

# Review on Autophagy: Mechanism of Cell House Keeping

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**Abstract:** Autophagy is a normal process occurring in our body to maintain cellular homeostasis but it is up-regulated during stress condition inside the cell. It is a highly conserved and regulated process which involves lysosomal mediated degradation of damaged and faulty components and recycle or reuses when needed. Despite its role in degradation it has its connection with various diseases like cancer, neurodegeneration, muscle disease, liver disease, cardiac disease and many more. The process of autophagy consist of various steps and each step is regulated by various regulatory proteins which is found to be deleted or mutated when present in disease. This review has focus on the process of autophagy and its regulation along with its connection with various diseases. Few examples might be paving a way for innovative remedy for treating many diseases and disorders.

**Keywords:** Autophagy, cancer, neurodegeneration, muscle disease, liver disease, cardiac disease.

## INTRODUCTION

Our metabolism is a continuous recycling process, where both catabolism and anabolism plays an important role in maintaining proper balance. In order to maintain proper health condition, cellular components that are vestigial should be eliminated from body and for this purpose body has few primary degradative pathways. One pathway is proteasome mediated which is responsible for the breakdown of short lived proteins, apoptosis and autophagy governs the degradation of majority of long-lived proteins, protein aggregates and whole organelles under nutrient limitation and cellular stress condition<sup>1</sup>. During autophagy the components of the cytoplasm are broken down into basic components and returned to the cytosol for reuse<sup>2</sup>. In a general sense autophagy can be said that it plays a role as housekeeping allowing cells to survive in response to multiple stress conditions<sup>3</sup>. In this review the overview of autophagy is discussed in detail.

## Definition:

The term autophagy was coined by Christian de Duve in 1963<sup>4</sup>. The word autophagy was derived for Greek word auto phagin which means self-eating<sup>5</sup>. It is a tightly regulated energy driven process occurring in living organism in which the cell undergoes a cellular suicide in a much programmed manner. Autophagy can be defined as complex process which allows degradation of superfluous (surplus) or damaged cellular components or whole organelles using hydrolytic enzymes in the lysosomes<sup>6</sup>. It can be defined as a regulated process of degradation and recycling of cellular constituents participating in organelle turnover, resulting in the bioenergetics management of starvation<sup>7</sup>. In general, basal autophagy is always in active state maintaining homeostasis of cells but in adverse conditions shown in fig1 autophagy is elevated to withstand those conditions. It is regulated by various proteins which are the products of autophagy related genes (ATG).

