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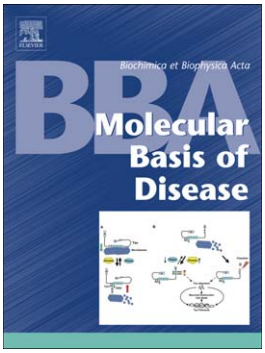

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Review

Is malfunction of the ubiquitin proteasome system the primary cause of α-synucleinopathies and other chronic human neurodegenerative disease?

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Summary

Neuropathological investigations have identified major hallmarks of chronic neurodegenerative disease. These include protein aggregates called Lewy bodies in dementia with Lewy bodies and Parkinson's disease. Mutations in the α-synuclein gene have been found in familial disease and this has lead to intense focused research *in vitro* and in transgenic animals to mimic and understand Parkinson's disease. A decade of transgenesis has lead to overexpression of wild type and mutated α-synuclein, but without faithful reproduction of human neuropathology and movement disorder. In particular, widespread regional neuronal cell death in the substantia nigra associated with human disease has not been described. The intraneuronal protein aggregates (inclusions) in all of the human chronic neurodegenerative diseases contain ubiquitylated proteins. There could be several reasons for the accumulation of ubiquitylated proteins, including malfunction of the ubiquitin proteasome system (UPS). This hypothesis has been genetically tested in mice by conditional deletion of a proteasomal regulatory ATPase gene. The consequences of gene ablation in the forebrain include extensive neuronal death and the production of Lewy-like bodies containing ubiquitylated proteins as in dementia with Lewy bodies. Gene deletion in catecholaminergic neurons, including in the

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substantia nigra, recapitulates the neuropathology of Parkinson's disease.

Keywords; ubiquitin, 26S proteasomes, neurodegeneration, dementia with Lewy bodies, Parkinson's disease

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Introduction

Walk in the street of any city in the world and you will notice more and more ageing people. Demographic predictions indicate that the ageing population is set to grow further and further, with centenarians becoming commonplace in many countries of the world [1]. The increasing numbers of the ageing population can be attributed to the control of childhood and adult infectious disease, public health improvements and good nutrition. This creates new problems for society, ranging from paying pensions for ever increasing periods to the construction of residential or nursing homes for the elderly. The only robust predictable correlation of dementing illness is age.

The brain has been described as a multi-organ system. Irrespective of this overarching notion, the brain shows profound structural and functional complexity as might be expected to control the behavioral and physiological activities of the body. There are several neurotransmitters that *via* different neuronal receptors regulate electrical activity in different parts of the brain. The neurotransmitters include glutamate, acetylcholine, serotonin, γ-amino butyric acid and dopamine.

Deficits in dopamine were detected many years ago in regions of the brain associated with Parkinson's disease [2, 3]. This lead to the use of levo-dopa and dopa decarboxylase inhibitors to treat Parkinson's disease. The use of these drugs and others to treat Parkinson's disease

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does not prevent neuronal death: disease progression is irreversible. The findings in Parkinson's disease led to the search for neurochemical deficits in the brain in other neurodegenerative diseases. Declining acetylcholine was found in certain parts of the brain in Alzheimer-related disorders, including Alzheimer's disease and dementia with Lewy bodies [4]. In turn, these observations led to the development of acetyl cholinesterase inhibitors to treat the symptoms of these diseases.

In order to try and gain an overall understanding of the molecular changes in chronic neurodegenerative disease it is necessary to remember that in the ageing brain there are a variety of neuropathological problems that are associated with cognitive decline and movement disorders, in addition to the neuronal death. These pathologies may appear as hallmarks that characterise a specific disease, e.g. proteinaceous aggregates such as Lewy bodies in neurons in the substania nigra in Parkinson's disease or neurofibrillary tangles in neurons in Alzheimer's disease. However, it is increasingly clear that these pathologies can be present together in the brains of many patients presenting with cognitive decline [5]. If drugs are to be developed to target fundamental defects in cellular physiology, it will be important to have diagnostic methods to determine which are present. The historical diagnosis and division of the major chronic neurodegenerative diseases into entities such as Parkinson's disease and Alzheimer's disease could

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be unhelpful because of the overlapping pathologies found in many patients. It is possibly more useful to think of co-exisiting neurofibrillary tangles, amyloid deposits, Lewy bodies and neuronal loss together with extensive gliosis (glial cells proliferate in association with advancing pathology in the brain).

