Chapter 1

UPS Activation in the Battle Against Aging and Aggregation-Related Diseases: An Extended Review

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Abstract

Aging is a biological process accompanied by gradual increase of damage in all cellular macromolecules, i.e., nucleic acids, lipids, and proteins. When the proteostasis network (chaperones and proteolytic systems) cannot reverse the damage load due to its excess as compared to cellular repair/regeneration capacity, failure of homeostasis is established. This failure is a major hallmark of aging and/or aggregation-related diseases. Dysfunction of the major cellular proteolytic machineries, namely the proteasome and the lysosome, has been reported during the progression of aging and aggregation-prone diseases. Therefore, activation of these pathways is considered as a possible preventive or therapeutic approach against the progression of these processes. This chapter focuses on UPS activation studies in cellular and organismal models and the effects of such activation on aging, longevity and disease prevention or reversal.

Key words Ubiquitin-proteasome system, Aging, Longevity, Aggregation-related diseases, Proteostasis, Proteasome activation

1 Aging and Aggregation-Related Diseases

1.1 Aging/Models of Aging

Aging is a multifactorial, natural process leading to gradual functional deterioration, continuing decline of self-defensive mechanisms, reduced homeostatic capacity of all tissues and an exponential accumulation of damage (in nucleic acids, proteins, and lipids) that leads to increased death incidence. The progression of aging is dynamically affected by both genetic and environmental factors. As long as equilibrium between cellular insults (mediated by stressors both from the micro- but also the macro-environment) and cellular repair/regeneration capacity is conserved, the cell/organism overcomes the damage that is produced without any fatal alterations in its phenotype and its physiology. However, once this balance is disturbed, the damaged molecules accumulate fast and multiple vicious circles of additional insults commence. As a result, an irreversible failure of homeostasis with compromised molecular pathways occurs. This failure eventually leads to aging and increased

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rates of morbidity and mortality [1, 2]. Given the effects of aging on a pleiad of key pathways, it is logical that it constitutes a major risk factor for several pathologies including aggregation-related disorders [3, 4].

The establishment of several short-lived model organisms, such as yeast, nematode worms, flies and rodents along with the use of primary mammalian cell cultures as well as the use of isolated tissues from donors of different ages are the main tools to investigate the aging process and to decipher its regulation. More specifically, the cellular and organismal models that are most commonly used in aging studies are:

The **replicative senescence model** is until now the most accepted cellular model to study human aging. The model is based on the notion that normal human fibroblasts may undergo a limited number of divisions in culture before they gradually reach a state of irreversible growth arrest. This process is termed as replicative senescence or Hayflick limit and it is believed to recapitulate most of the human aging features [5].

Saccharomyces cerevisiae (*S. cerevisiae*) is often used in the study of various molecular pathways that govern the aging progression. There are two types of life-span that can be dissected in this model, namely the replicative and the chronological. The replicative (mitotic) life-span is defined by the number of daughter cells that a single mother yeast cell produces, whereas chronological life-span or stationary phase (post-mitotic) is defined by the time period during which the nondividing yeast cells can remain viable. Given those two types of life-span, it is suggested that *S. cerevisiae* is an attractive model to study the life-span of various human cell types, and thus mitotically active types but also post-mitotic types [6].

The soil nematode ***Caenorhabditis elegans*** (*C. elegans*) is a post-mitotic multicellular eukaryotic model organism that due to its advantages is heavily used to study aging. *C. elegans* shares many fundamental cellular/molecular structures and biological properties with more advanced organisms (including humans with which *C. elegans* shares 40% homology), characteristics that nominate the nematode as an ideal model organism. Moreover, it is the first multicellular organism with known cell lineage and completely sequenced genome.

The fruit fly ***Drosophila melanogaster*** (*D. melanogaster*) has been used as a model organism for nearly a century. It is mostly composed of post-mitotic cells, it has a short life cycle/span and shows gradual aging. There is a 60% conservation of genes between flies and humans [7] while 77% of all known human disease genes have fly homologues [8]. Consequently, this insect is frequently used as a model organism in aging studies.

Rodents are frequently used in animal testing with mice and rats being the most used ones. The high degree of gene conservation between rodents and humans (i.e., humans share over 90%

homology with mice into corresponding regions of conserved synteny; [9]), the possibilities of genetic manipulation of their genomes but also their relative short life expectancy are few of the advantages in using those animals as models to study aging. On top of that, the so far obtained results from studies on caloric restriction (CR) and pharmacological anti-aging/prolongevity treatments that have revealed increased relevance to humans further advocate for the use of those animals in aging studies [10].

Using the abovementioned models, numerous genes, proteins, and functional networks have been identified so far, thus permitting to establish the current known hallmarks of aging [2].

1.2 Aggregation-Related Diseases

In general, most of the misfolded and/or aggregated proteins are subjected to degradation by the cellular proteolytic machineries. However, there are few proteins (native and mutant) that are resistant to the degradation systems due to their tendency to form β-sheet-enriched oligomers that are finally packed into inclusion bodies or extracellular plaques. This characteristic accumulation of protease-resistant aggregated proteins is a common feature in protein misfolding disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), and prion diseases (PrD).

1.2.1 Alzheimer's Disease

AD is the most known and common cause of dementia worldwide representing 65–75 % of all dementia cases [11]. It is a polygenic disorder that is characterized by loss of synaptic connections, extensive neurodegeneration and brain atrophy. AD patients can have an early onset mainly due to genetic mutations or a late onset, the latter being the most common case. The key hallmarks of AD are the deposition of intracellular, filamentous aggregates that consist of hyper-phosphorylated Tau protein (intracellular neurofibrillary tangles; NFTs) and amyloid-β (Aβ) extracellular plaques [12–14]. Aβ is produced through the presenilin-mediated cleavage of a transmembrane protein that normally regulates the synaptic function, namely Amyloid Precursor Protein (APP). Early onset of AD is characterized by the expression of both mutant APP (mAPP) and presenilin 1 and 2, which are required for the active function of γ-secretase to produce the Aβ peptide through APP breakdown [15–17]. Late onset of AD is induced by genetic and environmental factors with aging being one of the main risk factors. Mutation in the apolipoprotein E ε4 allele represents one pivotal genetic factor involved in this sporadic AD form [18]. Various other genes have been implicated to the sporadic late onset of AD as CLU, CR1, and PICALM [19]. The consecutive neurodegenerative alterations lead to a gradual decline in cognitive functions, especially in memory and visual-spatial orientation ending up to the individual's incapability to live functionally.

Most of the therapeutic approaches have focused so far on Aβ production, degradation, and prevention of its toxicity, on Tau formation and on general neuroprotection [20]. Various in vitro and in vivo models of the disease like neuroblastoma cell lines, mammals, *Aplysia*, zebra fish, fruit fly, and nematode mutant strains expressing the human Aβ peptide have been exploited [21, 22]. Here we summarize data regarding UPS activation as a promising therapeutic approach against AD.

1.2.2 Parkinson's Disease

PD is the second most common neurodegenerative disease characterized by muscular rigidity, bradykinesia, and uncontrollable tremor that worsen gradually in severity. The main pathologoanatomical feature of PD is the loss of a large portion of substantia nigra dopaminergic neurons [23, 24]. The gradual accumulation of inclusion bodies in the neuronal cytoplasm that consists of α-synuclein, parkin, UHC-L1, ubiquitin, and neurofilaments, namely Lewy bodies leads to irreversible neurodegeneration.

α-Synuclein is a 14 kDa protein that normally regulates vesicle trafficking during neurotransmission signaling through a chaperone-like activity [24]. Oligomeric and fibrillar conformations of α-synuclein (that polymerizes into fibrils in vitro) induce toxicity through (a) impairment of the function of several organelles, (b) alterations of the proper signal transmission through synapses, and (c) inhibition of the proteostasis mechanisms [24].

Parkin is the second important protein that exerts a distinct role on PD pathology while it is also responsible for autosomal recessive juvenile parkinsonism. It is a RING-domain E3 ligase that under normal conditions regulates the degradation of synaptic transmission-associated proteins and prevents the creation of aggregates while it is also essential for the regulation of mitophagy and mitochondrial equilibrium [25, 26]. Parkin mutations may lead to substrate recognition impairment and prevent the interaction with E2 enzymes. Lewy body inclusions in turn affect the normal function of Parkin by interfering to its normal ability to regulate degradation, thus leading to high toxicity [27].

Other molecules that have been identified to play a critical role in PD onset and progression are UCH-L1, PINK1, and DJ-1. UCH-L1 is a deubiquitinase, PINK1 is a serine/threonine kinase that acts protectively under conditions of proteasome inhibition, while DJ-1 has been shown to exert chaperone activity and protease activity both resulting in prevention of α-synuclein accumulation and aggregation [28]. It is obvious that the gene products targeted in familial PD are somehow associated to the UPS; either as UPS substrates (α-synuclein, parkin, synphilin-1, mutated DJ-1) or as components of the degradation pathway (parkin, ubiquitin, C-terminal hydrolase L1; [29]).

1.2.3 Huntington's Disease

HD is an autosomal dominant neurodegenerative disorder which is characterized by gradual degeneration of striatum neurons, affects muscle coordination, and causes mental decline and psychopathological problems [30]. Huntingtin (HTT) is the key protein involved in HD pathogenesis. More specifically, wild type (wt) huntingtin gene (htt) bears 6–35 CAG repeats in the N-terminus producing a polyglutamine (polyQ) tract. In contrast, in mutated htt gene the CAG triplet repeat stretch overpasses 36 repeats promoting a toxic gain of function, a feature that coincides with the onset of HD pathology [31]. The onset, progression, as well as severity of the disease are directly affected by the polyQ length. HD is a proteinopathy mainly characterized by intracellular inclusions bodies (IBs) formed by mutant HTT (mHTT) aggregates [32]. These IBs are gradually increasing in number and size thus impeding the normal function of neurons. Several studies have suggested that mHTT is cleaved to produce a shorter N-terminal fragment containing the polyQ expansion that eventually induces the protein fragment to misfold and form aggregates. Neurotoxicity has been linked to either the soluble and/or the aggregated form of the misfolded protein as well as to the aggregation process itself. The various forms of mHTT protein have been suggested to affect transcriptional regulation through the interaction with various transcription cofactors (activators or repressors), to promote apoptosis, to enhance the intracellular production of reactive oxygen species, to affect caspase activation, and to inhibit proteasome function.

1.2.4 Amyotrophic Lateral Sclerosis

ALS is a motor neuron degenerative disorder with severe symptoms and an expeditious progress from symptoms onset, ending to muscular atrophy, weakness, and eventually death due to degeneration of the respiratory muscles. The main cells that are affected are the pyramidal Betz cells in the motor cortex, the large anterior horn cells of the spinal cord, and the lower cranial motor nuclei of the brainstem [33]. ALS is mainly a sporadic disease but 10% of ALS cases are familial [34]. The pathologoanatomical signature of the disease is the accumulation of insoluble proteins that form intracellular aggregates (Skein-like inclusions, SLIs) as found in samples from human patients and animal models of ALS [35, 36].

Superoxide dismutase 1 (SOD1) missense mutations play a distinct role to most cases of the familial onset of the disease [37]. Toxic gain of function is believed to occur while increased levels of intracellular protein aggregates of mutant SOD1 (mSOD1) that disturb the unfolded protein response (UPR) and mitochondrial functionality are also revealed [38]. Several other proteins have been also implicated to ALS, including ALSIN, TDP-43, nuclear protein FUS, ubiquilin 2, p62, optineurin, and valosin-containing protein [39]. The causes are basically unknown in the absence of family history (sporadic ALS). C9ORF72 is one of the locuses on chromosome 9p identified to be involved in the sporadic ALS onset

together with UNC13A, a presynaptic protein that normally acts in the neurotransmission signaling procedure [34]. It was recently pointed that most of the involved proteins in both sporadic and familial ALS share aggregation-prone properties that may ultimately act toxically and inhibitory to the proteostasis network.

1.2.5 Prion Diseases

PrDs, also known as transmissible spongiform encephalopathies, are infectious neurodegenerative disorders with acute and severe symptoms including memory and movement control problems, visual dysfunction and cognitive inability [40, 41]. Severe neuronal loss in prion-affected sections leads to the development of a "spongy" architecture which is the main anatomical characteristic of the disease. The most known PrDs are divided into three groups: the sporadic group including Jakob-Creutzfeldt disease (JCD); the genetic group including genetic JCD, Gerstmann-Sträussler-Sneaker disease, and fatal familial insomnia; and the infectious group including Kuru, variant JCD, and iatrogenic JCD.

All known mammalian PrDs are caused by the scrapie prion protein (PrP^{Sc}) an abnormal form of the naturally occurring protein PrP^{C}, a cell surface membrane [42]. The role of PrP^{C} is not yet fully elucidated. PrP knockout mice exhibit only minor abnormalities but more recently, it was shown that that neuronal expression and regulated proteolysis of PrP^{C} are essential for myelin maintenance [43]. Moreover, mice devoid of PrP^{C} exhibit an altered hippocampal long-term potentiation [44] while it was also suggested that PrP^{c} is necessary for the self-renewal of long-term hematopoietic stem cells [45].

PrP^{Sc} is a β-sheet-enriched isoform [46] able to self-propagate and fold in a variety of distinct ways [47]. This self-replication mechanism leads to the formation of spontaneous extracellular aggregates (prion deposits; [48]). Prions are at least partially protease-resistant proteins and therefore they tend to constantly accumulate. Moreover, PrP^{Sc} has the ability to interact with PrP^{C} and change its conformation into the infectious isoform, thus initiating a vicious cycle that potentiates the disease progression. Even a small quantity of PrP^{Sc} is enough to trigger the conversion of PrP^{C} to PrP^{Sc} as shown in vitro [49] but also in vivo [50].

Apart from the PrP, additional proteins have been shown to share prion-like domains. These domains endow the proteins with the self-replicating ability that is necessary for the formation of amyloid-like deposits. For example, it has been shown that TDP-43 mutations facilitate the conversion of misfolded proteins to aggregation-prone prion-like conformation, resulting in the ALS-related aggregates found in many familial ALS cases [51]. The latter case is the so-called prion paradigm, where otherwise harmless proteins can be converted to a pathogenic form by a small number of misfolded, nucleating proteins [52]. Nevertheless, cautiousness should be attributed since with the exception of PrP, the rest of the aggregation-prone proteins are not infectious agents.

1.3 Proteostasis in Normal Aging and Aggregation-Related Diseases

The proteome is challenged constantly and proteome integrity (proteostasis) is one of the nodal points that needs to be preserved in order to maintain organismal homeostasis. Therefore, it is not surprising that a group of specific molecules is dedicated to preserve the cellular protein load and therefore the cellular proteostasis. A complicated surveillance network of cellular mechanisms that inspect every aspect of protein biology from synthesis and folding to trafficking and clearance is set as responsible for proteostasis [53]. One primary arsenal of this network is constituted by chaperones that assure the correct folding/function of proteins and their maintenance in a correctly folded/functional mode. If however this arm of the proteostasis network fails, the secondary arsenal takes over to degrade the damaged, unfolded, aggregated and in general unwanted proteins. This arm includes the ubiquitin-proteasome system (UPS; which is the theme of this chapter) and the autophagy-lysosome system (for a recent review refer to [54, 55]). Upon failure of all surveillance systems, failure of proteostasis occurs with detrimental effects on the cellular physiology and life. It is not thus astonishing that the loss of proteostasis is considered as one of the hallmarks of aging [2] and that this loss is strongly related to the onset and progression of aging and aggregation-related diseases.

2 Introduction: The Ubiquitin System

Ubiquitin is a highly conserved protein that covalently modifies proteins through the ubiquitination process. There are three main steps that are gradually followed in order for an ubiquitin moiety to be added on a protein. These three steps are characterized by the action of three different types of ligases, namely E1 (ubiquitin-activating enzymes), E2 (ubiquitin-conjugating enzymes), and E3 (ubiquitin-ligase enzymes). The cycles of ubiquitination for a given protein can occur once thus leading to mono-ubiquitination or can be repeated several times on the same lysine thus leading to polyubiquitination. Depending on the moieties of ubiquitin added on a protein along with the lysine residues used for this binding, the localization/intracellular trafficking, activity, protein-protein interactions, participation in different signaling pathways, and degradation either by the 26S proteasome or by autophagy-lysosome system can be signaled [56, 57]. Polyubiquitin chains with at least four moieties constitute the signal for the 26S proteasome-mediated recognition and degradation of the protein substrate with the most frequent signal being the K48-linked ubiquitin chain [58]. To prevent energy loss, once the tagged substrate is recognized by the proteasome for degradation, specific deubiquitinases (DUBs) remove the polyubiquitin chains; those ubiquitin molecules can be reused [59]. The abovementioned proteins constitute the UPS (Fig. 1).

