Chaperone-mediated autophagy: Role in disease and aging

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Abstract

This review focuses on chaperone-mediated autophagy (CMA), one of the proteolytic systems that contributes to degradation of intracellular proteins in lysosomes. CMA substrate proteins are selectively targeted to lysosomes and translocated into the lysosomal lumen through the coordinated action of chaperones located in both sides of the membrane and a dedicate protein translocation complex. The selectivity of CMA permits timed degradation of specific proteins with regulatory purposes supporting a modulatory role for CMA in enzymatic metabolic processes and subsets of the cellular transcriptional program. In addition, CMA contributes to cellular quality control through the removal of damaged or malfunctioning proteins. Here, we describe recent advances in the understanding of the molecular dynamics, regulation and physiology of CMA and discuss the evidences in support of the contribution of CMA dysfunction to severe human disorders such as neurodegeneration and cancer.

Introduction

Intracellular proteins are subjected to continuous turn-over through coordinated synthesis, degradation and recycling of their component amino acids. This constant renewal of the proteome assures its proper functioning and permits a tight control of intracellular levels of proteins as a way to modulate multiple intracellular processes [1, 2]. Proteins can undergo degradation by the proteasome or by lysosomes. Delivery of proteins to lysosomes for degradation, or autophagy, can occur through different mechanisms[3]. In some instances, such as macroautophagy, proteins are sequestered in vesicles that form in the cytosol and then fuse with lysosomes to transfer their contents for degradation. In other cases, such as microautophagy, proteins are trapped inside vesicles that form directly through the invagination of the lysosomal membrane. These vesicles then pinch off into the lysosomal lumen and are degraded by the proteases inside lysosomes. However, not all lysosomal delivery involves vesicles. Proteins can be targeted from the cytosol to the lysosomal membrane and then gain access to the lumen of this organelle by directly crossing its membrane. This process is known as chaperone-mediated autophagy (CMA) and constitutes the focus of this review [4].

One of the distinctive features of CMA is that proteins that undergo degradation by this autophagic pathway are selected individually through a recognition motif in their amino acid sequences [5]. This allows for the removal of specific proteins without disturbance of neighboring ones and makes CMA an efficient system for degradation of damaged or abnormal proteins and of surplus subunits of multi-protein complexes. In addition, this selectivity permits CMA to play a regulatory role in multiple cellular processes by contributing to modulate intracellular levels of enzymes, transcription factors and cell maintenance proteins [4].

In this review, we first summarize the main steps and components involved in CMA and the diversity of physiological functions attributed to this autophagy pathway, and then we discuss the consequences of CMA malfunctioning in the context of disease and aging.

How does CMA work?

CMA is a multi-step process that involves: I) substrate recognition and lysosomal targeting; II) substrate binding and unfolding; III) substrate translocation and IV) substrate degradation in the

lysosomal lumen (Fig. 1A).

Recognition of substrate proteins takes place in the cytosol through the binding of a constitutive chaperone, the heat shock cognate protein of 70KDa (hsc70), to a pentapeptide motif present in the amino acid sequences of all CMA substrates[6]. This motif consist of an invariant amino acid, a glutamine (Q) residue, at the beginning or end of the sequence, followed by one of the two positively charged amino acids, lysine (K) or arginine (R), one of the four hydrophobic amino acids, phenylalanine (F), valine (V), leucine (L) or isoleucine (I) and one of the two negatively charged amino acids, glutamic acid (E) or aspartic acid (D) [5]. The fifth amino acid in the sequence can be one of the positive or hydrophobic residues. The fact that the CMA motif is based on the charge of the amino acids makes it possible to create a motif out of an incomplete four amino acid motif through posttranslational modifications such as phosphorylation or acetylation. Motifs can also become accessible for chaperone recognition after protein unfolding - in the case of motifs buried in the core of the protein -, after proteins diassemble from multiprotein complexes - if the motif was hidden in the regions of protein-to-protein interaction- , or when proteins are released from the subcellular membranes – in those instances where the motif is in the region of binding to the membrane.

Once bound to the chaperone, the substrate is targeted to the surface of the lysosomes where it interacts with the cytosolic tail of the single span membrane protein lysosome-associated membrane protein type 2A (LAMP-2A[7])(Fig. 1A). This protein is one of the three variants that originate from the alternative splicing of a single gene (lamp2) and that have different cytosolic and transmembrane regions, but share a common luminal domain. LAMP-2A is present at the lysosomal membrane as monomers and in association with other proteins to form a multi-protein complex required for translocation[8]. The assembly of LAMP-2A into this complex is dynamic and is driven by the binding of the substrate to this receptor protein. During the transition from monomer to multimer, the stability of LAMP-2A is maintained through its interaction with a form of hsp90 located in the luminal side of the lysosomal membrane [8]. The substrate can bind to the receptor while still in a folded state, but in order to cross the lysosomal membrane, it required that the substrate undergos unfolding[9]. This process is likely mediated by hsc70 and some of its cochaperones detected at the lysosomal membrane and is completed before the LAMP-2A complex is fully assembled (Fig. 1A).

