

Chapter 2

Role of Chaperone-Mediated Autophagy in Ageing and Neurodegeneration

J. V. Ferreira, P. Pereira and H. Girao

Abstract Depending on the mechanism and molecular players involved in the targeting of a substrate to the lysosome, autophagy can be divided in three different subtypes: Macroautophagy, Microautophagy and Chaperone-Mediated Autophagy (CMA).

In contrast to other forms of autophagy, in CMA, soluble cytosolic proteins can be targeted selectively for degradation in lysosomes. Selectivity in CMA is conferred by the presence of a pentapeptide motif in the amino acid sequence of the substrate proteins, biochemically related to KFERQ, that is recognized by the cytosolic chaperone Hsc70, which results in the targeting of substrates to the lysosome. Once at the lysosomal surface, the substrate–chaperone complex binds to the membrane, and, after unfolding the substrate, is translocated into the lumen by LAMP2A, that acts as the resident a CMA “receptor”.

CMA has been implicated both in the elimination of parts of the proteome damaged by stressors, as well as, in the selective turnover of substrates directly related with several proteinopathies, most notably in neurodegenerative diseases. In this chapter we will focus on the role of CMA in age-related neurodegeneration and how CMA often becomes the target of the toxic effect of neurodegeneration-related aberrant substrates. The multifactorial nature of the CMA role in neurodegenerative disorders makes the careful analyses of the evidences gathered thus far instrumental for the understanding of CMA in the context of these diseases.

Keywords Autophagy · Chaperones · Neurodegeneration · LAMP2A · Proteolysis, proteostasis, aging · Lysosome

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Abbreviations

CMA	Chaperone mediated autophagy
Hsc	Heat shock cognate protein
LAMP2	Lysosomal associated protein 2
KDa	Kilodalton
HSP	Heat shock protein
Bag	Bcl 2 associate athanogene
Hop	Hsc70 Hsp90 organizing protein
Hip	Hsc70 interacting protein
GFAP	Glial fibrillary acidic protein
EF1 α	Elongation factor 1 alpha
Lys	Lysosomal
UPS	Ubiquitin proteasome system
HD	Huntinton's disease
PD	Parkinson's disease
RNA	Ribonucleic Acid
LRRK2	Leucine rich repeat kinase 2
UCH-L1	Ubiquitin C terminal hydrolase L1
IPS	Induced pluripotent stem cells
MEF	Myocyte enhancer factor
AD	Alzheimer's disease
RCAN1	Regulator of calcineurin 1
HTT	Huntingtin

Introduction

The term Autophagy broadly refers to a catabolic process that is canonically involved in the lysosomal degradation of cytoplasmic components, initially including organelles and soluble proteins, but now also some membrane proteins [1–4]. However, depending on the mechanism and molecular players involved in the targeting of a substrate to the lysosome, autophagy can be divided in three different subtypes: macroautophagy, microautophagy and chaperone-mediated autophagy. Although autophagy was initially thought as a degradation pathway, activated in response to external stimuli, it is now established that it can also act as a mechanism of quality control under basal conditions.

Autophagy plays a vital role in many physiological processes [5], including the response to starvation, cell growth and innate immunity. In contrast to the in-bulk sequestration of cytosolic components characteristic of macro and microautophagy, soluble cytosolic proteins can be targeted selectively for degradation in lysosomes by a process called Chaperone-Mediated Autophagy (CMA). Indeed, what distinguishes CMA from other forms of autophagy is the selective recognition of cargo by cytosolic chaperones and the fact that substrates are not engulfed, but, instead, translocated across the lysosomal membrane in a

receptor-mediated manner [6]. Although basal CMA activity can be detected in most types of mammalian cells, as in the case of macroautophagy, maximal activation of this pathway is triggered in response to stressors, such as long-term starvation, oxidative stress, or exposure to toxic compounds that induce abnormal conformational changes in cytosolic proteins [7].

Selectivity in CMA is conferred by the presence of a pentapeptide motif in the amino acid sequence of the substrate proteins, biochemically related to KFERQ, that when recognized by a cytosolic chaperone results in the targeting of substrates to lysosomes [8]. Similar to most targeting motifs, the KFERQ motif is degenerated and allows for a series of amino acid combinations as far as they follow this rule: Q should be the flanking amino acid but could be located at the beginning or at the end of the sequence; there could be up to two of the allowed hydrophobic residues (I, F, L or V) or two of the allowed positive residues (R or K), but only one negative charge provided either by E or D [8]. In certain proteins, Q can be replaced with N, but this exchange does not work in all proteins, suggesting that the surrounding amino acid context might be important in this case [9]. The “pure” KFERQ motif is only present in ribonuclease A, the first protein identified as a CMA substrate.

