

Protein Aggregation, the Ubiquitin-Proteasome System and Neurodegenerative Diseases

Protein aggregation is thought to be the pathological driving force responsible for neurodegenerative disorders such as Alzheimer's, Parkinson's, Huntington's and prion diseases.¹ However, it is not yet clear whether, or to what extent, the misfolded proteins are the cause of the disease rather than the consequence. The aggregated proteins that are characteristic of these diseases have in common the ability to undergo conformational changes and often form fibrillar structures (Figure 1). It is postulated that these proteins may share similar pathways of aggregation. Degradation of intracellular proteins via the ubiquitin-proteasome system (UPS) is a highly complex, temporally controlled and tightly regulated process that plays major roles in a variety of cellular processes and aberrations in this system have been implicated, either as a primary cause or secondary consequence, in the pathogenesis of neurodegenerative disease.² This review will give a brief overview of the possible role of proteasome dysfunction and protein aggregation in neurodegeneration.

Prion diseases

Transmissible spongiform encephalopathies (TSEs) or prion disorders are rare fatal neurodegenerative disorders which include Creutzfeldt-Jakob disease (CJD) in humans, bovine spongiform encephalopathy (BSE) in cattle and scrapie in sheep. A hallmark of these disorders is the accu-

mulation of abnormal prion protein (PrP^{Sc}) which is a misfolded form of the normal cellular host prion protein (PrP^C). The key pathological features of prion disorders are spongiform degeneration of the brain, massive neuronal loss, astrogliosis, and the accumulation in the CNS of PrP^{Sc}. However the precise cause of neurodegeneration in these disorders is not well understood, and a major gap exists in our understanding of how the conformational conversion of PrP^C to PrP^{Sc} ultimately kills neurons.

Lindquist and colleagues have suggested a novel mechanism involving inhibition of the UPS and altered trafficking of PrP^C that may account for prion-associated toxicity.³ They propose that in neurones misfolded PrP^C is retrogradely transported to the cytoplasm via ER-associated degradation (ERAD). Whereas normally PrP^C reaching the cytosol would be rapidly degraded, they demonstrated using inhibition of the UPS with high-dose proteasome inhibitors in mouse neuroblastoma N2a cells that cytoplasmic accumulation of large quantities of PrP^C resulted in aggregates in the cytoplasm, which acquired properties of partial protease-resistance with self-sustaining properties of replication,³ thereby inducing neuronal cell death. However other reports argue against this potential neurotoxic species by suggesting that PrP^C does not undergo ERAD.⁴ Paradoxically one group found retro-translocated cytoplasmic PrP^C was actually neuroprotective in primary cultured



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Table 1. Neurodegenerative diseases: aggregation in disease		
Disease	Protein deposit - major composition	Characteristic pathology
Prion disease	Prion protein	Extracellular amyloid plaques, intracellular deposits, and occasional synaptic and axonal deposits
Huntington's disease	Mutant Huntingtin	Intranuclear neuronal inclusions, cytoplasmic aggregates and fibrillar huntingtin fragments
Parkinson's disease	α -synuclein	Intracellular Lewy bodies, Lewy neurites, fibrillar α -synuclein
Alzheimer's disease	A β or tau	Extracellular neuritic plaques, intracellular neurofibrillary tangles of hyperphosphorylated tau
Amyotrophic lateral sclerosis	SOD-1	Intraneuronal inclusions, insoluble SOD

Figure 1: Aggregation in neurodegenerative diseases (A) Immunoreactive aggregates in neuronal nuclei (long arrows) and perikarya (short arrows) localised in Huntington's disease cortex (with permission from Gutekunst et al)⁶ (B) Hyperphosphorylated tau forms neurofibrillary tangles in neuronal cell bodies (Hippocampus of Alzheimer's disease brain) (C) Halo immunostaining of Lewy bodies in Parkinson's disease that predominantly comprises α -synuclein. The lighter granular material beside the Lewy body represents the neuromelanin of dopaminergic neurons of the substantia nigra (D) Extracellular plaque in sporadic prion disease stained with anti-PrP monoclonal antibody.

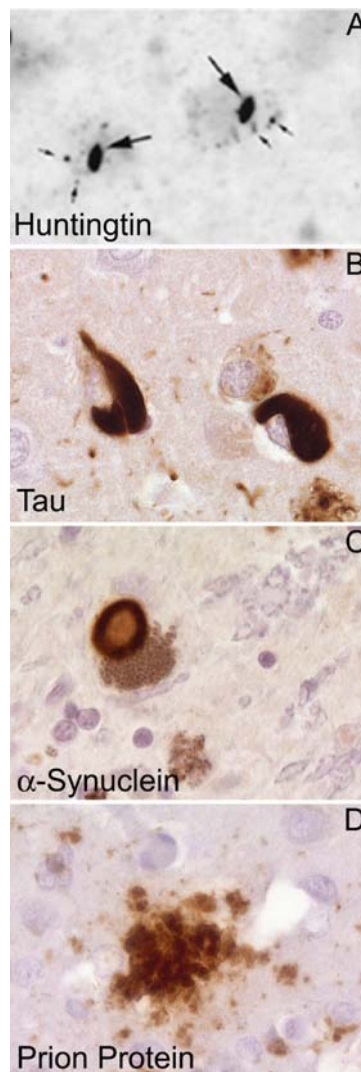


Figure 1



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human neurons by protecting against Bax-mediated cell death.⁵ It seems likely that some proportion of misfolded PrP^C undergoes ERAD-proteasome mediated degradation,⁶ but is likely to be rapidly degraded and a short-lived species;⁷ there is still clearly debate as to whether accumulation of this species is neurotoxic to the cell. It has been reported that proteasome dysfunction occurs *in vivo* in the brains of prion scrapie-infected mice; in this study they found that the level of ubiquitin protein conjugates increased significantly ~144 days post infection when clinical signs first became apparent and that this elevation correlated with a decline in proteasome function.⁸ It is likely therefore that proteasome impairment may be important in the pathogenesis of prion disease, but may be unrelated to the accumulation of the wild-type prion protein, and work is ongoing in our laboratory to address this question.

