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The coming of age of chaperone-mediated autophagy

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Abstract

Chaperone-mediated autophagy (CMA) was the first studied process that indicated that degradation of intracellular components by the lysosome can be selective — a concept that is now well accepted for other forms of autophagy. Lysosomes can degrade cellular cytosol in a nonspecific manner but can also discriminate what to target for degradation with the involvement of a degradation tag, a chaperone and a sophisticated mechanism to make the selected proteins cross the lysosomal membrane through a dedicated translocation complex. Recent studies modulating CMA activity in vivo using transgenic mouse models have demonstrated that selectivity confers on CMA the ability to participate in the regulation of multiple cellular functions. Timely degradation of specific cellular proteins by CMA modulates, for example, glucose and lipid metabolism, DNA repair, cellular reprogramming and the cellular response to stress. These findings expand the physiological relevance of CMA beyond its originally identified role in protein quality control and reveal that CMA failure with age may aggravate diseases, such as ageing-associated neurodegeneration and cancer.

Last year, Yoshimori Ohsumi received the Nobel Prize in Medicine or Physiology for his molecular discoveries in autophagy. Work from many groups since the discovery of lysosomes has paved the way to our current understanding of the autophagic mechanisms that mediate the delivery of cytosolic proteins and organelles to lysosomes for degradation^{1,2}. These new findings have proved that autophagy can be very selective in what it targets for degradation (cargo)³, an idea that originally encountered considerable resistance.

It was during this early resistance that the discovery of chaperone-mediated autophagy (CMA) occurred. At that time, the best-characterized form of autophagy was macroautophagy, a process during which cargo that is sequestered in double-membrane vesicles (autophagosomes) is delivered to lysosomes through vesicular fusion⁴ (Fig. 1). This

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apparent in bulk trapping of cytoplasm inside autophagosomes was not compatible with the idea of selective degradation. A similar in bulk principle applied to microautophagy, whereby invaginations at the lysosomal membrane (or the vacuole in yeast) internalize cytosolic cargo into small vesicles that then detach into the lumen for degradation⁵ (Fig. 1).

Macroautophagy and microautophagy are now known to be capable of exquisite cargo selectivity. Macroautophagy uses cytosolic receptor proteins (for example, sequestosome 1 (p62)), next to BRCA1 gene 1 protein (NBR1) and NIP3-like protein X (NIX; also known as BNIP3L), that bring cargo and the autophagy machinery together³. This spatial coincidence allows assembly of the autophagy proteins to form a limiting membrane around the cargo. This membrane grows and seals, giving rise to an autophagosome with the cargo and receptors inside. Selective organelle degradation by microautophagy occurs by interaction of organelle proteins with surface proteins at the vacuole, lysosomes or late endosomes^{6,7}, whereas a cytosolic chaperone, the heat shock cognate 71 kDa protein (HSC70; also known as HSPA8), mediates selective protein degradation by micro-autophagy (known as endosomal microautophagy in mammals)⁸ (Fig. 1).

Selectivity has been associated with CMA since its discovery because of the unique mechanism that underlies lysosomal cargo delivery. Proteins, the only cargo degraded by this pathway, cross the lysosomal membrane one by one. Not all proteins can undergo degradation via CMA. To be CMA substrates, proteins must contain a specific targeting motif in their amino acid sequence⁹. This motif binds to a cytosolic chaperone (HSC70), which brings the substrate protein to the lysosomal surface for internalization and rapid intralysosomal degradation.

The focus of CMA studies has shifted through the years. Early studies optimized systems to reconstitute CMA in vitro, which helped in understanding chaperone–cargo recognition and substrate lysosomal translocation^{10,11}. Molecular dissection of CMA has been slow because model systems, such as yeast, worms or flies, that accelerated discovery in macroautophagy are of no use in the study of CMA as one of the essential CMA components is absent in these species and, to date, has been described only in birds and mammals¹². The introduction of mammalian RNAi technology facilitated the identification of new components and regulators of the CMA pathway^{13–16}. The ability to genetically modulate CMA has been key, in recent years, to linking CMA malfunctioning to human diseases, such as neurodegeneration and cancer^{17,18}. Current studies on CMA at the cellular and organism level are providing a more comprehensive view of its physiological relevance by discovering previously unknown CMA functions^{19–21}. For example, genetic blockage of CMA in mouse liver has revealed a key role for CMA in the regulation of glucose and lipid metabolism, whereas mouse models with compromised CMA in T cells support the requirement for a fully functional CMA to reach maximal T cell activation.

In this Review, we describe recent advances in our understanding of the mechanisms of CMA and the finely tuned orchestration of CMA activity as part of the proteostasis networks. We also discuss new findings on the pathophysiological relevance of CMA, particularly in the context of ageing and two age-related disorders (neurodegeneration and cancer).

Mechanisms of CMA

Biochemical and genetic approaches have helped dissect the steps and molecular machinery involved in CMA. These studies have confirmed the unique characteristics of CMA regarding substrate identification and delivery inside lysosomes when compared to other types of autophagy.

The basis for cargo selectivity

The concept of selective lysosomal degradation by CMA originated from the finding that not all cytosolic proteins could undergo lysosomal degradation. Using ribonuclease A, which is one of the proteins that can be degraded, the late American biochemist and cell biologist Fred Dice and his team identified an 11-amino-acid region in the protein, later narrowed down to the pentapeptide KFERQ, which is necessary and sufficient to target proteins for lysosomal degradation²².

The properties of the residues that constitute the motif, rather than the specific amino acids, determine whether the CMA-targeting chaperone HSC70 can bind to this region⁹. The motif is always flanked by a glutamine on one of the sides (as the pentapeptide functions as a targeting sequence in both directions) and contains one or two of the positive residues K and R, one or two of the hydrophobic residues F, L, I or V and one of the negatively charged E or D residues. Approximately 40% of proteins in the mammalian proteome contain a canonical KFERQ-like motif. In addition, in some substrate proteins, the same targeting motif can be generated through post-translational modifications, thus expanding the number of potential CMA substrates. Phosphorylation of S, T or Y present in a motif containing only four of the canonical residues and missing the negatively charged one can complete the motif and convert the protein to a CMA substrate^{23–26}. In some instances, Q can be replaced by K, which upon acetylation acquires properties similar to Q, thus completing the motif^{27,28}. Ubiquitylation or acetylation of the same K could, in theory, become a switch between proteasomal and lysosomal degradation. Characteristics of canonical or putative CMA motifs and recommendations for motif validation are described in Box 1.

Post-translational modifications outside the motif can also modulate CMA targeting of proteins bearing canonical motifs by facilitating conformational changes that expose or mask the motif. For example, CMA-dependent degradation of hypoxia-inducible factor 1 α (HiF1 α), a validated CMA substrate, takes place only when it is ubiquitylated on Lys63 by the E3 ubiquitin-protein ligase STUB1 (REF.²⁹). By contrast, acetylation of mammalian Ste20-like kinase 1 (MST1; also known as STK4) in a residue far from the canonical CMA motif prevents its lysosomal degradation, and only upon deacetylation can HSC70 bind the canonical motif³⁰.

HSC70 involvement in CMA

The KFERQ-like motif provided the ‘bait’ to identify HSC70 as the cytosolic chaperone that, upon binding that region, targeted proteins for lysosomal degradation³¹. Although cochaperones, such as carboxyl terminus of HSC70-interacting protein (CHIP), heat shock protein 40 (HSP40; also known as DNABJ1) and HSP70–HSP90 organizing protein HOP

modulate substrate targeting to lysosomes in an HSC70-dependent manner^{32,33}, to date, HSC70 remains the only chaperone proved to directly bind the KFERQ-like motif. HSC70 participates in multiple cellular functions by facilitating folding of unfolded or misfolded proteins. HSC70 binds hydrophobic regions to assist in protein folding. In contrast, HSC70 binds KFERQ-like motifs to target proteins for degradation via CMA.

The relationship of cytosolic HSC70 with autophagy has expanded beyond CMA. In cooperation with the cochaperones BAG family molecular chaperone regulator 1 (BAG1) and BAG3, HSC70 participates in the selective degradation of ubiquitin-positive protein aggregates by a type of macroautophagy known as chaperone-assisted selective autophagy (CASA)³⁴ (Fig. 1). The search for a process in mammalian cells that is homologous to yeast microautophagy led to the discovery of endosomal microautophagy, in which cytosolic proteins enter endosomal compartments inside vesicles generated at the surface of late endosomes through the assembly of endosomal sorting complexes required for transport (ESCRT)⁸. Although cytosolic proteins are trapped in a nonselective manner as these vesicles form, a subset of cytosolic proteins are selectively targeted there by HSC70 (REF.⁸) (Fig. 1). As in CMA, HSC70 binds endosomal microautophagy cargo proteins through a KFERQ-like motif. HSC70 is thus at the centre of protein triage between three different types of autophagy in mammals.

Discovery of selective endosomal microautophagy⁸ has necessitated revising the criteria for bona fide CMA substrates. Dependence on HSC70 for lysosomal degradation, for many years a distinctive characteristic of CMA substrates, is now insufficient to attribute degradation to this pathway. Dependence on the lysosomal membrane receptor lysosome-associated membrane protein type 2A (LAMP2A, discussed below), is the best criteria to determine whether protein degradation occurs via CMA, as LAMP2A is not required for CASA³⁴ or endosomal-microautophagy⁸. Whereas HSC70 requires LAMP2A for lysosomal docking in CMA, the chaperone binds directly to lipids at the endosomal membrane in endosomal microautophagy⁸.

A fraction of cellular HSC70 that is constitutively present in lysosomes, both at their membrane cytosolic side and in the lysosomal lumen, also participates in CMA^{15,35} (Fig. 2). Membrane-bound HSC70 in a complex with the cochaperones HSP90, HSP40, HSP70-interacting protein (HIP) and HOP has been proposed to participate in substrate unfolding³³, which is required before lysosomal translocation³⁶. Membrane HSC70 is also required to sustain continuous cycles of CMA because after the substrate is internalized, HSC70 facilitates dissociation of the receptor protein from the CMA translocation complex to allow for binding of new substrates¹⁵.

The second form of lysosomal HSC70 is located in the lumen^{35,37} (Fig. 2). The stability of HSC70 in this acidic environment with high concentrations of proteases relies on specific properties of the luminal HSC70. Although both cytosolic and lysosomal HSC70 originate from the same gene, it was shown that luminal HSC70 has a highly acidic isoelectric point, which is very different from the neutral isoelectric point of the cytosolic chaperone³⁷. Luminal HSC70 becomes unstable with small changes in lysosomal acidification, which are physiologically used to modulate the percentage of lysosomes competent for CMA at a

given time³⁵. As luminal HSC70 is necessary to complete substrate translocation, lysosomes lacking this chaperone cannot perform CMA. An unresolved question is how HSC70 gains access to the lysosomal lumen. Blockage of macro-autophagy or CMA does not reduce the levels of luminal HSC70 (REF.^{13,38}), thus discarding the possibility that it might be internalized through these pathways. It is possible that HSC70 internalization from the cytosol occurs into late endosomes through microautophagy and that lysosome–endosome fusion facilitates HSC70 transfer to the lysosomal lumen.

