

Chaperone-mediated autophagy: a unique way to enter the lysosome world

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All cellular proteins undergo continuous synthesis and degradation. This permanent renewal is necessary to maintain a functional proteome and to allow rapid changes in levels of specific proteins with regulatory purposes. Although for a long time lysosomes were considered unable to contribute to the selective degradation of individual proteins, the discovery of chaperone-mediated autophagy (CMA) changed this notion. Here, we review the characteristics that set CMA apart from other types of lysosomal degradation and the subset of molecules that confer cells the capability to identify individual cytosolic proteins and direct them across the lysosomal membrane for degradation.

Autophagy: more than the 'cool' double membrane vesicles

Cells depend on an organelle, the lysosome, which is dedicated to the degradation of products coming from outside the cell that are internalized by endocytosis or phagocytosis [1]. In addition, lysosomes can also degrade intracellular components (proteins and organelles) through a process generically known as autophagy (see [Glossary](#)) [2]. Most cells can perform autophagy through different mechanisms depending on the way that the products to be degraded (or cargo) are delivered to lysosomes. In addition, recent studies have subdivided the main autophagic pathways depending on the nature of the cargo or the stimuli that promote their degradation [3,4]. In this review we focus on a type of selective autophagy, known as chaperone-mediated autophagy (CMA), so far only identified in mammalian cells, that was set apart from other types of autophagy due to the unique way in which its substrates, mainly cytosolic proteins, enter the lysosome [5]. Proteins degraded by CMA are identified one-by-one by a cytosolic chaperone that delivers them to the surface of the lysosomes. Once there, the substrate proteins unfold and cross the lysosomal membrane. These three events – (i) individual recognition of single proteins, (ii) unfolding before degradation, and (iii) translocation inside the lysosomes – constitute the trademark signature of CMA and the basis for its cellular functions.

Degradation of cytosolic material by lysosomes was described almost in parallel to the identification of

lysosomes as digestive organelles [6]. Shortly after the finding that cytosolic content could be found inside lysosomes, elegant morphological studies demonstrated two possible mechanisms for the arrival of this cytosolic cargo. The term macroautophagy was coined to describe the sequestration of regions of the cytosol inside double-membrane vesicles that then fuse with lysosomes to transfer their luminal content for degradation [7]. The term microautophagy was reserved to describe the internalization of cytosolic content into lysosomes upon sequestration by invaginations of the lysosomal membrane that then pinch off in the shape of small vesicles into the lysosomal lumen for degradation [8]. When these two types of autophagy were described, the idea of selective degradation of single cytosolic proteins was not contemplated because both macroautophagy and microautophagy delivered to the lysosomes regions of the cytosol containing pools of soluble proteins and organelles. However, later analysis of the proteins internalized and degraded in lysosomes in cultured cells demonstrated that removal of nutrients from the culture media resulted in a preference for the lysosomal degradation of particular subsets of proteins [9]. These findings set the

Glossary

Autophagy: degradation of intracellular proteins and organelles in lysosomes.

Cathepsins: proteases located inside lysosomes that reach maximal activity at acidic pH.

Chaperone: protein that assists other proteins in their folding, unfolding, and intracellular trafficking by preventing non-specific interactions with other surrounding proteins.

Chaperone-mediated autophagy: autophagic pathway in which cytosolic proteins are targeted one-by-one to the surface of the lysosome from where they reach the lumen by crossing the lysosomal membrane.

Endosomal microautophagy: degradation of cytosolic proteins in late endosomes after internalization by mechanisms that resemble those of microautophagy.

Lysosome: single-membrane-enclosed vesicles dedicated to the breakdown of cellular structures for recycling. They are characterized by their luminal acidic pH and their high abundance of hydrolases.

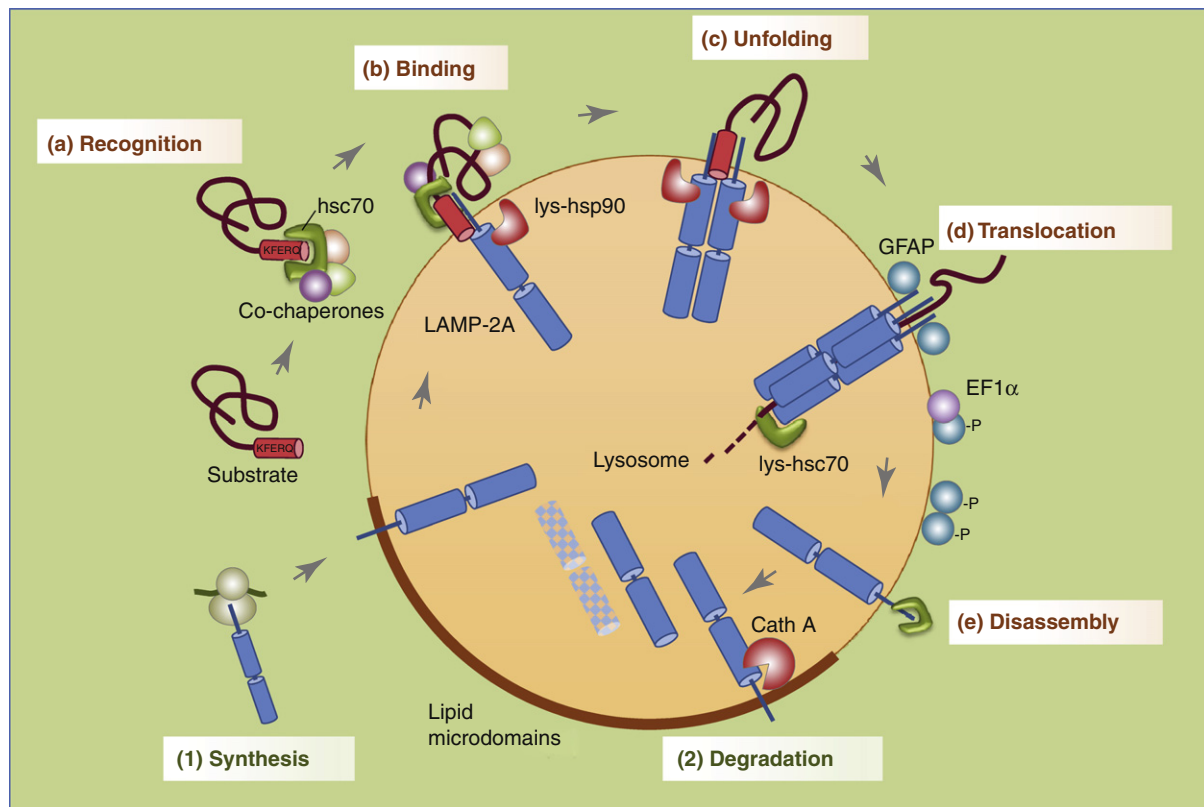
Lysosome-associated membrane proteins: a family of single-span membrane proteins preferentially located in lysosomes that display a short C-terminus (10–12 amino acids) at the cytosolic side of the lysosomal membrane. The luminal part of these proteins is heavily glycosylated to protect them from degradation.

