Abstract: For many years, the ubiquitin-26S proteasome degradation pathway was considered the primary route for proteasomal degradation. However, it is now becoming clear that proteins can also be targeted for degradation by the core 20S proteasome itself. Degradation by the 20S proteasome does not require ubiquitin tagging or the presence of the 19S regulatory particle; rather, it relies on the inherent structural disorder of the protein being degraded. Thus, proteins that contain unstructured regions due to oxidation, mutation, or aging, as well as naturally, intrinsically unfolded proteins, are susceptible to 20S degradation. Unlike the extensive knowledge acquired over the years concerning degradation by the 26S proteasome, relatively little is known about the means by which 20S-mediated proteolysis is controlled. Here, we describe our current understanding of the regulatory mechanisms that coordinate 20S proteasome-mediated degradation, and highlight the gaps in knowledge that remain to be bridged.

Keywords: protein degradation; 20S proteasome; intrinsically disordered proteins; oxidatively damaged proteins; regulatory mechanisms

1. Introduction

Cells must continuously adapt their proteome composition [1]. The constant flux of the proteome is reflected in the fact that most proteins have a half-life several fold shorter than cell generation time,
even for rapidly dividing cells [2]. Protein level adjustment in response to various conditions such as oxidative stress, cellular differentiation and fluctuating nutrient supply is critical for cell viability. The control of protein levels relies not only on the regulation of protein production and folding, but also on protein degradation. Failure or malfunction of this process may lead to various illnesses, including cancer and neurodegenerative diseases.

The ubiquitin-dependent 26S proteasomal degradation process is the major system that mediates the degradation of damaged and short-lived regulatory proteins (for reviews, see [3–5]) (Figure 1A). This active, ATP-dependent pathway involves a cascade of three different types of enzymes that tag substrate proteins with an ubiquitin chain, for their recognition and proteolysis by the 26S proteasome. The structure of the 26S proteasome consists of two entities: a 20S core particle, and a 19S regulatory particle (Figure 1A). The 20S complex carries out the proteolytic activities. It is composed of 28 subunits encoded by 14 different genes, 7α and 7β subunits, which form a highly conserved cylindrical structure of four rings, each containing seven subunits. The two outer rings contain α-type subunits whose function is to maintain a “gate” through which proteins enter the catalytic site [6]. The β-subunits form the two inner rings. Three of the β-subunits, β1, β2 and β5, are catalytically active, and responsible for the caspase-like (or peptidylglutamyl-peptide), trypsin-like and chymotrypsin-like hydrolyzing functions, respectively. Overall, this architecture creates a compartment whose proteolytic active sites are restricted to its interior, so that only proteins entering this chamber are degraded. The addition of a 19S regulator to either one or both ends of the 20S proteasome creates the 26S proteasome. The 19S particle coordinates the degradation process by recognizing the polyubiquitinated substrates, unfolding, deubiquitinating and translocating them into the interior of the 20S complex, where they are degraded to oligopeptides.

Recently, it was realized that proteins are also subject to proteasomal degradation in an ubiquitin-independent manner [7–9]. Both the 26S and the 20S proteasomes were shown to be capable of degrading proteins without the substrate selectivity achieved by ubiquitin tagging (Figure 1B). The primary requirement for degradation by this route is the presence of an unstructured region. In fact, the two alternative proteasomal degradation mechanisms, ubiquitin-dependent and ubiquitin-independent, are not mutually exclusive, and different populations of the same protein can be sent to degradation via either pathway [10]. Here, we will focus on the ubiquitin-independent degradation process that is mediated only by the 20S proteasome complex, without the assistance provided by the 19S particle.

Substrates of the 20S proteasome are made up of proteins that have become partially or completely unfolded due to aging, mutations or oxidation [8,11]. Native proteins containing large unstructured segments (>30 amino acids in length), referred to as intrinsically disordered regions (IDRs), or proteins with entirely disordered sequences (intrinsically disordered proteins, IDPs) [12], are also susceptible to degradation by the 20S proteasome (see summary in Table 1). This latter group of substrates is dominated by key regulatory and signaling proteins with essential roles in cell cycle progression, cellular growth control, and oncogenesis (as reflected in Table 1) [13]. Thus, their levels must be tightly controlled, as alterations in their abundance may lead to the development of various diseases [11].
Figure 1. A simplified representation of the (A) ubiquitin-dependent 26S proteasome; and (B) ubiquitin- and ATP-independent 26S or 20S proteasome, protein degradation pathways.

Table 1. A list of proteins with varying degrees of disorder that serve as 20S proteasome substrates.

| Protein     | Function                        | Associated Disease | % Disorder a | Reference |
|-------------|---------------------------------|--------------------|--------------|-----------|
| p21 cip1    | Cell cycle regulator            | Cancer             | 57 (64)      | [14,15]   |
| p27 kipl    | Cell cycle regulator            | Cancer             | 98 (104)     | [16]      |
| p33         | Tumor suppressor                | Cancer             | 77 (115)     | [17]      |
| p53         | Tumor suppressor                | Cancer             | 62 (128)     | [10]      |
| p63         | Tumor suppressor                | Cancer             | 51 (73)      | [18,19]   |
| p73         | Tumor suppressor                | Cancer             | 33 (57)      | [10]      |
| Rb          | Tumor suppressor                | Cancer             | 35 (73)      | [20]      |
| BIMEL       | Pro-apoptotic protein           | Cancer             | 38 (62)      | [21]      |
| MCL-1       | Anti-apoptotic protein          | Cancer             | 30 (105)     | [22]      |
| c-Fos       | Transcription factor            | Cancer             | 33 (88)      | [23]      |
| IxBa        | NF-κB inhibitor                 | Cancer             | 43 (77)      | [24]      |
| ODC         | Biosynthesis of polyamines      | Cancer             | 15 (32)      | [25]      |
| HIF-1α      | Transcription factor            | Cancer             | 55 (108)     | [26]      |
| C/EBPα      | Transcription factor            | Myeloproliferative disease | 40 (70) | [27]      |
| PGC-1α      | Transcription co-activator      | Neurological disorders | 96 (178) | [28]      |
| tau         | Neuronal protein, stabilizes microtubules | Alzheimer’s | 80 (165) | [29,30]      |
| α-synuclein | Presynaptic neuronal protein    | Parkinson’s        | 31 (44)      | [31]      |

a Values represent the percentage of disordered residues as predicted by the FoldIndex program [32]. Numbers in parenthesis indicate the amino-acid length of the longest unfolded stretch.
Considering that 44% of human protein-coding genes contain IDRs [12], it is reasonable to assume that in the future, the number of proteins recognized as substrates of the ubiquitin-independent pathway will continue to increase. That being said, it should be noted that establishing whether a protein is degraded in a 20S proteosome ubiquitin-independent manner is not a trivial task. *In vitro* assays showing that the non-ubiquitinated protein is degraded by the 20S proteosome cannot stand on their own, and should be complemented by cellular assays, as detailed in recent reviews [7,33]. In practice, however, even successful demonstration of *in vivo* ubiquitin-independent degradation cannot unambiguously distinguish between 20S- or 26S-mediated degradation. Results indicating that the protein of interest: (i) is not ubiquitinated upon destabilization or inhibition of the proteasome; (ii) is not stabilized following mutations in potential ubiquitin acceptor sites, or due to the excess of non-polymerizable ubiquitin; and (iii) is not stabilized when inhibiting the ubiquitination pathway, which imply that either 20S and/or 26S proteosome ubiquitin-independent degradation has taken place. In order to define which of the proteasomes is involved in the process, approaches indicating whether the 19S particle is involved in the process should be used.

Several recent findings emphasize the significance of the ubiquitin independent 20S proteosome degradation route. First, the majority of proteasomes in mammalian cells were found to be uncapped 20S proteasomes, whereas only about 20%–30% of the total number of mammalian proteasomes were identified as 26S proteasomes [34–37]. Secondly, more than 20% of the cellular proteins in mammalian cell extracts were shown to undergo 20S proteasomal cleavage [38], suggesting the extensive involvement of this process in cell viability. Moreover, under oxidizing conditions, the 20S proteasome was identified as the major degradation machinery (reviewed in [8,11,39]). The 20S proteasome was shown to be more resistant to oxidative stress and to maintain its activity under conditions in which protein damage occurs, while the 26S proteasome and the ubiquitination pathway are much more vulnerable to such oxidative stress conditions. In summary, these findings suggest that 20S-mediated proteolysis is not limited to rare cases, but rather represents a complementary degradation route that is critical for removing damaged, unfolded proteins, and for maintaining normal levels of proteins containing IDRs.