Diverse functions of the lysosomal receptor LAMP2A

LAMP2A was the first identified lysosomal component required for CMA³⁹. Participation of a receptor protein in CMA was inferred because substrate internalization in lysosomes was saturable and trypsinization of the surface of lysosomes (to remove protein components) ablated substrate binding and internalization^{35,40}. LAMP2A is one of the three splice variants of a single gene, *LAMP2*¹². LAMP2A, LAMP2B and LAMP2C have identical luminal regions but different transmembrane and cytosolic regions. LAMP2A is the only one of the three isoforms required for CMA^{39,41} (Fig. 2). Although LAMP2A may share with LAMP2B and LAMP2C some of the functions attributed to the luminal region that is identical in the three LAMP2 variants⁴², it is not the most abundant of the three proteins, and its blockage remains, to date, the most specific way to inhibit CMA. LAMP2A contributes to different steps of CMA.

Substrate binding.—The 12-amino-acid cytosolic tail of LAMP2A is required for lysosomal docking of HSC70– substrate complexes^{39,41}. HSC70 and the substrate can bind this tail at the same time, suggesting that substrate recognition and targeting are coupled processes^{39,41,43}; however, the KFERQ-like motif in the substrate is not required for LAMP2A binding³⁹. Three positive residues in the cytosolic tail of LAMP2A are essential to complete CMA of substrates⁴¹, leaving open the possibility that transferring of the unfolding substrate requires interaction only with negatively charged residues in the substrate protein. Studies using an unfoldable artificial substrate demonstrated that unfolding is not required for lysosomal surface binding but is necessary for its translocation³⁶.

LAMP2A assembly.—Multimerization of LAMP2A into a 700 kDa multimeric protein complex is essential for substrate translocation into the lumen^{14,15} (Fig. 2). Assembly of this translocation complex occurs step-wise and includes the formation of an intermediate LAMP2A homotrimer⁴⁴ that increases substrate affinity and probably prevents its aggregation as the substrate unfolds⁴⁴. Cytosolic HSC70 associates with LAMP2A monomers and homotrimers, but it is released before the fully functional translocation complex is assembled¹⁵. A form of HSP90 associated at the luminal part of the lysosomal membrane interacts with and stabilizes LAMP2A during this transition¹⁵ (Fig. 2). HSP90 may cover protease-sensitive regions of LAMP2A that are otherwise exposed during its conformational arrangement for multimeric assembly¹⁵.

Regulation of lysosomal levels of LAMP2A.—Luminal HSC70 is required for CMA, but LAMP2A is the rate-limiting component of this pathway. Changes in levels and dynamics of LAMP2A at the lysosomal membrane regulate CMA flux (that is, the rate at

which cargo is degraded). De novo synthesis of LAMP2A occurs during mild oxidative stress^{21,45}, genotoxic damage²⁴ or hypoxia^{32,46} – all conditions that induce CMA activation. This transcriptional upregulation of LAMP2A is still poorly understood. The transcription factor nuclear factor of activated T cells (NFAT1) has been shown to directly promote the transcriptional upregulation of LAMP2A during T cell activation²¹ (Fig. 3). Transcription of several CMA components, including LAMP2A, have been shown to be under the negative control of the nuclear retinoic acid receptor- α ⁴⁷. Although most lysosomal proteins, including other LAMPs, are part of the programme of the transcription factor EB (TFEB), *LAMP2* transcription is not regulated, at least directly, by this factor⁴⁸.

The increase in LAMP2A levels in other conditions that activate CMA, such as starvation, does not involve de novo synthesis of LAMP2A and is instead mediated through changes in LAMP2A degradation at the lysosomal membrane, which is a finely controlled process^{43,49} (Fig. 2). LAMP2A is usually well protected from the luminal proteases by its abundant glycosylation and unique spread conformation against the inner leaflet of the membrane⁵⁰. Degradation of LAMP2A requires its mobilization to lipid microdomains^{49,51}, where it undergoes a dual cleavage by cathepsin A and a yet unidentified metalloproteinase to be released into the lumen for rapid degradation⁵¹ (Fig. 2). Changes in the lipid composition of the lysosomal membrane with age or upon high-lipid dietary challenges accelerate this regulated degradation of LAMP2A because it becomes trapped in the expanded lipid microdomains^{52,53}. The half-life of LAMP2A in lysosomes is approximately 36 h, but it becomes more than 72 h in hepatocytes and in fibroblasts in culture during prolonged starvation⁵¹. This reduced degradation leads to a net increase in LAMP2A levels in lysosomes. Mobilization of a fraction of LAMP2A resident in the lysosomal lumen (likely in micelles) to the lysosomal membrane is responsible for the further increase in LAMP2A levels at the lysosomal membrane observed when starvation persists beyond 48 h (REF.⁴³).

There is no evidence yet that Golgi complex-to-lysosome trafficking of LAMP2A modulates CMA activity under physiological conditions. However, mis-targeting of LAMP2A has been identified as the basis of reduced CMA observed in the lysosomal storage disorder cystinosis⁵⁴. Defective trafficking seems specific for LAMP2A, as other LAMPs reach lysosomes normally in this disease, thus suggesting the existence of LAMP2A-dedicated Golgi-to-lysosomal transport machinery. The small GTPase Ras-related protein Rab-11A (RAB11) and the RAB7 effector Rab-interacting lysosomal protein (RILP) are likely part of this transport machinery, as correction of the lower levels observed for both proteins in patients is sufficient to correct LAMP2A mistargeting in disease cells⁵⁵. Small molecules that activate CMA by releasing the inhibitory effect of retinoic acid receptor- α (RAR α) on several CMA components⁴⁷ also correct LAMP2A trafficking⁵⁵. Altered endosome-to-Golgi retrieval of LAMPs has been found in cells bearing mutations in the retromer component vacuolar protein sorting-associated protein 35 (VPS35). These mutations have been identified in patients with Parkinson disease (PD)⁵⁶, which was the first neurodegenerative disorder associated with defective CMA activity¹⁷. However, contrary to the selectivity for LAMP2A observed in cystinosis, VPS35 mutations alter trafficking of multiple lysosomal proteins, suggesting a more general lysosomal malfunction in these conditions⁵⁶.

Dynamics of LAMP2A assembly.—Changes in rates of LAMP2A assembly and disassembly from the translocation complex impact overall CMA activity (Fig. 2). A pair of proteins, the monomeric form of the intermediate filament protein glial fibrillary acidic protein (GFAP) and its GTP-binding partner elongation factor 1- α (EF1 α) modulate the stability of multimeric LAMP2A in a GTP-dependent manner¹⁴. Part of GFAP at the lysosomal membrane is phosphorylated and bound to EF1 α , and part is unmodified and bound to the LAMP2A multimeric complex, conferring stability¹⁴. In the presence of GTP, EF1 α dissociates from phospho-GFAP, thus promoting mobilization of GFAP from the translocation complex to phospho-GFAP and the subsequent disassembly of LAMP2A¹⁴.

Conditions that change the lipid composition of the lysosomal membrane impact CMA. High-lipid-content diets change the lysosomal membrane lipidome, which compromises both LAMP2A stability and its ability to multimerize⁵². Interestingly, changes in the lysosomal membrane lipidome with age have comparable effects on LAMP2A stability and dynamics⁵². It is plausible that interventions to modify lipid membrane composition could be utilized to modulate CMA activity under pathological conditions.

Physiological regulation of CMA

CMA was initially described as part of the cellular response to stress, but basal CMA activity is detectable in almost all mammalian cells⁵⁷. The tools and assays currently available to study CMA are summarized in TABLES 1,2. Basal levels of CMA differ widely among cell types and tissues, suggesting possible differences in their dependence on CMA. CMA is upregulated in response to a large variety of stressors, such as starvation, oxidative stress, genotoxic insults, lipid challenges, hypoxia and radiation^{24,29,40,45,58,59}. Integration of CMA with the rest of the cellular stress response requires fine-tuning of CMA activity. Although much is yet to be discovered about physiological regulators of CMA, cues from cytosolic signalling pathways and signalling events at the lysosomal membrane have been shown to regulate CMA (Fig. 2).

NFAT and calcium signalling

The first signalling pathway identified in the activation of CMA was the calcineurin–NFAT pathway, required for CMA activation in T cells²¹, which also provided novel insights on CMA activation in response to oxidative stress⁴⁵. The transcription factor NFAT1 directly binds the *lamp2* proximal promoter region, which contains several putative NFAT1-binding sites. Generation of reactive oxygen species (ROS) during T cell activation promotes the nuclear translocation of NFAT1 and the subsequent increase in *lamp2a* expression. Both calcineurin inhibition by cyclosporine A or blocking ROS production ablate CMA activation in these cells²¹. T cell receptor (TCR)-induced CMA activation favours CMA degradation of TCR signalling inhibitors such as E3 ubiquitin-protein ligase Itchy homolog (ITCH) and regulator of calcineurin 1 (RCAN1) — a requirement for maintaining T cell activation in these cells²¹.

RAR α signaling

Signalling through RAR α , but not through other abundant members of this nuclear receptor family (such as RAR β , RAR γ or RXR), inhibits CMA⁴⁷. Genetic and chemical blockage of RAR α demonstrate that not only LAMP2A⁴⁷ but also proteins that facilitate LAMP2A trafficking to lysosomes, such as RAB11 or RILP, are also under the negative regulation of this receptor⁵⁵. It is thus likely that RAR α controls a discrete CMA-related transcriptional programme. In fact, structure-based chemical design has made possible generating synthetic derivatives of all-*trans* retinoic acid that block only the inhibitory effect of RAR α on CMA without affecting the RAR α -dependent transcriptional programme⁴⁷. Chemical upregulation of CMA with these compounds rendered cellular protection from oxidative stress and proteotoxicity⁴⁷. Given the ubiquitous expression of *RAR α* , these atypical RAR α inhibitors hold great promise in the systemic restoration of impaired CMA in ageing.

