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Chaperone-mediated autophagy dysfunction in the pathogenesis of neurodegeneration

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Abstract

Chaperone-mediated autophagy (CMA) contributes to selective degradation of individual soluble proteins in lysosomes. Unique to this type of autophagy is the fact that proteins reach the lysosomal lumen for degradation by directly crossing the lysosomal membrane, in contrast with the vesicle-mediated delivery characteristic of the other types of autophagy. These two characteristics - selective targeting and direct translocation of substrates - determine the contribution of CMA to different physiological functions and the type of pathological conditions associated with CMA dysfunction. In this review, we briefly revise recent findings on the molecular mechanisms behind CMA function, and describe the physiological relevance of the selective lysosomal degradation through this pathway. We also comment on the cellular consequences of CMA malfunction and on the connections already established between CMA dysfunction and different human disorders, with special emphasis on neurodegenerative diseases.

Keywords

aging; chaperones; lysosomes; neurodegeneration; Parkinson’s disease; proteases; proteotoxicity

Introduction[1]

Continuous turnover of proteins and organelles is essential for normal cell functioning and for maintenance of cellular homeostasis (Ciechanover, 2005; Koga et al., 2010; Mizushima et al., 2008). Modification of the proteome composition through degradation allows rapid cellular adaptation to the changing extracellular environment and assures the removal of altered and damaged cellular constituents that otherwise could become cytotoxic.

The lysosomal system is one of the major mechanisms involved in this continuous intracellular clearance through what is known as autophagy (Klionsky, 2005; Mizushima et al., 2008). Different types of autophagy have been described on the basis of the mechanisms used for delivery of cargo to lysosomes, their regulation and their participation in different

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[1]Abbreviations: CMA: chaperone-mediated autophagy; LAMP: lysosome-associated membrane protein; hsc: heat shock cognate protein; hsp: heat shock protein

cellular functions. Macroautophagy, microautophagy and chaperone-mediated autophagy (CMA) are the most common types of autophagy in mammalian cells (Cuervo, 2004). The first two autophagic pathways have been described in detail in previous works and constitute the main topic of other contributions in this special issue (Klionsky, 2005; Mizushima et al., 2008). This review focuses on chaperone-mediated autophagy, a selective form of autophagy by which individual cytosolic proteins are targeted one-by-one into lysosomes for degradation. Following, we will describe the new findings regarding the molecular mechanisms that contribute to substrate targeting, binding and translocation across the lysosomal membrane through this pathway, as well as the novel regulators of this autophagic process. In the second part of this review, we will describe the recent advances in our understanding of the physiological relevance of CMA and its unique role as part of the mechanisms activated in response to cellular stress. We will also review evidence supporting failure of this autophagic pathway described under conditions such as aging and certain age-related pathologies. In fact, CMA dysfunction has recently been linked to different neurodegenerative disorders. We will comment on the molecular mechanisms behind the CMA failure under these conditions and speculate on the possibility of developing CMA-targeted therapeutic strategies to prevent or slow down neurodegeneration.

General Characteristics of CMA

In contrast to the other autophagic pathways that are conserved from yeast to mammals, CMA has been described to date only in mammals (Fig. 1). Also different from these other autophagic pathways is the mechanism of cargo recognition and its delivery to lysosomes. While cargo is usually sequestered inside vesicular compartments, either free-standing in the cytosol (in the case of macroautophagy) or formed through invaginations of the lysosomal membrane (in the case of microautophagy), CMA substrate proteins are recognized in the cytosol by a chaperone complex and are delivered to the surface of the lysosomal membrane, where they bind to a receptor protein (Cuervo, 2010;Dice, 2007) (Fig. 1). Binding to this receptor protein makes CMA a saturable process. Furthermore, because in macroautophagy and microautophagy cargo is delivered to the lysosomal lumen inside vesicles, the folding state of the proteins destined for degradation is irrelevant. However, in the case of CMA, the cytosolic proteins presented to the lysosomal receptor cross the lysosomal membrane through a translocation complex that requires complete unfolding of the substrate (Salvador et al., 2000). CMA is thus a pathway for degradation of soluble proteins amenable to unfolding but not of proteins when they organize into irreversible oligomeric or multimeric complexes such as aggregates. For the same reason, whole organelles cannot be degraded through this pathway.

CMA activity is detectable in almost all types of mammalian cells, although levels of activity vary depending on the cell type and on the cellular conditions. CMA is maximally activated in most cells under conditions of stress, when it contributes to selective degradation of proteins no longer necessary under those conditions or of the proteins damaged by the stressors (Cuervo, 2010; Dice, 2007).

Molecular effectors of CMA

All CMA substrates contain in their amino acid sequence a pentapeptide motif (biochemically related to KFERQ) (Dice, 1990), which is selectively recognized by a chaperone complex consisting of the constitutive member of the 70kDa family of chaperones, hsc70, and some of its cochaperones (Chiang et al., 1989). Although the specific subset of cochaperones directly responsible for CMA targeting has not been characterized in detail, hsp90, hsp40, hip, hop and bag-1 have been shown to associate to the hsc70-substrate complex at some point during the substrate recognition and lysosomal

targeting process (Agarraberes and Dice, 2001). The KFERQ motif, as such, is only present in ribonuclease A, the first substrate identified for this pathway, but combinations of amino acids with the same biophysical properties are recognized by the hsc70 chaperone and have been demonstrated to be necessary for the degradation of the protein by CMA (Dice, 1990). Although, about 30% of cytosolic proteins can be retrieved with an antibody against the KFERQ motif, the pool of substrates for this pathway is likely larger, since post-translational modifications such as phosphorylation or alkylation could provide the missing charge to complete a CMA-targeting motif.