Over the past three decades, extensive research focusing on the ubiquitin-dependent 26S proteasome degradation pathway has uncovered hundreds of factors that regulate this process—including a series proteins that deliver the ubiquitinated substrates to the 26S proteasome, enzymes that antagonize degradation by trimming off the ubiquitin chain, and proteasome-associated ubiquitin ligases that enhance substrate turnover [3–6]. In contrast, relatively little is known about the mechanisms that coordinate protein degradation by the 20S proteasome. Here, we will discuss the emerging view of how the proteolytic capacity of the 20S proteasome is specifically controlled. In doing so, we will describe eight distinct mechanisms that enable either enhancement or inhibition of 20S proteolytic capacity, including: the disassembly of the 26S proteasome, gene regulation, subunit composition diversity, and associations with regulatory and substrate-targeting molecules, as well as post-translational modifications (PTMs) and divergence in cellular localization. We also examine how temporal regulation is critical for forming a tightly coordinated and dynamic system for combating oxidative stress.
2. Molecular Mechanisms that Regulate the Function of the 20S Proteasome

2.1. Disassembly of the 26S Proteasome

Cellular levels of the 20S proteasome can be increased through disassembly of the 26S proteasome into its 20S and 19S components. Several studies have shown that this, indeed, occurs following oxidative stress, at the time when there is an urgent need to enhance the degradation capacity of this complex so as to cope with increasing numbers of oxidatively damaged proteins [40–43]. Various proteins appear to be involved in the decomposition process. In the mammalian system, the chaperone Hsp70 was found to be important for the stabilization of the 19S regulator after its dissociation from the 20S proteasome, and for the reassembly of functional 26S proteasomes, once the oxidative stress is eliminated [41]. In yeast, H₂O₂-induced disassembly of 26S proteasomes is dependent on the proteasome-associated protein, Ecm29 [40]. Low levels of Hsp90 were also shown to cause almost complete disassembly of the yeast 26S proteasome, and an increase in the abundance of free 20S proteasomes [44]. Inevitably, the release of the 20S proteasome from the holo-26S complex is accompanied by a reduction in ATP-stimulated peptidase activity; however, this did not appear to influence the degree of resistance to oxidative conditions [42,45,46].

Recently, the levels of the 26S and 20S proteasomes were also shown to be dependent on the cellular metabolic state [43,47]. For example, a low cellular ratio of the cofactors NADH/NAD⁺ destabilizes the 26S proteasome, giving rise to free 20S proteasome particles [47]. Similarly, ATP deficiency associated with acute and long-term mitochondrial impairment leads to a reduction in 26S proteasome levels, in concert with an increase in 20S proteasomes [42]. Overall, the disassembly of the 26S proteasome is a straightforward mechanism for increasing 20S levels. However, further research is needed, in order to specifically indicate whether the increase in the 20S levels is accompanied by a concomitant rise in the catalytic capacity of the 20S proteasome. If true, such a process could ensure efficient degradation of damaged proteins and proteins with IDRs, without the energy expenditure associated with ubiquitin-dependent 26S-mediated degradation.

2.2. Alternative Proteasome Activators

More than 50% of cellular 20S proteasomes are found in a free form, and about 30% are associated with the 19S regulatory particle. The remaining 20S proteasome pool is associated with other proteasome activators (PAs)—the cytosolic PA28αβ (or 11S), as well as the nuclear PA28γ and PA200 regulators [35,48]—that enhance the proteolytic capacity of the complex in an ATP- and ubiquitin-independent manner. Both PA28αβ and PA28γ form hetero- or homo-heptameric rings, respectively, which interact with the α-rings of the 20S proteasome. This association activates the proteasome by triggering structural rearrangements that lead to the opening of the sealed gate structure of the complex [49–51]. PA200 is a monomer, but similar to the PA28 complexes, it docks onto the 20S proteasome orifice, and results in gate opening [52].

Activation of the 20S proteasome by PA28αβ was initially associated with the immune response since, like the immunoproteasome, PA28αβ is more abundant in immune tissues and is upregulated by interferon, suggesting that it has a role in antigen presentation [53]. The nuclear proteasome activator, PA28γ, was shown to activate the 20S both in vitro and in vivo, as demonstrated in the degradation
of the nuclear factor p21 [14,54] and the oncogenic steroid receptor coactivator-3 (SRC-3) [55]. This activation, though, appears to be selective, since PA28γ was unable to enhance the degradation of another unstructured cell cycle regulator, p27 [14].

More recently, it was realized that during adaptation to oxidative stress, the 20S immunoproteasome and the PA complexes were up-regulated, whereas the level of the 19S proteasome remained unchanged [55]. PA28 regulators were also tied to the clearance of oxidized proteins under stress conditions. PA28αβ and PA28γ increased the capacity of the 20S proteasome to degrade oxidized proteins, and PA28αβ could also induce the degradation of oxidized proteins by means of the immunoproteasome [43]. Similarly, in the process of embryonic stem cell differentiation, a significant increase in the levels and peptidase activity of the immunoproteasome and PA28αβ was seen, accompanied by reduced levels of carbonylated proteins, whereas silencing of PA28αβ caused accumulation of damaged proteins [56]. The role of PA200 in the regulation of 20S degradation is less straightforward, as on the one hand, PA200 was shown to enhance the 20S degradation of acetylated core histones [57], and the yeast ortholog of PA200, Blm10, enhanced degradation of the partially unstructured Dmm1 protein, a major regulator of mitochondrial morphology [58]. On the other hand, PA200 actually inhibited the proteolytic capacity of both the 20S proteasome and the immunoproteasome towards oxidized proteins [43]. Thus, it is possible that the PA200 particle and the PA28 regulators possess distinct functionalities.

The alternative proteasome activators exhibit yet another level of regulation, by forming highly complex interactions, as they appear to bind to the 20S proteasome, with or without the addition of a 19S regulator [36,43,59–61]. These associations generate multiple types of hybrid proteasomes that stimulate the degradation of non-ubiquitinated substrates [34,36,43,56,59,60,62,63] and possibly also ubiquitinated substrates. Future studies are likely to clarify how the structural diversity of the proteasome population enables compartments, cells or tissues to rapidly respond to various physiological conditions.

2.3. Gene Regulation

An increase in the proteolytic activity of the 20S proteasome can also be achieved by up-regulation of 20S subunit expression. Several studies involving Drosophila melanogaster, Caenorhabditis elegans, and mammalian cells have indicated that the increase in 20S proteasome expression is mediated by Nrf2, the master regulator of antioxidant transcriptional responses, following oxidative stress [64]. The synthesis of 20S proteasome subunits was shown to facilitate H2O2-induced adaptation to oxidative stress. A microarray-based survey, however, identified the Nrf2 pathway as enhancing the expression of both 19S and 20S proteasome subunits [65,66]. Further analysis indicated that sothiocyanate sulforaphane (SFN), an Nrf2 activator, specifically up-regulates the levels of the 20S proteasome’s catalytic β-subunits, leading to enhanced proteolytic activity in murine neuroblastoma cells [67]. Hence, while it is broadly accepted that stabilization of Nrf2 induces the expression and activation of 20S proteasomes, it remains to be determined whether increased expression of 19S subunits also plays a functional role in combating the oxidative stimulus.

Recently, another set of studies have demonstrated that induction of proteasome gene expression is also mediated by the related transcription factor, Nrf1 [68,69]. This transcription factor was shown to up-regulate the expression of both 20S and 19S genes in an Nrf2-independent manner, in response to
proteotoxic stress caused by proteasome inhibition. In the context of proteasomal gene activation, Nrf1 and Nrf2 appear to have only partially redundant roles, as their functions vary among cell types, tissues and cellular stresses [68–71]. Interestingly, Nrf1 is present in the cell in several alternative splicing forms, which have different cellular functions; furthermore, it was shown that the post-translational processing of Nrf1 is mediated by the 26S proteasome complex [72]. Thus, inhibition of the proteasome may result in a response favoring accumulation of the full-length variant, which in turn results in the induction of proteasome gene expression [72,73].

2.4. Variations in Subunit Composition

Another means to regulate the 20S proteasome function is through varying the building blocks of which it is composed. Indeed, a number of studies demonstrate that substantial diversity exists in the subunit composition of the 20S proteasome population. The most common variants are the immunoproteasome catalytic subunits: LMP2 (β1i), MECL-1 (β2i), and LMP7 (β5i), which can replace the conventional catalytic β-subunits (β1, β2 and β5) in different combinations (reviewed in [34,74]). Immunoproteasome subunits are constitutively expressed at low levels in various cell types, and at higher degrees in the immune and lymphatic tissues. In addition, they are up-regulated and incorporated into newly assembled 20S proteasomes in various tissues, in response to specific stimuli such as cytokine treatment, viral, fungal or bacterial infection, or injury, as well as ischemia reperfusion and muscle atrophy due to aging or diabetes ([62,75,76], and references within).

The major function of the immunoproteasome was traditionally thought to encompass the processing of specific peptides which would serve as antigenic peptides for MHC class I complex antigen presentation [75]. In recent years, however, the immunoproteasome has also emerged as an important player in the removal of damaged proteins in response to stress conditions [77–79]. This view is supported by the fact that the immunoproteasome was shown to be up-regulated during conditions of oxidative stress [78], and that depletion of the immunoproteasome from cells results in higher susceptibility and lower adaptation to such a state [77,78]. Moreover, when associated with 20S regulators such as the PA28αβ complex, immunoproteasomes display higher specific activity, as well as a greater capacity to degrade oxidized proteins [43].

In addition to the immunoproteasome, several other, tissue-specific 20S subunits have been identified, including the thymoproteasome and spermatoproteasome subunits [76]. The 20S proteasomes in the thymus predominantly contain a specific β6 subunit, and are thought to be primarily associated with antigenic peptide generation for positive selection of T lymphocytes [80], while the mammalian testis-specific proteasomes contain an alternative α6 subunit, implicated together with the nuclear PA200, in the degradation of acetylated histones during spermatogenesis and DNA repair [57,81,82]. Interestingly, both the thymo- and spermatoproteasome subunits can be found together with the immunoproteasome β3i and β2i subunits within the 20S proteasome (reviewed in [62]). These observations imply that various combinations of the 20S particle can be generated, however, the functional contributions of these divergent sets of proteasomes, and their unique, tissue-specific roles, if any, require further study.
2.5. Interacting Regulatory Proteins

Like other protein complexes, the function of the 20S proteasome can be modulated by interactions with regulatory proteins. To date, only a small number of studies demonstrating protein association-mediated enhancement or inactivation of the 20S proteasome’s proteolytic activity are available. These include the proteasome’s associations of NAD(P)H: quinone oxidoreductase 1 and 2 (NQO1 and NQO2), Hsp90, and Poly [ADP-ribose] polymerase 1 (PARP-1) (detailed below); however, our own research indicates that additional proteins that specifically regulate 20S proteasome function exist [83].