Huntington's disease

Huntington's disease (HD) is an autosomal dominant neurodegenerative disease with a prevalence of 4-10 per 100,000 in populations of Western European descent. In 1993, the gene defect associated with HD was identified as a CAG repeat expansion, encoding polyglutamine repeats, within a novel protein *huntingtin*. This highly polymorphic CAG repeat encoding polyglutamine is located in exon 1 and has been shown to range between 10 and 29 copies on normal chromosomes, whereas it is expanded to a range of 36-121 on HD chromosomes. HD is characterised by selective loss of neurons in the striatum and cerebral cortex, and by nuclear and cytoplasmic inclusion bodies of aggregated fragments of N-terminal mutant huntingtin, but it is not clear what role these aggregates play in the pathogenesis of HD. However, there is a striking correlation between the threshold for aggregation *in vitro* and the threshold for disease in humans, consistent with the idea that aggregation is related to pathogenesis.⁹ This supports the argument that self-association or aggregation of expanded polyglutamine underlies a toxic gain of function. Bence and colleagues found that mutant huntingtin fragments inhibited proteasome function in intact cells, and the formation of intracellular aggregated inclusions was associated with a further decline in UPS function resulting in a positive feedback mechanism that may explain the rapid loss of neuronal function in many neu-

rodegenerative diseases.¹⁰ Whether a decline in UPS dysfunction alone is sufficient to induce striatal neuronal damage in HD was recently investigated by Seo and colleagues, who found inhibition of the UPS in various brain regions from early and late-stage HD patients and also interestingly in their skin fibroblasts.¹¹ However, they report that UPS dysfunction is associated with neuronal pathology only when it occurs in parallel with other neurodegenerative pathways involving decreases in brain derived neurotrophic factor levels and defects in mitochondrial respiratory chain complex II, both of which are found in HD brains. A novel method of trying to ascertain whether HD inclusion bodies are pathogenic, incidental or a beneficial coping response has been reported recently by Arrasate and colleagues who developed an automated robotic microscope that returns to precisely the same neuron after arbitrary intervals.¹² They found by studying neurons expressing mutant huntingtin fragments that inclusion bodies were not essential for neuronal death. They argue that inclusion body formation can lead to decreased levels of mutant huntingtin and thus improve survival, and suggest that the toxic species may, in fact, be an intermediate soluble form of the protein.

Parkinson's disease

Parkinson's disease (PD) is a common age-related neurodegenerative disease that is characterised pathologically by extensive selective and progressive neurodegeneration in the substantia nigra (SN) of the midbrain with resultant loss of dopamine in the striatum, and formation of Lewy bodies in the remaining neurons in the SN as well as in a few other brain regions. As well as mitochondrial dysfunction and oxidative stress in sporadic PD, UPS impairment may also contribute to SN dopaminergic pathology. Lewy bodies are cytoplasmic inclusion bodies of fibrillar, misfolded proteins containing ubiquitinated α -synuclein and parkin as the major constituents although proteasome components are often found. Sporadic PD has been shown to be associated with impaired 26/20S proteasomal function and in 2002, McNaught and colleagues showed that inhibition of 26/20S proteasome function resulted in the specific accumulation of α -synuclein and ubiquitin, with the formation of cytoplasmic inclusions that were immunoreactive for these proteins, in dopaminergic neurons

in culture.¹³ More recently, they showed that proteasome inhibition alone is sufficient to produce a clinical syndrome in rats that closely recapitulates key features of PD and not more widespread neuronal damage.¹⁴ Whether such inclusions contribute to neuronal death or protect cells from the toxic effects of misfolded proteins remains controversial.¹⁵ Genetic defects in UPS protein components have been found linked to familial forms of PD. These cases, although rare, have yielded important information on basic pathogenetic mechanisms in PD² and suggest that failure of the UPS to degrade abnormal proteins may underlie nigral degeneration and Lewy body formation that occur in PD. For example, mutations in the gene encoding parkin, which is a ubiquitin protein ligase, have been found to inactivate its ubiquitin ligating activity whereas mutations in the gene encoding UCH-L1 have been shown to lead to a decrease in ubiquitin hydrolytic activity causing a shortage of free ubiquitin resulting in a general impairment of UPS function. α -synuclein has been implicated in both sporadic and familial forms of PD but whilst a direct link between α -synuclein and the UPS has not been firmly established, impairment of α -synuclein function via mutations may disturb ubiquitination of α -synuclein associated proteins that may lead to a disturbance in neuronal homeostasis. It has also been shown that the mutant form of α -synuclein is less susceptible to degradation by the proteasome and subsequent aggregation may lead to secondary neuronal damage by inhibiting the UPS.²

Conclusion

Several lines of evidence argue for a common mechanism of toxicity based on protein aggregation and the impairment of the UPS in neurodegeneration. However, it is still unclear whether protein aggregation and dysfunction of the UPS is the cause or consequence of neuronal loss in these disorders, and is still a subject of speculation and intense research interest. It may well be that proteasome inhibition occurs in these diseases but the pathophysiology of the neurodegenerative process per se in each disorder is likely to be multifactorial, and not a one-hit phenomenon. Therefore a key challenge in the development of disease-modifying treatments for this group of diseases is the design of therapeutic strategies that target many different cellular pathways.

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