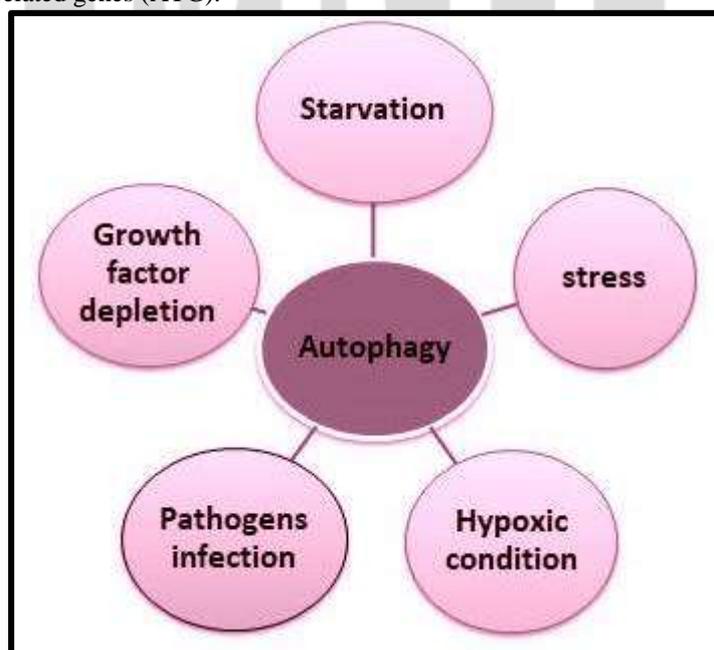


Fig 1 Factors inducing autophagy

### Importance of Autophagy:

Autophagy research is booming up because it plays a significant role in health and disease including cancer, other pathologies, inflammation, immunity, infection, neurodegeneration and aging<sup>8</sup>. Autophagy directly or indirectly involved in maintaining the quality of proteins and organelles<sup>9</sup>. It is associated with either cell survival or cell death<sup>10</sup>. Stress-responsive autophagy can enable adaptation and promote cell survival, whereas in certain models, autophagy has also been associated with cell death, representing either a failed attempt at survival or a mechanism that supports cell and tissue degradation<sup>11</sup>. Autophagy prevents the accumulation of random molecular damage in long-lived structures, particularly mitochondria, and more generally provides a means to reallocate cellular resources from one biochemical pathway to another<sup>12</sup>. Consequently, it is up-regulated in conditions where a cell is responding to stress signals, such as starvation, oxidative stress, and exercise-induced adaptation<sup>13</sup>. The balance between protein and lipid biosynthesis, and their eventual degradation and re-synthesis, is one critical component of cellular health<sup>14</sup>.

Degradation and recycling of macromolecules via autophagy provides a source of building blocks (amino acids, fatty acids, sugars) that allow temporal adaptation of cells to adverse conditions<sup>15</sup>. In addition to recycling, autophagy is required for the degradation of damaged or toxic material that can be generated as a result of ROS accumulation during oxidative stress<sup>16</sup>. Autophagy plays a direct or indirect role in health and disease, including control of embryonic and early postnatal development; tissue homeostasis (protein and cell organelle turnover); mitochondrial quality control; protection of cells from stresses; survival response to nutrient deprivation; cellular survival or physiological cell death during development; involvement in cell death upon treatment with chemotherapy and radiotherapy; tissue remodeling during differentiation and development, including regulation of number of cells and cell size, endocytosed gap junctions, villous trophoblasts, cellular house-cleaning, protein, glucose, and lipid metabolism; supply of energy; anti-aging; human malignancy, tumorigenesis, tumor maintenance, inflammation, cancer (pro and anti), ovarian cancer, nasopharyngeal carcinoma, melanoma, colon cancer and neutrophil differentiation of acute promyelocytic leukemia; lysosomal storage diseases; metabolic disorders; osteoarthritis; cardiovascular diseases; alcoholic cardiomyopathy, and steatosis in alcoholics (fatty degeneration of the heart); neurodegenerative diseases (Alzheimer's, Parkinson's, Huntington's, amyotrophic lateral sclerosis, and prion disease); muscular dystrophy; skeletal myopathy; atherosclerosis; diabetes; obesity; lipid degradation in the liver; alcoholic liver disease; pancreatitis; cellular quality control; protection of the genome; innate and adaptive immune responses to infection by microbial pathogens; defense against intracellular bacterial, parasitic, and viral infections; protection of intracellular pathogens; epileptogenesis; pompe disease; nephropathy; reduction of liver damage during ischemia-reperfusion; regression of the corpus luteum; protection of stem cells from apoptosis during stress. Neonates also adapt to transitive starvation by inducing autophagy.

### Classes of Autophagy:

Depending on the process of autophagy there are three classes.

#### 1. Microautophagy

#### 2. Macroautophagy

#### 3. Chaperone mediated autophagy:

##### 1. Microautophagy:

This is a non-selective autophagy where there is involvement of lysosomal enzymes to degrade the cytosolic component without forming autophagosome. The process involves the engulfment of components followed by degradation. This process of autophagy is further classified based on the cell organelle involved. For example micropexophagy in which peroxisomes are involved, mitophagy involves mitochondrion and piecemeal microautophagy involves nucleus which results in pinching-off or release of nonessential portions of nucleus.