CMA substrates are recognized first in the cytoplasm by the heat shock cognate protein of 70 kD (Hsc70), the constitutively expressed member of the 70-kD family of chaperones [6]. This is actually the same chaperone responsible for disassembly of clathrin from coated vesicles and for assisting in the folding of cytosolic proteins upon recognition of exposed hydrophobic regions. It is unknown what determines the multiplicity of functions of chaperones, but the particular array of cochaperones that bind to Hsc70 in each condition is probably behind the final fate of the substrate protein. A subset of cochaperones, Hsp90, Hsp40, Bcl-2 associate athanogene 1 (Bag-1), Hsc70-Hsp90 organizing protein (Hop), and Hsc70-interacting protein (Hip) have been shown to interact with the CMA substrate-chaperone complex at the lysosomal membrane. Some of the cochaperones may not be directly involved in substrate targeting, but rather participate in the unfolding step, required just before the substrate can translocate across the lysosomal membrane [10].

Once at the lysosomal surface, the substrate-chaperone complex binds to the membrane and, after unfolding the substrate, is translocated into its lumen. This recognition at the lysosomal membrane is mediated by the lysosome-associated membrane protein type 2A (LAMP2A) that acts as the resident a CMA “receptor” [11]. LAMP2A is a single-span membrane protein with a very heavily glycosylated luminal region and a short (12-amino acid) C-terminus tail exposed on the surface of the lysosomes, where substrate proteins bind. LAMP2A is one of the three splice variants of the *lamp2* gene, all of which contain identical luminal regions, but different transmembrane and cytosolic tails [12]. The mechanisms behind the translocation of substrate proteins across the lysosomal membrane are, as yet, poorly understood. Much evidence supports direct translocation across the lysosomal membrane, rather than engulfment by invaginations of the membrane. Indeed, invaginations have never been observed when this transport is reproduced in vitro. On the other hand conjugation or cross-linking of substrate proteins to bigger structures, such as gold particles, prevents their uptake. Moreover, several studies mainly

carried out by Cuervo and colleagues demonstrated that substrate proteins need to be completely unfolded before reaching the lysosomal lumen [9, 11, 13, 14]. By analogy with other protein translocation systems, it has been speculated that the translocation through the lysosomal membrane might include a multispan membrane protein to create a discontinuity in the lysosomal membrane. However, to date, proteomic analysis of proteins associated with LAMP2A at the lysosomal membrane has not rendered such a partner. Nevertheless, evidences point to the existence of a unique mechanism for translocation of substrate proteins across the lysosomal membrane via CMA that requires multimerization of LAMP2A [15]. In fact, binding of substrate proteins to the cytosolic tail of monomeric forms of LAMP2A drives its multimerization to form a 700-kDa complex at the lysosomal membrane [15]. Moreover, the presence of a lysosome-specific form of Hsp90 on the luminal side of the lysosomal membrane is essential to preserve the stability of LAMP2A while it undergoes these conformational changes at the lysosomal membrane [15]. Also, it seems that the CMA translocation complex forms only transiently and that, once the substrate crosses the membrane, LAMP2A rapidly disassembles in a process mediated by the Hsc70 present on the cytosolic side of the lysosomal membrane [15]. Cytosolic and lysosomal chaperones only associate with lower-order complexes of substrate and LAMP2A, but are no longer present in the 700-kD complex required for translocation. The regulation of CMA through changes in lysosomal LAMP2A highlights the importance of lateral mobility within the membrane, which has been shown to be determined by its dynamic association with lysosomal lipid microdomains [16]. In this context, upon conditions of low CMA activity, part of LAMP2A is recruited into regions of defined lipid composition, whereas the number of LAMP2A molecules in these lipid microdomains is markedly reduced when CMA is activated. Accordingly, an increase in microdomain size, by augmenting lysosomal cholesterol results in reduced CMA, whereas cholesterol-extracting drugs increase membrane levels of LAMP2A, activating CMA [16]. In fact, the regulated degradation of LAMP2A described above occurs in these lipid microdomains, as luminal cathepsin A preferentially associates to the lysosomal membrane in these regions. By contrast, binding of substrates to LAMP2A and its assembly into and disassembly from the multimeric CMA translocation complex only pertains to LAMP2A molecules outside these microdomains [16]. Also, CMA activation includes not only the relocation of LAMP2A outside the lipid microdomains, as well as, a luminal pool of intact LAMP2A that can be retrieved to the lysosomal membrane upon CMA stimulation [17]. Intrinsic properties of LAMP2A are required to modulate its membrane dynamics. In addition to the GxxG motif required for multimerization [15], a proline residue that is present at the interface between its transmembrane and luminal regions is absolutely required for the mobilization of LAMP2A into the lipid microdomains [16]. Other components at the lysosomal membrane that modulate LAMP2A dynamics are the intermediate filament protein glial fibrillary acidic protein (GFAP) and elongation factor 1 α (EF1 α), a pair of interacting proteins that modify the stability of the multimeric LAMP2A complex and the association of LAMP2A with the lipid microdomains in a GTP-dependent