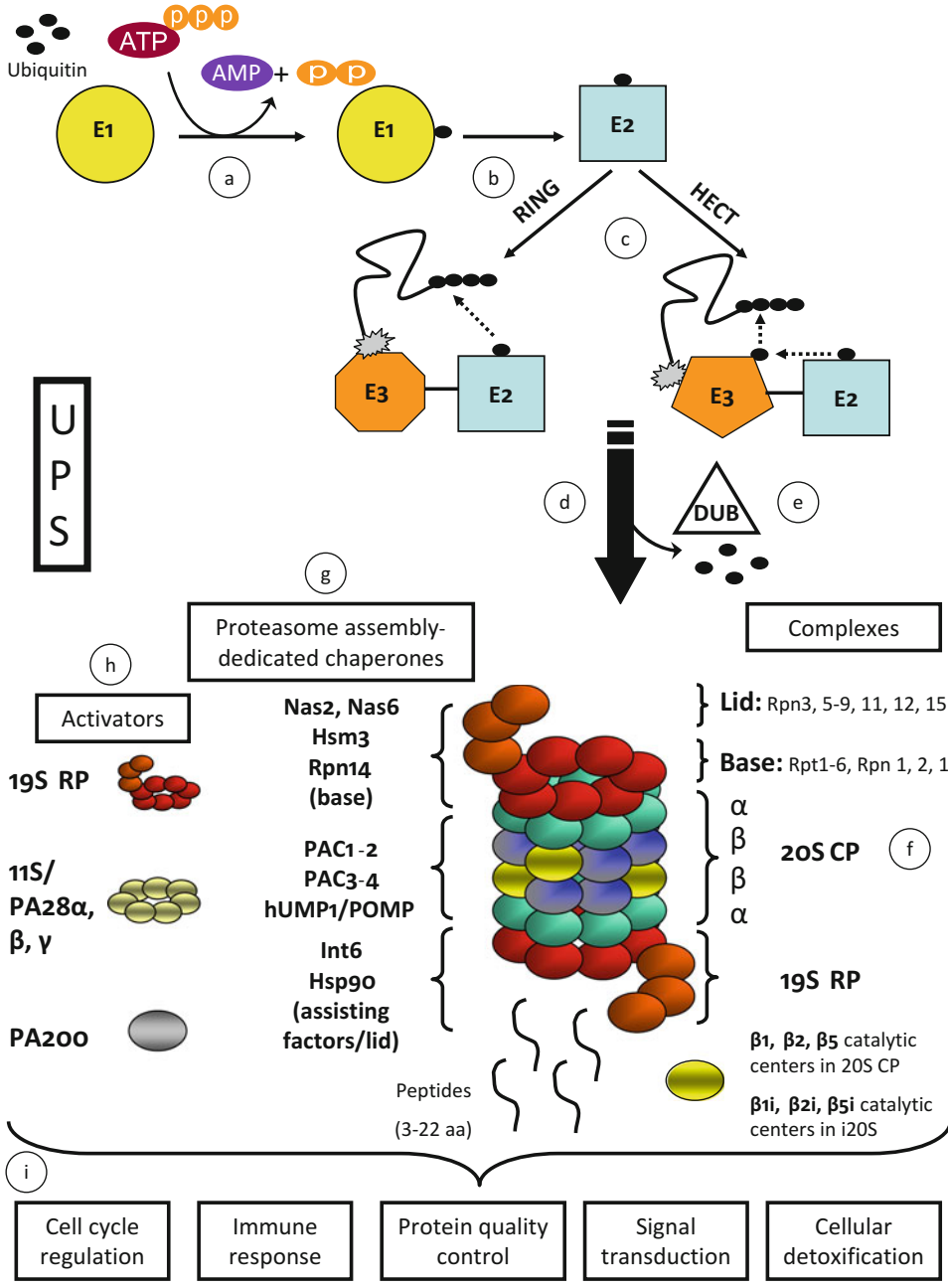

Fig. 1 The ubiquitin-proteasome system (UPS). **(a)** Ubiquitin activation through ubiquitin-activating enzyme (E1). **(b)** Activated ubiquitin is transferred to ubiquitin-conjugating enzyme (E2). **(c)** RING domain ligase: the ubiquitin-charged E2 binds to the E3 ligase that carries the substrate for degradation and ubiquitin is directly transferred to the substrate. HECT domain ligase: ubiquitin is firstly transferred from E2 to the E3 ligase that carries the substrate for degradation and then to the substrate. All three steps are repeated to result in substrate polyubiquitination. **(d)** Ubiquitinated protein is recognized by the proteasome, captured and processed for degradation. Short peptides (3–22 aa) are released at the end of the process. **(e)** Following substrate recognition, polyubiquitin chain is cleaved off through deubiquitinases (DUB) and free ubiquitin is released in order to be reused. **(f)** 26S proteasome structure; the constituent subunits appear for each subcomplex. In the case of 20S proteasome, β1, β2, and β5 subunits are the catalytic centers of the complex, whereas in the case of i20S these subunits are *de novo* substituted by β1i, β2i, and β5i subunits. **(g)** Proteasome assembly-dedicated chaperones (or assisting factors in the case of lid assembly). **(h)** Major proteasome activators that can be located on the top of 20S complex. **(i)** The various proteasome complexes are involved in multiple cellular pathways/processes

2.1 E1, E2, E3 Enzymes

The first two-step reaction in the ubiquitination process is catalyzed by the ubiquitin-activating enzymes (E1), in an ATP-dependent process that results in an activated ubiquitin molecule. More specifically, the E1 enzyme binds ATP and ubiquitin and catalyzes ubiquitin C-terminal acyl-adenylation. Ubiquitin is then transferred to the catalytic cysteine of the E1 enzyme producing a high-energy thioester bond and forming ubiquitin–E1 complex [60]. There are two human genes that have been so far identified to produce E1s, namely Uba1 and Uba6 [61, 62]. As expected, E1s can collaborate with multiple E2s.

The ubiquitin-conjugating enzymes (E2) catalyze the transfer of the activated ubiquitin from E1 to its own catalytic cysteine residue where a thioester bond is formed. So far, 35 E2 enzymes have been identified in humans while in other eukaryotes the number ranges between 16 and 35 [63]. Each E2 can activate a palette of E3 ligases in an hierarchical manner thus producing multiple different but specific E2–E3 combinations.

The final step of ubiquitination is catalyzed by E3 ligases forming an isopeptide bond between the C-terminal glycine of ubiquitin and a lysine of the target protein. The two main classes of E3 ligases (classified according to the domain that they possess) are the homologous to the E6-AP carboxyl terminus (HECT) domain proteins and the really interesting new gene (RING) domain proteins where one can find monomeric and multisubunit RING finger ligases [64]. The RING group of E3s along with the RING-related E3s, such as members of the U-box family, the plant homeodomain (PHD), and leukemia-associated protein (LAP) finger proteins, is the largest group of E3 ligases [65]. HECT-domain E3s firstly accept through a thioester linkage the ubiquitin moiety and then they transfer it to the protein substrate, whereas RING-domains E3s bind the cooperating E2 and they mediate the direct transfer of ubiquitin from E2 to the target protein [64] (Fig. 1). More than 600 E3 ligases have been annotated in humans [66] from which ~30 are HECT-domain E3 ligases. Most of the multisubunit RING E3s belong to the cullin RING ligase (CRL) superfamily [67] with SCF complex (consisting of S-phase phase kinase-associated protein 1/Skp1, cullin, and F-box protein) and anaphase-promoting complex (APC/C) being the most known complexes. Both complexes assure the correct cell cycle progression [68].

2.2 Deubiquitinases

Ubiquitination can be reversed through the act of specific proteases, namely deubiquitinating enzymes (DUBs; also known as deubiquitinases, deubiquitinating peptidases, ubiquitin isopeptidases, deubiquitinating isopeptidases, ubiquitin proteases, and ubiquitin hydrolases; [69]). DUBs cleave ubiquitin from protein substrates and other molecules and thus they act antagonistically to the ubiquitination process. Apart from their role in protein degradation they have been also implicated in several other pathways including

cell growth and differentiation, membrane protein trafficking, development, neuronal diseases, and transcriptional regulation while they are also responsible for ubiquitin activation and recycling [70, 71]. Approximately 100 DUBs have been annotated in humans, grouped into two classes: cysteine proteases and zinc-dependent metalloproteases. Cysteine proteases include ubiquitin-specific proteases (USPs), ovarian-tumor (OTU) domain proteases, ubiquitin C-terminal hydrolases (UCHs), and Machado-Josephin domain proteases (MJDs) while metalloproteases contain a Jab1/MPN metalloenzyme (JAMM) domain [69, 70].

3 Introduction: The Proteasome System

The proteasome is a large multisubunit enzyme complex hosting multiple catalytic centers and is responsible for the clearance of short-lived normal, regulatory proteins but also for the elimination of unwanted (misfolded, damaged, or in any way abnormal) proteins [72, 73]. The 20S core proteasome (CP) is the main complex that hosts the catalytic activities of the multienzyme while various regulators can be attached in either one or both ends of the 20S, giving rise to supra-proteasome complexes with 19S regulatory particle (RP) being the most common. The various proteasome complexes are thus engaged in the regulation of numerous biological processes including signal transduction, cell cycle control, cell differentiation, stress response, quality control, antigen presentation, and cellular detoxification [74].

3.1 20S Core Proteasome: Structure, Assembly, and Localization

3.1.1 Structure

The 20S CP is a barrel-like structure composed of 28 subunits (14 α-type and 14 β-type) arranged in four seven-membered rings with a molecular weight of 700 kDa and a diameter of 120–160 Å (Fig. 1). The α-type subunits form the two external rings and create an aperture of 10–15 Å through which the protein substrate enters to reach the three catalytic centers of the CP that are located in the inner β-rings. More specifically, β1, β2, and β5 subunits possess the caspase-like (C-L or PGPH), the trypsin-like (T-L), and chymotrypsin-like (CT-L) activities, respectively. The α-subunits also offer the matrix for the binding of the various regulators that modify the specific activity of the CP [74].

3.1.2 Assembly

The assembly of the eukaryotic 20S CP is highly orchestrated, assisted by several proteasome-dedicated chaperones. This assembly initiates with the α-ring formation that it then serves as a template for the incorporation of the β-subunits. Up to now, four different proteasome assembling chaperons (PACs), namely PAC1-PAC4 (Pba1-4 in yeast; [75]) and the proteasome maturation factor POMP (Ump1 in yeast; [76–78]) have been isolated. PAC1-PAC2 heterodimer is responsible for the α-ring formation

as well as for the prevention of incorrect dimerization. PAC3–PAC4 heterodimer assists the incorporation of pro-β2 subunit that is followed by the incorporation of β3, β4, pro-β5, pro-β6, pro-β1, and pro-β7 subunits. PAC3-PAC4 gets displaced once β4 and hUMP1/POMP join the complex. hUMP1/POMP then assists the serial incorporation of the rest β subunits [79]. The two half-CP are dimerized with the help of Hsc73 which is then released, the β-propeptides are self-cleaved, and UMP1/POMP is the first substrate of the newly assembled CP [80]. CP maturation induces an affinity switch mechanism that reduces its affinity for PAC1-PAC2 and thus enables the RP to dislocate the dimer and to get attached on the CP [81].

3.1.3 Localization

Intracellular proteasomes localize in the cytoplasm, the nucleus and the ER and can constitute approximately up to 5% of the total cellular protein content depending on the cell type [82]. However, the 20S core proteasome has been identified to get attached to the plasma membrane thus suggesting its potential release in the extracellular space, e.g., in the alveolar lining fluid, epididymal fluid and possibly during the acrosome reaction. Moreover, active (reported as circulating) proteasomes have been detected in normal human plasma but also in plasma from patients suffering from various forms of malignancies, autoimmune diseases, sepsis, and trauma [83]. It was lately shown that activated immune cells can export assembled proteasomes (fully functional) as microparticles, thus possibly revealing the mode of extracellular proteasomes generation. Moreover, 19S particles as well as the PA28 activator were also detected in these microparticles [84].

3.2 26S Proteasome: Structure and Assembly

3.2.1 Structure

One or two RP may bind in the CP ends; the RP-CP configuration is termed as 26S complex whereas the RP-CP-RP configuration is termed as 30S complex. The RP is responsible for the substrate recognition, unfolding, deubiquitination, and translocation. It is subdivided into two smaller complexes, namely the base and the lid [85, 86]. The base is composed of six AAA-ATPases (Rpt1-6) along with three non-ATPases namely Rpn1, Rpn2, and Rpn13. The ATPases are responsible for the unfolding of the protein substrate, the opening of the α-gated channel on the CP, and the translocation of the unfolded protein towards the inner proteolytic cavity of the proteasome. Both Rpn1-Rpn2 and the ATPases are necessary for substrate translocation and gating of the proteolytic channel [87], while Rpn13 together with Rpn10 act as integral ubiquitin receptors thus recognizing the tagged substrates [88, 89]. Moreover, Rpn10 acts as a "bridge" subunit that connects the base and the lid. The lid is composed by 9 Rpn subunits namely Rpn3, 5–9, 11, 12, and 15. Rpn11 serves as a deubiquitinating enzyme [90] while it stabilizes the otherwise weak interaction between the CP and the RP [91].

3.2.2 Assembly

The incorporation of the base subunits is the first step in the RP assembly. Rpn14, Nas6, Nas2, and Hsm3 (PAAF1, gankyrin/p28, p27, and S5b in human, respectively) are the yeast 19S-specific assembly factors assisting the RP assembly and not found on the mature 26S proteasome [92, 93]. These four factors can be also found named as RAC (RP assembling chaperones) 1, 2, 3, and 4, respectively [94]. Three intermediates are produced, namely RPN1-RPT2-RPT1-Hsm3, Nas6–RPT3–RPT6–RPN14, and Nas2–RPT5–RPT4. These intermediates form the base complex and Rpn2 and Rpn13 are finally added to give rise to the final base complex that will be bound to the lid through Rpn10. Following Rpn10 binding, the chaperones are detached from the base.

The lid assembly is not fully elucidated. Recent studies suggest that Rpn5, 6, 8, and 11 form an initial stable module where Rpn3, 7, and 15 then bind and the full lid is formed through the addition of Rpn12 [95]. Hsp90 [96] and Yin6 (ortholog of the mammalian Int6) [97] are two assisting factors identified in the lid formation in yeast.

3.3 Various Proteasome Forms

3.3.1 Immuno-proteasome

Upon interferon γ (IFNγ) stimulation, the constitutively expressed catalytic subunits are de novo replaced by their cytokine inducible counterparts, namely β1i (LPM2 or PSMB9), β2i (MECL-1 or PSMB10), and β5i (LPM7 or PSMB8), thus giving rise to the immunoproteasome or i20S [98]. Immunoproteasomes exhibit increased CT-L activity and decreased C-L activity, thus facilitating antigen presentation due to the generation of antigenic peptides with increased affinity for MHC class I clefts. Mice lacking immunoproteasomes display major alterations in antigen presentation [99]. Despite this particular role, increasing number of studies implicate immunoproteasomes in processes irrelevant to antigen presentation like the adaptive response of the cells to oxidative stressors in order to preserve homeostasis [100], aging [101, 102], and longevity [103].

The activities of the immunoproteasome can be altered through the binding of various activators like the RP but also the 11S complex (also known as PA28/REG/PA26), a heptameric IFNγ-inducible protein that induces the degradation of short peptides in an ATP-independent manner [104]. There are three 11S isoforms in higher eukaryotes, namely PA28α, β, and γ (or REGα, β, and γ; [105]).

3.3.2 Hybrid Proteasomes

Upon binding of an RP in one end of the CP and an 11S in the other end, hybrid proteasomes are produced [106]. It is believed that the RP serves at the substrate recognition while the 11S complex alters the proteolytic potential of the CP.

3.3.3 Thymo-proteasomes

A specific catalytic β5 subunit has been isolated in mouse cortical thymic epithelial cells, namely β5t [107]. A similar subunit with thymus-specific expression was then revealed in humans as well

[108]. More specifically, β5i subunit is substituted by the proteolytic active subunit β5t in the relative tissue, thus giving rise to the thymoproteasomes. Thymoproteasomes contain β1i and β2i along with β5t, but notably not the constitutive β subunits [108]. In contrast to β5i incorporation, β5t insertion leads to markedly decreased CT-L activity, a feature that was shown to be necessary for the positive selection of developing thymocytes [107, 109].

