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Impairment of the Ubiquitin-Proteasome System in Ageing

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Besides its role in normal protein turnover, the ubiquitin-proteasome system (UPS) functions as a cellular 'garbage-disposal' machinery by removing misfolded or oxidatively damaged proteins. The target proteins are covalently tagged with polyubiquitin, recognised by the proteasome and degraded. It has become increasingly clear that the UPS functions less efficiently with ageing due to multiple factors. These include altered transcription of the proteasomal subunits, lowered proteolytic activity, damage caused by oxidative stress and inhibition of proteasomes by misfolded, cross-linked proteins. The impaired proteasomal activity results in further accumulation of non-functional protein aggregates. Accumulation of such protein aggregates disrupts normal cellular activity in diverse ways and causes or exacerbates many disorders. A better understanding of the molecular events leading to the proteasomal dysfunction in ageing could yield more effective strategies to treat a variety of neurological diseases that are characterised by the presence of protein aggregates.

Key Words: Ubiquitin, Proteasomes, Protein degradation, Ageing, Ub+1, Molecular misreading, Oxidative damage

Introduction

The ubiquitin-proteasomal system (UPS) performs an essential role in a wide variety of cellular functions by degrading a specific subset of cellular proteins in a highly complex and temporally controlled process (Glickman & Ciechanover 2002). Their importance to normal cellular functioning is shown by the fact that both ubiquitin and proteasomal proteins are evolutionarily conserved. The UPS is involved in a wide variety of cellular functions such as cell cycle, differentiation, apoptosis, DNA repair and proper functioning of the immune system (Ciechanover 1998). It also helps to keep the cell functioning normally by degrading misfolded- or those proteins that are oxidatively damaged as a result of cellular ageing (Grune et al. 1997, Davies 2001). The target proteins are polyubiquitinated in a multi-step process by a series of enzymes, the tagged proteins are recognised by 26S proteasome and broken down into small peptides which are further degraded by cytoplasmic peptidases. Besides the proteasomes, endolysosomal system as well as calpains also play an important role in protein turnover (Perrin & Huttenlocher 2002, Pillay et al. 2002).

Several studies have documented age-related changes in proteasome structure and function in species from flies to humans (Bulteau et al. 2000, Ding & Keller 2001a, Merker et al. 2001, Carrard et al. 2002) Such changes occur in many cell types and tissues and the neurons seem to be particularly vulnerable (Conconi et al. 1996, Keller et al. 2000a,b, Keller et al. 2002). An important feature of the ageing process is the accumulation of oxidatively damaged proteins. As brilliantly proposed by Harman in 1956 (Harman 1956) the theory of free radical suggests that ageing is caused by the harmful and irreversible effects of free radical reactions (Droge 2002). Reactive oxygen species generated as a result of mitochondrial

Abbreviations: *Ub*, ubiquitin, *UPS*, ubiquitin proteasomal system

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respiratory chain (Lenaz et al. 2002) cannot be effectively neutralized by cellular antioxidant systems. As a result, reactive oxygen species accumulate in the cells and modify cellular lipids and proteins. The oxidatively damaged proteins form insoluble, cross-linked aggregates which inhibit the proteasome. In addition, although the ubiquitination machinery remains largely intact, it also shows a defect with ageing. This results in generation of a mutant form of Ubiquitin (Ub) termed Ub+1 which potently inhibits the proteasomal activity (Lam et al. 2000, van Leeuwen et al. 2000). Thus, many factors contribute to the age-related impairment in the ubiquitin proteasome system. Whereas some factors such as Ub+1 have only recently been identified, others such as oxidative damage have been characterised in greater details. This review summarizes the recent studies that shed light on the molecular basis of the proteasomal impairment in ageing. Several excellent review articles have described other aspects of ubiquitin and proteasomes (Ciechanover 1998, Voges et al. 1999, Pickart 2001a, Glickman & Ciechanover 2002, Hartmann-Petersen et al. 2003).