Once at the lysosomal membrane, the substrate/chaperone complex interacts with the lysosome-associated protein type 2A (LAMP-2A) which acts as a receptor for CMA (Cuervo and Dice, 1996). Binding of the substrate to monomeric forms of LAMP-2A at the lysosomal membrane promotes the organization of LAMP-2A into a 700kDa multimeric complex of which LAMP-2A is the predominant component (Bandyopadhyay et al., 2008) (Fig. 2). Formation of this complex is required for substrate translocation into lysosomes. A lysosomal form of hsp90 preserves LAMP-2A stability as it transitions from monomers to multimers. In fact, blockage of this lysosomal hsp90 with specific inhibitors results in rapid degradation of LAMP-2A by the luminal proteases (Bandyopadhyay et al., 2008).

Complete unfolding of the substrate protein is not required for binding to LAMP-2A but it is necessary to attain translocation of the substrate into the lysosomal lumen (Salvador et al., 2000). Once associated with the translocation complex, the substrate protein is unfolded and is translocated into the lysosomal lumen assisted by a chaperone (lys-hsc70) on the lumenal side of the lysosomal membrane (Agarraberes et al., 1997; Cuervo et al., 1997). Lys-hsc70 is resistant to lysosomal proteases when in an acidic environment but it is rapidly degraded if the pH rises above 5.6 (Cuervo et al., 1997). Once in the lysosomal matrix substrate proteins are degraded rapidly by proteases.

Recent studies have unveiled the unique nature of the CMA translocation complex at the lysosomal membrane. Thus, in contrast to other protein translocation systems in other membranes in which the minimal translocation components are stably organized into channels or pores, the formation of the CMA translocation complex at the lysosomal membrane is transient and only persists while the substrate is crossing the membrane (Bandyopadhyay et al., 2008) (Fig. 2). In fact, while in the presence of substrates, hsc70 facilitates substrate binding to LAMP-2A and promotes the formation of the CMA translocation complex, once the substrate has crossed the membrane, hsc70 actively mediates disassembly of LAMP-2A into monomeric forms (Bandyopadhyay et al., 2008). Continuous assembly and disassembly of the CMA translocation complex guarantees cycles of sequential binding and uptake of substrates by this pathway (Fig. 2).

Regulation of CMA

Binding to LAMP-2A is a limiting step in CMA (Cuervo and Dice, 2000b). Consequently, the activity of this autophagic pathway is in part regulated locally at the lysosomal membrane through tight control of the levels of LAMP-2A. Transcriptional modulation of LAMP-2A along with changes in its degradation, in its ability to multimerize and in the dynamic distribution of this receptor protein between the lysosomal membrane and lumen, all contribute to regulate the LAMP-2A content in lysosomes (Cuervo and Dice, 2000b). Transcriptional upregulation of LAMP-2A has been so far detected only in one condition, mild-oxidative stress, in which CMA is activated (Kiffin et al., 2004). Activation of CMA in most conditions, does not require *de novo* synthesis of LAMP-2A but rather the increase in LAMP-2A levels necessary to sustain CMA activity is attained by decreasing the degradation of this receptor at the lysosomal membrane, increasing its lateral mobility to

favor rapid cycles of assembly/disassembly and favoring mobilization of a pool of LAMP-2A usually resident in the lysosomal lumen toward the lysosomal membrane (Kiffin et al., 2004). Although the precise mechanisms that regulate the dynamics of LAMP-2A at the lysosomal membrane are currently under investigation, studies from our group support that the association of LAMP-2A to microdomains of discrete lipid composition (enriched in cholesterol and glycosphingolipids) at the lysosomal membrane is behind LAMP-2A regulation (Kaushik et al., 2006). Under basal conditions, when CMA activity is low, a higher percentage of LAMP-2A localizes in the discrete lipid microdomains where regulated degradation of LAMP-2A occurs. In contrast, during CMA activation LAMP-2A exits these regions, escaping in this way degradation and becoming amenable to multimerization. Changes in this subcompartmentalization of LAMP-2A contribute to modulate CMA activity. In fact, enhanced sequestration of LAMP-2A in lipid microdomains through treatments that increase cholesterol content at the lysosomal membrane lead to a reduction in CMA activity. Conversely, disruption of the lysosomal membrane microdomains with cholesterol extracting drugs enhances CMA activity (Kaushik et al., 2006). These findings support that pathological changes in the intracellular lipid content and specifically in the lipid composition at the lysosomal membrane could affect CMA activity.