Macroautophagy: autophagic pathway in which cytosolic proteins and organelles are sequestered into a double-membrane vesicle and then fuse with lysosomes to assure their degradation.

Microautophagy: internalization of cytosolic proteins and organelles into lysosomes through invaginations of the lysosomal membrane that then pinch off as single-membrane vesicles into the lysosomal lumen.

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TRENDS in Cell Biology

Figure 1. Steps and regulation of CMA. Steps: (a) Recognition of substrate proteins by hsc70/co-chaperones; (b) binding of substrate-chaperone complex to LAMP-2A; (c) unfolding of the substrate; (d) LAMP-2A multimerization, substrate translocation, and subsequent degradation; (e) disassembly of LAMP-2A multimer/translocon. Regulation of the levels of LAMP-2A at the lysosomal membrane is achieved through (1) *de novo* synthesis, and (2) degradation in specialized microdomains at the lysosomal membrane. Abbreviations: CMA, chaperone-mediated autophagy; EF1 α , elongation factor 1 α ; GFAP, glial fibrillary acidic protein; hsc70, heat shock cognate protein of 70 kDa; LAMP-2A, lysosome-associated membrane protein type 2A; lys-hsc70, lysosome-associated hsc70.

bases for the discovery and characterization of what is today known as CMA (Figure 1) [5].

CMA: the basics

The discovery that individual cytosolic proteins could be rerouted for lysosomal degradation during nutrient deprivation, motivated attempts to reconstitute this process *in vitro* using isolated lysosomes [10]. Taking as a model the protease-protection assays utilized to study the translocation of proteins inside other organelles, it was demonstrated that proteins presented to intact lysosomes in an assay tube could be detected inside these organelles after incubation at physiological temperatures [11].

Although in the beginning what was being reproduced in the test tube was thought to be some type of microautophagy, the unique characteristics of this transport provided support for a different mechanism of internalization. Lysosomal uptake occurred for some proteins but not for others, and this selectivity for individual proteins did not fit well with a model of internalization inside vesicles that more likely would contain a representative sample of the mixture of presented proteins [10,11]. In addition, attempts to detect vesicle formation at the surface of lysosomes during the internalization of proteins in the *in vitro* system were fruitless.

Studies with isolated lysosomes helped to characterize further the main properties of this new 'direct' lysosomal

uptake. Binding of substrates to the lysosomal membrane was saturable and addition of increasing concentrations of one substrate protein competed the binding of others, supporting the existence of specific substrate-binding sites at the lysosomal membrane [12]. This saturability suggested the participation of a membrane receptor, also supported by the fact that substrate proteins could no longer bind to lysosomal membranes previously exposed to proteases (to eliminate proteins or protein regions exposed on the cytosolic side of the lysosomal membrane) [12].

The use of biochemical and, later, genetic procedures has contributed to the current molecular characterization of this autophagic pathway, and the identification of a key role for chaperones in this direct lysosomal uptake motivated the renaming of this type of autophagy as chaperone-mediated autophagy or CMA.

Targeting cytosolic proteins one-by-one

All the proteins internalized in lysosomes through CMA contain in their amino acid sequence a pentapeptide motif that is necessary and sufficient for their targeting to lysosomes [13]. Studies with the first prototype substrate, ribonuclease A, demonstrated that removal of the pentapeptide KFERQ completely abolished its lysosomal degradation in response to nutrient deprivation [14]. Furthermore, attachment of this sequence to proteins that are not usually degraded by this lysosomal pathway is enough to induce

their degradation through CMA [15]. In fact, this dependence on the KFERQ peptide constitutes the basis for the recent fluorescent reporters developed to monitor CMA in intact cells [15].

The consensus motif is based on the physical properties of the amino acid residues, rather than on the specific amino acids [13], and has been defined as the combination of a positively and a negatively charged residue, a hydrophobic residue and a fourth amino acid that can be either positively charged or hydrophobic. These four residues are always flanked by a glutamine on either side (Box 1).

These pentapeptide motifs are recognized specifically by the cytosolic protein hsc70 (heat shock cognate protein of 70 kDa), the constitutively expressed member of the hsp70 family of cytosolic chaperones (Figure 1a) [16]. Hsc70 is a multifunctional chaperone that participates in many other cellular functions (i.e., protein refolding, disassembly of multiprotein complexes such as clathrin, protein targeting into other organelles). For many of these functions, hsc70

interacts with proteins on the basis of their hydrophobicity or other amino acid motifs; however, mutagenesis of the KFERQ-like motif in substrate proteins has demonstrated that this is the only binding region used by hsc70 for their delivery to lysosomes [16].

A very recent development has been the discovery that binding of hsc70 to the KFERQ region of cytosolic proteins can also mediate their delivery to late endosomes where they undergo degradation through endosomal microautophagy (e-MI) [17] (the characteristics of this pathway and the differences between CMA and e-MI are summarized in Box 2).

In contrast to other degradation tags, such as ubiquitin, that are added to a protein only when it needs to be degraded, the KFERQ motif is always present in the substrate sequence; how then is this degradation regulated? Regulation may depend, for the most part, on whether or not this motif is accessible to the chaperone. Partial unfolding may favor recognition of motifs buried in the core of the folded proteins, and the motif could also be masked by interacting proteins or by binding to intracellular membranes. Subcompartmentalization may also make targeting possible only when the proteins reach the cytosol. Recent studies have also revealed regulation by post-translational modifications in proteins missing only one of the residues necessary to complete a CMA-targeting motif. For example, phosphorylation can contribute a missing negatively charged residue, or acetylation of a lysine can provide a missing glutamine (acetylated lysine is comparable to glutamine). In fact, acetylation of the glycolytic enzyme embryonic M2 isoform of pyruvate kinase (PKM2) initiates its lysosomal degradation by CMA [18]. Likewise, degradation of the N-terminal region of huntingtin is initiated by phosphorylation followed by acetylation that completes a targeting motif [19]. Generation of CMA-targeting motifs through post-translational modifications expands the subset of cellular proteins that can undergo degradation by this pathway and provides an additional level of regulation for this type of degradation. A list of proteins carrying the KFERQ-like motif confirmed experimentally to be CMA substrates can be found in [20].