The Ubiquitination Cascade

The proteins destined for ATP-dependent degradation by the proteasomes are initially covalently tagged with ubiquitin (Ub)- a small protein of 76 residues- by a process that involves a multi-enzyme system. Ubiquitination usually involves the formation of an isopeptide bond between the C-terminal most glycine (Gly76) of Ub and a lysine residue on target protein. This reaction is orchestrated by sequential actions of three enzymes (figure 1) (Ciechanover 1998, Pickart 2001a). First, ubiquitin is activated by an enzyme called E1 which forms a thioester bond with the carboxyl group of Gly76. The activated Ub is then transferred to a cysteine residue of a family of enzymes termed E2 or ubiquitin conjugating proteins. Finally, a ligase (E3) catalyzes the formation of an isopeptide bond between Gly76 and a lysine on target protein. Addition of a single Ub moiety to the target protein is followed by transfer of more Ub by the concerted action of E1, E2 and E3 enzymes. Polyubiquitination involves

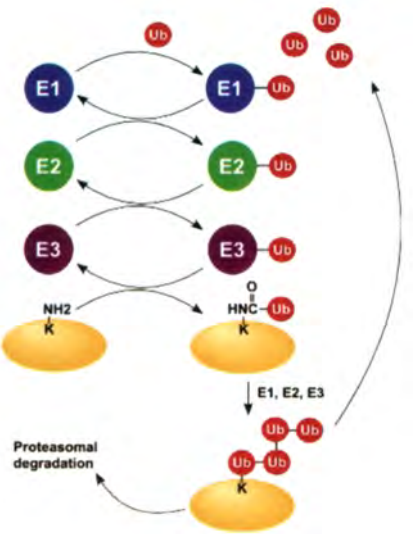

Figure 1 The ubiquitin conjugation cascade. Ubiquitin is activated by E1 by forming a thioester bond and the activated Ub is transferred to a member of E2 ubiquitin conjugating enzyme (UBC). A target specific E3 forms a ternary complex with E2 and the target protein and mediates the transfer of Ub to a lysine residue in target protein. More Ub units are added on to the attached Ub and this polyubiquitin chain triggers binding to and degradation by the proteasome. Intact Ub units are released in the cytoplasm and recycled for the next round of ubiquitination.

covalent attachment of Gly76 of another Ub to Lys48 of Ub already linked to target protein. Addition of four or more Ub forming Lys48-Gly76 isopeptide linkage triggers the binding of the tagged target proteins to the proteasome (Pickart 2001a). Upon binding, the Ub chains are removed and reutilized for the next round of ubiquitination while the target protein is inserted into the catalytic core of the proteasome and degraded by the associated proteolytic activities.

The chemistry of ubiquitination is fairly well understood and crystal structures of many E2 enzymes have been elucidated (Worthylake et al. 1998, Jiang & Basavappa 1999, Miura et al. 1999). In humans a single E1 Ub activating enzymes is responsible for catalysing the first step in the ubiquitination pathway (McGrath et al. 1991). E1 binds MgATP and Ub and catalyzes the formation of a covalent thioester bond. Although over a dozen or so E2 enzymes are conjugated by a single E1, the high catalytic efficiency of E1 provides sufficient quantities of activated Ub for all downstream reactions. All E2s share a conserved common motif of ~150 residues that contain a highly conserved active site cysteine. Although they are similar to one

another, different E2s also contain non-homologous domains which are used in interaction with specific E3 enzymes. Thus, different E2s regulate distinct pathways such as cell cycle or DNA repair via interaction with specific E3s. Some E2s are membrane-associated and are probably involved in degradation of misfolded secretory proteins as they emerge out of the endoplasmic reticulum lumen (Tsai et al. 2000).

E3s provide the substrate specificity by binding to the target protein via the ubiquitination signal (such as the 'PEST' domains or 'Destruction box') and mediating transfer of Ub from E2 to a lysine on the target. E3 also catalyzes the subsequent addition of Ub to the Lys48 of target linked Ub (polyubiquitination). In recent years, several E3s have been identified and the existence of more potential E3s has been indicated in available databases. Despite a lack of sequence homology among E3s, these ligases fall into one of the two classes. One family of E3s is characterized by the presence of HECT domain, a ~350 residue domain initially identified in E6-AP ligase. The HECT (Homologous to E6-APC-terminal region) domains contain a conserved cysteine that forms a thioester bond with Ub while a non-conserved, variable N-terminal domain in E3s interact with substrates. The other family of E3s contain a 'RING finger' -named such for Really Interesting New Gene- (Pickart 2001a) motif that comprise of characteristically spaced histidine and cysteine residues which bind Zn+2. Hundreds of 'RING finger' proteins are present in databases and they seem to be involved in a wide variety of functions (Deshaies 1999). RING finger proteins are likely to function as molecular scaffold that facilitate interaction between two proteins (Borden 2000). Some RING finger proteins are present in multisubunit complexes called as SCF ligase complex (Skp1-cullin-F-box) while other RING finger proteins act as Ub ligase independently for specific subunits (Deshaies 1999). In addition to these two families of E3 ligases, a large (~1.5 mDa) multisubunit complex called cyclosome or 'anaphase promoting complex' is shown to possesses ubiquitin ligase activity specifically towards proteins involved in cell cycle progression including cyclins, kinetochore proteins and spindle proteins (Peters 2002).

