

CLINICAL IMPLICATIONS OF BASIC RESEARCH

On Prions, Proteasomes, and Mad Cows

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All cells have the capacity to selectively degrade misfolded intracellular proteins, which, if they accumulated, could interfere with normal function and could be toxic. Such proteins may arise by mutation, errors in gene expression, failure to fold correctly, spontaneous denaturation, or postsynthetic damage (for example, by oxygen radicals). How often such events occur in cells is uncertain, largely because the ubiquitin–proteasome pathway rapidly degrades such aberrant proteins, including those that cause various inherited diseases, such as cystic fibrosis and certain hemoglobinopathies.¹

This pathway also protects against neurodegenerative diseases.^{1,2} The hallmarks of amyotrophic lateral sclerosis, Parkinson's disease, Lewy-body dementia, Huntington's disease, and Alzheimer's disease vary, but all are characterized by the presence of abnormal proteins in intracellular inclusions that are generally associated with ubiquitin and proteasomes.² These diseases seem to result from the accumulation of a toxic protein species, although there is increasing evidence that these amyloid inclusions are a correlate rather than the immediate cause of the disease^{2,3} and that the neuronal pathology results from smaller soluble microaggregates of the aberrant protein.

The mechanism by which these amyloid-like proteins interfere with normal function and eventually promote apoptosis remains uncertain. One often-proposed hypothesis is that the ubiquitin–proteasome pathway normally eliminates these misfolded toxic proteins, but they gradually accumulate, and eventually, especially in aged persons, overwhelm the capacity of this proteolytic system and interfere with its functioning. The further buildup of the misfolded proteins finally causes a failure of the proteolytic mechanisms of neurons that are necessary for normal cell function and survival.² Direct evidence that the abnormal toxic proteins in these various diseases can inhibit the

functioning of the proteasome pathway has been lacking.

Recently, however, Kristiansen et al.³ presented strong evidence that soluble aggregates of the toxic protein may cause prion disease by specifically inhibiting the function of the 26S proteasome. Prion diseases are a set of transmissible, fatal neurodegenerative diseases associated with spongiform encephalopathy, including the diseases that occur in humans, Creutzfeldt–Jakob disease, the Gerstmann–Sträussler–Scheinker syndrome, and kuru; in sheep, scrapie; and in cows, bovine spongiform encephalopathy (or “mad cow disease”).⁴ More than 150 people in Britain have died of a variant of Creutzfeldt–Jakob disease acquired through ingestion of prion-containing beef. These diseases had long represented a biologic mystery, primarily because the transmissible agent was shown to lack nucleic acids, but the seminal studies of Stanley Prusiner and Charles Weissmann established that the diseases are caused by a toxic form of the prion protein. This protein is able to enter cells and induce a conformational rearrangement of a normal soluble, monomeric cell constituent, the prion protein (PrP^C), into a toxic transmissible species (PrP^{Sc}).⁴ In this pathogenic conformation, the prion is primarily insoluble, is resistant to proteases or detergents, has more amyloid-forming β -sheet structure, and forms larger aggregates than the normal species. This conformational change and the resulting accumulation of PrP^{Sc} ultimately cause severe loss of neurons, gliosis, and spongiform appearance. Accordingly, mice that do not express the normal protein are not susceptible to the disease.^{3,4}

A major gap in our understanding has been how the conversion of PrP^C to PrP^{Sc} eventually kills neurons. To clarify the mechanism of neurotoxicity, Kristiansen et al. tested whether PrP^{Sc} interferes with proteasome function, using *in vivo* and

in vitro approaches. In the ubiquitin–proteasome pathway, proteins are targeted for rapid degradation by covalent linkage to a chain of ubiquitin molecules, which marks them for rapid hydrolysis by the large 26S proteasome complex. When tagged with a ubiquitin chain, substrates bind to the proteasome's 19S regulatory component, which disassembles the ubiquitin chain and recycles the ubiquitin molecules. Then through a multi-step ATP-dependent process that is only now beginning to be understood, the 19S particle's ATPases unfold the substrate and translocate it through a narrow, gated entry channel into the

20S proteasome for degradation (Fig. 1).⁵ Inside this hollow cylindrical particle, the doomed protein is cleaved to small peptides, which are then released from the proteasome and rapidly degraded to amino acids by cytosolic peptidases.

