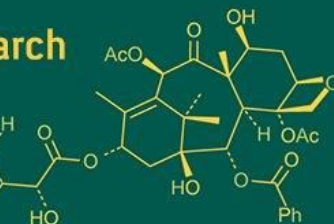


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; 10(2): 756-764
www.biochemjournal.com
Received: 15-11-2025
Accepted: 19-12-2025

Ghanshyam Sahu
Division of Biochemistry,
ICAR-IVRI, Izatnagar,
Uttar Pradesh, India

Vineet Kumar Pandey
Division of Biochemistry,
ICAR-IVRI, Izatnagar,
Uttar Pradesh, India

Uttam Kumar Sahu
Division of Animal
Reproduction, ICAR-IVRI,
Izatnagar, Uttar Pradesh,
India

Amrita Behera
Department of Veterinary
Biochemistry, Bihar
Veterinary College, Patna,
Bihar, India

Corresponding Author:
Ghanshyam Sahu
Division of Biochemistry,
ICAR-IVRI, Izatnagar,
Uttar Pradesh, India

Chaperone-mediated autophagy: Key mechanisms and its role in protein turnover

Ghanshyam Sahu, Vineet Kumar Pandey, Uttam Kumar Sahu and Amrita Behera

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i2j.7719>

Abstract

Chaperone-mediated autophagy (CMA) is a highly selective cellular degradation pathway responsible for the turnover of specific cytosolic proteins. This process relies on the recognition of protein substrates containing KFERQ-like motifs by the chaperone protein Hsc70, facilitating their translocation into lysosomes via LAMP-2A. CMA plays a pivotal role in maintaining protein homeostasis, particularly during cellular stress, and contributes to the removal of damaged or misfolded proteins. Dysregulation of CMA has been linked to various diseases, including neurodegenerative disorders and cancer. Understanding the intricate molecular mechanisms of CMA and its regulatory pathways is essential for developing potential therapeutic strategies. This work highlights the key aspects of CMA, the method to study chaperone-mediated autophagy, and its significance in protein turnover, emphasizing the importance of research into this process to address a range of human diseases.

Keywords: Chaperone-mediated autophagy, protein turnover, KFERQ-like motifs, Hsc70, LAMP-2A, cellular stress, neurodegenerative disorders, cancer, therapeutic strategies, protein homeostasis

1. Introduction

Autophagy, derived from the Greek term meaning "self-eating," is a meticulously regulated cellular process responsible for breaking down internal components, including individual large molecules and organelles, within lysosomes. In normal cellular conditions, there exists a precise equilibrium between the synthesis and degradation of proteins, ensuring the maintenance of cellular balance and the continual renewal of the proteome and organelles. However, it has been observed in experiments involving animal models or certain diseases that a malfunction in the autophagic system leads to the accumulation of abnormal proteins and dysfunctional organelles, resulting in impaired function and, often, cell death (Yamamoto *et al.*, 2023) [27]. Furthermore, autophagy is crucial in helping cells adapt to environmental changes and respond to external and internal stressors. This multifaceted role explains why autophagy is involved in processes such as cellular growth, differentiation, development, and defending against pathogens.

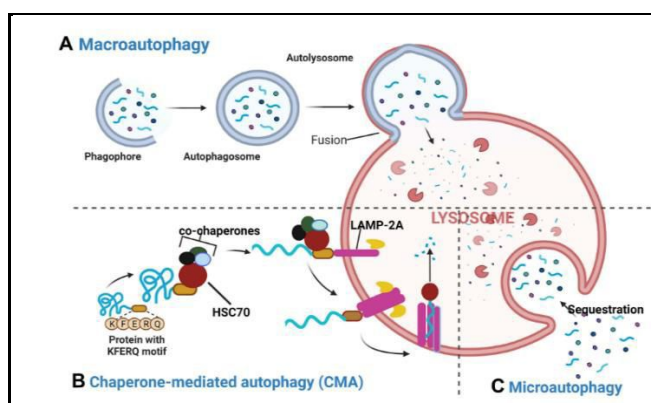


Fig 1: Lysosomal autophagy pathways.

Illustration of macroautophagy, chaperone-mediated autophagy (CMA), and microautophagy. Macroautophagy involves autophagosome formation and fusion with lysosomes, CMA selectively degrades KFERQ-containing proteins via HSC70 and LAMP-2A-mediated translocation, and microautophagy sequesters cytosolic material through direct lysosomal membrane invagination.

Various types of autophagy depend on the mechanism responsible for transporting cellular material to lysosomes for degradation (Figure 1). In mammals, the three primary types are macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA) (Assaye and Gizaw, 2022). Unlike the first two pathways, which are conserved across different species, growing evidence suggests that CMA emerged later in evolution, likely to fulfill specific requirements of evolving organisms. This review concentrates on the distinctive characteristics and cellular functions of CMA, the molecular components involved in this form of autophagy, and how CMA dysfunction is at the root of various human diseases and contributes to the aging phenotype.

1.1 Chaperone-Mediated Autophagy (CMA)

Overview

Chaperone-mediated autophagy (CMA) is a distinct pathway for lysosome-dependent protein degradation (Isenman and Dice, 1989) [15]. It sets itself apart from macroautophagy due to several key differences: (i) it doesn't involve the formation of autophagosomes and autolysosomes, (ii) its targets are cellular proteins rather than organelles, and (iii) the protein cargo is directly transported into the lysosomal lumen through interactions with HSC70 and LAMP-2A (Arias and Cuervo, 2011) [1]. HSC70 belongs to the Hsp70 protein family and is constitutively expressed, primarily participating in the CMA pathway but also in microautophagy. LAMP-2A is a specific isoform of Lamp-2 (lysosome-associated membrane protein type 2) crucial for regulating the CMA degradation process. Importantly, CMA exhibits selectivity in that all CMA substrate proteins contain a specific amino acid motif

closely related to the penta-peptide KFERQ. In the cytoplasm of the cell, HSC70 identifies the KFERQ sequence within target proteins, forming the HSC70-substrate complex. While the exact timing of this process is not fully understood, this complex subsequently interacts with the cytosolic tail of LAMP-2A, which facilitates the translocation of the target protein into the lysosome lumen (Rout *et al.*, 2015) [20]. All cells possess a basal level of CMA activity that contributes to the maintenance of cellular protein homeostasis. However, under certain conditions such as nutrient scarcity, serum deprivation, or cellular stress (e.g., protein aggregation), CMA activity is upregulated. This heightened state can be observed through indicators such as increased mRNA expression of LAMP-2A, the co-localization of HSC70 and LAMP-2A, and the presence of LAMP-2A-positive lysosomes with a more concentrated distribution near the cell nucleus (Kaushik and Cuervo, 2008) [17].

