Aim for the core: suitability of the ubiquitin-independent 20S proteasome as a drug target in neurodegeneration

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Neurodegenerative diseases are a class of age-associated proteopathies characterized by the accumulation of misfolded and/or aggregation-prone proteins. This imbalance has been attributed, in part, to an age-dependent decay in the capacity of protein turnover. Most proteins are degraded by the ubiquitin-proteasome system (UPS), which is composed of ubiquitin ligases and regulatory particles, such as the 19S, that deliver cargo to the proteolytically active 20S proteasome (20S) core. However, a subset of clients, especially intrinsically disordered proteins (IDPs), are also removed by the action of the ubiquitin-independent proteasome system (UIPS). What are the specific contributions of the UPS and UIPS in the context of neurodegeneration? Here, we explore how age-associated changes in the relative contribution of the UPS and UIPS, combined with the IDP-like structure of many neurodegenerative disease-associated proteins, might contribute. Strikingly, the 20S has been shown to predominate in older neurons and to preferentially act on relevant substrates, such as synuclein and tau. Moreover, pharmacological activation of the 20S has been shown to accelerate removal of aggregation-prone proteins in some models. Together, these recent studies are turning attention to the 20S and the UIPS as potential therapeutic targets in neurodegeneration. (Translational Research 2018; ■■:■■-■■)

Abbreviations: 20S = core 20S proteasome; AD = Alzheimer's disease; ALS = amyotrophic lateral sclerosis; ATP = adenosine triphosphate; BBB = blood-brain barrier; DUB = deubiquitinating enzyme; HbYX = hydrophobic-tyrosine-unspecified residue 'X'; IDP = intrinsically disordered protein; IDR = intrinsically-disordered region; LLVY-amc = succinyl-Leu-Leu-Val-Tyr-7-amino-4-methylcoumarin; NCC = NIH (National Institute of Health) clinical collection; NPL = Natural Product Library; PA = proteasome activators; PAINS = pan-assay interference compounds; PD = Parkinson's disease; ROS = reactive oxygen species; Rpt = regulatory particle of triple-ATPase; SAR = structure-activity relationship; tau = microtubule-associated protein tau (MAPT); TDP-43 = trans-activation response element (TAR) DNA-binding protein 43; Ub = ubiquitin; UIPS = ubiquitin-independent proteasome system; UPS = ubiquitin-proteasome system; USP-14 = ubiquitin-specific-processing protease

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INTRODUCTION TO THE PROTEASOME

The proteasome is a central protein degradation machine in eukaryotes.[1] Through hydrolysis activities, it removes damaged proteins and ensures the delivery of amino acids to support ongoing biosynthesis. In addition, the proteasome has been co-opted for more specialized tasks in regulating the cell cycle, differentiation, the inflammatory response, antigen presentation, and apoptosis.[2,3] To enable these functions, the proteasome makes up a staggering 1%−2% of the entire proteome in healthy cells. However, a decline in

proteasome activity has been broadly implicated in ageing and age-associated diseases, including neurodegeneration. Presumably, this decline contributes to a catastrophic imbalance in proteostasis and accumulation of damaged and/or misfolded proteins. In this review, we explore the structure-function of the proteasome and its implications in the onset and progression of neurodegenerative diseases. In addition, we focus on emerging therapeutic opportunities through pharmacological activation of this degradation machine.

The 20S proteasome (20S) is a barrel-shaped complex comprised of 4 heptameric rings: 2 stacked β-rings that are sandwiched by 2 α-rings (Fig 1, A). Three of the 7 subunits (β1, β2, and β5) that make up the β-ring are proteases that hydrolyze peptide bonds of substrates. These active sites are sequestered in the interior of the 20S chamber, such that substrates must first traverse through the exterior α-rings. In its closed state, the α-rings have a narrow pore that occludes the entry of most proteins.[4] Thus, 1 key to understanding proteasome regulation is to learn how substrates are granted access to the proteolytic chamber. Substrates are targeted to the proteasome through 2 major pathways, the ubiquitin-proteasome system (UPS) and the ubiquitin-independent proteasome system (UIPS). Proteasome activators (PA), which are predominantly multi-protein complexes, help facilitate degradation by the 20S. There are many types of PAs and the specific one that is bound determines whether that 20S is coupled to the UPS or UIPS (Fig 1, A). However, most of the PAs share a conserved tripeptide sequence, the HbYX (Hydrophobic-TYRosine-unspecified residue 'X'), at their C-termini that interacts with pockets in the α-rings of the 20S to allosterically open the pore.[5]

