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Pathogenic mechanisms of prion protein, amyloid-β and α-synuclein misfolding: the prion concept and neurotoxicity of protein oligomers

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Abstract

Proteinopathies represent a group of diseases characterized by the unregulated misfolding and aggregation of proteins. Accumulation of misfolded protein in the central nervous system (CNS) is associated with neurodegenerative diseases such as the transmissible spongiform encephalopathies (or prion diseases), Alzheimer's disease and the synucleinopathies (the most common of which is Parkinson's disease). Of these, the pathogenic mechanisms of prion diseases are particularly striking where the transmissible, causative agent of disease is the prion, or *proteinacious infectious particle*. Prions are composed almost exclusively of PrP^{Sc}; a misfolded isoform of the normal cellular protein, PrP^{C}, which is found accumulated in the CNS in disease.

Today, mounting evidence suggests other aggregating proteins such as amyloid-β (Aβ) and α-synuclein (αsyn), proteins associated with Alzheimer's disease and synucleinopathies respectively, share similar biophysical and biochemical properties with PrP^{Sc} that influences how they misfold, aggregate and propagate in disease. In this regard, the definition of a 'prion' may ultimately expand to include other pathogenic proteins. Unifying knowledge of folded proteins may also reveal common mechanisms associated with other features of disease that are less understood, such as neurotoxicity. This review discusses the common features Aβ and αsyn share with PrP and neurotoxic mechanisms associated with these misfolded proteins.

Abbreviations:

αsyn – alpha synuclein
APP - amyloid–β protein precursor
Aβ – amyloid beta
ApoE – apolipoprotein E
AD – Alzheimer's Disease
ALS – amyotrophic lateral sclerosis
CAA – cerebral amyloid angiopathy
CNS – central nervous system
CSF – cerebrospinal fluid
CJD – Creutzfeldt-Jakob disease
DLB - dementia with Lewy bodies
FTD – frontotemporal dementia
GPI - glycosylphosphatidylinositol
LB – Lewy body
LN – Lewy neurite
LTP - long term potentiation
LTD - long term depression
MSA - multiple system atrophy
PD – Parkinson's Disease
PrP^{C} – cellular prion protein
PrP^{Sc} – disease associated prion protein
PK – proteinase K
PMCA – protein misfolding cyclic amplification
TSE – transmissible spongiform encephalopathy

Introduction

Neurodegenerative proteinopathies are a group of disorders distinguished by the intra- or extracellular accumulation of specific proteins as β-sheet rich aggregates in the central nervous system (CNS) and associated neuronal vulnerability. They include Alzheimer's disease (AD),

synucleinopathies (the most common of which is Parkinson's disease, PD), transmissible spongiform encephalopathies (TSEs; also known as prion diseases), Amyotrophic Lateral Sclerosis (ALS) and frontotemporal dementia (FTD), among others. Although distinguished by disease-specific pathology and clinical presentations, these disorders share conspicuous similarities. They are largely age-related disorders with most cases being of sporadic origin. Currently they are incurable with therapeutic strategies only aimed at alleviating symptoms and despite extensive efforts, the normal physiological function of the proteins that aggregate in disease remains unknown. These proteins are, however, all intimately associated with their respective disorder, as mutations in the gene encoding the protein, or its precursor forms cause early-onset familial disease.

Of the proteinopathies, the unusual mechanisms governing the infectious nature of prion diseases are particularly striking. They represent a group of neurodegenerative diseases where the causative agent of disease is the prion or *proteinaceous infectious particle* (Prusiner 1982) that is composed almost exclusively of PrP^{Sc}; a misfolded isoform of the normal cellular prion protein, PrP^{C}. The prion hypothesis dictates that, in disease, aberrant PrP^{Sc} molecules aggregate together and induce conformational misfolding in susceptible PrP^{C} causing nucleation-dependent, autocatalytic amplification of transmissible prions. This continuous cycle of seeding allows prions to propagate within the host resulting in accumulation of β-sheet rich PrP^{Sc} in the CNS and widespread damage to neurons. During prion propagation, extension of the growing PrP^{Sc} aggregate can cause the aggregate to break, thus creating more nucleation seeds to amplify propagation (s 1). Prion propagation occurs during a long silent incubation period that can span decades, which is followed by the rapid onset of clinical disease and death.

Today, accumulating evidence suggests misfolded proteins associated with other proteinopathies share conserved molecular and biochemical properties with PrP^{Sc} that influences how they misfold, aggregate and propagate in disease. Some of the strongest evidence for prion-like behaviour exists for amyloid-β (Aβ) and α-synuclein (αsyn), proteins associated with AD and synucleinopathies (e.g. Multiple System Atrophy (MSA), Dementia with Lewy bodies (DLB), PD), respectively. Indeed, detailing of the stereotypical spread of these proteins in the CNS over the course of disease provided the first evidence for propagation of pathogenic αsyn and Aβ seeds (Braak *et al.* 2003, Braak & Braak 1991, Thal *et al.* 2002). The accumulating evidence of prion-like characteristics of Aβ and αsyn has led to suggestions of the expansion of the prion concept. This review discusses the characteristics Aβ and αsyn share with PrP^{Sc}, and explores how these common characteristics may also inform our understanding of neurotoxicity. Although outside the scope of this review, it is important to note that in addition to Aβ and αsyn, many other proteins associated with neurodegenerative proteinopathies have been shown to share similarities with

prions, including superoxide dismutase 1 (SOD1), huntingtin protein, and tau (Lee & Kim 2015, Holmes & Diamond 2014, Costanzo & Zurzolo 2013).

PrP^{Sc}: the conventional prion

Prion diseases represent a group of infectious, invariably lethal neurodegenerative diseases that affect both humans and animals. In humans, prion disease can be caused by mutations in the gene encoding PrP^{C}, *PRNP*, resulting in familial Creutzfeldt-Jakob disease (CJD), Gerstmann-Stäussler-Scheinker (GSS) syndrome or fatal familial insomnia (FFI). These diseases can also be contracted following exposure to prions and include variant CJD (vCJD), kuru and iatrogenic CJD (iCJD), or they may arise from an unknown origin (sporadic CJD). Non-human prion diseases include scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle and chronic wasting disease in deer and elk. The pathology of prion disease is characterized by extracellular deposition of aggregated PrP^{Sc} (Figure 2), along with astrogliosis and vacuolation including associated neuropathological spongiform change in the CNS.

The infectious nature of prions is well established to transmit disease both within and across species. Early studies showed brain homogenate from scrapie-infected sheep could successfully transmit disease to goats (Cuillé & Chelle 1936) and mice (Chandler 1961). Likewise, numerous studies demonstrated human prion disease could be transmitted to non-human primates (Gajdusek *et al.* 1966, Gibbs *et al.* 1968, Masters *et al.* 1981). In humans, acquired prion disease has been associated with several serious epidemics. Kuru, one of the most prominent forms of prion disease, afflicted the native Fore tribes of Papua New Guinea. In these populations, ritualistic cannibalism was identified as the route of transmission and cessation of this practice has virtually eradicated new cases of disease (Collinge & Alpers 2008). The finding that consumption of BSE-infected beef was responsible for vCJD (Will *et al.* 1996, Hill *et al.* 1997) led to one of the largest health crises of the 20th century, causing widespread economic damage to countries found to have a contaminated beef-industry and concern of large-scale public exposure to the pathogen due to the long incubation period of disease. Additionally various iCJD cases have been documented with routes of unintentional human-to-human transmission including the use of inappropriately sterilised surgical equipment (Bernoulli *et al.* 1977, El Hachimi *et al.* 1997), dura mater grafts (Miyashita *et al.* 1991, Willison *et al.* 1991), corneal grafting (Duffy *et al.* 1974, Heckmann *et al.* 1997), blood transfusion (Llewelyn *et al.* 2004, Peden *et al.* 2004) and use of human cadaveric pituitary-derived growth hormone or gonadotrophin (Cochius *et al.* 1990, Powell-Jackson *et al.* 1985) (for an extensive list of references on human-to-human transmission of prion disease, refer to: (Brown *et al.* 2006)). Of these, the highest number of iCJD cases are associated with dura mater grafts and hormone administration (Brown *et al.* 2012). These cases of acquired disease have led to major reform in the handling of biological samples in the food and medical industry to reduce contamination and prevent future outbreaks of prion disease.

The conversion of PrP^{C} to PrP^{Sc} is concomitant with substantial structural and biophysical alterations to the molecule. Upon misfolding, the α-helix rich PrP^{C} that is usually attached to the cellular membrane via its glycosylphosphatidylinositol (GPI) anchor, switches to one primarily composed of β-sheet (Borchelt *et al.* 1990, Pan *et al.* 1993). These changes lead to an increased resistance to degradation by heat and proteases; the latter feature of which is often exploited for its detection by incubation with proteinase K (PK). In addition, it is well accepted not all PrP^{Sc} are identical and rather, PrP^{Sc} encompass a range of various sized PrP^{Sc} quasi-species (Li *et al.* 2010) that harbour unique properties; for example, the most infectious prion particles appear to be those of small masses consisting of 14-28 molecules (Silveira *et al.* 2005, Simoneau *et al.* 2007).

Numerous data demonstrate that propagation of PrP^{Sc} via conformational misfolding of PrP^{C} is an essential component of disease pathogenesis. Transgenic PrP^{C} knockout mice are resistant to infection by prions (Bueler *et al.* 1993) and ablating PrP^{C} expression in prion-infected mice after the onset of clinical symptoms halts disease progression and rescues early neuropathological changes (Mallucci *et al.* 2003). The development of assays that study the conversion of PrP^{C} to PrP^{Sc} in cell free systems provides additional insight into the misfolding of the protein. In the cell free conversion assay, radio-labelled recombinant PrP^{C} adopts PrP^{Sc}-like features such as resistance to PK when incubated in the presence of partially denatured PrP^{Sc} derived from prion-infected hamster (Kocisko *et al.* 1994). Another cell free assay, the Protein Misfolding Cyclic Amplification (PMCA) assay, shows amplification of PK-resistant PrP occurs when excess PrP^{C} is subjected to repeated rounds of sonication and incubation in the presence of a small PrP^{Sc} seed (Saborio *et al.* 2001). When subjected to PMCA, normal brain homogenate seeded with a small amount of prion-infected brain homogenate amplifies PK-resistant, insoluble PrP^{Sc}-like species, that transmits disease when inoculated into wild-type mice (Castilla *et al.* 2005). Collectively these studies demonstrate the requirement of PrP^{C} in the generation of PrP^{Sc} and in the propagation and transmission of prions *in vivo*.

An important component to proving the prion hypothesis required demonstration that PrP^{Sc} alone was a transmissible element that could induce the pathology of prion disease. Studies showed recombinant PrP^{C} may be misfolded to form a PrP^{Sc}-like species that causes disease when inoculated into PrP-overexpressing mice (Legname *et al.* 2004) or in wild-type mice when the protein is misfolded in the presence of normal brain homogenate (Makarava *et al.* 2010), however variations existed in the pathology and long incubation period required for presentation of disease indicating PrP^{C} alone may not be the sole pathogenic agent in traditional prion disease. An explanation for this variation was suggested by the observation that additional factors, or co-factors, could contribute to the misfolding of PrP^{C}. Using PMCA, PrP^{Sc}-like species may be formed from normal brain homogenate or purified brain-derived PrP^{C} combined with RNA

species (Deleault *et al.* 2007, Barria *et al.* 2009), both that cause disease in wild-type rodents. The latter finding, along with others, show polyanionic and lipid species are required for efficient misfolding of PrP^{C} and infectivity (Deleault *et al.* 2005, Deleault et al. 2007, Deleault *et al.* 2003, Geoghegan *et al.* 2007, Wang *et al.* 2007). By the addition of co-factors, misfolding recombinant PrP in the presence of RNA and lipids produces *de novo* prions that are highly infectious *in vivo* and manifest disease in wild-type mice which faithfully replicates conventional prions (Wang *et al.* 2010). These studies confirm an altered conformation of PrP is the major component responsible for infectivity of prions, however the requirement of co-factors suggests other molecules may form a natural component of a transmissible prion.