1.2 Mechanism of chaperon-mediated autophagy

The process of Chaperone-Mediated Autophagy (CMA) initiates with the recognition of cytosolic proteins by the 70 kDa heat-shock protein (Hsc70), which belongs to the Hsp70 chaperone family, and it identifies proteins bearing the KFERQ pentapeptide motif (Figure 2). Numerous co-chaperones are involved in this process, including Hsp40, Hsp90, and Hip (Tang *et al.*, 2017) [23]. Once Hsc70 binds to the substrate proteins, they are directed to a subset of lysosomes associated with the autophagic pathway. Following their binding to the Hsc70 complex, substrate proteins are translocated to the lysosomal membrane surface, where the lysosomal receptor LAMP2A, with its 12 amino acid tail exposed in the cytoplasm, can bind to the substrate protein (Li *et al.*, 2019, Cuervo *et al.*, 2014) [18, 9]. LAMP2A plays a dual role in both substrate binding and lysosome internalization, with its levels and conformation being critical for the proper functioning of this mechanism. This phase is rate-limiting in the process and can be altered through synthesis, degradation, and redistribution (Hosaka *et al.*, 2021) [14].

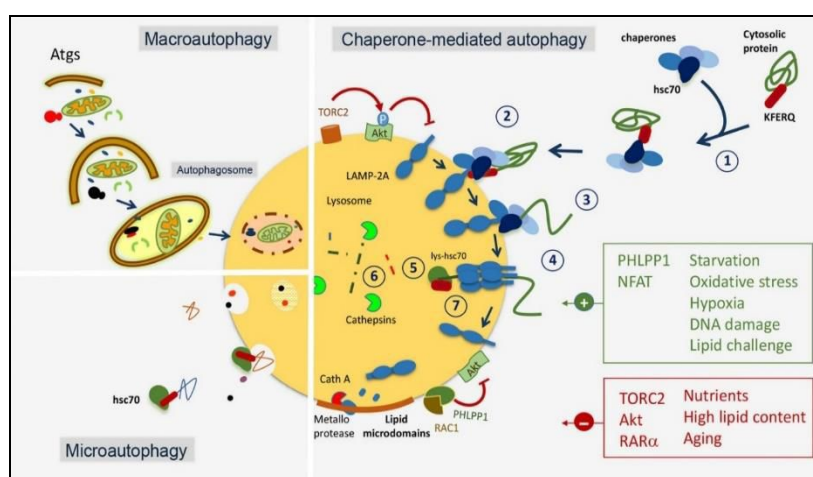


Fig 2: Overview of autophagic pathways with emphasis on chaperone-mediated autophagy (CMA).

Schematic representation of macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA). Unlike macro- and microautophagy, CMA selectively degrades cytosolic proteins containing KFERQ-like motifs via Hsc70 recognition and LAMP-2A-mediated translocation into lysosomes. Substrates are unfolded,

translocated, and degraded by lysosomal cathepsins. CMA activity is positively regulated by cellular stress (e.g., starvation, oxidative stress) and negatively regulated by nutrient-rich conditions through the mTORC2-Akt and RAR α pathways.

At the lysosomal membrane, LAMP2A exists as a monomer and forms a multimeric complex with other proteins. For substrate translocation into the lysosome lumen, the multimerization of LAMP2A, resulting in a 700 kDa protein complex and serving as a transmembrane protein channel, is necessary (Cai *et al.*, 2015) [8]. CMA substrates initially bind to monomeric LAMP2A at the lysosomal membrane, leading to the multimerization of LAMP2A, creating the essential 700-kDa complex for substrate translocation into the lysosome (Cuervo *et al.*, 2014) [9]. As the substrate undergoes unfolding, LAMP2A multimerization enhances substrate affinity and likely prevents aggregation. This protein complex facilitates the entry of the substrate protein into the lysosome lumen, where it is subsequently degraded by hydrolytic enzymes. While the substrate can initially bind to the receptor in its folded state, it must unfold to traverse the lysosomal membrane, a process likely mediated by Hsc70 and some of its co-chaperones, and it occurs prior to the complete assembly of the LAMP-2A complex

(Cuervo *et al.*, 2014) [9]. The presence of a luminal chaperone, lys-Hsc70, within the lysosome is also crucial for the complete internalization of the substrate. As LAMP-2A transitions from monomers to multimers, a lysosomal version of Hsp90 helps maintain its stability (30). Once within the lysosomal matrix, the substrate is broken down into amino acids by lysosomal hydrolytic enzymes, known as cathepsins. Lys-Hsc70 (lysosomal HSC70) promotes the disassembly of LAMP2A from its multimeric form into its monomeric form, allowing the next substrate protein to bind to monomeric LAMP2A in a new cycle. Subsequently, Lys-Hsc70 (lysosomal Hsc70) and EF1 α (Elongation Factor 1 α) induce the disassembly of LAMP2A multimers into the monomeric form, facilitating the initiation of a new degradation cycle with the binding to monomeric LAMP2A (Tang *et al.*, 2017) [23]. Notably, the activation of CMA does not necessitate the de novo synthesis of LAMP2A because its lysosomal abundance can be altered by modifying its stability, organization, and