Emphasis must be placed on the key, sometimes overlooked, major pathological finding in the ageing brain, neuronal death. This predominant event in the brain in Alzheimer's disease and Parkinson's disease has so-far not been recapitulated in transgenic models of disease. Animal models have been generated to over-express normal or mutated versions of the proteins that accumulate in aggregates in the diseases without extensive neurodegeneration [6]. There is much *in vitro* experimental evidence that fragments of the Alzheimer precursor protein can cause cell death in neuronal cell lines or primary neurons [7], but this does not generally occur in transgenic animals expressing large quantities of these so-called amyloidogenic proteins. Real understanding of chronic neurodegeneration will only come about when there is experimental insight into the mechanisms of neuronal cell death together with intraneuronal inclusion body and extraneuronal amyloid formation.

The Lewy bodies that are found in surviving neurons in the substantia nigra in Parkinson's disease and cortex in dementia with Lewy bodies contain α-synuclein and ubiquitylated proteins. Putative

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precursors of Lewy bodies (pale bodies) also contain mitochondria. The diseases are characterized by extensive neuronal cell death. How can this be explained? Furthermore for progress in understanding and treating disease can these neuropathological features be recapitulated in animal models?

The ubiquitin proteasome system

Protein ubiquitylation/deubiquitylation has moved from the periphery to centre stage in cell physiology, cooperating and competing with protein phosphorylation/dephosphorylation in the control of cellular activities. Ubiquitin was initially intensely studied as a tag for the degradation of target proteins by the 26S proteasome [8]. Regulated protein degradation is a major force in the cell to control the concentration of key regulatory proteins like transcription factors. Abnormal degradation of such molecules plays an important role in cancer [9]. However, protein ubiquitylation has many other roles in eukaryotic cells [10]. Proteins are monophosphorylated on serines, threonines and tyrosines to achieve regulatory diversity in the cell. Proteins can be monoubiquitylated on single lysine residues, polyubiquitylated on single or multiple lysines, e.g. p53 [11] or multiubiquitylated on different lysines to achieve regulatory diversity in the cell [12].

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The 26S proteasome consists of a cylindrical multi-subunit 20S catalytic core ($\alpha_7\beta_7\beta_7\alpha_7$) together with two 19S end caps. Each 19S particle consists of a nine subunit base and a lid containing multiple non-identical subunits, involved in functions including the recognition of polyubiquitylated proteins destined for degradation [13]. The base contains six related, but non-identical and non-redundant ATPases of the AAA superfamily of ATPases (**A**TPases of **A**lternative **A**ctivities). The putative heterohexameric ring of ATPases is a protein unfolding machine to feed string-like polypeptides into the catalytic core of the 26S proteasome [14] after deubiquitylation by proteasomal deubiquitylating enzymes. Two of the non-ATPases in the base appear to form a central "stub" composed of rpn2 attached to the 20S proteolytic core with rpn1 sitting on top of rpn2 [15]. The rpn2 protein interacts with the N-terminus of rpn13, which is a receptor for ubiquitylated proteins [16]. The C-terminus of rpn13 is attached to deubiquitylating enzyme Uch37. It is tempting to speculate that the "stub" acts as a central hub separated by an annulus from the putative hexameric ATPases. Target proteins could be unfolded in the central annulus between the ATPase ring and the central "stub" following deubiquitylation to produce the string-like polypeptides for insertion into the 20S catalytic core. Such mechanical coupling of ATPase activity to protein movement is

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reminiscent of the rotary biochemical mechanism of the mitochondrial F1-ATPase [17].

Fig. 1 (the 26S proteasome)

Other forms of proteasomes also exist in the cell [18] and it is increasingly seen that proteasomes have many roles. For example, in the regulation of transcription where 26S proteasomes, 20S proteasomes, 19S particles and sub-particles, ATPases independent of 20S (APIS) [19] are associated with transcriptional complexes for different genes [20-22].

The ubiquitin proteasome system (UPS) has many important roles in neuronal development and homeostasis [23, 24]. Synaptogenesis and synaptic plasticity are, in part, regulated by the UPS [25]. Synaptic functions and electrical activity are regulated by the combined processes of protein synthesis and protein degradation by the UPS [23, 26]. In turn, normal behaviors and memory [27] are regulated by the UPS. The functions and stability of neurotransmitter receptors and the organization of the post-synaptic density are controlled by the UPS [28]. Protein synthesis and degradation are not confined to the neuronal cell body but also occur in synapses to control synaptic morphology and electrical activity.