An unusual feature of prion disease is strain variability, whereby unique patterns of clinical presentation, incubation period, PrP^{Sc} deposition and neuronal vulnerability are displayed amongst prions (Morales *et al.* 2007). Unlike conventional pathogens where strain variability is a result of genomic alteration, it is unusual for multiple disease phenotypes to be enciphered by a polypeptide chain. Important knowledge into strain variability came from elegant studies on two hamster-adapted prion strains Hyper (HY) and Drowsy (DY). These two strains arose from the same original animal and were propagated through hamsters with identical *PRNP* sequence, however while hamsters inoculated with HY prions develop ataxia and hyperaesthesia, hamsters infected with DY prions show lethargy and a longer incubation period (Bessen & Marsh 1992a). These divergent clinical features are coupled with unique pathological profiles of protein aggregation between the two strains (Bessen & Marsh 1992a) and biochemical analysis of the brains of animals infected with HY or DY reveal differences in electrophoretic mobility of PK-resistant PrP (Bessen & Marsh 1992b), suggesting the structure of PrP^{Sc} is altered between the two strains. This observation is further supported by the finding that these strains also exhibit different infrared spectrum of absorption in the range that correlates to β-sheet content (Caughey *et al.* 1998), indicating the secondary structures of HY and DY are dissimilar. Structural variability of PrP^{Sc} is also likely to occur in the human condition, where PrP^{Sc} present in sporadic prion diseased brain are distinguished by changes in electrophoretic mobility which appears to correlate with pathological and clinical features of disease (Hill *et al.* 2003). Although the general consensus is that the conformer of the misfolded protein encodes its disease phenotype, the origin of unique strains and how these conformational changes incite the unique clinical and pathological profiles is still poorly understood.

Prion-like properties of Aβ

The accumulation of amyloid plaques primarily composed of Aβ in the extracellular space of the CNS is a common feature of Alzheimer's disease (Masters *et al.* 1985) (Figure 2). A naturally-occurring cleavage product of the integral membrane protein, amyloid–β protein precursor (APP) (Kang *et al.* 1987), Aβ is produced by the sequential cleavage by β- and γ- secretases that cleave

at various sites in APP, producing Aβ peptides which differ by several amino acid lengths. Along with Aβ deposition, AD is associated with the accumulation of neurofibrillary tangles consisting of hyperphosphorylated forms of the microtubule-associated protein, tau. Aside from age, the biggest known risk factor for AD is the expression of the ε4 allele of the lipoprotein ApoE (ApoE4) (Corder *et al.* 1993) that worsens the trajectory of cognitive decline in association with Aβ burden in preclinical AD individuals (Mormino *et al.* 2014, Lim *et al.* 2015a, Lim *et al.* 2015b).

Some of the first indications of prion-like behaviour of Aβ came from primate studies, where Aβ deposition was found in the CNS of marmosets 6-7 years after intracerebral inoculation of human AD tissue (Baker *et al.* 1993), suggesting pathogenic Aβ seeds may propagate within its host similar to prions. Further support for this concept came following the generation of transgenic animals. Mice expressing APP containing mutations associated with human familial AD spontaneously develop Aβ pathology and are used to model disease, with two of the most widely used transgenic mice Tg2576 (Hsiao *et al.* 1996) and APP23 (Sturchler-Pierrat *et al.* 1997) expressing the Swedish familial mutation K670M/N671L. Unilateral inoculation of dilute brain homogenate from human AD brain into the hippocampus and neocortex of young Tg2576 mice accelerates the deposition of Aβ, with extensive Aβ-pathology found 5 months post injection whilst limited or no deposition occurred in Tg2576 counterparts injected with age-matched non-AD or young non-AD brain extract (Kane *et al.* 2000). Under the same experimental conditions, a similar finding was observed by aging the mice to 12 months, where although Aβ deposition was present bilaterally, protein deposition was significantly more extensive in the hemisphere inoculated with AD brain (Walker *et al.* 2002). Consistent with the ability of Aβ-rich brain homogenate to induce this pathology, a similar phenotype may be achieved by inoculating young pre-symptomatic mice with brain homogenate from aged counterparts that have developed extensive Aβ deposition (Meyer-Luehmann *et al.* 2006).

Since these findings, numerous studies have highlighted the relevance of Aβ in accelerating pathology in AD-model mice. Removal of Aβ from aged APP23 brain homogenate via immunodepletion or immunization against Aβ abrogates the ability of extracts to trigger Aβ pathology (Meyer-Luehmann et al. 2006) and pre-treating human AD brain homogenate with magnetic beads coupled to a compound that binds misfolded protein decreases Aβ levels in the homogenate and reduces its seeding potential *in vivo* (Duran-Aniotz *et al.* 2014). Consistent with these observations, the concentration of Aβ in the seed and its expression in the brain determines the time and severity of Aβ deposition, and is independent of the age of the mouse when inoculated (Hamaguchi *et al.* 2012, Meyer-Luehmann et al. 2006). In AD-model mice, Aβ pathology may also be triggered by Aβ seeds derived from human brain of patients with amyloid burden without dementia (Duran-Aniotz *et al.* 2013) and synthetic misfolded Aβ (Stöhr *et al.*

2012, Stöhr *et al.* 2014). The development of Aβ-pathology in transgenic APP-model mice has been studied where Aβ propagation follows a systematic spread with induction of pathology corresponding to the limbic connectome (Ye *et al.* 2015). Collectively these studies give strong support for Aβ being the agent driving the accelerated Aβ pathology seen in AD-model mice and supports the concept that Aβ may propagate similar to prions.

The transmissible nature of Aβ seeds share several similarities with prions including their resistance to inactivation by treatment with formaldehyde (Fritschi *et al.* 2014a) and the ability to be transmitted on steel wires (Eisele *et al.* 2009); a concept developed to replicate transmission of prions via surgical equipment (Flechsig *et al.* 2001, Zobeley *et al.* 1999). It has been reported small aggregates of Aβ are the most infectious (Langer *et al.* 2011). Although synthetic Aβ may induce AD pathology *in vivo* (Stöhr et al. 2012, Stöhr et al. 2014), it is markedly less effective than brain homogenate (Stöhr et al. 2012), which may indicate biological co-factors are required for efficient propagation of Aβ *in vivo*. The propagation of Aβ *in vivo* has also been confirmed following injection of Aβ seeds via the intraperitoneal route (Eisele *et al.* 2010, Eisele *et al.* 2014) consistent with propagation of pathogenic seeds from the periphery to the CNS.

Although compelling, there are several caveats to using AD-model mice to study Aβ propagation. Acceleration of an existing Aβ pathology in transgenic mice after exposure to an Aβ extract does not necessarily prove that Aβ is infectious; however the generation of Aβ pathology in transgenic mice expressing wild-type human APP or mutant human APP that would not develop spontaneous Aβ pathology in the experimental timeframe does support the transmissible properties of Aβ aggregates (Morales *et al.* 2012, Rosen *et al.* 2012, Hamaguchi et al. 2012, Stöhr et al. 2012). Cerebral spinal fluid (CSF) from AD patients that contain higher levels of Aβ compared to brain extracts does not induce Aβ pathology in young APP23 mice (Fritschi *et al.* 2014b), which may indicate co-factors in the brain but not CSF influences the ability of Aβ to propagate pathology. An additional consideration using these transgenic mice is that although Aβ deposition can be accelerated by the addition of Aβ seeds, neurological dysfunction is absent, and thus the model of Aβ propagation does not model all features of AD.

Evidence of transmission of Aβ in humans has been reported where unusual deposition of Aβ was found in the brains of iCJD patients who contracted disease following human cadaveric pituitary-derived growth hormone treatment (Jaunmuktane *et al.* 2015). Of a small sample group of eight, four contained extensive deposition of Aβ in the CNS parenchyma and varying degrees of cerebral amyloid angiopathy (CAA); the pathological deposition of Aβ in blood vessels that occurs in approximately 80% of AD patients (Serrano-Pozo *et al.* 2011). An additional three cases had sparse Aβ deposition or Aβ deposition in association with PrP plaques. Given the young age of the patients (age range: 36 – 51 years), it is unlikely the Aβ pathology arose sporadically. A possible

explanation for this observation is that Aβ seeds were present in the cadaver-derived growth hormone. As evidence that Aβ deposition occurred through a transmission event, the growth-hormone recipients did not have any known factors that may have predisposed them to Aβ pathology, including known AD-associated genetic mutations and positive ApoE4 status. It is possible that the Aβ deposition was a result of cross-seeding by PrP^{Sc}, however in the majority of cases co-localization was not found and post mortem pathological assessment of a larger number (35) of patients with genetic or sporadic prion disease failed to identify Aβ pathology in comparable age groups, with the exception of two cases that were positive for the ApoE4 allele (Jaunmuktane et al. 2015). As proof-of-principle for the presence of Aβ in pituitary-derived growth hormone, deposited Aβ has been found in the pituitary gland of AD patients (Irwin *et al.* 2013, Jaunmuktane et al. 2015). Another study has reported unusual Aβ pathology in iCJD patients who contracted disease after dura mater grafting (Frontzek *et al.* 2016). Five out of seven patients (age range: 28 - 63 years) reported Aβ pathology and CAA consistent with AD, which was significantly greater than the frequency of Aβ pathology seen in 21 age-matched controls. In both cases, although it is intriguing to postulate iatrogenic transmission of Aβ has occurred, the overarching manifestation of iCJD makes it impossible to ascertain whether this transmission would have resulted in clinical AD.

Strain variation may also be a feature of Aβ. Differences are found in the pathological and biochemical profiles of Aβ isolated from different transgenic mutant APP mice such as APP23 (containing the APP Swedish mutation, K670M/N671L) (Sturchler-Pierrat et al. 1997) and APP/PS1 mice; a transgenic mouse line that contains the Swedish mutation along with a mutation in the AD-associated gene *PSEN1* at position L166P (Radde *et al.* 2006). The large Aβ deposits formed in the brain of APP23 mice are pathologically dissimilar to the Aβ aggregates in APP/PS1 mice which present as small, compact plaques (Sturchler-Pierrat et al. 1997, Radde et al. 2006). Aβ species derived from these transgenic mouse lines also harbour divergent Aβ peptide ratios and spectre upon incubation with luminescent amyloid-binding dyes (Heilbronner *et al.* 2013). Consistent with the concept of prion strains, inoculation of APP/PS1 or APP23 brain homogenate into young APP23 mice induces the formation of Aβ aggregates that are similar in morphology, binding affinity to amyloid dye and peptide ratio to that of the inoculum (Heilbronner et al. 2013). Similarly, distinct clinical and pathological phenotypes are found in the human condition, an example of which is seen in AD-patients carrying the Arctic or Swedish mutation in APP. The Arctic mutation (E693G) occurs within the Aβ sequence of APP and in humans disease is characterized by increased protofibril formation and the deposition of a specific Aβ peptide (Aβ38) preferentially surrounding blood vessels (Moro *et al.* 2012). Disease in patients carrying this mutation is associated with rapid cognitive decline and lower plasma levels of Aβ compared to what is typically seen in AD patients (Nilsberth *et al.* 2001). In contrast, the Swedish mutation (K670M/N671L) is located outside the Aβ sequence, causing disease associated with the

overproduction of wild-type Aβ and AD pathology that is similar to sporadic disease (Citron *et al.* 1992, Mullan *et al.* 1992). These mutations in APP confer unique biochemical profiles of Aβ species formed, where brain-derived Arctic Aβ species are more PK resistant than those isolated from Swedish or sporadic AD patients (Watts *et al.* 2014). Transgenic mice inoculated with human brain from patients carrying either the Arctic or Swedish mutation generate distinct profiles of Aβ species that reflect the human condition, where APP23 mice inoculated with human brain homogenate containing the Arctic mutation have a shorter incubation period and reduced ratio of Aβ peptides compared to mice inoculated with human homogenate from mutant Swedish or sporadic AD origin. In addition, Arctic-inoculated mice contain abundant Aβ38-positive CAA deposits in the thalamus that appeared furry by immunohistochemistry analysis, with Aβ aggregates extending outwards from the blood vessels (Watts et al. 2014). Strikingly, these apparently strain-specific features are conserved following serial passage in mice (Watts et al. 2014). These studies demonstrate that, similar to PrP^{Sc}, unique pathological and biochemical profiles of Aβ are found amongst cases of AD, which are conserved upon introduction in susceptible hosts.