The TORC2–AKT1–PHLPP1 axis

Physiological regulation of CMA activity also occurs directly at the lysosomal membrane. As previously mentioned, dynamics of the CMA translocation complex depend on GFAP phosphorylation status¹⁴, which determines its oligomeric state. Lysosomal GFAP phosphorylation is performed by a form of AKT1 that resides at the lysosomal membrane¹⁶. AKT1 activity is closely monitored by the phosphatase PH domain leucine-rich repeat-containing protein phosphatase 1 (PHLPP1) and the kinase TOR complex 2 (TORC2)¹⁶ at the lysosomal membrane (Fig. 2). Lysosomal TORC2 phosphorylates AKT1, which, by phosphorylating GFAP, puts a break on CMA activity. Contrary to TORC1, TORC2 does not shuttle and is always present at the membrane of CMA-active lysosomes¹⁶. TORC2 and AKT1 exert sustained inhibition on CMA as chemical TORC2 inhibitors such as torin (at concentrations that do not affect TORC1), knockdown of the TORC2 component rapamycin-insensitive companion of mTOR (RICTOR), or chemical or genetic blockage of AKT1 all lead to CMA induction¹⁶. The TORC2–AKT1-axis inhibition on CMA is physiologically released through RAC1-dependent recruitment of the phosphatase PHLPP1 to the lysosomal membrane whenever CMA activation is needed¹⁶. PHLPP1 dephosphorylates AKT1, and inactivation of this kinase makes dephosphorylated GFAP available to accelerate the rounds of LAMP2A assembly and disassembly¹⁶ (Fig. 2). Confining these signalling events to the lysosomal membrane may allow fine-tuning of CMA to rapidly adjust to the cellular needs. Interestingly, several of the CMA regulators at the lysosomal membrane (that is, GFAP, EF1 α , RICTOR and AKT1) contain CMA-targeting motifs and undergo some, albeit discrete, lysosomal degradation^{14,16}. It is tempting to speculate that the targeting motif may be used not only for degradation but, in some instances, for delivery of CMA regulators to the lysosomal membrane and that degradation of these regulators by CMA may be a way to terminate their stimulatory or inhibitory effect on CMA.

Physiological roles of CMA

The diverse nature of already identified CMA substrates and the additional potential CMA substrates that can be generated by post-translational modification justifies the variety of cellular processes impacted by CMA (Fig. 3). Early studies emphasized the role of CMA in protein quality control and considered abnormally synthesized or post-translationally

damaged KFERQ-containing proteins as main CMA substrates⁴⁵. However, the finding that during starvation most KFERQ-bearing proteins can undergo CMA degradation revealed that protein damage is not a requirement for CMA targeting. There is evidence that properly folded, fully functional proteins can become CMA substrates in specific cellular scenarios and that their timely selective degradation by CMA contributes to the regulation of the cellular pathways in which these proteins participate. Experimental approaches to designate a protein as a CMA substrate are described in TABLE 2.

CMA and protein quality control

CMA activation is often triggered by stressors that cause protein damage, such as mild oxidative stress⁴⁵, hypoxic stress^{32,58} or protein denaturation⁶⁰, and in fact, CMA-incompetent cells and organs accumulate oxidized and aggregated proteins^{13,20} (Fig. 3). CMA cannot degrade proteins once they aggregate, but it is part of the first line of defence against protein aggregation by mediating degradation of single proteins upon damage or partial unfolding^{17,61}. Interestingly, in tissues such as the liver, loss of proteostasis is not imminent upon CMA blockage because compensatory upregulation of macroautophagy and the proteasome can handle the load of CMA substrates¹⁹. However, ageing or additional stressors (that is, oxidative stress or lipid challenge) increase protein damage and aggregation in CMA-incompetent tissues, where the compensatory systems become insufficient to maintain proteostasis²⁰. In further support of the role for CMA in protein quality control, both genetic and chemical upregulation of CMA in vitro and in vivo reduce levels of oxidized and aggregated proteins and improve cellular resistance against proteotoxicity^{47,62}.

CMA in the response to starvation

Lack of nutrients activates CMA in most organs and cell types tested^{22,40,59}. Considering the role of autophagy in adaptation to starvation and the timing of CMA activation — after at least 8 h of starvation^{13,63}, when macroautophagy has already peaked — it was suggested that starvation-induced CMA provides cells with free amino acids for the generation of energy and for the synthesis of crucial proteins required for cell function (Fig. 3). In organs such as the liver, proteolysis through macroautophagy lasts for about 8 h of starvation and then switches to preferential degradation of lipids⁶⁴. In the face of nutrient deprivation beyond 10 h, the pool of intracellular amino acids may be mostly replenished through CMA, which is upregulated and sustained at this high level for up to three days in rodents⁴⁰. CMA generates amino acids that sustain protein synthesis but some amino acids, such as alanine and glutamine, also provide the carbon skeletons for gluconeogenesis. Interestingly, tissues with high gluconeogenesis rates, such as the liver and kidney, are very active for CMA. In support of the contribution of CMA to cellular energetics, in vitro and in vivo models with impaired CMA exhibit reduced levels of ATP during nutrient deprivation^{13,18,19}, while genetic restoration of CMA in aged mice normalizes their ATP levels⁶². The ability of CMA to generate free amino acids through degradation has been proposed to be utilized by pathogens, such as *Salmonella*, which recruits CMA components to gain host-derived peptides for its growth⁶⁵.

The signals that activate CMA during starvation are still for the most part unknown. Ketone bodies, the choice fuel in prolonged starvation, activate CMA⁵⁹, and hydrolysis of amino acids derived from CMA generate α -ketoacids themselves, thus establishing a positive feedback loop between CMA and energy production. Intriguingly, CMA activation by ketone bodies occurs through generation of ROS⁵⁹, suggesting that similar mechanisms to those described during T cell activation are in play.

An interesting combination of CMA-dependent quality control and cellular energetics has been recently described to occur through the CMA degradation of oxidatively damaged nonfunctional mitochondrial enzyme protein/nucleic acid deglycase DJ-1 (PARK7)⁶⁶. By efficiently degrading this mitochondrial protein, CMA helps preserve mitochondrial function and should have a positive impact on cellular energetics⁶⁶.

Recent studies in mice with CMA-incompetent livers have revealed a new level of complexity in the interplay between CMA and cellular energetics whereby, as described in the next section, CMA regulates the rates of metabolic pathways through timely selective degradation of key enzymes in these pathways^{19,67}.

Selective proteome remodelling by CMA

Protein degradation is extensively utilized by cells to downregulate cellular processes in which these proteins participate. The selectivity of CMA makes it suitable for this type of regulatory degradation, which underlies the basis of some of the recent novel physiological functions attributed to CMA (Fig. 3)

Regulation of metabolic pathways.—CMA contributes to the regulation of glucose and lipid metabolism through selective degradation of key enzymes in these pathways (Fig. 3). Studies in mice have revealed that basal degradation of many metabolic enzymes via CMA in the liver is accelerated several-fold during fasting^{19,40}. Timely CMA degradation of glycolytic enzymes contributes, for example, to shutting off hepatic glycolysis during starvation, and failure to do so leads to abnormally elevated rates of hepatic glycolysis and the subsequent energy deficiency in peripheral organs¹⁹. Comparative proteomic analysis of lysosomes from CMA-defective mouse livers and from control littermates confirmed that the majority of the glycolytic enzymes and some of the enzymes in the tricarboxylic acid cycle undergo CMA degradation during starvation¹⁹. Abnormal upregulation of CMA activity, such as that observed in patients with tripeptidyl peptidase II (*TPPII*) mutations⁶⁸ can also become deleterious for cellular energetics. In this case, CMA activation is secondary to the lysosomal expansion that occurs upon the loss of TPPII in an attempt to maintain the pool of free amino acids normally provided by this peptidase. The caveat, however, is that general lysosomal upregulation increases degradation via CMA of hexokinase 2, a key glycolytic enzyme, which further impairs cellular energetics in these patients⁶⁸.

CMA also contributes to regulate lipid metabolism (Fig. 3). Proteins involved in lipid metabolism — lipogenesis enzymes, lipid carriers and lipid droplet coat proteins — have been identified as bona fide CMA substrates^{19,67}. Control of both lipogenesis and lipolysis by CMA underlies the basis of the marked derangements in lipid usage and pronounced hepatosteatosis observed in CMA-incompetent mouse liver¹⁹. CMA facilitates lipolysis by

selective degradation of lipid droplet coat proteins, such as perilipins 2 and 3 (PLIN2 and PLIN3)⁶⁷. Selective removal of these proteins from the surface of the lipid droplets by CMA is required to facilitate access of cytosolic lipolytic enzymes and the macroautophagy machinery to the lipids stored in the core of the droplets to initiate both lipolysis and lipophagy⁶⁷. These findings place CMA upstream of both lipolytic pathways. Timely removal of lipid droplet coat proteins by CMA during starvation is triggered in part via 5'-AMP-activated protein kinase (AMPK)-dependent phosphorylation of PLIN2 (REF.²³). CMA contributes to keeping intracellular lipid levels in check by reducing lipogenesis and increasing lipolysis¹⁹. In fact, cells upregulate CMA in response to acute lipid challenges, and failure to do so results in cellular toxicity⁵².

Transcriptional regulation by CMA.—Additional regulatory roles for CMA, beyond the control of metabolic pathways, have been recently identified (Fig. 3). CMA regulates transcriptional programmes mediated by nuclear factor- κ B (NF- κ B) by degrading its inhibitor NF- κ B inhibitor- α ($\text{I}\kappa\text{B}\alpha$) during starvation⁶⁹. Timely CMA degradation of paired-box protein PAX2, a transcription factor important for cell proliferation and differentiation⁷⁰, maintains renal size and growth, whereas CMA protects neurons against stressors through selective degradation of the transcription factors myocyte-specific enhancer factor 2A (MEF2A) and MEF2D, which are required for neuronal survival^{71,72}.

CMA and the immune response.—CMA is important for CD4⁺ T cell activation because it selectively degrades two negative regulators of TCR signalling, namely, ITCH and RCAN1 (REF.²¹) (Fig. 3). Persistent high levels of these two factors in CMA-incompetent T cells are responsible for their reduced cell proliferation and cytokine secretion following activation. These observations unveil an important link between CMA activity and the immune response, further supported by the fact that mice with defective T cell CMA are compromised in their responses to immunization and to infection by pathogens such as *Listeria monocytogenes*²¹. Recent studies have revealed contribution of CMA to the regulation of innate immunity through degradation of stimulator of interferon genes protein (STING), a signalling protein in the response against cytosolic nucleic acids⁷³. The trigger for STING degradation is desumoylation, which unmask its KFERQ-like motif during the late phase of viral infection to shut off the innate immune response⁷³.