Cytosolic hsc70: the decision maker

Hsc70 is the only chaperone demonstrated to mediate substrate targeting for CMA [16]. The chaperone CHIP has been shown to mediate lysosomal degradation of proteins such as α -synuclein, previously identified as a CMA substrate [21], however, whether CHIP-mediated degradation occurs by CMA or other forms of autophagy requires further investigation.

Hsc70 also targets KFERQ-containing proteins to late endosomes for e-MI (Box 2) [17], and has been shown to interact with protein aggregates and mediate their degradation by macroautophagy through a process named chaperone-assisted selective autophagy (CASA) [22]. Whether or not CASA requires the KFERQ-motif is still a pending question. Of current interest is the identification of the factors that determine targeting through one type of autophagy or another following the initial interaction of hsc70 with a KFERQ-bearing protein. It is possible that different subsets of co-chaperones, when associated to hsc70, modulate targeting to one particular pathway. The fact that the

Box 1. Properties of the CMA-targeting motif

- **Composition:** all CMA-targeting motifs contain: (i) one or two of the following positively charged residues: K, R; (ii) one or two of the following hydrophobic residues: I, L, V, F; (iii) one of the following negatively charged residues: D, E; and (iv) one Q on either side of the pentapeptide [13].
- **Location:** the CMA-targeting motif can be located in the C terminus, N terminus, or in central regions of the protein, the only requirement being that it becomes exposed or accessible for chaperone binding. Scenarios that could promote exposure of the CMA-targeting motif include partial unfolding of the protein, dissociation of protein complexes in which interacting proteins mask the targeting motif, or protein release from intracellular membranes.
- **Number:** although several cytosolic proteins contain more than one targeting motif, experimental addition of tandem motifs does not make proteins better CMA substrates (one motif suffices for lysosomal targeting) [13]. In proteins with multiple targeting motifs it is plausible that different motifs become available for chaperone recognition depending on the stimuli that induce the degradation of the protein (i.e., partial unfolding versus dissociation from a protein complex when still fully folded). In proteins that undergo physiological cleavage to release functional peptides the presence of multiple motifs could guarantee selective removal of the resulting peptides by CMA.
- **Abundance:** sequence analysis reveals that about 30% of soluble cytosolic proteins contain a putative CMA-targeting motif [16]. However, the fact that post-translational modifications may generate additional motifs increases the number of possible substrates [18,19]. Motifs are only detected in soluble cytosolic proteins or in organelle-resident proteins that may enter the cytosolic compartment under specific conditions. Membrane proteins cannot be translocated into lysosomes, and consequently this pool of proteins lack CMA-targeting motifs. The only known exceptions are specific membrane proteins in which the targeting motif is present in regions of the membrane protein that can be released into the cytosol upon cleavage.
- **Selectivity:** although the presence of a CMA-targeting motif is a *sine qua non* requirement for all CMA substrates, the classification of a protein as a CMA substrate cannot be performed solely based on this criterion. This motif has recently been shown to be also utilized for targeting of proteins to late endosomes to undergo microautophagy [17]. In addition, it is possible that some KFERQ-like motifs are utilized for targeting of proteins that exert a function at the lysosomal membrane and do not necessarily undergo degradation in this compartment [28].

Box 2. CMA and microautophagy – how are they different?

Of the three autophagic pathways, microautophagy has been the least studied, with mainly morphological evidence demonstrating the entrapment of cytosolic components via invaginations of the lysosomal membrane, which subsequently pinch off and are then degraded [8]. Recently, a similar internalization of cytosolic components has been described to occur in late endosomes [17]. Interestingly, in addition to carrying out in-bulk entrapment of cargo, this process, now termed endosomal microautophagy (e-MI), can also mediate the selective internalization of cytosolic

proteins. Selective e-MI and CMA share the requirement for the KFERQ-like motif in their substrates, which are recognized by the cytosolic chaperone hsc70 [17]. However, e-MI does not require LAMP-2A for binding of the hsc70/substrate complex, which instead binds directly to the phospholipid phosphatidylserine at the endosomal membrane through specific residues in hsc70. Also, unfolding of substrate proteins is not a requirement for e-MI [17] (similarities and differences between e-MI and CMA are summarized in Figure 1).