3.3.4 Other Forms of Proteasomes

An additional tissue-specific subunit has been identified in *D. melanogaster* where Prosalpha6 subunit is replaced by the testis-specific subunit Prosalpha6T. It is suggested that this substitution is necessary for spermatogenesis [110, 111].

Finally, PA200/Blm10 (human/*S. cerevisiae*) is another activator that similarly to the 11S induces peptides degradation by the CP in an ATP-independent manner [112]. This activator has been implicated so far in various processes ranging from proteasome assembly [112] and inhibition [113], to DNA repair [114] and mitochondrial checkpoint regulation [115].

3.4 Regulation of the Proteasome Expression and Function

3.4.1 Transcriptional Regulation

Although the proteasome structure and function is extensively studied, the transcriptional regulation of the proteasome genes is still not fully elucidated. Rpn4 is a yeast transcription factor controlling the expression levels of the proteasomal genes bearing the proteasome-associated control element (PACE) in their promoters [116]. Rpn4 controls proteasome expression under both normal and stress conditions including proteasome inhibition and DNA damage [117]. Recently, a minimal hexamer "PACE-core" sequence that is responsive to Rpn4 was identified. These PACE-cores are present in many genes related to proteasome function (including the proteasome assembly chaperones), although they cannot substitute for the known PACE of the subunits [118]. Nevertheless, no human homologue of RPN4 has been identified thus far.

Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a transcription factor that has been implicated in the regulation of proteasome genes in mammals. Nrf2 is the main responsible for the expression of various antioxidant enzymes [119], including several components of the proteostasis network namely, chaperons and proteasome subunits under specific conditions [120]. Nrf2 belongs to the family of Cap'n'collar (Cnc) transcription factors. It is responsible for the cellular transcriptional response to oxidative stressors and electrophilic xenobiotics thus being nominated as the central mediator of a prominent antioxidant response system. Kelch-like ECH-associated protein 1 (Keap1) is the main regulator that keeps Nrf2 in the cytoplasm and mediates its proteasomal degradation [121]. Upon a stimulus, Nrf2 may become phosphorylated and/or Keap1 may be modified, resulting in the disruption of the Keap1-Nrf2 complex and the nuclear translocation of Nrf2 [122]. In the nucleus, Nrf2 heterodimerizes with small musculo-aponeurotic fibrosarcoma (Maf)

proteins and recognizes a cis-acting DNA element namely antioxidant response element (ARE) or electrophile responsive element (EpRE; 5′-TGA[C/T]NNNGC-3′) on its target genes, thereby conducting their transcription [123–125]. Several studies have reported the Nrf2-mediated proteasome induction as it will be discussed in various sections below. The nematode ortholog, SKN-1 has been also implicated in the regulation of proteasome genes. More specifically, depending on the redox conditions, proteasomal genes have been shown to be regulated by SKN-1 [126]. SKN-1 has been shown to exert pivotal role in longevity [126, 127] and resistance to oxidative stress. Moreover, it has been shown that proteasome deregulation/inhibition imposes SKN-1 translocation to the nucleus and promotes proteasome subunits upregulation [128–131]. We have also found that proteasome activation through the overexpression of *pbs-5* proteasome subunit and the consequent life-span extension is at least partially SKN-1-dependent [132].

Nuclear factor erythroid-derived 2-related factor 1 (Nrf1, also known as NFE2L1/LCRF1/TCF11) is also a member of the CNC family [133]. *NFE2L1* gene encodes two main isoforms [134]: Nrf1 (a short isoform) and TCF11 (a long isoform). TCF11 was shown to regulate the induction of proteasome genes, rather than Nrf2, after proteasome inhibition via an ERAD-dependent feedback loop [135, 136]. It was further elucidated that in normal conditions, proteasomes are active and they degrade Nrf1. In contrast, when there is a partial proteasome inhibition, proteasomes proceed to limited proteolysis thus releasing the processed Nrf1 (lacking its N-terminal region) from the ER which is also the active Nrf1 form that promotes gene expression [137]. Interestingly, if Nrf1 expression is lost in the brain, various proteasome subunits get downregulated and it was suggested that Nrf1 perturbations may be at least partially responsible for neurodegenerative diseases progression [59]. Given the interplay between Nrf1 and the proteasome, such possibility could also implicate the proteasome in this Nrf1-dependent process.

Finally, it was recently shown that the expression of β catalytic subunits and especially β5 subunit in mammals is regulated by constitutively activated signal transducer and activator of transcription 3 (STAT3; [138]). There is more available data for the transcription factors of the immunosubunits. More specifically, interferon regulatory factor-1 (IRF-1) has been suggested to be the master regulator for the concerted expression of immunoproteasome subunits [139, 140]. More recently, the transcription factor PU.1 was shown to bind and transactivate PSMB8, PSMB9 and PSMB10 (immunosubunits) promoters. Furthermore, PU.1-dependent transactivation and PU.1 expression were shown to be repressed by PML/RARα [141].

3.4.2 Posttranslational Modifications

The various proteasome regulators (RP/19S, PA28/11S and PA200/Blm10) that have been described above alter drastically the proteasome activities. Apart from this kind of proteasome activity

regulation, several posttranslational modifications (PTMs) such as oxidation, phosphorylation, ubiquitination, O-linked addition of N-acetylglucosamine, glycosylation, N-acetylation, and lipid peroxidation may also have an impact on proteasome function.

Rpt3 and Rpt5 are two subunits that have been shown to be carbonylated (oxidized) in human end-stage heart failure and experimental myocardial ischemia [142, 143]. In both cases, this oxidation leads to proteasome activities compromise.

Phosphorylation is one of the most frequent PTMs that have been detected in several CP and RP subunits. Two CP subunits namely, α7 and α3 subunits were initially identified to be phosphorylated and proteasomes with α7 phosphorylated subunit have elevated activity levels [144]. It was additionally found that α7 phosphorylation stabilizes 26S proteasomes and upon IFNγ treatment, 26S proteasomes are destabilized due to α7 dephosphorylation [145]. Caseine kinase II was identified to be the kinase responsible for this phosphorylation [146]. Calcium/calmodulin-dependent protein kinase II (CaMKII) and polo-like kinase (Plk) were also identified as proteasome-phosphorylating kinases. More specifically, CaMKII phosphorylates Rpt6 both in vitro and in vivo and consequently stimulates proteasome activity and plays a regulatory role in remodeling of synaptic connections [147]. Plk was found to interact with all α subunits but α2 and β1, β2, β3, β5, and β7 subunits, to phosphorylate α3 and α7 subunits in vivo and to enhance proteasome activities [148]. Using MS/MS, Kikuchi et al. [149] identified 33 Ser/Thr phosphorylation sites in 15 subunits of the yeast proteasome and showed that dephosphorylation of the 19S RP results in a 30% decrease in ATPase activity. Other groups have found additional subunits subjected to phosphorylation (α1, α2, α7, and β6 in mammalian proteasomes [150, 151]. In contrast to the abovementioned activating properties of the phosphorylation of proteasome subunits, diminished 26S activity in failing human hearts is suggested to be related to the impaired docking of the RP to the CP as a result of decreased Rpt subunit ATPase activity and α7 phosphorylation [152]. DNA damage induces phosphorylation of several α-subunits (α5, α6, α7), thus probably affecting protein-protein interactions and gate opening due to the increased net negative charge given by the phosphate groups [153].

Ubiquitinated forms of α5, α6, α7, and β5 have been identified following doxorubicin treatment. Ubiquitination of proteasome subunits inhibits CT-L and C-L activities in vitro while in vivo doxorubicin treatment enhances proteasome activities in parallel to the decreased levels of ubiquitination thus suggesting that the proteasome activities upon DNA damage are regulated by ubiquitination [153].

O-Linked addition of the monosaccharide N-acetylglucosamine (O-GlcNAc) has been shown to inhibit the 26S proteolytic activities but not the 20S activities. It was further shown that the ATPase activity is inhibited and Rpt2 is identified as a substrate for this

kind of PMT [154]. It was also suggested that the O-GlcNAc system may participate in neurodegeneration and this is at least partially linked with the inhibition of the proteasome [155]. In addition, O-GlcNAc-sites have been identified in CP subunits, namely α1 (Ser5), α4 (Ser130), α5 (Ser198), and α6 (Ser110) and the β subunit β6 (Ser57 and Ser208; [156]).

Subunits α1, α2, α3, β4, β5, and β6 of the murine cardiac 20S proteasome were identified to be glycosylated without however revealing whether this has a positive or a negative effect on proteasome activities [150].

N-Acetylation was also shown to affect proteasome subunits. More specifically, all α-type subunits and β3 and β4 subunits were found acetylated in yeast and CT-L activity was shown to be elevated in a mutant that cannot perform N-acetylation [157]. Rpt4, Rpt5, Rpt6, Rpn2, Rpn3, Rpn5, Rpn6, Rpn8, Rpt3, and Rpn11 were also found acetylated in yeast but nevertheless, the activities were not altered [158] whereas Rpt3 and 6 and Rpn1, 5 and 6 were found acetylated in murine proteasomes [150].

Proteasome subunits can also be subjected to modification by the lipid peroxidation product 4-hydroxy-2-nonenal (HNE). HNE modification of α1, α2, and α4 subunits during cardiac ischemia/reperfusion results in reduced peptidase activities [159, 160]. A similar decrease was also found in epidermis samples from old donors and HNE-modification of certain α-subunits was involved in the age-related decline of the proteasome function [161]. Accordingly, HNE modification promoted proteasome activity decline in neural PC6 cells [162].

Finally, other types of PMTs like N-myristoylation [158], S-glutathionylation [163], and nitrosylation [150] have also been identified. N-myristoylation of Rpt2 does not alter proteasome activities but it controls proteasome localization [164]. S-glutathionylation of α5 subunit promotes gate opening and therefore stimulation of 20S activity while the effects of nitrosylation are not yet elucidated. Despite the abovementioned changes of proteasome activity, several PTMs have been shown to indirectly alter the function of the proteasome through alterations in the ability of various RP subunits to directly interact with protein substrates (e.g., autoubiquitination of Rpn13; [165], monoubiquitination of Rpn10; [166], in situ ubiquitination of Rpn10 (S5a), Rpt5, and Uch37DUB; [167]).

3.5 Elimination of Proteasomes

Proteasomes are degraded through the lysosomal machinery. They have been found in autophagic vacuoles, thus suggesting that they follow the pathway of nonselective autophagy. Nevertheless, under starvation conditions, they follow the heat-shock cognate protein of 73 kDa (hsc73)-mediated transport [168].

4 Proteasome Status During Aging/Senescence in Cellular and Organismal Models

4.1 Cellular Senescence

Proteasome activities diminish upon progression of senescence of human fibroblasts [169]. Moreover, partial inhibition of the proteasome by 50% in young cells (in levels analogous to the levels normally found in senescent cells) elicits a premature senescence phenotype [170] in a p53-dependent process [171]. Elaborate analysis of the expression of the various proteasome subunits during the senescence progression has revealed the critical role of the β-catalytic subunits that have been suggested to act as the rate-limiting factors in the proteasome assembly pathway [169]. Additionally, senescent cells exhibit a reduced response to IFNγ, thus resulting in lower expression levels of immunosubunits [172]. Apart from expression and assembly alterations during senescence, the proteasomal function is also affected by the accumulation of damaged, aggregated, and cross-linked proteins as shown by the negative effect of lipofuscin on proteasome activities [173].

In contrast to senescent fibroblasts, fibroblasts derived from healthy centenarians exhibit proteasome activities similar to the ones exhibited by cells derived from younger donors. Both of these cultures differ significantly in terms of proteasome potential with the cultures derived from older donors that are not centenarians [174]. These results further advocate for the pivotal role of proteasome in cellular senescence and aging.

4.2 Model Organisms

4.2.1 Saccharomyces cerevisiae

Proteasomal function has been reported to deteriorate during stationary phase conditions [175] and the decreased proteolysis has been correlated with increased rates of 26S proteasomes disassembly [176]. Recently, the important role of Cdc48-Vms1 complex in the preservation of the 26S proteasome assembly was revealed [177]. Upon starvation, a relocalization of the proteasome subunits from the nucleus into cytoplasmic structures termed as proteasome storage granules (PSGs) occurs [178]. The nuclear-to-cytosolic proteasome relocalization upon starvation is affected by chronological aging since young cells efficiently relocalize the proteasomes and form PSGs in contrast to the old cells. This process is dependent on two of the three N-acetylation complexes [179]. PSG formation requires fully assembled 26S proteasomes and Rpn11 proteasome subunit is crucial for both PSGs formation and cell survival during stationary phase [180]. Finally, 20S core sequestration into PSGs is mediated by Blm10 whereas upon resumption of cell growth Blm10 facilitates nuclear import of the 20S particles [181].

4.2.2 Caenorhabditis elegans

Cell-specific photoconvertible reporters assaying proteasome activity in the nematodes have revealed an impaired UPS function in the dorsorectal neurons of 7-day-old worms as compared to the

one found in young adults. In contrast, no alterations are scored in body-wall muscle cells thus suggesting a cell type-specific decline of the proteasome in nematodes [182]. The pivotal role of the proteasome in the aging procedure of the animal is exhibited by the fact that deletion/knockdown of various 19S and 20S proteasome subunits elicited premature aging and shortened life-span [132, 183, 184]. Finally, increased ROS levels (that are strongly related to chronological age) are linked with impaired UPS activity and this in turn may potentiate disease progression [185].

4.2.3 *Drosophila melanogaster*

During the progression of aging, the proteasome function becomes gradually impaired in *D. melanogaster* fruits flies. More specifically, the 26S proteasome assembly has been shown to be impaired during aging. This impairment is also accompanied by a significant reduction of the endogenous ATP levels. In bright contrast, the 20S proteasome function is slightly increased, thus suggesting a possible compensatory mechanism in response to loss of 26S integrity [186]. Other studies have shown that the proteasome function is decreased in the somatic tissues upon the aging progression but however elevated proteasome activities are maintained in the gonads and the eggs of the aged flies [187].

4.2.4 *Rodents*

Proteasome activity and/or expression are compromised in various tissues in mice and rats including adipose [188], retina [189, 190], liver [191–193], lung [191], muscle [194], brain [192], spinal cord [162], heart [195, 196], hippocampus, and cortex [162]. On the other hand, increased levels of immunosubunit-containing proteasomes are usually detected in aging tissues [194, 197]. Nevertheless, several controversial results have been reported suggesting that proteasomal activity alterations in brain differ between species and brain regions [162, 198, 199]. In bright contrast, enhanced proteasome activity levels were found in the longest-living rodents, namely the naked mole rats [200].

A transgenic β5t-overexpressing mouse has decreased CT-L activity and eventually exhibits a premature senescent phenotype that leads to shortened life-span. Moreover, the animals accumulate polyubiquitinated and oxidized proteins while they are more prone to age-associated metabolic disorders [201]. Similar results were obtained in LMP2 (β1i) knockout mice [202]. Accordingly, PA28γ-deficient mice age prematurely [203]. Finally, CT-L proteasome activity is lower in the senescence-accelerated mouse prone 8 (SAMP8) as compared to the relative control SAMR1 that exhibits normal aging phenotype [204].

4.2.5 *Homo sapiens*

Decreased levels of proteasome expression and/or function has been revealed during the progression of aging in several human tissues including lymphocytes [205, 206], lens [207], skeletal muscle [208], and epidermis [161, 209], with controversial results for few tissues [210–212]. Additionally, compositional but not

functional alterations have been also suggested for tissues like liver [213]. In bright contrast, proteasome function is maintained in fibroblasts derived from healthy centenarians [174]. The effects of aging and cellular senescence on the various levels of proteasome regulation are summarized in Fig. 2.