Control of cell cycle.—Following genotoxic insults, CMA is required to initiate cell cycle progression after DNA repair has been completed²⁴. This effect is mediated in part by degradation of phosphorylated serine/ threonine-protein kinase CHK1 (also known as cell cycle checkpoint kinase), which in the absence of CMA, stays in the nucleus, interfering with DNA repair²⁴ (Fig. 3). CMA also degrades HIF1 α ^{32,46}, another regulator of cell cycle progression, thus adjusting its levels to the different phases of the cell cycle under conditions of hypoxia. This close regulation is possible through the opposite effects of a pair of cyclin-dependent kinases, whereby CDK1 blocks HIF1 α lysosomal degradation, and CDK2 promotes it⁴⁶. K63 ubiquitylation appears to be the trigger for degradation of HIF1 α via CMA²⁹.

Anti-ageing functions of CMA

Age-dependent decrease in CMA activity occurs in almost all cell types and tissues in rodents and in humans^{21,22,62,74,75}. Lower stability and therefore reduced levels of LAMP2A at the lysosomal membrane is one of the main causes of decline in CMA activity⁵³. Changes in the lipid composition of the lysosomal membrane with age are responsible for the loss of LAMP2A stability^{49,52,53}. Recently generated, tissue-specific AMP2A-knockout mice¹⁹ have begun to expand our understanding of the organism's reaction to CMA malfunctioning and the consequences of reduced function of this pathway with age. Studies in cultured cells have previously demonstrated crosstalk between macroautophagy and CMA, whereby cells respond to blockage of one of these pathways by upregulating the other^{13,38}. Similar positive compensation by macroautophagy has also been found in vivo in mouse models with selective blockage of CMA in liver or in T cells^{19,21}. However, studies in mouse retina suggest that compensation for CMA failure is not universal⁷⁵. While the age-related decline in retinal macroautophagy coincides with pronounced upregulation of CMA, blockage of CMA does not elicit a compensatory activation of macroautophagy in this organ⁷⁵, thus explaining its higher sensitivity to stress upon CMA blockage⁷⁵. In general, although macroautophagy and CMA can compensate for some of each other's functions, they are not redundant, and their loss of function, even when the other pathway is upregulated, becomes evident. For example, CMA-incompetent livers do not accumulate damaged or aggregated proteins because increases in macroautophagy and the proteasome take over some of the CMA protein quality control functions. However, owing to the different timing of activation and substrate-targeting mechanisms of these two pathways, they cannot compensate for CMA regulatory functions and problems with metabolic flux, DNA repair, and so on, which become evident in these animal livers¹⁹. Furthermore, upregulation of macroautophagy and the proteasome in CMA-incompetent livers is rapidly lost when animals are subjected to stressors (that is, oxidative stress and lipid challenges) and as the animals age²⁰. Compensation failure with age can explain the gradual loss of protein homeostasis and higher sensitivity to stress of aged AMP2A-knockout mice livers.

Genetic interventions to restore CMA function in old mouse liver have shown remarkable improvement in age-related changes in this organ. Expressing an inducible exogenous copy of *Lamp2a* protected aged livers from stressors and led to overall improvement in proteostasis and organ function⁶². Preventing the systemic decline of CMA with age may prove an attractive strategy against organism functional loss and age-associated disorders.

The contribution of CMA failure to human disease

A better understanding of CMA dynamics, the identification of novel CMA molecular effectors and regulators and the development of tools to track CMA (TABLES 1,2) have all contributed to linking CMA malfunctioning to a growing number of human diseases. We have selected two types of pathologies with defective CMA to illustrate examples in which diminished or increased CMA activity contributes to disease progression. A more comprehensive description of other CMA-related diseases, summarized in TABLE 3, can be found in recent reviews^{76,77}.

CMA failure in neurodegenerative disorders

The first connection between CMA malfunctioning and a human disease was with PD¹⁷. Since then, CMA failure has been linked to the pathogenesis of a growing number of neurodegenerative disorders (TABLE 3). CMA contributes to the degradation of several proteins that have a propensity to aggregate and, consequently, a decline in CMA activity leads to the accumulation of toxic aggregates.

The pathogenic variants of several CMA substrates are involved in PD, Alzheimer disease, frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Neurodegeneration-related proteins validated as CMA substrates include α -synuclein^{17,61,78–80}, PARK7 (REF.⁶⁶), leucine-rich repeat serine/threonine-protein kinase 2 (LRRK2)⁸¹ and ubiquitin carboxyl-terminal hydrolase isozyme L1 (UCHL1)^{82,83} (in PD), Tau protein⁸⁴, TAR DNA-binding protein 43 (TDP-43)⁸⁵ (in tauopathies such as Alzheimer disease or frontotemporal dementia) and huntingtin^{86–88} (in Huntington disease). However, it is possible that a larger number of proteins that are associated with neurodegeneration are CMA substrates, as many other proteins in these diseases contain the KFERQ-targeting motif.

A common characteristic of these neurodegeneration-related proteins is that, in most cases, the unmodified protein behaves as a conventional CMA substrate, whereas the pathogenic variants are targeted to lysosomes but fail to degrade and inhibit CMA (Fig. 4). In PD, pathogenic forms of α -synuclein^{17,61,89}, UCHL1 (REFS^{82,83}) and LRRK2 (REF.⁸¹) bind cytosolic HSC70 but, unlike their wild-type counterparts, interact abnormally with CMA components at the lysosomal membrane. Their obliterated entry into the lysosomal lumen often favours their organization into oligomeric complexes at the lysosomal surface, which negatively impacts CMA-mediated degradation of other substrates and further disrupts neuronal proteostasis^{17,61,78,80,82,85,90}. Failed assembly of LAMP2A into the CMA translocation complex upon binding of PD proteins to this receptor has been demonstrated as the basis of their toxic effect on CMA⁸¹. Accordingly, in vitro and in vivo upregulation of CMA with small molecules⁴⁷ or by viral-mediated *LAMP2A* expression⁹¹ has been shown to decrease α -synuclein levels and to protect against neurodegeneration⁹¹. Similarly, *Nrf2* (also known as *Nfe2l2*) expression in astrocytes slows down CMA decline in PD mice by as-yet unidentified mechanisms and has a similar neuroprotective effect⁹². CMA malfunction is becoming a common feature of most PD types, although CMA failure may originate through different mechanisms. Besides the direct toxic effect on CMA described for pathogenic α -synuclein, UCHL1 or LRRK2, PD-related proteins, such as mutant VPS35 (REF.⁵⁶) or GBA1 (REF.⁹³), may reduce CMA activity by affecting lysosomal biogenesis. Although direct measurement of CMA in PD patient brains is still not feasible, correlative evidence supporting CMA malfunctioning in PD patients has populated the literature in recent years (TABLE 3). Reduced LAMP2A levels have been reported in both familial and idiopathic PD patients' brains⁹⁴, especially in the areas more vulnerable to disease⁹⁵. PD-linked sequence variants in the *LAMP2* promoter⁹⁶ and increased levels of specific microRNA in PD patients⁹⁷ seem responsible, at least in part, for the changes in LAMP2A in PD brains. Early stage PD brains show reduced LAMP2A levels, even before α -synuclein accumulation, suggesting that CMA dysfunction is an early event in PD pathogenesis⁹⁵.

Several of the proteins associated with frontotemporal dementias and tauopathies, including Alzheimer disease, have also been validated as bona fide CMA substrates whose degradation through this pathway is reduced in the diseased brains^{84,85,98,99}. Similar to PD-related proteins, mutant forms of tau protein are targeted to lysosomes but fail to reach the lysosomal lumen⁸⁴. In this case, the mutant proteins do not disrupt LAMP2A multimerization, but their translocation through this complex is halted halfway into the lysosomal lumen⁸⁴ (Fig. 4). Incomplete translocation of mutant tau leads to its partial cleavage into highly amyloidogenic peptides that form irreversible oligomers at the lysosomal membrane, thus inhibiting CMA⁸⁴. CMA also contributes to the degradation of other neurodegeneration-related proteins such as RCAN1⁹⁹, ubiquilin 1 (REF.¹⁰⁰) and TDP-43 (REF.⁸⁵), but less information is available about the CMA of their pathogenic variants or their impact on CMA. An unexpected relation has been suggested between CMA and the processing of amyloid precursor protein (APP), a protein closely associated with Alzheimer disease⁹⁸. APP is a single-span membrane protein and, as such, is not a candidate for CMA degradation. However, APP undergoes physiological cleavage to release a short cytosolic stub where, coincidentally, the KFERQ motif is located. Although direct evidence for its CMA degradation is still missing, mutation of this motif led to cytosolic accumulation of this small, highly toxic peptide⁹⁸.

Despite most studies pointing towards faulty functioning of CMA in neurodegenerative disorders, in Huntington disease, CMA activity is upregulated as a compensatory response to macroautophagy failure⁸⁷. In fact, CMA can degrade both wild-type and mutant huntingtin, the protein that aggregates in the affected neurons, and artificial CMA targeting of mutant huntingtin has proved effective in reducing neurotoxicity in experimental mouse models^{86,88}. However, the therapeutic applicability of mutant huntingtin re-routing towards CMA may be limited by the fact that the normal age-related decline in CMA activity is accelerated in the Huntington disease brain⁸⁷.

Associations between CMA and other neuropathogenic proteins, such as prion proteins, and changes in CMA components in conditions, such as brain ischaemia or traumatic brain and spinal cord injury, have begun to appear (TABLE 3).

Accumulating evidence points towards a failure in CMA in several neurodegenerative diseases. A common theme that has also emerged is that the mechanism of this CMA inhibition varies on a case-by-case basis. Studying the pathological protein along with the wild-type version and delineating the exact step affected in CMA would be key in providing specific therapeutic interventions.