Protein degradation in all cells is not mediated by one mechanism. In addition to the UPS, which degrades cytosolic and nuclear proteins,

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there is the autophagic and endosome-lysosome system. Interestingly, autophagy is controlled by an enzyme cascade involving ubiquitons, cousins of ubiquitin [10]. The endosome-lysosome system is likewise regulated by ubiquitin, through the ubiquitylation of the tails of ligand-bound receptors to ensure internalization along with other post-translation modifications, e.g. phosphorylation [10, 29, 30]. Protein ubiquitylation is also needed for the delivery of cargo into multivesicular bodies – *via* the so-called ESCRT complexes (endosomal sorting complexes required for transport) for fusion with lysosomes and destruction of the contents of the multivesicular bodies [31]. It is interesting and perhaps evolutionarily predictable, that ubiquitons should have been used to control all the known protein degradation systems in the cell (except perhaps the mitochondrial degradation systems?).

The UPS and other protein degradation systems may have acquired particular importance in the nervous system because, in general, adult neurons do not divide and therefore protein elimination from neurons assumes particular importance in situations where proteins become damaged, e.g. oxidized or undergo structural changes such as conformational changes as in chronic neurodegenerative disease.

The first indications of the activity of the UPS in neurons in disease was the detection by ubiquitin immunohistochemistry of ubiquitylated proteins in protein inclusions in all the major chronic

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neurodegenerative diseases [reviewed in [32]]. Furthermore, the detection of cortical Lewy bodies by ubiquitin immunocytochemistry facilitated the identification and further clinical definition of dementia with Lewy bodies as a chronic neurodegenerative disease [33-36]. As is usual in biomedical science, the difficulty has been to explain why ubiquitylated proteins accumulate in protein aggregates in all the different chronic neurodegenerative diseases. There could be several explanations for accumulation of ubiquitylated proteins in inclusions, including malfunction or overwhelming of the 26S proteasome or ubiquitylation of proteins as part of a signaling pathway for cell survival or death. Ubiquitylated proteins may accumulate in inclusions as part of a mechanism, possibly related to the “aggresome” response, to protect neurons from cell death. Alternatively, as some believe, protein ubiquitylation may be a late event in inclusion biogenesis and perhaps an epiphenomenon in relation to disease progression and cell death.

The signaling pathways (neuronal and/or glial) involved in aggresome formation and neuronal survival or cell death are currently incompletely characterised.

Ubiquitylation has been shown to be part of a signaling pathway in afflicted neurons. Protein aggregate formation in tissue culture cells (not caused by disease-associated aggregate-prone proteins) causes transient expression of the MEK5 gene. The MEK5 kinase has only one known

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downstream substrate ERK5. This pathway has important developmental roles, e.g. in the heart [37], thymocytes [38] and during angiogenesis [39], but also functions in neuronal survival mechanisms in response to retrogradely transported nerve growth factor [40] and in response to neuronal oxidative damage [37]. Immunohistochemistry with antibodies to MEK5 and ERK5 showed that both neurofibrillary tangles and Lewy bodies contain MEK5 and the afflicted perikarya are enriched in ERK5 [41]. These findings provide evidence for a potential generic survival pathway in response to protein aggregates, which appears to take place in neurons containing the inclusions associated with chronic neurodegenerative disease. Neurofibrillary tangles and Lewy bodies contain the p62 protein, which is also involved in signaling in the cell, e.g. in the NFKB system [42, 43]. A direct molecular connection between the presence of ubiquitylated proteins in inclusions and MEK5 can be made through the p62. The p62 protein has a
C-terminal UBA domain that binds to ubiquitylated proteins for delivery to the 26S proteasome [44]. The p62 protein also belongs to a small family of proteins that have N-terminal PB1 domains that can either self-associate to give oligomeric signaling platforms or bind to other proteins with PB1 domains to form linked functional complexes in the cell. The MEK5 kinase has an N-terminal PB1 domain and binds to p62 [45, 46].Therefore, ubiquitylation of proteins will cause binding of p62 and in

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turn MEK5 that will phosphorylate ERK5 and activate transcription factors. If this then causes the expression of cell-survival genes in neurons in the human brain containing neurofibrillary tangles or Lewy bodies an adaptive response pathway can be proposed. The MEK5 kinase is also associated with inclusions in other neurodegenerative diseases; including frontotemporal dementia and progressive supranuclear palsy (Lowe & Mayer, unpublished). The p62 protein also has a key role in autophagy, which is also another potential reason explaining why p62 is in inclusions in chronic neurodegenerative disease [47, 48].