5 Proteasome Impairment During Aggregation-Related Diseases

5.1 Alzheimer's Disease

The link between UPS and the AD onset and progression was initially suggested when senile plaques were stained positively for ubiquitin [214] while elevated levels of UBB^{+1} (a mutated ubiquitin form) were detected in sporadic and familial AD [215]. When proteasome activities of different parts of the brains of AD patients were tested, diminished levels were detected, thus verifying the link between dysfunctional UPS and AD [216, 217]. A vicious circle exists since Aβ, paired helical filament-tau and the UBB^{+1} are all identified as inhibitors of the proteasome function [218–220] and this inhibition further leads to β-amyloid precursor protein (AβPP), Aβ, and tau accumulation [219, 221]. ER stress is induced in activated astrocytes from AD brains and autophagy is increased [222]. Nevertheless, marked inhibition of proteasome activities and impairment in the autophagic flux is monitored in cells overexpressing AβPP mutant isoform thus suggesting that the whole proteolysis network is affected during AD [223]. Finally, several E3 ligases such as parkin [224], HRD1 [225], and UCHL-1 [226] are downregulated in AD while E2-25K, a nontraditional ubiquitin-conjugating enzyme is accumulated in AD samples [227].

5.2 Parkinson's Disease

Aggregated and monomeric α-synuclein is deleterious for neurons viability due to its inhibitory role on both 20S and 26S proteasome activities. It was additionally shown that aggregated α-synuclein directly interacts with Rpt5 subunit [228]. In mutant α-synuclein transgenic mice a remarkable downregulation of proteasome activity is recorded [229]. Nevertheless, it was suggested that α-synuclein expression levels per se do not significantly affect proteasome activities, subunit expression, assembly, and function but additional mechanisms contributing to α-synuclein aggregation are central players in the deterioration of the UPS during PD [230].

Parkin has been identified as an interacting protein of various proteasome subunits such as α4 [231], Rpt6 [232], and Rpn10 [233]. Wild-type parkin has been shown to activate the 26S proteasome (*see* in Subheading 6.2.2) in contrast to PD-linked parkin mutants that lose this ability, thus impairing the 26S proteasome assembly [234]. In accordance, parkin knockout mice and flies exhibit reduced proteasome activity [234].

The 20S [235] but also the 26S [236] proteasome activities are diminished in the substantia nigra of PD patients while reduced levels of α-subunits, RP and 11S complexes have been revealed in

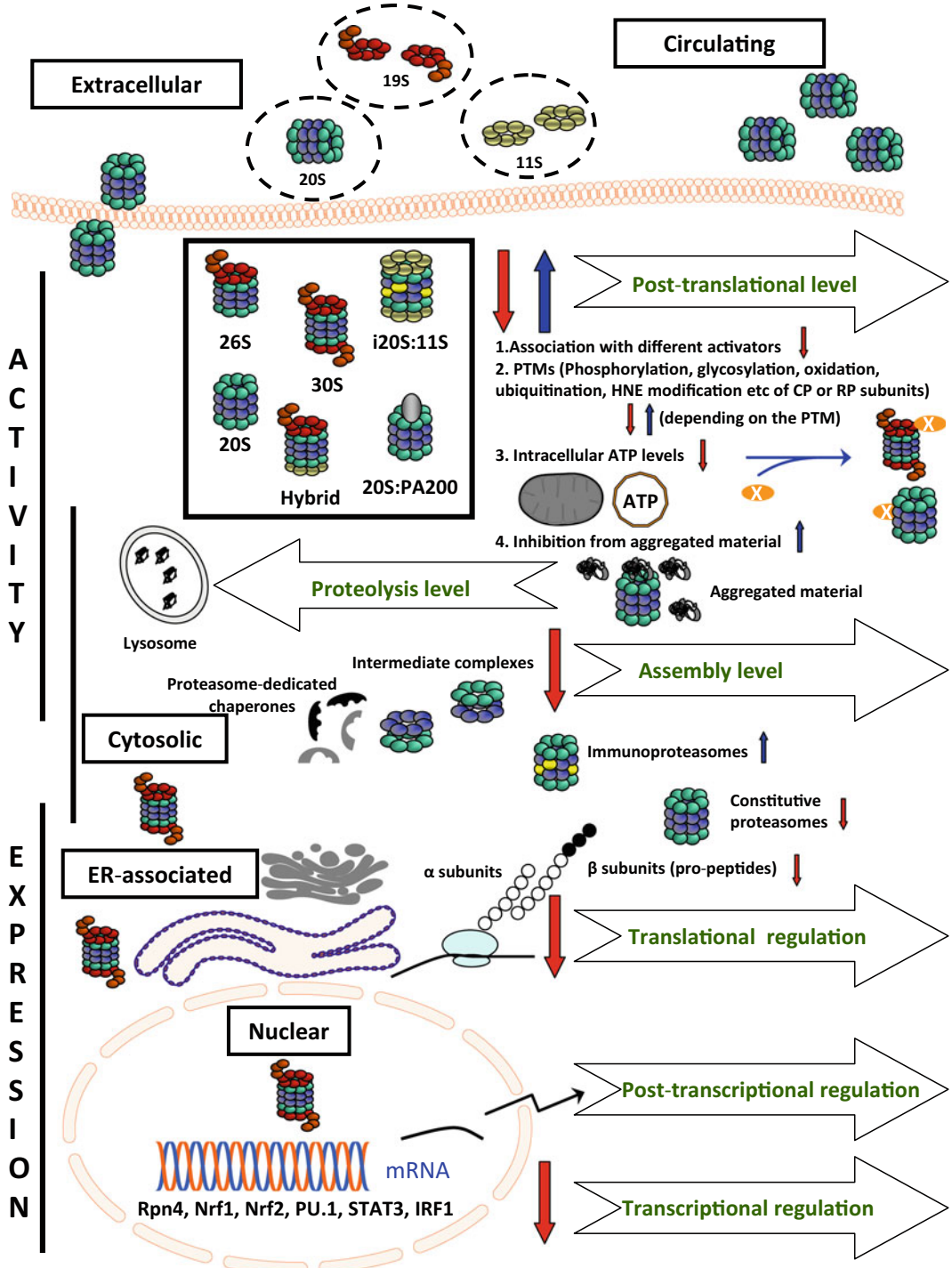

Fig. 2 The effects of aging on the various levels of proteasome regulation. Proteasomes can be found in the nucleus (nuclear), in the cytosol (cytosolic), attached to the endoplasmic reticulum mediating the ERAD (ER-associated) as well as in the extracellular space (named as extracellular or circulating). 30S complex appears in the various compartments in the figure for the scope of presentation but various complexes have been detected in the different compartments in vivo. However, so far only circulating 20S complexes have been isolated although 19S and 11S complexes have been also detected in various somatic fluids. The supra-complexes that constitute the proteasome potential include the constitutive proteasome (20S) and the immunoproteasome (i20S), the 26S and 30S complexes, the hybrid proteasomes, the i20S:11S as well as the 20S:PA200 complexes as shown in the

brain samples from sporadic PD patients [237, 238]. Finally, reduced proteasome activities are also detected in peripheral blood lymphocytes of patients with PD thus paving the way to the development of a potential peripheral biomarker of PD [239].

5.3 Huntington's Disease

As in all proteinopathies, the accumulation of aggregated proteins suggest a failure of the proteostasis network per se. PolyQ aggregates and ubiquitin co-localize in brain samples from HD patients [32], while mutant ubiquitin (UBB^{+1}) has been also detected in IBs [240]. Numerous studies have shown that mHTT inhibits proteasome function in cellular models as well as in vivo in animal models or patients thus suggesting choking or clogging of the proteasome by mHTT aggregates [241–244]. In an attempt to find the biochemical cause of proteasome inhibition, polyQ-containing proteins were shown to get kinetically trapped within proteasomes, thus inhibiting them [245, 246]. A selective inhibition of 26S proteasome but not 20S complex is also suggested and this is related to the interaction of HTT filaments with the 19S particles [247] as well as to ATP depletion due to the HD-induced dysfunction of mitochondria [248]. However, an indirect proteasome inhibition has been also suggested [249], while efficient degradation of expanded polyQ sequences without inhibitory effects on the proteasome has also been shown [250]. The abovementioned studies verify the contradictory results regarding UPS function and HD onset and progression. Furthermore, studies in HD mouse models challenge the concept of proteasome impairment during HD. Bett et al. [251] have revealed that overall proteasome function is not impaired by trapped mutant polyQ in R6/2 HD mice, while Maynard et al. [252] reported that although expression of N-mHtt caused a general UPS inhibition in PC12 cells, no inhibition was detected in the brains of R6/2 and R6/1 mice. Finally, dynamic recruitment of fully active proteasomes into IBs has been also suggested [253].

Fig. 2 (continued) inserted square. As potentially all proteins, the proteasome expression and function may be regulated in the following levels: transcriptional, posttranscriptional, translational, and posttranslational level. The two additional levels that appear in the figure, namely the assembly and the proteolysis level, constitute parts of post-regulation but given their importance in proteasome biology, we have included them here as additional regulatory levels. Multiple studies have already revealed an effect on proteasome expression and/or function/activity in several of those levels [e.g. identified transcription factors that regulate proteasomal RNA expression, regulative conditions for the shift between the expression of constitutive proteasome subunits or immunosubunits, chaperones that regulate its assembly, various PTMs (*X* in the figure represents the various groups that can be added or altered on the various proteasome subunits), association with different activators, alterations by aggregated material or alterations due to the energetic status of the cell]. During the progression of aging and senescence in organisms and cell cultures respectively, several of these regulatory levels are affected. The *red arrows* indicate decrease/downregulation and the *blue arrows* indicate increase/upregulation of pathways that have been shown to eventually affect the proteasome content and/or function during aging and senescence. Some of these regulatory levels affect mainly the proteasome content, some affect the proteasome activity without altering the content and some affect both as shown by the lines on the left of the figure. For more details, please refer to the text

5.4 Amyotrophic Lateral Sclerosis

The detection of ubiquitin and ubiquitin ligases within the ALS-related protein aggregates in ALS mutant mice [254] and in samples from ALS patients [255–257] indicates the possible involvement of UPS in ALS pathophysiology. Deposition of TDP-43 protein aggregates leads to proteasome inhibition [258]. Nevertheless inclusion bodies have been suggested to exert a possible neuroprotective role, given that monomeric and oligomeric misfolded ALS proteins are the actual toxic molecules in motor neurons [259]. Motor neuron-specific knockout mice for Rpt3 19S subunit possess inclusions with ALS-related proteins such as optineurin, ubiquilin 2, FUS, and TDP-43, thus indicating that decreased proteasome activity may result in ALS phenotype [260]. Accordingly, cells from rat spinal cords treated with lactacystin possess reduced proteasome activity and accumulate neurofilaments [261]. In line with these results, the UPS is found inhibited in terms of activity and/or expression in neuronal cell lines overexpressing human mSOD1 [262], in $SOD1^{G93A}$ transgenic mice [263, 264], as well as in samples from ALS patients [265–267]. Finally, upregulation of immunosubunits [268], PA28γ [268], and PA28αβ [269] occur in the motor neurons of $SOD1^{G93A}$ transgenic mice.

5.5 Prion Diseases

Accumulation and aggregation of prion and prion-like proteins in intracellular inclusions and extracellular plaques have been reported to impair protein homeostasis and to provoke cellular stress [270]. Prions cause severe ER stress [271] accompanied by the consequent downregulation of protein translation through chronic eIF2α phosphorylation [272] and impairment of ER protein translocation [273]. The above observations may link the proteolytic pathways to PrD. In addition, abnormal levels of ubiquitin and ubiquitinated proteins have been detected in intracellular inclusions located in the brain tissue, while PrP^{Sc} specifically inhibits the β-type proteasome subunits in two different neuronal cell lines and prion-infected mouse brain. Immunoblot analysis revealed no loss of subunits, while oligomeric inhibitory PrP species directly inhibit the activities of the 20S particle without affecting the 26S assembly. Collectively, the loss of proteolytic activity results from an inhibitory effect on the proteasome [274]. More recently, it was suggested that PrP aggregates inhibit the proteasome by stabilizing the closed conformation of the 20S proteasome and therefore obstruct the entry of the substrate [275]. Table 1 summarizes the proteostasis factors that have been found to be affected upon the progression of aggregation-related diseases.

Table 1
Proteostasis factors in neurodegenerative diseases

Neurodegenerative disease	(a) Impaired proteostasis factor (related to proteolysis)	(b) Manipulated proteostasis factor
Alzheimer's disease	1. High levels of UBB^{+1} 2. Decreased proteasome activities 3. Induced ER stress 4. Increased autophagy 5. Downregulation of E3 ligases	1. Overexpression of *pbs-5*, *aip-1* and key E3 ligases 2. Downregulation of VHL-1 ligase 3. Inhibition of USP14 DUB 4. Compound-mediated proteasome activation (CNB-001, apomorphine, quercetin, resveratrol, rasagiline, thioflavin T, methylene blue, geldamycin, polysaccharide PS5, ganoderic acid DM, lithium)
Parkinson's disease	1. Proteasome inhibition by aggregated and monomeric α-synuclein 2. α-Synuclein and RPT-5 interaction 3. Decreased proteasome activities 4. Proteasome assembly impairment by mutant parkin	1. Acceleration of 19S assembly and proteasome activation via wt parkin 2. Increased mitochondrial activity 3. Increased levels of K48-linked polyubiquitin 4. Upregulation of heat-shock proteins 5. Compound-mediated rescue of the PD-induced proteasome impairment (apomorphine, pramipexole, D3 receptor-preferring agonist D-264, rasagiline, coenzyme Q10) 6. Compound-mediated proteasome stimulation (sepiapterin, pueranin, n-butylidenephthalide, acetylcorynoline, rasagiline)
Huntington's disease	1. PolyQ aggregates and ubiquitin interaction 2. UBB^{+1} detection 3. Proteasome inhibition (chocking or clogging by polyQ aggregates, interaction with HTT filaments) 4. ATP depletion 5. Recruitment of fully active proteasomes into inclusion bodies	1. Overexpression of *pbs-5*, *rpn-6.1*, *rpn-11*, PA28γ, key E3 ligases and USP14 2. Downregulation of VHL-1 ligase 3. Activation of PKA, Akt, and CKB kinases 4. ROCKs inhibition 5. Overexpression of ubiquilins 6. Compound-mediated rescue of the HD-induced proteasome impairment (CGS21680 agonist, benzamil, baclofen, scyllo-inositol, sulforaphane, EGb 761 extract)
Amyotrophic lateral sclerosis	1. Ubiquitin and ubiquitin ligases interaction with aggregates 2. Proteasome inhibition 3. Decreased proteasome activities 4. Upregulation of immunoproteasome subunits	1. Overexpression of key E3 ligases 2. Overexpression of TorsinA, Derlin-1 and p62 3. Compound-mediated proteasome activation (PAP1, pyrazolone, bee venom, melittin, methyl pyruvate) 4. Nrf2 activation via CDDO-TFEA
Prion diseases	1. ER stress 2. Abnormal levels of ubiquitinated proteins 3. Inhibition of β-type subunits 4. Decreased 20S activities 5. Stabilization of 20S closed conformation	1. Congo red derivatives-mediated proteasome activation

Overview of the proteostasis factors (related to proteolysis) that have been (a) found altered in various neurodegenerative diseases and, (b) subjected to manipulation in the context of a potential therapeutic strategy

6 Proteasome Activation During Aging

Manipulation of several UPS-related factors in various cellular and organismal models results in an increase of the proteasome/UPS function with various effects in the cellular/animals life-span and stress resistance. The so far revealed factors include 20S and 19S proteasome subunits, other proteasome activators, E2 and E3 ligases and deubiquitinases. Moreover, the proteasome has been shown to get activated under various conditions and through several molecular pathways while there are also few compounds that have been shown to promote its activation. These factors/conditions/pathways in the various cellular and animal models that ultimately affect aging, longevity, and stress resistance are summarized below.

6.1 Saccharomyces cerevisiae

The yeast orthologs for α- and β-type proteasome subunits are PRE5/6/8/9/10, PUP2, SCL1 and PRE1/2/3/4/7, PUP1/3, respectively. Accordingly, the yeast 19S complex ATPases and non-ATPases are termed RPT1-6 and RPN1-12, respectively (Table 2).

6.1.1 20S and 19S Proteasome Subunits and Other Proteasome Activators

20S proteasome activity gets elevated upon α5 subunit S-glutathionylation and the consequent gate opening which results in increased ability of the yeast cells to degrade oxidized proteins [163].

Blm10 is an alternative proteasome activator identified in *S. cerevisiae* [276]. Enhanced degradation of peptide substrates is scored upon binding of Blm10 on the 20S core particle through a gate opening strategy [277].