NQO1 is a stress-inducible flavoprotein that, in addition to its role as an antioxidant enzyme, was shown to physically bind the 20S proteasome [84] and protect IDPs from degradation [10]. These include p33, p53, p63, p73α, c-Fos, C/EBPα and PGC-1α [10,17–19,23,27,28,85]. It seems that the regulatory role of NQO1 is conserved throughout evolution, as the yeast ortholog, Lot6, was also reported to bind the 20S proteasome and control degradation in a redox-dependent manner [86]. Apart from its association with the proteasome, NQO1 has been shown in several instances to bind the IDP substrates in an NADH-dependent manner [10,27,28], or even to compete with the 20S proteasome for substrate interaction [27]. However, the exact molecular mechanism underlying NQO1’s ability to inhibit substrate degradation remains uncertain.

Cellular levels of NQO1 greatly increase in response to various stimuli, including oxidative stress, UV light, and ionizing radiation [87]. This up-regulation of NQO1 seems to regulate the levels of proteins containing IDRs, as was shown for p53 and p73 [88] and, more recently, for the myeloid differentiation factor, C/EBPα, following exposure of mice to γ-radiation [27]. Thus, while a correlation exists between NQO1 and the levels of partially folded proteins, it is still not known how up-regulation of NQO1 influences the 20S proteasome-mediated degradation of oxidatively damaged proteins. Overloading the 20S proteasome with oxidatively damaged proteins may clog the proteasome and inhibit degradation. Proteasomal dysfunction may then lead to decreased degradation of misfolded proteins, resulting in accumulation of oxidized proteins, and subsequent protein aggregation. It is possible that by preventing protein degradation, NQO1 regulates the flux of proteins which are targeted to the 20S proteasome, so as to maintain proper proteasomal function. Thus, NQO1 may be part of a system that interrupts this cascade of events, which otherwise could lead to various ailments, especially neurodegenerative diseases.

Interestingly, another oxidoreductase, NRH:quinone oxidoreductase 2 (NQO2) was also shown to rescue proteins with IDRs from 20S proteasome degradation [89,90]. Like NQO1, NQO2 is also induced by stress, and its cellular levels are increased by exposure to carcinogens and ionizing radiation. Accordingly, its up-regulation leads to stabilization of regulatory proteins containing IDRs such as p53.
and C/EBPα, in dependence on its electron donor, dihydronicotinamide riboside (NRH). NQO2 is thought to inhibit degradation by interaction with substrates, and its protective role appears to be independent of NQO1 [89,90]. Thus, it is tempting to speculate that oxidoreductases and possibly other structurally-related proteins take part in the concerted regulation of the stability of proteins comprising IDRs.

An additional protein that controls the catalytic capacity of the 20S proteasome is Hsp90. This molecular chaperone is one of the most abundant proteins in eukaryotic cells, comprising 1%–2% of total cellular proteins, even under basal conditions [91]. Hsp90, besides its role in stabilizing damaged proteins and preventing their aggregation [92], directly binds the 20S proteasome and influences its activity [93–95]. This interaction has been implicated in promoting the degradation of oxidatively damaged proteins [93], and may play a role in protecting the 20S proteasome from oxidation inactivation [96,97]. How Hsp90 influences 20S proteasome activity, and whether other chaperones aid in the recognition and targeting of substrates to the 20S proteasome, is still unclear.

During oxidative stress, a vast increase in proteasomal activity is detected in the nucleus, as early as 15 min after exposure. This enhanced proteolytic function is correlated with the presence and activity of PARP-1, and mediated by poly(ADP-ribosyl)ation [98,99]. In response to DNA damage, especially single-strand DNA breaks, PARP-1 modifies nuclear proteins by poly ADP-ribosylation, probably as a means of DNA damage signaling, to induce its repair [100]. Polymers of ADP-ribose, as well as auto-modified PARP-1, were shown by in vitro studies to interact non-covalently with the 20S proteasome, leading to an increase in proteasomal peptidase activity [101]. Likewise, inhibition of PARP-1 activity leads to a decrease in nuclear proteolytic activity [98]. Still to be determined is whether, in addition to oxidative stress, other DNA damaging conditions may also lead to PARP-1-mediated 20S proteasome activation. Nevertheless, these studies implicate PARP-1 as an important player in the proteasome-dependent removal of oxidation-damaged proteins in the nucleus. This conclusion is supported by the fact that decreasing PARP-1 activity during aging correlates with the decline of nuclear proteasome activity, and accumulation of oxidized nuclear proteins [102].

2.6. Substrate-Targeting Proteins

Several accessory proteins that are either transiently or constitutively expressed, can recognize and target specific substrates for degradation by the 20S proteasome. This has been shown for the cell cycle regulator p21: due to its crucial cellular task, several mechanisms have evolved to tightly regulate its abundance, both by ubiquitin-dependent and -independent routes [15,103]. In the latter instance, the so-called accessory protein MDM2 was shown to promote the 20S proteasome-mediated degradation of p21, by enhancing the association between p21 and the proteasome α7 subunit [104–107]. Paradoxically, during this process, MDM2, an ubiquitin E3 ligase, actually promotes ubiquitin-independent degradation. Recently, it was discovered that the 14-3-3τ protein also facilitates this process [108]. The 14-3-3τ protein promotes complex formation with MDM2, p21, and the 20S proteasome α7 subunit, triggering the degradation of the free p21 pool during the G1 phase. Considering that enhanced degradation of p21 leads to unchecked cell cycle progression and proliferation, it is not surprising that overexpression of MDM2 and 14-3-3τ was found to be correlated with cancerous processes [108,109].
This strategy of targeting proteins to the 20S proteasome for their degradation has also been harnessed by viruses, which employ the host cell’s protein degradation machinery for their own use [7]. For example, IκBα, a regulator of NFκB-mediated transcription [110], is targeted for degradation by the viral protein Tax, which is encoded by the human T-cell leukemia virus (HTLV-1) [111,112]. Tax was found to promote the interaction between IκBα and two subunits of the 20S proteasome, α3 and β4, probably leading to the degradation of IκBα, and the transformation and survival of cells infected with HTLV-1. Similarly, Tax was shown to associate with the tumor suppressor retinoblastoma (Rb) protein and target it for proteasomal degradation; however, further investigation is necessary, to determine whether the 26S or the 20S proteasome is involved in this process [113].

2.7. Post-Translational Modifications that Influence 20S Proteasome Activity

An additional approach to modifying the function of protein complexes is through post-translational modifications (PTMs). Various proteasome subunits have been reported to undergo phosphorylation, as well as acetylation, myristoylation, ubiquitination, modification with O-linked N-acetyl-glucosamine (O-GlcNAc), S-glutathionylation, and oxidation [3,114]. However, here we will only describe PTMs that can specifically and reversibly modulate 20S proteasome function.

In this regard, the tyrosine kinases c-Abl and Arg were reported to interact with the proteasome α4 subunit and phosphorylate it, leading to inhibition of proteasomal peptidase activity [115]. In contrast, association of the α3 and α4 subunits with the polo-like kinase (Plk), resulting in their phosphorylation, seems to increase the 20S proteasome proteolytic activity [116]. Moreover, reduction in the phosphorylation levels of α3 and α7 was shown to promote the disassembly of the 26S proteasome into 19S and 20S particles [117,118]. In a similar fashion, stress induction by interferon-γ, which triggers a decrease in the phosphorylation of both 20S and 19S subunits, leads to the disassembly of 26S proteasomes, and enhanced 20S proteasomal peptidase activity [118].

S-glutathiolation of cysteine residues within α subunits was found to affect 20S proteasome activity in yeast [119–121]. It appears that oxidation of Cys residues to sulfenic acid species, followed by glutathiolation, induces gate opening in the 20S proteasome, and consequently enhances degradation of damaged and partially unstructured proteins. This post-translational modification can be reversed by oxidoreductases. In yeast, the major glutaredoxin, Grx2, as well as the cytoplasmic thioredoxins 1 and 2, deglutathionylate the 20S proteasome [121]. Overall, S-glutathiolation is emerging as an important mechanism for coping with stressful conditions, enabling transient and reversible adaptation of the proteolytic machinery.

2.8. Cellular Localization

One major regulatory mechanism within cells is compartmentalization, thereby regulating the sub-cellular localization of the various cellular components. The 20S proteasome is considered to be ubiquitously distributed within the cell; however, it specifically accumulates within different sub-cellular domains. The majority of 20S proteasomes are found in the cytoplasm; a smaller sub-fraction is also associated with the cytoskeleton [122,123] and the microsomal fractions [34,124,125]. A lesser amount of 20S proteasome is present in the nucleus, where it localizes within the nucleoplasm, but not within the nucleoli. In addition, 20S proteasomes are also associated with different nuclear domains,
such as perinuclear centrosome-associated structures [126], matrix-associated nuclear bodies [125], and interchromatin nuclear speckles [127].