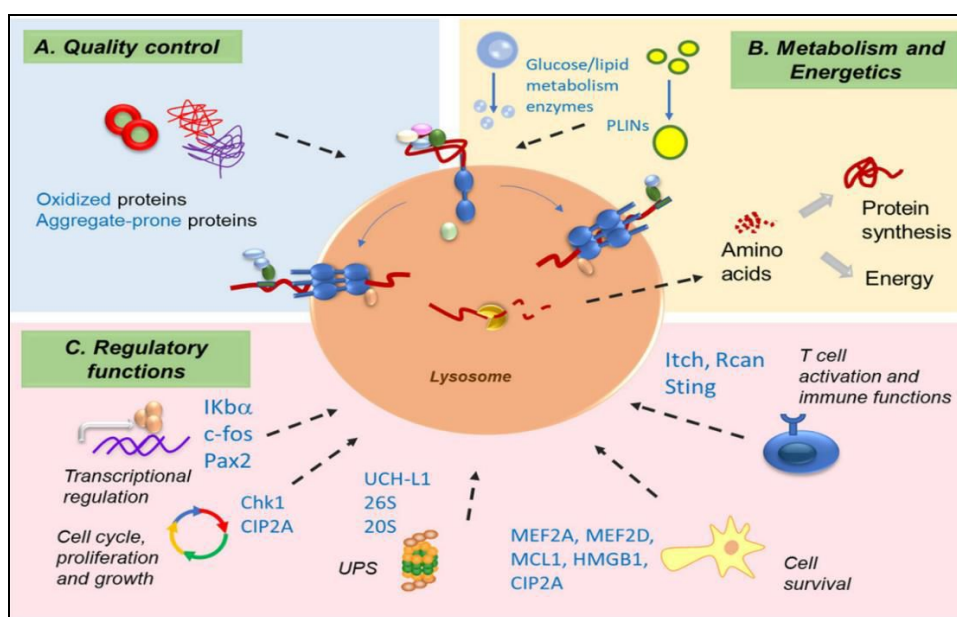


Fig 3: Physiological functions of chaperone-mediated autophagy (CMA).

Schematic overview of the major cellular roles of CMA. (A) In protein quality control, CMA selectively removes oxidized and aggregation-prone proteins. (B) In metabolism and energetics, CMA regulates enzymes of glucose and lipid metabolism, contributing to amino acid recycling, energy production, and protein synthesis. (C) In regulatory functions, CMA modulates transcription factors and signaling proteins involved in cell cycle control, immune responses, and cell survival, highlighting its broad impact on cellular homeostasis.

dynamics at the lysosomal membrane. These processes are meticulously regulated by lysosome-associated proteins, including the GFAP/EF1 α pair and the mTORC2/AKT1/PHLPP1 axis (Arias and Cuervo, 2019) [16].

1.3 Physiological Role of CMA

Chaperone-Mediated Autophagy (CMA) serves two general functions across various cell types: energy homeostasis and protein quality control. Energy homeostasis is achieved through the recycling of amino acids, while protein quality control involves the removal of damaged and prone-to-

aggregate proteins (Cuervo and Wong, 2014, Sahu *et al.*, 2011) [9, 21]. Additionally, CMA plays a role in cell type-specific functions, depending on the specific substrate proteins targeted for degradation by this pathway (Cuervo and Dice, 1996, Arias *et al.*, 2015) [10, 5]. In some cases, CMA has specialized functions in distinct cell types, often associated with the degradation of particular proteins or sets of proteins, in addition to its broader functions shared by all cells. For instance, in professional antigen-presenting cells, CMA contributes to the presentation of cytoplasmic peptides by class II molecules, which has implications for organism autoimmunity. Manipulation of hsc70 and hsp90 can reduce CMA rates and antigen presentation in these cells. In neurons, CMA enhances neuronal survival by degrading inactive versions of the transcription factor MEF2D (myocyte enhancer factor 2D). When CMA is compromised, inactivated MEF2D molecules accumulate in neurons, rendering them more susceptible to stresses (Arias *et al.*, 2015) [5]. In the kidney, CMA plays a role in controlling tubular cell growth through the degradation of the transcription factor Pax2. Dysfunction in CMA in kidney epithelial cells hampers the degradation of Pax2, a

protein crucial for cell proliferation and differentiation. Consequently, the failure to eliminate Pax2 leads to abnormal kidney cell proliferation and hypertrophy (Galluzzi *et al.*, 2017) ^[13].

1.4 The regulation of Chaperone-Mediated Autophagy (CMA) involves multiple mechanisms

1.4.1 Regulation via LAMP2A: LAMP2A, the CMA receptor on the lysosomal membrane, is a key target for CMA regulation. Its level directly correlates with CMA activity and can be altered by de novo synthesis, degradation, and redistribution. Changes in LAMP2A levels and its dynamic transition from monomers to oligomers to degradation at the lysosomal membrane modulate CMA flux (the rate of cargo degradation). For instance, during prolonged starvation, oxidative stress, or inhibition of other proteolytic pathways, up-regulation of CMA is accompanied by increased Lamp2a levels and the presence of Lamp2a-positive lysosomes. Conversely, reducing Lamp2a levels leads to decreased CMA activity (Aniento *et al.*, 1993) ^[11].

1.4.2 Regulation via HSC70: While LAMP2A is necessary for CMA, HSC70 also plays a role in CMA regulation. HSC70 requires LAMP2A for lysosomal docking in CMA, and its presence on the luminal side of the lysosomal membrane is essential for complete substrate translocation. The distribution and level of HSC70 directly reflect CMA activity and status, although the processes controlling its increase are not yet fully understood (Cuervo and Dice, 1996) ^[10].

1.4.3 Post-Translational Modifications: Post-translational modifications, such as phosphorylation, acetylation, or ubiquitination, can influence CMA substrate recognition. These modifications can create or expose the KFERQ-like motif on substrate proteins, making them eligible for CMA targeting. Conversely, modifications outside the motif can hinder substrate recognition. For instance, hypoxia-inducible factor 1 (HIF1) undergoes CMA-dependent degradation when ubiquitinated on Lys63 by the E3 ubiquitin-protein ligase STUB1, while acetylation of mammalian Ste20-like kinase 1 (MST1) prevents lysosomal degradation until it is deacetylated, allowing HSC70 to bind the canonical CMA motif (Cuervo *et al.*, 2004) ^[11].