Translocation of the substrate protein across the lysosomal membrane requires the presence

of a form of hsc70 (lys-hsc70) normally resident in lysosomes[10]. Although the specific way in which this chaperone contributes to translocation has not been elucidated yet, it has been proposed that it may function actively by pulling the substrate proteins in a ratchet-like manner, or alternatively may hold onto the substrate passively to prevent its return to the cytosol. Hsc70 bears in its amino acid sequence targeting motifs for CMA and can indeed behave as a substrate protein[11]. However, it is unlikely that lys-hsc70 reaches the lysosomal lumen through CMA since experimental blockage of CMA does not affect the content of hsc70 in lysosomes[12]. Likewise, blockage of macroautophagy does not reduce r the luminal content of hsc70 either, discarding that the chaperone could be delivered upon cytosolic sequestration through autophagosome-lysosome fusion[13]. It is possible that hsc70 is internalized as multivesicular bodies from on the surface of late endosomes and reaches the lysosomal compartment through endosomal-lysosomal fusion. Once in the lumen, hsc70 stability is highly dependent on the lysosomal pH, whereby small increases in pH are sufficient to promote its degradation in this compartment[11].

After the substrate is translocated into the lysosomal lumen, LAMP-2A is rapidly dissembled from the translocation complex into monomers where substrates can bind again[8]. Binding and translocation of substrates by CMA are coordinated steps but they can be dissociated under conditions that compromise assembly of LAMP-2A into the translocation complex. Consequently, the rate of CMA can be modulated by the rate of assembly/disassembly of the translocation complex [8]. Multiple factors may participate in the regulation of this step, such as, for example, changes in the fluidity of the lysosomal membrane that affect lateral mobility or changes in protein density of this membrane [14]. To date, a pair of proteins, GFAP and EF1α have been the first ones described to specifically modulate LAMP-2A assembly/disassembly in a GTP-dependent manner (since EF1α is a GTP binding protein) [15]. Association of GFAP to the translocation complex contributes to its stabilization. Once the substrate has passed through the complex, disassembly occurs by the mobilization of GFAP from the complex to phosphorylated forms of GFAP resident in the membrane and normally caped by EF1α.

Rates of CMA are also directly dependent on the content of LAMP-2A at the lysosomal membrane. Levels of LAMP-2A can be regulated through transcriptional upregulation, as in the case

of oxidative stress [16], or through changes in the degradation rate of LAMP-2A at the lysosomal membrane, as occurs when CMA is upregulated during prolonged starvation [17, 18]. Although, there is still relatively limited information about the signaling pathways that contribute to modulate CMA activity, a negative regulatory effect of the nuclear receptor retinoic acid receptor alpha on CMA has been recently described [19]. Signaling through this receptor inhibits, among others, LAMP-2A transcription and this blockage is released when CMA upregulation is required.

Validation of a protein as a CMA substrate requires more than the mere identification of a putative CMA targeting motif in its amino acid sequence, as this motif has also been recently shown to be utilized for targeting of proteins to late endosomes for endosomal microautophagy [20]. Although both CMA and endosomal microautophagy share hsc70 as the targeting chaperone, the dependence on LAMP-2A is exclusive for CMA. Ultimately, the best way to confirm that a protein is a CMA substrate is to reproduce its binding and translocation across the membrane of isolated lysosomes [21].

What are the physiological functions of CMA?

The first role proposed for CMA was to contribute to amino acid recycling during prolonged starvation, a condition in which CMA is maximally activated [22](Fig. 1B). In fact, although some levels of basal CMA activity can be detected in almost all cells, starvation has been one of the best characterized stimuli for CMA. In contrast to macroautophagy that becomes activated shortly after starvation and, reaches its maximal peak, at least for protein degradation, around 4-6 h, CMA is gradually activated after 8-10 h of starvation and persists at maximal activity for up to three days [12, 22]. Proteins degraded under these conditions were thought to be those proteins no longer necessary for cells under starvation which could be broken down for amino acid recycling to sustain synthesis in the absence of nutrients. However, it is also possible that this regulated degradation as starvation persists allows for changes in the proteome aimed at adapting the cell to the new conditions. For example, degradation of inhibitors of transcription factors by CMA during starvation has already been demonstrated for specific transcription programs [23]. Likewise, degradation of regulatory metabolic enzymes by CMA has been shown to contribute to the metabolic changes that allow, for example, for cancer cells to adapt to low nutrient conditions [24, 25].

The other important function that CMA fulfills in cells is quality control, directly linked to the

ability of this pathway to selectively remove single proteins from the cytosol (Fig. 1B). CMA is upregulated during oxidative stress, when it contributes to the degradation of oxidized proteins [16], and in fact, inability to upregulate CMA under those conditions results in marked accumulation of oxidative damage and reduced cellular viability [12]. It is also possible that CMA contributes to quality control of cytosolic assembled protein complexes by eliminating excess subunits. For example, different subunits of the catalytic core of the proteasome bear CMA-targeting motifs and have been shown to be selectively degraded in lysosomes by CMA [26]. Whether their degradation occurs pre-assembly or if subunits that already formed part of the proteasome are actively disassembled and targeted to CMA as a way to reduce overall proteasome activity, requires future investigation.

CMA is also upregulated in other conditions resulting in protein damage such as exposure to denaturing toxic compounds [22]. Activation of CMA has also been shown to support survival of retinal cells upon activation of a pro-apoptotic program in those cells [27]. Recent studies have demonstrated that CMA is activated during hypoxia and that this activation is required for cell survival [28]. Although the specific substrates degraded by CMA under these conditions are not fully elucidated the fact that the hypoxia-inducible factor 1 has been confirmed as a CMA[29] substrate supports a possible regulatory effect of CMA on the intensity and duration of the cellular response to hypoxia.