It should be noted that ubiquitination is a reversible modification and susceptible for the loss of Ub by deubiquitinating enzymes (Pickart 2000). It is thought that once bound to the target protein, E3s do not let go the substrate without completing several rounds of ubiquitination. It is not clear how E3s change their activity from 'target protein' ubiquitination to 'ubiquitin' ubiquitination. Recent studies have uncovered a novel use of ubiquitination in cell signaling. Some proteins undergo only monoubiquitination which acts as a signal in a diverse array of processes including endocytosis, modulation of protein activity and gene transcription (Pickart 2001b). Mono-ubiquitinated proteins are not substrates for proteasomal degradation. The target proteins can also be covalently modified with ubiquitin like proteins such as SUMO and Nedd8 (Yeh et al. 2000). The role of these modifications, which can take place on the same lysine residues that are targets for ubiquitination, has only recently begun to be understood. The chemistry of these modifications seems to be distinct from that of ubiquitination pathways and they seem to play a role in unique biological functions (Yeh et al. 2000). Finally, aberrant functioning of the UPS as a result of a process called 'molecular misreading' has been implicated in ageing (van Leeuwen et al. 2000) and may potentially play a significant role in neurodegenerative diseases.

The Proteasome

The 26S proteasome is a large, barrel shaped, multi-subunit protein complex (>1 mDa) that is ubiquitously expressed in all eukaryotic cells (Voges et al. 1999). It possesses proteolytic activities that cleave peptide bonds at various sites in a polyubiquitinated protein producing short peptides. It consists of a 20S catalytic core that performs proteolysis and a 19S regulatory complex that recognise the polyubiquitinated target proteins and keep the catalytic core in a latent form. The proteasome plays an essential role not only in the turnover of regulatory proteins, it also removes misfolded or damaged proteins (Tsai et al. 2002).

The 20S Catalytic Core

The 26S proteasome consist of a cylinder shaped 20S catalytic core bound with 19S or 11S regulatory

units at both ends of the cylinder. The proteasome is found both in the cytoplasm and the nucleus although a vast majority of proteasomal activity (85-90%) remains in the cytoplasmic compartment. The proteasomes are also found associated with the endoplasmic reticulum in perinuclear region (Kruger et al. 2001). The electron microscopic studies show that the catalytic core is made up of 4 stacked rings measuring 11 nm in diameter and 15 nm in length (figure 2). Each ring consists of seven monomers of 21-35 kDa peptides termed a- and b-subunits and two a- and two b-rings make up the 20S proteasome. The a-subunits form the top and bottom rings while the middle two rings, which also contain the non-lysosomal proteolytic activities, are comprised of b-subunits. The a-subunits provide the stability to the catalytic core and participate in binding to the 19S regulatory core. The b-subunits possess the three proteolytic activities that have been identified- a trypsin-like, a chymotrypsin-like and a peptidyl glutamyl peptide hydrolase activity.

The active site of the proteolytic activities of each b-subunits is directed towards the inner core of the complex. Upon binding to the 19S regulatory complex, the target proteins are first unfolded and then inserted into the narrow opening of the 20S catalytic core. The protein entry follows a specific orientation with the N-terminal end of protein entering the core (Mo et al. 1999, Benaroudj et al. 2001). The target protein is proteolysed inside the catalytic chamber in a processive manner into peptides of 3-20 residue in length which are released into the cytoplasm from the other end of the proteasome. The substrate specificity of the proteasome is varied; a chymotrypsin-like activity cleaves at hydrophobic residues, a trypsin like activity cleaves at basic residues and a peptidyl glutamyl peptide hydrolase activity cleaves at acidic residues (Rivett 1993, Tanaka & Chiba 1998, Voges et al. 1999). The released peptide fragments are further broken down by non-proteasomal peptidases (Coux et al. 1996, Tomkinson 1999). Based on the processivity of degradation and allosteric regulation of active sites, a 'bite-chew' model has been proposed for the mechanism of proteasomal degradation (Kisselev et al. 1999).