1.4.4 Regulation via Signal Pathways: Several signaling pathways influence CMA activity. These include the

calcineurin-NFAT pathway, which activates CMA in T cells and responds to oxidative stress; PAR α signaling, which inhibits CMA; and the TOR complex 2 (TORC2)-AKT1-PHLPP1 axis, which also inhibits CMA. Additionally, endoplasmic reticulum (ER) stress can activate CMA (Deter *et al.*, 1967) ^[12].

1.4.5 Regulation by Retinoic Acid Receptor Alpha (RAR α): RAR α signaling negatively regulates the transcription of CMA components, including LAMP2A. Inhibiting RAR α holds potential for restoring impaired CMA in aging.

1.4.6 Regulation via ER Stress: CMA and p38 mitogen-activated protein kinase (P38MAPK) are activated in response to ER stress. This involves PERK-dependent recruitment of mitogen-activated protein kinase kinase 4 (MKK4) and subsequent activation of a P38MAPK pool at the lysosomal membrane. LAMP2A is phosphorylated at Thr211 and Thr213 during P38MAPK activation, leading to improved stability and oligomerization at the lysosomal membrane. This connection is referred to as the "ERICA pathway," highlighting the link between ER stress and CMA.

2. Role In Protein Turnover and diseases

Chaperone-mediated autophagy (CMA) plays a pivotal role in maintaining protein homeostasis within neurons. As neurons age or encounter pathogenic proteins, CMA activity tends to decline, resulting in the accumulation of undegraded CMA substrates in the form of protein aggregates. These aggregates have significant consequences for neuronal function, leading to both a gain of toxic function due to their presence and a loss of function as functional proteins become entrapped within them. Moreover, the failure to timely degrade proteins through CMA can disrupt other essential neuronal processes. Conversely, activating CMA, even in the context of preexisting neuropathology, can restore proteostasis in neurons, preventing a shift of the proteome towards insolubility. This restoration of CMA activity contributes to the neuronal defense against proteotoxicity, helping clear accumulated protein aggregates and preserving cellular function. (Bourdenx *et al.*, 2021) ^[7] The lysosomal-associated membrane protein 2A (LAMP2A) plays a crucial role in mediating these processes.

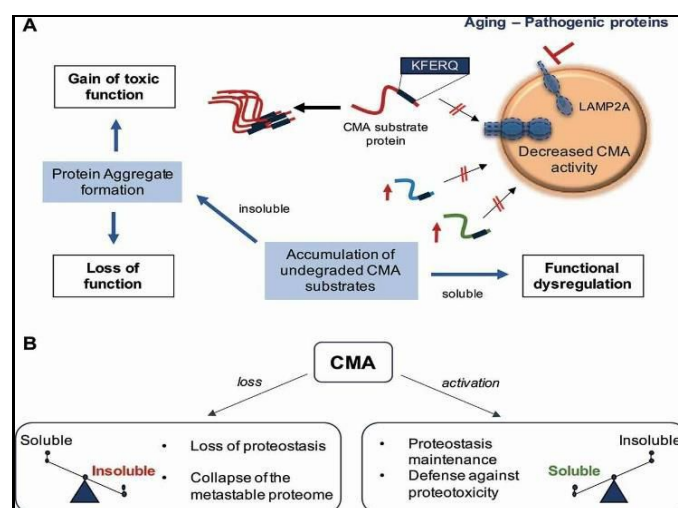


Fig 4: Consequences of altered chaperone-mediated autophagy (CMA) activity

(A) Aging or pathogenic conditions reduce CMA activity, leading to accumulation of undegraded CMA substrates, protein aggregation, toxic gain of function, and functional dysregulation. (B) Loss of CMA disrupts proteostasis and promotes insoluble protein buildup, whereas CMA activation maintains protein homeostasis and protects against proteotoxicity.

Dysregulation of proteolytic pathways is widely recognized as a contributing factor in the development and progression of neurodegenerative diseases. Many of these disorders are characterized by the abnormal accumulation of misfolded

and aggregated proteins within neurons. Neurons, being post-mitotic cells, rely heavily on efficient protein breakdown systems to manage cellular stress. When unfolded or damaged proteins are not properly eliminated, they tend to aggregate, a common hallmark in numerous neurodegenerative conditions. There is a growing body of evidence suggesting that dysregulation of the Chaperone-Mediated Autophagy (CMA) pathway plays a critical role in neurodegeneration. CMA plays a crucial role in degrading various proteins prone to aggregation, and a decrease in CMA activity leads to the buildup of toxic aggregates.

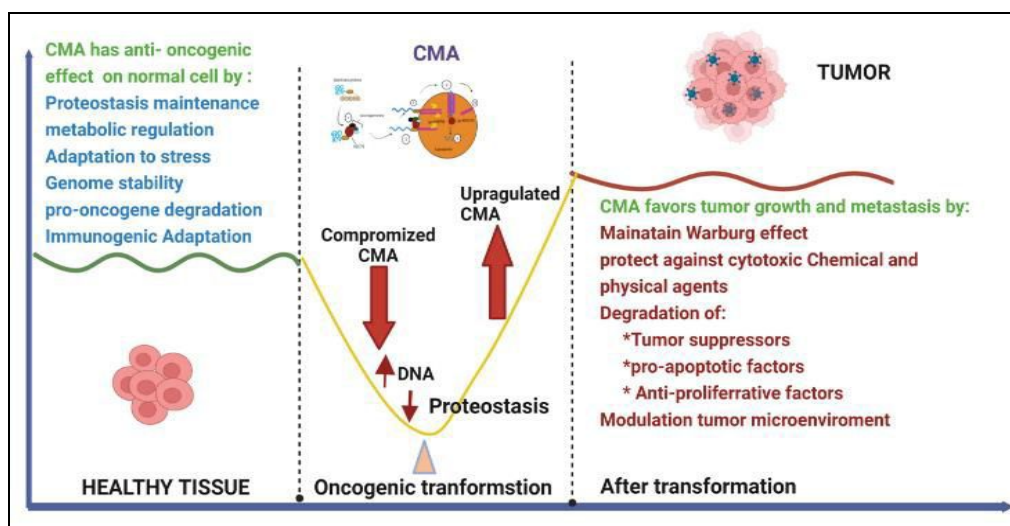


Fig 5: Dual role of CMA in cancer.