Notably, several studies have demonstrated the redistribution of proteasomes in response to different cues. In solid tumors, for example, stress conditions such as glucose starvation or hypoxia induce the recruitment of proteasomes to the nucleus [128], and in various types of cancerous cells, proteasomes were shown to accumulate on chromatin, together with PA200, in response to ionizing radiation [129]. Similarly, in MEF cells, proteasomes were shown to be recruited to mitochondria during mitochondrial depolarization [130]. Although such studies cannot always accurately differentiate between the 20S and the 26S proteasomes, it is only reasonable to assume that the cell possesses various regulatory mechanisms capable of relocating either particle towards specific locations, when needed.

Recent studies have also identified active 20S proteasomes in various extracellular compartments, such as serum, cerebrospinal fluid, and extracellular bronchoalveolar lavage fluids. Moreover, the levels of these so-called “circulating” or extracellular proteasomes are significantly elevated under stress conditions such as injury and various diseases, as well as in different types of cancer [131–136]. Circulating proteasomes were shown to be predominantly in the form of 20S particles but it remains to be established whether these extracellular proteasomes also act in association with regulatory complexes. The function of 20S proteasomes in the extracellular fluids is not clear, but they may be involved in the clearance of proteins outside of the cellular context, particularly in instances of stress, in the process of extracellular antigen processing, or even in a novel, “moonlighting” role, outside of the cellular boundaries. Taken together, the 20S proteasome appears to be impressively omnipresent both within and outside of the cellular context, and regulation of its localization is part of the cell’s ongoing efforts to maintain homeostasis, as well as to cope with stress.

3. Dynamic Regulation Following Oxidative Stress

Clearly, the aforementioned regulatory mechanisms are intertwined, as exemplified by their coordinated adaptation to oxidative stress [8,11]. Prior to the onset of stress, the 26S proteasome functions as the major degradation machinery. Following oxidative stress, the proteolytic capacity of the 20S proteasome is enhanced to rapidly remove oxidatively damaged proteins, thus preventing the pathogenicity caused by protein aggregates. Thus, within half an hour of oxidative stress, the activity of pre-existing 20S proteasomes is significantly elevated. In the nucleus, PARP-1 is involved in this process [98,99,101]; other activators may exist in the cytoplasm. A more persistent activation challenge (3–5 h) leads to a reduction in 26S proteasome activity, accumulation of ubiquitinated substrates and oxidized products, and an increase in 20S proteasome activity. The latter is due to the disassembly of the 26S proteasome, which, in turn, liberates active 20S particles [40,41]. After combating the oxidative stress, reassembly of the 26S proteasome takes place, and degradation of ubiquitinated substrates resumes [40].

Throughout sustained exposure to oxidative stress (at least 12 h), de novo synthesis of both constitutive and immuno-20S proteasomes occurs [78,79]. The newly generated 20S complexes can function on their own, or associate with the PAs and/or 19S regulatory complexes to form various proteasome assemblies [78]. Overall, temporal regulation enables a coordinated and accelerated response to take place, depending on the length and severity of the stress.
The fate of proteins comprising IDRs during this dynamic enhancement of 20S proteasome activity is still not known. It could be that their degradation is inevitable or, alternatively, that mechanisms have evolved to maintain their cellular abundance during the enhanced 20S catalytic process. The latter was recently demonstrated for α-synuclein, in which oxidation of two methionine residues under mild oxidation conditions prevented its degradation [137]. The 20S-mediated degradation of α-synuclein was restored, following enzymatic reversal of the oxidation. This observation might suggest that while oxidative conditions induce the transition from a natively folded to a partially unstructured state in some proteins, making them prone to 20S proteasome degradation, oxidation in IDPs may lead to structural stabilization, thus reducing their proteolytic susceptibility.

4. Conclusions and Perspectives

As research into the 20S proteasome activity and ubiquitin-independent degradation continues, new insights are helping to unravel the multiple mechanisms that coordinate this pathway (Figure 2). These intricate regulatory modes, and the key role played by the 20S proteasome system during oxidative stress, strongly suggest that 20S-mediated proteolysis is not an incidental occurrence, but rather an independent and highly complementary degradation route. It is reasonable to speculate that this degradation pathway is especially crucial at times when rapid elimination of proteins is required for maintaining cellular viability. Overcoming the enzymatic cascade required for ubiquitination not only enhances the rate of degradation, but also spares the energy costs associated with it. Thus, under basal conditions, proteolysis by the 20S proteasome may not constitute a dominant route for protein turnover; however, under stress conditions, the 20S proteasome degradation capacity is significantly enhanced, as well as assisted by its ATP and ubiquitin-independent regulators, PA28 αβ, PA28γ and PA200. Overall, there are probably multiple occasions in which switching off programmed degradation of native ubiquitin-tagged proteins by the 26S proteasome, thereby favoring 20S-mediated degradation, are essential for maintaining protein homeostasis.

Considering the narrow entrance of the 20S proteasome, and the lack of the 19S regulatory particle that causes proteins to unfold, substrates of this degradation route are primarily limited to partially unfolded proteins that can access its restricted opening. The two main groups of substrates that fit this category and were shown to be susceptible to 20S-mediated degradation are: the natively existing proteins with IDRs, as well as damaged proteins that have lost their tertiary structures. While the first group, IDR comprising proteins, mainly represents proteins with regulatory and signaling functions that coordinate key cellular processes [13], the second group comprises damaged proteins, which may lead to cytotoxicity and human pathologies and consequently, should be rapidly removed. It is not yet known whether there is any kind of selectivity between these two groups of substrates, and if there is, how is it achieved. Are partially unfolded proteins doomed to death when the 20S proteolytic capacity is enhanced? Several protective mechanisms have been discovered that rescue proteins with IDRs from degradation, such as interactions with “nanny” proteins that mask the unstructured regions [138], or oxidation-mediated structural stabilization [137]; however, the extent to which these processes are widespread is yet to be investigated. Furthermore, it is not yet known how NQO1, which rescues proteins with IDRs from degradation, influences the abundance of damaged, partially unfolded proteins. Similarly, while the presence of damaged proteins triggers 20S proteolytic capacity, it remains to be
discovered whether comparable mechanisms exist for regulating the abundance of partially unfolded proteins. Overall, the nature of the molecular cross-talk between 20S proteasome IDPs, and damaged proteins, requires further investigation.

**Figure 2.** Specific mechanisms that regulate the function of the 20S proteasome.

The importance of in-depth exploration of the 20S proteasome degradation pathway, also lies in its potential therapeutic benefits. To date, proteasome inhibitors as bortezomib and carfilzomib have been developed for treating certain cancers, especially multiple myeloma and mantle cell lymphoma [139–141], and many other such inhibitors are currently being tested for anti-tumor activities [142]. These drugs, however, target the chymotrypsin-like activity of the 20S proteasome, and inhibit the activities of both the 20S and 26S proteasomes. Thus, it remains to be explored whether selective drug intervention specifically inhibiting either proteasomes will improve the rates of cancer cell toxicity, and/or minimize the deleterious side effects of the current therapeutic regimens. Alternatively, 20S proteasomal activation, which is not only associated with increased lifespan, is also expected to demonstrate clinical utility, considering that oxidatively damaged proteins and protein aggregates are the cause of a large number of neurological diseases [3,8].
Acknowledgments

Michal Sharon is grateful for the financial support of a Starting Grant from the European Research Council (ERC) (FP7/2007-2013)/ERC Grant Agreement No. 239679, a Minerva Foundation Grant, an Acceleration Grant from the Israel Cancer Research Foundation and a grant from the Abisch-Frenkel Foundation. Michal Sharon is the incumbent of the Elaine Blond Career Development Chair.

Author Contributions

Both Gili Ben-Nissan and Michal Sharon contributed to reviewing the literature, and to the writing of this manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Powers, E.T.; Morimoto, R.I.; Dillin, A.; Kelly, J.W.; Balch, W.E. Biological and chemical approaches to diseases of proteostasis deficiency. *Annu. Rev. Biochem.* 2009, 78, 959–991.
2. Eden, E.; Geva-Zatorsky, N.; Issaeva, I.; Cohen, A.; Dekel, E.; Danon, T.; Cohen, L.; Mayo, A.; Alon, U. Proteome half-life dynamics in living human cells. *Science* 2011, 331, 764–768.
3. Schmidt, M.; Finley, D. Regulation of proteasome activity in health and disease. *Biochim. Biophys. Acta* 2014, 1843, 13–25.
4. Goldberg, A.L. Protein degradation and protection against misfolded or damaged proteins. *Nature* 2003, 426, 895–899.
5. Schwartz, A.L.; Ciechanover, A. Targeting proteins for destruction by the ubiquitin system: Implications for human pathobiology. *Annu. Rev. Pharmacol. Toxicol.* 2009, 49, 73–96.
6. Kish-Trier, E.; Hill, C.P. Structural biology of the proteasome. *Annu. Rev. Biophys.* 2013, 42, 29–49.
7. Hwang, J.; Winkler, L.; Kalejta, R.F. Ubiquitin-independent proteasomal degradation during oncogenic viral infections. *Biochim. Biophys. Acta* 2011, 1816, 147–157.
8. Pickering, A.M.; Davies, K.J. Degradation of damaged proteins: The main function of the 20S proteasome. *Prog. Mol. Biol. Transl. Sci.* 2012, 109, 227–248.
9. Erales, J.; Coffino, P. Ubiquitin-independent proteasomal degradation. *Biochim. Biophys. Acta* 2014, 1843, 216–221.
10. Asher, G.; Tsvetkov, P.; Kahana, C.; Shaul, Y. A mechanism of ubiquitin-independent proteasomal degradation of the tumor suppressors p53 and p73. *Genes Dev.* 2005, 19, 316–321.
11. Aiken, C.T.; Kaake, R.M.; Wang, X.; Huang, L. Oxidative stress-mediated regulation of proteasome complexes. *Mol. Cell. Proteomics* 2011, doi:10.1074/mcp.M110.006924.
12. Van der Lee, R.; Buljan, M.; Lang, B.; Weatheritt, R.J.; Daughdrill, G.W.; Dunker, A.K.; Fuxreiter, M.; Gough, J.; Gsponer, J.; Jones, D.T.; et al. Classification of intrinsically disordered regions and proteins. *Chem. Rev.* 2014, 114, 6589–6631.
13. Dyson, H.J.; Wright, P.E. Intrinsically unstructured proteins and their functions. *Nat. Rev. Mol. Cell Biol.* 2005, 6, 197–208.