Strain variability in prion disease is attributed to alterations in the conformation of PrP^{Sc}, and likewise, evidence suggests the conformation of Aβ may encipher unique pathological and clinical profiles in AD. Transmission electron microscopy and solid-state NMR has been used to model Aβ species where fibrillization of monomeric Aβ seeded with human brain from different AD patients produces discrete populations of fibrils with unique morphology (Lu *et al.* 2013). The ability of AD brain to seed fibrils that are not polymorphic suggests that a single nucleation event may be responsible for the spread of Aβ in disease and, given that the patients from which the Aβ seeds were derived exhibited different clinical and pathological profiles, the conformational properties of Aβ may translate the clinical presentation in human AD. Consistent with this, producing recombinant Aβ fibrils in the absence of presence of detergent produces distinct species of Aβ that induce differential plaque characteristics and Aβ peptide ratios in mouse brain (Stöhr et al. 2014). Hence the conformation of the Aβ species appears to be a contributing factor to the clinical and pathological development of disease.

Prion-like properties of α-synuclein

The deposition of misfolded αsyn in the CNS is a pathological hallmark of synucleinopathies and includes PD, DLB and MSA, among others. Although overlapping clinical presentations can make correct diagnosis difficult, synucleinopathies are distinguished by unique profiles of cell types and brain regions susceptible to develop αsyn pathology and the locality of neuronal vulnerability. For example, the pathology of PD and DLB is characterized by intraneuronal deposits of αsyn called Lewy bodies (LB) or Lewy neurites (LN) whereas MSA-associated αsyn is distinguished

predominantly by cytoplasmic inclusions called glial cytoplasmic inclusions (GCIs) which predominately occur in oligodendroglia (Figure 2). The expression of αsyn protein is associated with disease as duplications or triplications in the gene encoding αsyn, *SNCA*, and various point mutations (such as A53T, A30P, E46K) cause early onset familial PD. Similarly, transgenic animal models that express human wild-type αsyn, or αsyn containing PD-associated mutations, spontaneously develop αsyn pathology and neurological illness and are used to model disease (Masliah *et al.* 2000, Kahle *et al.* 2000, Feany & Bender 2000, van der Putten *et al.* 2000). Transgenic animal models expressing mutant αsyn are typically used to study disease associated with protein aggregation, however many other transgenic systems have been developed to study other features of disease such as mitochondrial dysfunction and motor impairment (Chesselet & Richter 2011).

Some of the earliest evidence that αsyn may have the characteristics of a prion came from autopsy of PD patients who, as part of a clinical trial, had fetal nigral dopaminergic nerve cells grafted into their brain. The presence of αsyn deposits in the transplanted tissue suggested in the 11-16 years post-surgery, αsyn transmission occurred from the host to the grafted tissue (Kordower *et al.* 2008, Li *et al.* 2008). Contrasting reports on fetal transplants in PD patients have described grafted regions remain dopaminergic and absent of αsyn pathology for up to 14 years post surgery (Mendez *et al.* 2008), however numerous *in vitro* data support propagation of αsyn seeds. In immortalized cells overexpressing A53T αsyn protein, exposure to misfolded αsyn species leads to punctate staining of αsyn suggestive of propagation of disease-associated inclusions (Danzer *et al.* 2009). Similarly, using cationic-liposomes to aid transfection, fluorescently-tagged fibrillar αsyn has been shown to enter immortalized cells overexpressing αsyn and induce changes in endogenous protein consistent with pathogenic αsyn such as phosphorylation, ubiquitination and insolubility (Luk *et al.* 2009). A caveat to these studies is the requirement for αsyn to be overexpressed in the cells, which does not model disease where the majority of synucleinopathies express normal levels of αsyn protein, and the use of artificial vehicle reagents to aid the delivery of misfolded protein into the cell. However a similar finding has also been found following extracellular exposure of primary neuronal cultures from wild-type mice to fibrillar αsyn species without the aid of vehicle reagents, which resulted in the recruitment of endogenous αsyn into insoluble LB-like inclusions and associated changes to neuronal vulnerability including decreases in synaptic proteins and impairment in neuronal excitability (Volpicelli-Daley *et al.* 2011).

Several other studies support *in vitro* propagation of αsyn. Co-culturing two cell lines expressing αsyn containing different fluorescent tags results in the progressive emergence of double-labelled cells (Hansen *et al.* 2011) and treating tagged αsyn cells with misfolded αsyn tagged with a different probe leads to significant co-localization in cultured cells (Bousset *et al.* 2013). In immortalized cells expressing tagged-αsyn, incubation with human brain derived from MSA

patients causes the tagged-αsyn to aggregate (Prusiner *et al.* 2015, Woerman *et al.* 2015). In these cultures, αsyn aggregates have been shown to transverse in both retrograde and anterograde direction and transfer to neighbouring neurons and glial cells (Hansen et al. 2011, Desplats *et al.* 2009, Danzer et al. 2009). Collectively these studies give strong evidence for prion-like transmission of αsyn *in vitro*.

Prion-like propagation of αsyn is further supported by *in vivo* studies inoculating αsyn seeds into susceptible mice. In transgenic mice expressing homozygous mutant A53T αsyn ($M83^{+/+}$), inoculation of fibrillar recombinant αsyn or brain homogenate from aged, terminal mice, into the brain of young pre-symptomatic mice accelerates the development of αsyn CNS pathology and clinical disease (Luk *et al.* 2012b, Mougenot *et al.* 2012, Peelaerts *et al.* 2015, Sacino *et al.* 2014c, Rutherford *et al.* 2015, Bétemps *et al.* 2014, Sacino *et al.* 2013, Sacino *et al.* 2014a). A similar observation is found following intramuscular or intravenous injection of αsyn seeds (Sacino *et al.* 2014b, Peelaerts et al. 2015). In these studies, similar to the human condition, aggregated αsyn is largely phosphorylated and other pathological features of disease present, including reactive astrogliosis and microgliosis. Consistent with the observations in human studies, grafting dopaminergic neurons into transgenic mice overexpressing human αsyn leads to the identification of αsyn aggregation in the grafted tissue (Hansen 2011).

Seeded propagation has also been reported after inoculation of misfolded recombinant αsyn in wild-type rodents (Luk *et al.* 2012a, Paumier *et al.* 2015, Masuda-Suzukake *et al.* 2014, Masuda-Suzukake *et al.* 2013, Tran *et al.* 2014, Sacino et al. 2013) and transgenic mice overexpressing wild-type αsyn ($M20^{+/+}$) that do not spontaneously develop inclusions over their lifetime (Sacino et al. 2013, Sacino et al. 2014a). In some studies, motor impairment has been reported to accompany propagation (Tran et al. 2014, Luk et al. 2012a), and accordingly, treating mice with antibodies targeting misfolded αsyn via intraperitoneal injection reduces neuronal loss and delays the onset of clinical disease (Tran et al. 2014). Similarly, human MSA brain may induce propagation of αsyn and clinical disease in hemizygous $M83^{+/-}$ mice, which do not spontaneously develop inclusions or clinical disease in most experimental timeframes (Prusiner et al. 2015, Watts *et al.* 2013). Although propagation of αsyn has been extensively reported, with αsyn pathology shown to extend along white matter tracks to regions distant from the injection site, it has recently been shown well-used antibodies targeting phosphorylated αsyn (pSer128/81A) cross-reacts with neurofilament subunit L that is abundant in white matter (Sacino et al. 2014c), hence previously reported models of propagation may include artefacts of phosphorylated αsyn expression associated with using these antibodies. This observation may be a matter of dose threshold of the antibody, as others have found no cross-reactivity (Luk et al. 2012a, Paumier et al. 2015, Masuda-Suzukake et al. 2013, Tran et al. 2014, Guo *et al.* 2013), including minimal pSer128/81A positive staining in transgenic αsyn knock-out mouse brain and primary neuronal cultures (Luk et al.

2012a, Volpicelli-Daley et al. 2011). Regardless, the specificity of these antibodies requires further investigation and is an important technical issue to clarify.

Although the evidence for prion-like propagation of αsyn is extensive, variation exists in the incubation period, pattern of αsyn pathology and mouse line used. For example, whilst $M83^{+/+}$ mice develop extensive αsyn pathology following injection with recombinant fibrillar αsyn, in other transgenic mouse lines, such as $M47^{+/+}$ (expressing human αsyn E46K mutation), αsyn deposition is largely restricted to the injection site (Sacino et al. 2014c). This may indicate artefacts associated with αsyn aggregation with the expression of certain transgenes, or that the normal wild-type αsyn cannot be patterned and amplified using the E46K mutated αsyn. Furthermore, it has recently been reported injection of spinal cord homogenate from wild-type mice induces robust CNS αsyn pathology in $M83^{+/-}$ mice (Sacino *et al.* 2015), suggesting inherent characteristics of the transgenic M83 mouse may contribute to αsyn pathology. Although propagation of αsyn pathology has also been reported in wild-type animals (Luk et al. 2012a, Paumier et al. 2015, Masuda-Suzukake et al. 2014, Masuda-Suzukake et al. 2013, Tran et al. 2014, Sacino et al. 2013, Sacino et al. 2014a), similar results are unable to be achieved in other studies (Sacino et al. 2014c) which may reflect the type of seed injected being unable to interact with the native αsyn. An important consideration is that mouse αsyn contains a threonine at position 53 and given that most studies using transgenic αsyn mice express the transgene on a wild-type background, species-specific contributions by endogenous αsyn may be a confounding factor in these studies. However recently it has been shown transgenic mice expressing wild-type human αsyn on a knockout αsyn background are also conducive to CNS αsyn propagation following injection of human brain homogenate from DLB and MSA patients (Bernis *et al.* 2015), giving evidence for αsyn propagation being a conserved observation in various genetic backgrounds.

Similar to prions, lipids may represent a co-factor to enhance the fibrillization of αsyn; lipid-rich exosomes accelerate the fibrillization of monomeric αsyn (Grey *et al.* 2015) and appear to act by stimulating primary nucleation (Galvagnion *et al.* 2015). Misfolded αsyn species may be produced using PMCA (Herva *et al.* 2014), however unlike PrP misfolding, aggregation of αsyn may be induced easily from recombinant wild-type protein in the absence of co-factors or serial rounds of propagation. Prions do not exclusively form amyloid and may exist as various soluble and insoluble species, and similarly the capacity of αsyn to trigger misfolding is conserved in both fibrillar and non-amyloidogenic seeds (Sacino et al. 2013). The ability to generate various αsyn species from the same starting material *in vitro* is reminiscent of strain variation amongst prions. Two populations of αsyn, ribbons and fibrils, exert differential abilities to cross-seed fibrillization of monomeric αsyn or tau protein and induce toxicity in immortalized cells (Bousset et al. 2013, Guo et al. 2013). Following injection into rat brain, ribbons induce more abundant deposition of phosphorylated αsyn, whilst fibrils were shown to represent the most toxic species, causing cell

death and associated motor impairment after injection (Peelaerts et al. 2015). Brain tissue from MSA patients exhibited differences in triggering aggregation of αsyn *in vitro* (Woerman et al. 2015) and αsyn isolated from human PD brain show differential PK-induced cleavage products (Guo et al. 2013). Intriguingly, recent evidence also demonstrates differences in transmissibility between αsyn derived from various human conditions. In $M83^{+/-}$ mice, inoculation with human MSA, but not PD brain homogenate induces disease consisting of deposited phosphorylated αsyn, progressive neurologic dysfunction, astrogliosis and microgliosis. The aggregation of αsyn in these mice following inoculation with MSA brain extract was predominantly observed as cytoplasmic inclusions located in neuronal populations, which is in contrast to its deposition in oligodendroglia characteristic of MSA (Prusiner et al. 2015). The pathological phenotype induced in these animals is independent of total αsyn protein and instead correlates to the capacity of the brain homogenate to induce aggregation in immortalized cells expressing tagged-A53T αsyn. This observation is in contrast to PD brain tissue, which was unable to induce aggregation in the same model of transgenic cells or transmit disease to $M83^{+/-}$ mice. The specific biochemical and biophysical differences between MSA and PD-derived αsyn that translate these pathogenic properties is unknown. Furthermore, the disease that develops in MSA-infected mice is transmissible, which causes disease when inoculated into young $M83^{+/-}$ mice.