The specific molecular components that mediate incorporation and exit of LAMP-2A from the membrane microdomains remain for the most part unknown. However, recently a pair of proteins previously unknown to associate with lysosomes have been revealed as possible modulators of LAMP-2A. A lysosome-associated form of the glial fibrillary acidic protein (GFAP), a component of the intermediate filament network, associates to LAMP-2A once it is organized into multimers and contributes to stabilize the CMA translocation complex against the disassembling activity of hsc70 (Bandhyopadhyay et al., 2010) (Fig. 2). GFAP is retrieved out of the LAMP-2A multimeric complex by interaction with a phosphorylated form of this protein that sits at the lysosomal membrane. An increase in the total amount of GFAP at the lysosomal membrane favors self-assembly over binding to LAMP-2A, and results in a net decrease in the amount of CMA translocation complexes present at a given time in lysosomes. Self-assembly of GFAP at the lysosomal membrane is normally prevented through binding of elongation factor 1 alpha to the phosphorylated form of GFAP (Bandhyopadhyay et al., 2010). This GTP-binding protein is released from the membrane in the presence of GTP, leaving accessible the regions on GFAP required for its dimerization. Consequently, GTP exerts a net inhibitory effect on CMA because it decreases binding of GFAP to LAMP-2A in the translocation complex which results in disassembly of LAMP-2A from this complex and its mobilization to lipid microdomains where multimerization is no longer possible. Thus, GFAP modulates the dynamics of LAMP-2A between the multimeric and monomeric state.

In contrast to the growing understanding of the local regulation of CMA through changes in the levels and dynamics of LAMP-2A at the lysosomal compartment, the signaling networks that participate in activation or inactivation of this autophagic pathway are unknown. Further efforts should be aimed at the characterization of the intracellular pathways that modulate CMA.

Physiological role of CMA

The selective removal of cytosolic proteins mediated by CMA contributes to maintenance of cellular homeostasis particularly under conditions of stress (Cuervo, 2010; Dice, 2007) (Fig. 3). In fact, CMA constitutes part of the mechanisms of the cellular response to stress. Although degradation of proteins through CMA occurs, to some extent, under basal conditions in most cells, CMA is maximally activated in stressful conditions such as prolonged starvation, oxidative stress or stress mediated by exposure to toxic compounds

(Cuervo, 2010; Dice, 2007). During the early periods of starvation, cells obtain the essential amino acids required to maintain protein synthesis, through activation of macroautophagy. However, in many cells and tissues, macroautophagy activity peaks at 4-6 hours post-starvation and rapidly decreases past this time (Finn and Dice, 2005; Massey et al., 2006). If starvation persists, CMA activity increases progressively, reaching maximum levels around 12-20 hours, and it remains active as long as the nutritional stress persists (Cuervo et al., 1995). The large capacity of macroautophagy accommodates the substantial amino acid demand required to synthesize the proteins involved in the first front of defense against a nutrient-limiting environment. As starvation persists, replacement with the more selective degradation that occurs via CMA could prevent the degradation of critical proteins while unnecessary ones (i.e., enzymes of metabolic pathways turned off during starvation) are preferentially degraded. This time-dependent switch from one type of autophagy to another, indirectly suggests the existence of some level of cross-talk between these two autophagic pathways. In fact, as described in detail later, blockage of CMA is associated with compensatory activation of macroautophagy and vice versa (Kaushik et al., 2008b; Massey et al., 2006).

A second general function of CMA common to all cells is its participation in quality control of the proteome (Fig. 3). Oxidized, misfolded or truncated proteins bearing the CMA-targeting motif are readily removed through this autophagic pathway thus preventing their intracellular accumulation (Cuervo et al., 1999;Kiffin et al., 2004). CMA is activated during mild oxidative stress, when it contributes to the efficient removal of oxidized proteins, and also upon exposure of cells to protein denaturing compounds (Cuervo et al., 1999;Kiffin et al., 2004).

In addition to the general functions of CMA common to all cells, this autophagic pathway also participates in other functions unique to particular cell types or linked to the degradation of a specific protein (Fig. 3). For example, CMA has been proposed to contribute to presentation of endogenous antigens mediated by MHC class II in professional antigen-presenting cells (Crotzer et al., 2010;Zhou et al., 2005), to the preservation of neuronal viability by degradation of the MEF2D survival factor (Yang and Mao, 2009) and to the control of cellular proliferation in kidney through the regulated degradation of the growth factor Pax-2 (Sooparb et al., 2004).

Consequences of CMA failure

A better understanding of the cellular consequences of reduced CMA activity has been gained by stably down-regulating LAMP-2A expression in cultured cells (Massey et al., 2008; Massey et al., 2006). Cells with impaired CMA maintain normal rates of protein degradation under basal conditions but have lower ability to respond to stress. Thus, exposure of CMA-deficient cells to different types of stress results in higher rates of apoptotic cell death when compared with control cells, supporting an essential role of CMA in the cellular response to stress. Accumulation of oxidized and misfolded proteins, frequently as intracellular cytosolic inclusions, is commonly observed upon CMA blockage and could contribute to the described enhanced rates of cell death (Massey et al., 2008; Massey et al., 2006).

