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AUTOPHAGY AS A CELL-REPAIR MECHANISM: ACTIVATION OF CHAPERONE-MEDIATED AUTOPHAGY DURING OXIDATIVE STRESS

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Abstract

Proper removal of oxidized proteins is an important determinant of success when evaluating the ability of cells to handle oxidative stress. The ubiquitin/proteasome system has been considered the main responsible mechanism for the removal of oxidized proteins, as it can discriminate between normal and altered proteins, and selectively target the last ones for degradation. A possible role for lysosomes, the other major intracellular proteolytic system, in the removal of oxidized proteins has been often refused, mostly on the basis of the lack of selectivity of this system. Although most of the degradation of intracellular components in lysosomes (autophagy) takes place through “in bulk” sequestration of complete cytosolic regions, selective targeting of proteins to lysosomes for their degradation is also possible via what is known as chaperone-mediated autophagy (CMA). In this work, we review recent evidence supporting the participation of CMA in the clearance of oxidized proteins in the forefront of the cellular response to oxidative stress. The consequences of an impairment in CMA activity, observed during aging and in some age-related disorders, are also discussed.

Keywords

Aging; autophagy; Chaperone-mediated autophagy; chaperones; lysosomes; oxidative stress; protein aggregation

Introduction: oxidative stress and lysosomes

Cells activate different mechanisms in response to oxidative stress in order to prevent damage to intracellular components by the free radicals generated under these conditions. However, this display of defensive mechanisms is often not enough to completely avoid cellular injury, and a second front of defence, aimed at the repair and removal of damaged components, is required. The ubiquitin/proteasome system and lysosomes are the main systems responsible for the turnover of intracellular components and for cell clearance. Degradation of oxidized proteins by the proteasome has been well documented both in vitro and in cultured cells (for review see (Shringarpure et al., 2001; Grune et al., 2003; Keller et al., 2004). In contrast, for a long time, lysosomes have been considered “the bad guys” in the context of oxidative stress,

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as they are a common target for the damaging effect of free radicals. The action of these reactive oxygen species on lysosomes is double. On one hand, through promoting cross-linking of protein components at the lysosomal membrane, they increase the lysosomal proton permeability consequently raising the pH of the lysosome (Wan et al., 2001). Because lysosomal hydrolases are maximally active at very low pH, these changes in lysosomal pH decrease the ability of lysosomes to degrade the internalized substrates, which with time, become resistant to degradation (due to nonspecific cross-linking and oxidation) and accumulate inside lysosomes in the form of an autofluorescent pigment known as lipofuscin (Terman and Brunk, 2004). On the other hand, free radicals can also directly damage the lysosomal membrane to an extent that results in leakage of lysosomal hydrolases out to the cytosol. These free hydrolases contribute to further organelle damage, leading, in extreme situations, to activation of the apoptotic program and cell death (Olejnicka et al., 1999; Brunk and Terman, 2002). Despite this negative picture of the role of lysosomes during oxidative stress, emerging evidence suggests that these catastrophic effects occur only during massive acute oxidative stress, and that, in the early stages of oxidation and in circumstances associated to mild-oxidative stress, the lysosomal system instead plays a protective role because it contributes to the removal of oxidized and damaged intracellular components via autophagy. This review focuses on the beneficial effect of the activation of a particular type of autophagy, chaperone-mediated autophagy, as part of the cellular response to oxidative stress.

Selective autophagy via chaperone-mediated autophagy

Three different types of autophagy have been described in mammalian cells, based on the mechanisms utilized for the delivery of cargo to lysosomes (Cuervo, 2004; Yorimitsu and Klionsky, 2005). Chaperone-mediated autophagy (CMA) is the type of autophagy wherein a particular pool of soluble cytosolic proteins is selectively targeted to lysosomes for degradation (Majeski and Dice, 2004; Massey et al., 2004). The substrate proteins are recognised by a chaperone-cochaperone complex which delivers them to the lysosomal membrane (Chiang et al., 1989). Here they bind to a receptor protein, the lysosomal-associated membrane protein type 2A (LAMP-2A) (Cuervo and Dice, 1996), and after unfolding (Salvador et al., 2000), the substrate proteins are translocated across the lysosomal membrane assisted by a lysosomal-resident chaperone (Agarraberes et al., 1997), following which they are degraded in the hydrolase-rich lumen (Figure 1).

In addition to this peculiar form of delivery of substrates to lysosomes, the type of substrates degraded through this pathway, as well as the mechanisms of activation and regulation, set CMA apart from the other two forms of autophagy- macroautophagy and microautophagy. These last two types of autophagy involve non-selective “in bulk” engulfment of complete cytosolic regions, including both organelles and soluble proteins (Cuervo, 2004; Yorimitsu and Klionsky, 2005), while CMA caters to soluble cytosolic proteins only. On this respect, a feature unique to CMA is the selectivity in the degradation of these cytosolic proteins. In fact, CMA can only degrade cytosolic proteins which contain a CMA-targeting motif, biochemically related to the pentapeptide KFERQ (Dice, 1990). CMA's intrinsic selectivity seems beneficial under particular conditions in which discrimination between different types of proteins for degradation is required. For example, although macroautophagy is induced early in starvation to generate essential amino acids (otherwise supplied in the diet) through the breakdown of existing organelles and proteins (Mizushima, 2005), this random degradation can only be maintained during a relatively short period of time (10h), beyond which it needs to be replaced by the selective degradation provided by CMA (Wing et al., 1991; Cuervo et al., 1995). Activation of CMA under these conditions favors degradation of unnecessary proteins against that of proteins essential for cell survival.

Main players in CMA

Both cytosolic and lysosomal chaperones are required for completion of CMA. The cytosolic chaperone, hsc70, the constitutive member of the hsp70 family of chaperones, recognizes and binds to the targeting motif in the substrates and, by still poorly understood mechanisms, promotes delivery of substrates to the lysosomal membrane (Chiang et al., 1989). The interaction between substrate and hsc70 is modulated by other cytosolic co-chaperones including hsp90, hsp40, hip, hop and bag-1 (Agarraberes and Dice, 2001). The specific role of each of these cochaperones in CMA remains unknown but, by extension of what is known about their participation in other cellular processes, it is likely that they modulate substrate/chaperone interaction and unfolding of the substrate protein at the lysosomal membrane by regulating the ATP/ADP hydrolysis cycles of hsc70.

The lysosomal counterpart of cytosolic hsc70, namely the lysosomal hsc70 (lys-hsc70), is absolutely necessary for substrate translocation across the lysosomal membrane (Agarraberes et al., 1997). In fact, the presence or not of this chaperone in the lysosomal lumen determines whether or not a particular lysosome is capable of CMA (Cuervo et al., 1997). Many questions still remain unanswered about this chaperone. For example, it is not known how it accesses the lysosomal lumen, if it requires other cochaperones to modulate its function inside lysosomes, or what is the mechanism by which it facilitates substrate translocation.

The limiting step in CMA is the interaction of the substrate/chaperone complex with the lysosomal receptor (Cuervo and Dice, 2000a). LAMP-2A is a single-span transmembrane protein whose short cytosolic tail contains positively charged residues essential for the binding of the substrates (Cuervo and Dice, 1996; Cuervo and Dice, 2000b). CMA activity directly correlates with levels of LAMP-2A in the lysosomal membrane (Cuervo and Dice, 2000b). These levels are tightly regulated by at least three different mechanisms including changes in the degradation of LAMP-2A at the lysosomal membrane (Cuervo et al., 2003), in its distribution between the lysosomal membrane and lumen (Cuervo and Dice, 2000a) and also through *de novo* synthesis of this receptor (Kiffin et al., 2004). Recently, LAMP-2A has been shown to dynamically associate to particular lipid microdomains at the lysosomal membrane, further identified as the specific sites where degradation of this receptor occurs (Kaushik, S., Massey, A.C. and Cuervo, A.M. *submitted*). LAMP-2A organizes in the membrane as CMA-active multimeric complexes (Cuervo and Dice, 2000b), but its localization inside the lipid microdomains prevents this multimerization.

CMA as part of the cellular response to stress

Along with macroautophagy, CMA is considered to be a stress-induced pathway. As discussed before, CMA provides the essential amino acids critical for cellular survival when the dietary supply of nutrients is limited for extended periods of time, while also preventing the degradation of proteins essential during these stress conditions (Cuervo et al., 1995). The signal transduction events that result in this activation of CMA are still unclear. The sequential activation of macroautophagy followed by CMA during starvation has led to hypothesize that degradation via macroautophagy of a yet unknown inhibitor of CMA could be behind CMA activation under these conditions. Activation of CMA is associated with particular changes in lysosomes: enrichment in hsc70, increased levels of LAMP-2A that multimerizes to form CMA-active complexes and relocation of lysosomes to the perinuclear region (Cuervo et al., 1995; Cuervo and Dice, 2000b). The significance of lysosomal relocation remains unclear, but it could favor homotypic fusions between lysosomes, thus promoting the transfer of part of hsc70 from CMA-active to CMA-inactive lysosomes, in order to recruit them for CMA.

In addition to nutritional stress, other conditions known to activate this pathway include mild-oxidative stress (as discussed in more detail below) (Kiffin et al., 2004) and stress induced by

exposure to toxic compounds (Cuervo et al., 1999). In this last condition, CMA activation is required for the selective removal of proteins directly altered by the chemical compounds, which otherwise would accumulate as toxic multimeric complexes inside cells.

Experimental proof for the essential character of CMA as part of the cellular response to stress has been recently provided in cells in which CMA was selectively blocked (Massey et al., 2006). While cells with impaired CMA maintain normal survival rates under normal conditions and are able to up-regulate other autophagic pathways, namely macroautophagy, to preserve normal rates of protein degradation, the blockage of CMA makes them extremely vulnerable to stressors. Exposure of CMA-impaired cells to different pro-oxidants, oxidants and to U.V. results in dramatic decrease in cell viability, activation of the apoptotic program and cell death (Massey et al., 2006).

CMA and oxidative stress

To further characterize this proposed role of CMA in the cellular response to stress, we have evaluated the participation of this autophagic pathway in the removal of oxidized proteins. We have found that induction of mild-oxidative stress in rodents and culture cells increases the degradation of proteins via CMA (Kiffin et al., 2004). In fact, elevated amounts of oxidized proteins can be detected under these conditions in the lumen of CMA-active lysosomes.

Part of the enhanced CMA directly results from the oxidative modification of the CMA substrates, which are more readily degraded through this pathway compared to their unmodified counterparts (Kiffin et al., 2004; Finn and Dice, 2005)(Fig. 2, A). It is possible that partial unfolding, typically associated with oxidative damage, could expose hidden CMA-targeting motifs, facilitating their recognition by the cytosolic chaperone complex. Substrate unfolding could also accelerate translocation across the lysosomal membrane by eliminating the unfolding step (Fig. 2, A). Independent of this effect on the substrate, changes in the lysosomal compartment also contributes to increased CMA activity during oxidative stress. Thus, we have found that levels of both the receptor and the lysosomal chaperone (lys70) increase, resulting in a higher number of translocation units per lysosome under these conditions (Kiffin et al., 2004) (Fig. 2, B).

Regarding the magnitude and the consequences of CMA activation in the context of oxidative stress, it is anticipated that only proteins bearing the CMA-targeting motif, about 30% of cytosolic proteins, would be removed by this pathway under these conditions. However, taking into account that the CMA-targeting motif is not sequence-linked, but it depends primarily on the physical properties of the constituent amino acids, it has been proposed that oxidation could convert a non-targeting motif into a CMA-targeting motif by modifying one or more of the amino acid residues (Gracy et al., 1998). Experimental evidence supporting this possibility is still missing.

Including the “aging” factor in the CMA/oxidation equation

Poor cellular response to oxidative stress underlies the basis of many chronic disorders and of aging (Simpson et al., 2003; Keller et al., 2004). CMA activity has been shown to decrease with age in rodents and in senescent human fibroblasts in culture (Dice, 1982; Cuervo and Dice, 2000c). Impaired CMA activity could contribute in part to the accumulation of oxidized and damaged proteins in aged organisms.

In contrast to the dramatic changes in the lysosomal system described in old organisms (enlargement of lysosome-related structures, intralysosomal accumulation of undegraded products in the form of lipofuscin, increased lysosomal membrane lability) (Hochschild, 1970; Noda and Suzuki, 1980), the group of lysosomes active for CMA show no major

morphological changes and preserve proteolytic activity with age (Cuervo and Dice, 2000c). In fact, once CMA substrates access the lumen of old rodents' lysosomes they display similar kinetics of degradation to those observed in lysosomes from young animals. Likewise, binding of substrate proteins to the cytosolic chaperone and their targeting to the lysosomal membrane also seems to be unaffected with age (Cuervo and Dice, 2000c). The main age-related deficiency seems to be in the binding and uptake of the substrates in the lysosomal membrane. This defect is due to a decline in the levels of LAMP-2A at the lysosomal membrane with age (Cuervo and Dice, 2000). Lower levels of LAMP-2A are initially compensated by increasing the number of lysosomes active for this pathway (those containing lys-hsc70). However, with advancing age, the low levels of LAMP-2A decrease to such an extent that the chaperone compensation is not enough, leading to the impaired binding and uptake of the substrate proteins into lysosomes (Cuervo and Dice, 2000c). Recent findings from our laboratory suggest that altered turnover of the receptor itself could be behind its reduced levels with age and the consequent decrease in CMA activity (Zeng, M., Kaushik, S., Massey, A., Bandyopadhyay, U., Kiffin, R., Cuervo, A.M. *in preparation*). Based on the findings in cultured cells with experimentally blocked CMA (Massey et al., 2006), it could be inferred that the decrease in CMA activity with age would leave cells vulnerable to different stressors, in particular those resulting in the generation of reactive oxygen species and protein damage/misfolding. Because of the proposed role of CMA in the selective removal of oxidized and damaged proteins, failure of this pathway with age could contribute to the abnormal accumulation of these altered products observed in aged organisms.