In addition to these general functions of CMA that are, for the most part, cell type-independent, there are also other instances in which activation of CMA contributes to modulate cell type-specific functions (Fig. 1B). For example, degradation of the growth factor Pax-1 by CMA in kidney is important to control tubular cells growth[30] and explains why in conditions such as diabetes when CMA is compromised, kidneys undergo pronounced hypertrophy[31]. Selective degradation of a neuronal survival factor, transcription factor myocyte enhancer factor 2D (MEF2D), has been shown to occur, at least in part by CMA, and it is essential to assure proper neuronal response to injury[32]. In this case, intuitively a reduction of CMA activity could be considered beneficial as it should contribute to increase cellular levels of MEF2D. However, the MEF2D removed by CMA is the no longer functional one, which, if it remains in neurons will compromise the functioning of active MEF2D. This selective turnover of the inactive forms of proteins by CMA has also been shown in the case of specific cancer cells for the PMK2 enzyme, which, once inactive, is targeted for removal by CMA upon acetylation[24]. In this case too, removal of the non-functional enzyme is required to maintain the functional pool.

Although the purpose of CMA in most cells is the complete degradation of proteins into their constituent amino acids, in some specialized cells such as antigen presenting professional cells, a role for CMA in antigen presentation has been proposed[33]. Future studies are needed to determine if partial degradation of CMA substrates can also occur in other cell types. Study of the consequences of CMA blockage in different cells types could help to identify additional cell-specific functions. However, to date, the best way to block this autophagic pathway is through knock-down or knock-out of LAMP-2A, because manipulation of any of the other CMA components (hsc70, hsp90, GFAP or EF1α), is usually more difficult to interpret due to the additional cellular functions of these proteins. Although chemical activators of CMA are now available [19], considerably less progress has been made in the identification of specific inhibitors of this pathway. Unfortunately, some of the small-molecules initially described to modulate this pathway [34] have proven to be rather non-specific and to affect the activity of other cellular quality control mechanisms, again making interpretation of results obtained with these drugs difficult.

How do alterations in CMA contribute to disease?

Increasing evidence demonstrates that malfunction of CMA plays a key role in the pathogenesis of severe human disorders [35-37]. Often, the mechanisms underlying the alterations of CMA in these pathologies involve perturbations in the functioning of the CMA translocation complex. Both diminished and enhanced CMA activity have been shown to associate with diseases, which highlights the importance of a tight regulation of CMA activity. In this review, we have selected neurodegenerative diseases as an example of pathologies associated with reduced CMA activity, and oncongenic processes as an example of pathological conditions in which enhanced CMA activity facilitates disease progression.

Reduced CMA and neurodegeneration

A common theme unifying different neurodegenerative pathologies is the failure of the proteolytic systems to adequately dispose of deleterious proteins[2, 38]. Such mishandling of aberrant proteins alters proteostasis and often leads to the precipitation of protein aggregates that contribute to neuronal demise[39]. The involvement of CMA in neurodegeneration is two-fold, as it contributes to the

elimination of pathogenic proteins, but also often, becomes victim of the toxic effect of these aberrant proteins[40-43]. This dual role of CMA in neurodegenerative disorders makes it necessary to analyze the status of this autophagy pathway in disease in order to determine whether interventions aimed at enhancing CMA activity could be of potential value or not, depending on the degree of compromise of this pathway and the reversibility of the CMA blockage.

Parkinson's disease (PD). Impairment of CMA is intimately linked to the pathogenesis of Parkinson's disease (PD)- the most prevalent neurodegenerative movement disorder that is characterized by the selective loss of dopaminergic neurons and subsequent motor deficits. Dysfunction in CMA has been described in both familial [40, 42, 44, 45] and sporadic [41, 42] PD. In the case of familial PD, sequence analysis reveals the presence of CMA targeting motifs in the majority of PD-related proteins, supporting an important role for CMA in the control of their intracellular levels (Fig. 2A). Indeed, the two most commonly mutated proteins in patients with familial PD, α-synuclein and leucine-rich repeat kinase 2 (LRRK2), have both been demonstrated to undergo degradation in lysosomes via CMA using various experimental systems such as isolated lysosomes, primary mouse neuronal cultures, mouse models of PD and even neuronal-differentiated induced pluripotent stem cells (iPSc) and brains from familial[40, 42, 44, 45] and sporadic [41, 42] PD patients. In contrast, pathogenic mutant variants of α-synuclein (eg: A30P and A53T mutants) and of LRRK2 (eg: G2019S and R1441C mutants), despite being recognized by cytosolic hsc70 and successfully delivered to the lysosomal membrane, fail to reach the lysosomal lumen to be degraded by CMA[40, 42] (Fig. 2A). Internalization of α-synuclein and LRRK2 mutants into lysosomes is obstructed due to aberrant interactions of these toxic proteins with the LAMP-2A receptors. Pathogenic α-synuclein mutants bind to LAMP-2A receptors with abnormally high affinity thus preventing its translocation across the lysosomal membrane[40] (Fig. 2A). Similarly, LRRK2 mutant proteins show enhanced lysosomal binding in the presence of other CMA substrates, which in the process interferes with the proper organization of the active CMA translocon[42] (Fig. 2A). Both toxic interactions of α-synuclein and LRRK2 mutants with the CMA transporter preclude not only their own degradation, but also inhibit the degradation of other CMA substrates[40, 42].