##### 2. Chaperone mediated autophagy:

This type of autophagy is commonly seen in higher eukaryotes, where there is involvement of chaperone like heat shock proteins (hsc70), that forms a complex with the cell components that has to be degraded<sup>62</sup>. This method is temperature and energy dependent. The chaperone recognizes the consensus sequences similar to KFERQ motif present in the substrate<sup>63</sup>. Further the degradation of substrate takes place by involving lysosomal enzymes. In order to bind to the lysosome there is a recognition receptor called 2A (LAMP-2A) present on lysosome that complexes with chaperone and substrate protein<sup>64</sup>. Once the complex is formed the lysosomal enzymes show activity on substrate protein leading to its degradation.

##### 3. Macroautophagy:

In this type of autophagy, once any protein that has to be degraded is detected the host cell initiated the formation of phagophores. These phagophores mask the target by elongating its membrane around the target. The phagophores along with the target is known as autophagosome which is a double membrane vesicle<sup>67</sup>. Further autophagosome changes to amphisome by fusing with endosome, which later on fuses with lysosome to provoke the degradation process of target involving lysosomal enzymes ( fig:2). This autophagy may be selective for specific organelles or proteins involving receptors or non-selective by directly encapsulating bulk cytoplasmic contents, selective mechanism involves receptors.

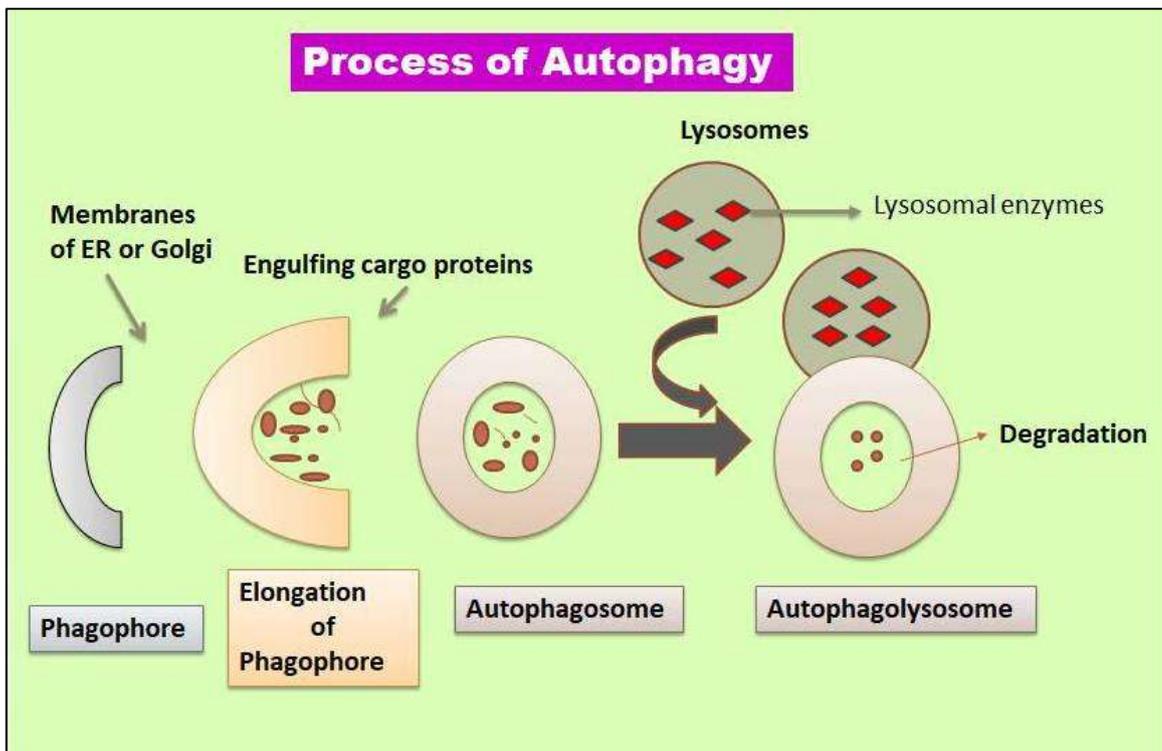


Fig.2 Process of Autophagy

**Mechanism of autophagy:**

**There are primarily 3 steps involved in autophagy**

- 1) Vesicle nucleation
- 2) Vesicle elongation
- 3) Vesicle completion.