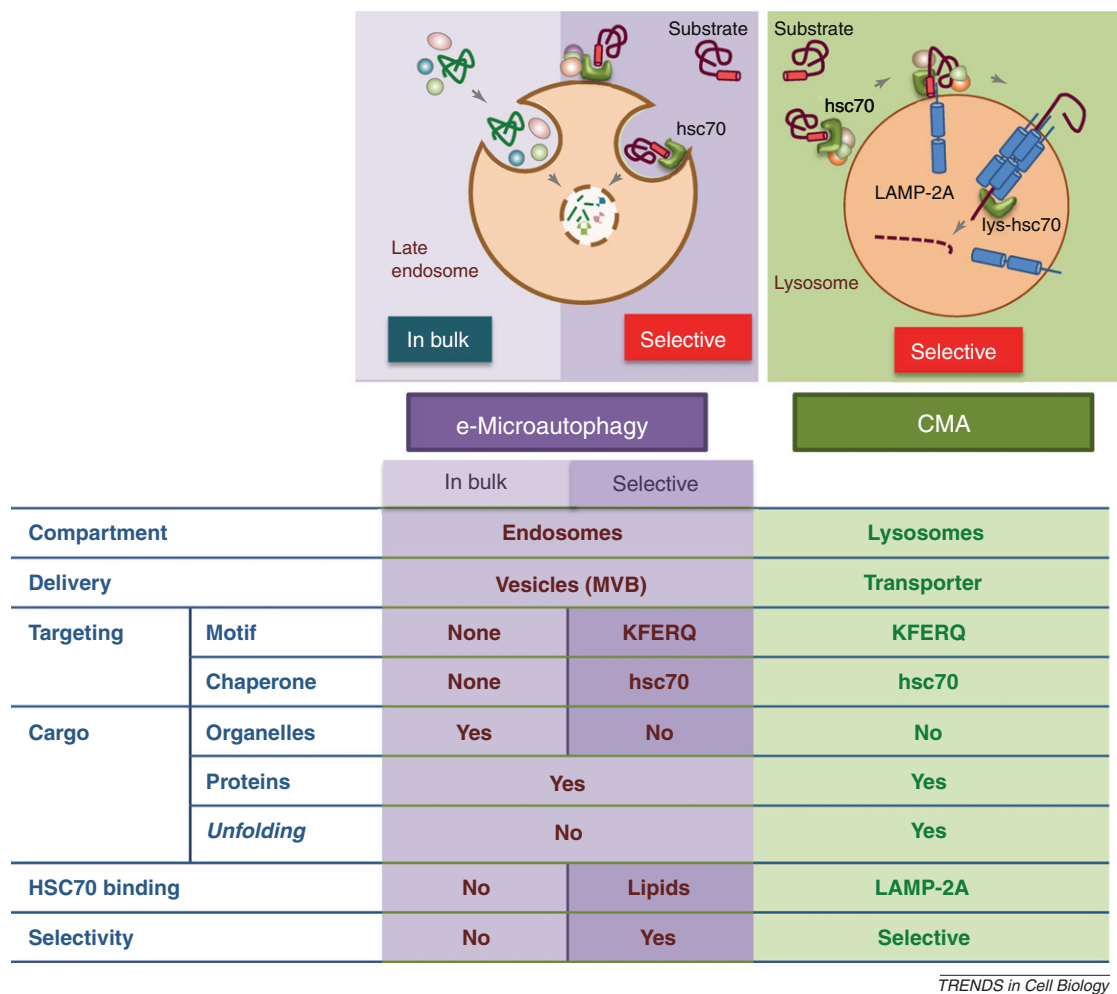


Figure 1. Comparison of endosomal microautophagy (e-MI) and chaperone-mediated autophagy (CMA).

KFERQ-motif is necessary and sufficient for CMA, and only necessary but not sufficient for e-MI supports the possible participation of additional proteins/factors in e-MI targeting [17]. Alternatively, properties of the substrate protein such as its ability to undergo complete unfolding, an absolute requirement for CMA [23] but not for e-MI [17], may also determine its degradative fate. Cytosolic hsc70 and its associated co-chaperones may participate in steps of CMA beyond substrate targeting (Figure 1). For example, substrate unfolding could be the responsibility of the same hsc70 and co-chaperones involved in the targeting of substrate to lysosomes. However, it is also possible that chaperones resident at the cytosolic side of the lysosomal membrane are the main unfoldases of

the CMA substrates because unfolding is not required for membrane binding, but only for translocation [23].

LAMP-2A at lysosomal entry

Binding of substrate proteins to the lysosomal membrane occurs at the cytosolic tail of the single-span membrane protein LAMP-2A (lysosome-associated membrane protein type 2A) (Figure 1b) [24]. This protein is one of the three splice variants encoded by the *lamp2* gene that share identical luminal regions and different transmembrane and cytosolic tails [25]. Substrate binding to LAMP-2A is limiting for CMA and, in fact, cells use changes in the levels of this membrane protein to upregulate or downregulate CMA rapidly [26]. Changes in the rates of LAMP-2A

synthesis, its regulated degradation at the lysosomal membrane, and its subcompartmentalization in this organelle all contribute to modulate CMA activity (Figure 1).

Binding of substrate proteins to monomeric forms of LAMP-2A promotes its assembly into a high molecular weight complex of about 700 kDa at the lysosomal membrane (Figure 1c,d) [27]. Multimerization is required for substrate translocation because LAMP-2A mutants unable to associate into this complex still bind substrate proteins but prevent translocation. Unexpectedly, the translocation complex is not stable at the lysosomal membrane and, once the substrate has been released into the lysosomal lumen, LAMP-2A dissociates into monomers (Figure 1e) [27]. Disassembly is an active process mediated by hsc70 that generates monomeric forms of LAMP-2A required for substrate binding to sustain cycles of binding and uptake.

Different subsets of proteins have been described to modulate the stability of the CMA translocation complex. In addition of the disassembling function of hsc70, the pair of proteins glial fibrillary acidic protein (GFAP) and elongation factor 1 α (EF1 α) work in a coordinated manner to regulate the amount of translocation complex at the lysosomal membrane [27]. GFAP associates to the lysosomal membrane in two forms, a non-phosphorylated variant that binds and stabilizes LAMP-2A in the multimeric complex, and phosphorylated GFAP (pGFAP) that binds to the lysosomal membrane outside this complex, and that is usually associated to EF1 α (Figure 1d). Binding to this GTP-binding protein prevents pGFAP from dimerizing with GFAP. However, in the presence of GTP, EF1 α is released from the membrane and GFAP abandons the translocation complex to associate with pGFAP, resulting in the disassembly of the translocation complex and the subsequent decrease in CMA [28]. Oscillatory changes in GTP concentration in the proximity of the lysosome may contribute to the regulation of CMA activity under physiological conditions.

Membrane lipids also exert a modulatory effect on CMA by affecting the stability of LAMP-2A at the lysosomal membrane. Animals subjected to diets with high lipid-content show reduced CMA activity due to the instability of LAMP-2A in their lysosomes [29]. Comparative lipidomic analysis revealed that these diets induce qualitative and quantitative changes in the lipid compositions of the lysosomal membrane that alter LAMP-2A multimerization and favor its recruitment to specific membrane microdomains where this protein is normally degraded [30] (Figure 1). Interestingly, the changes in the lipid profile resemble those observed during physiological aging, a condition also associated with reduced CMA activity.

Lysosomal chaperones in CMA

In addition to the cytosolic form of hsc70, variants of this protein associated with lysosomes also contribute to CMA. Levels of lysosome-associated hsc70 (lys-hsc70) increase proportionally to the increase in CMA activity and become limiting when LAMP-2A is in excess. Part of lys-hsc70 is localized in the lumen of lysosomes, and blockage of this intralysosomal hsc70, with antibodies delivered by endocytosis, does not affect membrane binding of substrate proteins but abolishes their internalization [31]. The presence or absence of luminal lys-hsc70 has helped to identify

lysosomal populations of different competence for CMA [32]. Although LAMP-2A is present in all types of lysosomes, only the subset of lysosomes that contain lys-hsc70 are competent for substrate uptake. The percentage of CMA-active lysosomes (containing both hsc70 and CMA substrates) increases in response to the stressors that upregulate this pathway (i.e., starvation, oxidative stress) and can change from 20%, in resting conditions, to up to 80% of the total cellular lysosomes when CMA is maximally active [32].