14. Li, X.; Amazit, L.; Long, W.; Lonard, D.M.; Monaco, J.J.; O’Malley, B.W. Ubiquitin- and ATP-independent proteolytic turnover of p21 by the reggamma-proteasome pathway. *Mol. Cell* 2007, 26, 831–842.

15. Sheaff, R.J.; Singer, J.D.; Swanger, J.; Smitherman, M.; Roberts, J.M.; Clurman, B.E. Proteasomal turnover of p21CIP1 does not require p21CIP1 ubiquitination. *Mol. Cell* 2000, 5, 403–410.

16. Tambyrajah, W.S.; Bowler, L.D.; Medina-Palazon, C.; Sinclair, A.J. Cell cycle-dependent caspase-like activity that cleaves p27(KIP1) is the beta(1) subunit of the 20S proteasome. *Arch. Biochem. Biophys.* 2007, 466, 186–193.

17. Garate, M.; Wong, R.P.; Campos, E.I.; Wang, Y.; Li, G. NAD(P)H quinone oxidoreductase 1 inhibits the proteasomal degradation of the tumour suppressor p33(ING1b). *EMBO Rep.* 2008, 9, 576–581.

18. Rokah, O.H.; Shpilberg, O.; Granot, G. NAD(P)H quinone oxidoreductase protects TAp63gamma from proteasomal degradation and regulates TAp63gamma-dependent growth arrest. *PLoS One* 2010, 5, e11401.

19. Patrick, B.A.; Gong, X.; Jaiswal, A.K. Disruption of NAD(P)H:Quinone oxidoreductase 1 gene in mice leads to 20S proteasomal degradation of p63 resulting in thinning of epithelium and chemical-induced skin cancer. *Oncogene* 2011, 30, 1098–1107.

20. Kalejta, R.F.; Shenk, T. Proteasome-dependent, ubiquitin-independent degradation of the Rb family of tumor suppressors by the human cytomegalovirus pp71 protein. *Proc. Natl. Acad. Sci. USA* 2003, 100, 3263–3268.

21. Wiggins, C.M.; Tsvetkov, P.; Johnson, M.; Joyce, C.L.; Lamb, C.A.; Bryant, N.J.; Komander, D.; Shaul, Y.; Cook, S.J. BIM(EL), an intrinsically disordered protein, is degraded by 20S proteasomes in the absence of poly-ubiquitylation. *J. Cell Sci.* 2011, 124, 969–977.

22. Stewart, D.P.; Koss, B.; Bathina, M.; Perciavalle, R.M.; Bisanz, K.; Opferman, J.T. Ubiquitin-independent degradation of antiapoptotic MCL-1. *Mol. Cell. Biol.* 2010, 30, 3099–3110.

23. Adler, J.; Reuven, N.; Kahana, C.; Shaul, Y. c-Fos proteasomal degradation is activated by a default mechanism, and its regulation by NAD(P)H:Quinone oxidoreductase 1 determines c-Fos serum response kinetics. *Mol. Cell. Biol.* 2010, 30, 3767–3778.

24. Alvarez-Castelao, B.; Castano, J.G. Mechanism of direct degradation of Ikappabalpha by 20S proteasome. *FEBS Lett.* 2005, 579, 4797–4802.

25. Asher, G.; Bercovich, Z.; Tsvetkov, P.; Shaul, Y.; Kahana, C. 20S proteasomal degradation of ornithine decarboxylase is regulated by NQO1. *Mol. Cell* 2005, 17, 645–655.

26. Kong, X.; Alvarez-Castelao, B.; Lin, Z.; Castano, J.G.; Caro, J. Constitutive/hypoxic degradation of HIF-alpha proteins by the proteasome is independent of von hippel lindau protein ubiquitylation and the transactivation activity of the protein. *J. Biol. Chem.* 2007, 282, 15498–15505.

27. Xu, J.; Jaiswal, A.K. NAD(P)H:Quinone oxidoreductase 1 (NQO1) competes with 20S proteasome for binding with C/EBPAlpha leading to its stabilization and protection against radiation-induced myeloproliferative disease. *J. Biol. Chem.* 2012, 287, 41608–41618.
28. Adamovich, Y.; Shlomai, A.; Tsvetkov, P.; Umansky, K.B.; Reuven, N.; Estall, J.L.; Spiegelman, B.M.; Shaul, Y. The protein level of PGC-1alpha, a key metabolic regulator, is controlled by NADH-NQO1. *Mol. Cell. Biol.* **2013**, *33*, 2603–2613.

29. David, D.C.; Layfield, R.; Serpell, L.; Narain, Y.; Goedert, M.; Spillantini, M.G. Proteasomal degradation of tau protein. *J. Neurochem.* **2002**, *83*, 176–185.

30. Cardozo, C.; Michaud, C. Proteasome-mediated degradation of tau proteins occurs independently of the chymotrypsin-like activity by a nonprocessive pathway. *Arch. Biochem. Biophys.* **2002**, *408*, 103–110.

31. Tofaris, G.K.; Layfield, R.; Spillantini, M.G. Alpha-synuclein metabolism and aggregation is linked to ubiquitin-independent degradation by the proteasome. *FEBS Lett.* **2001**, *509*, 22–26.

32. Prilusky, J.; Felder, C.E.; Zeev-Ben-Mordehai, T.; Rydberg, E.H.; Man, O.; Beckmann, J.S.; Silman, I.; Sussman, J.L. Foldindex: A simple tool to predict whether a given protein sequence is intrinsically unfolded. *Bioinformatics* **2005**, *21*, 3435–3438.

33. Jariel-Encontre, I.; Bossis, G.; Piechaczyk, M. Ubiquitin-independent degradation of proteins by the proteasome. *Biochim. Biophys. Acta* **2008**, *1786*, 153–177.

34. Fabre, B.; Lambour, T.; Delobel, J.; Amalric, F.; Monsarrat, B.; Burlet-Schiltz, O.; Bousquet-Dubouch, M.P. Subcellular distribution and dynamics of active proteasome complexes unraveled by a workflow combining *in vivo* complex cross-linking and quantitative proteomics. *Mol. Cell. Proteomics* **2012**, *12*, 687–699.

35. Babbitt, S.E.; Kiss, A.; Deffenbaugh, A.E.; Chang, Y.H.; Bailly, E.; Erdjument-Bromage, H.; Tempst, P.; Buranda, T.; Sklar, L.A.; Baumler, J.; *et al.* ATP hydrolysis-dependent disassembly of the 26S proteasome is part of the catalytic cycle. *Cell* **2005**, *121*, 553–565.

36. Tanahashi, N.; Murakami, Y.; Minami, Y.; Shimbara, N.; Hendil, K.B.; Tanaka, K. Hybrid proteasomes. Induction by interferon-gamma and contribution to ATP-dependent proteolysis. *J. Biol. Chem.* **2000**, *275*, 14336–14345.

37. Brooks, P.; Fuertes, G.; Murray, R.Z.; Bose, S.; Knecht, E.; Rechsteiner, M.C.; Hendil, K.B.; Tanaka, K.; Dyson, J.; Rivett, J. Subcellular localization of proteasomes and their regulatory complexes in mammalian cells. *Biochem. J.* **2000**, *346*, 155–161.

38. Baugh, J.M.; Viktorova, E.G.; Pilipenko, E.V. Proteasomes can degrade a significant proportion of cellular proteins independent of ubiquitination. *J. Mol. Biol.* **2009**, *386*, 814–827.

39. Hohn, T.J.; Grune, T. The proteasome and the degradation of oxidized proteins: Part III-redox regulation of the proteasomal system. *Redox Biol.* **2014**, *2*, 388–394.

40. Wang, X.; Yen, J.; Kaiser, P.; Huang, L. Regulation of the 26S proteasome complex during oxidative stress. *Sci. Signal.* **2010**, *3*, ra88.

41. Grune, T.; Catalgol, B.; Licht, A.; Ermak, G.; Pickering, A.M.; Ngo, J.K.; Davies, K.J. HSP70 mediates dissociation and reassociation of the 26S proteasome during adaptation to oxidative stress. *Free Radic. Biol. Med.* **2011**, *51*, 1355–1364.