Complex role of CMA in cancer biology

Despite connections between CMA and cancer having been first established little more than 6 years ago¹⁸, some common key features of this interplay have since emerged. CMA activity is markedly upregulated in most cancer cell lines, and levels of CMA components, mainly LAMP2A, are elevated in a large array of human tumours^{18,30,101} (TABLE 3). Blocking CMA in cancer cells adversely affects cell survival and decreases their tumorigenicity, whereas blocking CMA in preformed xenografts causes tumour shrinkage and reduces metastasis¹⁸. The bases for the pro-oncogenic effect of CMA are probably

multiple and depend on the type and stage of cancer (Fig. 4). The contribution of CMA to protein quality control explains why upregulating CMA provides the cancer cells several survival benefits, for example, resistance to hypoxia, oxidative stress and DNA damage — all key elements of a growing tumour microenvironment and of most anti-oncogenic interventions^{26,101–108}. The role of CMA in tumour growth goes beyond quality control of damaged proteins and has been linked to selective degradation of specific regulatory proteins. Thus, CMA activity is required in some cancers to sustain the Warburg effect — aerobic glycolysis important for tumour progression^{18,27}. CMA degradation of some glycolytic enzymes, such as acetylated pyruvate kinase PKM2, results in increased levels of glycolytic intermediates, which promote growth and proliferation²⁷. Additionally, CMA indirectly reduces p53 levels, thus releasing its transcriptional inhibition on key glycolytic enzymes and resulting in higher glycolytic rates¹⁸. CMA also sustains tumour growth by degrading antiproliferation proteins (such as Rho-related GTP-binding protein RhoE (RND3)²⁶), tumour suppressor proteins (such as MST1 (REF.³⁰), 15 kDa phosphoprotein enriched in astrocytes (PEA-15; also known as PED)²⁵ and mutant p53 (REF.¹⁰⁹)) or pro-apoptotic proteins (such as Bcl-2-binding component 3 (BBC3; also known as PUMA¹¹⁰)), and by indirectly contributing to stabilize prosurvival proteins such as the induced myeloid leukaemia cell differentiation protein MCL1 (REF.¹¹¹). Accordingly, blockage of CMA in cancer cells causes oxidative stress and higher susceptibility to chemotherapeutic agents and apoptosis¹⁰¹, and is being pursued as a possible antitumorigenic intervention. Upregulation of CMA — beyond that already occurring in the tumour — after blocking macroautophagy and the proteasome promotes cell death in specific tumours through metabolic collapse due to rapid degradation of glycolytic enzymes via CMA^{112,113}.

Despite this protumorigenic role of CMA in cancer cells, it is important to note that the role of CMA in non-transformed cells is remarkably the opposite and that all evidence supports an anti-oncogenic role for CMA under physiological conditions (Fig. 4). CMA plays a tumour suppressive role in nontumorigenic cells by accelerating MYC's proteasomal degradation and thus abolishing its oncogenic activity¹¹⁴ by directly degrading oncoproteins such as E3 ubiquitin-protein ligase MDM2 (REF.¹¹⁵), and by perhaps facilitating immunogenic cell death¹¹⁶. CMA may also protect against malignant transformation by assuring genome stability through its role in efficient DNA repair²⁴. Progressive decrease in CMA with age may thus contribute to the higher risk of malignant transformation in ageing. In fact, mice with hepatic blockage of CMA develop spontaneous tumours²⁰. Therefore, interventions aiming to preserve or restore CMA activity in ageing individuals may have potential value in cancer prevention.

Conclusions and perspectives

Research on CMA has a long way to go to reach the wealth of knowledge accumulated on other forms of autophagy during the past 2 decades. However, the identification of the essential CMA molecular players, the better understanding of the unique dynamics of CMA substrate degradation and the in vivo validation of the physiological relevance of CMA through the study of transgenic mouse models have all contributed to the coming of age of CMA.

Discovery of the regulated LAMP2A multimerization at the lysosomal membrane was key in understanding how CMA is regulated at the mechanistic level, but in the cellular context, the number of signalling pathways known in CMA regulation is still discrete, especially when considering the variety of CMA-activating stimuli present. Whether endosomal microautophagy is the CMA alternative in species where LAMP2A is not present or whether there could be yet another CMA-equivalent pathway in these species needs exploration. The growing evidence in support of a close interplay between CMA and macroautophagy makes it a priority to understand the molecular players involved in this crosstalk. Ongoing studies using high-resolution technologies on CMA reconstituted translocation units should provide new insights on the energetic requirements, driving forces and roles of cochaperones and chaperones at each side of the lysosomal membrane.

CMA bears functions common to all cell types (that is, protein quality control) and also more cell-type- specific regulatory functions that stem from its ability to selectively degrade single still-functional proteins. The introduction of mouse models with increased or reduced CMA and the future expansion of the tissue-specific conditional models will help us better understand these CMA regulatory functions and how tissue-specific changes in CMA impact whole organism function. Similarly, despite the already impressive list of connections between CMA malfunctioning and disease, a large number of them are still correlative, and experimental evidence of changes in CMA activity, the CMA step affected and the molecular mechanisms by which CMA contributes to pathogenesis is still missing. Is the toxic effect of α -synuclein or tau protein on CMA reversible? What are the more vulnerable CMA steps and/or components? Is CMA upregulation in these disease conditions always possible, or is there a 'point of no return'? Multiple questions also surround the dual role of CMA as an anti-oncogenic mechanism in normal cells but a protumorigenic one in transformed cells. What mediates the switch from low to high CMA during transformation? How do oncogenic proteins protect themselves from CMA degradation in the cancer cell? As our knowledge of the contribution of CMA malfunction to disease grows, the need for good chemical modulators of CMA will increase. Despite recent success in the development of selective CMA activators⁴⁷, the field still lacks selective inhibitors of CMA beyond those that inhibit lysosomal proteolysis and thus disrupt all forms of autophagy. Research on CMA has reached a mature stage, and the recent advances in understanding its cellular functions and uncovering new links between CMA failure and human diseases open new avenues for basic and clinical research.

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Chaperone

A protein that assists during folding of other proteins until they reach their functional conformations. Chaperone substrates include both de novo synthesized native proteins and previously folded proteins that undergo partial unfolding.

Proteostasis networks

Intracellular components, mainly chaperones and proteolytic systems, that control each of the processes that occur from protein synthesis to degradation to prevent protein aggregation and ensure maintenance of a stable proteome.

HIF1 α

The hypoxia-inducible factor 1 α is a transcription factor that modulates the cellular response to hypoxia. HIF1 α orchestrates the transcription of a large set of genes involved in cell proliferation, cell survival, and glucose and iron metabolism.

E3 ubiquitin-protein ligase

Intracellular enzyme that participates in the covalent attachment of ubiquitin moieties to cargo proteins. This tagging is commonly used for the targeting of the protein to the proteasome system for degradation.

MST1

The mammalian STE20-like protein kinase 1 is a protein component of the Hippo signalling pathway that modulates cell proliferation and differentiation.

Cochaperones

Proteins that assist chaperones in their functions by modulating their ATP hydrolytic capability.

HSP90

Heat shock protein 90 is a chaperone that stabilizes proteins in transient conformations and facilitates their final folding. HSP90 often acts in conjunction with other intracellular chaperones such as HSC70.

Lysosome-associated membrane protein (LAMP).

Single-span membrane proteins at the lysosomal membrane with a short C-terminus (12 amino acids) exposed to the cytosol while the rest of the protein is in the lysosomal lumen.

Isoelectric point

The pH at which the net charge on a protein is zero.

Nuclear retinoic acid receptor- α

Nuclear receptor activated by retinoic acid that activates or represses gene expression. its wide range of targets include genes involved in development, apoptosis, differentiation, autophagy and circadian regulation.

Lipid microdomains

Cholesterol and/or glycosphingolipid-rich regions in membranes that present higher order and density than the surrounding membrane. They can be transient and highly dynamic in terms of resident proteins and lipids and in their size.

Cathepsin

Protease located in the lysosomal lumen that is maximally active at acidic pH.

Cystinosis

Lysosomal storage disorder characterized by the abnormal accumulation of cysteine inside lysosomes owing to a defect in its normal export from this organelle.

Retromer

Multiprotein complex made up of membrane-associated sorting nexin and vacuolar-protein-sorting proteins that recycle transmembrane proteins from endosomes to the golgi complex and the plasma membrane.

AKT1

Intracellular kinase that participates in signalling pathways that regulate a wide array of intracellular processes, including proliferation, cell survival, metabolism, growth and angiogenesis.

TOR complex 2 (TORC2).

One of the two functional multiprotein complexes containing the nutrient sensing kinase ToR. ToRC2 plays regulatory roles in actin cytoskeleton dynamics, proliferation, growth and metabolism.

Perilipins

Proteins that cover the surface of lipid droplets and shield the hydrophobic lipid core from the aqueous cytosol. Perilipins also regulate the rate of consumption of the lipids (lipolysis) in the lipid droplet.

Warburg effect

Unique metabolic characteristic of many cancer cells whereby they sustain very high levels of glycolysis followed by lactic acid fermentation rather than the common usage of oxidation of pyruvate in mitochondria that follows glycolysis in most cells.

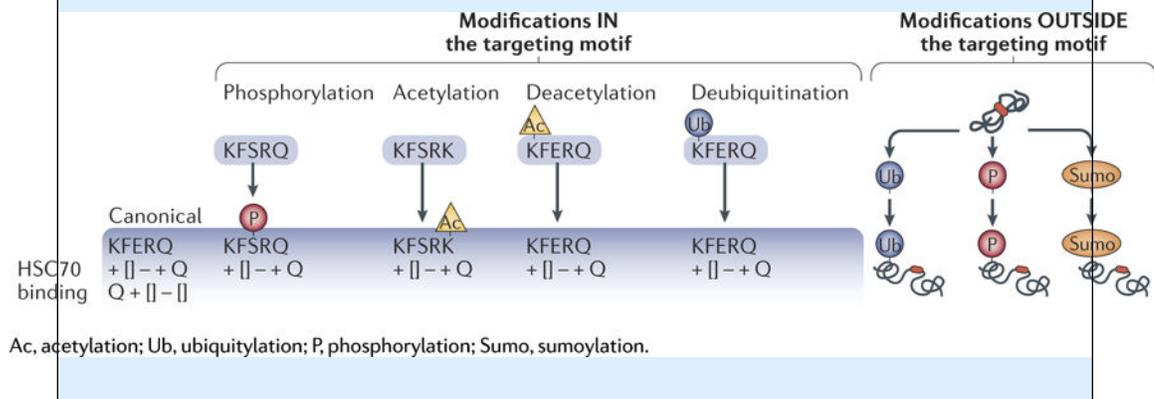
Immunogenic cell death

Functionally peculiar variant of regulated cell death that — in immunocompetent syngeneic hosts — is sufficient to activate an adaptive immune response against dead cell-associated antigens.

Box 1**Validation of KFERQ-like motifs for cMa targeting**

Despite the dynamic nature that post-translational modifications have conferred to the KFERQ-like motif, it is important to notice that the specific requirements for a motif to function in chaperone-mediated autophagy (CMA) targeting have been established and validated experimentally. Several published studies have proposed as CMA-targeting motifs what appear to be randomly chosen sequences. In order to maintain consistency and avoid confusion in the field, it is important that any new proposed motif is validated experimentally according to the following original criteria: elimination of the motif abolishes lysosomal degradation of the protein independent of macroautophagy; insertion of the proposed motif in a well-characterized non-CMA substrate makes it amenable to CMA degradation; and elimination or mutation of residues in the motif suppresses binding of the protein to heat shock cognate 71 kDa protein (Hsc70; also known as HspA8), or if there is still Hsc70 binding, it can no longer compete with a KFERQ-containing peptide.