6.1.2 E1, E2, and E3 Ligases

The Mub1/Ubr2 ubiquitin ligase complex is responsible for Rpn4 (the yeast transcription factor controlling the expression of proteasome genes) tagging for proteasomal degradation [278, 279]. Loss of *UBR2* and *MUB1* results in stabilization and increase of Rpn4 levels and a consequent induction of 20S and 26S subunits expression levels. The elevated protein levels are accompanied by enhanced activity levels that eventually lead to life-span extension. This extension is exclusively related to the increased proteasome function and the downstream degradation of unstable proteins [280].

6.1.3 Deubiquitinases

Low ubiquitin levels in yeast are sensed and trigger the expression of Ubp6, a proteasome-associated DUB. As a consequence, increased numbers of proteasomes loaded with Ubp6 are monitored with parallel alterations in proteasome function and ultimately, the restoration of the ubiquitin pool [281]. More recently, it was shown that the ubiquitin chain of ubiquitinated proteins is bound to the 26S-associated DUB, Ubp6, and this interaction promotes ATP hydrolysis and enhancement of their own degradation [282].

Table 2
Proteasome subunit nomenclature in different organisms

		Gene name				
Protein type definition		***S. cerevisiae***	***C. elegans***	***D. melanogaster***	***M. musculus***	***H. sapiens***
Reference	**Subunit**	**[451]**	**[452]**	**[7]**	**[9]**	**[453]**
20S proteasome subunits						
Proteasome subunit, alpha type, 1	α6	PRE5	pas-6	Prosalpha6	Psma1	PSMA1
Proteasome subunit, alpha type, 2	α2	PRE8	pas-2	Prosalpha2	Psma2	PSMA2
Proteasome subunit, alpha type, 3	α7	PRE10	pas-7	Prosalpha7	Psma3	PSMA3
Proteasome subunit, alpha type, 4	α3	PRE9	pas-3	Prosalpha3T	Psma4	PSMA4
Proteasome subunit, alpha type, 5	α5	PUP2	pas-5	Prosalpha5	Psma5	PSMA5
Proteasome subunit, alpha type, 6	α1	SCL1	pas-1	Prosalpha1	Psma6	PSMA6
Proteasome subunit, alpha type, 7	α4	PRE6	pas-4	Prosalpha4T1	Psma7	PSMA7
Proteasome subunit, beta type, 1	β6	PRE7	pbs-6	Prosbeta6	Psmb1	PSMB1
Proteasome subunit, beta type, 2	β4	PRE1	pbs-4	Prosbeta4R1	Psmb2	PSMB2
Proteasome subunit, beta type, 3	β3	PUP3	pbs-3	Prosbeta3	Psmb3	PSMB3
Proteasome subunit, beta type, 4	β7	PRE4	pbs-7	Prosbeta7	Psmb4	PSMB4
Proteasome subunit, beta type, 5	β5	PRE2	pbs-5	Prosbeta5	Psmb5	PSMB5
Proteasome subunit, beta type, 6	β1	PRE3	pbs-1	Prosbeta1	Psmb6	PSMB6
Proteasome subunit, beta type, 7	β2	PUP1	pbs-2	Prosbeta2R2	Psmb7	PSMB7

(continued)

Table 2
(continued)

		Gene name				
Protein type definition		***S. cerevisiae***	***C. elegans***	***D. melanogaster***	***M. musculus***	***H. sapiens***
Reference	**Subunit**	**[451]**	**[452]**	**[7]**	**[9]**	**[453]**
19S proteasome subunits						
Proteasome 26S subunit, ATPase, 1	rpt2	rpt2	rpt-2	rpt2	Psmc1	PSMC1
Proteasome 26S subunit, ATPase, 2	rpt1	rpt1	rpt-1	rpt1	Psmc2	PSMC2
Proteasome 26S subunit, ATPase, 3	rpt5	rpt5	rpt-5	rpt5	Psmc3	PSMC3
Proteasome 26S subunit, ATPase, 4	rpt3	rpt3	rpt-3	rpt3	Psmc4	PSMC4
Proteasome 26S subunit, ATPase, 5	rpt6	rpt6	rpt-6	rpt6	Psmc5	PSMC5
Proteasome 26S subunit, ATPase, 6	rpt4	rpt4	rpt-4	rpt4	Psmc6	PSMC6
Proteasome 26S subunit, non-ATPase, 1	rpn2	rpn2	rpn-2	rpn2	Psmd1	PSMD1
Proteasome 26S subunit, non-ATPase, 2	rpn1	rpn1	rpn-1	rpn1	Psmd2	PSMD2
Proteasome 26S subunit, non-ATPase, 3	rpn3	rpn3	rpn-3	rpn3	Psmd3	PSMD3
Proteasome 26S subunit, non-ATPase, 4	rpn10	rpn10	rpn-10	rpn10	Psmd4	PSMD4
Proteasome 26S subunit, non-ATPase, 6	rpn7	rpn7	rpn-7	rpn7	Psmd6	PSMD6
Proteasome 26S subunit, non-ATPase, 7	rpn8	rpn8	rpn-8	rpn8	Psmd7	PSMD7
proteasome 26S subunit, non-ATPase, 8	rpn12	rpn12	rpn-12	rpn12	Psmd8	PSMD8
Proteasome 26S subunit, non-ATPase, 11	rpn6	rpn6	rpn-6.1	rpn6	Psmd11	PSMD11
Proteasome 26S subunit, non-ATPase, 12	rpn5	rpn5	rpn-5	rpn5	Psmd12	PSMD12
Proteasome 26S subunit, non-ATPase, 13	rpn9	rpn9	rpn-9	rpn9	Psmd13	PSMD13
Proteasome 26S subunit, non-ATPase, 14	rpn11	rpn11	rpn-11	rpn11	Psmd14	PSMD14

Ubp3 is a conserved DUB that suppresses accelerated replicative aging and heat-stress sensitivity through the induction of proteasome-mediated degradation of cytotoxic proteins or (depending on the stage at which the damaged protein is committed for destruction) through their rescue from destruction [283].

6.1.4 Other Conditions and Compounds

Ump1 is a proteasome-dedicated assembly chaperone in yeast. Upon its overexpression, yeast cells exhibit increased resistance to various oxidative stressors, enhanced degradation rates of oxidized proteins, and elongated chronological life-span. All those effects are positively correlated with the elevated levels of CT-L activity exhibited by the overexpressors [229]. In accordance, deletion of *UMP1* gene results in increased levels of protein oxidation and reduced survival during stationary phase [175].

Overexpression of the heat-shock protein Hsp104 drives to elevated levels of disaggregase activity resulting in lower levels of protein aggregates and importantly in restored levels of UPS activity in aged yeast cells. Nevertheless, under those conditions the proteasome levels are unaffected and the cellular life-span is not altered [284].

PAP1 peptide (proteasome-activating peptide 1) activates the 20S proteasome activity through α-gate opening. Yeast cells are then able to effectuate more sufficient clearance of the oxidized proteins and therefore to exhibit an increased resistance to oxidative stress [285].

CR extends the replicative and chronological life-span [286, 287]. Increased levels of CT-L activity are scored in CR yeast cells accompanied by decreased levels of oxidized/ carbonylated proteins. Young CR yeast cells carry lower amounts of ubiquitinated proteins as compared to the control cells while CR preserves the ubiquitinylating ability of aged yeast cells, thus resulting in increased viability of the CR cells [288].

Adc17 is a newly identified chaperone that has been suggested to adjust proteasome assembly upon increased demand. It interacts with Rpt6 subunit (without being part of the proteasome) to assist an early step during proteasome assembly in yeast and it is induced upon conditions of proteasomes deficiency. As a result, Adc17 is important for biogenesis of adequate proteasome levels during stress and consequently for cell viability [289].

Finally, it was recently shown that the proteasome-mediated life-span extension is partially correlated to the deregulation of the AMPK signaling pathway. More specifically, increased proteasome activity is linked to premature activation of respiration that induces a mitohormetic response with beneficial impact on yeast life-span [290].

6.2 *Caenorhabditis elegans*

The nematode orthologs for α- and β-type proteasome subunits are PAS1-7 and PBS1-7, respectively. Accordingly, the nematode 19S complex ATPases and non-ATPases are termed RPT1-6 and RPN1-12, respectively (Table 2).

6.2.1 20S and 19S Proteasome Subunits

We have recently shown that overexpression of *pbs-5* catalytic subunit results in proteasome activation in terms of both content and activity. As a result, *pbs-5*-overexpressing animals exhibit extended life-span and ameliorated healthspan while they are more resistant to oxidative stress [132]. A similar phenotype has been achieved through the overexpression of a 19S subunit, namely *rpn-6*. Transgenic nematodes possess elevated proteasome activities that lead to increased survival to oxidative and mild heat stress and ameliorated response to proteotoxicity [291]. This particular subunit has been correlated with the increased proteasome activity that is detected in the long-lived *glp-1* mutants. Interestingly, *pbs-5* subunit is the only other proteasome subunit that is moderately increased in those animals [291]. A similar induction is also observed for the ortholog of *rpn-6*, namely PSMD11, and the ortholog of *pbs-5*, namely β5, in human embryonic stem cells [292] and in human embryonic fibroblasts [293], respectively, as described in Subheading 6.5.1.

AIP-1 (homologue of mammalian AIRAP) is a non-constitutive 19S proteasome subunit that is induced following exposure to arsenite. Upon *aip-1* overexpression, the nematodes conduct a more effective degradation of damaged proteins in stress response conditions, e.g., following arsenic treatment, and fumarylacetoacetate or maleylacetoacetate treatment [294, 295]. In contrast, silencing of *aip-1* results in shorter life-span [184]. As described in Subheading 6.5.1, its mammalian homologue, AIRAP, promotes 20S proteasome activation that enables the cells to cope with proteotoxic stress induced by an environmental toxin like arsenite [296].

6.2.2 E1, E2, and E3 Ligases

Modulation of several E3 ligases has been shown to result in life-span extension mainly through the enhanced degradation of key components for longevity and stress resistance. For example, the conserved insulin/IGF-1 signaling (IIS) pathway is a major pathway that governs the nematodes growth and differentiation [297] with DAF-16 (transcription factor of the FOXO family that is the downstream regulator of the IIS pathway) being the central player [298]. The results regarding DAF-16 effects on proteasome activities per se are controversial. The wt form of the main IIS receptor, DAF-2, has been shown to positively affect the activities of the proteasome since *daf-2* mutants (where *daf-16* expression is elevated), possess lower proteasomal activity [299]. In contrast, Vilchez et al. [291] have suggested that in *glp-1* mutants CT-L proteasome activity is increased through DAF-16 activation while we have also revealed a DAF-16 positive dependence in the *pbs-5*-overexpressing nematodes [132]. A similar positive dependence was also suggested by Holmberg's group using an in vivo reporter system for UPS activity [300].

EGF pathway has been also implicated with the UPS. A positive regulation of the UPS activity via Ras-MAPK pathway and the EOR-1 and EOR-2 transcription factors has been suggested [301].

This increase is correlated with SKR-5, a Skp-1-like protein, upon the loss of which, no UPS activation is observed while shorter life-span is monitored [301].

Proteasome subunit expression is induced through SKN-1 upon proteasome deregulation or inhibition [128–131]. H_2O_2 pretreatment of nematodes leads to SKN-1-mediated 20S proteasome activity elevation but notably not to alteration of the 26S activity [302, 303]. Moreover, it has been shown that IIS affects proteasome activity in a SKN-1-dependent manner [304]. Loss of a WD40 repeat protein, namely WDR-23, is accompanied by accumulation of SKN-1 in the nucleus and subsequent extension of life-span and increased resistance to stress. WDR-23 interacts with CUL4/DDB-1 ubiquitin ligase in order to target SKN-1 for degradation [128]. It is however noteworthy that UPS-independent regulation of SKN-1 through WDR-23 has been also suggested [305].

Elevated levels of proteasome activity accompanied by increased levels of various proteasome subunits have been also revealed in various dietary restriction (DR) nematode models [291, 306]. WWP-1 is a HECT E3 ligase that has been shown to be indispensable for the DR-mediated life-span extension [307]. Moreover, its overexpression in ad libitum-fed nematodes promotes a moderate but still significant 20% life-span extension in a FOXA transcription factor *pha-4*-dependent way. Ubiquitination of specific substrates that are pivotal for DR-related longevity has been suggested as the mode of action of WWP-1 and the crucial E2 ligase that collaborates with WWP-1, namely UBC-18 has been also identified [307]. In agreement, overexpression of the human WWP1 delays the progression of cellular senescence in human fibroblasts, while irreversible premature senescence is established upon its knockdown [308] (*see* Subheading 6.5.1).

6.2.3 Deubiquitinases

Modulation of UBH-4 DUB in *C. elegans* has been implicated with alterations in proteasome activities and with notable effects in stress/proteotoxicity resistance and longevity. More specifically, *ubh-4* silencing results in proteasome activity induction without alterations of the relative expression levels. *Ubh-4* was identified as a DAF-16 target gene that may slightly affect life-span of wt animals with no effects on animals with suppressed IIS pathway [300]. Accordingly, when *uchl5*, the human ortholog of *ubh-4*, is knocked down, increased UPS activity is monitored [309] (*see* Subheading 6.5.1).

6.2.4 Other Conditions and Compounds

Stress adaptation has been shown to occur in nematodes following repeated exposure to mild heat shock or mild doses of oxidants and this hormetic effect has been linked to enhanced longevity [310, 311]. More recently, it was revealed that the mild adaptive stress induced by exposure to H_2O_2 results in elevated proteasome activity [302].

Exposure of nematodes to UV increases UPS function via the activation of the innate immune system with a consequent increased proteostasis and systemic stress resistance [312].

Protein aggregation has been also shown to affect proteasome function and activity. Increased RNA expression levels of key UPS-relevant genes (i.e., *pdr-1*, *ubc-7*, *pas-5*, *pbs-4*, *rpt-2*, and *psmd9*) are detected in transgenic animals overexpressing A53T human synuclein, an aggregation-prone protein found in cellular inclusions in PD, Lewy body dementia, and multiple system atrophy [313].

Although several compounds have been described to promote proteasome activation in cells in vitro [314], only few of them have been examined for their proteasome-activating properties in *C. elegans* and their downstream effects in life-span. Quercetin, a known polyphenolic compound, induces proteasome activation and consequently inhibits $A\beta_{1-42}$-induced paralysis in nematodes [315]. Given that quercetin is a life-span-extending compound [316], one cannot rule out the possibility that this is also related to the induced proteasome activation. Several plant extracts were recently tested in *C. elegans* subjected to high glucose levels for reversal of the glucose-induced survival reduction. Extracts from hibiscus, elderberries, jiaogulan, and blackberries leaves have been identified as potent rescuers while they also promote proteasome activation thus suggesting an efficient degradation of glucose-impaired proteins [317]. Additionally, quercetin prevents glucose-induced reduction of survival through SIR-2.1, DAF-12, and MDT-15 that activate UPR and proteasomal degradation [318]. More recently, a catechin-enriched green tea extract was shown to completely reverse the glucose-induced decrease of life-span. Furthermore, it was shown that the recorded survival extension was dependent on *sir-2.1* and most importantly on *uba-1* that encodes for the unique E1-ubiquitin-activating enzyme in *C. elegans*. This extract stimulates the proteasome activities and thus reverses the glucose-mediated damage through the activation of adaptive responses that include proteasomal degradation [319]. Enhanced activity accompanied by elevated levels of *rpn-5* is monitored following treatment with acetylcorynoline, a Chinese herb-derived alkaloid component [320]. We have also recently shown that feeding of wt *C. elegans* with 18α-glycyrrhetinic acid, a triterpenoid, promotes life-span extension that is dependent on proteasome activation [321]. Finally, osmotic stress caused by NaCl treatment, leads to elevated levels of proteasome degradation as a protective action against stress-induced accumulation of damaged proteins [322].

6.3 *Drosophila melanogaster*

The fly orthologs for α- and β-type proteasome subunits are Prosalpha1-7 and Prosbeta1-7, respectively. Accordingly, the *Drosophila* 19S complex ATPases and non-ATPases are termed RPT1-6 and RPN1-12, respectively (Table 2).

6.3.1 20S and 19S Proteasome Subunits

Ectopic overexpression of *Rpn11* 19S complex subunit attenuates the age-related decline of proteasome activities. As a consequence, the flies exhibit an elongated life-span [323].