42. Huang, Q.; Wang, H.; Perry, S.W.; Figueiredo-Pereira, M.E. Negative regulation of 26S proteasome stability via calpain-mediated cleavage of Rpn10 subunit upon mitochondrial dysfunction in neurons. *J. Biol. Chem.* **2013**, *288*, 12161–12174.
43. Pickering, A.M.; Davies, K.J. Differential roles of proteasome and immunoproteasome regulators Pa28alphabeta, Pa28gamma and Pa200 in the degradation of oxidized proteins. *Arch. Biochem. Biophys.* 2012, 523, 181–190.

44. Imai, J.; Maruya, M.; Yashirola, H.; Yahara, I.; Tanaka, K. The molecular chaperone Hsp90 plays a role in the assembly and maintenance of the 26S proteasome. *EMBO J.* 2003, 22, 3557–3567.

45. Inai, Y.; Nishikimi, M. Increased degradation of oxidized proteins in yeast defective in 26S proteasome assembly. *Arch. Biochem. Biophys.* 2002, 404, 279–284.

46. Shringarpure, R.; Grune, T.; Mehlhase, J.; Davies, K.J. Ubiquitin conjugation is not required for the degradation of oxidized proteins by proteasome. *J. Biol. Chem.* 2003, 278, 311–318.

47. Tsvetkov, P.; Myers, N.; Eliav, R.; Adamovich, Y.; Hagai, T.; Adler, J.; Navon, A.; Shaul, Y. NADH binds and stabilizes the 26S proteasomes independent of ATP. *J. Biol. Chem.* 2014, 289, 11272–11281.

48. Ma, C.P.; Slaughter, C.A.; DeMartino, G.N. Identification, purification, and characterization of a protein activator (PA28) of the 20S proteasome (macropain). *J. Biol. Chem.* 1992, 267, 10515–10523.

49. Stadtmueller, B.M.; Hill, C.P. Proteasome activators. *Mol. Cell* 2011, 41, 8–19.

50. Sugiyama, M.; Sahashi, H.; Kurimoto, E.; Takata, S.; Yagi, H.; Kanai, K.; Sakata, E.; Minami, Y.; Tanaka, K.; Kato, K. Spatial arrangement and functional role of alpha subunits of proteasome activator PA28 in hetero-oligomeric form. *Biochem. Biophys. Res. Commun.* 2013, 432, 141–145.

51. Whitby, F.G.; Masters, E.I.; Kramer, L.; Knowlton, J.R.; Yao, Y.; Wang, C.C.; Hill, C.P. Structural basis for the activation of 20S proteasomes by 11S regulators. *Nature* 2000, 408, 115–120.

52. Ortega, J.; Heymann, J.B.; Kajava, A.V.; Ustrell, V.; Rechsteiner, M.; Steven, A.C. The axial channel of the 20S proteasome opens upon binding of the PA200 activator. *J. Mol. Biol.* 2005, 346, 1221–1227.

53. Sijts, A.; Sun, Y.; Janek, K.; Kral, S.; Paschen, A.; Schadendorf, D.; Kloetzel, P.M. The role of the proteasome activator PA28 in MHC class I antigen processing. *Mol. Immunol.* 2002, 39, 165–169.

54. Chen, X.; Barton, L.F.; Chi, Y.; Clurman, B.E.; Roberts, J.M. Ubiquitin-independent degradation of cell-cycle inhibitors by the reggamma proteasome. *Mol. Cell* 2007, 26, 843–852.

55. Li, X.; Lonard, D.M.; Jung, S.Y.; Malovannaya, A.; Feng, Q.; Qin, J.; Tsai, S.Y.; Tsai, M.J.; O’Malley, B.W. The SRC-3/AIB1 coactivator is degraded in a ubiquitin- and ATP-independent manner by the REGgamma proteasome. *Cell* 2006, 124, 381–392.

56. Hernebring, M.; Fredriksson, A.; Liljevald, M.; Cvijovic, M.; Norman, K.; Wiseman, J.; Semb, H.; Nystrom, T. Removal of damaged proteins during ES cell fate specification requires the proteasome activator PA28. *Sci. Rep.* 2013, doi:10.1038/srep01381.

57. Qian, M.X.; Pang, Y.; Liu, C.H.; Haratake, K.; Du, B.Y.; Ji, D.Y.; Wang, G.F.; Zhu, Q.Q.; Song, W.; Yu, Y.; et al. Acetylation-mediated proteasomal degradation of core histones during DNA repair and spermatogenesis. *Cell* 2013, 153, 1012–1024.

58. Tar, K.; Dange, T.; Yang, C.; Yao, Y.; Bulteau, A.L.; Salcedo, E.F.; Braigen, S.; Bouillaud, F.; Finley, D.; Schmidt, M. Proteasomes associated with the Blm10 activator protein antagonize mitochondrial fission through degradation of the fission protein Dnm1. *J. Biol. Chem.* 2014, 289, 12145–12156.
59. Cascio, P.; Call, M.; Petre, B.M.; Walz, T.; Goldberg, A.L. Properties of the hybrid form of the 26S proteasome containing both 19S and PA28 complexes. *EMBO J.* 2002, 21, 2636–2645.

60. Li, J.; Horak, K.M.; Su, H.; Sanbe, A.; Robbins, J.; Wang, X. Enhancement of proteasomal function protects against cardiac proteinopathy and ischemia/reperfusion injury in mice. *J. Clin. Investig.* 2011, 121, 3689–3700.

61. Blickwedehl, J.; Agarwal, M.; Seong, C.; Pandita, R.K.; Melendy, T.; Sung, P.; Pandita, T.K.; Bangia, N. Role for proteasome activator PA200 and postglutamyl proteasome activity in genomic stability. *Proc. Natl. Acad. Sci. USA* 2008, 105, 16165–16170.

62. Meiners, S.; Keller, I.E.; Semren, N.; Caniard, A. Regulation of the proteasome: Evaluating the lung proteasome as a new therapeutic target. *Antioxid. Redox Signal.* 2014, doi:10.1089/ars.2013.5798.

63. Schmidt, M.; Haas, W.; Crosas, B.; Santamaria, P.G.; Gygi, S.P.; Walz, T.; Finley, D. The HEAT repeat protein Blm10 regulates the yeast proteasome by capping the core particle. *Nat. Struct. Mol. Biol.* 2005, 12, 294–303.

64. Pickering, A.M.; Linder, R.A.; Zhang, H.; Forman, H.J.; Davies, K.J. Nrf2-dependent induction of proteasome and Pa28alphabeta regulator are required for adaptation to oxidative stress. *J. Biol. Chem.* 2012, 287, 10021–10031.

65. Kwak, M.K.; Wakabayashi, N.; Itoh, K.; Motohashi, H.; Yamamoto, M.; Kensler, T.W. Modulation of gene expression by cancer chemopreventive dithiolethiones through the Keap1-Nrf2 pathway. Identification of novel gene clusters for cell survival. *J. Biol. Chem.* 2003, 278, 8135–8145.

66. Kwak, M.K.; Wakabayashi, N.; Greenlaw, J.L.; Yamamoto, M.; Kensler, T.W. Antioxidants enhance mammalian proteasome expression through the Keap1-Nrf2 signaling pathway. *Mol. Cell. Biol.* 2003, 23, 8786–8794.

67. Kwak, M.K.; Cho, J.M.; Huang, B.; Shin, S.; Kensler, T.W. Role of increased expression of the proteasome in the protective effects of sulforaphane against hydrogen peroxide-mediated cytotoxicity in murine neuroblastoma cells. *Free Radic. Biol. Med.* 2007, 43, 809–817.

68. Lee, C.S.; Lee, C.; Hu, T.; Nguyen, J.M.; Zhang, J.; Martin, M.V.; Vawter, M.P.; Huang, E.J.; Chan, J.Y. Loss of nuclear factor E2-related factor 1 in the brain leads to dysregulation of proteasome gene expression and neurodegeneration. *Proc. Natl. Acad. Sci. USA* 2011, 108, 8408–8413.

69. Radhakrishnan, S.K.; Lee, C.S.; Young, P.; Beskow, A.; Chan, J.Y.; Deshaies, R.J. Transcription factor Nrf1 mediates the proteasome recovery pathway after proteasome inhibition in mammalian cells. *Mol. Cell* 2010, 38, 17–28.

70. Lee, C.S.; Ho, D.V.; Chan, J.Y. Nuclear factor-erythroid 2-related factor 1 regulates expression of proteasome genes in hepatocytes and protects against endoplasmic reticulum stress and steatosis in mice. *FEBS J.* 2013, 280, 3609–3620.

71. Tsuchiya, Y.; Taniguchi, H.; Ito, Y.; Morita, T.; Karim, M.R.; Ohtake, N.; Fukagai, K.; Ito, T.; Okamuro, S.; Iemura, S.; et al. The casein kinase 2-Nrf1 axis controls the clearance of ubiquitinated proteins by regulating proteasome gene expression. *Mol. Cell Biol.* 2013, 33, 3461–3472.
72. Chepelev, N.L.; Bennitz, J.D.; Huang, T.; McBride, S.; Willmore, W.G. The Nrf1 CNC-bZIP protein is regulated by the proteasome and activated by hypoxia. *PLoS One* **2011**, *6*, e29167.

73. Steffen, J.; Seeger, M.; Koch, A.; Kruger, E. Proteasomal degradation is transcriptionally controlled by TCF11 via an ERAD-dependent feedback loop. *Mol. Cell* **2010**, *40*, 147–158.

74. Ferrington, D.A.; Gregerson, D.S. Immunoproteasomes: Structure, function, and antigen presentation. *Prog. Mol. Biol. Transl. Sci.* **2012**, *109*, 75–112.