The electrophoretic patterns of cytosolic and lys-hsc70 are markedly different (nine cytosolic isoforms and only two in lysosomes) [31], but the fact that cytosolic hsc70 substitutes for lys-hsc70 when artificially introduced inside lysosomes incompetent for CMA [32] suggests that both forms of hsc70 probably originate from the same gene, and that only modified forms of hsc70 can reach the lysosomal lumen through as yet unknown mechanisms. Further studies are needed to identify the basis for the resistance of hsc70 to degradation in this compartment. Other lysosome-resident proteins avoid digestion through assembly into semi-aggregated complexes from which they are released in a pH-dependent manner. It is interesting that higher acidification is a distinctive characteristic of CMA-competent lysosomes and that a small increase in their pH is enough to destabilize and rapidly degrade lys-hsc70 [32].

Hsc70 also associates with the cytosolic side of the lysosomal membrane where it may contribute to unfolding of the substrate proteins [33] and active disassembly of the LAMP-2A translocation complex [27]. Hsp90 localizes on both sides of the lysosomal membrane, but the luminal form is tightly bound to the lysosomal membrane [27] (Figure 1b,c). This luminal variant stabilizes LAMP-2A when transitioning from a monomeric to a multimeric state. To date, the specific co-chaperones modulating the activity of the membrane-associated hsc70 and hsp90 have not been identified. In addition, it is not known if there are luminal lysosome-specific co-chaperones or how the intralysosomal chaperones obtain the ATP required for their function.

Physiology of CMA

Independent of the cell type, CMA contributes to two crucial functions in cellular physiology – maintenance of energy homeostasis and protein quality control. In addition, CMA also takes part in cell type-specific functions that depend on the substrate protein being degraded by this pathway (Figure 2).

General functions

In almost all cell types, nutrient scarcity maximally activates CMA [34,35]. Degradation of proteins through this autophagic pathway provides free amino acids to sustain synthesis of other proteins (Figure 2a). Activation of CMA is initiated after approximately 10 h of starvation and follows the upregulation of macroautophagy that typically occurs approximately 30 min into the starvation and does not last more than 8–10 h. The selectivity of CMA for single soluble cytosolic proteins might allow the degradation of proteins that are no longer required (such as glycolytic enzymes [11]

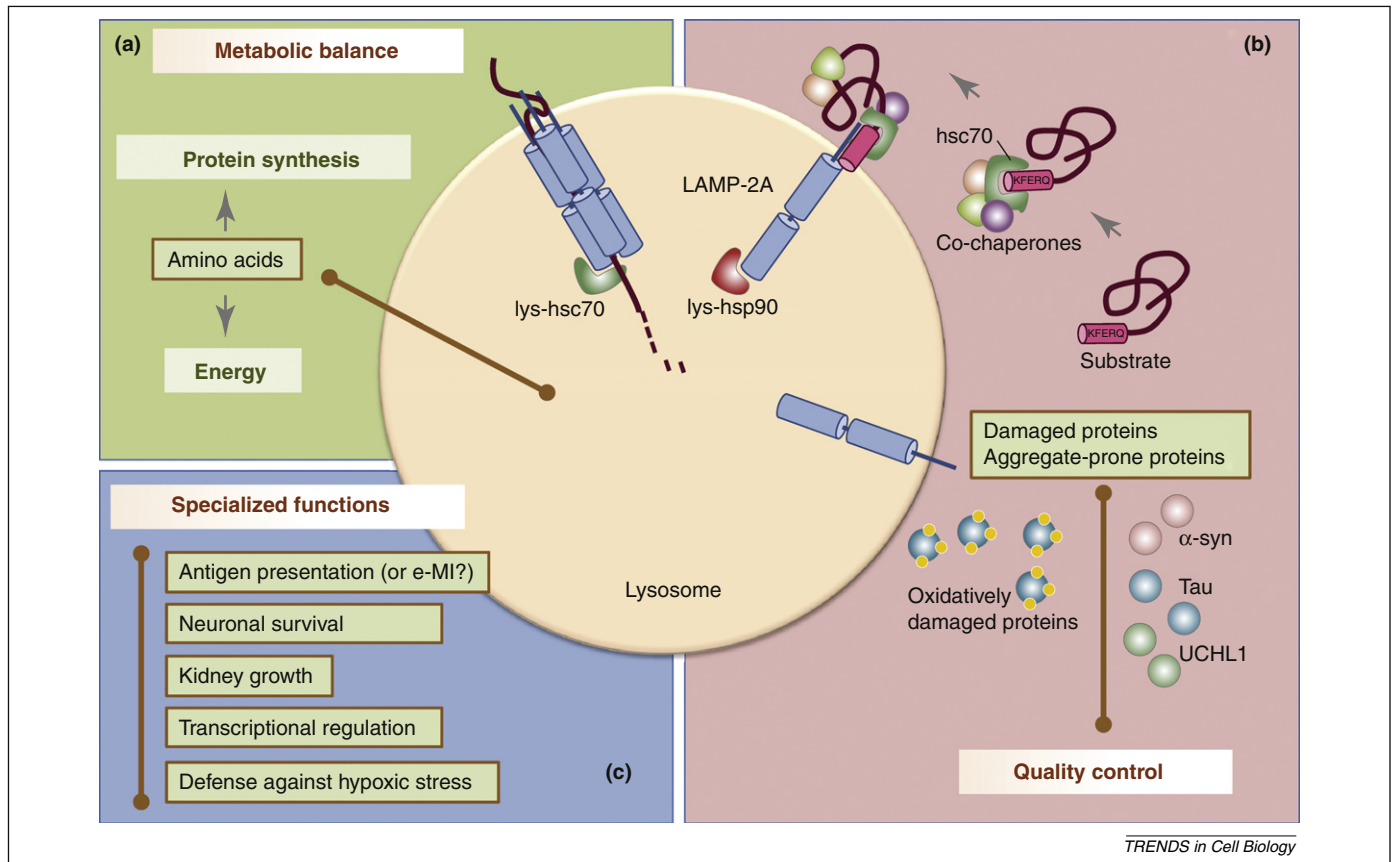


Figure 2. Functions of CMA. (a) By degrading no longer needed proteins during nutrient deprivation, CMA provides amino acids for the synthesis of needed proteins and generates energy. (b) By degrading damaged and prone-to-aggregate proteins, CMA is an essential component of the cellular quality control system. (c) CMA also has specialized functions, depending on the substrate protein and cells/tissues involved. Some examples discussed in the text are listed. Abbreviations: CMA, chaperone-mediated autophagy; e-MI, endosomal microautophagy; hsc70, heat shock cognate protein of 70 kDa; LAMP-2A, lysosome-associated membrane protein type 2A; lys-hsc70, lysosome-associated hsc70; α syn, α -synuclein.

or catalytic subunits of the 20S proteasome [36]) and channel their amino acids for the synthesis of proteins needed [37]. In tissues such as liver, CMA activity plateaus at approximately 36 h of starvation and remains active until three days [34]. In addition to assuring protein synthesis, part of the amino acids provided through CMA can be utilized by the cells to generate energy, because CMA-deficient cells have reduced levels of ATP when deprived of nutrients [38].