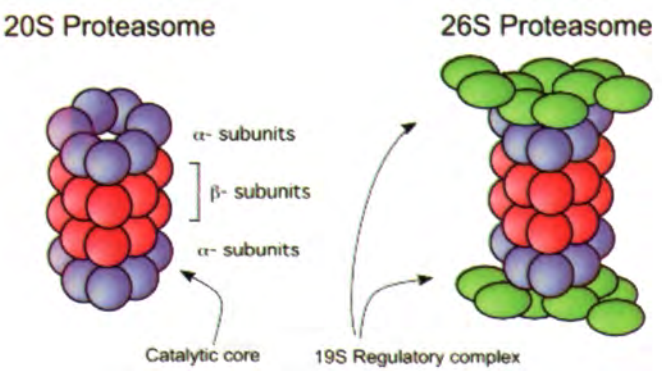

Figure 2 Schematic representation of the proteasomes. Left, the 20S catalytic core is composed of two rings of a b-subunit heptamer (red) that is sandwiched between heptameric a-subunit rings (blue). Right, the 26S proteasome consists of the 20S catalytic core associated with 19S or 11S regulatory complex (green). The 19S complex provides a docking site for the polyubiquitinated proteins and also helps in protein unfolding as it enters the catalytic core.

The 20S proteasome is a dynamic structure whose proteolytic activity can be altered by various stimuli (Fruh et al. 1994, Stohwasser & Kloetzel 1996). Since each of the protease-like activity functions in a sequential manner and associated with individual b-subunits, the proteasomal activity can be varied by regulating subunit composition of the proteasomes. The size and spacing of individual b-subunits also play a role in protein degradation. The proteasome subunits are constitutively expressed, however, expression of specific subunits can be induced by inflammatory signals (Stohwasser et al. 2000, Ding & Keller 2001b) showing that proteasome composition and therefore its activity can be dramatically altered in response to incoming signals (Fruh et al. 1994, Stohwasser & Kloetzel 1996). Also, neuronal tissues seem to exhibit unique proteasomal composition and activity profile suggesting that the proteasomes can be altered to accommodate specific needs of the tissue (Noda et al. 2000).

Regulatory Subunits

The 20S proteasome is found associated with 11S or 19S cap-like protein complexes to give rise to the 26S proteasomal complex (figure 2). The 20S proteasome associates with one or two 19S subunit in ATP-dependent manner to form a 'single-capped' or 'double-capped' structures. A population of 'mixed' proteasomes containing 20S catalytic core associated with both an 11S and 19S regulatory subunits has been reported (Tanahashi et al. 2000). The regulatory subunits are thought to be involved

in target protein recognition and protein unfolding (Rivett 1993, DeMartino & Slaughter 1999) since many constituents of the 11S and 19S complexes contain heat shock protein binding motifs (Luders et al. 2000). It is believed that the catalytic core of the 20S proteasome remains closed in non-active form by association with 11S and 19S regulatory units and can be turned on to the active form under stressful conditions such as heat treatment (Tanaka et al. 1986).

The 19S proteasome is a large protein complex of more than 18 subunits of varying molecular mass. The various subunits form a 'base' and 'lid' of the regulatory subunit. The base of 19S complex is made up of 6 ATPase subunits (Rpt1-Rpt6) which bind to the 20S proteasome (Rubin et al. 1998). These ATPase subunits are thought to unfold the target proteins prior to their insertion into the catalytic core (Glickman et al. 1998, Hershko & Ciechanover 1998, Braun et al. 1999, Glickman et al. 1999, Kloetzel 2001). The remaining 12-13 subunits (Rpn1-Rpn12, (Takeuchi & Toh-e 1999) form the lid structure and a subunit, Rpn10, that contains 'ubiquitin-binding' sequences is involved in target protein recognition (Deveraux et al. 1994, van Nocker et al. 1996, Wilkinson et al. 2000). The proteasomal protein degradation occurs in a step-wise fashion; a) an ubiquitinated target protein first associates with the 'mouth' of the proteasome, b) the target protein undergoes unfolding and is inserted into the proteolytic chamber through the narrow opening, c) the proteolytic activities cleave the target into 3-20 residue-long peptides, and d) the short peptides are released from the catalytic chamber into the cytoplasm and further degraded by cytoplasmic peptidases.

