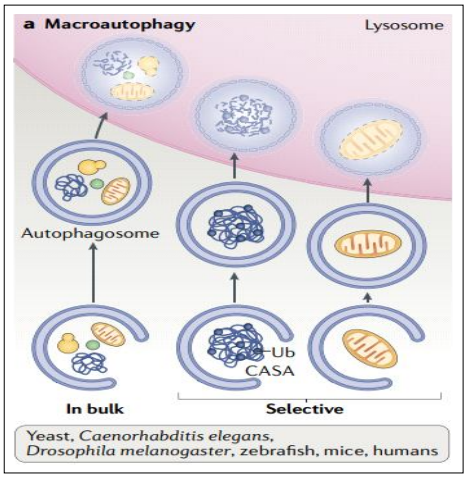


**Fig 3:** Mechanism of macroautopahgy.

material is trapped in lysosome/vacuole by a random processof membrane invagination (Fig 4).

Like macroautophagy it is important for survival of cells understarvation, nitrogen deprivation, or after treatment with rapamycin.

**Table 2:** List of selective autophagy types and target cargo.

| Name | Target cargo |
|---|---|
| Allophagy | Paternal organelles |
| Mitophagy | Mitochondria |
| Lipophagy | Lipid droplets |
| Chlorophagy | Chloroplasts |
| Peroxyphagy | Perioxisomes |
| Ribophagy | Ribosomes |
| ER-phagy | Endoplasmic reticulum |
| Lysophagy | Lysosome |
| Nucleophagy | Nucleus |
| Xenophagy | Cellular pathogens |
| Aggrephagy | Abnormal protein aggregates |

Source: (Tadashi *et al*., 2020).

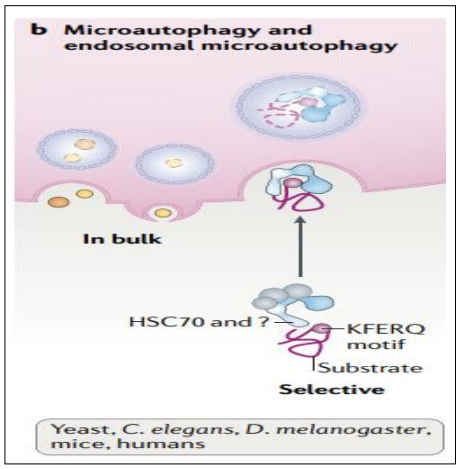


**Fig 4:** Mechanism of microautopahgy and endosomal microautophagy.

There are three special cases of a selective microautophagic pathway: Micropexophagy, Piecemeal microautophagy of the nucleus and Micromitophagy activated only under a specific condition.

Micro-and macroautophagy are both highly conserved from yeast to mammals.

## Chaperone-mediated autophagy (CMA)

This pathway involves chaperone-dependent selection of soluble cytosolic proteins then targetedto lysosomes and directly translocated across lysosome membrane for degradation. Unique features of this type are selection of proteins degraded and direct shuttling across lysosomal membrane without requirement for formation of additional vesicles.

Proteins, are the only cargo degraded by this pathway. But not all proteins can undergo degradation *via* CMA. To be CMA substrates, proteins must contain a specific targeting motif in their amino acid sequence. This motif binds to a cytosolic chaperone and involves formation of a complex between a cytosolic "substrate" protein and "chaperone" protein whichthen interacts with lysosomal membrane receptor for translocation into a lysosome. CMA is largely confined to mammalian systems (Kaushik and Cuervo, 2018) (Fig 5).

## Crinophagy

It is least known form of autophagic process. The word crinophagy derived from Greek,meaning- krino (secretion) and fagein (eating). The term was coined by Christian de Duve and described it as a process of selective degradation of secretory granules. It occurs in all cells that produce secreted material and is particularly important in glandular cells (exocrine, endocrine and neuroendocrine) specialized in secretion. This pathway involves fusion of unreleased secretory vesicles directly with late endosomes or lysosomes. After that there is rapid digestion and reuse of degraded contents. Fusion of secretory vesicles and lysosomes results in formation of Crinosome which is a digestive secondary lysosome (Tamas and Juhasz, 2020).

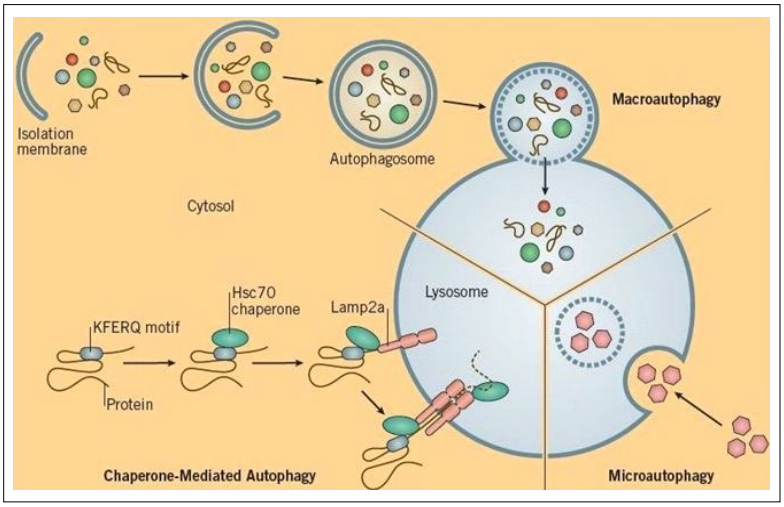


**Fig 5:** Mechanism of chaperone-mediated autophagy.

**Table 3:** Role of autophagy.

| Cancer | Dual role-Defense as well as promote |
|---|---|
| Infection | Degradation-Mycobacteria, Shigella, *etc* |
| Neurodegenerative diseases | Dysregulation of autophagy and accelerates neurodegeneration |
| Inflammatory bowel disease | Ulcerative colitis and Crohn's disease (Ileitis) |

(Saha *et al*., 2018).

## Role of autophagy

Autophagy is important in normal development and responds to changing environmental stimuli. Defects in autophagy have been attributed to cancer, neurodegeneration, muscle and heart disease, infectious disease, as well as aging. There have been several recent reviews about autophagy as it relates to cancer and other diseases. In addition to its role in cancer, it is important in numerous diseases, including bacterial and viral infections, Diabetes mellitus, Inflammatory bowel diseases, neurodegenerative disorders, several myopathies and cardiovascular diseases (Table 3).

## Autophagy in stem cell maintenance

Adult stem cells, which can be long-lived cells that own the specific capability to self-renew and differentiate into specialized cells at some stage in the body, have awesome metabolic necessities. Research in a diffusion of stem cell types have set up that autophagy plays essential roles in stem cellular quiescence, activation, differentiation and self-renewal.

## CONCLUSION

Autophagy is a process of engulfing one's own cytoplasmic proteins or organelles and coating them into vesicles, fusing with lysosomes to form autophagic lysosomes and degrading the contents of the lysosomes, thereby fulfilling the metabolic needs of the cells and the renewal of certain organelles in case of various stressful conditions. According to the different ways of transporting cellular material to the lysosome, autophagy is divided into macroautophagy, microautophagy, chaperone-mediated autophagy and recently recognized form *i.e*., crinophagy. Autophagy is seen in the body's physiological and pathological processes and has a complex regulatory mechanism. Its role is positive as well as negative depending upon the environment.

### Conflict of interest

All authors declared that there is no conflict of interest.

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