Another important function of CMA is protein quality control via the selective degradation of damaged or altered proteins (Figure 2b). CMA is upregulated in response to stressors that cause protein damage, such as mild oxidative stress [39,40], or protein denaturing compounds [41]. Partial unfolding or the formation of protein adducts can upregulate degradation of particular proteins via CMA, preventing their aggregation into insoluble protein inclusions [42,43]. In fact, oxidized and aggregated proteins accumulate in CMA-incompetent cells, rendering them more susceptible to a variety of stressors, and further supporting the contribution of CMA to the maintenance of proteome stability [38].

Recent studies have reported CMA activation in response to hypoxic stress and the need for functional CMA under these conditions to guarantee neuronal survival [44]. Future studies are needed to clarify whether CMA is required to maintain the energetic cellular balance or to facilitate

removal of altered components during hypoxia. However, the fact that CMA activation occurs 7 days after ischemic injury is more supportive of the latter possibility.

Specific functions

CMA participates in specialized functions, in a cell type-specific context, depending on the substrate proteins being degraded by this pathway (Figure 2c).

Several well-characterized CMA substrates are transcription factors or inhibitors of transcription factors, making transcriptional regulation an important function of CMA. For example, during nutrient deprivation, CMA degrades a fraction of cellular I κ B α , the endogenous inhibitor of the transcription factor NF- κ B, allowing NF- κ B to activate the transcriptional program required for adaptation to prolonged starvation [45]. Changes in the oxidative status of I κ B α during starvation are the trigger for its degradation by CMA. In other instances, CMA controls changes in transcription factors only in specific tissues. For example, malfunctioning CMA in kidney epithelial cells has been shown to compromise the degradation of the paired box gene 2 (Pax2), a protein important for cell proliferation and differentiation [46]. Failure to degrade Pax2 in these cells results in abnormal growth of kidney cells and kidney hypertrophy. Similarly, functional compromise of CMA in neurons favors accumulation of inactive molecules of the myocyte-specific enhancer factor

2D (MEF2D), a transcription factor required for neuronal survival, rendering cells more susceptible to stressors [47].

CMA also contributes to MHC class II antigen presentation in professional antigen-presenting cells (macrophages) (Figure 2c). Manipulations in hsc70 and hsp90 in these cells reduce their CMA rates and antigen presentation [48,49]. In light of the recently described participation of hsc70 in the delivery of cytosolic proteins to late endosomes, a compartment classically associated with antigen presentation, it now becomes of great interest to elucidate the relative contribution of CMA and e-MI to this immune function.

More CMA functions will undoubtedly be added to the already growing list as new substrates for CMA are identified.

CMA in pathology and aging

Neurodegeneration

Over the past years, defects in CMA have been linked to the pathogenesis of some severe neurodegenerative disorders. Accumulation of pathogenic proteins, in the form of insoluble inclusions, is a common factor underlying all these diseases. Many of these pathogenic proteins bear CMA-targeting motifs making them potential CMA substrates. For example, KFERQ-like sequences have been identified in Parkinson's disease (PD)-associated proteins α -synuclein, parkin, UCHL1 and pink-1; the Alzheimer's disease (AD)-associated proteins APP and tau; and the protein mutated in

Huntington's disease (HD), huntingtin. These proteins can be degraded by CMA only when they are in soluble forms; once the insoluble inclusions are formed, they can only be degraded via other proteolytic pathways [50].

Interestingly, in many instances not only is CMA unable to degrade the pathogenic variants of these proteins, but they also exert an inhibitory effect on CMA function. Studies with isolated lysosomes [42], neuronal cultured cells [42,43,51], and mouse models of PD [52] demonstrated that wild-type α -synuclein is a CMA substrate, but its pathogenic variants are less amenable to this autophagic degradation. Pathogenic α -synucleins are properly recognized by cytosolic hsc70 and targeted to lysosomes, but they fail to cross the lysosomal membrane due to their organization into irreversible oligomeric complexes, which at the same time, block the degradation of other CMA substrates via steric hindrance (Figure 3a) [42,43]. Similar abnormal interaction with CMA components has been shown for the mutant forms of UCHL1 [53].

Two proteins implicated in AD, tau [54] and regulator of calcineurin 1 (RCAN1) [55], have been confirmed as CMA substrates. Mutant variants of tau demonstrate impaired CMA activity, in this case due to their partial translocation across the membrane, that results in their cleavage while still bound to the membrane and the generation of toxic amyloidogenic peptides that disrupt lysosomes [54]. Blocking CMA of this tau mutant by preventing its binding to