75. Ebstein, F.; Kloetzel, P.M.; Kruger, E.; Seifert, U. Emerging roles of immunoproteasomes beyond MHC class I antigen processing. *Cell. Mol. Life Sci.* **2012**, *69*, 2543–2558.

76. Kniepert, A.; Groettrup, M. The unique functions of tissue-specific proteasomes. *Trends Biochem. Sci.* **2014**, *39*, 17–24.

77. Ethen, C.M.; Hussong, S.A.; Reilly, C.; Feng, X.; Olsen, T.W.; Ferrington, D.A. Transformation of the proteasome with age-related macular degeneration. *FEBS Lett.* **2007**, *581*, 885–890.

78. Pickering, A.M.; Koop, A.L.; Teoh, C.Y.; Ermlak, G.; Grune, T.; Davies, K.J. The immunoproteasome, the 20S proteasome and the PA28alphabeta proteasome regulator are oxidative-stress-adaptive proteolytic complexes. *Biochem. J.* **2010**, *432*, 585–594.

79. Seifert, U.; Bialy, L.P.; Ebstein, F.; Bech-Otschir, D.; Voigt, A.; Schroter, F.; Prozorovski, T.; Lange, N.; Steffen, J.; Rieger, M.; et al. Immunoproteasomes preserve protein homeostasis upon interferon-induced oxidative stress. *Cell* **2010**, *142*, 613–624.

80. Murata, S.; Sasaki, K.; Kishimoto, T.; Niwa, S.; Hayashi, H.; Takahama, Y.; Tanaka, K. Regulation of CD8+ T cell development by thymus-specific proteasomes. *Science* **2007**, *316*, 1349–1353.

81. Klein, L.; Hinterberger, M.; Wirnsberger, G.; Kyewski, B. Antigen presentation in the thymus for positive selection and central tolerance induction. *Nat. Rev. Immunol.* **2009**, *9*, 833–844.

82. Zhong, L.; Belote, J.M. The testis-specific proteasome subunit Prosalpha6T of *D. Melanogaster* is required for individualization and nuclear maturation during spermatogenesis. *Development* **2007**, *134*, 3517–3525.

83. Moscovitz, M.; Ben-Nissan, G.; Fainer, I.; Polack, D.; Mizrachi, L.; Sharon, M. Department of Biological Chemistry, Weizmann Institute of Science, Rehovot, Israel. Unpublished work, 2014.

84. Moscovitz, O.; Tsvetkov, P.; Hazan, N.; Michaelevski, I.; Keisar, H.; Ben-Nissan, G.; Shaul, Y.; Sharon, M. A mutually inhibitory feedback loop between the 20S proteasome and its regulator, NQO1. *Mol. Cell* **2012**, *47*, 76–86.

85. Patrick, B.A.; Jaiswal, A.K. Stress-induced NQO1 controls stability of C/EBPalpha against 20S proteasomal degradation to regulate p63 expression with implications in protection against chemical-induced skin cancer. *Oncogene* **2012**, *31*, 4362–4371.

86. Sollner, S.; Schober, M.; Wagner, A.; Prem, A.; Lorkova, L.; Palfey, B.A.; Groll, M.; Macheroux, P. Quinone reductase acts as a redox switch of the 20S yeast proteasome. *EMBO Rep.* **2009**, *10*, 65–70.

87. De Long, M.J.; Prochaska, H.J.; Talalay, P. Induction of NAD(P)H:Quinone reductase in murine hepatoma cells by phenolic antioxidants, azo dyes, and other chemoprotectors: A model system for the study of anticarcinogens. *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 787–791.
88. Asher, G.; Dym, O.; Tsvetkov, P.; Adler, J.; Shaul, Y. The crystal structure of NAD(P)H quinone oxidoreductase 1 in complex with its potent inhibitor dicoumarol. *Biochemistry* **2006**, *45*, 6372–6378.

89. Xu, J.; Patrick, B.A.; Jaiswal, A.K. NRH:Quinone oxidoreductase 2 (NQO2) protein competes with the 20S proteasome to stabilize transcription factor CCAAT enhancer-binding protein alpha (C/EBPalpha), leading to protection against gamma radiation-induced myeloproliferative disease. *J. Biol. Chem.* **2013**, *288*, 34799–34808.

90. Gong, X.; Kole, L.; Iskander, K.; Jaiswal, A.K. NRH:Quinone oxidoreductase 2 and NAD(P)H:Quinone oxidoreductase 1 protect tumor suppressor p53 against 20S proteasomal degradation leading to stabilization and activation of p53. *Cancer Res.* **2007**, *67*, 5380–5388.

91. Csermely, P.; Schnaider, T.; Soti, C.; Prohaszka, Z.; Nardai, G. The 90-kDa molecular chaperone family: Structure, function, and clinical applications. A comprehensive review. *Pharmacol. Ther.* **1998**, *79*, 129–168.

92. Daturpalli, S.; Waudby, C.A.; Meehan, S.; Jackson, S.E. Hsp90 inhibits alpha-synuclein aggregation by interacting with soluble oligomers. *J. Mol. Biol.* **2013**, *425*, 4614–4628.

93. Whittier, J.E.; Xiong, Y.; Rechsteiner, M.C.; Squier, T.C. Hsp90 enhances degradation of oxidized calmodulin by the 20S proteasome. *J. Biol. Chem.* **2004**, *279*, 46135–46142.

94. Eleuteri, A.M.; Cuccioloni, M.; Bellesi, J.; Lupidi, G.; Fioretti, E.; Angeletti, M. Interaction of Hsp90 with 20S proteasome: Thermodynamic and kinetic characterization. *Proteins* **2002**, *48*, 169–177.

95. Wagner, B.J.; Margolis, J.W. Age-dependent association of isolated bovine lens multicatalytic proteinase complex (proteasome) with heat-shock protein 90, an endogenous inhibitor. *Arch. Biochem. Biophys.* **1995**, *323*, 455–462.

96. Conconi, M.; Szweda, L.I.; Levine, R.L.; Stadtman, E.R.; Friguet, B. Age-related decline of rat liver multicatalytic proteinase activity and protection from oxidative inactivation by heat-shock protein 90. *Arch. Biochem. Biophys.* **1996**, *331*, 232–240.

97. Conconi, M.; Petropoulos, I.; Emod, I.; Turlin, E.; Biville, F.; Friguet, B. Protection from oxidative inactivation of the 20S proteasome by heat-shock protein 90. *Biochem. J.* **1998**, *333*, 407–415.

98. Ullrich, O.; Reinheckel, T.; Sitte, N.; Hass, R.; Grune, T.; Davies, K.J. Poly-ADP ribose polymerase activates nuclear proteasome to degrade oxidatively damaged histones. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 6223–6228.

99. Catalgol, B.; Wendt, B.; Grimm, S.; Breusing, N.; Ozer, N.K.; Grune, T. Chromatin repair after oxidative stress: Role of PARP-mediated proteasome activation. *Free Radic. Biol. Med.* **2010**, *48*, 673–680.

100. Banasik, M.; Komura, H.; Shimoyama, M.; Ueda, K. Specific inhibitors of poly(ADP-ribose) synthetase and mono(ADP-ribosyl)transferase. *J. Biol. Chem.* **1992**, *267*, 1569–1575.

101. Mayer-Kuckuk, P.; Ullrich, O.; Ziegler, M.; Grune, T.; Schweiger, M. Functional interaction of poly(ADP-ribose) with the 20S proteasome in vitro. *Biochem. Biophys. Res. Commun.* **1999**, *259*, 576–581.
102. Bakondi, E.; Catalgol, B.; Bak, I.; Jung, T.; Bozaykut, P.; Bayramicli, M.; Ozer, N.K.; Grune, T. Age-related loss of stress-induced nuclear proteasome activation is due to low PARP-1 activity. *Free Radic. Biol. Med.* 2011, 50, 86–92.

103. Abbas, T.; Sivaprasad, U.; Terai, K.; Amador, V.; Pagano, M.; Dutta, A. PCNA-dependent regulation of p21 ubiquitylation and degradation via the CRL4Cdt2 ubiquitin ligase complex. *Genes Dev.* 2008, 22, 2496–2506.

104. Xu, H.; Zhang, Z.; Li, M.; Zhang, R. MDM2 promotes proteasomal degradation of p21WAF1 via a conformation change. *J. Biol. Chem.* 2010, 285, 18407–18414.

105. Jin, Y.; Lee, H.; Zeng, S.X.; Dai, M.S.; Lu, H. MDM2 promotes p21WAF1/CIP1 proteasomal turnover independently of ubiquitylation. *EMBO J.* 2003, 22, 6365–6377.

106. Zhang, Z.; Wang, H.; Li, M.; Agrawal, S.; Chen, X.; Zhang, R. MDM2 is a negative regulator of p21WAF1/CIP1, independent of p53. *J. Biol. Chem.* 2004, 279, 16000–16006.

107. Toutou, R.; Richardson, J.; Bose, S.; Nakanishi, M.; Rivett, J.; Allday, M.J. A degradation signal located in the C-terminus of p21WAF1/CIP1 is a binding site for the C8 alpha-subunit of the 20S proteasome. *EMBO J.* 2001, 20, 2367–2375.

108. Wang, B.; Liu, K.; Lin, H.Y.; Bellam, N.; Ling, S.; Lin, W.C. 14-3-3-Tau regulates ubiquitin-independent proteasomal degradation of p21, a novel mechanism of p21 downregulation in breast cancer. *Mol. Cell. Biol.* 2010, 30, 1508–1527.