Particularly noteworthy is that mutations in one of the PD-related proteins also interfere with

the degradation A of other pathogenic proteins by CMA [42]. For example, mutant LRRK2 exacerbates the intracellular accumulation of α-synuclein, in part by preventing its clearance through CMA. Although α-synuclein is still delivered to lysosomes it fails to translocate inside lysosomes due to the disruption of the CMA translocation complex by LRRK2 (Fig. 2A). Interestingly, the persistence of α-synuclein bound to the lysosomal membrane, promotes its multimerization into toxic oligomers that further compromise CMA activity and could in principle contribute to the seeding of protein aggregates characteristic of this disorder. These dual pathogenic effects of reduced elimination of the pathogenic protein and additional CMA blockage should considerably trigger a more severe PD pathology in LRRK2 mediated PD cases. Similar abnormal interaction with CMA components has also been described for another PD-associated protein, the ubiquitin C-terminal hydrolase L1 (UCH-L1). Wild-type UCH-L1 interacts with CMA-related chaperones and LAMP-2A, but the levels of these interactions are abnormally increased by the PD-linked I93M mutation in UCH-L1 leading to blockage of α-synuclein degradation by CMA[46] (Fig. 2A).

Alterations of CMA have also been implicated in sporadic PD, which accounts for the majority of the PD cases [41, 42]. Perturbation of α-synuclein degradation as a consequence of unfavorable posttranslational modifications caused by environmental or cellular stressors (eg. pesticides, oxidative stress, etc) is a key event in the pathogenesis of sporadic PD and various synucleinopathies[47]. Among the different posttranslational modifications described for the α-synuclein proteins that accumulate in inclusions in PD neurons, dopamine (DA)-modified α-synuclein has shown reduced susceptibility to CMA degradation in a manner similar to that of familial α-synuclein mutants[41]. Like α-synuclein mutants, the tight binding yet inefficient translocation of DA-modified α-synuclein by the CMA translocation complex inhibits the degradation of DA-modified α-synuclein as well as other CMA substrates. As for the mutant variants, the persistence of the posttranslationally modified forms of α-synuclein bound to the lysosomal membrane, and likely the arrival of other CMA substrates at the lysosomal surface, promote the formation of highly toxic α-synuclein oligomers or protofibrils at the lysosomal membrane[41]. Recent studies support that even in the absence of noticeable posttranslational modifications, an increase in the cellular levels of either α-synuclein [40] or LRRK2[42] beyond a tolerable threshold, has very similar inhibitory effects on CMA activity. In fact, these two

proteins seems to potentiate each other's toxic effect on CMA[42].

The proposed involvement of CMA malfunction in PD pathogenesis is further supported by the overt changes in the CMA components seen in the post-mortem brain samples from PD patients. Increased levels of LAMP-2A have been observed in the early stages of PD both in mouse models [44] and in brains of PD patients[42]. However, in advance stages, reduced levels of LAMP-2A and hsc70 have been detected instead in the dopaminergic neurons of human brain regions [48]. In fact, there seems to be good correlation between regional deficiency in LAMP-2A, used as surrogate marker for CMA function, and the selective vulnerability of the brain regions to α-synuclein aggregation[49]. Although, as described in later sections, age-related changes in the LAMP-2A protein itself, could be behind the gradual reduction in CMA and subsequent acceleration of the disease in the older patients, a recent study shows that deregulation of microRNA reported in PD brains may underscore the down-regulation of some CMA components in the affected neurons[50]. In addition, a sequence variation in the promoter region of LAMP-2 identified recently in a PD patient[51], now opens up the possibility that alterations in CMA components may be behind some forms of PD. The fact that both chemical[19] and genetic[52] upregulation of CMA have been shown capable of alleviating cellular toxicity associated with pathogenic forms of α-synuclein supports that the changes in CMA observed in PD are not a consequence of the disease, but that rather they contribute to pathogenesis.

Alzheimer's disease (AD). The compromise of CMA function as a consequence of blockage of this autophagic pathway by the pathogenic variants of proteins that normally undergo degradation by this pathway is not limited to PD. In fact, CMA blockage also plays a role in influencing the levels of neurofibrillary tangles arising from the aggregation of mutant tau proteins associate with Alzheimer's disease (AD) and tauopathies[43]. As in the case of the PD-related proteins, normal tau undergoes degradation by CMA upon hsc70 recognition of one of the two targeting motifs in its C-terminus [43] (Fig. 2B). In contrast, mutant tau variants exhibit peculiar lysosomal processing by CMA. The mutant tau protein, once bound to LAMP-2A, is only partially internalized and the portion of the protein that gains entry into the lysosomal lumen is trimmed, resulting in the formation of smaller amyloidogenic tau fragments at the lysosomal membrane[43]. These fragments oligomerize directly at the surface of

lysosomes, resulting in disruption of the lysosomal membrane integrity and blockage of normal CMA function. Release of these toxic mutant tau oligomers from the lysosomes upon membrane rupture could further seed tau aggregation in the cytosol by acting as a nucleating center (Fig. 3B). Besides tau, a connection between CMA and a second AD-related protein, the regulator of calcineurin 1 (RCAN1) has also been established. CMA degrades RCAN1, a protein whose high expression in AD brains has been linked to neuronal demise[53]. The possible contribution of the blockage of CMA by pathogenic tau to the elevation of RCAN1 levels in the affected neurons awaits evaluation. In light of the described interplay between different PD-pathogenic proteins in the context of CMA it is plausible that the pathogenic effect of mutant tau and the aberrant levels of RCAN1 are interconnected and contribute to determine the severity of the AD phenotype.