6.3.2 E1, E2, and E3 Ligases

Loss-of-function mutations of the *Drosophila* Ubiquitin Activating Enzyme, Uba1 results in reduced life-span and in severe motility defects. Even loss of one of the two alleles results in a significant life-span reduction [324]. Parkin is an E3 ubiquitin ligase that dictates the degradation of various proteins via the UPS [325] while *parkin* mutations are involved in autosomal-recessive PD [326]. Overexpression of *parkin* in flies is accompanied by increased levels of proteasome activity [234], in accordance with in vitro results [231, 234, 327]. This parkin-mediated proteasome activation is independent of parkin's E3 ligase activity. The proteasome function enhancement is related to parkin-mediated enhanced interactions between the 19S complex subunits. In accordance, parkin-null *Drosophila* exhibit decreased proteasome activity [234]. A more recent study has revealed that both ubiquitous and neuron-specific *parkin* overexpression results in elongated mean as well as maximum life-span. Moreover, those long-lived flies also exhibit decreased protein aggregation levels during the progression of aging [328].

6.3.3 Deubiquitinases

The DUB Leon/USP5 is essential for viability and tissue maintenance during *Drosophila* development. Leon mutants exhibit abnormal ubiquitin homeostasis, characterized by increased tissue disorder and augmented death incidents. Notably in those mutants, protein expression levels of proteasome subunits along with the relative enzymatic activities are elevated as a compensation mechanism in response to aberrant ubiquitin homeostasis [329]. Nevertheless, impaired degradation levels of ubiquitinated substrates are monitored.

USP2 DUB prevents uncontrollable activation of the fly immune response in unchallenged conditions by controlling the proteasomal degradation of Imd, an NF-κB-like *Drosophila* factor. Apart from the obvious action of USP2 related to the K48-ubiquitin chain cleavage from Imd, a synergistic binding of USP2 and Imd on the proteasome further alters proteasome-mediated Imd degradation [330].

6.3.4 Other Conditions and Compounds

DmPI31 is the *Drosophila* homolog of the mammalian PI31, a known inhibitor of the 20S proteasome [331, 332]. As opposed to the mammalian homolog, DmPI31 functions as an activator of 26S proteasomes in vitro but also in vivo, since its overexpression in flies suppresses the phenotypes that are caused by dominant temperature-sensitive proteasome alleles (rough eye phenotype; [333]).

Basic leucine zipper protein CncC has been shown to be a transcriptional regulator of the *Drosophila* 26S proteasome [334]. Impaired proteasome function triggers a CncC-mediated upregulation of the proteasome subunits. Conversely, induction of CncC leads to elevated proteasome expression and activity. Nevertheless,

prolonged CncC overexpression results in shorter life-span [335]. Exposure of female flies to low H_2O_2 doses promotes increase of proteasome activity and 20S proteasome expression in a CncC-dependent manner [302].

Finally, several proteasome subunits have been shown to be induced upon exposure of flies to low doses of γ-irradiation and to lead to life-span extension [336, 337].

6.4 Rodents

The rodent orthologs for α- and β-type proteasome subunits are Psma1-7 and Psmb1-7, respectively. Accordingly, the rodent 19S complex ATPases and non-ATPases are termed Psmc1-6 and Psmd1-14, respectively (Table 2).

6.4.1 20S and 19S Proteasome Subunits and Other Proteasome Activators and Components

PA28α is the only proteasome component that has been so far manipulated. More specifically, transgenic mice with cardiomyocyte-restricted PA28α overexpression exhibit diminished aberrant protein aggregation in their hearts. This results in decreased levels of cardiac hypertrophy and consequently, in increased life-span. Therefore, PA28α overexpression may promote protection from cardiac proteinopathy following ischemia [338].

6.4.2 Other Conditions and Compounds

The naked mole rat (*Heterocephalus glaber*) is a nice model of exceptional life-span since it is the longest-living rodent known (~31 years maximum life-span). The proteasomal activities of this rodent are 1.5-fold higher than the ones exhibited by the "normal" mice while they are also maintained in high levels upon the progression of aging. Moreover, they exhibit attenuated age-dependent accumulation of ubiquitinated proteins and cysteine oxidation [200]. In the liver of these animals, more active 20S and 26S proteasomes accompanied by an enhanced proportion of immunoproteasomes are scored [103]. A cytosolic protein factor was shown to interact with the proteasome and to stimulate its activity. Heat shock proteins 72 and 40 were identified as some of the constituents of the unknown factor which however is still not totally characterized. Upon exposure of proteasomes isolated from yeast, mouse and human samples to the cytosolic proteasome-depleted fractions from the naked-mole rat, induction of proteasome activity occurs, thus suggesting a conserved action of this factor across species [339]. A theory that long-lived species may have superior mechanisms to ensure protein quality has been also suggested recently following analysis of protein quality control players in rodents, marsupials and bats [340].

High levels of 20S and 26S proteasome activities are scored in the frontal cortex of transgenic mice overproducing IGF-1 with PI3-kinase/mTOR signaling being involved. The same stimulation is also detected in cell cultures upon IGF-1 stimulation [341].

Late-onset DR in mice and rats is beneficial since it promotes restoration of proteasome activation and reduction of oxidative

damage [342]. In other tissues like the rat spleen, DR does not induce proteasome activity but nevertheless, in the same samples, DR leads to decreased levels of ubiquitinated proteins [343]. Lifelong CR induces T-L proteasome activity but not CT-L and this increase is suggested to be related to the elevated levels of Hsp90 that are revealed in CR animals [344]. Mild CR counteracts the age-related decrease of proteasome activity in rats liver [345] while increased proteasome biogenesis occurs in the same tissue in response to DR [192]. Short-term food deprivation induces UPS function through induced expression of E3 ubiquitin ligases, muscle RING-finger protein-1 (*Murf1*), and muscle atrophy F-box protein or Atrogin-1 (*Fbxo32*) [346]. Treatment of rats with T3 induces the expression of Atrogin-1 and MuRF1 and enhances the proteasome activities by ~40% whereas the UPS remains activated during extended periods of untreated hyperthyroidism [347]. Finally, gene expression analysis in mice subjected to DR revealed the induction of Psmc3 19S subunit and PA28α [348].

Sulforaphane and 3H-1,2-dithiole-3-thione (D3T) are natural compounds that are capable of activating genes that bear the antioxidant response element (ARE) in their promoters through Nrf2 induction [123, 349]. Nineteen proteasome subunits are upregulated by D3T in wt mice as opposed to *nrf2*-disrupted mice. This upregulation is followed by increased proteasome activities [120] and is tissue-specific [350]. 26S/20S proteasome subunits, including PSMB5, the subunit that is responsible for the CT-L proteasome activity are identified among the gene clusters that are under the Nrf2-mediated regulation [351]. Several additional compounds have been shown to alter proteasome activities in mouse models for various diseases. These compounds will be presented in the relative sections. Finally, proteolysis-inducing factor (PIF) is a glycoprotein firstly identified in cancer patients that acts as an enhancer of the proteasome subunits expression and activities in skeletal muscle in vivo [352].

6.5 Mammalian Cells

6.5.1 20S and 19S Proteasome Subunits and Other Proteasome Activators and Components

Since β5 catalytic subunit is the catalytic center for the CT-L activity, many groups have attempted its overexpression in several cell lines. In the stable transfectants, enhanced proteasome activities and/or expression and/or assembly are monitored. Furthermore, β5 overexpression (a) in WI-38/T and IMR90 human fibroblast cell lines and in HL-60 human promyelocytic leukemia cells endows cells with an increased capacity to cope with various oxidants (EtOH, tBHP, H_2O_2, and $FeCl_3$) while human primary cells overexpressing β5 subunit exhibit a ~15–20% life-span extension [293], (b) in dermal fibroblasts from elderly donors results in diminished levels of aging markers such as oxidized and ubiquitinated proteins, SA-β-galactosidase activity and p21 content [353], (c) in lens epithelial cells leads to increased capacity to cope with oxidative stress [354], (d) in human bone marrow stromal cells restores their

capacity for growth while they remain pluripotent for longer [355], and (e) in murine neuroblastoma leads to increased resistance against H_2O_2 toxicity and protein oxidation [350]. Similar results were also obtained upon overexpression of β1 subunit which is the catalytic center for C-L activity [293, 353], while β1 overexpression in human bronchial epithelial cells promotes a protection from cigarette smoke-induced ER stress through enhanced proteasome activities [356]. Accordingly, β5i immunosubunit overexpression in lymphoblasts and HeLa cells leads to elevated CT-L and T-L activities [357], while T-L activity is induced following overexpression of the β1i immunosubunit [358].

With regard to 19S proteasome subunits, human embryonic stem cells (hESCs) overexpressing the 19S PSMD11 subunit have more 26S proteasomes with potential effects in their pluripotency and differentiation capacity [291]. Overexpression of AIRAP, an inducible 19S subunit, promotes proteasome activation upon exposure to an environmental toxic factor, namely arsenite and confers protection in primary mouse embryonic fibroblasts (MEFs) and primary cells of the murine proximal tubule epithelia [359]. AIRAP association on the 19S cap promotes changes in the assembly of the various proteasome complexes favoring the stability of hybrid proteasomes [296]. A similar protection is observed in nematodes by overexpression of its homologue, AIP-1, as described in Subheading 6.2.1.

The association of PA28 activator with the proteasome has been shown to play a role in antigen presentation. Nevertheless, it was recently shown that PA28α overexpression in rat cardiomyocytes results in stabilization and increase of 11S proteasomes that leads to increased resistance to oxidative stress [338].

Finally, proteasome activation has been achieved in human fibroblasts through overexpression of hUMP1/POMP proteasome assembly chaperone. More specifically, overexpression of hUMP1/POMP in WI-38/T fibroblasts leads to enhanced proteasome activities and assembly that ultimately lead to resistance to oxidative stressors [360].

6.5.2 E1, E2, and E3 Ligases

Several E3 ligases have been modulated in various cell lines and have been shown to exert pro-longevity effects, mainly through the induced degradation of their target proteins that inhibit cell growth. Nevertheless, there are no reports showing a simultaneous modulation of proteasome activity. We will just report here the overexpression of two ubiquitin ligases that have been correlated with aging and proteasome degradation: WWP1 and CHIP ligase. We refer to the human WW domain-containing E3 ubiquitin protein ligase 1 (WWP1) as (a) it is implicated in cellular senescence [308], (b) its nematode ortholog has been shown to be essential for the DR-mediated life-span extension [307], and (c) DR has been shown to induce proteasome expression and activities [344, 348]. Therefore, there is a potential link between WWP1 with the

proteasome activities that is however still unrevealed. WWP1 overexpression delays cellular senescence in human diploid fibroblasts through the enhanced degradation of p27(Kip1) while its knockdown leads to premature senescence [308].

The ubiquitin ligase CHIP (carboxyl terminus of HSP70-interacting protein) has been shown to regulate protein quality control and to affect longevity. More specifically, it has been shown that CHIP-deficient mice possess lower levels of proteasome activities and increased levels of oligomerized proteins that eventually lead to reduced life-span and premature aging phenotypes [361]. It was recently revealed that CHIP saves SirT6 (a lysine deacetylase/ADR ribosylase, member of the sirtuin family) from degradation through noncanonical ubiquitination. CHIP overexpression leads to SirT6 stabilization that endows cells with resistance to cellular stress and elevated DNA repair capacity [362]. CHIP overexpression remains to be shown if it may induce proteasome activities/function.

6.5.3 Deubiquitinases

Knockdown of UCHL5 (UCH37) promotes the clearance of aggregation-prone proteins in human U-2OS osteosarcoma cells through increased UPS function similarly to its nematode ortholog UBH-4 [300]. A similar effect is observed upon silencing of UCHL5 in HeLa cells where increased degradation rates of ubiquitinated proteins are scored but notably not enhanced hydrolytic proteasome capacity [309]. Given that silencing of its ortholog in nematodes promotes life-span extension, it would be interesting to see whether modulation of UCHL5 has the same effects in cells and higher eukaryotes.

USP14, another DUB, inhibits the degradation of ubiquitinated proteins both in vitro and in vivo. In agreement with the results from UCHL5 silencing, treatment of MEFs with a selective and reversible inhibitor of USP14, namely IU1, accelerates the degradation of ubiquitinated or oxidized proteins through proteasome activation [363].

It was recently shown that occupancy of Usp14 (a DUB reversibly associated with 26S proteasomes; [309]) or Uch37 (a constitutive DUB of the 26S proteasomes; [309]) by the polyubiquitin chains of tagged proteins leads to enhanced degradation of these substrates through stimulation of ATP hydrolysis [282].

6.5.4 Other Conditions and Compounds

Several natural or synthetic compounds have been shown to stimulate proteasome activities and function in mammalian cell cultures. Oleuropein, the most abundant constituent found in *Olea europea* leaves, olives, and olive oil, has been shown to stimulate the proteasome activities and function in various human embryonic fibroblasts. This induction is accompanied by reduced levels of oxidized proteins, while long-term treatment promotes cellular life-span extension [364]. Various phenolic and flavonoid constituents of the bee pollen induce CT-L proteasome activity in HFL-1 human fibroblasts [365]. Curcumin is a natural phenol that

positively alters proteasome activities in human keratinocytes [366]. An algae extract protects human keratinocytes from the UV-mediated proteasome inactivation [195]. More recently, the synthetic peptide, PAP1 was shown to stimulate CT-L activity in fibroblasts and consequently to protect from oxidative damage and protein aggregation [367].

D3T activates Nrf2 and leads to induction of proteasome subunit protein levels and activity in wt MEFs. This induction is lost upon Nrf2 knockout [120]. A similar enhancement was revealed upon treatment of murine neuroblastoma cells with sulforaphane, a bioactive molecule within the isothiocyanate group of organosulfur compounds [350] as well as in HeLa cells [368]. We have also identified a proteasome-activating compound, namely the triterpenoid 18α-glycyrrhetinic acid [369]. Long-term treatment of human fibroblasts with this compound results in stimulation of the proteasome activities/assembly and function and ultimately in cellular life-span extension and increased resistance to oxidative stress. A similar phenotype was revealed upon chronic treatment of human fibroblasts with the flavonoid quercetin [370]. Although we have not checked whether this proteasome activation is Nrf2-dependent, this possibility cannot be excluded given that quercetin is a known Nrf2 activator [371]. Proteasome activation has been also achieved in Hepa1c1c7 mouse hepatocytes by zerumbone (a sesquiterpene isolated from the plant *Zingiber zerumbet*; [372]). Finally, Nrf2 and proteasome have been shown to be key mediators of human embryonic stem cells (hESCs) physiology. Nrf2 expression decreases upon differentiation while Nrf2 activation delays it through regulation of the proteasome activity. Accordingly, treatment of hESCs with t-BHQ or sulforaphane results in Nrf2-dependent increase of proteasome activities and in delayed differentiation and preservation of cellular pluripotency for longer [373].

Various cardiovascular diseases are characterized by proteasome functional insufficiency and protein control failure. Elevated levels of cGMP along with the downstream activation of cGMP-dependent protein kinase (PKG) have been demonstrated to prevent and reverse already existing hypertrophy and to inhibit the pathways related to hypertrophy [374]. Therefore, while seeking for a potential link between PKG and the UPS pathway, it was shown that overexpression of the protein kinase G (PKG) in rat ventricular myocytes induces proteasome activities resulting in enhanced clearance of misfolded proteins, thus protecting from cardiac proteinopathies [375].

20S levels and activity are augmented upon calpain-mediated processing of the 26S subunit Rpn10. More specifically, upon mitochondrial impairment, Rpn10 is cleaved by calpain, thus resulting in 26S disassembly with a concurrent increase of 20S levels [376].

Treatment of cells with IGF-1 results in elevated levels of CT-L activity in rat glioblastoma cells and WI38 human fibroblasts with an

initial peak at 15 min of stimulation. Activities remain elevated for 24 h following IGF-1 addition but no quantitative alterations are observed with the exception of a slight increase of β5 expression. This induction is abolished in knockout cells for IGF-1 receptor. Accordingly, the Akt/PI3-kinase/mTOR cascade signaling is also involved given that in the presence of the relative inhibitors, proteasome activation by IGF-1 is significantly reduced [341]. Finally, PIF was also shown to enhance the proteasome potential in murine myoblasts in vitro through the induction of NF-κB [352, 377].

6.6 Homo sapiens

The human orthologs for α- and β-type proteasome subunits are PSMA1-7 and PSMB1-7, respectively. Accordingly, the human 19S complex ATPases and non-ATPases are termed PSMC1-6 and PSMD1-14, respectively (Table 2).