**Vesicle nucleation**

Initially phagophore formation was initiated by recruiting Atg proteins required for the formation of phagophore assembly structure (PAS)<sup>72</sup>. Based on the literature, in order to initiate the process of phagophore formation there is an involvement of phosphatidylinositol 3-kinase (PtdIns3K) complex activation that helps in phagophore formation<sup>73</sup>. The formation of phagophore is influenced by some adverse conditions like nutrient starvation. Any lack of essential nutrient can trigger autophagy. There was a hypothesis stating that phagophore membrane is thought to be originated from membrane of various organelle like mitochondria, endoplasmic reticulum and Golgi apparatus<sup>74</sup>. **MTOR** (mammalian target of rapamycin) is a key regulator of autophagy<sup>75</sup>. It is a serine threonine kinase conserved in eukaryotes<sup>75</sup>. It forms two complexes in eukaryotes mTORC1 and mTORC2. Of these mTORC1 plays a key role in regulation of autophagy. This kinase belongs to phosphatidylinositol kinase related kinase family. The mTORC1 complex contains other proteins like Raptor, GβL/mLst8, PRAS40, DEPTOR while mTORC2 contains proteins like Sin1, rictor, PRR5/protor, DEPTOR, GβL<sup>76-94</sup>. mTORC1 plays a central role in sensing nutrient availability, mitogenic signal and energy status<sup>77</sup>. Thus it controls cell growth, protein translation and cell proliferation<sup>78</sup>. It is inversely correlated with induction in autophagy. Under high nutrient condition, mTOR phosphorylates Atg13 and inhibits its interaction with that of ULK1 and hence complex is not formed<sup>79</sup>. Under starvation condition mTOR is inactivated thus it fails to phosphorylate Atg13 instead ULK1 phosphorylates it and complex is formed, ultimately autophagy is induced in it<sup>79</sup>. Similarly, there are other mechanism where autophagy is induced in mTOR independent manner