The expression of 11S regulatory complex (also known as PA28) is induced by g-interferon (Chu-Ping et al. 1992, Dubiel et al. 1992). This cytoplasmic complex consist of PA28a and PA28b subunits which associate to form a ring like structure (Mott et al. 1994, Ahn et al. 1995). The 26S proteasome containing the 11S regulatory complex plays an essential role in antigen presentation and immune response by regulating the length of peptides produced for MHC presentation (Ma et al. 1993, Preckel et al. 1999, Rechsteiner et al. 2000). The 11S protein subunits lack the ubiquitin-binding motifs and consequently, such proteasomal units cannot degrade the ubiquitinated proteins. The induction with g-interferon also causes expression of proteasomes with altered proteolytic activities (Akiyama et al. 1994, Belich & Trowsdale 1995, Hisamatsu et al. 1996) which are believed to cause the production of peptides with higher affinity to the MHC class I complex (Gaczynska et al. 1993).

Proteasomal Impairment in Ageing

Several factors seem to be responsible for the impairment of proteasomal function in ageing. These can be altered transcription of proteasome subunits, oxidative damage to proteasome itself or accumulation of aberrant proteins that inhibit proteasomal activity.

A Reduction in the Proteasomal Activity

Several studies have indicated that the proteasomal degradation pathway becomes progressively impaired with ageing. A loss or reduction in proteasome activity has been observed in vitro during the ageing of cultured cells as well as in vivo in aged animals (Carney et al. 1991, Conconi et al. 1996, Keller et al. 2000b, Sitte et al. 2000a,b,c). On the other hand, some studies have reported no alterations in proteasomal function with ageing (Agarwal & Sohal 1994, Keller et al. 2000a). The reasons for this discrepancy are not clear (Keller et al. 2002). The proteasomal activity seems to decline with age in a variety of tissues, although, some of the changes can be extremely cell-type and tissue-type specific (Sitte et al. 2000a, c,d). For example, some parts aged rat brain show a decreased chymotrypsin like activity which remains unaltered in other parts of the central nervous system (Keller et al. 2000a,b). Also, senescent human fibroblasts show a differential loss of peptidyl glutamyl peptidase activity without any significant changes in the total levels of proteasomal activity (Sitte et al. 2000a,b,c,d). Such changes probably reflect age-dependent changes in the expression pattern of specific proteasomal subunits. However it should be pointed out that the significance of senescent cells for aging in vivo has been questioned (Bandyopadhyay et al. 2001, Campisi 2001). Thus, although an impairment of the proteasomal function is not universally observed among all the tissues, certain cells and tissues seem to be more susceptible.

Decreased Proteasomal Levels

The decrease in proteasomal activity in ageing can be due to a number of factors. An age-related decline in the mRNA levels of several subunits of both 20S and 26S proteasomes has been reported for both mitotic and post-mitotic cells (Lee et al. 1999, Ly et al. 2000). Decreased proteasome subunit expression has also been reported in fibroblasts from donors of different ages (Chondrogianni et al. 2000). In addition, a decrease in proteasomal activity can also result from oxidative damage to the proteasomal enzymes. Consistent with this, the proteolytic activities of the b-subunits of 20S proteasome are inhibited as a result of metal-dependent oxidation (Conconi et al. 1996) and the 26S proteasome is reported to be more sensitive to oxidation damage than the 20S proteasome (Reinheckel et al. 1998). Oxidative stress induced by ischemia reperfusion in brain is shown to cause impairment of proteasome function (Keller et al. 2000c). Biochemical characterization of purified 20S proteasome from a variety cells and tissues indicate that the age-related post-translational modification as well as altered subunit expression could be responsible for the decreased proteasomal activity (Anselmi et al. 1998, Bulteau et al. 2000, 2002).

Accumulation of Oxidatively Damaged Proteins

In addition to the age-related modification of proteasomal subunits themselves, the proteasomal activity is also inhibited by the generation of oxidatively damaged proteins which seem to 'clog-up' the system. Covalent modification of a reporter protein by HNE (4-hydroxy-2-nonenal) results in intramolecularly cross-linked protein product that has been shown to act as a non-competitive inhibitor of the 20S proteasome (Friguet et al. 1994, Friguet & Szweda 1997). Lipid peroxidation products, including HNE are formed during oxidative stress and have been shown to be potent inhibitors of proteasome activity (Friguet & Szweda 1997, Keller et al. 2000a,b, Shringarpure et al. 2000). Using GFP-chimeric proteins, a recent study elegantly showed that protein aggregation directly impaired the function of the UPS (Bence et al. 2001). It is believed that the oxidatively damaged proteins form cross-linked aggregates that block the entry of protein substrates into the catalytic chamber of the proteasome and thus inhibit its function (figure 3).