109. Onel, K.; Cordon-Cardo, C. MDM2 and prognosis. *Mol. Cancer Res.* 2004, 2, 1–8.

110. Oezdinghaus, A.; Ghosh, S. The NF-κB family of transcription factors and its regulation. *Cold Spring Harb. Perspect. Biol.* 2009, doi:10.1101/cshperspect.a000034.

111. Petropoulos, L.; Hiscott, J. Association between HTLV-1 Tax and I kappa B alpha is dependent on the I kappa B alpha phosphorylation state. *Virology* 1998, 252, 189–199.

112. Rousset, R.; Desbois, C.; Bantignies, F.; Jalinot, P. Effects on NF-kappa B1/p105 processing of the interaction between the HTLV-1 transactivator Tax and the proteasome. *Nature* 1996, 381, 328–331.

113. Kehn, K.; Fuente Cde, L.; Strouss, K.; Berro, R.; Jiang, H.; Brady, J.; Mahieux, R.; Pumfery, A.; Bottazzi, M.E.; Kashanchi, F. The HTLV-I Tax oncoprotein targets the retinoblastoma protein for proteasomal degradation. *Oncogene* 2005, 24, 525–540.

114. Cui, Z.; Scruggs, S.B.; Gilda, J.E.; Ping, P.; Gomes, A.V. Regulation of cardiac proteasomes by ubiquitination, SUMOylation, and beyond. *J. Mol. Cell. Cardiol.* 2013, 71, 32–42.

115. Liu, X.; Huang, W.; Li, C.; Li, P.; Yuan, J.; Li, X.; Qiu, X.B.; Ma, Q.; Cao, C. Interaction between c-Abl and Arg tyrosine kinases and proteasome subunit PSMA7 regulates proteasome degradation. *Mol. Cell* 2006, 22, 317–327.

116. Feng, Y.; Longo, D.L.; Ferris, D.K. Polo-like kinase interacts with proteasomes and regulates their activity. *Cell Growth Differ.* 2001, 12, 29–37.

117. Day, S.M.; Divald, A.; Wang, P.; Davis, F.; Bartolone, S.; Jones, R.; Powell, S.R. Impaired assembly and post-translational regulation of 26S proteasome in human end-stage heart failure. *Circ. Heart Fail.* 2013, 6, 544–549.

118. Bose, S.; Stratford, F.L.; Broadfoot, K.I.; Mason, G.G.; Rivett, A.J. Phosphorylation of 20S proteasome alpha subunit C8 (alpha7) stabilizes the 26S proteasome and plays a role in the regulation of proteasome complexes by gamma-interferon. *Biochem. J.* 2004, 378, 177–184.
119. Silva, G.M.; Netto, L.E.; Simoes, V.; Santos, L.F.; Gozzo, F.C.; Demasi, M.A.; Oliveira, C.L.; Bicev, R.N.; Klitzke, C.F.; Sogayar, M.C.; et al. Redox control of 20S proteasome gating. *Antioxid. Redox Signal.* 2012, 16, 1183–1194.

120. Demasi, M.; Silva, G.M.; Netto, L.E. 20S proteasome from *Saccharomyces cerevisiae* is responsive to redox modifications and is S-glutathionylated. *J. Biol. Chem.* 2003, 278, 679–685.

121. Silva, G.M.; Netto, L.E.; Discola, K.F.; Piassa-Filho, G.M.; Pimenta, D.C.; Barcena, J.A.; Demasi, M. Role of glutaredoxin 2 and cytosolic thioredoxins in cysteiny1-based redox modification of the 20S proteasome. *FEBS J.* 2008, 275, 2942–2955.

122. De Sa, M.F.G.; de Sa, C.M.; Harper, F.; Olink-Coux, M.; Huesca, M.; Scherrer, K. The association of prosomes with some of the intermediate filament networks of the animal cell. *J. Cell Biol.* 1988, 107, 1517–1530.

123. Olink-Coux, M.; Arcangeletti, C.; Pinardi, F.; Minisini, R.; Huesca, M.; Chezzi, C.; Scherrer, K. Cytolocaiton of prosome antigens on intermediate filament subnetworks of cytokeratin, vimentin and desmin type. *J. Cell Sci.* 1994, 107, 353–366.

124. Brooks, P.; Murray, R.Z.; Mason, G.G.; Hendil, K.B.; Rivett, A.J. Association of immunoproteasomes with the endoplasmic reticulum. *Biochem. J.* 2000, 352, 611–615.

125. Wojcik, C.; DeMartino, G.N. Intracellular localization of proteasomes. *Int. J. Biochem. Cell Biol.* 2003, 35, 579–589.

126. Wigley, W.C.; Fabunmi, R.P.; Lee, M.G.; Marino, C.R.; Muallem, S.; DeMartino, G.N.; Thomas, P.J. Dynamic association of proteasomal machinery with the centrosome. *J. Cell Biol.* 1999, 145, 481–490.

127. Baldin, V.; Militello, M.; Thomas, Y.; Doucet, C.; Fic, W.; Boireau, S.; Jariel-Encontre, I.; Picchaczyk, M.; Bertrand, E.; Tazi, J.; et al. A novel role for PA28γ-proteasome in nuclear speckle organization and SR protein trafficking. *Mol. Biol. Cell* 2008, 19, 1706–1716.

128. Ogiso, Y.; Tomida, A.; Tsuruo, T. Nuclear localization of proteasomes participates in stress-inducible resistance of solid tumor cells to topoisomerase II-directed drugs. *Cancer Res.* 2002, 62, 5008–5012.

129. Blickwedehl, J.; McEvoy, S.; Wong, I.; Kousis, P.; Clements, J.; Elliott, R.; Cresswell, P.; Liang, P.; Bangia, N. Proteasomes and proteasome activator 200 kDa (PA200) accumulate on chromatin in response to ionizing radiation. *Radiat. Res.* 2007, 167, 663–674.

130. Yoshii, S.R.; Kishi, C.; Ishihara, N.; Mizushima, N. Parkin mediates proteasome-dependent protein degradation and rupture of the outer mitochondrial membrane. *J. Biol. Chem.* 2011, 286, 19630–19640.

131. Bochmann, I.; Ebstein, F.; Lehmann, A.; Wohlschlaeger, J.; Sixt, S.U.; Kloetzel, P.M.; Dahlmann, B. T lymphocytes export proteasomes by way of microparticles: A possible mechanism for generation of extracellular proteasomes. *J. Cell. Mol. Med.* 2014, 18, 59–68.

132. Majetschak, M.; Perez, M.; Sorell, L.T.; Lam, J.; Maldonado, M.E.; Hoffman, R.W. Circulating 20S proteasome levels in patients with mixed connective tissue disease and systemic lupus erythematosus. *Clin. Vaccine Immunol.* 2008, 15, 1489–1493.

133. Majetschak, M.; Zedler, S.; Romero, J.; Albright, J.M.; Kraft, R.; Kovacs, E.J.; Faist, E.; Gamelli, R.L. Circulating proteasomes after burn injury. *J. Burn Care Res.* 2010, 31, 243–250.
134. Mueller, O.; Anlasik, T.; Wiedemann, J.; Thomassen, J.; Wohlschlaeger, J.; Hagel, V.; Keyvani, K.; Schwieger, I.; Dahlmann, B.; Sure, U.; et al. Circulating extracellular proteasome in the cerebrospinal fluid: A study on concentration and proteolytic activity. *J. Mol. Neurosci.* 2012, 46, 509–515.

135. Roth, G.A.; Moser, B.; Krenn, C.; Roth-Walter, F.; Hetz, H.; Richter, S.; Brunner, M.; Jensen-Jarolim, E.; Wolner, E.; Hoetzenecker, K.; et al. Heightened levels of circulating 20S proteasome in critically ill patients. *Eur. J. Clin. Investig.* 2005, 35, 399–403.

136. Sixt, S.U.; Dahlmann, B. Extracellular, circulating proteasomes and ubiquitin-incidence and relevance. *Biochim. Biophys. Acta* 2008, 1782, 817–823.

137. Alvarez-Castelao, B.; Goethals, M.; Vandekerckhove, J.; Castano, J.G. Mechanism of cleavage of alpha-synuclein by the 20S proteasome and modulation of its degradation by the redox state of the N-terminal methionines. *Biochim. Biophys. Acta* 2014, 1843, 352–365.

138. Tsvetkov, P.; Reuven, N.; Shaul, Y. The nanny model for IDPs. *Nat. Chem. Biol.* 2009, 5, 778–781.

139. Frankland-Searby, S.; Bhaumik, S.R. The 26S proteasome complex: An attractive target for cancer therapy. *Biochim. Biophys. Acta* 2012, 1825, 64–76.

140. Kisselev, A.F.; Goldberg, A.L. Proteasome inhibitors: From research tools to drug candidates. *Chem. Biol.* 2001, 8, 739–758.

141. Bross, P.F.; Kane, R.; Farrell, A.T.; Abraham, S.; Benson, K.; Brower, M.E.; Bradley, S.; Gobburu, J.V.; Goheer, A.; Lee, S.L.; et al. Approval summary for bortezomib for injection in the treatment of multiple myeloma. *Clin. Cancer Res.* 2004, 10, 3954–3964.

142. Stein, M.L.; Groll, M. Applied techniques for mining natural proteasome inhibitors. *Biochim. Biophys. Acta* 2014, 1843, 26–38.

© 2014 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/3.0/).