Kristiansen et al. showed that neurons and neuroblastoma cells infected with prions have reduced proteasomal activity against model substrates. The 20S proteasome contains within its central chamber six proteolytic sites: two prefer to cleave proteins immediately after hydrophobic amino acids; two, after basic residues; and two, after acidic ones.^{1,2} Using peptide substrates spe-

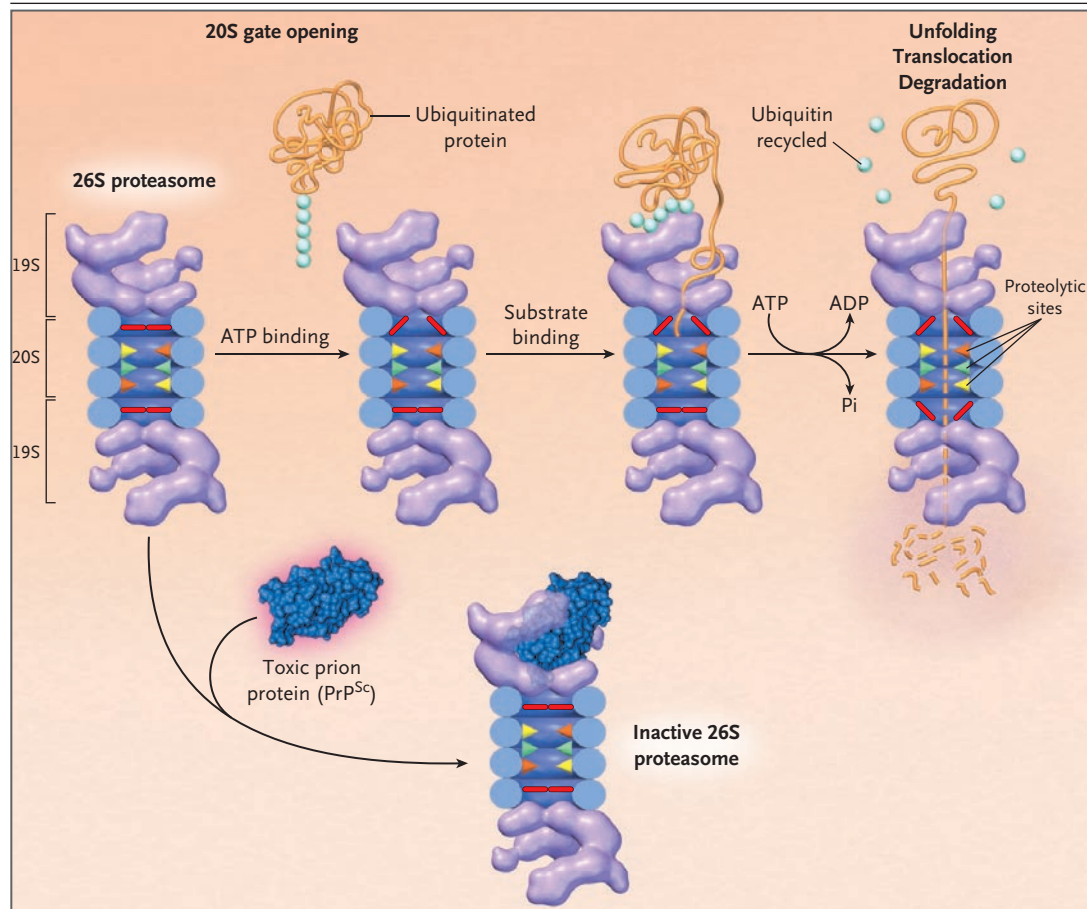


Figure 1. The Ubiquitin–Proteasome Pathway and the Toxic Prion Protein.

Protein degradation by the 26S proteasome involves multiple ATP-dependent steps,⁵ including the opening of the gated channel for substrate entry into the 20S particle, binding of the ubiquitinated substrate, disassembly of the ubiquitinated substrate, and the unfolding and translocation of the protein through the ATPase ring and the open channel. Binding of the oligomers of the toxic prion protein (PrP^{Sc}) to the ATPases can explain recent observations by Kristiansen et al.³ that in PrP^{Sc}-infected cells, the degradation of protein and peptide substrates by proteasomes is inhibited and ubiquitinated proteins (blue circles) accumulate. ATP denotes adenosine triphosphate, ADP adenosine diphosphate, and Pi inorganic phosphate.

cific for each site, Kristiansen et al. showed that all three activities were reduced in prion-infected neurons. Furthermore, they showed in infected neurons in culture that some PrP^{Sc} was present in the cytosol and that these cells had a decreased capacity to degrade a transfected fluorescent protein that is a substrate of the ubiquitin–proteasome pathway. Moreover, “curing” these cells of prions restored normal rates of degradation.