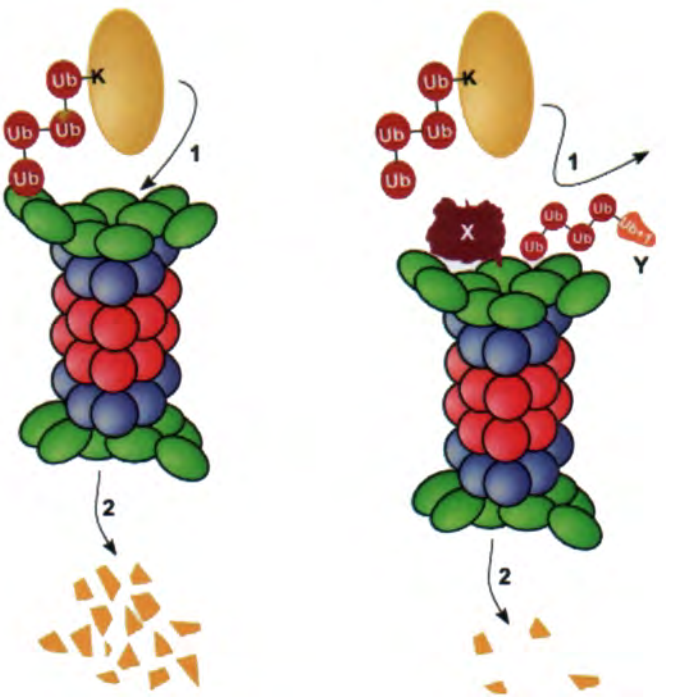

Figure 3 Age-related impairment of Proteasomal function. Left, in young organisms a polyubiquitinated target protein binds the 19S regulatory complex, the target protein unfolds and is inserted into the catalytic core (1) for degradation by the proteolytic activities associated with the b-subunits. The substrate is degraded to short peptides which are released into the cytoplasm (2) for further degradation by cytosolic peptidases. Right, In old organisms, cross-linked insoluble proteins (shown by 'X') are formed as a result of the damage by reactive oxygen species. In addition, the process of 'molecular misreading' may generate mutant Ub+1. These aberrant proteins block the proteasome and prevent the entry of target proteins in to the catalytic core (1). As a result the misfolded proteins are inefficiently processed (2) and accumulate in the cells further exacerbating the toxic effects of non-functional damaged proteins. In addition, proteasome subunits themselves can be altered by changes in expression or rendered non-functional by oxidative damage.

As discussed above, oxidative stress is likely to, be a crucial factor in proteasomal impairment. Interestingly, the proteasomal activity is shown to be induced as a response to mild forms of oxidative stress which helps prevent the accumulation of inhibitory oxidized proteins (Grune et al. 1997, Davies 2001). However, a condition of sustained oxidative stress produces crosslinked protein aggregates that inhibit the proteasomal activity. This in turn causes inefficient removal of the damaged proteins and results in accelerated accumulation of the protein aggregates. Also, in addition to the changes in the basal proteasomal function, ageing has been shown to impair the ability of the proteasome to respond to stress (Merker et al. 2000).

In summary, although the proteasome can undergo oxidative damage and still function normally under some conditions (Keller et al. 2000b), there is increasing evidence that oxidative stress is one of the leading causes of proteasomal impairment (Conconi et al. 1996, Keller et al. 2000a).

Ub+1 and Molecular Misreading

Recent studies have uncovered a new aberrant phenomenon termed as 'molecular misreading' which seems to arise due to errors in gene transcription (van Leeuwen et al. 2000). It has been reported that a dinucleotide deletion (mostly GA or GU) occurs in many genes that bear a GAGAGA rich region. Some of these aberrant transcript fail to be detected and are translated into protein. This results in translation in +1 frame from the point of deletion and therefore these proteins are called "+1proteins". Such proteins have the normal wild-type sequence up to the point of deletion but contain a different sequence downstream of the dinucleotide deletion. This phenomenon was initially reported in the vasopressin gene (Evans et al. 1994) in Brattleboro rat suffering from hypothalamic diabetes insipidus and the occurrence of dinucleotide deletion has been shown to increase with the age (van Leeuwen et al. 2002).