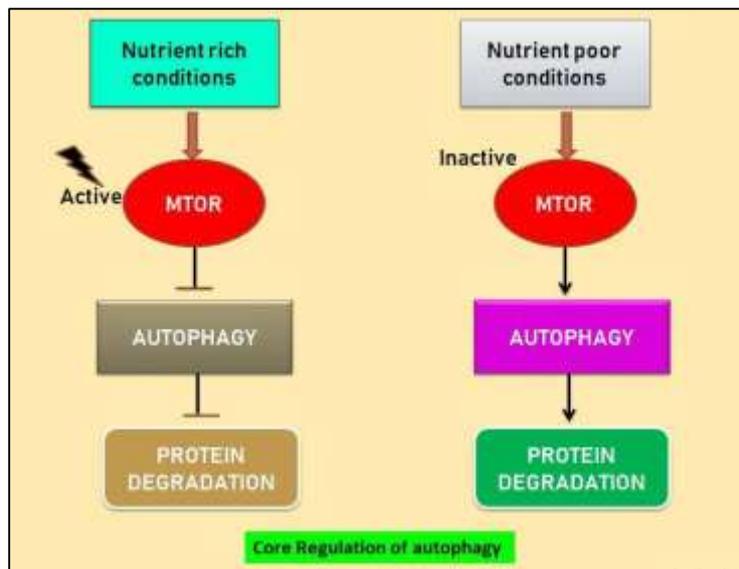


Fig.3 (a) Core Regulation of autophagy

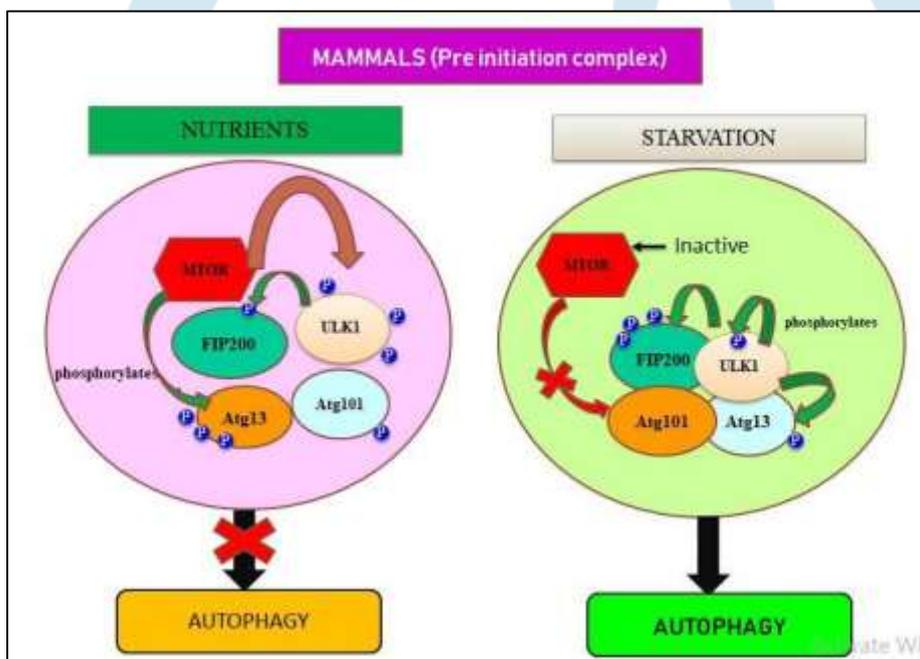


Fig.3 (b) Core regulation of autophagy

**2. Elongation:**

The phagophore membrane starts to elongate to form a double membrane structure called autophagosome. Two ubiquitin like conjugation system is involved which regulates this process

1) In first step, the Atg12 ubiquitin like protein is activated in an ATP-dependent reaction with Atg7, E1 like enzyme. Atg12 is then conjugated to Atg5 mediated by Atg10, an E2-like enzyme. Atg16L then forms a complex with Atg12-Atg5 conjugate. This complex is of 800kDa which is essential for autophagosome formation<sup>80</sup>. This components gets dissociates and return to cytoplasm when elongation is complete.

2) In second ubiquitin-like conjugation the modification of MAP1LC3 (microtubule associated protein 1 Light chain 3) occurs. Atg4B, one of the four mammalian Atg4 homologs cleaves the C-terminal 22 residues of precursor LC3 producing LC31. Cytoplasmic LC31 is then conjugated with phosphotidylethanolamine (PE) by Atg7 and Atg3, an E2 like enzyme<sup>80</sup>. LC3-II is selectively arranged in forming autophagosomal membrane which makes it a perfect autophagy marker. Thus, the double membrane autophagosome forms and now it proceeds towards maturation and fusion.

**3. Maturation and fusion of autophagosome:**

Once autophagosome is formed it travels towards endocytic system to fuse and deliver its Cargo proteins for degradation. Loss of the presence of the protein complexes which are formed on the surface of autophagosomal membrane during initiation gives a signal to fusion Proteins to recognize and allow binding with lysosome. Inside the lysosome various proteases are involved for degradation and then the simple monomeric subunit are exported outside the cytosol for reuse again.

**Autophagy and disease:****Autophagy and Cancer:**

In the initial stage of cancer autophagy hinders the initiation of cancer cell development by restricting the availability of nutrients. Initially it was reported that in many cases of cancer there was low or nil expression of BECN1 gene that encodes beclin protein which is one of the important component in regulating autophagy<sup>82</sup>. Based on the studies of Liang et al. 1999, it was stated that when the Beclin protein was over expressed in colon cancer cell lines it had led to the activation of autophagy resulting in decrease of proliferation and inhibition of tumor genesis<sup>83</sup>.

Liu and Ryan, 2012 reported number of autophagy regulating genes such as BECN1, UVRAG, Bif-1, Atg2B, Atg5, Atg9B, Atg12, RAB7A have been deleted in many cancer developing cells<sup>84</sup>. In 2009 Kang *et al.*, stated that mutations in Atg2B, Atg5, Atg9B, and Atg12 in gastric and colorectal cancers enhanced their growth regulation<sup>85</sup>. Takahashi *et al.*, 2010 reported on expression of Bif-1 has been down regulated in gastric and prostate cancers, Knaevelsrud *et al.*, 2010 has also reported mutations of UVRAG have been found in colon cancer. Thus it is clear that if the expression of genes that regulate autophagy are revived there will be less chance of cancer cell to grow. Hence some researchers have stated that autophagy may increase the effectiveness of anticancer therapies.