The connection between the UPS and chronic neurodegenerative diseases was enhanced by identification of the parkin gene which is mutated in autosomal recessive juvenile onset Parkinson's disease. Parkin is a ubiquitin protein ligase [49]. Normal parkin accumulates in Lewy bodies in the disease [50]. Interestingly, Lewy bodies do not form when parkin is mutated suggesting that the ligase has a direct role in Lewy body formation [49]. The connection between the UPS and chronic neurodegenerative disease is also shown by mutations of a deubiquityling enzyme UCHL-1 (PGP9.5) in a small group of patients with familial Parkinson's disease [51]. It is possible that Parkin and UCHL-1 are important upstream players in synucleinopathies. Genome-wide linkage analysis of a pedigree of parkinsonian-pyramidal syndrome has

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shown linkage to chromosome 22 and sequencing of candidate genes showed disease-associated homozygous variation in an F-box protein, which is a substrate-receptor for a ubiquitin protein ligase [52]. Given the importance of the UPS for neuronal development and homeostasis, it is not surprising that mutations in genes for proteins of the UPS cause chronic neurodegenerative disease. Neurological abnormalities are seen in a variety of transgenic mouse models with deficits in the UPS [53].

Whereas genetics identifies mutant proteins associated with disease it is more difficult to prove the role of a pathway in disease. Some indirect evidence for malfunction of the proteasome in chronic neurodegenerative disease comes from the observation that proteasomal catalytic activity is reduced in the substantia nigra in Parkinson's disease [54]. Similarly, the ubiquitin proteasome system appears impaired in the ageing brain [55] and particularly so in Huntington's disease [56], Creutzfeld-Jakob disease, Alzheimer's disease [57, 58], as well as in brain ischaemia [59, 60]

The systemic administration of proteasomal inhibitors has been reported to cause Parkinson-like neuropathological changes, including the formation of Lewy-like inclusions in rodents [61]. However, these observations are controversial with some laboratories being unable to reproduce the original findings [62-65]. Interestingly, α-synuclein

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protofilaments, but not monomers or dimers, inhibit the catalytic activity of the 26S proteasome *in vitro* [66].

In summary, the neuropathological insight and conclusions drawn from these findings pertaining to chronic neurodegenerative disease are;

- In neurodegenerative disease neuronal inclusions contain different proteins
- Inclusions have a primary role in disease
- Overexpression of proteins found in inclusions in mouse models may mimic disease (or overexpression of extracellular amyloid)
- The majority of intraneuronal inclusions contain ubiquitylated proteins
- Genetic insight into human neurodegenerative disease supports involvement of the UPS
- Impairment of the UPS may cause disease
- The role of the UPS is uncertain, e.g. is the UPS the primary response to protein aggregation (cf autophagy) and is the system cytoprotective?

There is clearly a need for the genetic proof or otherwise of the importance of the UPS in the chronic neurodegenerative diseases.

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Proteasomal Gene targeting

A decade of transgenesis in which disease-associated proteins are over-expressed singly or together in the brain has produced some, but not all features of human disease: neurodegeneration has been conspicuously absent in models of Alzheimer's disease [6] and Parkinson's disease [67]. For example, mice overexpressing mutant human α-synuclein in the brain show loss of motor neurons in the spinal cord, but not neuronal loss in the brain [68]. These findings have been rationalized by the notion that mice are short lived animals (longevity of 2-3 years), whereas human disease occurs much later in life. In idiopathic cases, usually in the fifth to eight decades. However, plaques can form remarkably rapidly, within 24h, in a transgenic amyloid over-expressing mouse model [69]. In general, the prevailing view is that mice are not men and may be of limited value in seeking the molecular key to chronic neurodegenerative disease [6, 67].

The alternative to transgenic over-expression of disease-related proteins is gene targeting, where candidate genes are deleted to see if neurodegeneration ensues. The targeted deletion of two autophagy-related genes in the brain results in neurodegeneration, but not the neuropathological features of human disease [70, 71]. Clearly, as expected, genes for proteins involved in autophagic protein degradation are important for neuronal homeostasis and when deleted result in

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neurodegenerative disease. Such models can be further refined by conditional gene targeting, gene ablation in specific regions of the brain to reflect selective vulnerability seen in neurological illnesses. There is, of course, the dilemma of the choice of the appropriate gene for deletion.

Based on the finding of abundant ubiquitylated proteins in inclusions in all the human chronic neurodegenerative diseases, it was logical to hypothesise that conditional deletion of a proteasomal protein in the brain may recapitulate the neuropathological and behavioral abnormalities of different human neurodegenerative diseases. In addition, it is wiser to delete a 19S regulator gene than a 20S core gene, in order to be able to examine a corollary hypothesis that 20S catalytic activity may be sufficient to prevent neurodegeneration. *In vitro*, 20S proteasomes have been shown to degrade α-synuclein and *tau* [72, 73]. Affecting a 19S proteasomal gene would demonstrate if ubiquitylated proteins could accumulate in the cell and be followed by recapitulation of any of the neuronal inclusions found in human disease. The six proteasomal ATPases are non-redundant [74] and putative hexameric ring formation will not occur in neurons lacking an individual ATPase. An attractive candidate gene is the Psmc1 ATPase gene (S4/rpt2), since studies in yeast and *in vitro* show that this ATPase has a central role in connecting protein unfolding to the proteolytic activity of the 20S proteasome core [75, 76]. Conditional targeted deletion of an ATPase

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gene in mouse brain might be expected to recapitulate the elusive neuronal cell death, which is so prevalent in human chronic neurodegenerative disease and, in the process, produce inclusions characteristic of human disease.