Recently, the +1 frame shift has been reported in the ubiquitin RNA in patients from Alzheimer's disease (van Leeuwen et al. 1998). A GAGAG motif is present in Ub gene at residue position 75 just before the critical Gly76 which conjugates to the target protein. A dinucleotide deletion in this region results in the mutation of the Gly76 residue and allows continued translation through the stop codon generating mutant Ub+1 that has an extension of 20 residues at the C-terminus. The frequency of molecular misreading is low (less than 5%) since the 'proof-reading' mechanisms remove the defective transcripts. Thus, a small fraction of mutant '+1proteins' would normally not be harmful to the cells. However, in case of Ub+1, this small fraction seems to act in a dominant negative manner. Since Ub+1 lacks a C-terminal Gly (Lam et al. 2000, van Leeuwen et al. 2000), it cannot be conjugated to substrate proteins. However, Ub+1 can be polyubiquitinated at Lys48 by wild-type Ub molecules that terminate in Gly76. It has been observed that a polyubiquitinated chain bearing Ub+1 is resistant to deubiquitination (Lam et al. 2000, Lindsten et al. 2002) and acts as a potent inhibitor of the proteasomal activity perhaps by blocking an entrance to the proteasome (figure 3). Ub+1 polyubiquitin is relatively stable and thus able to effectively prevent degradation of misfolded proteins by proteasome (van Leeuwen et al. 2000). Also, we have observed that coexpression of Ub+1 in HEK293 cells increases the steady state levels of several proteins that are usually rapidly turned over (Gao & Pimplikar, unpublished observations). Thus, accumulation of Ub+1 as a result of ageing can potently impair the proteasomal function which in turn seems to contribute to a variety of disorders (Sherman & Goldberg 2001, McPhaul et al. 2002, van Leeuwen et al. 2002).

Conclusions and Future Perspectives

It has become abundantly clear that the proteasome function is impaired as a result of ageing. The factors responsible for the impairment seem to be the i) age-related alterations in subunit epression, ii) oxidative damage to the proteasomes, iii) inhibition of proteasome activity by oxidatively damaged proteins, and iv) accumulation of Ub+1 as a result of the ageing. Ironically, proteasomal inhibition by cross-linked, misfolded proteins causes further accumulation of the damaged proteins. The results in an ever escalating cycle of increasing protein aggregation and decreasing proteasomal activity. This view is consistent with the general observations that symptoms of ageing or neurodegenerative disorders increase exponentially - not linearly - with time.

A major consequence of proteasomal dysfunction is the accumulation of toxic protein aggregates that disrupt normal cellular functions. A direct demonstration that such aggregates do exert toxic effects on cells in normal ageing is lacking. However, the neurotoxicity of protein aggregates in disorders such as Alzheimer's disease, Parkinson's disease and Huntington's disease has been well established (Sherman & Goldberg 2001). Recent animal studies suggest that immunological approaches to remove the Ab aggregates in animal models of Alzheimer's disease caused significant improvement in memory functions in transgenic

animals (DeMattos et al. 2001, Kotilinek et al. 2002, Morgan et al. 2000). These observations raise a possibility that pharmacological induction of proteasomal system could reduce the burden of protein aggregates and also improve cellular functioning. If so, this could be a potential therapeutic approach to reduce the intracellular aggregates that can not be accessed by antibody vaccines. The observations that the memory functions can be improved by reducing Ab load suggest that removal of protein aggregates by stimulating proteasomes could result in marked improvement in cell functions.

Of course, accumulation of protein aggregates is not the only outcome of a dysfunctional UPS. As discussed earlier, the UPS is involved in multiple cellular processes including cell growth and differentiation, response to stress and inflammation, DNA repair. A decline in the proteasomal efficiency is likely to adversely affect these cellular functions as well. Thus, restoring the proteasomal function by pharmacological intervention could also improve the cellular functioning independent of its action on protein aggregates. As we gain a better understanding of the molecular basis of proteasomal impairment in ageing and identify the molecular players that are involved in this process, it will become more likely that an effective therapeutic strategy based on the use of proteasomal stimulator can be developed. Such a strategy is likely to be helpful not only in various neurodegenerative disorders but also in normal ageing.

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