Mounting evidence appears to draw parallels between αsyn, Aβ and PrP^{Sc}, with studies supporting prion-like seeding, propagation and transmissibility of pathogenic proteins causing disease, however caveats exist associated with the use of transgenic mice to adequately model disease. For example, the possibility of other agents aside from αsyn to causes aggregation raises important considerations when evoking prion-like mechanisms and reflects hazards associated with using transgenic mice where aggregation of protein is an inherent characteristic of that model. Studies that use transgenic mice that remain asymptomatic may alleviate these issues, however it is not clear whether expressing lower levels of the transgene are entirely innocuous. In prion disease, transmission of disease into susceptible wild-type animals may readily be achieved, and represent *bona fide* models of disease. Therefore the gold-standard for investigating prion-like propagation and transmission of misfolded protein would require the use of non-transgenic animals. Numerous data support the ability of certain αsyn species to propagate and transmit disease in wild-type mice (Luk et al. 2012a, Paumier et al. 2015, Masuda-Suzukake et al. 2014, Masuda-Suzukake et al. 2013, Tran et al. 2014, Sacino et al. 2013), however in the case of Aβ, equivalent studies require the use of transgenic APP animals (Figure 3).

If conclusive evidence arises showing other misfolded proteins constitute a prion, there would undoubtedly be substantial implications on the health and safety regulations of these diseases. However it is important to bear in mind that prions derived from different proteins will likely have different biochemical characteristics. Recently it has been shown misfolded αsyn are markedly

easier to decontaminate from surfaces and do not require the stringent techniques required for decontamination of prions (Bousset *et al.* 2015), and as such the potential for untoward public health risks, such as unintentional transmission of misfolded αsyn, seems unlikely. This observation reflects fundamental differences in the structure of these proteins and stability of the misfolded isoform. Although Aβ and αsyn are largely unstructured proteins, the GPI-anchored PrP^{C} has a well-defined tertiary structure, and PrP^{C} and PrP^{Sc} are stable proteins; as such, the activation energy required for misfolding of PrP^{C} into PrP^{Sc} is high. This is in contrast to αsyn and Aβ where the activation energy required is low and the prion-like 'misfolding' of protein reflects the aggregation of protein and its ability to form amyloid. In this regard, the size of the infectious unit of PrP may differ from αsyn and Aβ.

Neurotoxicity of misfolded protein

A consistent feature that accompanies the accumulation of protein in the CNS is the progressive damage and loss of neurons. Although disease-specific differences are found in the tempo and localization of neurotoxicity between proteinopathies, similarities are found which may indicate conserved mechanisms underlie the generation of cell death. This may reflect conserved fibrillization properties, where fibril formation involves the misfolding of monomeric protein and extension into various species (oligomers, protofibrils) prior to its maturation into mature fibrils (Figure 1). Given the current attention on the prion concept in proteinopathies, extrapolation of knowledge of folded proteins may prove rewarding in deciphering general neurotoxic mechanisms.

Historically, the presence of misfolded proteins in the brain led to the belief that the sheer aggregation of protein was responsible for neurotoxicity in proteinopathies, however today this is considered an oversimplification. In prion disease, large variation exists in the protein deposition seen in the CNS at post mortem, where in some cases neurodegeneration occurs with minimal PrP^{Sc} load (Medori *et al.* 1992, Collinge *et al.* 1990). Transgenic mice expressing mutations associated with familial prion disease have less aggregated protein in their brain at terminal stage disease compared to prion-infected wild-type mice (Manson *et al.* 1999), or PrP^{Sc} is entirely absent (Hsiao *et al.* 1990). Prion infection in mice expressing PrP^{C} lacking a GPI-anchor causes disease characterized by a long incubation period prior to the development of clinical symptoms, despite large amounts of PrP^{Sc} accumulation in the CNS (Chesebro *et al.* 2010). In addition, under certain conditions, subclinical infection can occur where PrP^{Sc} propagation occurs without the development of clinical symptoms or neurodegeneration. Mice infected with hamster prions remain asymptomatic despite having prion titres in their brain equivalent to end-stage disease in conventional mice (Hill *et al.* 2000). Other states of subclinical infection are achieved when low inoculum or oral infection routes are used (Thackray *et al.* 2002, Thackray *et al.* 2003, Collins *et al.* 2005). The PERK arm of the unfolded protein response (UPR) has emerged as a pertinent

signalling pathway associated with neurodegeneration in prion disease (Moreno *et al.* 2012) and treating prion-infected mice with compounds targeting this pathway reverses cognitive deficits and improves synaptic markers independent of amyloid load (Moreno *et al.* 2013, Halliday *et al.* 2015). Kinetic studies also reveal a divergence between infectivity and toxicity, with the two occurring in separate phases in disease (Sandberg *et al.* 2011, Sandberg *et al.* 2014, Mays *et al.* 2015).

Disassociation between protein aggregation and neurotoxicity is similarly observed in AD and synucleinopathies. In human AD brain, cell loss is not associated with regions of protein deposition (Giannakopoulos *et al.* 2003, Bennett *et al.* 2004) and in certain transgenic animal models of AD, death can occur in the absence of amyloid formation (Carlson *et al.* 1997). Likewise, insoluble αsyn does not correlate well with the degree of neurodegeneration in human brain (Hughes *et al.* 1992, Tompkins & Hill 1997, Parkkinen *et al.* 2005). This observation extends to animal models where in transgenic rodent and fly models of PD, cell loss can occur in the absence of protein aggregation (Auluck *et al.* 2002, Masliah et al. 2000). These studies suggest that the deposition of misfolded protein is unlikely to be the toxic agent and instead may be an unrelated endpoint to disease pathogenesis.

In prion disease, toxicity is it not considered associated with a loss of function of the protein because PrP knockout mice exhibit no neurodegenerative phenotype (Bueler *et al.* 1992, Mallucci *et al.* 2002). Instead neurotoxicity is linked to prion replication, in view of the finding that only cells that express PrP^{C} may be damaged by PrP^{Sc}. Prion-infected PrP knockout mice hosting grafted PrP-expressing tissue only develop neurodegeneration in the PrP-expressing tissue, whilst PrP deposition is widespread across the brain (Brandner *et al.* 1996). Similarly, ablation of PrP in mice with established prion infection terminates disease development and reverses clinical symptoms, despite these mice harbouring levels of PrP^{Sc} in the brain equivalent to end-stage disease (Mallucci et al. 2003). These studies give strong evidence for the conversion of PrP^{C} to PrP^{Sc}, and not the sheer aggregation of protein, being a major component to neurotoxicity. These findings may be relevant to neurotoxic mechanisms of αsyn and Aβ misfolding, and thus unifying knowledge may help shed light on general toxic mechanisms of folded protein.

Toxic oligomers

Some of the strongest evidence exists for small soluble oligomers of misfolded protein being the neurotoxic agent in proteinopathies. These species are highly unstable compared to the organized β-sheet rich structures formed by mature fibrils and by virtue of size, smaller aggregates have a higher relative exposed surface area than larger mature aggregates (Chiti & Dobson 2006). Indeed, it has been suggested the recruitment of misfolded protein into aggregates is a mechanism

employed by the cell to sequester small toxic species away from vital cellular compartments where they cause substantial damage. This may represent a common mechanism shared amongst the various proteinopathies.

Aβ oligomers

In human AD brain, neurodegeneration has been correlated to the pool of soluble Aβ species (McLean *et al.* 1999, Mc Donald *et al.* 2010); a finding which is consistent with the fact the particularly aggressive AD-associated Arctic mutation produces larger quantities of prefibrillar species (Nilsberth et al. 2001). The toxicity of Aβ in cultured cells is enhanced in heterogeneous populations of Aβ species compared to discrete species, with the toxicity of protofibrils being linked to the expression of monomeric protein (Jan *et al.* 2011). This finding suggests ongoing fibril formation involving interactions between misfolded species and monomeric Aβ is pertinent to toxicity. In wild-type mice, oligomeric Aβ preparations, but not insoluble amyloid plaque cores, alters synaptic plasticity by blocking long term potentiation (LTP) and activating long term depression (LTD) (Walsh *et al.* 2002, Shankar *et al.* 2008). They also trigger dendritic spine retraction (Lacor *et al.* 2007, Shankar *et al.* 2007), reduce dendritic spine density (Shankar et al. 2008) and cause memory deficits (Shankar et al. 2008, Lesne *et al.* 2006). Collectively these studies give strong evidence for oligomeric Aβ being the neurotoxic species.

The mechanisms underlying Aβ oligomeric-induced toxicity has also been studied, where the N-Methyl D-Aspartate (NMDA) and metabotrophic glutamate-5 (mGlu5) receptors have been associated with the development of LTP and LTD respectively (Shankar et al. 2007, Shankar et al. 2008). Interestingly, the modulation of the mGlu5 receptor appears to involve PrP^{C}, which acts as a receptor for Aβ oligomers and complexes with mGlu5 (Lauren *et al.* 2009). Accordingly, PrP-directed antibodies inhibit Aβ-oligomer induced toxicity (Klyubin *et al.* 2014). It has been reported PrP knockout mice are resistant to toxicity elicited by Aβ oligomers (Lauren et al. 2009), however follow up studies have been unable to observe such a striking effect (Balducci *et al.* 2010, Calella *et al.* 2010, Cisse *et al.* 2011, Kessels *et al.* 2010). Aside from acting on neuronal receptors, Aβ oligomers may also cause toxicity by inducing hyperphosphorylation of tau, leading to collapse of microtubule cytoskeleton and neuritic dystrophy (Jin *et al.* 2011).

The conformation of the toxic Aβ species is still unclear. Investigations into the toxicity of structurally defined oligomers demonstrate neurotoxicity in cultured cells increases with oligomer order for species larger than a monomer (Ono *et al.* 2009); a finding which is supported by studies showing the smallest toxic Aβ species is the dimer (Shankar et al. 2008, Jin et al. 2011). However others report dimers correlate poorly with toxicity in cultured neurons and instead trimers and tetramers may be the smallest toxic agents (Jana *et al.* 2015). Trimers are also believed to be the basic component of Aβ*56 (Lesne et al. 2006); a non-fibrillar 56-kDa Aβ oligomeric species that

has also been implicated in toxicity. This species is naturally present in human brain and CSF, where it correlates with soluble tau and negatively correlates with several synaptic proteins in cognitively intact individuals (Handoko *et al.* 2013, Lesne *et al.* 2013). In rats, Aβ*56 induces transient cognitive dysfunction independent of amyloid deposition and neuronal loss (Lesne et al. 2006) and as such Aβ*56 may contribute to the early deficits seen in AD, prior to the accumulation of protein indicative of disease. Given Aβ*56 is likely to be a dodecamer (based on its apparent molecular weight on SDS-PAGE), further support for it being the toxic agent comes from studies highlighting the role of large oligomeric structures of Aβ as potent toxic oligomers (Gong *et al.* 2003, Bitan *et al.* 2003, Bernstein *et al.* 2009). However given that there is no evidence Aβ*56 alone can induce persistent neurological dysfunction, its generation is not considered necessary for toxicity associated with Aβ accumulation.