A formidable and technically successful method of gene deletion involves the use of the Cre/loxP system [77]. In this approach, the gene of interest is modified by the insertion of intronic loxP sequences, such that in the presence of Cre recombinase, the loxP flanked region is deleted. Once mice carrying the “floxed” target gene have been established these are mated with appropriate “Cre-deletor” mice to cause spatiotemporal ablation of the gene of interest. In theory, the gene of interest can be deleted in any cell type in the body of the “floxed” mice with an appropriate cell-specific Cre recombinase.

Deletor strains of mice are available so that ablation of the Psmc1 gene will only occur in catecholaminergic neurons [78], including dopaminergic neurons of the substantia nigra, or in the forebrain (cortex, hippocampus, striatum and amygdala) [79, 80]. Deletion of the gene in the substantia nigra may cause the neuropathology of Parkinson’s disease, while deletion of the gene in the forebrain may cause an Alzheimer-related disorder e.g. dementia with Lewy bodies.

This experimental approach has been successful [81]. Crosses of “floxed” Psmc1 mice with tyrosine hydroxylase (TH)-driven Cre

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recombinase mice leads to the death of nigrostriatal neurons and the appearance of Lewy-like bodies in the substantia nigra, pathognomic characteristics of Parkinson's disease. Nissl staining shows loss of neuronal RNA-containing rough endoplasmic reticulum in surviving neurons. Statistical evidence for enlarged neuronal nuclei (nucleomegaly) perhaps suggests that S4-depleted neurons instigate the cell cycle as part of the neuronal death process (Bedford & Mayer, unpublished). This has been demonstrated in the nigra in Parkinson's disease [82] and in models of neuronal loss in the brain [83].

Using a calcium calmodulin-dependent protein kinase IIa (CamKIIa)-expressing Cre recombinase mouse results in Psmc1 inactivation in forebrain regions. Neuronal cell death and Lewy-like bodies characteristic of dementia with Lewy bodies are found in the forebrain, together with some behavioral abnormalities of the disease [81]. The neuropathology is relatively rapid in these mouse models, within 12 weeks. Chronic neurodegeneration may occur in old people but can be reproduced in a matter of weeks in these models [81].

The sequence of events in the "Lewy mouse" begins with the accumulation of ubiquitylated proteins in neurons following 26S proteasomal depletion. Selective 26S depletion, with retention of 20S activity, was confirmed by glycerol gradient analyses. The appearance of α-synuclein, ubiquitin and p62 positive Lewy-like inclusions and

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progressive neurodegeneration and gliosis follow rapidly. Neuronal death is indicative of apoptotic mechanisms. Ablation of the ATPase gene in catecholaminergic neurons occurs throughout the autonomic nervous system and in the neuroendocrine adrenal medulla, resulting in the premature death of these animals. The neurodegeneration in the nigrostriatal pathway is accompanied by a corresponding loss of catecholamines, including dopamine in appropriate regions of the brain, but not indolamines. Following forebrain-specific deletion of PSMC1, comprehensive analyses indicate progressive behavioral and memory deficits, including a decline in visuospatial memory functions that may mimic those seen in dementia with Lewy bodies. Details of these behavioural abnormalities can be found in the supplementary information of Bedford et al. (2008). These neuropathological changes and behavioral abnormalities occur after deletion of 26S proteasomes without any evidence for the accumulation of amyloid plaques or neurofibrillary tangles. Therefore, extensive neuronal cell death and Lewy-like body deposition can occur in the brain in the absence of the neuropathological hallmarks of Alzheimer's disease. The immunohistochemical and morphological features of neurons in 26S proteasome-depleted mice are indicative of an "aggresomal" pathway of neurodegeneration, involving α-synuclein, protein ubiquitylation, p62 and other proteins together as

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seen in human disease. Additionally, mitochondria are a prominent feature of the inclusions in surviving neurons.

The occurrence of α-synuclein in Lewy-like bodies after 26S proteasome deletion is interesting and suggests that α-synuclein is not only involved in phospholipid and synaptic vesicle function [84], but may also have a primary role in Lewy body formation. This would explain why α-synuclein is present in Lewy bodies. An accepted notion [85] is that the aggregate-prone α-synuclein (and mutant forms) is deposited in Lewy bodies deliberately as a sequestration pathway to remove the protein from the cytoplasm (in addition to being degraded by the UPS and autophagy). However, if α-synuclein is necessary for Lewy body formation and the Lewy body is a specific cytoprotective response, then this gives a primary and functional reason for the incorporation of α-synuclein (and mutant forms?) into Lewy bodies.