Of particular interest is the fact that maintained CMA blockage results in upregulation of macroautophagy even under basal cellular conditions, explaining thus why protein degradation rates are not reduced in these cells (Massey et al., 2006). However, in light of the increased cellular death of CMA-deficient cells upon exposure to stressors, macroautophagy cannot completely compensate for CMA as part of the cellular response to stress. Interestingly, the cross-talk between these two autophagic pathways functions in both

directions, as blockage of macroautophagy leads to up-regulation of CMA in most cells (Kaushik et al., 2008a). Growing evidence shows that this cross-talk does not seem to be limited to the autophagic system but extends to other major intracellular proteolytic systems such as the ubiquitin/proteasome system (UPS). For example, alterations in some of the proteolytic activities of the proteasome and in the clearance of polyubiquitinated proteins has been described in the early stages of CMA blockage (Massey et al., 2008).

CMA in disease and neurodegeneration

Altered CMA function has been described in different human diseases, but due to the nature of this special issue here we particularly emphasize the evidence supporting a connection between CMA malfunctioning and neurodegenerative disorders. Besides the central nervous system, different kidney afflictions have also been linked to CMA activity (Fig. 3). CMA upregulation has been detected in tubular kidney cells upon exposure to toxic compounds that alter lipophilic proteins, previously validated as CMA substrates (Cuervo et al., 1999). Decrease of CMA beyond physiological limits has been linked to the pathogenesis of renal hypertrophy, distinctive from the diabetic kidney (Sooparb, 2004).

Altered CMA has also been described in two lysosomal storage disorders, galactosialidosis and mucolipidosis type IV (Fig. 3). CMA is abnormally upregulated in the former one, due to reduced degradation of LAMP-2A in lysosomes from the affected cells (Cuervo et al., 2003), whereas failure to activate CMA has been described in mucolipidosis type IV, likely due to abnormal interactions with the protein mutated in this disease and CMA chaperones (Venugopal et al., 2009). The possible connections between CMA and other common human disorders such as cancer, metabolic disorders, liver disease, etc. are currently under investigation.

Cellular toxicity resulting from the accumulation of altered proteins (or proteotoxicity) is a common feature of a large number of diseases generically known as protein conformational disorders (Liberek et al., 2008; Michalik and Van Broeckhoven, 2003; Robinson, 2008). Although these diseases can affect almost every tissue in the organism, the contribution of proteotoxicity to alterations of the central nervous system and neurodegeneration has received particular attention lately. This is due, in part, to the high prevalence of these pathologies, and in part as a result of the severe consequences that the loss of cellular homeostasis associated to the presence of protein deposits has on differentiated cells such as neurons.

In neurons, as in most other cell types, misfolded or damaged cytosolic proteins are often detected by chaperones in the cytosol (Liberek et al., 2008). If unfolding or repair of the altered protein by these chaperones is not possible, the same chaperone determines if the protein’s degradation will occur through the proteasome or the autophagic system. Most soluble monomeric proteins in the cytosol can be substrates for the ubiquitin/proteasome system or for CMA (Koga et al., 2010). However, once organized into irreversible oligomers or aggregates unable to undergo complete unfolding, the only degradative option feasible is macroautophagy. Alterations in both lysosomal and proteasomal protein degradation have been described as the basis of different neurodegenerative disorders (Larsen and Sulzer, 2002). In this review, we comment only on the relation of CMA with neurodegeneration, and direct readers to other works focused on other autophagic and proteolytic pathways.

CMA in the central nervous system during aging

In addition to the previously mentioned role of CMA in neuronal survival through the degradation of MEF2D (Yang and Mao, 2009), and its contribution of CMA to the maintenance of cellular homeostasis, the active role of this pathway in the cellular response

to stress and the fact that CMA activity declines with age in most cell and tissues types underlies the importance of this autophagic pathway in neuronal biology and its connections to neurodegeneration.

Decreased activity with age is not exclusive to CMA, as other autophagic pathways also malfunction in old organisms (Cuervo, 2008). In fact, the lysosomal system itself is noticeably affected by aging, revealing an impaired regulation of lysosomal pH, changes in the activity of lysosomal hydrolases, decreased stability of the lysosomal membrane and accumulation of undegraded products in the form of lipofuscin (Cuervo et al., 2005). In addition to these changes intrinsic to the lysosomal compartment and bound to affect all types of autophagic activity, specific changes with age in the effectors and regulators of CMA have been described (Cuervo and Dice, 2000a; Kiffin et al., 2007). Although binding of substrate proteins to chaperones and their targeting to the lysosomal membrane seem preserved until late in life, binding to the lysosomal receptor and translocation into the lysosomal lumen are severely impaired at advanced ages. A progressive decrease of the lysosomal levels of LAMP-2A with age has been identified as the main reason behind reduced substrate binding and translocation (Cuervo and Dice, 2000a; Kiffin et al., 2007) (Fig. 4). Reduced LAMP-2A levels do not result from an age-dependent transcriptional downregulation of the *lamp2* gene, but instead, originate primarily from changes in the lysosomal membrane, which alter the stability of LAMP-2A in this compartment (Kiffin et al., 2007). Disrupted LAMP-2A dynamics lead to a larger percentage of LAMP-2A being retained in the lysosomal lumen where it undergoes rapid unregulated degradation by luminal proteases. Although further studies are required to elucidate the reasons behind these altered LAMP-2A dynamics, our ongoing investigations support that primary changes in the lipid composition of the lysosomal membrane with age may contribute to the LAMP-2A alterations.