manner [18]. Concerning GFAP it was shown that a lysosome specific variant of the protein associates with LAMP2A multimers, enhancing the stability of the complex and counteracting the disassembly-promoting effect of Hsc70. Lysosomal GFAP partitions into two subpopulations; unphosphorylated GFAP that binds to multimers of LAMP2A and phosphorylated GFAP (GFAP-P), the latter of which is usually bound to the GTP-binding protein EF1 α . Moreover, unphosphorylated GFAP has higher affinity for GFAP-P than for LAMP2A. However, the formation of GFAP–GFAP-P dimers is usually prevented by the presence of EF1 α bound to GFAP-P. In the presence of GTP, EF1 α is released from the lysosomal membrane allowing the dissociation of GFAP from the translocation complex and its binding to GFAP-P [18]. This dissociation favors the rapid disassembly of the LAMP2A multimeric complex and its active mobilization to lipid microdomains for degradation. Changes in the levels of GFAP–GFAP-P, EF1 α present at the lysosomal membrane, as well as of intracellular GTP or intra-lysosomal Ca²⁺ (facilitating association of cathepsin A to lipid microdomains) can all contribute to modulation of CMA activity.

The final step in substrate translocation into the lysosome appears to involve a form of Hsc70 resident in the lysosomal lumen (lys-Hsc70) [19]. Only those lysosomes containing Hsc70 in their lumen are competent for uptake of CMA substrates. Interestingly, the percentage of Hsc70-containing lysosomes, which is no more than 40% under resting conditions, escalates to 80% in liver under conditions in which CMA is up-regulated, such as during prolonged starvation or mild oxidative stress [20, 21]. The mechanism by which lys-Hsc70 mediates substrate translocation remains unclear. This chaperone can act either actively, by facilitating substrate internalization in an energy-dependent manner, or passively, by binding the portion of substrate already translocated and preventing its retrograde movement to the cytoplasm. Also unknown is the pathway followed by lys-Hsc70 to reach the lysosomal lumen. It is possible that Hsc70 reaches the lysosome through fusion with late endocytic compartments, where Hsc70 has also been detected. Whether other luminal chaperones are required for substrate translocation is currently unknown.

Sequence analysis of the cytosolic proteome has revealed that about 30% of cytosolic proteins might be putative substrates for CMA [8]. However, it is possible that this amount is an underestimation, because particular post-translational modifications, such as deamidation, phosphorylation, acetylation, etc., could provide the charge missing in a four-amino acid sequence [10]. This possibility of modulating chaperone recognition of the substrates by post-translational modifications adds an additional level of regulation to CMA. Another interesting fact is the existence of substrates that can be degraded either by the proteasome or by the lysosome, through CMA, suggesting that an interplay between UPS and CMA may exist. Indeed, some canonical proteasomal substrates were shown to have KFERQ motifs and, consequently, get degraded in the lysosome by CMA [4, 22–25]. However the mechanisms and signalling pathways that direct these substrates to either degradative pathway remain unclear.

CMA and Pathology

CMA's Role in Proteostasis

The accumulation of degradation-resistant proteins that become toxic has been consistently associated with various pathological conditions. Moreover, it is consensual that intracellular accumulation of aberrant proteins plays a major role in the aging process and constitutes a key feature shared by a large number of human age-related diseases. The most notorious among these are the neurodegenerative diseases where abnormal proteins accumulate in the form of inclusions in the affected neurons.