Abnormally enhanced CMA and cancer

While CMA deficiency characterizes many neurodegenerative pathologies, upregulation of CMA has been linked to the survival and proliferation of cancer cells [24, 25, 54]. Examination of CMA activity in a wide array of cancer cell lines and human tumor biopsies has demonstrated a consistent increase in basal CMA activity[25]. Activation of CMA is mostly due to an increase in the LAMP-2A levels in these cancer cells and tumors. Genetic knock-down of LAMP-2A in cancer cells helped to establish that CMA is required for cancer cell proliferation, optimal tumor growth and metastasis[25].

The beneficial effect associated with upregulated CMA may be different depending on the cell type. The enhanced CMA in these cells has been shown necessary to sustain enhanced glycolysis to meet the bioenergetic demand of rapid cancer cell growth and proliferation. Selective blockage of CMA in cancer cells results in transcriptional attenuation of several rate-limiting glycolytic enzymes, and the subsequent reduction in glycolysis and ATP production [25]. In other cancers, the decrease in glycolysis observed upon CMA blockage is not due to changes at the transcriptional level, but rather at the protein level. As mentioned in previous sections, the inactive forms of PMK2, one of the limiting glycolytic enzymes, are eliminated via CMA [24]. Blockage of CMA in cancer cells leads to the accumulation of these non-functional forms of PKM2 and the subsequent decrease in glycolysis and energetic compromise. Lastly, it is possible that in other tumors part of the negative consequences of

CMA blockage are due to reduced quality control. While in many cancer cell types, prolonged blockage of CMA elicits upregulation of the ubiquitin proteasome system (UPS), thus preventing accumulation of damaged substrates degraded by CMA [25], in other types of cancers, accumulation of oxidized and misfolded proteins has been proposed to underlie the toxic effect of CMA blockage [54, 55]. The upregulated CMA in these latter cells helps to ameliorate intracellular stress and promotes the activation of oncogenic survival pathways[55]. The mechanism behind induction of CMA in tumorgenesis is still unclear, although it is tempting to postulate that microRNA deregulation may underlie the cancer related increase in LAMP-2A expression as seen in the aberrant microRNA regulation of CMA components in neurodegeneration [50].

Although the dependence of cancer cells on CMA suggest a pro-oncogenic function for CMA, the effect of CMA in normal cells seems to be quite the opposite, as it protects cells from the damage caused by extracellular and intracellular injuries, which, if allow to accumulate could facilitate oncogenesis. In addition, a recent study shows that CMA takes on an anti-oncogenic role in non-proliferating, mutant p53 accumulating tumor cells by reducing the cellular levels of mutant p53 through CMA degradation [56]. Furthermore, CMA has also been shown to control proteolysis of another pro-oncogenic protein, the epidermal growth factor receptor pathway substrate 8 (Eps8) implicated in solid malignancies [57].

In summary, a complex relationship exists between CMA and cancer biology which warrants further studies to better understand the multifaceted roles of CMA in tumorgenesis. However, from the therapeutic point of view, manipulation of CMA is highly promising based on the fact that blockage of CMA in human tumor explants in mice through knock-down of LAMP-2A has proven effective in not only reducing tumor growth and metastasis but also in inducing tumor shrinkage through cancer cell necrotic dead [25].

How does aging affect CMA?

Alterations in CMA are not only limited to pathological conditions. Functional decline in CMA also occurs with physiological aging. Reduced CMA activity has been observed in many cell types and tissues of old rodents as well as in cells derived from aged individuals [58, 59]. Age-dependent decay in CMA appears to be caused by age-related changes in the lipid constituents of lysosomal membrane

that alter the dynamics and stability of the LAMP-2A receptor in the lysosomes of old organisms [14, 59, 60]. While the transcription, synthesis and lysosomal targeting of the LAMP-2A protein during lysosome biogenesis remain unchanged from young to old individuals, the stability of LAMP-2A at the lysosomal membrane is greatly compromised with increasing age [59]. Undesired changes in the lipid composition of the lysosomal membrane abnormally enhance the degradation of LAMP-2A in the lysosomal lumen, and as a result, the binding and translocation of substrate proteins by lysosomes is markedly reduced in older organisms [60]. Interestingly, similar lipid changes can be induced at the lysosomal membrane through diets with high lipidic content, thus underscoring the importance of the diet in the control of this autophagic pathway and the possible acceleration of its decline with age.

Experimental blockage of CMA activity in cultured cells suggests that a direct consequence of the age-dependent failure in CMA is the loss of CMA mediated homeostasis such as the removal of oxidatively damaged proteins and the ability to respond to stressors [61]. Consequently, age-dependent decline in CMA could be a major aggravating factor in accelerating the pathological changes in many age-related disorders. Genetic manipulation to preserve CMA function in old rodents by expressing an exogenous copy of LAMP-2A in mouse liver has proven effective in improving the healthspan of aged animals [62]. Restored CMA functions in the LAMP-2A transgenic animals result in improved cellular homeostasis, enhanced resistance to different stressors and preservation of organ functions. Such pronounced beneficial effects in prolonging healthspan underscore CMA as an important anti-aging mechanism.