In light of the described functions of CMA, defective CMA in aged organisms is anticipated to contribute to the accumulation of altered proteins, abnormal cellular homeostasis and poor ability to respond to stress (Cuervo, 2008). Due to the distinctive characteristics of neuronal cells (negligible rates of cell division, cytoplasm that extends into rather large cellular expansions – dendrites and axons – and their need for a highly dynamic vesicular trafficking) elimination of damaged cellular components is key for preservation of normal neuronal function, and consequently, CMA malfunction with age would be particularly harmful to neurons. In fact, independent of the primary defects in CMA described for some neurodegenerative diseases, the age-dependent decline in the activity of this and other autophagic pathways is likely to contribute to disease progression in almost all neurodegenerative disorders.

CMA and Parkinson's disease

Parkinson's disease (PD) and the groups of related diseases known as Parkinson syndrome all share as a common characteristic the presence of proteinaceous inclusions – known as Lewy bodies – in the affected neurons (Schapira, 2009; Yang, 2009). Although most forms of PD are idiopathic, mutations in 10 different genes have been described in familial forms of PD. Interestingly, analysis of the amino acid sequence of the protein products of these genes has revealed motifs compatible with the CMA-targeting motif in several of them. Connections of three of these proteins with CMA have already been already established, and it is likely that future studies will reveal additional interactions between other PD proteins and CMA (Fig. 4).

The first PD protein for which association to CMA was shown is α-synuclein (Cuervo et al., 2004b). *In vitro* and *in vivo* studies support that α-synuclein, the main component of Lewy bodies both in familial and also in idiopathic PD brains, is a *bona fide* CMA substrate

(Cuervo et al., 2004a; Mak et al., 2010; Martinez-Vicente et al., 2008; Vogiatzi et al., 2008). Wild type α-synuclein is taken up and degraded via CMA by intact lysosomes in a manner dependent on hsc70 and LAMP-2A and on the presence of the CMA-targeting motif in its sequence (Cuervo et al., 2004a). Recent studies in brain of PD mouse models have also confirmed the preferential association and degradation of α-synuclein in CMA-active lysosomes (Mak et al., 2010). Pathogenic mutant forms of α-synuclein are still recognized by the cytosolic chaperone and are targeted to the lysosomal surface where they bind tightly to the CMA receptor, however the amount of these proteins that translocates into the lysosomal lumen is negligible (Cuervo et al., 2004a). Further characterization of lysosomes exposed to mutant α-synucleins has revealed that binding of the pathogenic proteins prevents other common CMA substrates from being degraded by this pathway (Cuervo et al., 2004a; Martinez-Vicente et al., 2008; Xilouri et al., 2009). In fact, rates of proteins undergoing degradation through CMA are noticeably reduced in different types of cells expressing mutant α-synucleins, supporting that the pathogenic proteins act as blockers to inhibit the degradation of other proteins via this pathway (Cuervo et al., 2004a)(Fig. 4).

Based on the previous described functions of CMA, blockage of this pathway in the PD-affected neurons could contribute to their altered homeostasis. However, one of the initial caveats to support that altered CMA underlies the bases of PD, was the fact that mutation in α- synuclein only occur in a small percentage of patients, whereas this protein accumulates in all forms of PD. Analysis of the degradation of wild type α-synuclein after undergoing different types of post-translational modifications provided an answer to this conundrum. Although phosphorylation, oxidation, nitration and irreversible multimerization reduced, to some extent, degradation of α-synuclein by CMA, none of these modified forms of the protein exerted a significant effect on the uptake of other CMA substrates. In contrast, dopamine-adducts of α-synuclein behaved very much like the pathogenic mutant forms of the protein: they bound tightly to the CMA receptor but did not translocate, resulting in blockage of CMA both in isolated lysosomes and in cultured dopaminergic cell lines and ventral midbrain neurons (Martinez-Vicente et al., 2008).

The mechanism behind the inhibitory effect of pathogenic α-synucleins on CMA remains unknown, but studies with isolated lysosomes have revealed the organization of the pathogenic proteins into irreversible oligomeric complexes at the lysosomal membrane. How the presence of these complexes at the lysosomal membrane interferes with the CMA machinery is currently subject of investigation (Martinez-Vicente et al., 2008). Also of particular interest is the role that other cytosolic chaperones may play in the targeting of α-synuclein to lysosomes for CMA degradation. Although until date only hsc70 has been demonstrated to be involved in CMA targeting, recent studies show that overexpression of carboxyl terminus of hsp70-interacting protein (CHIP) inhibits α-synuclein inclusion formation, reduces α-synuclein protein levels and enhances the association of this protein to lysosomes (Shin et al., 2005). The tetratricopeptide domain at the amino terminus of CHIP is critical for proteasome targeting of substrates, whereas the U-box domain at the carboxyl terminus has been proposed sufficient to direct α-synuclein towards the lysosomal degradation pathway. Although the type of autophagy responsible for the degradation of CHIP-targeted α-synuclein has not been elucidated yet, the interrelation of CHIP with hsc70 makes it likely that CMA could be involved.