In this context CMA has been deemed as an efficient pathway for removing abnormal or otherwise damaged proteins. The first physiological stressor found to activate CMA was prolonged starvation, where the selective breakdown of superfluous proteins through CMA would contribute with the necessary amino acids required for protein synthesis, thus helping cells to cope with nutrient deprivation. Additionally, CMA is activated in circumstances associated with cytosolic protein damage, such as oxidative stress. In fact, exposure of cells to oxidative stress induces a rapid activation of CMA through transcriptional up-regulation of LAMP2A [20]. Moreover, oxidized proteins bearing the CMA-targeting motif are readily identified by Hsc70 and delivered to lysosomes for degradation. Interestingly, uptake of these oxidized proteins is faster than for their unmodified counterparts, suggesting that some oxidative induced unfolding of the substrates promotes its lysosomal targeting in the cytosol [20]. Consistently, experimental blockage of CMA has been shown to lead to the accumulation of oxidized cytosolic proteins in different cellular models [26], thus ascribing a role to CMA in the disposal of a particular subset of proteins that, after their oxidation, have CMA degradation favored over their removal through other pathways. In addition, the type and source of the oxidative stress may also determine the preference for one proteolytic system or another [27].

In the context of the ageing process, CMA-dependent degradation of oxidized proteins has been shown to decrease with age in most organs in rodents, as well as, in primary skin fibroblasts and peripheral leucocytes of healthy human subjects [28, 29], resulting in the accumulation of aggregation-prone oxidized proteins. This decrease was attributed to an age related LAMP2A reduction at the lysosomal membrane [30, 31]. Notably, restoration of normal levels of the CMA receptor in aged animals through genetic manipulation recuperates oxidized proteins levels of aging tissues to pre-ageing levels [32], suggesting a relevant role to CMA in the cellular defense against oxidative damage. These and other evidences strongly support the hypothesis that age is inversely correlated with CMA activity [28]. Indeed it has been suggested that this age-related decline in CMA activity contributes to the accumulation of abnormal and dysfunctional proteins and exacerbates disease progression of age-related pathologies. Logically, growing evidences also support CMA activation in response to oxidative stress in the central nervous system. For example, an increase in CMA markers has been observed in response to

6-hydroxydopamine-induced lesions in the nigrostriatal pathway [33]. Likewise, levels of LAMP2A have been shown to increase in response to seizures and status epilepticus, conditions associated with excessive production of reactive oxygen species [34].

CMA is also part of the cellular response to other stressors such as hypoxia, where CMA up-regulation enhances cell survival [35]. The fact that HIF-1 α (hypoxia-inducible factor 1 α) has recently been identified as a CMA substrate places this autophagic pathway at the centre of the regulation of the cellular response to hypoxia [22]. On the other hand, up-regulation of CMA components has been described in response to the activation of the pro-apoptotic programme in a common form of rod–cone dystrophy, indicating that CMA is part of the pro-survival response in retinal cells [36]. In fact, the response to stress in cone photoreceptors seems to rely, in a large part, on CMA activity, which explains the increased sensitivity of these cells to different stressors when CMA is compromised [37].

Crosstalk between CMA and Other Proteolytic Pathways

Another important aspect of CMA's role in proteostasis is the fact that its activity is regulated and tightly coordinated, not only with other forms of autophagy, but also with the Ubiquitin Proteasome System (UPS) [38, 39]. Crosstalk between CMA and macroautophagy is exemplified by the observed constitutive activation of CMA in cells deficient in macroautophagy [40]. Conversely, macroautophagy is highly induced in response to CMA blockage [26]. In the case of the CMA and macroautophagy interplay, though the roles of CMA and macroautophagy are considered similar but non-redundant, they can compensate for each other to sustain cell survival, though it may not be sufficient to allow efficient adaptation of cells to a certain stress.