Does CMA communicate with other proteolytic systems to maintain proteostasis?

An important aspect to keep in mind when thinking about the contribution of CMA to cellular physiology and disease is the fact that CMA does not function in isolation or completely independently of other degradative systems. On the contrary, CMA activity is tightly coordinated not only with that of other forms of autophagy and with the UPS(reviewed in [63, 64]). The points of interaction between CMA and these systems are multiple and seem to function in different directions.

Compensatory upregulation of CMA has been described to limit the severity of damages caused by primary defects in macroautophagy and UPS in cultured cells (Fig. 3). Cross-talk between

CMA and macroautophagy is exemplified by the observed constitutive activation of CMA in cells deficient in macroautophagy [65]. Similarly, many cells respond to chemical blockage of the proteasome by upregulating CMA [66]. Conversely, macroautophagy is highly induced in response to CMA blockage [12]. Although the functions of macroautophagy and CMA are distinct and non-redundant, they can still compensate for each other to sustain cell survival under normal conditions. However in the event of stressors, this compensatory response may not suffice to allow cells to cope well. Aditionally, compromised CMA perturbs functioning of the UPS, at least during the early stages of acute CMA blockage [67], likely by affecting the turnover of specific proteasome subunits [68] which in turn may alter proteasome assembly. Interestingly, the maintenance of efficient CMA functions in old rodents has also shown to positively preserve UPS activity [62].

This coordinated nature of the proteolytic responses could explain the late onset of many age-related disorders that originate from loss of proteostasis. For example, recent studies have revealed that constitutive upregulation of CMA [69] compensates for the dual failure of macroautophagy [70, 71] and UPS [72] seen in mouse models of Huntington's disease (HD). Activation of CMA in HD is achieved through both enhanced transcription and stability of LAMP-2A in the affected cells[69]. However the ability of CMA to compensate for the severe proteolytic deficiency in HD cells is limited by the progressive functional decline in CMA with age (Fig. 3).

Synergy between macroautophagy and CMA is also crucial in PD and in certain tauopathies where the blockage of CMA is often compensated by activation of macroautophagy [43, 73-75]. This upregulation of macroautophagy is crucial for removing the toxic α-synuclein and tau oligomers to alleviate these conditions [73-75].

Understanding the molecular determinants that efficiently reroute a protein substrate from one degradative pathway to another or upregulating the activity of one pathway over the other would allow us to better manipulate the various proteolytic systems to efficiently correct proteostasis deficiencies and, in this way, slow down disease progression. Molecules such as ubiquitin could become interesting therapeutic targets in lieu of their putative important role in the cross-talk among autophagic pathways. For example, added to the already well-characterized role of ubiquitin in proteasome-mediated degradation, cells utilize specific ubiquitin topology to promote aggregation of

pathogenic proteins in transitory aggresomal structure for removal by macroautophagy[76]. The recent identification of enhancers that promote this type of aggrephagy of pathogenic proteins and of determinants that differentiate their degradation by basal or inducible macroautophagy[74] may allow, in the future, to upregulate specific stress coping mechanisms to prevent overloading and subsequent blockage of other proteostasis components.

Concluding remarks

In recent years, the identification of a plethora of new CMA substrates and of deficiencies in CMA associated with diverse human pathologies have expanded our understanding of the importance of CMA in multiple cellular functions. These findings have also provided a wider perspective of the repercussion that CMA deregulation has on cellular and organismal homeostasis and function. The growing number of connections between CMA and human diseases has generated interest in modulating CMA activity for therapeutic purposes. Genetic manipulation to enhance CMA has proved useful in order to mitigate mutant α-synuclein-induced neurodegeneration in mouse models of PD[52]. Similarly, experimental upregulation of CMA also attenuates the toxicity associated with HD in brain slice cultures [77] and interventions that enhance targeting of the HD toxic protein to CMA have also succeeded in slowing down neurodegeneration in HD mouse models[78]. Interestingly, rather than targeting the pathogenic proteins that induce CMA blockage in each of these diseases, the promising results from attempting to slow down the decline of CMA with age by preserving normal levels of the CMA receptor until late in life, supports that restoration of CMA in old organisms could be of value to prevent a myriad of age-related diseases.

While genetic interventions may be unfeasible or highly challenging in elderly, recent studies have provided more amenable intervention options through the modulation of dietary lipid intake and the development of retinoic acid derivatives that specifically regulate CMA without affecting the other autophagic variants [19, 60]. Inverse interventions may be considered under those instances such as cancer in which blockage of CMA may be useful. However, the challenge remains in administrating selective blockage of CMA only in cancer cells without compromising normal CMA functions in healthy cells. Also of potential therapeutic value are interventions targeting the cross-talk among different

autophagic pathways, although additional research in this area is required before these types of interventions can be implemented. Thus far, little is known about the molecular modulators of this cross-talk and the universality of these processes. For example, while studies in fibroblasts, hepatocytes and neurons support that compensation between CMA and macroautophagy in these cells is bidirectional, recent studies show that retinal cells can respond to the blockage of macroautophagy by upregulating CMA, although this compensation does not work in the opposite direction [79], highlighting a critical role of CMA in the maintenance of retinal homeostasis.