Mutant forms of a second PD-related protein, the ubiquitin carboxyl-terminal esterase L1 (UCHL-1), have also been shown to interact abnormally with CMA components (Fig. 4). Although degradation of UCHL-1 via CMA has not yet been demonstrated, this protein also contains a CMA-targeting motif in its sequence and interacts with hsc70, hsp90 and LAMP-2A (Kabuta and Wada, 2008). An abnormally enhanced binding to LAMP-2A has

been found for the UCHL-1 mutant described in some forms of familial PD. Whether or not this aberrant binding results in impaired CMA requires further investigation.

In addition to the degradation of PD-related proteins by CMA, further connections between this autophagic pathway and PD have been recently proposed upon the identification that the myocyte enhancer factor 2D (MEF2D), a transcription factor required for neuronal survival, is a *bona fide* CMA substrate (Yang et al., 2009) (Fig. 4). Interestingly, the cytoplasmic pool of MEF2D is increased in brains of α-synuclein transgenic mice and of PD patients compared to unaffected patients (Smith et al., 2006), and a similar increase of a nonfunctional pool of MEF2D has been attained experimentally in neurons upon partial blockage of CMA (Yang et al., 2009). It is thus possible that the compromised neuronal viability observed in PD results, at least in part, from CMA dysfunction.

CMA and Alzheimer's disease

Connections between CMA with Alzheimer's disease and AD-related disorders have recently been established after the discovery that some mutant forms of Tau, the cytoskeletal protein that organizes into tangles in this disease, are targeted to lysosomes via CMA. In fact, although Tau bears a CMA-targeting motif in its sequence, the amount of wild type Tau degraded by CMA is negligible, at least under normal conditions (Wang et al., 2009). Pathology associated to certain Tau mutants results from an abnormal cleavage of the mutant protein into peptides with amyloidogenic properties (Fig. 4). The first cleavage occurs in the cytosol, whereas the second and third cleavage are dependent on lysosomal proteases and on the acidification of the lysosomal lumen, supporting that they occur inside this compartment (Wang et al., 2009). However, although macroautophagy has been shown to degrade Tau containing protein inclusions, blockage of this autophagic pathway did not prevent the pathologic cleavage of the Tau mutants, supporting that the truncated protein reached the lysosomal lumen by a different mechanism. Studies *in vitro* and in cultured neuronal cells have shown that after the cytosolic cleavage, the truncated c-terminus region of Tau is recognized by hsc70 and this results in its delivery to the CMA receptor. However, despite efficient binding to this receptor, translocation of the truncated Tau is aborted at an early stage, allowing entry into the lysosomal lumen of only the most c-terminal part of the protein. This is the part that undergoes the second and third cleavage in the hands of the lysosomal cathepsins and that renders the amyloidogenic products (Fig. 4). Some of these products organize as oligomeric structures at the lysosomal membrane where they interfere with CMA functioning (Wang et al., 2009). With time, these oligomers can promote disruption of the lysosomal membrane and subsequent leakage of lysosomal enzymes into the cytosol. These incipient oligomers could also act as seeding for the formation of amyloid through association with full length Tau in the cytosol (Wang et al., 2009). Although the process depicted here resembles the one described for the pathogenic forms of α-synuclein in CMA, two major differences should be noted. First, disruption of the lysosomal translocation of α-synuclein seems to occur at an earlier step than for Tau, since there is no evidence of any portion of the former reaching the lysosomal lumen. And second, whereas accumulation of oligomers of Tau in lysosomes often leads to their disruption, oligomers of α-synuclein, at least under the conditions analyzed so far, do not affect lysosomal stability. Whether other mutant forms of Tau can undergo similar lysosomal cleavage, or if conditions interfering with CMA activity could favor formation of the amyloidogenic peptides even in absence of mutations in Tau requires further investigation.

In the case of Amyloid Precursor Protein (APP), the other pathogenic protein associated with AD, a direct connection with CMA is not easily inferred, as membrane proteins are not amenable to CMA. However, the cytoplasmic tail of APP, where the toxic C31 fragment originates after APP cleavage (Park et al., 2009), bears a CMA-targeting motif. It is thus conceivable that CMA could be one of the mechanisms for the normal elimination of this

cytotoxic product, and that alteration of CMA could impair its clearance and contribute to toxicity.

Connections between CMA and a particular degenerating disease, are not always through the degradation of the pathogenic proteins detected in the protein inclusions, plaque and tangles, but instead, can be through the degradation of regulatory proteins relevant for the disease. Thus, CMA degradation of the regulator of calcineurin 1 (RCAN1), a gene implied in AD, has been recently reported (Liu et al., 2009). Although how abnormal degradation of RCAN1 contributes to AD pathogenesis has not yet been explored, it is anticipated that conditions with impaired CMA, such as the declined activity observed with aging, could contribute to progression of the disease through changes in cytosolic levels of RCAN1.