CMA suppresses tumor initiation in normal cells by maintaining proteostasis and genomic stability, but in established tumors its upregulation promotes growth, survival, and metastasis.

Neurodegenerative diseases have been associated with both increased and decreased CMA activity. In general, these pathologies are characterized by the failure of proteolytic systems, resulting in the harmful accumulation of transformed proteins. Alzheimer's (AD), Parkinson's (PD), and Huntington's (HD) diseases, among others, share a common pathological feature: the presence of aggregated protein deposits in specific brain regions. These pathogenic protein accumulations take the form of insoluble inclusions and become highly resistant to CMA-mediated degradation once they have formed. Importantly, these aggregates can disrupt CMA by causing a "clogging effect" at the lysosomal membrane, hindering the degradation of other cytosolic substrate proteins. Numerous pathogenic proteins implicated in these diseases, including alpha-synuclein (α -syn), PARK7/DJ-1 (Parkinsonism-associated deglycase), LRRK2 (leucine-rich repeat serine/threonine-protein kinase 2), UCHL1 (ubiquitin carboxyl-terminal hydrolase isozyme L1) in PD, tau protein and RCAN1 (regulator of calcineurin) in AD, and HTT (huntingtin) in HD, contain KFERQ motifs in their amino acid sequences and have been identified as CMA substrates (E Arias, 2017) ^[4]. Remarkably, while the unmodified forms of these proteins behave as conventional CMA substrates, their pathogenic variants are targeted to lysosomes but fail to degrade, ultimately inhibiting the CMA process. This interplay between CMA dysregulation and the accumulation of pathogenic proteins underscores the significance of CMA in the pathogenesis of neurodegenerative diseases. (Assaye and Gizaw, 2022) ^[6]

3. Methods to Monitor Chaperone-Mediated Autophagy

3.1 Experimental Models

Autophagy is a process found in all eukaryotic organisms. However, Chaperone-Mediated Autophagy (CMA) is exclusive to mammals, with the key component, LAMP2A, being present in birds and mammals but absent in yeast, worms, flies, and fish. CMA activity has been observed in various mammalian cell types, including fibroblasts, hepatocytes, astrocytes, neurons, macrophages, dendritic cells, T-cells, retinal cells, and various cancer cells. The liver is the most extensively studied organ for CMA research (Patel and Cuervo, 2015) ^[19].

3.2 Steady-State Readouts of CMA

Changes in the levels of critical CMA components and the presence of CMA-active lysosomes provide indirect ways to assess CMA. Common methods to analyze changes in these components include immunoblotting and imaging, as changes are typically posttranslational. LAMP2A levels in lysosomes correlate with CMA activity, but it's not sufficient to confirm lysosomal activity for CMA. To identify active CMA lysosomes, the presence of both LAMP2A and lys-hsc70 is necessary. Only a fraction of lysosomes contain both, which is typically less than 30% under normal conditions. Antibodies that specifically recognize the cytosolic tail of LAMP2A are vital. Additionally, measuring LAMP2A mRNA levels may correlate with CMA activity, but the absence of mRNA changes doesn't rule out changes in CMA activity. (Patel and Cuervo, 2015) ^[19]

Levels of lysosomal-hsc70 are also proportional to CMA activity, but total cellular lysate immunoblotting is not informative due to the abundance of hsc70. For CMA

assessment, hsc70 in lysosome-enriched fractions or colocalization with lysosomal markers is more informative. The subcellular mobilization of hsc70-positive lysosomes toward the perinuclear region is an indirect indicator of CMA activation.

There are other proteins like glial fibrillary acidic protein, elongation factor 1 α , lysosomal hsp90, and cathepsin A found in the lysosomal compartment, but they are not commonly used to assess CMA activity. The magnitude of

changes in the levels of LAMP2A and lys-hsc70 in lysosomes varies by cell type, so comparative studies are more valuable than absolute values.

When working with isolated lysosomes from cells or tissues, an increase in well-known CMA substrates is a good indicator of CMA. Comparing lysosomal levels of CMA substrates in cells or animals treated with lysosomal protease inhibitors, like leupeptin, with untreated samples allows measuring flux through CMA