**Autophagy and Neurodegenerative Diseases:**

Autophagy plays an important role in curing neurodegenerative disease. It was observed that deletion of core autophagy genes results in neonatal and embryonic lethality stating that impairment results in accumulation of damaged proteins which are usually found to be the substrates of autophagy. For proper functioning of nervous system and homeostasis autophagy is required. Many neurodegenerative are associated with the accumulation of damaged proteins like in Alzheimer's disease which results in accumulation of A $\beta$  plaques extracellular<sup>88</sup>. Amyotrophic lateral sclerosis is another neurodegenerative disease which results in accumulation of misfolded proteins such as superoxide dismutase (SOD1), TAR- DNA binding protein 43<sup>89</sup>. As autophagy deregulation is associated with neurodegeneration its up regulation can have a significant therapeutic potential.

**Autophagy and Muscle Disease:**

Autophagy is crucial for normal muscle function and muscle remodeling<sup>94</sup>. A condition of massive muscular accumulation of autophagic-like vesicles which is known as vacuolar myopathies affects the physical properties of the skeletal muscle. In many studies of myopathy the responsive genes identified connects with autophagy morphologically. In genetic analysis of Danon's disease the mutation was found to be in gene for lysosome associated membrane protein (Lamp2)<sup>95</sup>. The increased accumulation of autophagic vesicles in myopathy might be either due to a defect in autophagy, such as a malfunction in autophagosome maturation, or to a defect in lysosomal protein degradation<sup>95</sup>.

**Autophagy and Liver Disease**

Sihyung Wang et al., 2017 has studied the TSG-6-mediated autophagy in the injured hepatocytes and liver. Tumor necrosis factor-inducible gene 6 protein (TSG-6) is one of the cytokines released from mesenchymal stem cells (MSC) that helps in protecting the damaged tissues by autophagy mediated mechanism<sup>93</sup>. Based on the studies hepatocyte survival and decrease of liver injury was observed by inducing autophagy influx both *in vivo* and *in vitro*. These findings suggest that TSG-6-regulated autophagy may be a viable therapeutic target for developing pharmacologic agents that treat chronic liver disease<sup>93</sup>.

**Autophagy and Cardiac Disease:**

Autophagy plays a crucial role in the shape and structure of heart. Weakened autophagy has been found in number of heart diseases. Exaggerated and lower activity of autophagy both leads to heart failure and improper heart function thus a balance is required for proper cardiac homeostasis<sup>91</sup>.

**Autophagy in Obesity and Diabetes**

Autophagy markers such as Atg5, Atg12-Atg5 complex, and the lipidated/cleaved form of LC3 (LC3 II) are up-regulated in human adipose tissue (AT) in obesity, especially in visceral fat<sup>92</sup>. Increased expression of autophagy genes correlates with the degree of obesity, visceral fat distribution, and adipocyte hypertrophy<sup>92</sup>.

**Conclusion:** Human body is a pack of many metabolic machinery pathways. Each and every second number of waste molecules and nonfunctional proteins are piling up in our body, if these products are not removed they may cause adverse effects leading to abnormalities of normal metabolism. In order to scavenge these waste materials various mechanisms like ubiquitinylation, apoptosis, autophagy etc. are involved. One of the mechanism that has been coming into limelight is an Autophagy. Activation of autophagy involves many autophagy related genes (ATG). In the process of autophagy the cell debris is degraded by involving many lysosomal enzymes either formation of autophagosome a double membrane structure or by direct involvement of lysosome. Autophagy plays a key role in many diseases like cancer, neurodegeneration, muscle disease, liver disease, cardiac disease and many more. In most of the cases the up regulation of autophagy regulating genes that have been deregulated can be a key role for the future therapeutic aspect.

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