Ultrastructural observations in the “Lewy mice” show that the perinuclear Lewy-like bodies are built around a core of mitochondria, surrounded by filamentous material with a periphery of membranes and autophagolysomes. This picture is the same as in Epstein-Barr virus transformed lymphoblastoid cells expressing a small number of viral proteins including the latent membrane protein [86]. In the mouse, inclusions containing mitochondria may be the equivalent of “pale bodies” seen in human disease, which contain mitochondria and may be

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an early form of Lewy body [81, 87]. In the mouse models, early neuronal death will prevent the formation of mature Lewy bodies, containing predominately aggregated filamentous α-synuclein [81].

The 26S proteasome-depleted models for understanding and treatment of Parkinson's disease and Dementia with Lewy bodies

The outcomes of conditional Psmc1 gene deletion in the nigrostriatal pathway and forebrain support the hypothesis that the 26S proteasome is essential for normal neuronal homeostasis. Depletion of 26S proteasomes will cause chronic neurodegenerative disease, resembling both Parkinson's disease and dementia with Lewy bodies [81]. Additionally, residual 20S proteasomes are insufficient for neuronal survival. The findings have considerable implications for human diseases, which are generally thought of as diseases of old-age with neuropathological changes occurring over periods of years.

There is increasing evidence that the pathologies associated with dementia with Lewy bodies, Parkinson's disease and Alzheimer's disease co-exist in the ageing brain to varying degrees and at the same time [5]. For example, most patients with Parkinson's disease eventually become demented with extensive cortical and brain stem Lewy bodies [88]. Patients with Alzheimer's disease have Lewy body pathology, with as many as 60% having Lewy bodies in the amygdala [89]. Clinically, the diseases are seen to be heterogeneous, a mixture of pathologies,

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neurochemical deficits and cognitive decline, making treatment through neurochemical replacement or receptor agonists difficult. There are only small “windows of opportunity” for treatment with existing drugs and complex responses depending on the pathological nature and extent of disease in the brains of individual patients [5]. Hence the need for new approaches that will come from studies on models including the “Lewy” mice. What is already clear from these mice is that neuronal death can be triggered by depletion of 26S proteasomes in the absence of neurofibrillary tangles or plaque amyloid. Yet, the opposite is not true in the transgenic overexpressing models, abundant amyloid and/or tangles or α-synuclein deposits do not give rise to rapid or extensive neuronal death in the brain [6, 68].

One scenario is that neurofibrillary tangles and plaque amyloid increase in amounts in the ageing brain without causing neuronal death or cognitive impairment. If, however, there is concurrent proteasomal malfunction or impairment in the same brains, further pathology will occur with Lewy body formation, followed by extensive neuronal death and irreversible cognitive decline and florid dementing illness. This “UPS critical failure” model could explain the transition from normal aging to disease. Whether 26S proteasomal dysfunction always occurs in human chronic neurodegenerative disease is not proven: that 26S proteasomal depletion causes neurodegeneration in the mouse is proven [81].

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It is not yet clear what the exact role of the neuronal Lewy body is in this situation. Opinion on the role of the Lewy body swings like a pendulum from protector to villain. The current position of the pendulum favours a cytoprotective role, particularly if the Lewy body is seen as an "aggresome" [90]. There are only three known mechanisms for the disposal of problem proteins in the cell: degradation by the UPS, degradation by autophagy or incorporation into aggresomes [10]. Degradation by the endosome-lysosome system is also available for proteins internalized from the cell surface [91]. It is now clear from gene targeting studies that ablation of autophagy genes in the brain, while leading to neurodegeneration with accumulation of ubiquitylated proteins, does not cause features of human disease such as Lewy bodies [70, 71]. Ablation of the proteasomal PSMC1 ATPase does recapitulate human neuropathology including Lewy-like bodies. In the 26S proteasome-deficient neurons proteins accumulate in perinuclear structures most likely built around the microtubule organizing centre (centrosome) like in EBV-virus transformed cells [86]. These structures are very similar to the aggresomes seen in different experimental models with aggregate-prone proteins. For operational purposes the Lewy body is almost certainly derived from a neuronal aggresome. Experimental aggresomes contain vimentin filaments and components of the UPS as well as peripheral autophagolysosomes presumably trying to degrade the

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aggresomal proteins [92]. When the UPS was in its infancy, protein microinjection studies into several tissue culture cell types showed that injected proteins were transported to a perinuclear "protein sequestration site associated with the microtubule organizing centre for protein degradation by autophagy" [93, 94], like in EBV-transformed lymphoblastoid cells [86]. Protein overload, whether in the cytosol or plasma membrane, sent proteins to this site quickly followed by slow autophagolysosomal degradation of the proteins (24-96 hours). In the substantia nigra, ablation of the expression of the proteasomal Psmc1 gene results in relatively rapid Lewy body formation (within 2 weeks).