The figure summarizes the types of post-translational modifications to take into consideration as possible enablers or disruptors of Hsc70 binding to substrate proteins through the KFERQ-like motif (details in the main text). Examples of canonical motifs and how post-translational modifications can complete a motif are shown in the left panel, whereas the right panel illustrates how post-translational modifications in a protein outside the motif can change the protein conformation and unmask the KFERQ-like motif.



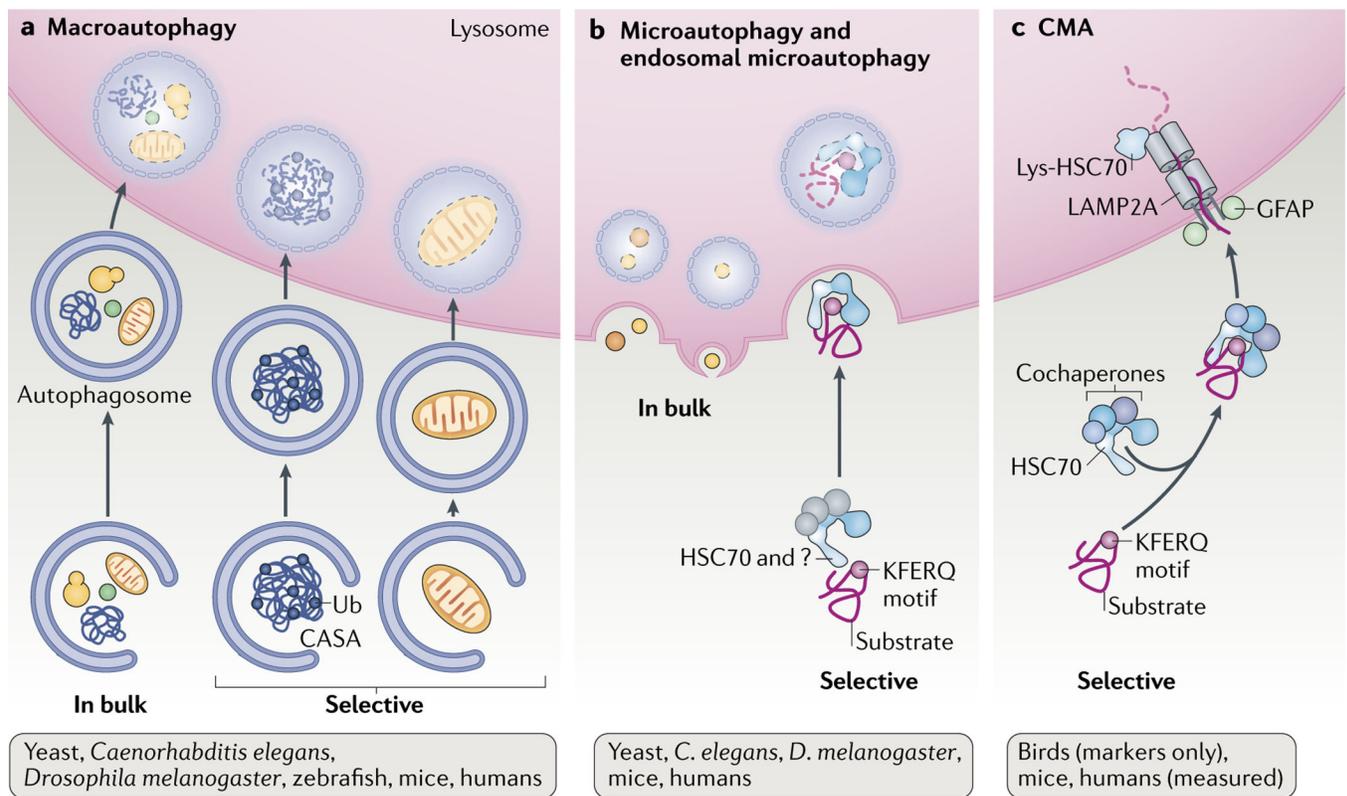


Fig. 1. Autophagic pathways in mammals.

a Macroautophagy sequesters cytosolic cargo by a delimiting membrane that forms through conjugation of specific proteins among themselves and with lipids in a complex multistep process. The membrane then seals into an autophagosome that is trafficked by microtubules. Fusion of autophagosomes with lysosomes mediates degradation of the trapped cargo. Macroautophagy can be in bulk or selective depending on the cargo sequestered. **b** Microautophagy entraps cytosolic cargo in small vesicles formed by invagination of the lysosomal membrane either in bulk or selectively via recognition and targeting by heat shock cognate 71 kDa protein (HSC70; also known as HSPA8) and cochaperones that are yet to be determined. **c** Chaperone-mediated autophagy (CMA) involves the selective degradation of KFERQ-like motif-bearing proteins delivered to the lysosomes via chaperone HSC70 and cochaperones, such as carboxyl terminus of HSC70-interacting protein (CHIP), heat shock protein 40 (HSP40; also known as DNABJ1) and HSP70–HSP90 organizing protein (HOP), and their internalization in lysosomes via the receptor lysosome-associated membrane protein type 2A (LAMP2A). Bottom: evolutionary conservation of each autophagy pathway. CASA, chaperone-assisted selective autophagy; GFAP, glial fibrillary acidic protein; lys-HSC70, lysosomal HSC70; Ub, ubiquitin.

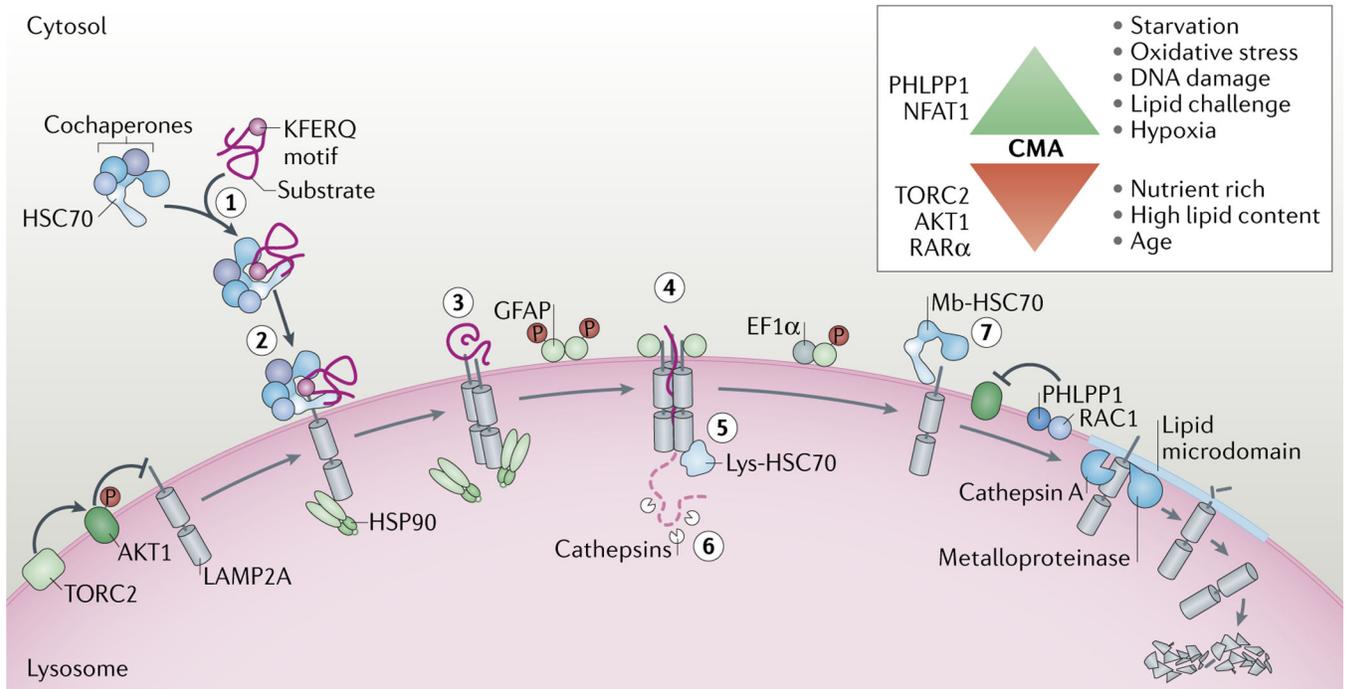


Fig. 2. Lysosomal effectors and regulators of CMA.

Steps in selective degradation of proteins by chaperone-mediated autophagy (CMA): recognition of the KFERQ-like motif in the substrate by heat shock cognate 71 kDa protein (HSC70; also known as HSPA8) (step 1); binding of the substrate–chaperone complex to lysosome-associated membrane protein type 2A (LAMP2A) (step 2); unfolding of the substrate by the chaperone complex (step 3); formation of the CMA translocation complex (step 4); substrate translocation mediated by lysosomal HSC70 (lys-HSC70) (step 5); substrate degradation by lysosomal proteases (step 6); and dissociation of LAMP2A from the translocation complex (step 7). CMA regulation via cytosolic and lysosomal signalling events is shown. Turnover of LAMP2A occurs in lipid microdomains by the dual action of cathepsin A and a metalloproteinase. Top right: activators and inhibitors of CMA. EF1 α , elongation factor 1- α ; GFAP, glial fibrillary acidic protein; HSP90, heat shock protein 90; mb-HSC70, membrane-associated HSC70; NFAT1, nuclear factor of activated T cells 1; PHLPP1, PH domain leucine-rich repeat-containing protein phosphatase 1; RAR, retinoic acid receptor; TORC2, TOR complex 2.