6.6.1 Other Conditions and Compounds

There are so far no population studies examining the possibility of proteasome activation. The only report comes from a study where volunteers were supplemented with zinc. More specifically, zinc supplementation for 7 weeks promoted the stimulation of both CT-L proteasome activity and MSR (methionine sulfoxide reductase) activity. In accordance, zinc supplemented donors exhibited reduced levels of oxidized protein thus suggesting the possible role of proteasome activation as an anti-aging strategy in vivo [378]. The various means of proteasome activation in cellular and organismal models are summarized in Fig. 3.

7 Proteasome Activation During Aggregation-Related Diseases

Proteasome activation has been attempted in several cellular and organismal models of aggregation-related diseases. Table 1 summarizes the proteostasis factors that have been subjected to various types of manipulation in the context of a potential therapeutic strategy.

7.1 Alzheimer's Disease (AD)

Given that UPS regulates the presynaptic protein turnover in the nervous system [379], it is not surprising that proteasome inhibition severely affects AD progression and normal synaptic function [22]. It is not additionally unexpected to attempt UPS activation as a therapeutic approach for AD.

7.1.1 20S and 19S Proteasome Subunits

Using a temperature-inducible *C. elegans* strain that expresses human $A\beta_{1-42}$ in muscle cells and that eventually is driven to paralysis [380], we have shown that *pbs-5* overexpression results in proteasome-mediated decreased levels of total but also oligomeric Aβ. This decrease is accompanied by significantly lower paralysis rates [132]. In the same nematode AD model, AIP-1 overexpression (an inducible 19S subunit) results in reduced Aβ levels, aggregation, and toxicity [295].

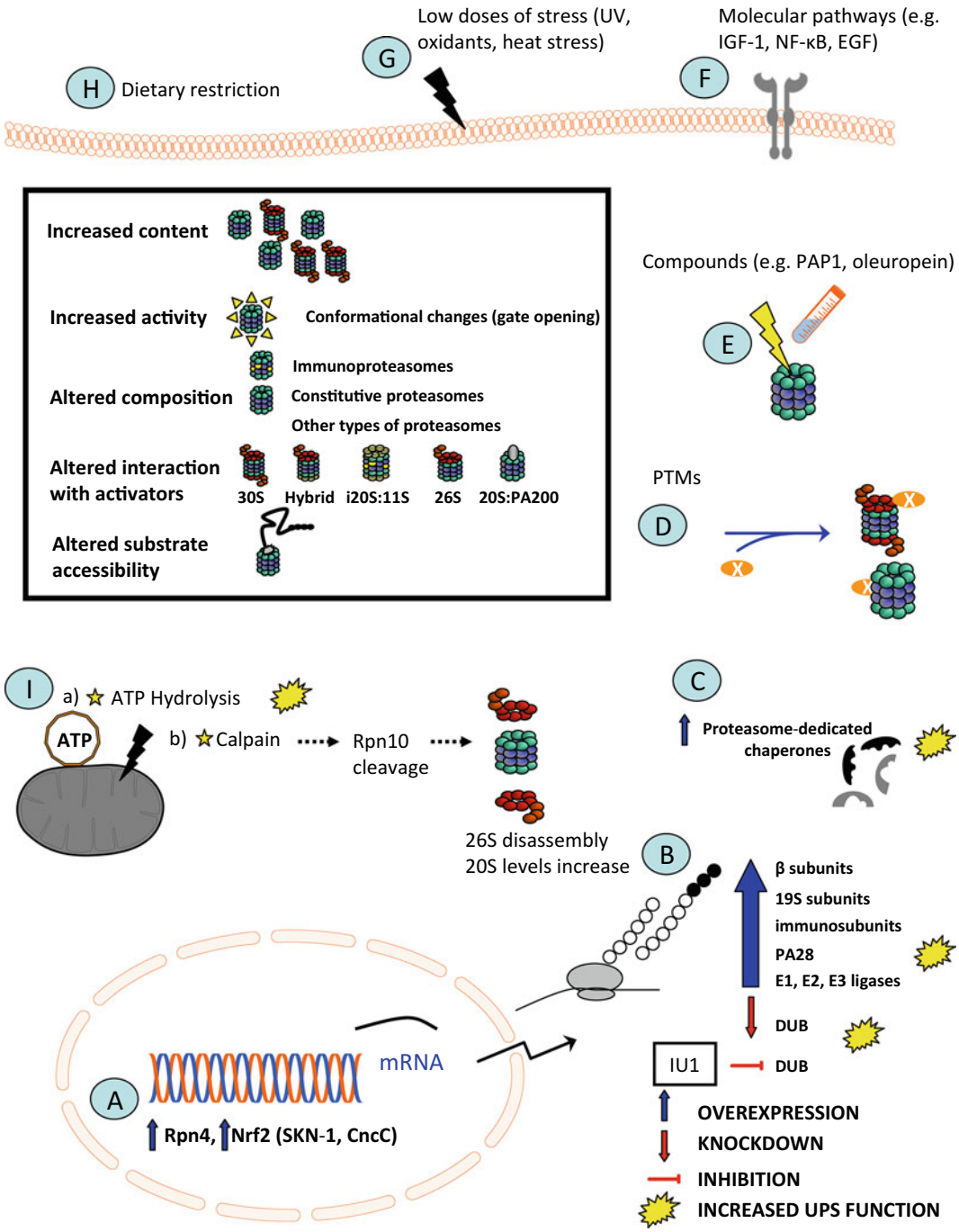

Fig. 3 Means of UPS activation. UPS has been shown to be enhanced through the manipulation of various constituents. UPS activation may refer to increased proteasome content, increased activity (with or without quantitative alterations) that may occur following conformational changes of the complexes that result in α-gate opening, altered ratios of proteasome types (constitutive proteasomes, immunoproteasomes, or other proteasome types), altered interactions with the various activators and altered substrate accessibility as shown in the inserted *square*. The end result of such alterations is the enhancement of UPS activity/function. The so far investigated means of UPS activation include: (*A*) Activation of proteasome-related transcription factors such as Rpn4 in yeast and Nrf2/SKN-1/CncC in mammals/nematodes/flies, respectively. This activation may occur following treatment with a specific compound or following genetic manipulation of these factors. (*B*) Overexpression of UPS components

7.1.2 E1, E2, and E3 Ligases

UPS activation during AD has been attempted through the induction/overexpression of key ligases. More specifically, co-transfection of APP and HRD1 (an ubiquitin ligase that induces proteasome-mediated degradation of ubiquitinated APP) results in reduced Aβ levels and aggregation in HEK293 cells [381]. Overexpression of Fbx2 (an SCF(Fbx2)-E3 ligase) in the primary cortical and hippocampal neurons of transgenic mice overexpressing mAPP (Tg2576), reduces the levels of BACE1, the β-secretase that induces β-amyloidogenesis, and consequently the Aβ levels and ameliorates the synaptic function in vivo [382]. CHIP E3 ligase also drives BACE1 to proteasome-dependent degradation and in parallel regulates p53-mediated trans-repression of BACE1 at both transcriptional and posttranslational level. As a result, reduced Aβ levels are monitored [383]. In an AD model of *C. elegans*, loss of VHL-1 (Hippel-Lindau tumor-suppressor homolog; an E3 ligase for HIF-1 transcription factor) results in delayed paralysis rates and resistance to Aβ proteotoxicity [384].

7.1.3 Deubiquitinases

Inhibition of USP14 DUB by the specific inhibitor IU1, an active-site-directed thiol protease inhibitor, leads to enhanced tau degradation via increased proteasome activities [363].

7.1.4 Other Conditions and Compounds

CNB-001 is a 5-lipoxygenase (5-LOX) inhibitor. Treatment of APP/PS1 AD transgenic mice with CNB-001 activates the eIF2α/ATF4 arm of the UPR that eventually activates both proteasome and autophagic flux and eventually promotes increased rates of Aβ clearance, thus resulting in ameliorated memory function [385].

Treatment of AD transgenic mice with a dopamine receptor agonist, namely apomorphine results in stimulation of proteasome activities and enhanced removal of Aβ and hyper-phosphorylated tau. As a consequence, ameliorated memory function is observed [386].

Quercetin is a proteasome activator and treatment of a transgenic nematode AD model with this polyphenol results in lower levels of Aβ aggregates and to decelerated paralysis rates [315]. Another polyphenol, namely resveratrol also reduces Aβ levels

Fig. 3 (continued) such as β-type 20S subunits, 19S subunits, immunosubunits, PA28 activator, various E1, E2, and E3 ligases or knockdown (or compound-mediated inhibition) of UPS components such as DUBs. (*C*) Enhancement of proteasome assembly through manipulation of proteasome-dedicated chaperones. (*D*) Enhancement of proteasome activity through various PTMs (*X* in the figure represents the various groups that can be added or altered on the various proteasome subunits; please refer to the text for details). (*E*) Direct allosteric alterations of the proteasome structure through the direct binding/interaction of specific natural or chemical compounds. (*F*) Activation of specific pathways that ultimately affect UPS content and/or function such as IGF-1, NF-κB or EGF. (*G*) Exposure to low doses of stress such as UV, oxidants, or heat stress that promote hormetic response that may finally promote UPS activation. (*H*) Effects of dietary protocols such as dietary restriction. (*I*) Cellular energy alterations that ultimately affect: (*a*) ATP hydrolysis and thus proteasome activity or, (*b*) proteases that are responsive to energy alterations and may regulate proteasome assembly/activity/function

[387]. Given that red grapes and red wine are characterized by increased resveratrol concentrations, these results coincide with epidemiological studies suggesting a reverse correlation between red wine intake and AD incidence [388]. More recently, we have also shown that constant feeding of various AD nematode models with the triterpenoid 18α-glycyrrhetinic acid (a previously identified proteasome activator) confers lower paralysis rates accompanied by decreased Aβ deposits, thus ultimately leading to deceleration of the AD phenotype progression. More importantly, similar positive outcomes were also scored in human and murine cells of nervous origin that were subjected to 18α-glycyrrhetinic acid treatment [321].

Rasagiline is an inhibitor of cholinesterase and MAO-A and B that has been shown to stimulate the proteasome activities. Its derivative, namely TV3326 was shown to be neuroprotective and anti-apoptotic in SH-SY5Y and PC-12 cells treated with exogenous Aβ peptide. One cannot rule out the possibility of a link between these positive outcomes and proteasome stimulation [389, 390]. Thioflavin T (ThT) has been shown to reduce Aβ aggregation in vivo in nematodes and this anti-aggregation activity was related to alterations in proteasome function, autophagy and molecular chaperones [391]. Methylene blue, a member of phenothiazines family enhances CT-L and T-L proteasome activities in the brain. This increased proteasome function was linked to the reduced Aβ levels in transgenic mice under chronic methylene blue supplementation and the downstream improved learning and memory functions [392]. Treatment of cells expressing the double truncated $Tau_{151–391}$ with geldamycin, a natural inhibitor of HSP90, results in decreased $Tau_{151–391}$ half-life due to enhanced proteasome degradation [393]. Finally, cellular treatment with polysaccharide PS5 derived from *Rubia cordifolia* and the organic compound ganoderic acid DM leads to an enhanced proteasome-mediated clearance of the intracellular Aβ aggregates [394].

Acetylcholinesterase (AChE) is an enzyme that inactivates acetylcholine at synapses and neuromuscular junctions and is downregulated in AD brains but notably it is still present and activated in amyloid plaques and tangle formations. Cell treatment with lithium results in rapid enhancement of synaptic AChE proteasome-mediated degradation [395].

7.2 Parkinson's Disease (PD)

Given the link between all the PD-associated genes with the UPS in one or the other way, UPS activation may serve as a potential anti-PD approach.

7.2.1 20S and 19S Proteasome Subunits

Overexpression of either 20S or 19S proteasome subunits has not been investigated so far in relation to PD progression. However, the importance of the proper proteasome function in PD was exhibited when upon conditional overexpression of mutated Rpt2 subunit in mice 26S proteasome malfunction occurs and ultimately formation of Lewy-like inclusions and neurodegeneration are established [396].

7.2.2 E1, E2, and E3 Ligases and Ubiquitin

Wt parkin (a key E3 ligase in PD) has been shown to activate the 26S proteasome in an E3 ligase activity-independent manner with an N-terminal ubiquitin-like domain within parkin being critical for this activation through enhancement of the interaction between 19S proteasomal subunits. As a result, wt parkin accelerates the assembly of the 19S RP and thus proteasome activity [234]. In accordance, parkin overexpression in neuroepithelioma cells has been shown to enhance proteasome activity [327] while 26S proteasome activity is upregulated in transgenic flies overexpressing wt parkin or any other form of parkin that possesses the N-terminal parkin fragment containing the necessary for activation UBL domain [234]. Upregulation of wt parkin extends the flies' life-span through decreased levels of protein aggregates, increased levels of K48-linked polyubiquitin and increased turnover of mitofusin (a mitochondrial fusion-promoting factor) followed by changes in mitochondrial morphology and an increase in mitochondrial activity [328].

In a PD *Drosophila* model that overexpresses wt α-synuclein in the eye [397], co-expression of wt Ub protects against α-synuclein-induced toxicity (eye degeneration, locomotor dysfunction, and dopaminergic neurodegeneration) in a K48-polyubiquitin linkage-dependent manner [398], thus suggesting that UPS upregulation might be an attractive anti-PD strategy.

7.2.3 Other Conditions and Compounds

Upregulation of heat-shock proteins protects neuroblastoma cells from the 1-methyl-4-phenylpyridinium ion (MPP^+)-induced neurotoxicity through inhibition of α-synuclein expression and UPS activation in terms of both ubiquitination rates and proteasome activities [399]. In an attempt to elucidate the molecular mechanism of MPP+ toxicity, Shang et al. [400] revealed that overexpression of neuronal nitric oxide synthase (nNOS) significantly enhances proteasome activity with a consequent reduction of apoptosis rates. In the same study, sepiapterin treatment resulted to nNOS activity restoration (that is negatively affected upon MPP+-induced oxidative stress) with the downstream inhibition of superoxide formation, the enhancement of proteasome activity accompanied by decreased levels of ubiquitinated proteins and the attenuation of apoptosis in MPP+-treated cells [400]. Using the same PD model, pretreatment with pueranin results in attenuation of the MPP(+)-induced dysfunction of the proteasome with a consequent delay of apoptosis [401].

Using a PD *C. elegans* model (α-synuclein overexpression in muscle cells), Fu et al. [320, 402] have shown that treatment with n-butylidenephthalide (a naturally occurring component derived from the chloroform extract of *Angelica sinensis*; [402]) or treatment with acetylcorynoline (the major alkaloid component derived from the traditional Chinese medical herb *Corydalis bungeana*; [320]) decreases 6-hydroxydopamine-mediated dopaminergic neuron degeneration, prevents α-synuclein aggregation, recovers lipid content, restores food-sensing behavior and dopamine levels,

and prolongs life-span. In both treatments, proteasome activity enhancement is suggested through the upregulation of *rpn-6* [402] and *rpn-5* [320], respectively. Rasagiline, the inhibitor of monoamine oxidase MAO-B, is a phase 3 anti-PD drug that has been shown to improve pathology symptoms like motor dysfunction [403]. Rasagiline enhances proteasome activity levels in neuroblastoma cells, thus leading to high anti-apoptotic activity [390]. DNA array studies indicate that rasagiline increases the expression of the genes coding mitochondrial energy synthesis, inhibitors of apoptosis, transcription factors, kinases and UPS, sequentially in a time-dependent way [404].

Systemic administration of proteasome inhibitors in the brain of rats or mice results in progressive PD development and has been suggested to constitute an appropriate model of the PD onset and progression [405, 406]). Apomorphine has been found to ameliorate motor activity probably through rescuing proteasome-mediated degradation in mice treated with lactacystin [235]. Pramipexole alleviates lactacystin-mediated proteasome dysfunction resulting in attenuation of the dopaminergic neuronal death in lactacystin-treated mice [407]. Pretreatment with the D3 receptor-preferring agonist D-264 totally blocks the proteasome inhibition and microglial activation in the substantia nigra thus improving behavioral performance and attenuating both MPTP- and lactacystin-induced DA neuron loss [408]. A similar protection was also revealed for rasagiline [409] and for coenzyme Q10 (that protects against proteasome impairment through induction of ATP production and therefore through enhancement of UPS function; [410]).

7.3 Huntington's Disease (HD)

In a conditional mouse model of HD, reversal of neuropathology and motor dysfunction was exhibited with a disappearance of inclusions upon blockade of the constant influx of the mHTT [411]. Therefore, HD pathology might be reversible and a link with the proteostasis network is revealed suggesting that UPS activation could be a potential anti-HD approach.