αsyn oligomers

In human DLB brain, the presence of αsyn aggregates at presynaptic terminals, rather than LBs, correlates with decreases in presynaptic markers and dendritic spine loss (Kramer & Schulz-Schaeffer 2007). Similarly, soluble oligomers are found enriched in regions of PD brain found to have deregulated markers of protein synthesis (Garcia-Esparcia *et al.* 2015). In rodents, the expression of mutant αsyn that preferentially forms oligomers is more toxic than mutations which more readily form fibrils (Winner *et al.* 2011). In primary neuronal cultures and transgenic invertebrate (*Caenorhabditis elegans and Drosphilia melanogaster*) model systems, the expression of variants of αsyn that increase its propensity to oligomerize and decrease the formation of fibrils are more toxic than wild-type αsyn (Karpinar *et al.* 2009). Misfolded αsyn species produced in the presence of a compound that promotes formation of large fibrillar aggregates and reduces oligomer species exhibits reduced seeding potential and toxicity to cultured cells compared to untreated fibrils (Lam *et al.* 2016). Various oligomeric species produced from synthetic wild-type αsyn display varying degrees of toxicity to cultured cells (Danzer *et al.* 2007) and compared to preparations of fibrillar αsyn, primary neuronal cultures treated with purified oligomeric species activate higher levels of reactive oxygen species (Chen *et al.* 2015). Collectively these studies give strong evidence for oligomers being the most toxic αsyn species.

Studies have investigated the mechanism of toxicity by αsyn species. Prefibrillar αsyn oligomers have been shown to cause mitochondrial impairment (Luth *et al.* 2014) and golgi fragmentation and trafficking malfunction in association with reduced cell viability (Gosavi *et al.* 2002) *in vitro*. The identification of mitochondria as a target for αsyn oligomers aligns with a plethora of data linking mitochondria dysfunction to synucleinopathies; in human PD brain deficits are found in mitochondrial complex I activity (Schapira *et al.* 1989, Parker *et al.* 1989, Janetzky *et al.* 1994) and several genes that cause familial PD are associated with mitochondria functioning, including

PINK1, *LRRK2*, *Parkin* and *DJ-1* (Camilleri & Vassallo 2014). Additionally chemicals that induce mitochondria dysfunction are capable of manifesting PD in humans and in animal models in the absence of αsyn deposition (Langston & Ballard 1983, Burns *et al.* 1983, Betarbet *et al.* 2000). Therefore it is possible that mitochondria dysfunction is a central mechanism associated with toxicity in the various subsets of synucleinopathies.

Another potential site for αsyn oligomer-induced damage is the cellular membrane. It is well known αsyn has a high affinity to bind to lipid species (Ruiperez *et al.* 2010) and in cultured neurons exogenously added αsyn oligomers can form clusters on the cell membrane and associate with the α3 subunit of Na^+/K^+-ATPase, causing alterations to the distribution of the receptor and deficits in Na^+ gradient and pumping potential (Shrivastava *et al.* 2015). This finding aligns with other *in vitro* studies suggesting pore formation or leak channel as toxic mechanisms by αsyn oligomer or protofibril species (Danzer et al. 2007, Volles *et al.* 2001). Given that mutations in the gene encoding the α3 subunit of Na^+/K^+-ATPase are associated with rapid-onset dystonia Parkinsonism (de Carvalho Aguiar *et al.* 2004, Rodacker *et al.* 2006, Clapcote *et al.* 2009), an adverse interaction with αsyn and the receptor is likely to be relevant to at least a subset of human synucleinopathy conditions.

Although αsyn oligomers have been shown to be toxic to neurons, they may not represent the only toxic αsyn species. Fibrils also form clusters on neuronal membranes and associate with the α3 subunit of Na^+/K^+-ATPase with higher affinity than oligomers (Shrivastava et al. 2015). They are also reported to exhibit the highest degree of toxicity compared to oligomers and ribbons when injected into rat brain (Peelaerts et al. 2015); an observation that is supported by several *in vitro* studies (Bousset et al. 2013, Pieri *et al.* 2012). Therefore the relevance of oligomeric, and larger αsyn species to toxicity needs further investigation.

PrP oligomers

Although difficulties in producing and isolating oligomeric PrP species have hampered studies on toxic PrP-oligomers, it is likely the structure of oligomers are conserved amongst amyloid-forming proteins. Support for this comes from the finding that synthetic antibodies are capable of binding oligomeric species of amyloid-forming protein irrespective of primary sequence including PrP, αsyn, Aβ and islet amyloid polypeptide (iAPP) (Kayed *et al.* 2003). Similarly, compounds that bind to and inhibit oligomer formation have been reported efficacious to both prion and PD-animal models (Wagner *et al.* 2013).

Studies using synthetic PrP show strong support for small aggregates being the toxic species. Species reminiscent of oligomers have increased toxicity *in vitro* (Kazlauskaite *et al.* 2005, Novitskaya *et al.* 2006, Simoneau et al. 2007) and *in vivo* (Simoneau et al. 2007) compared to

larger fibrillar species. During PrP^{C} misfolding, it is believed monomeric PrP adopts an α-helical conformation prior to its dimerization and extension into oligomers (Jansen *et al.* 2001). Monomeric recombinant α-helical PrP is highly toxic to murine neuronal but not fibroblast cell cultures and causes toxicity in a dose-dependent manner when injected into mouse brain (Zhou *et al.* 2012). These studies are supported by kinetic studies showing oligomeric species increase with the development of clinical disease in prion-infected mice (Mays et al. 2015).

Although the evidence for oligomers being the toxic agent is compelling, no common mechanism of action has been identified. This may be a result of the differential endogenous localization of the proteins and/or different biochemical or morphological properties of the putative toxic species. The latter factor is particularly relevant given that 'oligomers' refers to essentially any species larger than a monomer and thus represents a wide range of various sized species. Given that these species exist in equilibrium with each other, distinguishing one species as toxic over another will be challenging. This was reflected by studies showing the reported putative toxic Aβ dimer rapidly aggregates into metastable protofibrils (O'Nuallain *et al.* 2010) that may be more toxic in nature. An additional consideration is the potential for artificial manipulation of fibrillization by laboratory processes or reagents. The popular laboratory solvent SDS is capable of inducing artificial aggregation of Aβ (Bitan *et al.* 2005) and αsyn (Giehm *et al.* 2010); which in the case of αsyn follows an atypical fibrillization pathway. Thus the *in vitro* generation of oligomers or preparations of tissue are important considerations when deciphering the toxic species.

Conclusion

A growing body of evidence is finding parallels between Aβ, αsyn and the well-studied PrP^{Sc}. Studies show that similar to prions, seeds of Aβ and αsyn are capable of inducing aggregation in normal protein that propagate within the CNS, that in some cases leads to disease in susceptible animals. The concept of strain variation may also be a conserved feature of these proteins, where differences in structure of misfolded species incite unique disease phenotypes. Current evidence suggests certain αsyn species are more prion-like than others, where in contrast to αsyn derived from PD brain, MSA-associated αsyn is efficient at propagating *in vitro* and *in vivo*, causing disease in susceptible transgenic mice (Prusiner et al. 2015). This is an interesting finding given that, similar to prion disease, the clinical progression of MSA is rapid in onset, whereas AD and PD are slower progressing disorders. Although compelling, there are experimental considerations in current studies, such as the ability of transgenic animals to accurately model disease and assess prion-like characteristics of aberrant proteins. Therefore, important future studies should include the use wild-type animals and robust biochemical techniques that investigate the different species of pathogenic protein aggregates, to define those that are most proficient at propagating, seeding

and causing toxicity. Further examination will, no doubt, shed important light on these issues which may ultimately lead to the expansion of the prion concept.

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References

Auluck, P. K., Chan, H. Y. E., Trojanowski, J. Q., Lee, V. M. Y. and Bonini, N. M. (2002) Chaperone suppression of α-synuclein toxicity in a Drosophila model for Parkinson's disease. *Science,* **295,** 865-868.

Baker, H. F., Ridley, R. M., Duchen, L. W., Crow, T. J. and Bruton, C. J. (1993) Evidence for the experimental transmission of cerebral β-amyloidosis to primates. *International journal of experimental pathology,* **74,** 441-454.

Balducci, C., Beeg, M., Stravalaci, M. et al. (2010) Synthetic amyloid-β oligomers impair long-term memory independently of cellular prion protein. *Proceedings of the National Academy of Sciences of the United States of America,* **107,** 2295-2300.

Barria, M. A., Mukherjee, A., Gonzalez-Romero, D., Morales, R. and Soto, C. (2009) De novo generation of infectious prions in vitro produces a new disease phenotype. *Plos Pathogens,* **5**.

Bennett, D. A., Schneider, J. A., Wilson, R. S., Bienias, J. L. and Arnold, S. E. (2004) Neurofibrillary tangles mediate the association of amyloid load with clinical Alzheimer disease and level of cognitive function. *Archives of Neurology,* **61,** 378-384.

Bernis, M. E., Babila, J. T., Breid, S., Wusten, K. A., Wullner, U. and Tamguney, G. (2015) Prion-like propagation of human brain-derived α-synuclein in transgenic mice expressing human wild-type α-synuclein. *Acta Neuropathol Commun,* **3,** 75.

Bernoulli, C., Siegfried, J., Baumgartner, G., Regli, F., Rabinowicz, T., Gajdusek, D. C. and Gibbs, C. J. (1977) Danger of accidental person-to-person transmission of Creutzfeldt-Jakob disease by surgery. *Lancet,* **1,** 478-479.

Bernstein, S. L., Dupuis, N. F., Lazo, N. D. et al. (2009) Amyloid-β protein oligomerization and the importance of tetramers and dodecamers in the aetiology of Alzheimer's disease. *Nature Chemistry,* **1,** 326-331.

Bessen, R. A. and Marsh, R. F. (1992a) Biochemical and physical properties of the prion protein from two strains of the transmissible mink encephalopathy agent. *J Virol,* **66,** 2096-2101.

Bessen, R. A. and Marsh, R. F. (1992b) Identification of two biologically distinct strains of transmissible mink encephalopathy in hamsters. *The Journal of general virology,* **73 (Pt 2),** 329-334.

Betarbet, R., Sherer, T. B., MacKenzie, G., Garcia-Osuna, M., Panov, A. V. and Greenamyre, J. T. (2000) Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nature Neuroscience,* **3,** 1301-1306.

Bétemps, D., Verchère, J., Brot, S., Morignat, E., Bousset, L., Gaillard, D., Lakhdar, L., Melki, R. and Baron, T. (2014) α-synuclein spreading in M83 mice brain revealed by detection of pathological α-synuclein by enhanced ELISA. *Acta Neuropathologica Communications,* **2,** 1-14.

Bitan, G., Fradinger, E. A., Spring, S. M. and Teplow, D. B. (2005) Neurotoxic protein oligomers - what you see is not always what you get. *Amyloid-Journal of Protein Folding Disorders,* **12,** 88-95.

Bitan, G., Kirkitadze, M. D., Lomakin, A., Vollers, S. S., Benedek, G. B. and Teplow, D. B. (2003) Amyloid β-protein (Aβ) assembly: Aβ40 and Aβ42 oligomerize through distinct pathways. *Proceedings of the National Academy of Sciences of the United States of America,* **100,** 330-335.

Borchelt, D. R., Scott, M., Taraboulos, A., Stahl, N. and Prusiner, S. B. (1990) Scrapie and cellular prion proteins differ in their kinetic of synthesis and topology in cultured-cells. *Journal of Cell Biology,* **110,** 743-752.