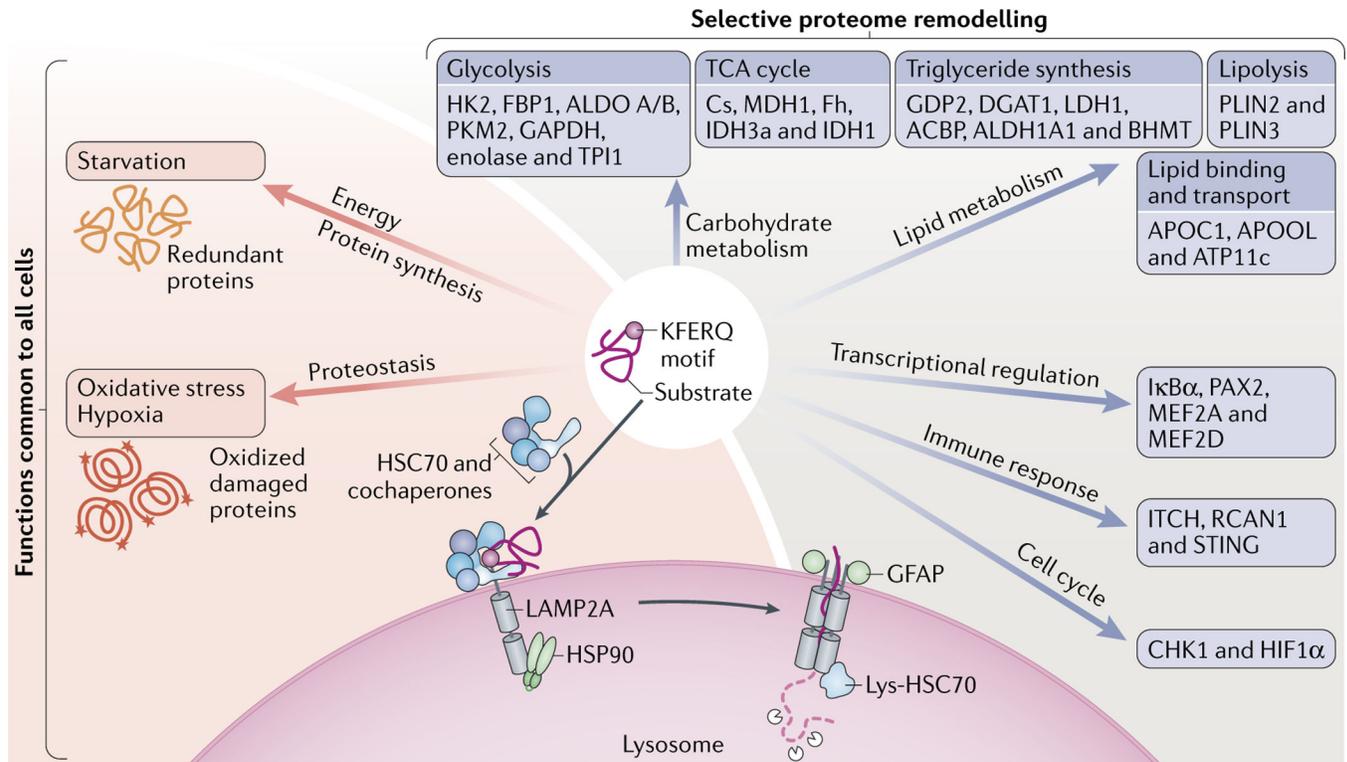


Fig. 3. Main physiological roles of CMA.

In most cells, chaperone-mediated autophagy (CMA) participates in protein quality control by degrading oxidized and damaged proteins under stress conditions and also contributes amino acids through degradation of proteins at advanced times of starvation. In addition, depending on the protein substrate degraded, CMA has a modulatory role in multiple cellular pathways. This CMA-mediated selective remodelling of the proteome has recently demonstrated a role for CMA in modulation of carbohydrate and lipid metabolism, transcriptional programmes, immune responses and the cell cycle. The selective CMA substrates linked to those pathways are shown in blue boxes. GFAP, glial fibrillary acidic protein; HSC70, heat shock cognate 71 kDa protein; HSP90, heat shock protein 90; LAMP2A, lysosome-associated membrane protein type 2A; lys-HSC70, lysosomal HSC70; TCA, tricarboxylic acid.

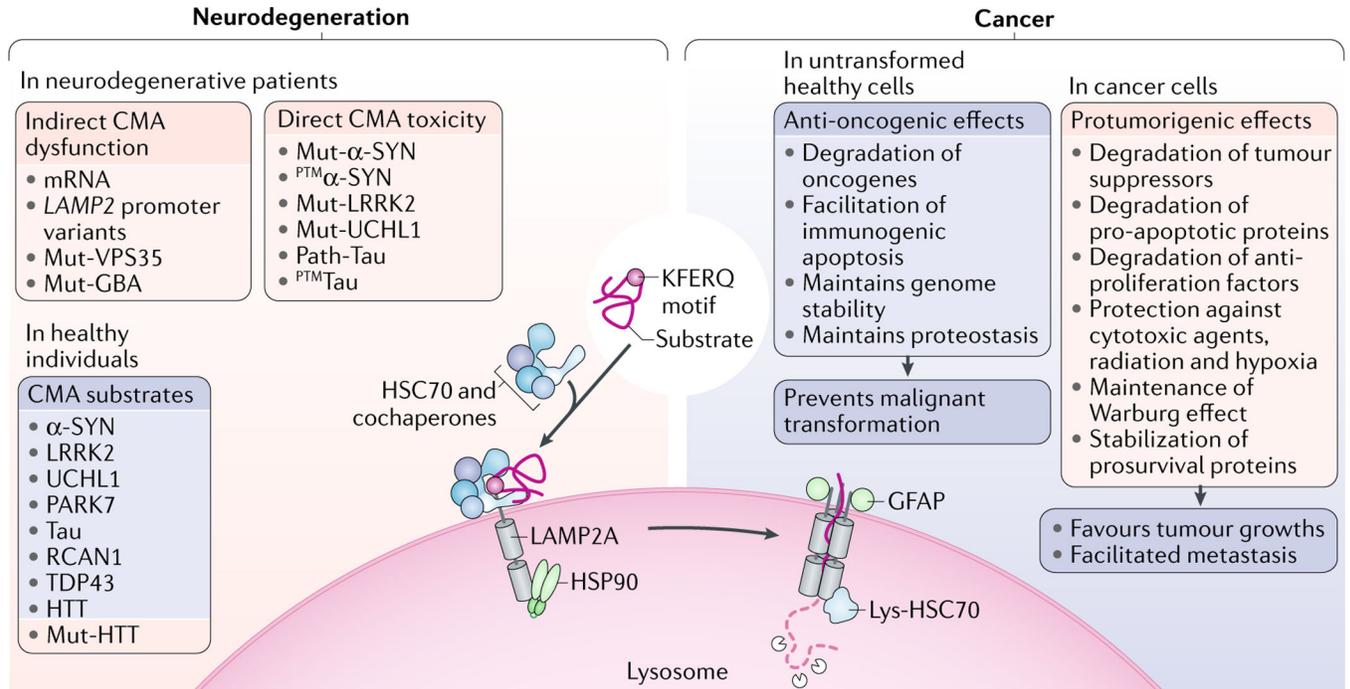


Fig. 4. CMA in neurodegeneration and cancer.

Chaperone-mediated autophagy (CMA) malfunctioning has been observed in multiple human disorders. Depicted here are two conditions, neurodegeneration and cancer, for which stronger experimental evidence of CMA involvement has been obtained. In neurodegeneration, pathogenic proteins can exert a toxic effect on CMA directly by disrupting the dynamics of the CMA translocation systems or indirectly by affecting levels of CMA effectors or lysosomal biogenesis. Most studies support that CMA has an anti-oncogenic role in normal untransformed cells and prevents malignant transformation, at least in part, through the mechanisms depicted here. In contrast, CMA has a protumorigenic effect in cancer cells by favouring their replication and growth and protecting them from extracellular insults. GFAP, glial fibrillary acidic protein; HSC70, heat shock cognate 71 kDa protein (also known as HSPA8); HSP90, heat shock protein 90; LAMP2A, lysosome-associated membrane protein type 2A; lys-HSC70, lysosomal HSC70.

Table 1

The CMA toolkit: overview of the approaches that can be used to measure CMA activity

assay	Principle and description	Key features
CMA reporter ^a	Uses photoconvertible and photoactivable fluorescent reporters that upon conversion change colour or upon activation start to fluoresce. CMA activation is detected as a change in the fluorescence pattern, from cytosolic diffuse to lysosomal punctate pattern, and is quantified as number of puncta per cell	<ul style="list-style-type: none"> • Pulse-chase experiments • CMA activity measured in intact cells in culture • Test sample size is not a limitation
Lysosomal uptake ^a	Intact isolated lysosomes pretreated or not with protease inhibitors are incubated with a known CMA substrate, and centrifugation after incubation and immunoblot is used to measure the amount of substrate associated with lysosomes	<ul style="list-style-type: none"> • In vitro assay • Direct measure of CMA activity • Binding and uptake can be separately measured
Lysosomal protein degradation ^a	Intact isolated lysosomes are incubated with radiolabelled protein substrates	<ul style="list-style-type: none"> • In vitro assay • Direct measure of CMA activity • Reconstitutes binding, uptake and degradation
Dynamics of CMA translocation complex	Isolated lysosomes are subjected to blue-native electrophoresis (immunoblot) to assess the amount of lysosomal LAMP2A present in the 700 kDa multimeric translocation complex	<ul style="list-style-type: none"> • In vitro assay • Provides information on CMA dynamics • Useful to determine possible failures in this CMA step
Protein degradation by CMA	Metabolic labelling of intact cells and measurement of the conversion of labelled protein to free-labelled amino acids in the presence or absence of lysosomal inhibitors (to block all forms of autophagy) and of macroautophagy inhibitors	<ul style="list-style-type: none"> • CMA activity measured in intact cells • Possible compensatory changes • Assesses other forms of autophagy in the same experiment
Immunofluorescence	CMA-active lysosomes are labelled by costaining with antibodies against LAMP2A and HSC70.	<ul style="list-style-type: none"> • Indirect measurement of CMA • Measures number of lysosomes performing CMA • Useful to determine changes in CMA-related compartments

For comprehensive details on assays and methodology to measure CMA, see REF. 117. CMA, chaperone-mediated autophagy; HSC70, heat shock cognate 71 kDa protein; LAMP2A, lysosome-associated membrane protein type 2A.

^aGold-standard assays in the field.

The CMA toolkit: overview of the approaches that can be used to identify CMA substrates

Table 2

Substrate feature	Description of the assay	Additional notes and features
Presence of KFERQ motif	<ul style="list-style-type: none"> • Scan for the motif in the amino acid sequence of the protein of interest • Incomplete motifs could be converted to a complete motif by post-translational modifications (Box 1) 	Also present in eMI substrates
Association with lysosomes	<ul style="list-style-type: none"> • Colocalization with CMA-active lysosomes (HSC70⁻) in intact cells • Immunoblot in isolated lysosomes to demonstrate preferential association with CMA-active lysosomes 	Proteins can associate with lysosomes to be functional
Degradation in lysosomes	Increased association of the protein of interest with CMA-active lysosomes upon lysosomal inhibition assayed by either immunofluorescence in intact cells or immunoblot of isolated lysosomes	Levels of substrates for other autophagic pathways also increase
Change in protein levels and degradation when CMA activity is modified	<ul style="list-style-type: none"> • Increased levels of the protein of interest or reduced degradation rates upon blockage of CMA (knockdown or knockout for LAMP2A) • Reduced levels of the protein of interest or increased degradation rates upon activation of CMA (overexpression of LAMP2A or AR7 compound) 	Compensatory upregulation of other forms of autophagy might mask this effect
Interaction with CMA components	Coimmunoprecipitation of the protein of interest with HSC70 in cytosolic fractions or LAMP2A in lysosomal fractions	Binding to HSC70 is not a sufficient criterion as it can occur because of unfolding or targeting to other pathways
Lysosomal uptake	In vitro assay with intact isolated lysosomes pretreated or not with protease inhibitors incubated with the protein of interest	Most conclusive proof of being a CMA substrate

For comprehensive details on assays and methodology to validate a protein as a CMA substrate, see REF. 117. CMA, chaperone-mediated autophagy; eMI, endosomal microautophagy; HSC70, heat shock cognate 71 kDa protein (also known as HSPA8); LAMP2A, lysosome-associated membrane protein type 2A.