In the 26S proteasome deficient models, electron microscopy shows that Lewy-like bodies are filled with mitochondria. In the EBV-transformed cells mitochondria were also a prominent feature of the inclusions [86]. There have been few reports of mitochondria in Lewy bodies [87, 95], but the mitochondrial inner-membrane PINK kinase is found experimentally in Lewy bodies [96]. The PINK kinase is mutated in familial Parkinson's disease and appears to act in mitochondria upstream of mitochondrial parkin [97, 98]. The PINK kinase and parkin seem to control mitochondrial fission [99]. The proportion of neuronal mitochondria that are incorporated into Lewy bodies is unknown. However, mitochondria have a central role in chronic neurodegenerative diseases, most strikingly in Parkinson's disease [96]. If Lewy bodies

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prolong the life of the neuron then could the ability to form Lewy bodies be dependent on normal mitochondria? Lewy bodies generally do not form in Parkinson's disease associated with parkin mutations [100]. Mitochondria are integral to the intrinsic pathway of apoptosis by releasing cytochrome c, triggering apoptosome formation and the activation of caspases 9 and 3 [101]. It is tempting to speculate that if mitochondria in early forms (pale bodies) of Lewy bodies [102] (which may be in the early inclusions to provide localized ATP for the biogenesis of early forms of Lewy bodies) are not subsequently removed these mitochondria may act as apoptotic "detonators" which trigger apoptosis by mechanisms including the localized release of cytochrome c. Surviving neurons, in which mitochondria are removed from maturing inclusions, will escape such a process and display the mature Lewy bodies.

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A unifying intraneuronal mechanism involving 26S proteasome malfunction and cell death in neurodegeneration

Critical failure of the 26S proteasome may be at the heart of an escalating process of neurodegeneration, in which adverse stimuli combine to increase the magnitude of regional neurodegeneration in the brain. Upstream events involving parkin and a-synuclein may also be major players in the disease process.

Fig. 2 (escalating cascade of neurodegeneration)

This process would be initiated by the accumulation of different types of intraneuronal proteins, triggering chaperone, UPS, autophagic and cell survival responses followed by inclusion body biogenesis. As a consequence of 26S proteasome inhibition by aggregate-prone proteins [56, 103, 104], or as shown genetically in the mouse models, 26S proteasome malfunction or depletion triggers neuronal death, which, because of the interacting neuronal pathways and projections, escalates to result in the extensive regional neurodegeneration characteristic of human disease.

Clearly, the 26S proteasome is central for neuronal survival and depletion of 26S proteasomes by ablation of the PSMC1 ATPase in appropriate specific regions of the brain recapitulates neuropathological

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and behavioral features of two major human chronic neurodegenerative diseases.

What now in 26S proteasome-depleted animals?

There are several predictions and hypotheses that can now be made as a result of the generation of the Lewy mouse and ensuing recapitulation of the pathology of human neurodegenerative disease including;

- The α-synuclein protein is necessary for the Lewy-like response in neurons
- Early involvement of mitochondria is a key feature of the Lewy body response
- Failure to abrogate the causative stimulus for Lewy body formation leads to mitochondrial-dependent apoptosis: this is a critical factor during Lewy body biogenesis and equates with the “pale body” seen in human disease

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Legends

Fig. 1 The 26S proteasome: showing the 20S catalytic core ($\alpha_7\beta_7\beta_7\alpha_7$) and one of the two 19S regulator complexes bound to a polyubiquitylated target protein. Another 19S regulator complex can be found at the other end of the 20S core.

Fig. 2 A dysfunctional 26S proteasome-dependent pathway for neurodegeneration. The pathway is triggered by neuronal protein aggregation and activation of cell survival genes coupled with attempts to eliminate aggregate-prone proteins and inclusion body formation. Eventual 26S proteasomal failure is caused by protein oligomers that trigger neuronal death by a mechanism based on expression of apoptotic genes and mitochondrial apoptosome-dependent cell death.