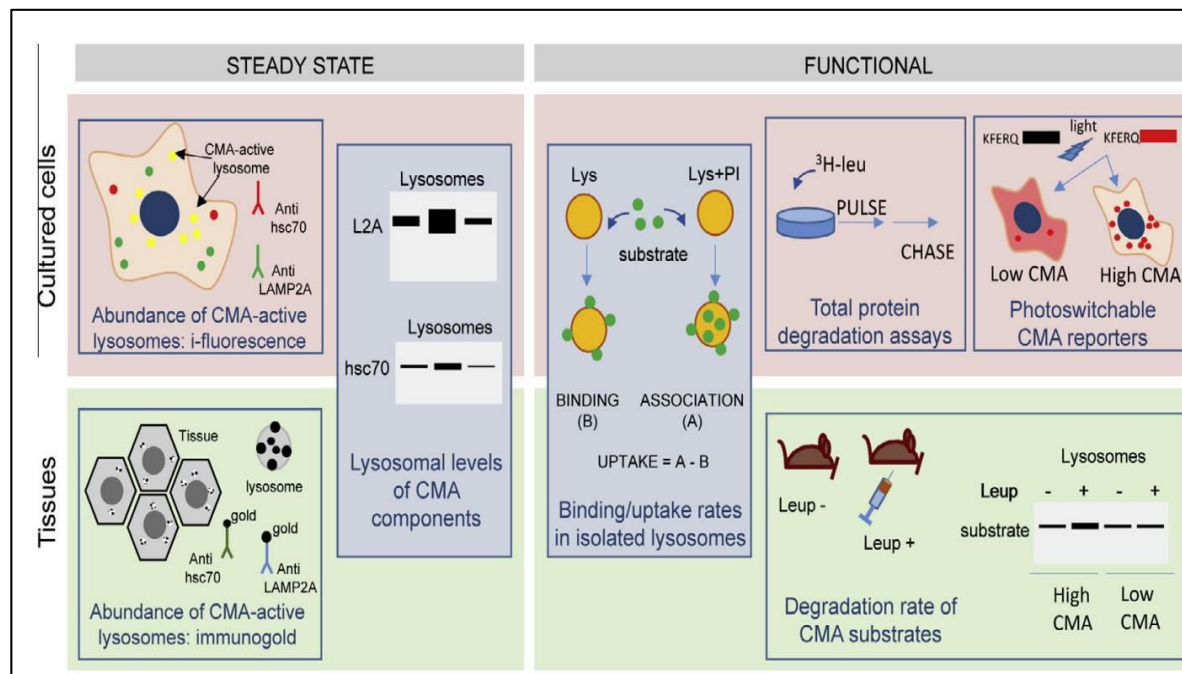


Fig 6: Experimental approaches to assess chaperone-mediated autophagy (CMA).

Overview of methods to measure CMA under steady-state and functional conditions in cultured cells and tissues, including quantification of CMA-active lysosomes, lysosomal levels of LAMP-2A and hsc70, substrate binding and uptake assays in isolated lysosomes, total protein degradation assays, CMA reporters, and substrate degradation rates.

3.3 Functional Assays

Several functional assays allow tracking CMA activity over time in cells, tissues, and isolated organelles.

3.3.1 Intracellular Protein Degradation Assessment

Approximately 30% of cytosolic proteins have the potential for CMA-mediated degradation, with the fraction degraded at a given time varying by cell type and conditions. Measuring the pool of proteins degraded through CMA is a common way to assess CMA activity. Pulse and chase experiments involving radiolabeled amino acids and inhibitors of lysosomal proteases or other autophagic pathways are used to determine which proteins undergo degradation through CMA. Pulse duration is ideally 48 hours to preferentially label long-lived proteins. After labeling, cells are incubated for an additional 2 hours to allow for the degradation of short-lived proteins. To calculate the fraction of long-lived proteins degraded in lysosomes, values in untreated cells are compared with those in cells treated with general inhibitors of lysosomal proteolysis, like leupeptin and NH₄Cl. 3-Methyladenine (3MA) can be used to estimate lysosomal degradation

through macroautophagy, but prolonged blockage of macroautophagy can lead to upregulation of CMA, potentially underestimating the fraction of proteins degraded through CMA (Patel and Cuervo, 2015) [19].

3.3.2 Photoconvertible CMA Reporters

To track the progress of Chaperone-Mediated Autophagy (CMA), a method involves using artificial fluorescent CMA reporters. These reporters carry a specific pentapeptide motif (KFERQ) that marks proteins, including fluorescent ones, for CMA degradation. However, the high cytosolic levels of these proteins often mask their association with lysosomes because they must fully unfold to translocate across the lysosomal membrane, causing them to lose fluorescence inside lysosomes.

To address this challenge, researchers have started using photoconvertible fluorescent reporters. These reporters can be switched or activated by exposure to specific wavelengths of light. By inducing this change at a particular time, it becomes possible to monitor the association of the converted protein with lysosomes in a different fluorescence channel than the original protein. Changes in the number of fluorescent puncta per cell serve as an indicator of CMA activation, with the degree of change varying by cell type. However, these reporters have limitations, as they only track lysosomal binding and cannot detect changes in degradation inside lysosomes or problems with protein translocation. To obtain a comprehensive view of CMA activity, other methods should be used in conjunction with these reporters, including the analysis of CMA substrate flux and *in vitro*

reconstitution with lysosomes isolated from the respective cells or tissues.

3.3.3 *In vitro* Reconstitution of CMA with Isolated Lysosomes

To overcome the limitations of *in vivo* assays, *in vitro* reconstitution using isolated lysosomes is a valuable method for studying Chaperone-Mediated Autophagy (CMA). Given that not all lysosomes are competent for CMA, it's crucial to isolate those specifically involved in this process. Isolation techniques that preserve lysosomal membrane integrity are preferred.

By isolating CMA-active lysosomes, researchers can analyze their content of endogenous CMA substrates at the moment of isolation. This provides insights into the flux of substrates through CMA. Additionally, isolated lysosomes allow for the reconstitution of CMA *in vitro*, enabling the separate tracking of various steps, including substrate binding, lysosomal uptake, and degradation.

Methods for assessing lysosomal binding and uptake of CMA substrates involve radiolabeled substrates and immunoblot-based assays. Isolated lysosomal fractions also facilitate comparisons of changes in the content and organization of CMA components at the lysosomal membrane, aiding in the assessment of CMA activity. (Patel and Cuervo, 2015)^[19]

4. Methods to Identify CMA Substrates

When investigating the fate of specific proteins under various cellular conditions or in pathological contexts, it's essential to consider whether they can be cleared through Chaperone-Mediated Autophagy (CMA). About 30% of cytosolic proteins contain KFERQ-like CMA targeting motifs, indicating their potential to be degraded by CMA. However, the mere presence of this targeting motif does not confirm continuous degradation through CMA, as various factors can limit accessibility to the chaperone. Therefore, additional evidence is needed to determine if a protein is a CMA substrate. Several approaches can be employed to identify CMA substrates based on changes in the protein's cellular location, levels, and degradation rate upon CMA modulation.