Bousset, L., Brundin, P., Bockmann, A., Meier, B. and Melki, R. (2015) An efficient procedure for removal and inactivation of α-synuclein assemblies from laboratory materials. *Journal of Parkinson's disease*.

Bousset, L., Pieri, L., Ruiz-Arlandis, G. et al. (2013) Structural and functional characterization of two α-synuclein strains. *Nature Communications,* **4,** 2575-2575.

Braak, H. and Braak, E. (1991) Neuropathological staging of Alzheimer-related changes. *Acta Neuropathologica,* **82,** 239-259.

Braak, H., Del Tredici, K., Rub, U., de Vos, R. A. I., Steur, E. and Braak, E. (2003) Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiology of Aging,* **24,** 197-211.

Brandner, S., Isenmann, S., Raeber, A., Fischer, M., Sailer, A., Kobayashi, Y., Marino, S., Weissmann, C. and Aguzzi, A. (1996) Normal host prion protein necessary for scrapie-induced neurotoxicity. *Nature,* **379,** 339-343.

Brown, P., Brandel, J. P., Preese, M. and Sato, T. (2006) Iatrogenic Creutzfeldt-Jakob disease - The waning of an era. *Neurology,* **67,** 389-393.

Brown, P., Brandel, J. P., Sato, T. et al. (2012) latrogenic Creutzfeldt-Jakob disease, final assessment. *Emerging Infectious Diseases,* **18,** 901-907.

Bueler, H., Aguzzi, A., Sailer, A., Greiner, R. A., Autenried, P., Aguet, M. and Weissmann, C. (1993) Mice devoid of PrP are resistant to scrapie. *Cell,* **73,** 1339-1347.

Bueler, H., Fischer, M., Lang, Y., Bluethmann, H., Lipp, H. P., Dearmond, S. J., Prusiner, S. B., Aguet, M. and Weissmann, C. (1992) Normal development and behavior of mice lacking the neuronal cell-surface PrP protein. *Nature,* **356,** 577-582.

Burns, R. S., Chiueh, C. C., Markey, S. P., Ebert, M. H., Jacobowitz, D. M. and Kopin, I. J. (1983) A primate model of Parkinsonism: Selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by n-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Proceedings of the National Academy of Sciences of the United States of America-Biological Sciences,* **80,** 4546-4550.

Calella, A. M., Farinelli, M., Nuvolone, M., Mirante, O., Moos, R., Falsig, J., Mansuy, I. M. and Aguzzi, A. (2010) Prion protein and Aβ-related synaptic toxicity impairment. *Embo Molecular Medicine,* **2,** 306-314.

Camilleri, A. and Vassallo, N. (2014) The centrality of mitochondria in the pathogenesis and treatment of Parkinson's disease. *Cns Neuroscience & Therapeutics,* **20,** 591-602.

Carlson, G. A., Borchelt, D. R., Dake, A. et al. (1997) Genetic modification of the phenotypes produced by amyloid precursor protein overexpression in transgenic mice. *Human Molecular Genetics,* **6,** 1951-1959.

Castilla, J., Saa, P., Hetz, C. and Soto, C. (2005) In vitro generation of infectious scrapie prions. *Cell,* **121,** 195-206.

Caughey, B., Raymond, G. J. and Bessen, R. A. (1998) Strain-dependent differences in β-sheet conformations of abnormal prion protein. *Journal of Biological Chemistry,* **273,** 32230.

Chandler, R. L. (1961) Encephalopathy in mice produced by inoculation with scrapie brain material. *Lancet,* **1,** 1378-1379.

Chen, S. W., Drakulic, S., Deas, E. et al. (2015) Structural characterization of toxic oligomers that are kinetically trapped during α-synuclein fibril formation. *Proceedings of the National Academy of Sciences of the United States of America,* **112,** E1994-E2003.

Chesebro, B., Race, B., Meade-White, K. et al. (2010) Fatal transmissible amyloid encephalopathy: A new type of prion disease associated with lack of prion protein membrane anchoring. *Plos Pathogens,* **6**.

Chesselet, M. F. and Richter, F. (2011) Modelling of Parkinson's disease in mice. *Lancet Neurology,* **10,** 1108-1118.

Chiti, F. and Dobson, C. M. (2006) Protein misfolding, functional amyloid, and human disease. In: *Annual Review of Biochemistry*, Vol. 75, pp. 333-366.

Cisse, M., Sanchez, P. E., Kim, D. H., Ho, K., Yu, G. Q. and Mucke, L. (2011) Ablation of cellular prion protein does not ameliorate abnormal neural network activity or cognitive dysfunction in the J20 line of human amyloid precursor protein transgenic mice. *Journal of Neuroscience,* **31,** 10427-10431.

Citron, M., Oltersdorf, T., Haass, C., McConlogue, L., Hung, A. Y., Seubert, P., Vigo-Pelfrey, C., Lieberburg, I. and Selkoe, D. J. (1992) Mutation of the β-amyloid precursor protein in familial Alzheimer's disease increases β-protein production. *Nature,* **360,** 672-674.

Clapcote, S. J., Duffy, S., Xie, G. et al. (2009) Mutation I810N in the α3 isoform of Na+,K+-ATPase causes impairments in the sodium pump and hyperexcitability in the CNS. *Proceedings Of The National Academy Of Sciences Of The United States Of America,* **106,** 14085-14090.

Cochius, J. I., Burns, R. J., Blumbergs, P. C., Mack, K. and Alderman, C. P. (1990) Creutzfeldt-Jakob disease in a recipient of human pituitary-derived gonadotropin. *Australian and New Zealand Journal of Medicine,* **20,** 592-593.

Collinge, J. and Alpers, M. P. (2008) Introduction. The end of kuru: 50 years of research into an extraordinary disease. *Philosophical Transactions of the Royal Society B: Biological Sciences,* **363,** 3607-3612.

Collinge, J., Owen, F., Poulter, M. et al. (1990) Prion dementia without characteristic pathology. *Lancet,* **336,** 7-9.

Collins, S. J., Lewis, V., Brazier, M. W., Hill, A. F., Lawson, V. A., Klug, G. M. and Masters, C. L. (2005) Extended period of asymptomatic prion disease after low dose inoculation: Assessment of detection methods and implications for infection control. *Neurobiology of Disease,* **20,** 336-346.

Corder, E. H., Saunders, A. M., Strittmatter, W. J., Schmechel, D. E., Gaskell, P. C., Small, G. W., Roses, A. D., Haines, J. L. and Pericakvance, M. A. (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science,* **261,** 921-923.

Costanzo, M. and Zurzolo, C. (2013) The cell biology of prion-like spread of protein aggregates: mechanisms and implication in neurodegeneration. *The Biochemical Journal,* **452,** 1-17.

Cuillé, J. and Chelle, P. L. (1936) La maladie dite tremblante du mouton est-elle inocuable? *Comptes Rendus Hebdomadaires Des Seances De L Academie Des Sciences,* **203,** 1552-1554.

Danzer, K. M., Haasen, D., Karow, A. R., Moussaud, S., Habeck, M., Giese, A., Kretzschmar, H., Hengerer, B. and Kostka, M. (2007) Different species of α-synuclein oligomers induce calcium influx and seeding. *Journal of Neuroscience,* **27,** 9220-9232.

Danzer, K. M., Krebs, S. K., Wolff, M., Birk, G. and Hengerer, B. (2009) Seeding induced by α-synuclein oligomers provides evidence for spreading of α-synuclein pathology. *Journal of Neurochemistry,* **111,** 192-203.

de Carvalho Aguiar, P., Sweadner, K. J., Penniston, J. T. et al. (2004) Mutations in the Na+/K+ ATPase α3 gene ATP1A3 are associated with rapid-onset dystonia parkinsonism. *Neuron,* **43,** 169-175.

Deleault, N. R., Geoghegan, J. C., Nishina, K., Kascsak, R., Williamson, R. A. and Supattapone, S. (2005) Protease-resistant prion protein amplification reconstituted with partially purified substrates and synthetic polyanions. *J Biol Chem,* **280,** 26873-26879.

Deleault, N. R., Harris, B. T., Rees, J. R. and Supattapone, S. (2007) Formation of native prions from minimal components in vitro. pp. 9741. National Academy of Sciences.

Deleault, N. R., Lucassen, R. W. and Supattapone, S. (2003) RNA molecules stimulate prion protein conversion. *Nature,* **425,** 717-720.

Desplats, P., Lee, H. J., Bae, E. J., Patrick, C., Rockenstein, E., Crews, L., Spencer, B., Masliah, E. and Lee, S. J. (2009) Inclusion formation and neuronal cell death through neuron-to-neuron transmission of α-synuclein. *Proceedings of the National Academy of Sciences of the United States of America,* **106,** 13010-13015.

Duffy, P., Wolf, J., Collins, G., Devoe, A. G., Streeten, B. and Cowen, D. (1974) Possible person-to-person transmission of Creutzfeldt-Jakob disease. *New England Journal of Medicine,* **290,** 692-693.

Duran-Aniotz, C., Morales, R., Moreno-Gonzalez, I., Hu, P. P., Fedynyshyn, J. and Soto, C. (2014) Aggregate-depleted brain fails to induce Aβ deposition in a mouse model of Alzheimer's disease. *PLoS ONE,* **9,** e89014.

Duran-Aniotz, C., Morales, R., Moreno-Gonzalez, I., Hu, P. P. and Soto, C. (2013) Brains from non-Alzheimer's individuals containing amyloid deposits accelerate Aβ deposition in vivo. *Acta Neuropathol Commun,* **1,** 76.

Eisele, Y. S., Bolmont, T., Heikenwalder, M. et al. (2009) Induction of cerebral β-amyloidosis: Intracerebral versus systemic Aβ inoculation. *Proceedings of the National Academy of Sciences of the United States of America,* **106,** 12926-12931.

Eisele, Y. S., Fritschi, S. K., Hamaguchi, T. et al. (2014) Multiple factors contribute to the peripheral induction of cerebral β-amyloidosis. *The Journal of neuroscience : the official journal of the Society for Neuroscience,* **34,** 10264-10273.

Eisele, Y. S., Obermuller, U., Heilbronner, G. et al. (2010) Peripherally applied Aβ-containing inoculates induce cerebral β-amyloidosis. *Science,* **330,** 980-982.

El Hachimi, K. H., Chaunu, M. P., Cervenakova, L., Brown, P. and Foncin, J. F. (1997) Putative neurosurgical transmission of Creutzfeldt-Jakob disease with analysis of donor and recipient: agent strains. *Comptes Rendus De L Academie Des Sciences Serie Iii-Sciences De La Vie-Life Sciences,* **320,** 319-328.

Feany, M. B. and Bender, W. W. (2000) A Drosophila model of Parkinson's disease. *Nature,* **404,** 394-398.

Flechsig, E., Hegyi, I., Enari, M., Schwarz, P., Collinge, J. and Weissmann, C. (2001) Transmission of scrapie by steel-surface-bound prions. *Molecular Medicine,* **7,** 679-684.

Fritschi, S. K., Cintron, A., Ye, L. et al. (2014a) Aβ seeds resist inactivation by formaldehyde. *Acta Neuropathol,* **128,** 477-484.

Fritschi, S. K., Langer, F., Kaeser, S. A. et al. (2014b) Highly potent soluble amyloid-β seeds in human Alzheimer brain but not cerebrospinal fluid. *Brain,* **137,** 2909-2915.

Frontzek, K., Lutz, M. I., Aguzzi, A., Kovacs, G. G. and Budka, H. (2016) Amyloid-β pathology and cerebral amyloid angiopathy are frequent in iatrogenic Creutzfeldt-Jakob disease after dural grafting. *Swiss medical weekly,* **146,** w14287.

Gajdusek, D. C., Gibbs, C. J. and Alpers, M. (1966) Experimental transmission of a Kuru-like syndrome to chimpanzees. *Nature,* **209,** 794-796.

Galvagnion, C., Buell, A. K., Meisl, G., Michaels, T. C. T., Vendruscolo, M., Knowles, T. P. J. and Dobson, C. M. (2015) Lipid vesicles trigger α-synuclein aggregation by stimulating primary nucleation. *Nat Chem Biol,* **11,** 229-234.

Garcia-Esparcia, P., Hernandez-Ortega, K., Koneti, A., Gil, L., Delgado-Morales, R., Castano, E., Carmona, M. and Ferrer, I. (2015) Altered machinery of protein synthesis is region- and stage-dependent and is associated with α-synuclein oligomers in Parkinson's disease. *Acta Neuropathol Commun,* **3,** 76.

Geoghegan, J. C., Valdes, P. A., Orem, N. R., Deleault, N. R., Williamson, R. A., Harris, B. T. and Supattapone, S. (2007) Selective incorporation of polyanionic molecules into hamster prions. *J Biol Chem,* **282,** 36341-36353.

Giannakopoulos, P., Herrmann, F. R., Bussiere, T., Bouras, C., Kovari, E., Perl, D. P., Morrison, J. H., Gold, G. and Hof, P. R. (2003) Tangle and neuron numbers, but not amyloid load, predict cognitive status in Alzheimer's disease. *Neurology,* **60,** 1495-1500.

Gibbs, C. J., Gajdusek, D. C., Asher, D. M., Alpers, M. P., Beck, E., Daniel, P. M. and Matthews, W. B. (1968) Creutzfeldt-Jakob disease (Spongiform Encephalopathy) - transmission to chimpanzee. *Science,* **161,** 388-&.

Giehm, L., Pinto Oliveira, C. L., Christiansen, G., Pedersen, J. S. and Otzen, D. E. (2010) SDS-induced fibrillation of α-synuclein: An alternative fibrillation pathway. *Journal of Molecular Biology,* **401,** 115-133.

Gong, Y. S., Chang, L., Viola, K. L., Lacor, P. N., Lambert, M. P., Finch, C. E., Krafft, G. A. and Klein, W. L. (2003) Alzheimer's disease-affected brain: Presence of oligomeric A β ligands (ADDLs) suggests a molecular basis for reversible memory loss. *Proceedings of the National Academy of Sciences of the United States of America,* **100,** 10417-10422.

Gosavi, N., Lee, H. J., Lee, J. S., Patel, S. and Lee, S. J. (2002) Golgi fragmentation occurs in the cells with prefibrillar α-synuclein aggregates and precedes the formation of fibrillar inclusion. *Journal of Biological Chemistry,* **277,** 48984-48992.

Grey, M., Dunning, C. J., Gaspar, R., Grey, C., Brundin, P., Sparr, E. and Linse, S. (2015) Acceleration of α-synuclein aggregation by exosomes. *J Biol Chem,* **290,** 2969-2982.

Guo, J. L., Covell, D. J., Daniels, J. P. et al. (2013) Distinct α-synuclein strains differentially promote tau inclusions in neurons. *Cell,* **154,** 103-117.

Halliday, M., Radford, H., Sekine, Y. et al. (2015) Partial restoration of protein synthesis rates by the small molecule ISRIB prevents neurodegeneration without pancreatic toxicity. *Cell death & disease*, **6,** e1672-e1672.

Hamaguchi, T., Eisele, Y. S., Varvel, N. H., Lamb, B. T., Walker, L. C. and Jucker, M. (2012) The presence of Aβ seeds, and not age per se, is critical to the initiation of Aβ deposition in the brain. *Acta Neuropathologica*, **123,** 31-37.

Handoko, M., Grant, M., Kuskowski, M., Zahs, K. R., Wallin, A., Blennow, K. and Ashe, K. H. (2013) Correlation of specific amyloid-β oligomers with tau in cerebrospinal fluid from cognitively normal older adults. *Jama Neurology*, **70,** 594-599.

Hansen, C., Angot, E., Bergstrom, A. L. et al. (2011) α-synuclein propagates from mouse brain to grafted dopaminergic neurons and seeds aggregation in cultured human cells. *Journal of Clinical Investigation*, **121,** 715-725.

Heckmann, J. G., Lang, C. J. G., Petruch, F., Druschky, A., Erb, C., Brown, P. and Neundorfer, B. (1997) Transmission of Creutzfeldt-Jakob disease via a corneal transplant. *Journal of Neurology Neurosurgery and Psychiatry*, **63,** 388-390.

Heilbronner, G. A., Eisele, Y. S. A., Langer, F. A. et al. (2013) Seeded strain-like transmission of β-amyloid morphotypes in APP transgenic mice. *EMBO Reports***,** 1017.

Herva, M. E., Zibaee, S., Fraser, G., Barker, R. A., Goedert, M. and Spillantini, M. G. (2014) Anti-amyloid compounds inhibit α-synuclein aggregation induced by Protein Misfolding Cyclic Amplification (PMCA). *The Journal Of Biological Chemistry*.

Hill, A. F., Desbruslais, M., Joiner, S., Sidle, K. C. L., Gowland, I., Collinge, J., Doey, L. J. and Lantos, P. (1997) The same prion strain causes vCJD and BSE. *Nature*, **389,** 448-450.

Hill, A. F., Joiner, S., Linehan, J., Desbruslais, M., Lantos, P. L. and Collinge, J. (2000) Species-barrier-independent prion replication in apparently resistant species. *Proceedings of the National Academy of Sciences of the United States of America*, **97,** 10248-10253.

Hill, A. F., Joiner, S., Wadsworth, J. D., Sidle, K. C., Bell, J. E., Budka, H., Ironside, J. W. and Collinge, J. (2003) Molecular classification of sporadic Creutzfeldt-Jakob disease. *Brain*, **126,** 1333-1346.

Holmes, B. B. and Diamond, M. I. (2014) Prion-like properties of tau protein: the importance of extracellular tau as a therapeutic target. *The Journal Of Biological Chemistry*, **289,** 19855-19861.

Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., Yang, F. S. and Cole, G. (1996) Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice. *Science*, **274,** 99-102.

Hsiao, K. K., Scott, M., Foster, D., Groth, D. F., Dearmond, S. J. and Prusiner, S. B. (1990) Spontaneous neurodegeneration in transgenic mice with mutant prion protein. *Science*, **250,** 1587-1590.

Hughes, A. J., Daniel, S. E., Kilford, L. and Lees, A. J. (1992) Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinicopathological study of 100 cases. *Journal of Neurology Neurosurgery and Psychiatry,* **55,** 181-184.

Irwin, D. J., Abrams, J. Y., Schonberger, L. B., Leschek, E. W., Mills, J. L., Lee, V. M. and Trojanowski, J. Q. (2013) Evaluation of potential infectivity of Alzheimer and Parkinson disease proteins in recipients of cadaver-derived human growth hormone. *JAMA Neurol,* **70,** 462-468.

Jan, A., Adolfsson, O., Allaman, I., Buccarello, A. L., Magistretti, P. J., Pfeifer, A., Muhs, A. and Lashuel, H. A. (2011) Aβ42 neurotoxicity is mediated by ongoing nucleated polymerization process rather than by discrete Aβ42 species. *Journal of Biological Chemistry,* **286,** 8585-8596.

Jana, M. K., Cappai, R., Pham, C. L. L. and Ciccotosto, G. D. (2015) Membrane bound tetramer and trimer Aβ oligomeric species correlate with toxicity towards cultured neurons. *Journal of Neurochemistry,* **136,** 594-608.

Janetzky, B., Hauck, S., Youdim, M. B. H., Riederer, P., Jellinger, K., Pantucek, F., Zochling, R., Boissl, K. W. and Reichmann, H. (1994) Unaltered aconitase activity, but decreased complex I activity in substantia nigra pars compacta of patients with Parkinson's disease. *Neuroscience Letters,* **169,** 126-128.

Jansen, K., Schafer, O., Birkmann, E., Post, K., Serban, H., Prusiner, S. B. and Riesner, D. (2001) Structural intermediates in the putative pathway from the cellular prion protein to the pathogenic form. *Biological Chemistry,* **382,** 683-691.

Jaunmuktane, Z., Mead, S., Ellis, M. et al. (2015) Evidence for human transmission of amyloid-β pathology and cerebral amyloid angiopathy. *Nature,* **525,** 247-250.

Jin, M., Shepardson, N., Yang, T., Chen, G., Walsh, D. and Selkoe, D. J. (2011) Soluble amyloid β-protein dimers isolated from Alzheimer cortex directly induce tau hyperphosphorylation and neuritic degeneration. *Proceedings of the National Academy of Sciences of the United States of America,* **108,** 5819-5824.

Kahle, P. J., Neumann, M., Ozmen, L. et al. (2000) Subcellular localization of wild-type and Parkinson's disease-associated mutant α-synuclein in human and transgenic mouse brain. *Journal of Neuroscience,* **20,** 6365-6373.

Kane, M. D., Lipinski, W. J., Callahan, M. J., Bian, F., Durham, R. A., Schwarz, R. D., Roher, A. E. and Walker, L. C. (2000) Evidence for seeding of β-amyloid by intracerebral infusion of Alzheimer brain extracts in β-amyloid precursor protein transgenic mice. *Journal of Neuroscience,* **20,** 3606-3611.

Kang, J., Lemaire, H. G., Unterbeck, A., Salbaum, J. M., Masters, C. L., Grzeschik, K. H., Multhaup, G., Beyreuther, K. and Mullerhill, B. (1987) The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature,* **325,** 733-736.

Karpinar, D. P., Balija, M. B. G., Kugler, S. et al. (2009) Pre-fibrillar α-synuclein variants with impaired β-structure increase neurotoxicity in Parkinson's disease models. *Embo Journal,* **28,** 3256-3268.

Kayed, R., Head, E., Thompson, J. L., McIntire, T. M., Milton, S. C., Cotman, C. W. and Glabe, C. G. (2003) Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science,* **300,** 486-489.

Kazlauskaite, J., Young, A., Gardner, C. E., Macpherson, J. V., Venien-Bryan, C. and Pinheiro, T. J. T. (2005) An unusual soluble β-turn-rich conformation of prion is involved in fibril formation and toxic to neuronal cells. *Biochemical and Biophysical Research Communications,* **328,** 292-305.

Kessels, H. W., Nguyen, L. N., Nabavi, S. and Malinow, R. (2010) The prion protein as a receptor for amyloid-β. *Nature,* **466,** E3-E4.

Klyubin, I., Nicoll, A. J., Khalili-Shirazi, A. et al. (2014) Peripheral administration of a humanized anti-PrP antibody blocks Alzheimer's disease Aβ synaptotoxicity. *Journal of Neuroscience,* **34,** 6140-6145.

Kocisko, D. A., Come, J. H., Priola, S. A., Chesebro, B., Raymond, G. J., Lansbury, P. T. and Caughey, B. (1994) Cell-free formation of protease-resistant prion protein. *Nature,* **370,** 471-474.

Kordower, J. H., Chu, Y. P., Hauser, R. A., Freeman, T. B. and Olanow, C. W. (2008) Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. *Nature Medicine,* **14,** 504-506.

Kramer, M. L. and Schulz-Schaeffer, W. J. (2007) Presynaptic α-synuclein aggregates, not Lewy bodies, cause neurodegeneration in dementia with Lewy bodies. *Journal of Neuroscience,* **27,** 1405-1410.

Lacor, P. N., Buniel, M. C., Furlow, P. W., Clemente, A. S., Velasco, P. T., Wood, M., Viola, K. L. and Klein, W. L. (2007) Aβ oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer's disease. *Journal of Neuroscience,* **27,** 796-807.

Lam, H. T., Graber, M. C., Gentry, K. A. and Bieschke, J. (2016) Stabilization of α-synuclein fibril clusters prevents fragmentation and reduces seeding activity and toxicity. *Biochemistry*.

Langer, F., Eisele, Y. S., Fritschi, S. K., Staufenbiel, M., Walker, L. C. and Jucker, M. (2011) Soluble Aβ seeds are potent inducers of cerebral β-amyloid deposition. *Journal of Neuroscience,* **31,** 14488-14495.