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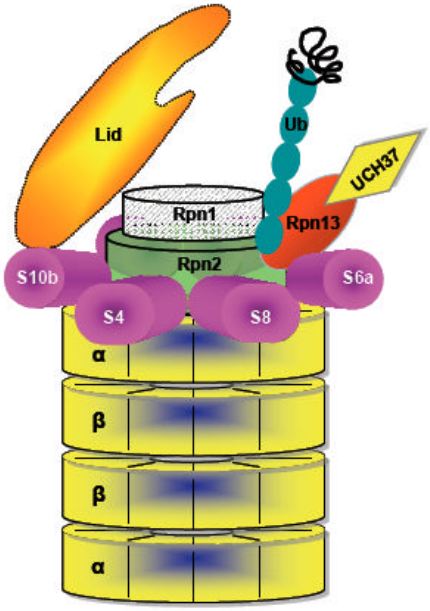

Fig 1:

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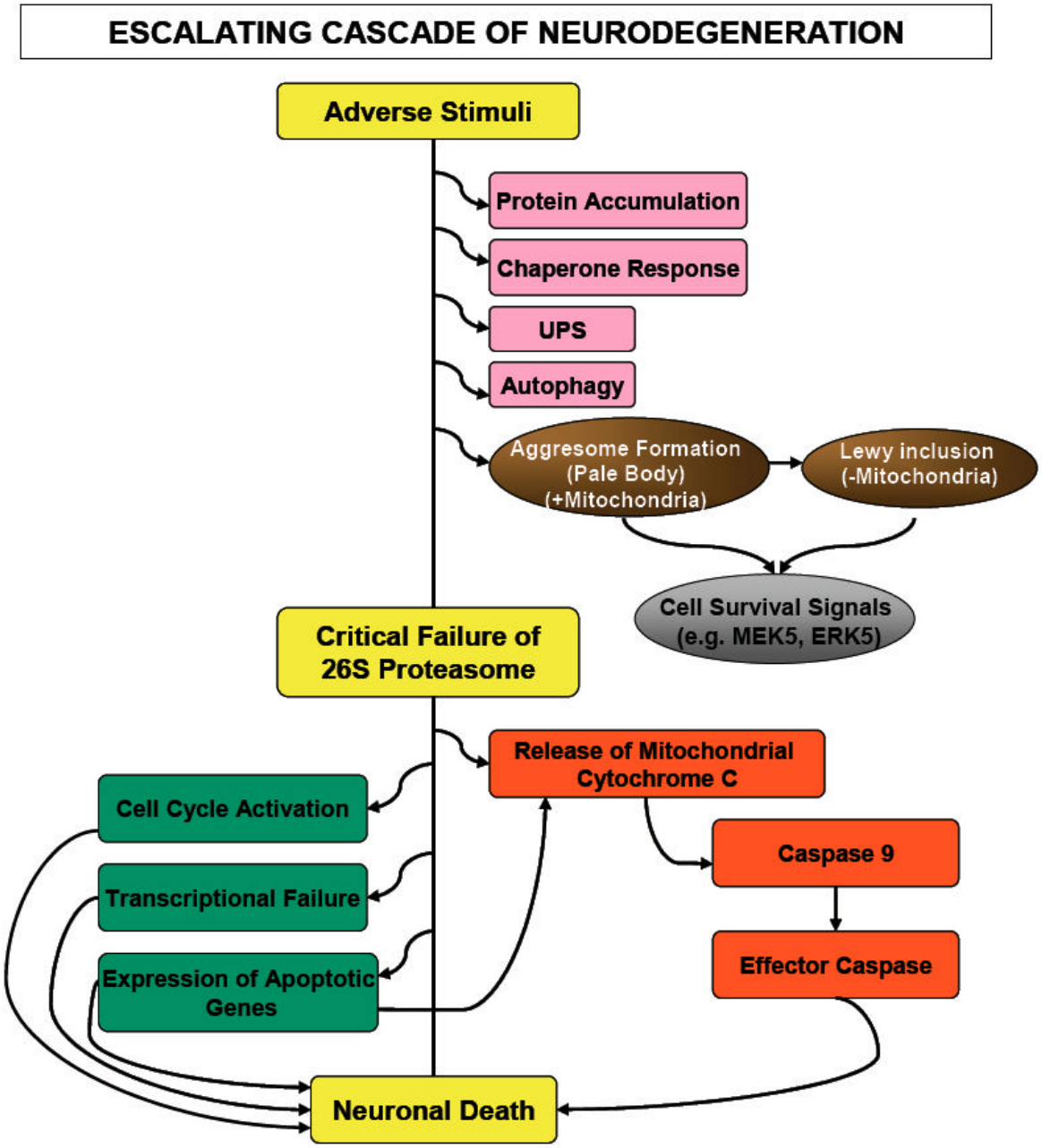

Fig 2: