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Mini-Review

Protein degradation and aging

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Abstract

Continuous turnover of intracellular proteins is essential for the maintenance of cellular homeostasis and for the regulation of multiple cellular functions. The first reports showing a decrease in total rates of protein degradation with age are dated more than 50 years ago, when the major players in protein degradation where still to be discovered. The current advances in the molecular characterization of the two main intracellular proteolytic systems, the lysosomal and the ubiquitin proteasome system, offer now the possibility of a systematic search for the defect(s) that lead to the declined activity of these systems in old organisms. We discuss here, in light of the current findings, how malfunctioning of these two proteolytic systems can contribute to different aspects of the phenotype of aging and to the pathogenesis of some age-related diseases.

Keywords: Proteolysis; Proteases; Lysosomes; Autophagy; Proteasome; Ubiquitin

1. Intracellular protein degradation: constant destruction for continuous rejuvenation

All intracellular proteins undergo continuous synthesis and degradation (Mortimore et al., 1989; Schimke, 1970). This constant protein turnover, among other functions, helps reduce, to a minimum, the time a particular protein is exposed to the hazardous cellular environment, and consequently, the probability of being damaged or altered. At a first sight, this constant renewal of cellular components before they lose functionality may appear a tremendous waste of cellular resources. However, it is well justified considering the detrimental consequences that the accumulation of damaged intracellular components has on cell function and survival (Goldberg, 2003). Furthermore, protein degradation rather than mere destruction is indeed a recycling process, as the constituent amino acids of the degraded protein are reutilized for the synthesis of new proteins (Mortimore et al., 1989; Schimke, 1970). The rates at which different proteins are synthesized and degraded inside cells are different and can change in response to different stimuli or under different conditions. This balance between protein synthesis and degradation also allows cells to rapidly modify intracellular levels of proteins to adapt to changes in the extracellular environment. Proper protein degradation is also essential for cell survival under conditions resulting in extensive cellular damage. In fact, activation of the intracellular proteolytic systems occurs frequently as part of the cellular response to stress (recently reviewed in Cuervo, 2004b; Goldberg, 2003). In this role as 'quality control' systems, the proteolytic systems are assisted by molecular chaperones, which ultimately determine the fate of the damaged/unfolded protein (Fig. 1). Damaged proteins are first recognized by molecular chaperones, which facilitate protein refolding/repairing. If the damage is too extensive, or under conditions unfavorable for protein repair, damaged proteins are targeted for degradation. Protein degradation is also essential during major cellular remodeling (i.e. embryogenesis, morphogenesis, cell differentiation), and as a defensive mechanism against harmful agents and pathogens (recently reviewed in Cuervo, 2004a; Klionsky, 2005).

Two major proteolytic systems are responsible for most intracellular protein turnover: the lysosomal system and the ubiquitin-proteasome system (reviewed in Ciechanover, 2005; Cuervo, 2004b; Goldberg, 2003). Although lysosomes were the first proteolytic system discovered, the recent

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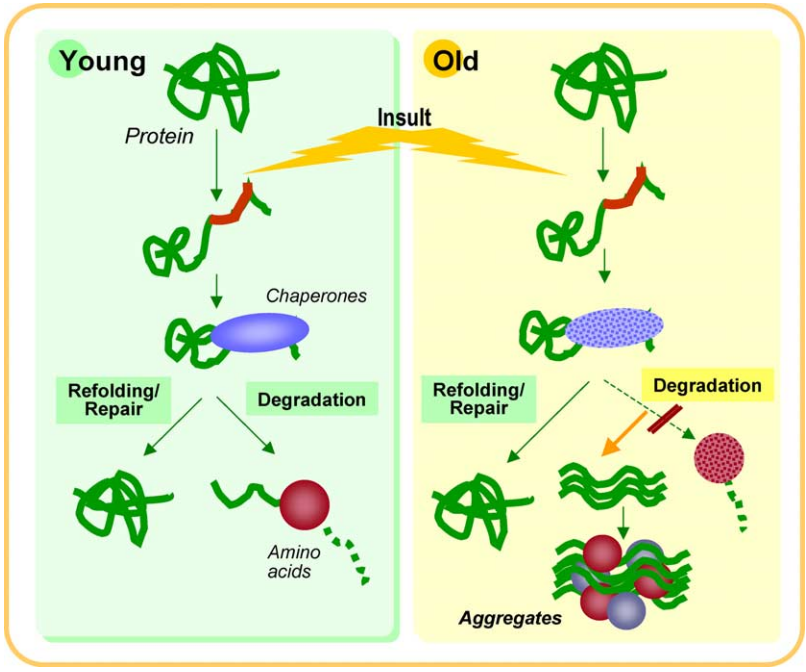

Fig. 1. Intracellular fate of altered proteins. In normally functioning cells (Young) damaged proteins are recognized by molecular chaperones that attempt to repair (refold) them. If refolding is not possible, chaperones target the damaged protein for degradation by the intracellular proteolytic systems. As cells age (Old) the defective activity of the major proteolytic systems leads to chaperone 'overload' and to the intracellular accumulation of damaged/unfolded protein products. Missfolded proteins often aggregate, probably as a defensive mechanism, sequestering in this aggregates chaperones, proteases and other neighboring proteins. Protein aggregates slowly accumulate in all cells through the life, but their formation can be precipitate under particular cellular conditions or in certain pathologies.

advances on the molecular characterization of this system have motivated their come back to the spot light (Cuervo, 2004b; Klionsky, 2005). The entry of the ubiquitin-proteasome system into the scene, back in the early 80s, resulted in a complete change of mind, expanding the role of protein degradation from mere housekeeping to regulator of major intracellular processes, such as cell cycle and cell division (Ciechanover, 2005).

The first reports showing a decrease in total rates of protein degradation with age are dated more than 30 years ago, when the major players in protein degradation where still to be discovered (Makrides, 1983). Since then, this age-related decline in proteolytic activity has been observed in almost all organisms analyzed, and specific defects in the different proteolytic systems with age have been reported. Keeping in mind the myriad of intracellular functions in which protein degradation participates, it is not surprising that the consequences of the age-related alterations in the proteolytic systems are widespread and contribute to a broad variety of pathologies (reviewed in Cuervo, 2004a; Keller et al., 2004; Shintani and Klionsky, 2004; Ward, 2002). We briefly recapitulate here some of the main characteristics of these two major proteolytic systems, highlighting recent findings that have contributed to our current understanding of their functioning, and discuss the major changes described in these systems with age and their consequences in aging and in some age-related pathologies.

2. The lysosomal/autophagic system: the return of the big giant

The term autophagy refers to any process resulting in the degradation of intracellular components inside lysosomes or the vacuole (the equivalent to lysosomes in yeast) (reviewed in Cuervo, 2004a,b; Klionsky, 2005). Lysosomes are single membrane organelles, which contain a large assortment of hydrolases capable of degrading any kind of macromolecules. Extracellular macromolecules can also be internalized and degraded in lysosomes through what is known as heterophagy (details about the main forms of heterophagy—endocytosis and phagocytosis—can be found in D'Hondt et al., 2000). Lastly, some extracellular proteins, such as secretory proteins, can undergo lysosomal degradation in the cells in which they were synthesized, by fusion of secretory vesicles with lysosomes instead of the plasma membrane. This process, known as crinophagy, is a common mechanism used by secretory cells to modulate secretion rates (reviewed in (Cuervo, 2004b). The lysosome becomes thus the 'end terminal' of a variety of pathways that carry intra- or extracellular components for complete

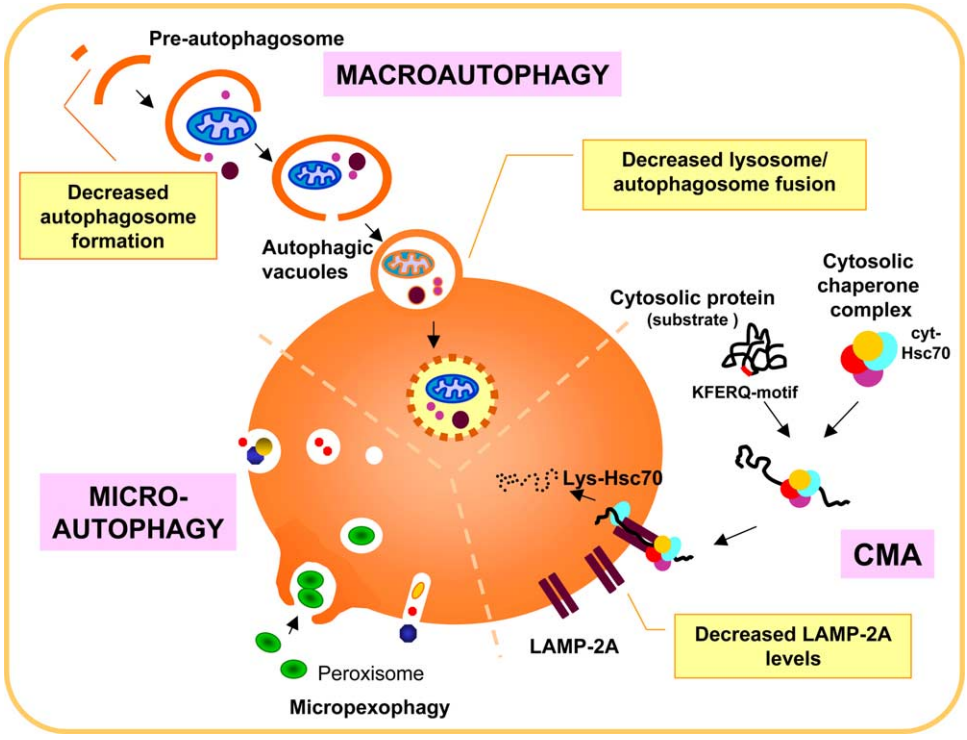

Fig. 2. Schematic model of the main forms of autophagy in mammalian cells and their changes in aging. Internalization of complete regions of cytosol first into autophagosomes that fuse then with lysosomes (macroautophagy) or directly by the lysosomal membrane (microautophagy) contrast with the selective uptake in a molecule-by-molecule basis of cytosolic proteins via chaperone-mediated autophagy (CMA). A variant of microautophagy that selectively removes peroxisomes from the cytosol (micropexophagy) is also shown. Callouts summarize the age-related defects identified until date in the different autophagic pathways. Abbreviations: hsc70, heat-shock cognate protein of 70 KDa; LAMP-2A, lysosome associate membrane protein type 2A.

degradation. The scope of this review is in the turnover of intracellular components, and consequently, only autophagy, main responsible for the turnover of organelles and a large number of long-lived cytosolic proteins, is discussed.

2.1. Types of autophagy

The best characterized autophagic pathways are macroautophagy, microautophagy and chaperone-mediated autophagy (Fig. 2) (Cuervo, 2004b; Klionsky, 2005). They differ in the way in which they deliver substrates into the lysosomal lumen, the types of substrates carried for degradation and their activity/regulation.

2.1.1. Macroautophagy

Macroautophagy (MATG) is an inducible form of autophagy responsible for the degradation of both long-lived soluble proteins and complete organelles under stress conditions (Klionsky, 2005; Shintani and Klionsky, 2004). The stressor best characterized as a MATG activator is starvation (nutritional stress). In MATG, a portion of cytosol, often including whole organelles, is surrounded by a de novo formed membrane (limiting membrane), which seals to generate a double-membrane organelle called autophagic vacuole or autophagosome (Ohsumi and Mizushima, 2004). Fusion of lysosomes with these autophagic vacuoles provides them with the enzymes required for the degradation of the sequestered material (Fig. 2). Recent genetic studies in yeast have facilitated the identification of a series of genes related to autophagy (autophagy genes or ATG), providing also key information about the regulatory mechanisms for this pathway (Klionsky, 2005; Ohsumi and Mizushima, 2004). Most of the genes identified in yeast are conserved in mammals and other organisms, confirming that MATG is a highly conserved process in eukaryotes.

The MATG machinery is controlled by two ubiquitin-like conjugation cascades (a protein–protein and a protein–lipid conjugation) and by two phosphorylation complexes (reviewed in Klionsky, 2005; Ohsumi and Mizushima, 2004). Conjugation among particular ATG proteins mediates their recruitment and interaction with pre-formed lipid micelles in the cytosol, most likely originated from the endoplasmic reticulum, and initiates the nucleation to form the limiting membrane. A series of phosphorylation events regulate the elongation of this isolation membrane and the size of the vesicle formed by resealing (Fig. 2). Less information is available about the events that mediate the fusion of the autophagic vacuole to the lysosome. MATG is regulated through a signaling cascade in response to changes in the circulating levels of amino acids,

glucocorticosteroids and other hormones (reviewed in Meijer and Codogno, 2004). MTOR-kinase, a nutrient and ATP sensor, is the key player in this signaling, acting as a negative regulator. MATG is activated in the early stages of nutrient deprivation to provide amino acids and other essential components required to maintain protein synthesis in those stress conditions. Although this pathway seems to be non-selective regarding the degradation of soluble cytosolic proteins, certain level of selectivity exists in the degradation of organelles such as mitochondria and peroxisomes via MATG. Although, still not clear how the MATG machinery distinguishes between a functional and a deteriorated organelle, recent studies have identified proteins in mitochondria and in peroxisomes required for their autophagic degradation (Kiel et al., 2003; Rodriguez-Enriquez et al., 2004).

Classical methods for measuring MATG activity are the morphometric quantification of the changes in the autophagic vacuolar compartment in electron microscopy sections, or the measurement of the degradation of long-lived proteins during the first hours of nutritional deprivation. The newly identified MATG molecular players now allow tracking MATG by following their levels and intracellular distribution (Ohsumi and Mizushima, 2004).

2.1.2. Microautophagy

Microautophagy (mATG) involves the engulfment and degradation of complete regions of the cytosol, including cytosolic proteins and organelles, directly by lysosomes, without requiring the formation of an intermediate autophagic vacuole (Farre and Subramani, 2004; Klionsky, 2005; Mortimore et al., 1988). The lysosomal membrane invaginates or projects finger-like protrusions to sequester cytosolic components in intralysosomal vesicles (Fig. 2). Once the vesicle is inside the lysosome, the membrane and the sequestered material are degraded. Morphologically, mATG was first described in mammalian cells as the presence of lysosome-like organelles with multiple vesicles trapped in their lumen (Mortimore et al., 1988). Although less characterized at the molecular level than MATG, recent studies on the degradation of peroxisomes by mATG (micropexophagy) in certain yeast strains, have provided new insights on this process (Farre and Subramani, 2004). Both exclusive mATG-related genes and genes shared with MATG have been shown to participate in mATG (Farre and Subramani, 2004). In addition to this role in the degradation of particular organelles, mATG has been traditionally considered as the form of autophagy constitutively active to guarantee proper turnover of long-lived proteins under basal conditions (Mortimore et al., 1988). The current lack of good experimental approaches to measure mATG in mammalian system is the major limitation to clearly addressing its physiological role and possible relation to particular diseases.

2.1.3. Chaperone-mediated autophagy

Most mammalian cell types can also degrade soluble cytosolic proteins through a third form of autophagy known as chaperone-mediated autophagy (CMA) (Cuervo, 2004b; Majeski and Dice, 2004; Massey et al., 2004). Distinctive of CMA are its selectivity toward a particular group of cytosolic proteins and the fact that these substrates are directly translocated across the lysosomal membrane, without requiring formation of intermediate vesicles or membrane deformation. All CMA substrates contain a sequence, biochemically related to the pentapeptide KFERQ, that targets them to the lysosome (Majeski and Dice, 2004). This motif is recognized in the cytosol by a chaperone, the heat shock cognate protein of 70 kDa (hsc70) and its cochaperones (Chiang et al., 1989) (Fig. 2). The substrate/chaperone complex is then targeted to the lysosomal membrane where it binds to a receptor protein, the lysosome-associated membrane protein type 2a (LAMP-2A). Assisted by a form of hsc70 present in the lysosomal lumen, the translocation of the previously unfolded protein is completed, followed by its rapid degradation inside lysosomes (Agarraberes and Dice, 2001; Cuervo, 2004b; Majeski and Dice, 2004).

CMA is modulated through changes in the levels of the receptor, LAMP-2A, in the lysosomal membrane (reviewed in (Majeski and Dice, 2004; Massey et al., 2004). Depending on the cellular conditions, transcriptional upregulation of LAMP-2A, blockage of LAMP-2A degradation or/and dynamic redistribution of LAMP-2A from the lysosomal lumen to the membrane can be used to enhance CMA activity. Similar to MATG, CMA is activated by stress conditions such as nutrient deprivation, toxic exposure and oxidative stress (Massey et al., 2004). In contrast to the activation of MATG in the early stages of starvation (4–6 h in liver), maximal CMA activation occurs after prolonged starvation (>6 h), concomitant with the decrease in MATG (Cuervo, 2004b). This sequential activation of first a non-selective and then a selective autophagy pathway may be intended to avoid the degradation of essential cellular components after extended fasting.

Both changes in intracellular proteolysis after prolonged nutritional deprivation, as well as, in the cellular distribution of the group of lysosomes active for CMA, are used to track this particular form of autophagy (Massey et al., 2004).

2.2. Physiological role of the different autophagic pathways

Autophagy contributes to the turnover of cellular components and can be stimulated in response to changes in the environmental conditions and different stressors, such as starvation, accumulation of misfolded proteins, oxidative stress, change in cell volume, and fluctuation in hormonal levels (reviewed in Cuervo, 2004a; Shintani and Klionsky, 2004). The identification of the ATG genes has recently allowed the establishment of a connection between

autophagy and diverse physiological processes, including cell differentiation and development, cell growth, programmed cell death, innate immunity, pathogen infection defense, and aging (Cuervo, 2004b; Shintani and Klionsky, 2004). Genetic evidence has helped in revealing the importance of MATG during the development of multicellular organisms and in tissue remodeling and repair. MATG is also involved in programmed cell death type II (*autophagic cell death*) (reviewed in Shintani and Klionsky, 2004) characterized by accumulation of large autophagic vesicles inside the dying cell. In contrast to the initial idea that autophagy was 'killing' the cells under these conditions, recent studies support instead a protective role. Thus, activation of MATG during cellular stress seems aimed at limiting cell death, by removing damaged organelles that could otherwise activate apoptosis (Lum et al., 2005). It is only the persistent overactivation of this initially protective mechanism that leads to cellular death (Lum et al., 2005). A critical role for MATG has been described as part of the host response against invasion by bacteria and viruses. Although not a new concept, it is only recently that evidence for the requirement of the MATG machinery in the fight against pathogens has been presented (Gutierrez et al., 2004; Shintani and Klionsky, 2004).

The large capability of the autophagic vacuoles and the rapid upregulation of MATG in response to stress are key for the role of MATG in the above mentioned functions. On the other hand, the unique selective character of CMA makes this form of autophagy the one preferentially activated when discriminating amongst the proteins to be degraded is important. For example, activation of CMA during oxidative stress or after toxic exposure allows the removal of damaged proteins, but not of their unmodified counterparts, in lysosomes (Kiffin et al., 2004; Massey et al., 2004). During prolonged nutritional stress, activation of CMA provides cells with the required amino acids for maintenance of protein synthesis, without compromising essential cytosolic proteins.

Although mATG has been classically associated with cell maintenance, the current lack of methods to track mATG in mammals renders difficult the determination of its possible physiological roles.

2.3. Aging of the lysosomal/autophagic system

2.3.1. Physiological aging

The lysosomal system undergoes striking changes as cells age (increase in lysosome volume, decrease of lysosomal stability, changes in some hydrolases activities, intralysosomal accumulation of indigestible materials (lipofuscin or 'aging pigment') and impaired regulation of lysosomal pH) (reviewed in Terman and Brunk, 2004a). The immediate functional consequence of these changes is a decrease in the rates of degradation of long-lived proteins in most tissues of aged organisms. MATG and CMA are altered in aging (Fig. 2, see callouts) (Bergamini et al., 2004; Cuervo and Dice, 2000). Both decrease in autophagic vacuoles formation, but predominantly in their fusion with the lysosomes, are behind the decreased MATG activity in aging (Terman and Brunk, 2004a). Now that the major MATG players have been identified, it is only a matter of time until the molecular defects responsible for the malfunctioning of MATG in old organisms are elucidated. Studies in old rodents and senescent cells in culture have revealed reduced rates of translocation of substrate proteins into lysosomes through CMA (Cuervo and Dice, 2000). A decrease in the levels of the CMA lysosomal receptor with age seems the main defect in this pathway. Once again, mATG falls under the 'unknown category' regarding possible age-related changes.

Based on the described functions for autophagy, two possible immediate consequences of declined MATG and CMA activity in aging are an inefficient turnover of intracellular components (proteins and organelles) and the inability of cells to properly adapt to changes in the extracellular environment (reviewed in Ward, 2002). Of particular interest for the aging phenotype is the recent proposed role for autophagy as part of the oxidative stress response. Failure to orchestrate this response has been involved in aging and in the pathogenesis of many age-related diseases (Keller et al., 2004). Because CMA is activated during mild oxidative stress (Kiffin et al., 2004), the age-dependent decrease in CMA activity is likely to contribute to the intracellular accumulation of oxidized proteins in aged organisms. Likewise, MATG plays a main role in the oxidative stress response, by selectively eliminating damaged mitochondria, the most important source of free radicals in the cell (Terman and Brunk, 2004a).

2.3.2. Age-related pathologies

The decreased efficiency of the autophagic system with age has gained renewed attention as a result of the increasing number of reports supporting a role for defective autophagy in the pathogenesis of different age-related diseases (neurodegenerative disorders, cancer, myophaties, diabetes, retinopathies among others) (reviewed in Bergamini et al., 2004; Cuervo, 2004a; Keller et al., 2004; Shintani and Klionsky, 2004).

Neurodegenerative disorders. Accumulation of autophagic vesicles and inclusion bodies (protein aggregates or aggresomes) is common to many protein-conformation disorders, among which several neurodegenerative pathologies (Parkinson's (PD), Huntington's (HD), and Alzheimer's (AD) diseases) are best characterized. In all these disorders, insoluble oligomeric complexes of misfolded or unfolded proteins accumulate in the cytosol as aggregates. Protein aggregation is probably cytoprotective, as aggregates sequester the toxic forms of the altered protein (those that because of their particular conformational changes expose highly hydrophobic

regions, prone to unspecific interactions with other intracellular components, or form structures with pore-like properties, able to destabilize intracellular membranes). Recent evidence supports a role for MATG in the clearance of these protein aggregates. Pharmacological induction of MATG can reduce mutant huntingtin aggregation and toxicity in a mouse model for Huntington's disease (Ravikumar et al., 2003), while blocking macroautophagy increases the number of cells bearing mutant huntingtin aggregates (Qin et al., 2003). Thus, activation of MATG in the early stages of neurodegeneration could facilitate the removal of the protein aggregates, and this probably explains the described upregulation of MATG early in AD (Nixon et al., 2005). However, the neuroprotective function of MATG gets compromised as the disorder progresses, leading to poor clearance and accumulation of immature autophagosomes in the affected neurons, and the consequent alteration in cell function. This failure of MATG has been proven particularly detrimental in the case of AD, where the accumulated autophagic vacuoles become a new compartment for the generation of Aβ-amyloid peptide (a toxic proteolytic product in AD) hence contributing to β-amyloid deposition in brain (Nixon et al., 2005). An aspect that would need further clarification is whether overactivation of MATG to eliminate aggregates could in some instances lead to the removal of critical cellular components and cell death.

A connection between dysfunctional CMA and some familial forms of PD has been recently established (Cuervo et al., 2004). α-synuclein, a cytosolic presynaptic protein often found in the protein aggregates of the affected neurons in PD, can be eliminated selectively in lysosomes by CMA. Mutant forms of α-synuclein, identified in some familial forms of PD, bind to the lysosomal membrane with high affinity but are not translocated, blocking as well the uptake and degradation of other CMA substrates (Cuervo et al., 2004).

Cancer. Opposite roles in both the promotion and prevention of cancer have been described for MATG, depending on the stage of tumor progression, the type of tumor, and therapeutic interventions attempted (Ogier--Denis and Codogno, 2003). Impaired ability to activate MATG is a common feature of several types of breast and ovarian cancer (Qu et al., 2003). This imbalance towards an anabolic (more protein synthesis than degradation) versus a catabolic state (more protein degradation than synthesis) may ensure tumor growth. Activation of MATG can act thus as tumor suppressor in the early stages of tumor progression. Also, in some forms of cancer that have evolved to suppress apoptosis and consequently cell death after certain therapeutic interventions, activation of MATG could lead to autophagic cell death and elimination of the malignant cell (Ogier-Denis and Codogno, 2003). However, in other types of cancer, selective inactivation of a novel cell death pathway that combines apoptosis and autophagy has been shown to allow cancer cell survival in the earliest stages of development (Thorburn et al., 2005). Tumors can also use autophagy for their own benefit. Activation of MATG after anti-oncogenic interventions facilitates the removal of damaged organelles from the cytosol of the malignant cell and guarantees cell survival in certain cancer types. In a similar way, in advance stages of solid tumors, MATG degradation may provide the essential amino acids to allow cancer cells survival under low-vascularized tumor conditions (reviewed in Shintani and Klionsky, 2004). No relationship has been established yet between the types of cancer preferentially affecting elders and their ability to orchestrate or not an autophagic response. However, it is likely that the decline in autophagic activity with age may favor the development of some type of tumors and could explain their increased resistant to certain anti-cancer treatments.

Myopathies and muscular disorders. Massive accumulation of autophagic vacuoles is characteristic of some types of vacuolar-myopathies (Nishino, 2003). Mutations in different autophagy-related proteins, such as the lysosomal membrane protein type 2, myotubularin (a phosphatase involved in vesicular trafficking and autophagy modulation) and acid α-glucosidase (a lysosomal enzyme) have been described in these myopathies. Unrelated to these genetic defects, one of the most dramatic changes in the aged muscle, the accumulation of morphologically and functionally altered mitochondria, is believed to result from their impaired turnover as muscle age (Terman and Brunk, 2004b). Whether the poor turnover is consequence of the decreased MATG activity in old cells, diminished susceptibility of aged-modified mitochondria for degradation, or of both, remains to be elucidated.

Lipofuscin and retinopathies. Lipofuscin accumulation, often used as a hallmark of aging, has been associated with various retinal diseases including age-related macular degeneration (Terman and Brunk, 2004a). Lipofuscin is an intralysosomal non-degradable pigment, primarily composed of cross-linked protein residues, which when accumulates in lysosomes decreases their ability to fuse to autophagosomes and alters other lysosomal properties. Although the molecular basis for this inhibitory effect is unknown, A2-E, the major lipofuscin fluorophore, has been shown to inhibit the lysosomal ATP-driven proton pump. This inhibition results in an increase in the lysosomal pH, the subsequent inhibition of lysosomal hydrolases, and therefore, impairment of all types of lysosomal-dependent degradation (Bergmann et al., 2004).

Diabetes. Reduced CMA contributes to the accumulation of proteins characteristic of the diabetic-induced renal hypertrophy. The preferential accumulation of substrate proteins for CMA in the hypertropic kidney and the decreased levels of hsc70 and LAMP-2A, the two proteins that regulate this pathway, helped to establish a link between CMA and diabetes (Sooparb et al., 2004). The

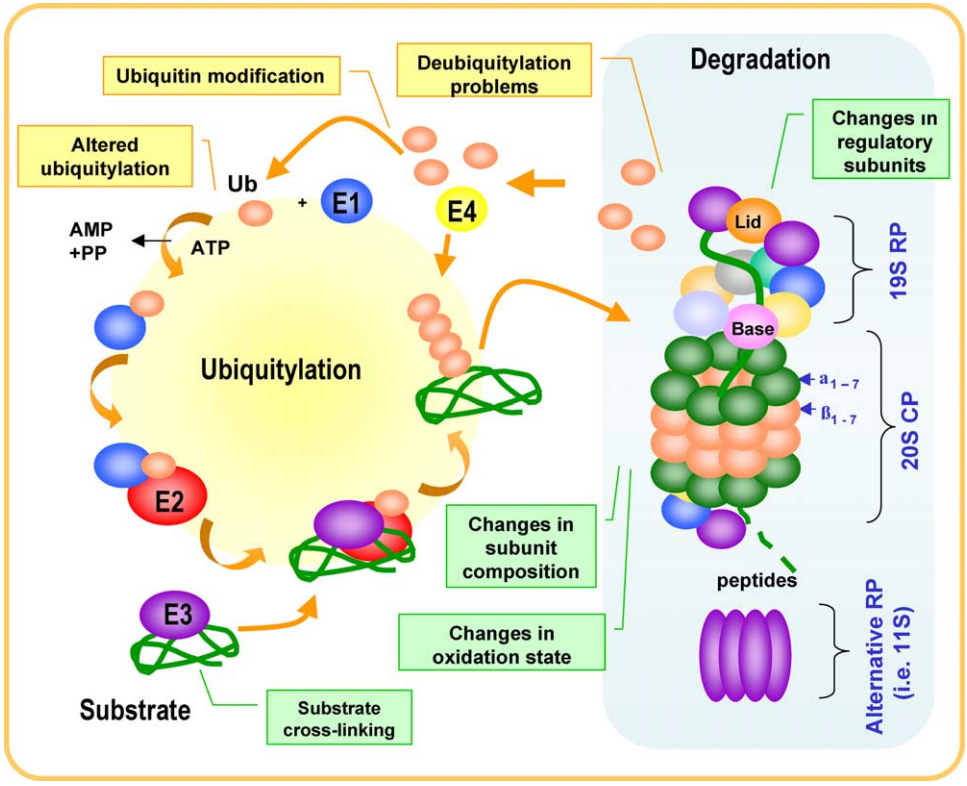

Fig. 3. The UPS system and its changes in aging. Two major steps, ubiquitylation (left) and degradation (right), contribute to the removal of cytosolic proteins by the UPS. The multienzyme-mediated attachment of several ubiquitin molecules to a protein is the most universal tag for targeting to the proteasome. The proteasome is a multicatalytic complex formed by a catalytic core (20S) and regulatory subunits (19 and 11S). Already identified age-related changes in the UPS (green callouts) and other possible changes that could explain the defective UPS activit in aging (yellow callouts) are shown. Abbreviations: Ub, ubiquitin; E1, ub-activating enzyme; E2, ub-conjugating enzyme; E3, ub-ligase; E4, ub-elongating factor; CP, catalytic proteasome; RP, regulatory proteasome.

age-related decline in CMA activity could presumably aggravate this process, explaining, at least in part, the higher incidence of diabetic complications as organisms age. Furthermore, accumulation of oxidized and glycated proteins, common protein modifications associated with diabetes, could be in part attributed to defective autophagy, in light of the recently described role for CMA in the removal of modified proteins (Kiffin et al., 2004).

3. The ubiquitin proteasome system

The ubiquitin proteasome system (UPS) is the other major proteolytic system in eukaryotic cells (Ciechanover, 2005; Wolf and Hilt, 2004). Two major steps, the tagging of the substrates for their degradation, and their actual proteolytic cleavage, are attained through the coordinate function of its main components, the ubiquitin conjugating cascade and the proteasome or degradation nano-machine (Fig. 3). The UPS contributes to the maintenance of cellular homeostasis and protein quality control, but it is its role as regulator of essential intracellular processes such as, cell cycle progression, cell division, transcription and signaling that makes proper UPS functioning an essential requirement for cell survival (Goldberg, 2003; Wolf and Hilt, 2004).

3.1. The proteasome

The proteolytic component of the UPS is the proteasome, a multicatalytic enzymatic complex conserved in prokaryotes and eukaryotes (reviewed in Wolf and Hilt, 2004). Different combinations of a catalytic core (the 20S proteasome) and several types of regulatory subunits (the 19S and the 11S) give rise to the various types of intracellular proteasomes. The minimal functional proteasome, composed of the catalytic core alone (20S), has been proposed to degrade untagged proteins in an ATP independent manner. This catalytic core is a barrel shaped compartment made up of four stacked rings of seven different α_{1-7} (the outer two rings) or β_{1-7} subunits (the inner two rings) (Fig. 3, right). Three of the two inner subunits ($\beta_{1,2,5}$) bear the proteolytic active sites, while the outer α subunits stabilize the holoenzyme (Wolf and Hilt, 2004).

The best-characterized type of proteasome is the 26S, which results from the assembly of the catalytic core and two flanking 19S regulatory subunits. Most polyubiquitin-

tagged proteins are recognized and degraded by the 26S proteasome (Ciechanover, 2005). Different components of the 19S regulatory subunit participate in substrate recognition, untagging and unfolding. They also induce conformational modifications toward an 'open' state of the α ring and, often, provide the force that drives the substrate into the catalytic core (Pickart and Cohen, 2004). The 19S subunits organize in a base and a lid stabilized together by other subunits.

Other proteasome variants, the 22S proteasomes or 'immunoproteasomes', have acquired particular relevance for their role in the processing of antigens for presentation by the MHC class I pathways (reviewed in Kloetzel, 2001). In these specialized proteasomes, the catalytic core, in which three of the seven β subunits are replaced by other β subunits, is flanked by two 11S regulatory subunits. Immunoproteasome assembly and synthesis of the 11S regulator are strongly dependent on interferon-gamma. The distinct subcellular distribution of the immunoproteasomes (mostly enriched at the ER surface) and subtle differences in cleavage specificity determine the efficiency of production of MHC class I binding peptides. The functional relevance of other proteasome variants (association of the catalytic core with two different regulatory subunits (20S + 11S + 19S) or catalytic cores flanked by only one regulatory subunit) is unknown.

3.2. The ubiquitylation machinery

In most instances, proteins need to be 'tagged' in order to be recognized and degraded by the proteasome. Phosphorylation and covalent attachment of ubiquitin (ubiquitylation) are the most common tags used for proteasome targeting. Three different classes of enzyme, E1, E2 and E3 are responsible for the conjugation of ubiquitin (Ub)—a small (76 residue), heat-stable protein- to the protein designated to be degraded (Fig. 3, left) (Ciechanover, 2005; Pickart and Cohen, 2004). In an ATP-dependent reaction, the Ub-activating enzyme, E1, activates Ub to a high energy E1-thiol-ester–Ub intermediate protein; this activated Ub is covalently attached to the target protein by the Ub-conjugating proteins, E2s; the third group of enzymes, E3s or Ub-protein ligases, 'present' the substrate to E2s for conjugation. E3s have thus two functional domains, one that facilitates the specific recognition of the substrate, and a second one directly involved in the binding of the substrate to E2 (Ciechanover, 2005; Pickart and Cohen, 2004). Attachment of new Ub molecules to the initial one to form polyUb chains is normally attained by repetitive passage through the E1–E2–E3 catalyzed cycle, but can also be directly catalyzed by the Ub chain assembly/elongator factors (E4) (Ciechanover, 2005; Pickart and Cohen, 2004). Although novel roles independent of degradation (signaling, enzyme activation, regulation of membrane dynamics, subcellular location, etc) have been identified for protein monoubiquitylation, chains of Ub seem to be the preferential target for degradation by the 26S proteasome. Phosphorylation is also a key signal for ubiquitylation and proteasomal catabolism of many proteins. Recruitment of the specific E3 to the substrate protein is often mediated by phosphorylation/dephosphorylation events on the substrate protein.

There are still many unanswered questions about the mechanisms that mediate targeting and recognition of the polyubiquitylated protein by the 26S proteasome. Recently, a sequential hand-off of ubiquitylated proteins from a substrate-recruiting complex to a multi-Ub chain assembly complex, and from this to a proteasomal targeting complex, which ultimately delivers the substrate to the proteasome has been described (Richly et al., 2004). Once recognized by the regulatory components, ubiquitin is removed and the substrate unfolded to enable its translocation into the gated catalytic chamber (Wolf and Hilt, 2004).

There are other mechanisms less well characterized by which substrate can be targeted to the proteasome for degradation. A set of proteins that simultaneously bind polyUb chains and proteasomes, have been proposed to function as delivery factors, although they can also protect against degradation by preventing proteasomes from accessing substrate-linked polyUb chains. In addition, some substrates bind directly to the proteasomes in an ubiquitin-independent manner promoting gating of the 20S proteasome (endoproteolysis) (reviewed in Grune et al., 2003; Wolf and Hilt, 2004).

3.3. Regulation and physiological role of the UPS

The UPS system is regulated at different levels (Hamel et al., 2004). The best-characterized regulation is through the association of the catalytic core to the regulatory subunits (19S and 11S). Recently, novel regulatory mechanisms for proteasome activity have been proposed, all sharing faster control ability over proteasome activity. Nutrients can control the expression of different components required for proper ubiquitylation. An increase in the RNA encoding different components of the ubiquitylating machinery has been described under starvation conditions, while amino acids and insulin have the opposite effect (Hamel et al., 2004). Furthermore, allosteric modulation of the catalytic core by different small molecules is also possible. For example, certain amino acids and fatty acids can directly inhibit the proteasome.

The UPS plays a critical role as part of the cellular protein quality control system for both cytosolic and secretory proteins (Ciechanover, 2005; Goldberg, 2003). Unfolded proteins, largely newly synthesized cytosolic proteins that cannot reach their proper folded conformation, and a large subset of post-translationally damaged proteins (oxidized, glycated, etc.) are removed from the cytosol via the UPS system. Secretory proteins that fail to fold properly in the endoplasmic reticulum are translocated into the cytosol and also degraded by the 26S proteasome after

ubiquitylation (Goldberg, 2003). Activation of this process, known as endoplasmic reticulum associated degradation (ERAD), has been proposed as a possible therapeutic approach for different protein conformational disorders in which the altered protein gets stuck in the endoplasmic reticulum. Finally, components of the UPS are also normally present in the nucleus where, in addition to a regulatory function, they participate in the removal of damaged proteins from this compartment. For instance, upregulation of nuclear proteasome during oxidative stress has been shown necessary for the elimination of glycoxidate nuclear histones (Cervantes-Laurean et al., 2005). Removal of mild-oxidized proteins is an important function of the proteasome system (Grune et al., 2003). Despite being susceptible to proteolytic cleavage by the proteasome, oxidized proteins are poor ubiquitylation substrates (Shringarpure et al., 2003). Extensive literature supports the existence of recognition signals other than ubiquitin, most probably hydrophobic patches exposed in the partially unfolded oxidized proteins, which can directly bind to the 20S proteasome and promote ATP-independent opening of the proteolytic chamber (Grune et al., 2003).

The ubiquitin/proteasome system is also responsible for the regulated degradation of short-lived proteins with key intracellular functions (Hamel et al., 2004; Wolf and Hilt, 2004). This confers the proteasome a crucial role in the control of many cellular processes, including cell cycle progression, cell differentiation, gene expression, signal transduction, trafficking, and apoptosis.

Lastly, the UPS has an important function in the immune and inflammatory response. The subset of proteasomes known as immunoproteasomes are the major source of antigenic peptides presented to the immune system by MHC class I molecules (Kloetzel, 2001). Furthermore, the UPS is directly responsible for the activation of the nuclear factor-kappa B—the central transcription factor of the immune system.

3.4. Aging of the UPS

3.4.1. Physiological aging

Many reports have shown different degrees of decrease in the activity of the UPS with age in several tissues, although, in contrast to the lysosomal system, the decline in activity with age does not seem to be universal (Carrard et al., 2002; Ferrington et al., 2005; Keller et al., 2004; Ward, 2002). The development of methods to directly analyze the different steps in UPS degradation has shed new light on the aging of this system, revealing that qualitative rather than quantitative changes set the basis for UPS malfunctioning in aging.

Of the two major steps for UPS dependent degradation, ubiquitylation and degradation, the former does not seem to be particularly affected by age. Studies in mouse liver or human fibroblasts have revealed no changes with age in levels of Ub, Ub mRNA and of E1, E2 or different E3s. Consequently, the accumulation of Ub-conjugated substrates, common in most aged tissues and in different age-related disorders, is likely to result from a decrease in their efficient removal by the proteasome (Carrard et al., 2002). Despite the original studies showing contradictory changes in proteasome proteolytic abilities with age, it is now accepted that the proteolytic ability of the proteasome is modulated in vivo by multiple factors, and that age-dependent modifications in these factors are probably responsible for altered proteasome activity (Fig. 3, callouts) (Carrard et al., 2002; Ferrington et al., 2005). In fact, taking advantage of the comprehensive molecular characterization of the UPS and its regulators, a recent study in aged muscle has revealed an increase in the content of the 20S proteasome with age (mainly due to increase in immuno-proteasome), concomitant with a severe decrease in the content of regulatory proteins (Ferrington et al., 2005). This deficit of the regulatory subunits is responsible for the inadequate activation of the 20S proteasome with age. Changes in the oxidation state of the proteasome subunits (oxidation, glycation and conjugation with peroxidized lipid products) increase with age and are likely to result in changes in UPS regulation (Carrard et al., 2002). In addition, oxidized proteins and crosslinked-proteins and lipids (all abundant in lipofuscin) can directly inhibit the proteasome (Terman and Brunk, 2004a). In summary, failure of proteasome function with age could be due to changes in the protease (decreased proteasome expression; alterations and/or replacement of proteasome subunits), changes in the modulatory molecules (endogenous regulatory subunits and their partners) and changes in the proteasome substrates (decreased proteolytic susceptibility, cross-linking of proteins, etc) (Fig. 3, green callouts). Possible changes with age in the ability of the substrates to be ubiquitylated, in the modulatory role of amino acids and hormones, and in the ability of the proteasome to recognize the tagged proteins or to remove the polyUb chains prior to degradation still need to be explored (Fig. 3, yellow callouts).

3.4.2. Age-related pathologies

Alterations in the UPS have been implicated in the pathogenesis of many diseases including malignancies, neurodegenerative disorders, hereditary syndromes and pathologies of the inflammatory and immune response. Some of the alterations in the UPS activity have a genetic origin. Different mutation in ubiquitylation enzymes, mostly in E3s, have been described, where the particular phenotype depends on the subset of substrates requiring the mutated enzyme (i.e. in Franconi anemia the E3 mutation associates to impaired DNA repair, while some forms of cervical cancer originate from a mutation in the E3 ligase required for the degradation of the tumor suppressor p53). Here, we discuss UPS pathologies particularly related to aging.

Neurodegenerative disorders. Changes in the UPS in the different neurodegenerative disorders (Section 2.3.2) were reported before any alteration in autophagy was described.

In fact, Ub and other UPS proteins are common components of the aggregates and inclusion bodies in these pathologies and, interactions of the mutant proteins with different subunits of the proteasome are well documented (Flood et al., 2005). However, the initial idea that aggregate proteins can have a toxic effect on UPS activity by sequestering some of their components, no longer stands, because the amount of UPS elements trapped in the aggregates is a relatively small percent of the total (Bennett et al., 2005). Although the specific reasons for the declined UPS activity in these pathologies remain unknown, mechanisms other than UPS depletion should contribute to decreased function. Some of the mutant proteins in these pathologies exert a direct inhibitory effect on the proteolytic activity of the 20S proteasome (i.e. tau-based paired helical filament (in AD), mutant synuclein (in PD), and huntingtin polyglutamine fragments (in HD)) (Bennett et al., 2005). On the other hand, altered proteasome activity (inhibition) seems to favor the formation of aggregates, and in some cases, such as in AD, increases the production of the toxic product (Flood et al., 2005). Interestingly, mutations in different UPS components have been described in both of the two most common neurodegenerative disorders, AD and PD. In AD, a posttranscriptional dinucleotide deletion in Ub generates an aberrant protein (UBB^{+1}) that accumulates in the affected cells. Parkin and UHCL-1, an E3 and a deubiquitylase enzyme, respectively, are mutated in some forms of PD. Although the particular substrates that these enzymes act on, why they accumulate in the aggregates of the affected neurons, and their role in the pathogenesis of the disease remains to be elucidated, experimental evidence supported a neuroprotective role for these UPS components.

Alteration in the UPS-mediated degradation of proteins retrotranslocated from the ER (ERAD) has also been implicated in neurodegeneration. In AD, mutations of a component of the protease complex that cleaves Aβ protein, lead to misassembly and degradation of other component of this complex via ERAD (Bergman et al., 2004). However, if proteasome activity is impaired (as in advanced AD or in aging) the protein accumulates in the ER resulting in ER stress.

The described role for the UPS as part of the oxidative stress response supports that the accumulation of oxidized proteins and lipid products in the affected neurons in neurodegenearative disorders could be a direct consequence of the decreased activity of this proteolytic system in these pathologies (Keller et al., 2004). Similarly, chronic inflammation, common in most of the neurodegenerative disorders and in aging, may contribute to perpetuate the disorder by maintaining some level of blockage on the UPS (Mishto et al., 2003).

Aging is, actually, the main risk factor for neurodegeneration. The described defective activity of the two major proteolytic systems with age maybe behind this increased risk to neurodegeneration in elders. Even though in many of these disorders the defect is present at birth, it is not clear why symptoms do not appear until advanced ages. It is possible that decreased protein degradation leads to accumulation of damaged products and the corresponding increase of oxidative stress, which could trigger the neuropathology. On the other hand, the increased production of free radicals associated to age may extenuate the proteolytic systems through the affluence of damaged proteins, and this could favor aggregation of the mutated proteins otherwise properly removed. A third scenario is also possible in which it is the progressive accumulation of the damaged protein, initially asymptomatic, that with time leads to extenuation or inhibition of the proteolytic systems, loss in the ability to remove damaged proteins and increased oxidative stress. In any case, as for many other aspects of aging, it is likely that the confluence of a discrete functional failure in several systems over time ends up triggering neurotoxicity.

Immunosenescence. A severe decline in cell mediated immunity, particularly because of major changes in T cell function, along with an increase in autoantibody frequency and decrease in antibody production and affinity, are hallmarks of the aged immune system (Franceschi et al., 2000). The UPS can indirectly contribute to some of those changes by altering the degradation of critical regulatory molecules. Reduced IkB degradation, as consequence of impaired proteasome activity with age, could explain the poor NF-B activation after TNF treatment in T cells from elderly donors, and consequently the weak anti-apoptotic response of these cells (reviewed in Mishto et al., 2003). Furthermore, the important role that the immunoproteasomes play in processing and presentation of MHC class I antigens makes the UPS an attractive candidate for the immunosenescence process. In fact, changes in immunoproteasome activity could have considerable effect in the quality and quantity of epitopes presented to the T cells, and consequently lead to a consistent modification of the immune response against self and exogenous antigens. Carrard et al. (2003) have shown structural alteration in purified 26S proteasomes from T and B lymphocytes, and studies on gene expression in aged brain and muscle have revealed decreased expression of constitutive proteasome subunits along with an increase in expression of immunoproteasome subunits (Lee et al., 1999). However, this is not a generalized change since studies in heart and blood revealed no changes in constitutive to immunoproteasome ratio (Carrard et al., 2003). Future studies directly addressing changes with age in immunoproteasome activity and function of the regulatory components (11S) are needed.

Other aging pathologies. Direct or indirect connections between the defective function of the UPS and other age-related disorders have been established. In some instances, it is the impaired ability to degrade a critical regulatory component via UPS that precipitates the pathology. In other cases, it is the poor degradation of altered proteins, predominantly oxidized, that leads to organ or system

malfunctioning. For example, it is well established that the UPS affects cell-cycle progression in part by controlling degradation of cyclins. Alterations in the UPS in aged skeletal muscle inhibit cell cycle entry and prevent cell division. This growth suppression in muscle satellite cells may be the reason for the poor healing potential of old skeletal muscles (Cai et al., 2004). On the other hand, decreased proteasomal activity with age in organs such as the heart, lung, liver, kidney and central nervous system is likely to contribute to the accumulation of damaged proteins in these organs and to the diseases associated to this accumulation (Keller et al., 2004). Thus, UPS malfunction in myocytes could be a major player in the inadequate response to ischemic stress in the aged heart and in the increased susceptibility to cardiovascular failure (Bulteau et al., 2002). Likewise, accumulation of carboxymethylated and ubiquitylated proteins in the aged lens as consequence of reduced UPS activity, often results in the formation of cataracts (Viteri et al., 2004).

Contrary to the lysosomal system for which all connections with age-related disorders originate from reduced lysosomal activity in aging, in the case of the UPS there are conditions in which exacerbated proteasome activity sets the basis for the age-related disorder, and blockage of this hyperactivation maybe desirable. For example sarcopernia, or the loss of muscle associated to aging, seems consequence of an imbalance between anabolism and catabolism, and the proteasome is likely to contribute to the increased protein degradation (Carrard et al., 2002). Also, proteasome inhibitors are useful in the prevention of ischemia-reperfusion injury of brain, heart and kidney.

4. Concluding remarks

Alterations in both the lysosomal system and the UPS are common in most tissues of old organisms. The numerous intracellular processes in which these proteolytic systems participate make comprehensible why their failure with age has been proposed as key in the pathogenesis of numerous age-related pathologies. The recent advances in the molecular dissection of autophagy and of the regulatory components of the UPS should help, in the coming years, the identification of the defect(s) responsible for the altered function of these systems in aging. Future studies would require, however, a change in mindset, since it has become evident that the different proteolytic systems and their multiple variants do not act as independent units but instead, growing evidence supports the existence of an intricate cross-talk among different proteolytic systems. Critical for any future restorative effort would be to understand how these systems balance their activities and the rules that dictate the eliciting of compensatory mechanisms after failure of one of these systems.

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