4.1 Association with the Lysosomal Compartment

Immunofluorescence: Initial assessments often involve colocalization of a potential CMA substrate with lysosomes through immunofluorescence. Authentic CMA substrates are delivered to lysosomes for degradation. Common lysosomal markers used for colocalization include LAMPs (LAMP1, LAMP2) or cathepsins (D or B). It's crucial to exclude colocalization with late endosomal/MVB markers, such as rab9 or ESCRT proteins, as these are utilized for endosomal microautophagy. This approach becomes more challenging when dealing with intact tissues.

Immunoblot analysis: In cases involving intact tissues, immunoblot analysis with isolated lysosomes from the tissues of interest can be performed. Enrichment of a specific protein in CMA-active lysosomes can suggest it may be a CMA substrate. Confirmation of degradation can be achieved by observing an increase in lysosomal association in the presence of lysosomal inhibitors. Comprehensive proteomic analysis of CMA-active lysosomes isolated from untreated and leupeptin-treated subjects is an effective method to identify proteins

undergoing CMA degradation.

4.2 Changes in Levels/Rates upon CMA Activation

Protease inhibitors: An increase in cellular levels of a potential CMA substrate is commonly observed when lysosomal degradation is inhibited by protease inhibitors. However, to confirm CMA degradation, further evidence is needed, as inhibition of lysosomal degradation can lead to increased cellular levels of proteins degraded by other autophagic pathways and endocytosis.

Indirect indications: CMA upregulation often occurs in response to cellular stress, such as nutrient deprivation, oxidative stress, and proteo- or lipotoxicity. An increase in the rate of lysosomal degradation of a protein under these stress conditions can provide an indirect indication of CMA degradation.

Direct modulation of CMA: The ultimate proof of a protein being a CMA substrate involves the direct modulation of CMA activity through genetic or chemical procedures.

4.2.1 Methods to Activate CMA

Activation of Chaperone-Mediated Autophagy (CMA) can be achieved through various methods:

4.2.1.1 Genetic Overexpression of LAMP2A: Increasing the expression of lysosome-associated membrane protein 2A (LAMP2A) can genetically upregulate CMA.

4.2.1.2 Pharmacological Activators: Earlier studies suggested the potential use of pharmacological agents like 6-aminonicotinamide (6-AN) and the Hsp90 inhibitor geldanamycin to activate CMA. However, their efficacy and specificity are now in question. 6-AN affects multiple lysosomal degradation pathways and has a limited effect on CMA when more direct assays are employed. Geldanamycin's effect on CMA appears to be highly cell-type dependent and biphasic, leading to early activation followed by CMA inhibition.

4.2.1.3 Novel CMA Activators: Recent research has identified novel atypical antagonists of the retinoic acid receptor alpha as potent and selective CMA activators. These novel activators can specifically increase CMA activity without affecting other degradation pathways. When a protein is a CMA substrate, its cellular levels are expected to decrease upon treatment with these activators.

4.2.1.4 Radiolabeling: Given the long half-life of CMA substrates, detecting a decrease in overall cellular levels by immunoblot can be challenging when the protein is abundant. In such cases, radiolabeling of intracellular proteins followed by immunoprecipitation at different times can offer more accurate evidence of accelerated degradation upon CMA activation.

4.2.2 Methods to Inhibit CMA

As of now, there are no well-established chemical methods to selectively block CMA. Early studies suggested that CMA can be inhibited by protein synthesis inhibitors, but these inhibitors have many broad effects on the cell, making them unsuitable for specific CMA inhibition.

The primary selective and efficient way to inhibit CMA is by knocking down or knocking out LAMP2A, the CMA receptor. This approach prevents substrate binding and

translocation into lysosomes. It is preferred over blocking other CMA components, such as hsc70, which have essential cellular functions beyond CMA. Knocking down LAMP2A does not significantly affect lysosomal stability and function and is particularly suitable for distinguishing between CMA and endosomal microautophagy (e-MI) degradation, as both pathways require hsc70, but only CMA depends on LAMP2A (Patel and Cuervo, 2015)^[19].

One limitation of genetic approaches to inhibit CMA is the relatively long half-life of LAMP2A, which requires a waiting period of at least five days before significant reductions in cellular levels are observed. Therefore, it may not be suitable for studying the acute effects of CMA inhibition.

Additional Consideration: When manipulating degradation through CMA, it's important to note that other forms of autophagy can be upregulated when CMA is compromised. As a result, the accumulation of a CMA substrate upon CMA blockage might be missed. In such cases, it's essential to investigate whether there is a shift towards degradation through macroautophagy and/or endosomal microautophagy. The contribution of these pathways to the degradation of the protein of interest can be estimated by knocking down essential components for macroautophagy (e.g., ATG7 or ATG5) or endosomal microautophagy (e.g., Vps4) (Patel and Cuervo, 2015)^[19].

5. Conclusion

Chaperone-Mediated Autophagy (CMA) is a highly selective cellular process responsible for degrading specific proteins in the cell. It distinguishes itself through its precise targeting of proteins bearing a KFERQ-like motif, which acts as a molecular tag for identification. The involvement of chaperone proteins, particularly Hsc70, is pivotal in this process. These chaperones recognize and bind to the target proteins, facilitating their translocation into lysosomes for degradation. The essential lysosomal receptor LAMP2A plays a crucial role in CMA. It assists in the translocation of chaperone-bound proteins into the lysosome, ensuring their efficient degradation. One of CMA's primary functions is to regulate protein turnover within the cell. It helps to remove damaged or surplus proteins, preventing their accumulation, which can be detrimental to cellular health. This role in protein quality control contributes significantly to maintaining cellular homeostasis. Particularly during periods of stress, such as nutrient deprivation or oxidative stress, CMA becomes more active to help manage cellular energy levels and protein load effectively. Dysregulation of CMA has been linked to various diseases, including neurodegenerative disorders and certain cancers. Understanding the molecular mechanisms of CMA and its involvement in disease pathogenesis offers promising opportunities for the development of therapeutic strategies. Targeting CMA pathways has the potential to address conditions associated with protein misfolding and aggregation, presenting a novel approach to treat these challenging diseases.