Different pathways for a common purpose

Activation of CMA during oxidative stress seems to obey the need for selective removal of the damaged proteins without affecting the functional neighboring ones. However, degradation of oxidized proteins also occurs through the ubiquitin/proteasome system. Why this need for redundant pathways? One possibility is that oxidized proteins follow the same degradation pathways than their unmodified forms, but in an accelerated manner. In this case, when oxidized, CMA substrates will be degraded by CMA, and proteasome substrates will be degraded by the proteasome. However, the concept that a particular protein only follows a determined proteolytic pathway has become obsolete, as numerous examples now support that the same protein can be degraded by different proteolytic systems depending on the cellular conditions. It is thus possible that activation of the ubiquitin/proteasome system during oxidative stress provides for the rapid removal of oxidized proteins, but as the oxidation conditions persist, oxidized proteins are instead delivered to lysosomes via CMA, in order to devote proteasomal activity to critical regulatory tasks. However, we cannot discard that both systems are simultaneously active during oxidation, and that, depending on the cell type and particular conditions, different percentages of oxidized proteins are delivered to one system or the other. The main limitation to accurately quantify the amount of proteins that follow one or the other proteolytic pathway is the fact that most of the available assays rely on the use of blockers of these pathways. However, there is growing evidence that supports the existence of a cross-talk between different proteolytic systems, and the eliciting of compensatory mechanisms among them in response to functional failure. Consequently, most of the attempts to quantify degradation using blockers of one pathway are likely to enhance the proteolytic activity of other pathway to compensate for this failure, leading to erroneous calculations. New approaches need to be devised to directly track within cells the fate of multiple proteins and their delivery to one proteolytic system or another, without perturbing the activity of these systems.

Concluding remarks and pending questions

Opposed to the classical view that depicted lysosomes as one of the main reasons responsible for cellular damage during oxidative stress, recent evidence supports a protective role for the lysosomal system in the early phases of the oxidative insult and in conditions leading to mild-oxidative stress. The selectivity intrinsic to CMA allows lysosomes to discriminate between altered and unmodified proteins, promoting the removal only of the former. The decline in CMA activity with age can contribute to the accumulation of damaged proteins typical of old organisms. However, the consequences of decreased CMA activity could be different in different tissues, depending on the rate of protein turnover, the contribution of other proteolytic pathways and their particular susceptibility to stress. Also unknown is the reason for the redundancy in this task of CMA and the ubiquitin/proteasome system. How do cells discriminate where to send the oxidized proteins? Are particular damaged proteins delivered to one system and others to the other? Are both pathways activated simultaneously or sequentially? Is this dual activation common for all types of cells? And in regards to the degradation of these altered proteins by CMA, different types of protein-damage have been described during aging and in chronic disorders (i.e. nitration, glycation, modification by products of lipid peroxidation), do all types of protein damage facilitate degradation, at least of CMA substrates, by this pathway, or are there particular conformational modifications-associated to particular types of protein damage that determine their suitability for CMA degradation? These and other questions require further clarification.

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Fig. 1.

Hypothetical model for chaperone-mediated autophagy. Certain cytosolic proteins bearing a particular targeting motif are recognized by a cytosolic chaperone/cochaperone complex which delivers them to the lysosomal membrane. Here this complex binds to a receptor protein at the lysosomal membrane, and after undergoing unfolding, substrate proteins are translocated into the lysosomal lumen, in a process mediated by a lysosomal resident chaperone. Once in the lumen, substrates are rapidly degraded by the potent combination of lysosomal proteases.

Fig. 2.

Activation of CMA as part of the oxidative stress response. Different mechanisms contribute to the enhanced degradation of proteins via CMA during mild oxidative stress. A) *Effect on the substrates:* exposure of hidden CMA-targeting motifs, partial unfolding and generation of CMA-targeting motifs in usually non-substrate proteins, could all contribute to facilitate substrate delivery and translocation into lysosomes. B) *Effect on the lysosomal system:* mild-oxidative stress results in an increase in the lysosomal levels of the major components of the CMA-translocation machinery at the lysosomal membrane.