CMA and polyQ disorders

PolyQ disorders results from an abnormal increase in the length of glutamine stretches in specific proteins which transform them to become pathogenic. In the case of Huntington's disease (HD), the polyQ expansion occurs in huntingtin (htt), the main component of the nuclear and cytosolic proteinaceous inclusions detected in HD-affected neurons. Strong evidences support that macroautophagy contributes to the removal of htt aggregates (Sarkar et al., 2009a; Sarkar et al., 2009b), whereas the mechanism responsible for clearance of the soluble protein likely involves different autophagic and non-autophagic pathways. Although htt contains in its sequence 5 different CMA-targeting motifs, the amount of full size protein degraded through this pathway under normal conditions is minimal. It is however possible that these motifs could be utilized for degradation of the functional peptides that originate through regulated cleavage of htt by calpains and caspases (Schilling et al., 2006). In fact, precedent has been recently described for an N-terminal caspase-7-generated fragment of ataxin-7, the protein bearing the polyQ stretch in spinocerebellar ataxia 7 (Duncan et al., 2010). While the physiological fragment is cleared in lysosomes, turnover of the fragment resulting from the pathogenic protein is impaired (Duncan et al., 2010). Further studies are required to determine the autophagic pathway behind this lysosomal degradation.

Interestingly, although the N-terminus of htt (commonly known as hexon-1) does not contain a *bona fide* CMA-targeting motif in its sequence, upregulation of CMA components, LAMP-2A or hsc70, decreases the toxicity associated to expression of mutant hexon-1 htt. Reduced toxicity results from increased clearance of this pathogenic protein both in lysosomes and proteasome (Thompson et al., 2009). This clearance is regulated by IKK-dependent phosphorylation of htt which has been shown to regulate additional posttranslational modifications in htt (ubiquitinization, SUMOylation, acetylation). It is thus plausible that one of these modifications completes a partial CMA-targeting motif and contributes to delivery of the protein to lysosomes.

Independently of whether htt is usually a CMA substrate or not, a recent report has shown that CMA degradation of mutant htt can be forced to manifest beneficial effects (Bauer et al., 2010). This intervention takes advantage of a peptide known as polyglutamine binding peptide 1 (QBP1) that binds expanded polyQ tracks but not the track present in the wild type protein. By including two different hsc70-binding motifs in QBP1, the mutant htt is targeted to lysosomes where it undergoes CMA (Fig. 4). Theoretically, this strategy would have been predicted to fail, because in this case, the hsc70-interacting region is not provided by the substrate but by a second protein, that should be disassembled from htt before transport through the CMA translocon takes place. However, the fact that this has been an experimental success suggests that once the QBP1-htt complex reaches the lysosomal membrane, it is possible that the endogenous CMA-targeting motifs in htt become accessible to the chaperones. A second possibility is that because those motifs are only required for interaction with the chaperone, but not with LAMP-2A, once the protein is close to the

receptor it can bind to it without further requirement for the chaperone interaction. Independently of the mechanism, this intervention could have important therapeutic potential, in particular considering that HD-affected cells, at least during the early stages of the disease, have increased CMA activity (Martinez-Vicente, Koga and Cuervo, submitted), likely to compensate for the macroautophagic defect identified in these cells (Martinez-Vicente et al., 2010).

Concluding remarks

The better understanding of the molecular basis for CMA gained during the last decade, allows now for the manipulation of this autophagic pathway in whole cells and for the subsequent analysis of the cellular consequences of changes in CMA activity. These types of approaches have unveiled previously unknown functions of this pathway in cellular physiology. The contribution of CMA to maintenance of cellular homeostasis and to the cellular response to stress, along with the growing number of specialized functions for this autophagic pathway, have been a natural preamble for the recent connections established between CMA dysfunction and human diseases. In this respect, advances have been particularly rapid in the dissection of the contribution of CMA to neurodegenerative diseases. These findings have revealed that as important as the contribution of CMA in the removal of pathogenic proteins, is the fact that CMA is often a target of these pathogenic proteins. Impaired CMA functions may be thus a common feature to, otherwise very different neurodegenerative disorders. With that in mind, interventions to improve CMA activity could become an efficient way to slow down the progression of these disorders.

This fast developing field is, of course, in need of novel tools that allow not only a better dissection of this process but also a more efficient mechanism of modulation. The use of genetic manipulation to modulate CMA has been successful in better understanding the consequences of changes in the activity of this pathway (Cuervo and Dice, 1996; Massey et al., 2006; Zhang and Cuervo, 2008), but it will not be possible using it for therapeutic purposes in elders, main targets of these neurodegenerative disorders. The development of pharmacological CMA modulators should be the next step toward the implementation of therapeutic strategies aimed to improve neuronal homeostasis through manipulations on CMA.

Research Highlights

- Chaperone-mediated autophagy (CMA) contributes to selective degradation of proteins
- CMA is required for maintenance of cellular homeostasis and in the response to stress
- Dysfunction of CMA occurs in different neurodegenerative disorders and with age

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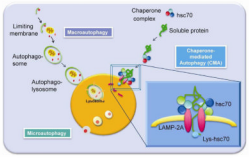

Figure 1. Chaperone-mediated autophagy in the context of mammalian autophagy

Macroautophagy, microautophagy and chaperone-mediated autophagy (CMA) are the principal forms of lysosomal degradation. In contrast to the sequestration of cytosol that occurs in the two former pathways, in CMA cytosolic substrate proteins are degraded one by one. CMA substrates are recognized by a cytosolic chaperone (hsc70) and its co-chaperones. This complex is targeted to the lysosome-associated membrane protein type 2A (LAMP-2A), the lysosomal receptor for this pathway. After unfolding, substrate proteins cross the lysosomal membrane assisted by a luminal chaperone (Lys-hsc70) and are rapidly degraded.