Similarly, many cells respond to chemical blockage of the proteasome by upregulating CMA [22, 41]. On the other hand an impairment of CMA activity perturbs UPS functioning at least during the early stages of acute CMA blockage [42], most likely by affecting the turnover of specific proteasome subunits [43]. In agreement, reports show that CMA blockage may alter proteasome assembly as exemplified by the fact that the maintenance of CMA efficiency allows for better preservation of UPS activity in old rodents [32]. Strikingly, as seen in a mouse model of Huntington's disease (HD), such a constitutive upregulation of CMA compensates for the dual failure of macroautophagy [44, 45] and UPS [46]. This activation of CMA in HD is achieved through both enhanced transcription and increased stability of LAMP2A in the affected cells [47]. 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 ageing. In the opposite direction crosstalk between macroautophagy and CMA is crucial in Parkinsons disease (PD) and in certain tauopathies, where the blockage of CMA is often compensated by activation of macroautophagy

[48–51]. In this case, macroautophagy activation is instrumental in promoting the removal of the toxic α -synuclein and tau oligomers. In any case, despite the significant advances in the last few years concerning the elucidation of the mechanisms that govern the crosstalk between CMA and other proteolytic pathways more studies are still needed to establish the molecular determinants that can switch a given substrate from one particular degradative pathway to the other. On this subject we have recently demonstrated that the ubiquitin ligase CHIP can reroute HIF-1A from the proteasome to CMA [22]. This finding opens the way for an involvement of ubiquitination and different ubiquitin chain topologies in diverting the substrates between CMA and other degradative pathways. Additionally, in the future, we may learn to upregulate these specific mechanisms to prevent overloading and subsequent blockage of other components of the proteostasis system.

Ageing and CMA

Reduced or otherwise altered CMA activity is not an exclusive of a pathological condition, but is also closely related with physiological ageing. An age-related reduction in the activity of CMA has been observed in many cell types and tissues [28, 30]. It is known that the transcription, synthesis and lysosomal targeting of the LAMP2A protein to the lysosome remains unchanged throughout lifespan. Nonetheless, the stability of LAMP2A at the lysosomal membrane is severely decreased with ageing [30]. This reduction is most probably linked with changes in the lipid constituents of the lysosomal membrane, which are important in maintaining the dynamics and stability of LAMP2A in the lysosomes. Molecularly, ageing brings changes into the lipid membrane composition of lysosomes, which in turn increases degradation of LAMP2A in the lysosomal lumen [16, 30, 31]. Consequently, the binding and translocation of substrates into the lysosomes by CMA is progressively unpaired during ageing [31]. Similar changes in the lipid composition of lysosomal membranes can be reproduced 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.

Up to this date, the main consequence of the age-related failure of CMA is proteostasis impairment, which induces a defective removal of oxidatively damaged proteins or the ability to respond to stressors [26]. Consequently, it is today largely accepted that age-dependent decline in CMA might constitute a contributing factor in the pathological changes in many age-related disorders. The most striking evidence in support of this theory is that genetic manipulation in old rodents, by expressing an exogenous copy of LAMP2A in mouse liver to preserve CMA function, has proven effective in improving the healthspan of aged animals [32]. Indeed, restored CMA functions in the LAMP2A transgenic animals results in improved cellular homeostasis, enhanced resistance to different stressors and preservation of organ function. Such pronounced beneficial effects in prolonging healthspan makes CMA a potential and promising anti-ageing target mechanism.

CMA in Neurodegenerative Diseases

In neurodegeneration CMA is implicated both in the elimination of parts of the proteome damaged by stressors as well as in the selective turnover of substrates directly related with neurodegenerative diseases. Furthermore, CMA often becomes the target of the toxic effect of these aberrant substrates, thus creating a vicious cycle that propagates and aggravates the effect of ageing. The multifactorial nature of the CMA role in neurodegenerative disorders makes the careful analyses of the evidences gathered thus far instrumental for the understanding of CMA in the context of these diseases.