Table 3

Human diseases associated with defects in CMA

Disease subtype	CMA substrate	CMA activity	Observations	Refs
<i>Cancer</i>				
Malignant transformation	CIP2A	Reduced	CMA regulates levels of <i>MYC</i> oncogene	114
	TCTP (acetylated)	Reduced	CMA regulates levels of TCTP	28
Tumour survival and/or growth	MDM2	Reduced	CMA degrades the oncogene <i>MDM2</i>	115
	Unknown	Reduced	CMA is required for efficient exposure of ecto-CRT (eat me signal) in immunogenic cell death	116
	Unknown	Increased	CMA required for Warburg effect	18
	PEA-15	n.d.	CMA degrades the anti-oncogenic variant	25
	PKM2	Increased	CMA required for Warburg effect	27
	BBC3 ^a	Increased	CMA degrades this pro-apoptotic protein, reducing cancer cell apoptosis	110
Breast cancer	MST1 (de-acetylated)	Increased	CMA favours degradation of tumour suppressor MST1	30
	Oxidized proteins	Increased	CMA provides cancer cells chemoresistance and defence against ROS	101
Colorectal cancer	HSD17B4 (acetylated)	Increased	Failure to acetylate makes the protein resistant to CMA; favours cancer	102
	P300	Increased	CMA of acetyltransferase p300 confers, 5-fluorouracil resistance	103
	RND3	Increased	CMA degradation of RND3 sustains proliferation	26
Hepatocellular carcinoma	Unknown	Increased	LAMP2A blockage reduces cancer cell viability	104
	HMGBl	n.d.	CMA degrades HMGBl to downregulate p53, and this confers resistance to irradiation-induced apoptosis	105
Non-small-cell lung cancer	Cyclin D1?	Increased	Macrophage-secreted IL-17 increases LAMP2A — proposed mechanism for oxaliplatin resistance	107b
	Unknown	Increased	Increased LAMP2A levels proposed to promote cancer cell survival in the cirrhotic liver	106b
Myeloid leukaemia	MCL1	n.d.	CMA stabilizes the prosurvival protein MCL1	111
	NCOR	n.d.	CMA degrades misfolded NCOR reducing ER stress	108
Neurodegenerative disorders	AF1Q ^a	n.d.	CMA degrades the poor prognosis biomarker AF1Q	118
	Hexokinase II	n.d.	CMA depletes hexokinase and induces metabolic catastrophe upon blockage of macroautophagy	112
General neuronal degeneration	MEF2A	n.d.	CMA degradation of oxidized MEF2A protects primary neurons from oxidative stress	72

Disease subtype	CMA substrate	CMA activity	Observations	Refs
	MEF2D ^a	n.d.	CMA degradation of oxidized MEF2D protects primary neurons from oxidative stress	119
	Unknown	Reduced	Blocking CMA leads to dopaminergic neurodegeneration	90
	Unknown	n.d.	Inverse correlation between LAMP2A levels in brain regions and their susceptibility to protein aggregation	79 b
Parkinson disease	α -Synuclein	Reduced	α -Synuclein is degraded by CMA but pathogenic α -synuclein blocks CMA	17,61,78,89
	α -Synuclein	Reduced	Blockage of LAMP2A in substantia nigra in rats leads to neurodegeneration	90
	LRRK2	Reduced	LRRK2 is degraded by CMA but pathogenic LRRK2 blocks CMA	81
	PARK7 (oxidized)	n.d.	CMA protects mitochondrial function by degrading nonfunctional PARK7	66
	UCHL1	Reduced	Pathogenic UCHL1 blocks CMA	82,83
	VPS35 mutant	Reduced	Pathogenic VPS35 impairs endosome-to-Golgi complex retrieval of LAMP2A and accelerates LAMP2A degradation	56
	GBA1 mutant	Reduced	GBA1 mutants prevent autophagy lysosomal reformation, affecting all forms of autophagy	93
	α -Synuclein mutant	n.d.	<i>Nrf2</i> expression in astrocytes slows down CMA decline in PD mice	92
	Unknown	Increased	LAMP2A upregulation (AAV) protects PD neurons	91 b
	Unknown	Reduced	Reduced levels of LAMP2A in PD patient brains	94 b
	Unknown	Reduced	Reduced <i>LAMP2A</i> and <i>HSC70</i> transcripts in PD brains due to increase of six miRNAs that control <i>LAMP2A</i> expression	97 b
	α -Synuclein	Reduced	miRNA against HSC70 (miR-320a) reduces α -synuclein degradation	120 b
	α -Synuclein	Reduced	Reduced LAMP2A protein in early stage PD brains (even before α -synuclein accumulation). CMA dysfunction is an early event	95 b
	Unknown	Reduced	GBA-affected brain regions have reduced LAMP2A levels	95 b
	Unknown	Reduced	Reduced <i>LAMP2A</i> or <i>HSC70</i> mRNA and LAMP2A and HSC70 protein levels in leukocytes from PD patients	121,122 b
	Unknown	n.d.	Sequence variants and SNPs in the <i>LAMP2</i> promoter in PD patients	96 b
Prion diseases	Prion protein	Increased	Upregulation of LAMP2A and HSC70 (protective)	123 b
Alzheimer disease	Tau	Decreased	Inefficient CMA	84
	APP c-term	n.d.	Eliminating KFERQ from APP leads to C-terminal fragment accumulation	98
Frontotemporal dementia	TDP-43	n.d.	CMA contributes to degradation of TDP-43	85
Huntington disease	Huntingtin	n.d.	CMA contributes to degradation of huntingtin	86-88

Disease subtype	CMA substrate	CMA activity	Observations	Refs
Heart diseases				
Heart failure	Ryanodine R2 (oxidized)	Reduced	CMA removes oxidized ryanodine receptor type 2; inability to do so may lead to heart failure (hypothesis)	124
Danon disease	Unknown	Reduced	No LAMP2A, undegraded proteins attributed to low CMA	125 ^b
Eye diseases				
Leber congenital amaurosis	Unknown	Increased	Upregulation of total LAMP2 expression suggested to protect against early apoptotic events	126 ^b
Liver diseases				
Acute liver failure	Unknown	Reduced	Reduced LAMP2A levels	127 ^b
NASH	Unknown	Reduced	Increased LAMP2A expression (compensatory)	128 ^b
Hepatosteatosis	Unknown	Reduced	Reduced LAMP2A levels	129 ^b
Alcoholic fatty liver	Unknown	Reduced	Reduced LAMP2A levels	130 ^b
Lung diseases				
Emphysema (cigarette smoke)	Unknown	Increased	CMA upregulated, protective against apoptosis	131
Kidney diseases				
Diabetes	PAX2	Reduced	Reduced PAX2 CMA leads to kidney hypertrophy	132
Muscle diseases				
Sporadic inclusion-body myositis	Unknown	Increased	LAMP2A mRNA and protein upregulated (likely in response to macroautophagy and proteasome decrease)	133 ^b
Neuronal diseases				
Spinal cord injury	ROS damaged proteins?	Increased	HDAC6 upregulates CMA (protective)	134
Brain ischaemia	No specific	Increased	CMA upregulation (protective)	58
Traumatic brain injury	Damaged proteins	Increased	LAMP2A levels increase during the recovery time (protective)	135 ^b
Immune system				
Immunosenescence	ITCH, RCAN1	Reduced	Failure to activate T cells because of reduced CMA; LAMP2A expression restores T cell response	21
Innate antiviral response	STING (desumoylated)	n.d.	CMA degrades STING in the late phase of infection to shut down the antiviral response	73
Salmonella	Unknown	Increased?	Recruits LAMP2A and HSC70 (CMA for nutrients?)	65
Lupus	Unknown	Increased	Increased LAMP2A expression	136 ^b
Lysosomal storage disorders				

Disease subtype	CMA substrate	CMA activity	Observations	Refs
Galactosialidosis	Unknown	Increased	Higher CMA by stabilizing LAMP2A	51
Cystinosis	Unknown	Reduced	Compromised LAMP2A trafficking to lysosomes	54,55
Mucopolipidosis	Unknown	Reduced	Patient cells have lower CMA activity	137

APP, amyloid precursor protein; AAV, adeno-associated virus; BBC3, Bcl-2-binding component 3 (also known as PUMA); CIP2A, protein CIP2A (also known as KIAA1524); CMA, chaperone-mediated autophagy; ecto-CRT, surface-exposed calreticulin; ER, endoplasmic reticulum; GBA, glucocerebrosidase; HDAC6, histone deacetylase 6; HMGBI, high mobility group protein B1; HSC70, heat shock cognate 71 kDa protein (also known as HSPA8); HSD17B4, peroxisomal multifunctional enzyme type 2; IL-17, interleukin-17; ITCH, E3 ubiquitin-protein ligase Itchy homolog; LAMP2A, lysosome-associated membrane protein type 2A; LRRK2, leucine-rich repeat serine/threonine-protein kinase 2; MEF, myocyte-specific enhancer factor; miRNA, microRNA; MST1, mammalian Ste20-like kinase 1 (also known as STK4); MYC, MYC proto-oncogene protein; NASH, nonalcoholic steatohepatitis; n.d., not determined; NCOR, nuclear receptor corepressor; PD, Parkinson disease; PEA-15, 15 kDa phosphoprotein enriched in astrocytes (also known as PED); PARK7, protein/nucleic acid deglycase DJ-1; RCAN1, regulator of calcineurin 1; RND3, Rho-related GTP-binding protein RhoE; ROS, reactive oxygen species; SNPs, single nucleotide polymorphisms; STING, stimulator of interferon genes protein; Tau, tau protein; TCTP, translationally-controlled tumour protein; TDP-43, TAR DNA-binding protein 43; UCHL1, ubiquitin carboxyl-terminal hydrolase isozyme L1; VPS35, vacuolar protein sorting-associated protein 35.

^aIndicates no bona-fide KFERQ-like motif has been identified upon sequence analysis.

^bIndicates studies in which changes in CMA are proposed on the basis of LAMP2A level measurements but CMA activity has not yet been analysed.