7.3.1 20S and 19S Proteasome Subunits

Upregulation of *pbs-5* subunit in *C. elegans* leads to enhanced proteasome activities and in turn to reduced polyQ toxicity and improved motility in transgenic worms expressing Q35 in body wall muscle cells or Q40 in neurons [132]. Accordingly, overexpression of *rpn-6.1* 19S subunit results in reduced polyQ toxicity and aggregates levels [291]. Overexpression of PA28γ in HD cells results in recovered proteasome function and in improved cell viability. However, overexpression of *rpn-10* did not result in either proteasome activation or neuroprotection [412]. Ectopic overexpression of a 19S complex subunit, namely *Rpn11*, was shown to attenuate the age-related decline of the proteasome activity in *Drosophila*. As a consequence, the flies exhibit an elongated life-span. Accordingly, *Rpn11* overexpression leads to decreased polyQ-induced toxicity and neurodegeneration [323].

7.3.2 E1, E2, and E3 Ligases

E6-AP E3 ubiquitin ligase promotes the degradation of misfolded polyQ proteins resulting in a suppression of aggregate formation and cell death in cellular HD model [413]. CHIP overexpression suppresses the formation of insoluble aggregates by mutant polyQ proteins in differentiated neuronal cells as well as in an HD zebrafish model [414]. Hrd1 is an endoplasmic reticulum (ER) membrane-E3 ligase with its catalytic active RING finger facing the cytosol that is upregulated in cells overexpressing the N-terminal fragment of htt containing an expanded polyQ tract (httN). Enhanced expression of Hrd1 results in increased degradation of httN and in decreased levels of httN-induced cell death [415]. Similar results are obtained upon overexpression of Parkin [232]. In an HD *C. elegans* model, loss of VHL-1 (a HIF E3 ligase) results in elevated resistance to polyQ toxicity with concomitant decreased paralysis rates [384]. Accordingly, increased resistance to proteotoxic stress is also observed upon loss of Mub1/Ubr2 ubiquitin ligase complex that results in Rpn4 stabilization [280].

7.3.3 Deubiquitinases

Overexpression of the USP14 DUB in mHTT-expressing cells leads to diminished levels of cellular aggregates mainly via the UPS. Specifically, the serine-threonine kinase IRE1 is an ER stress-associated protein that is activated during mHTT toxicity. USP14 overexpression counteracts the IRE1 activation thus leading to reduced rates of cell degeneration [416].

7.3.4 Other Conditions and Compounds

Activation of protein kinase A (PKA) confers Rpt6 phosphorylation that in turn results in increased proteasome activity, reduced mHTT aggregates and improved motor capacity of an HD mouse model [203]. Proteasome impairment through HTT aggregates has been shown to be alleviated by Akt kinase [417] as well as by brain-type creatine kinase (CKB) [203]. Inhibition of Rho-associated kinases (ROCKs) in cellular models of HD reduces the aggregation levels of mHTT via activation of the UPS and macroautophagy [418].

Ubiquilins are proteins that are speculated to function as shuttle factors to transfer misfolded proteins to the proteasome since they have the ability to bind ubiquitin moieties conjugated onto proteins via their UBA domain and subunits of the proteasome via their UBL domain [419]. Overexpression of ubiquilin-1 suppresses polyQ toxicity in cell culture and *C. elegans* models of HD [420], as well as in an HD mouse model where extension of life-span, delayed htt inclusions formation and attenuated ER stress in the hippocampus are scored. Nevertheless, motor defects are not ameliorated [421]. Overexpression of NUB1, a negative regulator of ubiquitin-like protein 1 results in elevated degradation rates of mHTT and thus in lower levels of aggregates and neuronal survival [422].

Various compounds have been identified to alleviate the mHTT-related proteasome impairment like an agonist of the A(2A) adenosine receptor (A(2A) receptor), namely CGS21680

[423], benzamil, an amiloride derivative [424], baclofen, a $GABA_B$ receptor agonist [425], and scyllo-inositol [426]. Finally, sulforaphane, a natural compound derived from broccoli and other vegetables, is a potent activator of both proteasome and autophagy in mice. Sulforaphane treatment enhances the proteasomal degradation of mHTT and induces cell survival in HD cell models [427]. A similar increase in proteasome activity accompanied by more efficient degradation of pathologic polyQ variants is also exerted by the antioxidant *Ginkgo biloba* extract EGb 761 [428].

7.4 Amyotrophic Lateral Sclerosis (ALS)

Given the link between UPS and ALS onset and progression, proteasome activation could be an ALS-targeted therapeutic strategy.

7.4.1 E1, E2, and E3 Ligases

Overexpression of various E3 ligases that target mSOD1 for degradation has shown promising results as potential targets for ALS therapy. More specifically, overexpression of dorphin (identified to promote the proteasome-mediated degradation of mSOD1 and to prevent neurotoxicity; [429]) ameliorates the ALS phenotype in the relevant transgenic mice [430]. Accordingly, overexpression of the ERAD E3 ubiquitin ligase Gp78 targets mSOD1 for ERAD resulting in increased cell viability and reduced SOD1 aggregation levels [431]. Finally, a mitochondrial ubiquitin ligase, namely MITOL, interacts with ubiquitinated mSOD1 but not wt SOD1 and its overexpression results in the enhanced clearance of mSOD1 and in the suppression of mitochondrial accumulation of mSOD1 [432].

7.4.2 Other Conditions and Compounds

Activation of UPR has been shown to be beneficial in conditions of ALS pathology. TorsinA is an AAA+ family member with molecular chaperone-like activity. TorsinA overexpression rescues an ALS *C. elegans* model from the mSOD1-specific ER stress increase and restores normal neuronal function. These positive effects are mediated through enhanced mSOD1 targeting for proteasome degradation [433]. Accordingly, overexpression of the ER-resident factor Derlin-1 results in suppression of the activation of ER stress and in increased proteasomal and autophagosomal turnover of mSOD1 [434].

Overexpression of p62 (sequestosome 1), an adaptor protein for the autophagy pathway, reduces TDP-43 aggregates through enhanced proteasome and autophagy function [435].

Treatment of human neuroblastoma $SOD1^{G93A}$ cells with the synthetic peptide PAP1 leads to decreased levels of mSOD1 aggregates and enhanced cytoprotection through the enhanced proteasome activities mediated via conformational alterations of the proteasome gate [367]. Two proteasome subunits (PSMC1 and PSMC4) have been identified as target proteins of pyrazolone (a five-membered-ring lactam). Treatment of PC12-$SOD1^{G93A}$ cells with pyrazolone results in proteasome activation and the downstream delay of ALS progression [436].

Bee venom and its anti-inflammatory component, melittin, alleviate proteasome activity impairment in human SOD1^{G85R}-expressing NSC34 motor neuron cells and in human ALS SOD1^{G93A} mouse model, respectively [437, 438]. Similar results are obtained following treatment of mouse N2A cells overexpressing mutant SIGMAR1 (a gene involved in familial ALS; [439]) with methyl pyruvate, a mitochondrial TCA cycle substrate. The proteasome activity is restored, mitochondrial ATP production is enhanced and aggregation-prone TDP-43 mislocalization is prevented [440].

Malfunction of Nrf2 pathway has been revealed in few ALS patients [441] and in cultures of SOD1^{G93A} motor neurons from the relevant transgenic mice [442]. Use of an Nrf2 activator, namely CDDO trifluoroethylamide (CDDO-TFEA), results in activation of Nrf2 and in deceleration of neurodegeneration [443]. Given that proteasome genes are Nrf2 target genes [120], one cannot rule out the possibility that proteasome activation might also occur and contribute to this neuroprotection.

7.5 Prion Diseases

Prion clearance and the relative proteolytic pathways may constitute a potential therapeutic target for PrDs given that (a) the pathogenesis of the disease is directly related to constant PrP^{Sc} aggregation [444] and (b) diminished PrP^{Sc} levels result in reversal of cognitive deficits and neurophysiological dysfunction of prion-infected mice [445, 446]. The so far collected data suggest that both lysosomal and proteasomal degradation may play significant roles in prion degradation [447]. Nevertheless, scarce data exist regarding the modulation of UPS as an anti-prion therapeutic approach.

7.5.1 E1, E2, and E3 Ligases

The responsible E3 ligase for the unglycosylated PrP (ugPrP) has been identified: Hrd1-Hrd3 in yeast [448] and Gp78 in mammalian cells [449]. Although overexpression of either of those ligases has not been attempted in relation to PrD progression, potential positive results may be expected similarly to what has been shown in ALS with Gp78 (see above).

7.5.2 Other Conditions and Compounds

Congo red derivatives WSP774 and WSP677 have been shown to enhance the proteasome-mediated degradation of PrP^{Sc} in infected cells and thus to alleviate the inhibitory effect of PrP^{Sc} on proteasome function [450]. The efficacy of other proteasome activating compounds like sulforaphane, quercetin, the DUB inhibitor IU1 and all the other molecules that have been so far investigated in various aggregation-prone diseases as mentioned above, remain untested in relation to PrD. Therefore, one cannot rule out the possibility that they could be potential anti-prion candidates. The effects of aggregation-related diseases on the proteasome and the outcome of UPS activation are summarized in Fig. 4.

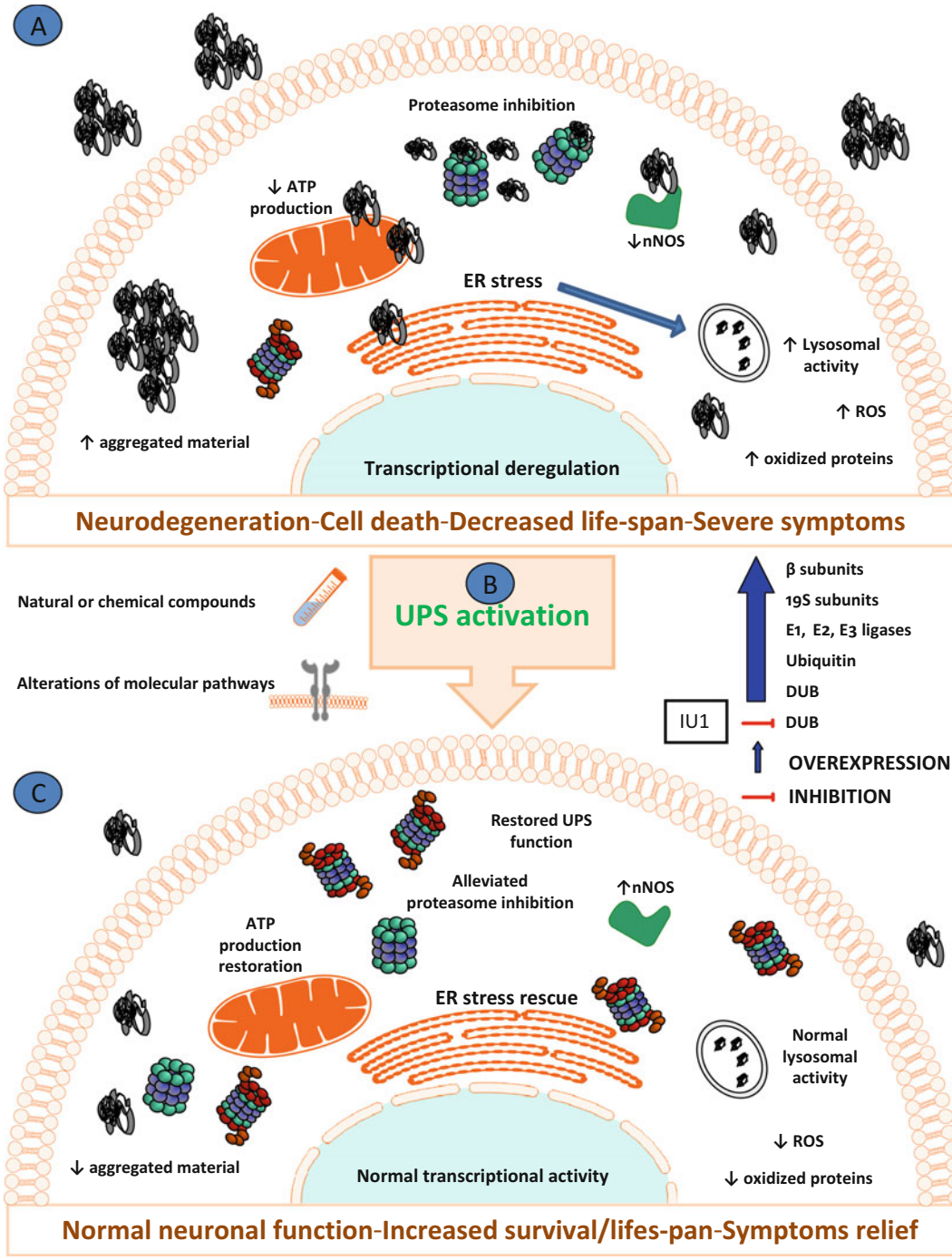

Fig. 4 The effects of aggregation-related diseases on the proteasome and the outcome of UPS activation. Aggregation-related diseases are characterized by increased amount of protease-resistant misfolded/aggregated proteins. (*A*) In neuronal cells from organisms suffering from an aggregation-related disease, aggregated material induces (among others) transcriptional deregulation, inhibition of several key enzymes including nNOS and the proteasome, defects in mitochondria that lead to decreased ATP production that further affects UPS function and ER stress due to the enhanced aggregate load and the inhibition of the normal proteasome function. These defects initiate a vicious circle of constant accumulation of aggregates, additional proteasome inhibition, and constant oxidative stress. The autophagy-lysosome system is induced to compensate for the reduced UPS activity but the end result includes neurodegeneration, cell death and decreased life-span. (*B*) UPS

8 Concluding Remarks

Life is linked to conditions of increased stress (e.g., oxidative stress due to respiration, UV stress by sun exposure). Nevertheless, excessive stress is not compatible with survival. Therefore, proteostasis mechanisms (with proteolytic modules forming the ultimate arsenal) have been evolved to assure the balance between the inevitable stress conditions and cellular/organismal homeostasis. Upon malfunction of these mechanisms due to intrinsic (e.g., mutations, loss- or gain-of-function alterations) or extrinsic (e.g., environmental stress factors) causes, this balance is destroyed. Therefore, the preservation or even the enhancement of proteostasis mechanisms function seems to be beneficial for cellular survival. This is further supported by the fact that most of the pro-longevity factors and pathways enhance the function of proteolysis modules leading to extended life-span, ameliorated response to stress and alleviation of aggregation-related disease phenotypes. Most of these studies have been performed in lower eukaryotes. Therefore additional studies in higher eukaryotes followed by human population studies (wherever possible) are necessary. These studies will finally validate the correlation between aging/aggregation-related diseases and enhanced proteostasis mechanisms.

In the case of the UPS and its potential enhancement, further studies are needed to fully elucidate the regulatory mechanisms behind such activation. For example, although genetic and compound-mediated UPS activation has been successful, the molecular mechanisms behind such modulation are not fully investigated. Questions that remain to be elucidated include regulation of transcription, assembly, trafficking, and elimination as well as posttranslational regulation of the various UPS components. The same mechanisms should be then thoroughly examined in the context of aging or a given aggregation-related disease as age- or disease-specific alterations might be expected. Given that overactivation might also prove to be detrimental, highly orchestrated UPS activation is necessary in order to be able to suggest a manipulation as an anti-aging/anti-aggregation preventive/therapeutic strategy. In the case of activating compounds, one should be very cautious with the translation of results, considering the possible (but still uncovered) side targets of a given molecule. Identification of activating molecules that are constituents of human regular diet should be also explored since they provide extra advantages;

Fig. 4 (continued) activation has been achieved in cellular and organismal models of aggregation-related diseases through the genetic manipulation of several UPS constituents, through treatment with natural or chemical compounds as well as through alterations of specific molecular pathways (please refer to text for details). (*C*) The abovementioned manipulations result in alleviated proteasome inhibition, ER stress rescue and restoration of (a) ATP production, (b) transcriptional activity, (c) lysosomal activity, and (d) UPS activity, among others. The cellular aggregate load decreases promoting normal neuronal function and increased survival/life-span

beneficial UPS activation or preservation should probably commence before heavily aggregated proteins get established in the cellular milieu thus in a young age before we can even detect such alterations. Therefore, diet constituents might be ideal for such approach. Addressing these questions will further pave the way to the establishment of therapeutic but also preventive strategies in the battle against aging and age-related diseases.

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