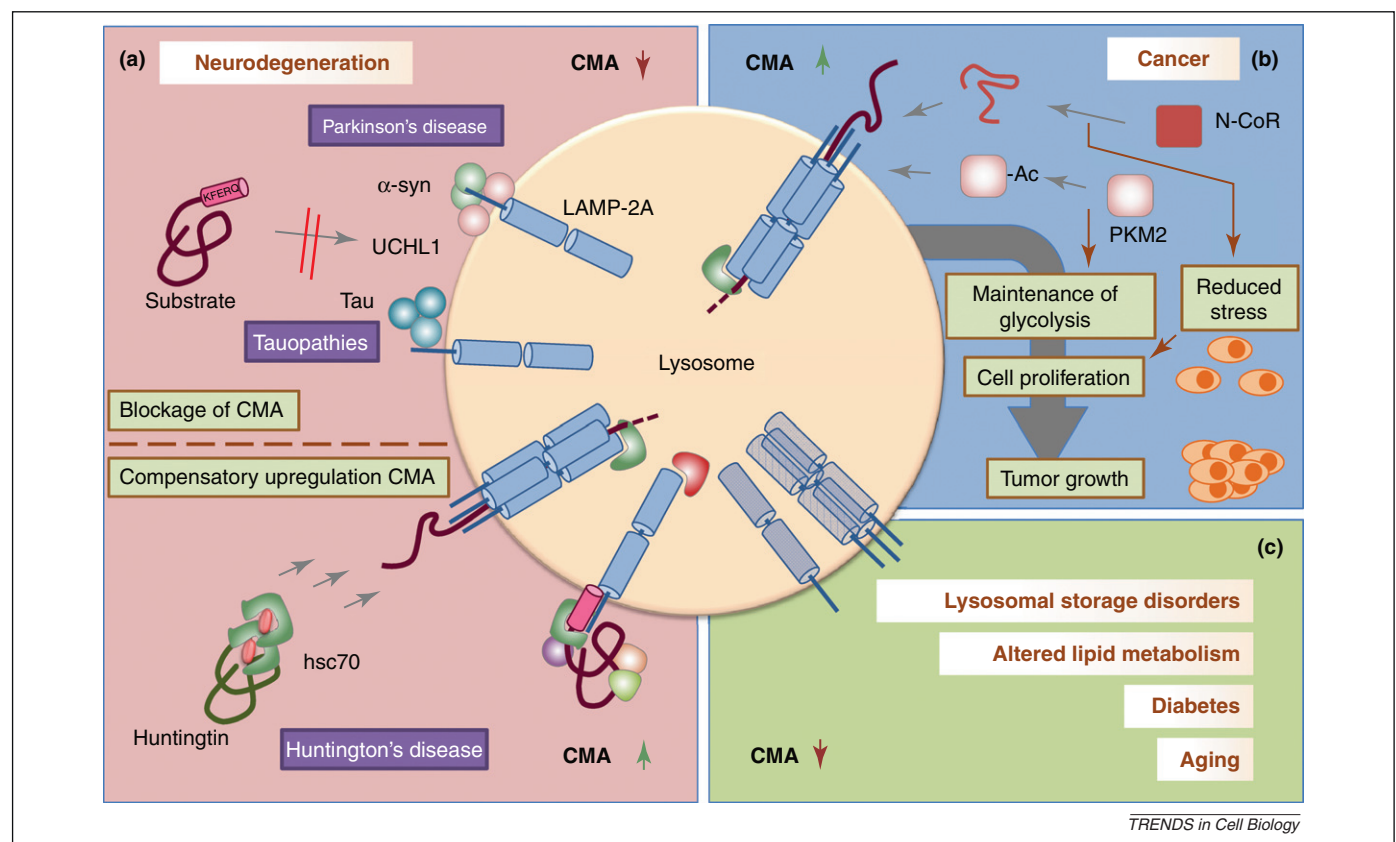


Figure 3. Pathology of CMA. (a) Top: CMA is primarily compromised in some neurodegenerative diseases due to direct blockage of the translocation machinery by the pathogenic proteins. Bottom: upregulation of CMA activity in Huntington's disease compensates for the blockage in other degradative pathways and has been utilized for artificial targeting of the toxic protein. (b) Cancer cells depend on CMA to sustain cellular proliferation and tumor growth. Continuous CMA activation maintains glycolytic activity and reduces stress in cancer cells. (c) CMA activity is compromised in disorders that compromise lysosomal function and in metabolic diseases such as those related to alterations in lipid metabolism and diabetes. CMA activity also decreases with age. Abbreviations: Ac, active; CMA, chaperone-mediated autophagy; hsc70, heat shock cognate protein of 70 kDa; LAMP-2A, lysosome-associated membrane protein type 2A; N-CoR, nuclear receptor corepressor; PKM2, M2 isoform of pyruvate kinase; α syn, α -synuclein.

Box 3. CMA in the context of other proteolytic systems

Autophagy and the ubiquitin-proteasome system coexist in all mammalian cells and growing evidence supports some level of crosstalk between them. As part of the autophagic system, CMA actively interacts with other autophagic pathways and with the ubiquitin-proteasome system.

Crosstalk with macroautophagy

Cultured cells knocked down for the CMA receptor, LAMP-2A, show compensatory upregulation of macroautophagy [38]. Even though macroautophagy and CMA are distinct and not redundant in terms of specific functions, they can compensate for each other. Cells respond to blockage of one of these pathways by upregulating the other; in the absence of any added stress, this compensation is enough to maintain normal cell viability. However, when the cells are exposed to stressors (i.e., mild oxidation, UV light, toxic compounds), which increase the abundance of damaged proteins that are normally degraded by CMA, the cells with compromised CMA become significantly less viable, despite macroautophagic activation [38]. Conversely, cells with abrogated macroautophagic activity demonstrate constitutive upregulation of CMA [73], highlighting the bidirectional nature of the crosstalk between these two pathways. Compensatory activation of CMA in response to macroautophagic compromise has been confirmed *in vivo* in an HD mouse model, highlighting the importance of the autophagic crosstalk in the prevention of pathological manifestations [59]. Although the mechanisms that modulate the intercommunication

between macroautophagy and CMA remain for the most part unknown, in the HD setting the increase in CMA was attained through both transcriptional upregulation and downregulation of degradation of LAMP-2A to increase the levels of this receptor [59]. Increased CMA in HD seems to be initially sufficient to maintain proteomic stability, but the functional decline in this pathway with age accelerates pathological manifestations of this neurodegenerative disorder.

Crosstalk with the ubiquitin-proteasome system

The crosstalk between the autophagic pathways is also extended to the ubiquitin-proteasome system. Acute blockage of the proteasome results in both activation of macroautophagy [74] and of CMA [15], whereas chronic blockage leads to macroautophagic deregulation [75]. By contrast, inhibition of macroautophagy seems to exert a negative effect on the proteasome, in part through competition for the enzymes that remove the ubiquitin tag from the substrates [76]. The blockage of these two major proteolytic pathways under these conditions makes even more relevant the compensatory activation of CMA in response to blockage of either. Specific proteasome activities are also compromised in situations with chronic blockage of CMA [38]. It is possible that the decrease in the proteolytic activities of the proteasome observed in cells with compromised CMA is a consequence of their poor turnover because some of the subunits of the proteasome have been previously identified as *bona fide* CMA substrates [36].

hsc70 improves cell viability because it reduces lysosomal toxicity [54].

Interestingly, in the case of HD, CMA acts more as a rescue compensatory pathway for the primary defects in the ubiquitin-proteasome system and in macroautophagy that occur in the affected cells [56–58]. HD models and cells from patients display constitutive activation of CMA [59], and experimental upregulation of CMA decreases toxicity in HD brain slices [19]. This compensatory activation is possible because of the close interrelation of CMA with the other proteolytic systems (Box 3). Interestingly, despite the presence of multiple KFERQ-like motifs in huntingtin, the protein mutated in HD patients, its degradation via CMA under normal conditions is negligible [59]. However, recent studies have succeeded in artificially enhancing the targeting of mutant huntingtin for CMA degradation [60], suggesting that the constitutive upregulation of CMA in this disorder could be exploited for therapeutic purposes (Figure 3a).

