The aggravating role of the ubiquitin–proteasome system in neurodegenerative disease

C.-C. Hung, E.J. Davison, P.A. Robinson and H.C. Ardley
Section of Ophthalmology and Neurosciences, Leeds Institute of Molecular Medicine, St James’s University Hospital, University of Leeds, Leeds LS9 7TF, U.K.

Abstract
Intraneuronal inclusion bodies are key pathological features of most age-related neurodegenerative disorders including Parkinson’s disease and Alzheimer’s disease. These inclusions are commonly characterized both by the presence of ubiquitinated proteins and the sequestration of components of the UPS (ubiquitin–proteasome system). Unfortunately, as we age, the efficiency of the UPS declines, suggesting that the presence of ubiquitinated proteins and UPS components in inclusions may reflect unsuccessful attempts by the UPS to remove the aggregating proteins. Whether the physical presence of inclusions causes cell death or, conversely, whether they are non-toxic and their presence reflects a cellular protective mechanism remains highly controversial. Animal and in vitro model systems that allow detailed characterization of the inclusions and their effects on the cell have been developed by us and others. Identification of the mechanisms involved in inclusion formation is already aiding the development of novel therapeutic strategies to prevent or alleviate aggregate-associated neurodegenerative diseases.

Introduction
Neurodegenerative disorders including AD (Alzheimer’s disease), PD (Parkinson’s disease) and HD (Huntington’s disease) are characterized by a selective loss of neurons in disease-specific regions of the brain. Such a neuronal cell loss causes disruption to motor, sensory or cognitive systems, resulting in severe disability of the patient.

Association of protein inclusions or aggregates within brain tissues of patients with neurodegenerative disorders has been widely reported [1]. In general, these ‘clumps’ of proteins consist of insoluble, unfolded, ubiquitinated polypeptides that fail to be targeted and degraded by the 26 S proteasome [1]. Such observations have led to the proposition that the UPS (ubiquitin–proteasome system) has a direct role in their formation. Indeed the presence of ubiquitinated proteins and UPS components in inclusions may reflect unsuccessful attempts by the UPS to remove aggregating proteins (Figure 1). Unfortunately, as we age, the efficacy of the UPS decreases [2]. In addition, progressive impairment of the chaperone system may lead to an intracellular accumulation of incorrectly folded molecules, which will then not be processed as efficiently by an aging UPS (Figure 1). These inclusions may eventually reach such a size as to trigger the death of the cell and the resulting neuronal loss in the patient’s brain.

Key words: aggresome, aging, inclusion, neurodegeneration, therapeutic strategy, ubiquitin–proteasome system.

Abbreviations used: AD, Alzheimer’s disease; HD, Huntington’s disease; PD, Parkinson’s disease; UPS, ubiquitin–proteasome system.

1To whom correspondence should be addressed (email h.c.ardley@leeds.ac.uk).

Inclusion formation in neurodegenerative disorders
Intracellular inclusions are thought to form when the capacity of the cellular proteasomes, the cell’s major proteolytic machine, becomes overwhelmed [3] (Figure 1). In the case of familial neurodegeneration, this may occur due to the presence of a mutant protein. For example, protein misfolding and subsequent loss of function due to an A53T substitution in α-synuclein in some families with PD, or the expansion of amino acid repeats as is observed in polyglutamine disorders such as HD, cause the rapid aggregation of these core proteins into inclusions. In addition to the core protein, inclusions commonly contain many other proteins that are thought to be sequestered into inclusions as they form. These latter components often include chaperones, UPS components and cytoskeletal elements [1,4–6] (Figure 1). In sporadic disease, the slow age-related decline in cellular chaperone and UPS activities is likely to affect the equilibrium between protein synthesis and degradation. In these cases, unfolded proteins, such as α-synuclein in PD and tau in AD, become more prone to aggregation and form inclusions. Because the neurones of the brain are terminally differentiated, non-renewing cell populations, they are particularly affected by such changes in cellular metabolism.

Whether inclusions represent the result of a protective cellular mechanism or whether their presence causes toxicity and death to the cells remains a hotly debated topic within the field of neurodegeneration. Indeed, studies have shown that pre-inclusion or protofibrillar forms of many neurodegenerative disease-associated proteins may be the actual cause of rapid cell death (hence, the earlier onset of inherited forms of these disorders than the sporadic forms) and not the mature inclusions. If these protofibrils can be stimulated to
form inclusions, the cell becomes somewhat protected from the damage that these toxic entities cause and may even be able to clear the aggregate via the proteasome or autophagy [7,8].

Current animal and cellular models
Multiple models of neurodegeneration have been developed both in vivo and in vitro.