By infecting transgenic mice expressing this reporter protein with prions, Kristiansen et al. showed that PrP^{Sc} induced similar defects in the brain’s capacity to degrade this fluorescent protein. In infected neurons of these mice, ubiquitinated deposits accumulated in the cytosol, reminiscent of those found in various adult-onset neurodegenerative diseases, and in cells treated with pharmacologic inhibitors of the proteasome.³ Although the decreased degradative capacity is consistent with a loss of proteasomal activity, it could also be due to defects in ubiquitination or other steps in the pathway. However, the authors obtained further supporting data using purified 26S proteasomes and PrP^{Sc} isolated from infected mouse brain, recombinant mouse PrP folded into an α -helical structure resembling the native PrP^C, or a predominantly β -sheet species that mimics the properties of PrP^{Sc}. A clear reduction in the activity of the purified proteasomes (as well as those in neurons) occurred only with the aggregated forms of PrP^{Sc}. This inhibition was shown to result from a stoichiometric association of the oligomeric species with the proteasomes.

Although it is now clear that cytosolic PrP^{Sc} can interfere with the proteasome’s peptidase activities and that infected neurons are defective in protein degradation, there is no proof that this is the only toxic mechanism, or even the critical one leading to neuronal loss in prion diseases. Loss of proteasomal function affects many critical cellular processes and can induce apoptosis. In addition to serving as a quality-control system, this pathway is critical in regulating cell signaling networks and metabolic pathways and achieves exquisite selectivity because of the presence in humans of hundreds of different ubiquitin ligases that func-

tion in the removal of different proteins.¹ Not surprisingly, in cellular models of all neurodegenerative diseases, treatment with inhibitors of the proteasome promotes pathogenesis and neuronal death. The proteolytic sites of the 20S proteasome are the target of the anticancer drug bortezomib (Velcade), which is widely used in the treatment of multiple myeloma — a cancer that seems to be particularly dependent on proteasomes for survival and for the degradation of misfolded immunoglobulins. An important feature of this drug’s selectivity is that it does not enter the central nervous system, where it would probably accelerate the onset of neurodegenerative disease.

It also remains unclear exactly how small aggregates of the PrP^{Sc} inhibit the cleavage of peptides by the proteasome. The “decision” of which proteins are translocated into the particle and degraded depends on the ring of ATPases in the 19S regulatory component, which is probably the site where PrP^{Sc} oligomers bind (Fig. 1). Normally, cell proteins that are destined for degradation bind to and are unfolded by these ATPases before being translocated into the 20S particle. However, the exceptional stability of PrP^{Sc} makes it unlikely that these structures can be disassembled similarly, and the microaggregates of PrP^{Sc} are probably too large to traverse the ATPase ring and the narrower, gated pore into the 20S particle. Thus, they may behave like a sticky cork that interferes with the entry of other substrates into the 20S particle. Such a simple mechanism is now amenable to testing.

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1. Goldberg AL. Protein degradation and protection against misfolded or damaged proteins. *Nature* 2003;426:895-9.
2. Rubinsztein DC. The roles of intracellular protein degradation pathways in neurodegeneration. *Nature* 2006;443:780-6.
3. Kristiansen M, Deriziotis P, Dimcheff DE, et al. Disease-associated prion protein oligomers inhibit the 26S proteasome. *Mol Cell* 2007;26:175-88.
4. Prusiner SB. *Prion biology and diseases*. New York: Cold Spring Harbor Laboratory Press, 2004.
5. Smith DM, Kafri G, Cheng Y, Ng D, Walz T, Goldberg AL. ATP binding to PAN or the 26S ATPases causes association with the 20S proteasome, gate opening, and translocation of unfolded polypeptides. *Mol Cell* 2005;20:687-98.

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