Cancer

Growing numbers of connections have been recently established between CMA and cancer biology. CMA has been shown to be upregulated in a wide collection of cancer cell lines, and levels of LAMP-2A, a surrogate for CMA activation, are also consistently higher in human tumor samples than in the corresponding healthy tissues [61]. Genetic manipulations have revealed that CMA is essential for cancer cell proliferation, tumor growth and generation of metastasis, and that blockage of CMA in already established tumors induces their shrinkage [61]. The favorable effect of CMA on cancer cell proliferation probably results from the combination of several mechanisms (Figure 3b). So far, the strongest candidate is the connection between CMA and glucose metabolism. Cancer cells are highly dependent on glycolysis for the generation of ATP and intermediate metabolites that promote cell proliferation. CMA activation in cancer cells is required to maintain

upregulated transcriptional expression of rate-limiting glycolytic enzymes contributing to increased flux through this pathway [61]. In addition, CMA specifically regulates the balance between the active and inactive form of PKM2, an enzyme highly expressed in tumor cells, recently identified to be a CMA substrate [18]. Interestingly, CMA reduced the levels of the inactive enzyme upon its tagging by acetylation and thus maintains a positive balance for the active form [18].

Beyond the glycolytic control, in specific types of cancer CMA reduces the cellular stress of the rapidly growing tumor. For example, degradation by CMA of the unfolded protein nuclear receptor corepressor (N-CoR) that is highly expressed in some types of lung cancer ameliorates ER stress and promotes the activation of survival pathways in these cells (Figure 3b) [62].

Despite this apparent favorable impact of CMA in cancer progression, CMA could exert an anti-oncogenic effect in normal cells by maintaining the cellular response to stressors and reducing cellular damage. In addition, CMA contributes to the degradation of at least one pro-oncogenic protein, the epidermal growth factor receptor pathway substrate 8 (Eps8) that is associated with promotion of numerous solid malignancies [63]. Future studies are needed to characterize better the contribution of CMA to the prevention of cancer.

Other pathologies

Changes in CMA have been described in different toxic-induced kidney pathologies. CMA is initially upregulated to eliminate the altered proteins generated by the toxic insult, but eventually becomes insufficient to fight against toxicity [41]. The kidney is also the organ predominantly affected by the decrease in CMA observed in diabetic mouse models, where compromised CMA contributes to the diabetes-induced nephropathy [64]. Diseases affecting lysosomes (i.e., lysosomal storage disorders) also impact

upon CMA indirectly because the undegraded material that accumulates in lysosomes in these disorders interferes with normal lysosomal functioning. In addition, CMA is primarily affected in lysosomal disorders such as galactosialidosis or mucopolipidosis because the protein mutated in these conditions also has a function in CMA [65,66].

Aging

Decline in CMA activity with age has been described in almost all cell types and tissues, both in rodents and in cells derived from human subjects [67,68]. This decrease is primarily due to a reduction in the levels of LAMP-2A at the lysosomal membrane [67]. Transcription, synthesis, and targeting of LAMP-2A to lysosomes once synthesized take place at similar rates in young and old individuals. However, the stability of LAMP-2A at the lysosomal membrane is markedly reduced with age, resulting in a decrease in the net content of this protein in lysosomes [69]. Changes in the lipid composition of the lysosomal membrane with age are one of the primary reasons for the reduced stability of LAMP-2A [29,30]. Genetic manipulations aimed at preventing the decrease of LAMP-2A with age, by expressing an exogenous copy of this transgene in mouse liver, have proven sufficient to restore CMA activity, improve cellular homeostasis, and preserve the function of this organ in aged rodents [70]. These studies support the notion of an active anti-aging function for CMA and the possible therapeutic value of modulating levels of LAMP-2A in aged organisms to extend their health-span.

Concluding remarks

In summary, the identification of selective degradation of proteins by CMA has expanded the number of regulatory functions attributable to the breakdown of proteins in lysosomes. Despite the number of outstanding questions about this autophagic pathway (Box 4), recent studies have stressed that cytosolic hsc70 determines which proteins are degraded by CMA and when, but the rate of degradation depends on the unique characteristics of the transient translocation system. The methods currently available to study CMA allow measurement of both the overall activity of this pathway as well as changes in its individual steps [71,72]. A combination of both types of approaches should be useful to identify additional pathological conditions in which CMA is altered and to dissect the steps of CMA responsible for its failure.

Together, the array of cellular functions in which CMA participates highlights the importance of this autophagic pathway at the forefront of the response to cellular stress. The ability of CMA to identify and degrade single proteins, a characteristic shared with the proteasome, makes it a preferred route when removal of specific proteins is needed (selective degradation of proteins has been described for macroautophagy but only when they form part of large protein complexes). However, as for other cellular processes, the strength of CMA (the one-by-one translocation of single proteins to allow selective removal) also becomes its weakness under pathological conditions, due to the ease of clogging of this translocation system by abnormal proteins.

Interventions to prevent CMA blockage and to improve its functioning in old organisms may have therapeutic

Box 4. Outstanding questions

Despite recent advances in our understanding of the molecular mechanisms that govern CMA and its contribution to cellular physiology, there are still important pending questions about this autophagic pathway:

- What are the groups of CMA substrates that reach this pathway upon post-translational modifications to complete a CMA-targeting motif? Are there dedicated kinases and acetylases to modulate their CMA targeting?
- How does hsc70 discriminate between targeting for CMA and e-MI? What are the subsets of cytosolic and lysosomal co-chaperones that modulate hsc70 function in CMA?
- What are the changes in the lipidome and proteome of the lysosomal membrane that physiologically contribute to regulation of CMA?
- What are the energetic requirements and driving forces of substrate translocation through the LAMP-2A-enriched multimeric complex?
- What are the molecular regulators of the crosstalk of CMA with other proteolytic pathways? Does this crosstalk expand to e-MI?
- Is the age-related defect of CMA universal, or are there cell type-specific differences in the steps and components of this pathway that are responsible for its functional decline in aging?
- Does CMA contribute to cellular functions common for other autophagic pathways such as host defense against pathogens, cellular remodeling, protein secretion, and programmed cell death?

value in age-related diseases. By contrast, the recently described dependence of cancer cells on CMA suggests that inhibition of this pathway may be of use as an anti-oncogenic therapy. In this respect, it is possible that neutralizers of lysosomal pH such as chloroquine, currently in use in anti-cancer clinical trials, exert their effect in part by compromising CMA, because the lysosome is shared by the different autophagic pathways. However, the challenge remains in designing more selective ways to compromise CMA activity without affecting the other types of autophagy.

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