Animal studies mostly in rodents and flies have lead to somewhat conflicting results on the importance of inclusions in neurodegenerative disorders because many of the transgenic animals do not develop brain-specific protein aggregations. This has led many to dismiss inclusions as simply a by-product of disease rather than of pathological significance. However, it seems somewhat strange that a stressed cell would spend so much of its time and energy developing these inclusions if they were not somehow beneficial. Animal models may not replicate the encoded protein behaviors. Nevertheless, some animal models do successfully produce neuronal inclusions. For example, overexpression of the human A53T isoform of α-synuclein in mice causes inclusion formation and axonal dysfunction [9,10]. Furthermore, exposure to proteasome inhibitors (i.e. mimicking the effects of an aging UPS system) in rats causes progressive parkinsonism with dopaminergic cell loss [11].

In vitro cell-culture systems have also proved to be a valuable resource as they are able to replicate many properties of neuronal inclusion formation. Using such systems, neurodegeneration-like inclusions have been observed when over-expressing a number of different disease-associated proteins, including Presenilin-1, Parkin and Huntingtin (associated with AD, PD and HD respectively) [3,12,13] (Figure 2). Moreover, their disease-associated mutant counterparts tend to be much more prone to aggregation than the wild-type proteins.

These in vitro inclusions have been characterized as ‘aggresomes’ [3] and share many properties of endogenous protein inclusions [3,12,13]. Most aggresomes are delivered in a microtubule-dependent manner to the microtubule-organizing centre, where they become surrounded by a vimentin ‘cage’ [3]. McNaught et al. [14] have proposed that the Lewy body inclusions associated with PD represent a specialized aggresome-related inclusion specific to dopaminergic neurons. This perhaps suggests that all neurodegenerative inclusions may be specialized forms of aggresomes, specific to their own particular disease.

Using inclusion models to develop therapeutic applications
The use of animal and cellular inclusion models is now becoming invaluable in the search for putative therapies targeted against the potential harmful effects of inclusion bodies. However, as discussed above, whether the presence of inclusions is toxic or protective remains unresolved. So, do we want to promote or prevent inclusion formation? Again, the current literature provides us with conflicting results. Some studies have demonstrated that treatments including addition of chaperones, small molecule inhibitors and even antibiotics such as tetracycline and dietary intake of the antioxidants found in red wine and green tea may prevent or clear inclusions without toxicity to cells [15–19]. Conversely, a recent chemical screening study suggests that promotion of inclusion formation may be beneficial in HD and PD [20].

Clearly, there is still much to be resolved regarding the importance of inclusions in neurodegenerative disorders and the mechanisms leading to their formation and removal. The UPS plays a significant role in this process, but it is becoming apparent that it is not the only mechanism involved. In addition to decreased chaperone activity, impaired electron transport chain activity is also likely to play a role in neurodegenerative pathology. Only by understanding the complex interplay between these important cellular systems and establishing whether there is a set order in which these
Figure 2 | In vitro models of neurodegeneration-associated inclusions

Overexpression of neurodegenerative disease-associated proteins often leads to aggresome formation in vitro. Proteins with disease-associated mutations or expansions readily aggregate (a), whereas wild-type proteins usually require additional proteasome inhibition for inclusions to form (b–f). Most proteins form classical compact aggresomes which are microtubule-dependent for their formation (a, c–e). Other proteins, including Parkin and UCH-L1 (b and f), form looser, more robust structures known as ribbon-like aggresomes which are not microtubule-dependent. Polyglutamine (polyQ) expansions are associated with HD, Presenilin-1 with AD, and Parkin, α-synuclein, synphilin-1 and UCH-L1 with PD. Huntington Q(103)–GFP (green fluorescent protein) (a), FLAG–Parkin (b), Presenilin-1 (c), α-synuclein–Myc/His (d), synphilin-1–V5 (e) or UCHL1–HA (f) constructs were transfected into COS-7 cells. At 28 h post-transfection, cells were incubated for 16 h in the presence of 5 μM MG132 (b–f only) prior to fixation with methanol. Cells were immunostained with antibodies specific to the protein or the appropriate tag (green) and counterstained with DAPI (4′,6-diamidino-2-phenylindole) to visualize nuclei (blue). Arrows indicate the inclusions.

systems fail, we will appreciate how, why and when neurodegenerative inclusions form and whether their presence is essential to disease progression.

H.C.A. is a recipient of a Research Councils (U.K.) academic fellowship award. Our work, which has contributed to this review, has been supported by Research into Ageing (personal fellowship to H.C.A.) and The Parkinson’s Disease Society (U.K.). We thank David Rubinsztein (Cambridge Institute for Medical Research, Cambridge, U.K.), Chris Miller (Institute of Psychiatry, University of London, London, U.K.) and Pam McLean (Department of Neurology, Harvard Medical School, Boston, MA, U.S.A.) for the gifts of the Huntington Q(103)–GFP (green fluorescent protein), Presenilin-1 and synphilin-1–V5 constructs used to generate Figure 2.

References

Received 6 July 2006