Ubiquitin-proteasome system. Proteasomal degradation by the UPS first requires the conjugation of multiple ubiquitin (Ub) proteins onto the substrate, generating the polyUb signal that designates it as a substrate of the proteasome. Recent work has shown that conjugation of 2 or more polyUb chains is needed on the tagged substrate to efficiently interact with the UPS machine.[6] Thus, regulation of this pathway by the activity of the E1, E2, and E3 Ub ligases is a critical component of its function,[7] but will not be described in detail here. The canonical regulatory particle of the UPS is PA700 (or 19S), which is a 700 kDa PA complex that associates with the 20S to create the 26S proteasome (26S).[8] PA700 is comprised of a "base" and a "lid." The lid contains subunits that bind to polyUb chains, as well as deubiquitinating enzymes that regulate association with the particle. The base contains the HbYX motifs that interact with the α-rings, and ATPases that unfold the substrate so that it can access the proteolytic chamber.[9] Recent reviews provide additional information about the structure of the 26S and its biological fuction.[6]

Ub-independent-proteasome system. Ub-independent degradation is coordinated by the 20S and may be amplified with UIPS-specific PAs, including PA200 and the heptameric PA28.[10] PA200 is a monomeric protein that uses a C-terminal HbYX motif to bind to and activate the 20S. PA28 is composed of multiple, different subunits (alpha, beta, and gamma) and it relies on an alternative (eg, nonHbYX) motif for association with the 20S.[11,12] The UIPS-specific PAs typically lack the unfolding activity of PA700; rather, they open the α-ring gate through a binding-induced conformational change and increase the flux of suitable substrates into the proteolytic chamber.[13] As discussed below, this mechanism restricts UIPS substrates to unfolded proteins that can fit into the channel without an active unfoldase. However, PA700-bound 26S has an open α-ring gate too and is thus capable of facilitating Ub-independent substrate turnover.[14] The relative contributions of the 26S in the UPS and UIPS pathways remain unclear (Fig 1, B); and, for simplicity, we will only mention the contributions of the 26S to the UIPS in passing in this review. Finally, the free 20S (not PA) is likely to be a contributor to the UIPS. Although the 20S has relatively low enzymatic activity in the absence of PAs (see below), some small or unfolded substrates may be able to traverse the closed gates and be degraded by the minimal machine.

Substrate-targeting by the UPS and UIPS. Over 90% of the human proteome is regulated by the UPS.[15] These substrates include a vast array of structured (or folded) proteins, intrinsically disordered proteins (IDPs) and proteins containing intrinsically disordered regions (IDRs). Structured proteins must be unfolded prior to their degradation and can therefore only be cleared by the UPS.[16,17] Essentially, folded proteins cannot fit through the narrow axial pore of the 20S, making them inaccessible to degradation by the UIPS particles.[18] However, IDPs and IDR-containing proteins, which lack this 3-dimensional structure, are thought to readily traverse the α-ring gate.[19] Twenty percent of cellular proteins are classified as IDPs and as many as 41% of the eukaryotic proteome is predicted to contain IDRs,[20,21] suggesting that the substrate pool of the UIPS may be considerably large. These substrates are particularly relevant for this discussion because they include the proteins that accumulate in neurodegenerative disorders, such as amyloid beta, tau, TDP-43, and α-synuclein (Table I).[22,23]

In cells, IDPs typically have shorter half-lives relative to structured proteins.[24] The UPS and UIPS have both been shown to facilitate the rapid proteasomal degradation of IDPs, such as p53 and p73.[25]