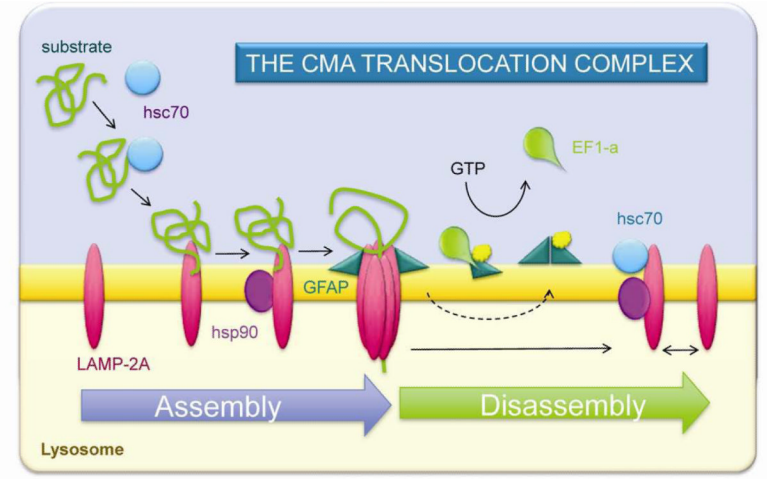

Figure 2. Dynamics of the CMA translocation complex at the lysosomal membrane

Substrate proteins bind to monomers of LAMP-2A initiating their sequential organization into a multimeric complex. Formation of this multimeric complex is required for substrate translocation into the lysosomal lumen. A form of hsp90 located in the luminal side of the lysosomal membrane stabilizes LAMP-2A while transitioning toward complexes. Hsc70 has two recognized roles in CMA: 1) it promotes the interaction of substrates with LAMP-2A and 2) facilitates disassembly of LAMP-2A from the multimeric complex. A pair of proteins (GFAP and EF1α) modulates the stability of the CMA translocation complex. GFAP binds to LAMP-2A at the complex and stabilizes it. In the presence of GTP, LAMP-2A bound GFAP exchanges with EF1α, which is released from the complex that it forms with phosphorylated GFAP at the lysosomal membrane.

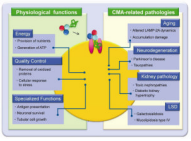

Figure 3. Roles of CMA in physiological process and connections of CMA dysfunction with disease

Left: Cellular functions in which participation of CMA has been demonstrated. **Right:** Partial list of human pathologies/conditions in which altered CMA has been described.

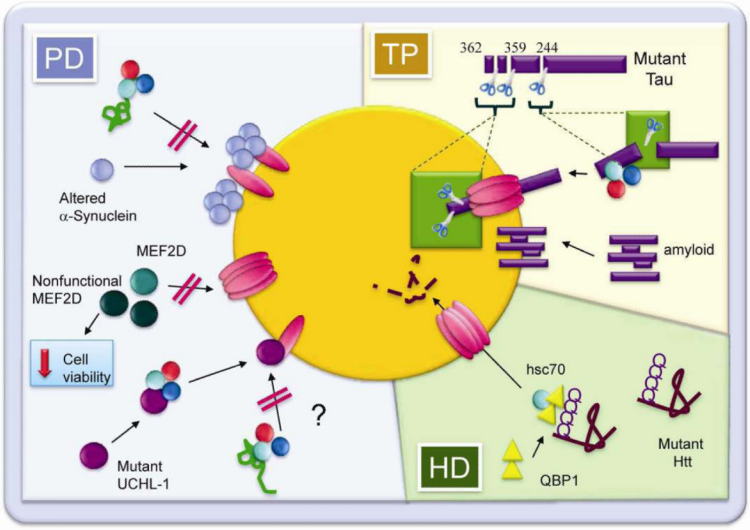

Figure 4. CMA and neurodegenerative disorders

Schematic model of the evidence supporting connections between CMA function and three neurodegenerative disorders: Parkinson's disease (PD), Tauopathies (TP) and Huntington's disease (HD). PD: pathogenic synucleins interact abnormally with CMA components blocking degradation of other substrates. Aberrant interactions with CMA components have been described for UCHL-1. Reduced CMA activity in PD results in cytosolic accumulation of non functional MEF2D which decreases cell survival. TP: mutant forms of Tau undergo abnormal cleavage into amyloidogenic peptides. After a first cytosolic cut, the truncated protein is targeted to lysosomes via CMA, but fails to translocate. However, the part of Tau already in the lumen undergoes two additional cuts that generate the amyloidogenic peptides. HD: mutant forms of huntingtin (htt) have been successfully delivered to lysosomes via CMA for degradation by overexpression of a recombinant dimeric form of QBP1 with hsc70 binding sites.