6. Future prospective

Chaperone-Mediated Autophagy (CMA) research offers exciting future prospects. It holds potential for drug development, especially in addressing diseases linked to protein misfolding and aggregation. CMA may enable

precision medicine, tailoring treatment strategies based on individual CMA status. Additionally, understanding CMA's role in aging could lead to insights into healthy aging. In the realm of neurodegenerative diseases, CMA-focused research may unveil therapies for conditions like Alzheimer's and Parkinson's disease. Moreover, CMA modulation has the potential to enhance cancer treatment by targeting specific proteins in cancer cells. As CMA research progresses, it will provide deeper molecular insights, and CMA-related biomarkers may emerge, aiding in disease diagnosis and prognosis.

7. Acknowledgments

I would like to express my sincere gratitude to my supervisor for continuous guidance, support, and encouragement throughout this work. I am also thankful to my colleagues and lab members for their valuable discussions and assistance. Finally, I acknowledge my institution for providing the facilities and resources necessary to complete this study.

References

1. Aniento F, Roche E, Cuervo AM, Knecht E. Uptake and degradation of glyceraldehyde-3-phosphate dehydrogenase by rat liver lysosomes. *J Biol Chem.* 1993;268(14):10463-10470.
2. Arias E, Cuervo AM. Chaperone-mediated autophagy in protein quality control. *Curr Opin Cell Biol.* 2011;23(2):184-189.
3. Arias E, Cuervo AM. Pros and cons of chaperone-mediated autophagy in cancer biology. *Trends Endocrinol Metab.* 2020;31(1):53-66.
4. Arias E. Methods to study chaperone-mediated autophagy. *Methods Enzymol.* 2017;588:283-305.
5. Arias E, Koga H, Diaz A, Mocholi E, Patel B, Cuervo AM. Lysosomal mTORC2/PHLPP1/Akt regulate chaperone-mediated autophagy. *Mol Cell.* 2015;59(2):270-284.
6. Assaye MA, Gizaw ST. Chaperone-mediated autophagy and its implications for neurodegeneration and cancer. *Int J Gen Med.* 2022;15:5635-5649.
7. Bourdenx M, Gavathiotis E, Cuervo AM. Chaperone-mediated autophagy: a gatekeeper of neuronal proteostasis. *Autophagy.* 2021;17(8):2040-2042.
8. Cai Z, Zeng W, Tao K, *et al.* Chaperone-mediated autophagy: roles in neuroprotection. *Neurosci Bull.* 2015;31(4):452-458.
9. Cuervo AM, Wong E. Chaperone-mediated autophagy: roles in disease and aging. *Cell Res.* 2014;24(1):92-104.
10. Cuervo AM, Dice JF. A receptor for the selective uptake and degradation of proteins by lysosomes. *Science.* 1996;273(5274):501-503.
11. Cuervo AM, Stefanis L, Fredenburg R, Lansbury PT, Sulzer D. Impaired degradation of mutant α -synuclein by chaperone-mediated autophagy. *Science.* 2004;305(5688):1292-1295.
12. Deter RL, Baudhuin P, de Duve C. Participation of lysosomes in cellular autophagy induced in rat liver by glucagon. *J Cell Biol.* 1967;35(2):C11-C16.
13. Galluzzi L, Baehrecke EH, Ballabio A, Boya P, Bravo-San Pedro JM, Cecconi F, *et al.* Molecular definitions of autophagy and related processes. *EMBO J.* 2017;36(13):1811-1836.

14. Hosaka Y, Araya J, Fujita Y, *et al.* Role of chaperone-mediated autophagy in the pathophysiology including pulmonary disorders. *Inflamm Regen.* 2021;41(1):1-10.
15. Isenman LD, Dice JF. Secretion of intact proteins and peptide fragments by lysosomal pathways of protein degradation. *J Biol Chem.* 1989;264(36):21591-21596.
16. Juste YR, Cuervo AM. Analysis of chaperone-mediated autophagy. *Methods Mol Biol.* 2019;1880:703-727.
17. Kaushik S, Cuervo AM. Chaperone-mediated autophagy. *Methods Mol Biol.* 2008;445:227-244.
18. Li W, Nie T, Xu H, *et al.* Chaperone-mediated autophagy: advances from bench to bedside. *Neurobiol Dis.* 2019;122:41-48.
19. Patel B, Cuervo AM. Methods to study chaperone-mediated autophagy. *Methods.* 2015;75:133-140.
20. Rout AK, Strub MP, Piszczek G, Tjandra N. Structure of transmembrane domain of lysosome-associated membrane protein type 2a (LAMP2A) reveals key features for substrate specificity in chaperone-mediated autophagy. *J Biol Chem.* 2014;289(51):35111-35123.
21. Sahu R, Kaushik S, Clement CC, Cannizzo ES, Scharf B, Follenzi A, *et al.* Microautophagy of cytosolic proteins by late endosomes. *Dev Cell.* 2011;20(1):131-139.
22. Skibo YV, Vodounon CA, Legba BB, Abramov SN, Evtugyn VG, Reshetnikova ID, *et al.* Morpho-biochemical characteristics of autophagy of lymphocytes from peripheral blood of patients with moderate and severe bronchial asthma. *Am J Biomed Res.* 2021;9(1):10-18.
23. Tang Y, Wang XW, Liu ZH, *et al.* Chaperone-mediated autophagy substrate proteins in cancer. *Oncotarget.* 2017;8(31):51970-51985.
24. Tasset I, Cuervo AM. Role of chaperone-mediated autophagy in metabolism. *FEBS J.* 2016;283(13):2403-2413.
25. Tekirdag K, Cuervo AM. Chaperone-mediated autophagy and endosomal microautophagy: jointed by a chaperone. *J Biol Chem.* 2018;293(15):5414-5424.
26. Varshney V, Majee M. Emerging roles of the ubiquitin-proteasome pathway in enhancing crop yield by optimizing seed agronomic traits. *Plant Cell Rep.* 2022;41(9):1805-1826.
27. Yamamoto H, Zhang S, Mizushima N. Autophagy genes in biology and disease. *Nat Rev Genet.* 2023;24(??):1-19.