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Legend to Figures

Figure 1. Steps and physiological functions of CMA. A) Proteins degraded by CMA are identified in the cytosol by a chaperone complex that, upon binding to the targeting motif in the substrate protein, (1) brings it to the surface of lysosomes (2). Binding of the substrate to the cytosolic tail of the receptor protein LAMP-2A promotes its multimerization to form a translocation complex (3). Upon unfolding, sustrate proteins cross the lysosomal membrane (4) assisted by a lumenal chaperone and reach the lysosomal matrix where they undergo complete degradation (5). B) General and cell-type specific functions of CMA and consequences of CMA failure in different organs and systems.

Figure 2. Impairment of CMA by pathogenic proteins contributes to neurodegeneration. A) Mechanisms of CMA failure in Parkinson's disease (PD). Many PD-related proteins bear CMA-targeting motifs (α-synuclein, UCH-LI and LRRK2 shown here) (top). LRRK2 has 8 CMA-targeting motifs but only the sequence of the most commonly used is shown. Both wild-type (WT) α-synuclein and LRRK2 are degraded by CMA. Mutant forms of these proteins and of UCH-L1 bind abnormally to CMA receptor LAMP-2A, albeit via different mechanisms, leading to blockage of their own degradation as well as degradation of other CMA substrates. Dopamine modified α-synuclein (DA-syn) and abnormally high levels of WT LRRK2 also impair CMA. CMA failure causes accumulation and aggregation of these toxic proteins that could contribute to Lewy body formation in PD. Alterations of CMA by mutant LRRK2 and UCH-L1 show converging toxic effects on α-synuclein aggregation. B) Perturbation of CMA by mutant tau in tauopathies. WT tau protein is a *bona fide* CMA substrate carrying two CMA-targeting motifs (top). Pathogenic variants of tau fail to translocate fully into the lysosomal lumen. Such inefficient translocation promotes formation of tau oligomers at the lysosomal membrane resulting in destabilization of lysosomal membrane and lysosomal leakage. Release of lysosomal tau oligomers into the cytosol may act as a precursor for further tau aggregation.

Figure 3. Cross-talk between different proteolytic systems. Increasing evidence shows that different proteolytic systems are wired through multi-levels of interactions to maintain cellular homeostasis. Examples of cross-talk implicated in neurodegenerative diseases are highlighted in grey boxes. PD, Parkinson's disease; HD, Huntington's disease; SMA, Spinal muscular atrophy.

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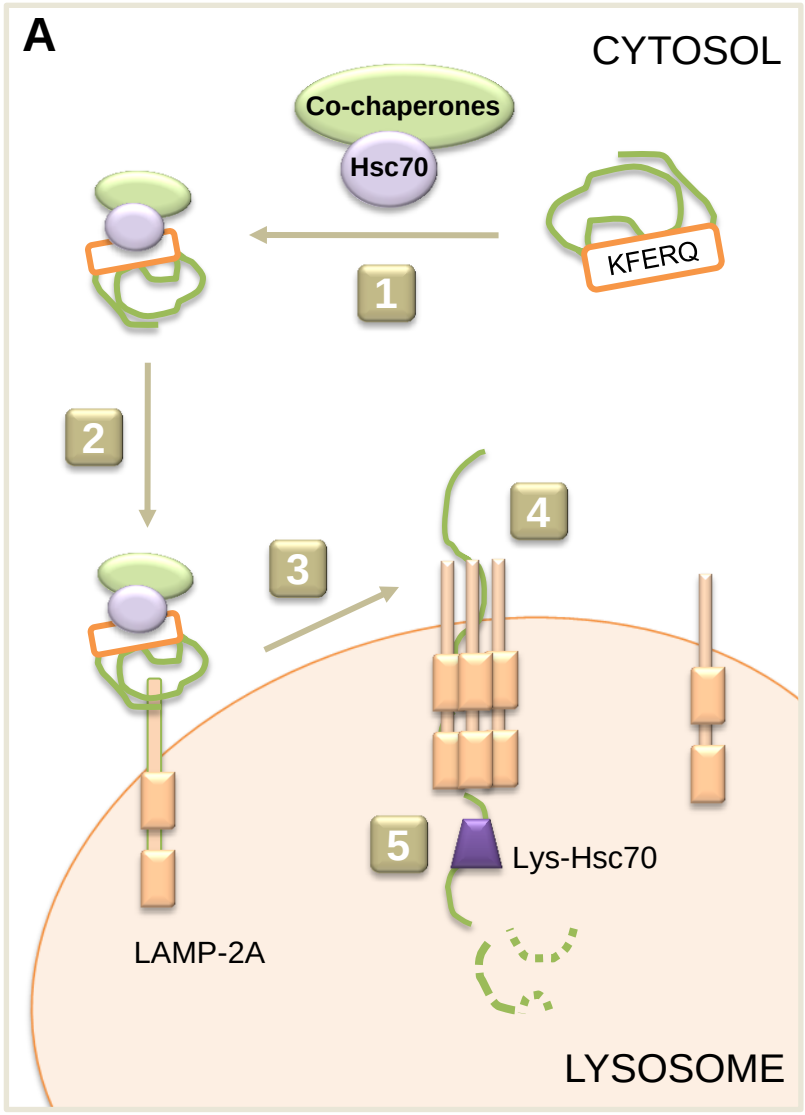
FIGURE 1