Parkinson's Disease

Since LAMP2A suffers an age-related reduction at the lysosomal membrane it is conceivable that CMA can play a role in neurodegenerative disorders that have ageing as a risk factor. Up until now, the most compelling evidences on the role of CMA malfunction in neurodegenerative diseases have arisen from PD related research. Indeed, several studies have consistently suggested that the gradual decrease in LAMP2A levels with age constitutes a major contributor to PD progression in older patients [28, 52]. In agreement, very recent data showed that some of the microRNAs that are deregulated in PD brains may underlie the down-regulation of some CMA components in the affected neurons [53]. Furthermore, a sequence variation in the promoter region of LAMP2 has been recently identified in a PD patient [54], which might indicate that genetic induced variations in CMA components could be a causative factor for some forms of the disease. The association between PD and CMA is further suggested in studies in which both chemical [55] and genetic [56] upregulation of CMA were demonstrated to be sufficient to alleviate cellular toxicity associated with pathogenic forms of α -synuclein in a model of dopaminergic neuronal degeneration. Interestingly, CMA seems to be involved both in the familial and the sporadic forms of PD. In the familial form of PD [24, 57–59] sequence analysis reveals the presence of CMA-targeting motifs in the majority of PD-related proteins (i.e. α -synuclein, the ubiquitin C-terminal hydrolase L1 (UCH-L1) and the leucine-rich repeat kinase 2 (LRRK2)). Consistently, using a number of experimental approaches such as isolated lysosomes, primary mouse neuronal cultures, mouse models of PD, neuronal-differentiated induced pluripotent stem cells as well as brains from familial and sporadic PD patients, it was shown that the two most commonly mutated proteins in patients with familial PD, α -synuclein and LRRK2, are *bona fide* CMA substrates. Moreover, several pathogenic mutants of such proteins as α -synuclein (A30P and A53T) and of LRRK2 (G2019S and R1441C), have a direct toxic effect in CMA through aberrant interactions of these mutants with LAMP2A, such that these interactions induce CMA inhibition. In fact, these mutants bind to LAMP2A with an abnormally high affinity, which prevents their translocation across the lysosomal membrane [60], thus precluding access of other substrates to the lysosome to get degraded. Consequently, not only the mutant

and toxic forms of α -synuclein and LRRK2 will have decreased degradation but they will also impair other CMA substrates degradation [60]. In accordance, it was shown that accumulation of α -synuclein can be caused by an aberrant interaction of mutant LRRK2 with LAMP2A. In fact, LRRK2 mutants impedes LAMP2A organization into complexes and leaves increased levels of monomeric forms of LAMP2A at the lysosomal membrane, increasing lysosomal association with α -synuclein that cannot be degraded [57]. Even with wild-type α -synuclein, the presence of mutant LRRK2 is sufficient to promote toxic oligomerization of α -synuclein at the lysosomal membrane due to a compromise in lysosomal translocation. The presence of these oligomeric forms of α -synuclein stuck at the lysosomal membrane further impairs CMA activity and might even contribute to the seeding of protein aggregates characteristic of this disorder [57]. Interestingly, artificial mutations that prevent delivery of α -synuclein to the lysosomal membrane improve cell survival, partially because it no longer impedes CMA function [59]. These dual pathogenic effects of reduced elimination of the pathogenic protein and additional CMA blockage should contribute significantly to a more severe PD pathology in LRRK2-mediated PD cases.

In addition to LRRK2, also mutated forms of UCH-L1 have been associated with CMA impairment. Wild-type UCH-L1 binds to CMA-related chaperones and LAMP2A. But similarly to LRRK2, the levels of these interactions are abnormally increased by the PD-linked I93M mutation in UCH-L1, leading once again to blockage of α -synuclein degradation by CMA [61].

Besides the familiar form of the disease, there are also evidences linking CMA to sporadic PD [57, 60]. In general, an inhibition of α -synuclein degradation by post-translational modifications caused by stressors such as pesticides or oxidative stress, are instrumental in the development of sporadic PD and other synucleinopathies [62]. In this context, dopamine-modified α -synuclein shows reduced susceptibility to CMA degradation similarly to that of hereditary α -synuclein mutants [60], where the tight binding but inefficient translocation by CMA of dopamine-modified α -synuclein inhibits its degradation, alongside other CMA substrates. A perpetuation of this scenario will promote the formation of highly toxic α -synuclein oligomers or protofibrils at the lysosomal membrane [60]. Also, very recently, reports showed that even in the absence of post-translational modifications, increased levels of either α -synuclein [24] or LRRK2 [57], beyond a certain threshold, are sufficient for CMA inhibition in a manner where either one of these proteins can potentiate each other toxic effect on CMA [57].

Compelling evidences of the involvement of CMA in PD have also arisen from post-mortem brain samples from PD patients. The analysis of these samples has made clear that in the early stages of PD, LAMP2A is increased [57]. Furthermore, studies in iPS (induced pluripotent stem) cells from a PD patient have also revealed that the initial up-regulation of CMA is followed by a marked decrease in CMA activity as the disease progresses [57]. Late down-regulation of this pathway is also supported by the reduced expression of LAMP2A and Hsc70 identified in peripheral leucocytes in idiopathic forms of PD [63] and in the substantia nigra pars compacta of PD brains [64].

The involvement of CMA in PD has also been associated with the myocyte enhancer factor 2D (MEF2D) since it is a substrate for CMA [65]. MEFs transcription

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