B

General functions	Consequences of failure
Energy during Starvation Amino acids	• Energetic compromise • Susceptibility to stress
Quality Control Damaged proteins	• Proteotoxicity • Cellular degeneration • Susceptibility to stress

Cell-type specific functions	Consequences of failure
Neuronal Survival MEF2D	• Neurodegeneration
Kidney Growth Pax2	• Kidney disorders
Antigen Presentation Cytosolic proteins	• Altered immunity
Transcription regulation FOX, IκBα	• Altered cellular processes

FIGURE 2

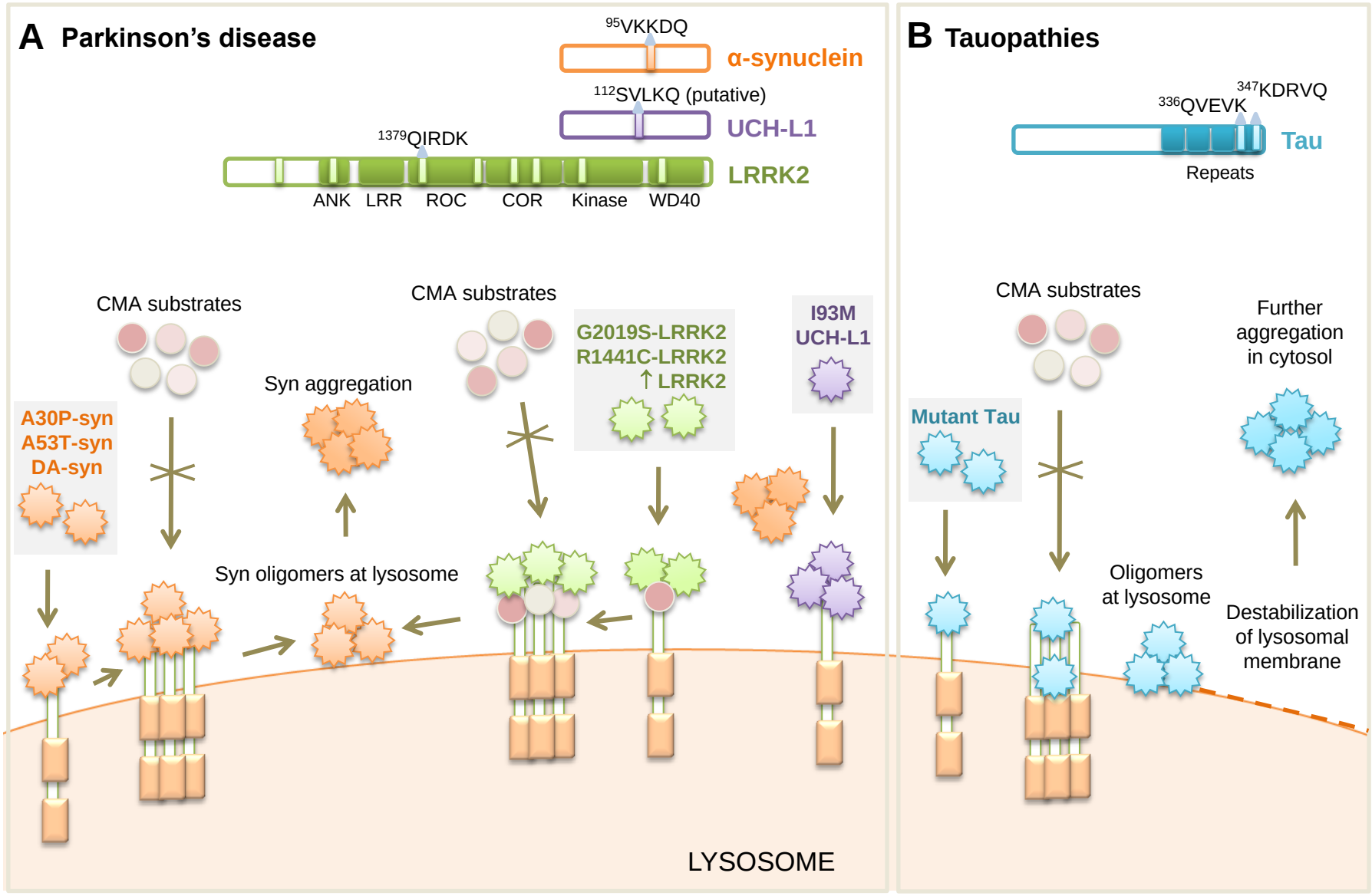

A Parkinson's disease
95VKKDQ
α-synuclein
112SVLKQ (putative)
UCH-L1
1379QIRDK
LRRK2
ANK LRR ROC COR Kinase WD40
CMA substrates
CMA substrates
G2019S-LRRK2
R1441C-LRRK2
↑ LRRK2
I93M
UCH-L1
A30P-syn
A53T-syn
DA-syn
Syn aggregation
Syn oligomers at lysosome
LYSOSOME
B Tauopathies
347KDRVQ
336QVEVK
Tau
Repeats
CMA substrates
Further
aggregation
in cytosol
Mutant Tau
Oligomers
at lysosome
Destabilization
of lysosomal
membrane

FIGURE 3