Langston, J. W. and Ballard, P. A. (1983) Parkinson's disease in a chemist working with 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. *New England Journal of Medicine,* **309,** 310-310.

Lauren, J., Gimbel, D. A., Nygaard, H. B., Gilbert, J. W. and Strittmatter, S. M. (2009) Cellular prion protein mediates impairment of synaptic plasticity by amyloid-β oligomers. *Nature,* **457,** 1128-U1184.

Lee, S. and Kim, H.-J. (2015) Prion-like mechanism in Amyotrophic Lateral Sclerosis: are protein aggregates the key? *Experimental Neurobiology,* **24,** 1-7.

Legname, G., Baskakov, I. V., Nguyen, H. O. B., Riesner, D., Cohen, F. E., DeArmond, S. J. and Prusiner, S. B. (2004) Synthetic mammalian prions. *Science,* **305,** 673-676.

Lesne, S., Koh, M. T., Kotilinek, L., Kayed, R., Glabe, C. G., Yang, A., Gallagher, M. and Ashe, K. H. (2006) A specific amyloid-β protein assembly in the brain impairs memory. *Nature,* **440,** 352-357.

Lesne, S. E., Sherman, M. A., Grant, M., Kuskowski, M., Schneider, J. A., Bennett, D. A. and Ashe, K. H. (2013) Brain amyloid-β oligomers in ageing and Alzheimer's disease. *Brain,* **136,** 1383-1398.

Li, J. L., Browning, S., Mahal, S. P., Oelschlegel, A. M. and Weissmann, C. (2010) Darwinian evolution of prions in cell culture. *Science,* **327,** 869-872.

Li, J. Y., Englund, E., Holton, J. L. et al. (2008) Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. *Nature Medicine,* **14,** 501-503.

Lim, Y. Y., Villemagne, V. L., Laws, S. M. et al. (2015a) APOE and BDNF polymorphisms moderate amyloid β-related cognitive decline in preclinical Alzheimer's disease. *Molecular Psychiatry,* **20,** 1322-1328.

Lim, Y. Y., Villemagne, V. L., Pietrzak, R. H. et al. (2015b) APOE epsilon 4 moderates amyloid-related memory decline in preclinical Alzheimer's disease. *Neurobiology of Aging,* **36,** 1239-1244.

Llewelyn, C. A., Hewitt, P. E., Knight, R. S. G., Amar, K., Cousens, S., Mackenzie, J. and Will, R. G. (2004) Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. *Lancet,* **363,** 417-421.

Lu, J. X., Qiang, W., Yau, W. M., Schwieters, C. D., Meredith, S. C. and Tycko, R. (2013) Molecular structure of β-amyloid fibrils in Alzheimer's disease brain tissue. *Cell,* **154,** 1257-1268.

Luk, K. C., Kehm, V., Carroll, J., Zhang, B., O'Brien, P., Trojanowski, J. Q. and Lee, V. M. Y. (2012a) Pathological α-Synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. *Science,* **338,** 949-953.

Luk, K. C., Kehm, V. M., Zhang, B., O'Brien, P., Trojanowski, J. Q. and Lee, V. M. Y. (2012b) Intracerebral inoculation of pathological α-synuclein initiates a rapidly progressive neurodegenerative α-synucleinopathy in mice. *Journal of Experimental Medicine,* **209,** 975-986.

Luk, K. C., Song, C., O'Brien, P., Stieber, A., Branch, J. R., Brunden, K. R., Trojanowski, J. Q. and Lee, V. M. Y. (2009) Exogenous α-synuclein fibrils seed the formation of Lewy body-like intracellular inclusions in cultured cells. *Proceedings of the National Academy of Sciences of the United States of America,* **106,** 20051-20056.

Luth, E. S., Stavrovskaya, I. G., Bartels, T., Kristal, B. S. and Selkoe, D. J. (2014) Soluble, prefibrillar α-synuclein oligomers promote complex I-dependent, Ca2+-induced mitochondrial dysfunction. *Journal of Biological Chemistry,* **289,** 21490-21507.

Makarava, N., Kovacs, G. G., Bocharova, O., Savtchenko, R., Alexeeva, I., Budka, H., Rohwer, R. G. and Baskakov, I. V. (2010) Recombinant prion protein induces a new transmissible prion disease in wild-type animals. *Acta Neuropathol,* **119,** 177-187.

Mallucci, G., Dickinson, A., Linehan, J., Klohn, P. C., Brandner, S. and Collinge, J. (2003) Depleting neuronal PrP in prion infection prevents disease and reverses spongiosis. *Science,* **302,** 871-874.

Mallucci, G. R., Ratte, S., Asante, E. A., Linehan, J., Gowland, I., Jefferys, J. G. R. and Collinge, J. (2002) Post-natal knockout of prion protein alters hippocampal CA1 properties, but does not result in neurodegeneration. *Embo Journal,* **21,** 202-210.

Manson, J. C., Jamieson, E., Baybutt, H. et al. (1999) A single amino acid alteration (101L) introduced into murine PrP dramatically alters incubation time of transmissible spongiform encephalopathy. *Embo Journal,* **18,** 6855-6864.

Masliah, E., Rockenstein, E., Veinbergs, I., Mallory, M., Hashimoto, M., Takeda, A., Sagara, Y., Sisk, A. and Mucke, L. (2000) Dopaminergic loss and inclusion body formation in α-synuclein mice: Implications for neurodegenerative disorders. *Science,* **287,** 1265-1269.

Masters, C. L., Gajdusek, D. C. and Gibbs, C. J., Jr. (1981) Creutzfeldt-Jakob disease virus isolations from the Gerstmann-Sträussler syndrome with an analysis of the various forms of amyloid plaque deposition in the virus-induced spongiform encephalopathies. *Brain,* **104,** 559-588.

Masters, C. L., Simms, G., Weinman, N. A., Multhaup, G., McDonald, B. L. and Beyreuther, K. (1985) Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proceedings of the National Academy of Sciences of the United States of America,* **82,** 4245-4249.

Masuda-Suzukake, M., Nonaka, T., Hosokawa, M., Kubo, M., Shimozawa, A., Akiyama, H. and Hasegawa, M. (2014) Pathological α-synuclein propagates through neural networks. *Acta Neuropathol Commun,* **2,** 88.

Masuda-Suzukake, M., Nonaka, T., Hosokawa, M., Oikawa, T., Arai, T., Akiyama, H., Mann, D. M. A. and Hasegawa, M. (2013) Prion-like spreading of pathological α-synuclein in brain. *Brain,* **136,** 1128-1138.

Mays, C. E., van der Merwe, J., Kim, C., Haldiman, T., McKenzie, D., Safar, J. G. and Westaway, D. (2015) Prion infectivity plateaus and conversion to symptomatic disease originate

from falling precursor levels and increased oligomeric PrPSc species. *Journal Of Virology*.

Mc Donald, J. M., Savva, G. M., Brayne, C. et al. (2010) The presence of sodium dodecyl sulphate-stable Aβ dimers is strongly associated with Alzheimer-type dementia. *Brain,* **133,** 1328-1341.

McLean, C. A., Cherny, R. A., Fraser, F. W., Fuller, S. J., Smith, M. J., Beyreuther, K., Bush, A. I. and Masters, C. L. (1999) Soluble pool of Aβ amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. *Annals of Neurology,* **46,** 860-866.

Medori, R., Tritschler, H. J., Leblanc, A. et al. (1992) Fatal familial insomnia, a prion disease with a mutation at codon-178 of the prion protein gene. *New England Journal of Medicine,* **326,** 444-449.

Mendez, I., Vinuela, A., Astradsson, A. et al. (2008) Dopamine neurons implanted into people with Parkinson's disease survive without pathology for 14 years. *Nature Medicine,* **14,** 507-509.

Meyer-Luehmann, M., Coomaraswamy, J., Bolmont, T. et al. (2006) Exogenous induction of cerebral β-amyloidogenesis is governed by agent and host. *Science,* **313,** 1781-1784.

Miyashita, K., Inuzuka, T., Kondo, H. et al. (1991) Creutzfeldt-Jakob disease in a patient with a cadaveric dural graft. *Neurology,* **41,** 940-941.

Morales, R., Abid, K. and Soto, C. (2007) The prion strain phenomenon: molecular basis and unprecedented features. *Biochimica et biophysica acta,* **1772,** 681-691.

Morales, R., Duran-Aniotz, C., Castilla, J., Estrada, L. D. and Soto, C. (2012) De novo induction of amyloid-β deposition in vivo. *Mol Psychiatry,* **17,** 1347-1353.

Moreno, J. A., Halliday, M., Molloy, C. et al. (2013) Oral treatment targeting the unfolded protein response prevents neurodegeneration and clinical disease in prion-infected mice. *Science Translational Medicine,* **5**.

Moreno, J. A., Radford, H., Peretti, D. et al. (2012) Sustained translational repression by eIF2 α-P mediates prion neurodegeneration. *Nature,* **485,** 507-U119.

Mormino, E. C., Betensky, R. A., Hedden, T. et al. (2014) Amyloid and APOE epsilon 4 interact to influence short-term decline in preclinical Alzheimer disease. *Neurology,* **82,** 1760-1767.

Moro, M. L., Giaccone, G., Lombardi, R. et al. (2012) APP mutations in the Aβ coding region are associated with abundant cerebral deposition of Aβ38. *Acta Neuropathol,* **124,** 809-821.

Mougenot, A. L., Nicot, S., Bencsik, A., Morignat, E., Verchere, J., Lakhdar, L., Legastelois, S. and Baron, T. (2012) Prion-like acceleration of a synucleinopathy in a transgenic mouse model. *Neurobiology of Aging,* **33,** 2225-2228.

Mullan, M., Crawford, F., Axelman, K., Houlden, H., Lilius, L., Winblad, B. and Lannfelt, L. (1992) A pathogenic mutation for probable Alzheimer's-Disease in the APP gene at the N-terminus of β-amyloid. *Nature Genetics,* **1,** 345-347.

Nilsberth, C., Westlind-Danielsson, A., Eckman, C. B. et al. (2001) The 'Arctic' APP mutation (E693G) causes Alzheimer's disease by enhanced Aβ protofibril formation. *Nature Neuroscience,* **4,** 887-893.

Novitskaya, V., Bocharova, O. V., Bronstein, I. and Baskakov, I. V. (2006) Amyloid fibrils of mammalian prion protein are highly toxic to cultured cells and primary neurons. *Journal of Biological Chemistry,* **281,** 13828-13836.

O'Nuallain, B., Freir, D. B., Nicoll, A. J., Risse, E., Ferguson, N., Herron, C. E., Collinge, J. and Walsh, D. M. (2010) Amyloid β-protein dimers rapidly form stable synaptotoxic protofibrils. *Journal of Neuroscience,* **30,** 14411-14419.

Ono, K., Condron, M. M. and Teplow, D. B. (2009) Structure-neurotoxicity relationships of amyloid β-protein oligomers. *Proceedings of the National Academy of Sciences of the United States of America,* **106,** 14745-14750.

Pan, K. M., Baldwin, M., Nguyen, J. et al. (1993) Conversion of α-helices into β-sheets features in the formation of the scrapie prion proteins. *Proceedings of the National Academy of Sciences of the United States of America,* **90,** 10962-10966.

Parker, W. D., Boyson, S. J. and Parks, J. K. (1989) Abnormalities of the electron transport chain in idiopathic Parkinson's disease. *Annals of Neurology,* **26,** 719-723.

Parkkinen, L., Kauppinen, T., Pirttila, T., Autere, J. M. and Alafuzoff, I. (2005) α-synuclein pathology does not predict extrapyramidal symptoms or dementia. *Annals of Neurology,* **57,** 82-91.

Paumier, K. L., Luk, K. C., Manfredsson, F. P. et al. (2015) Intrastriatal injection of pre-formed mouse α-synuclein fibrils into rats triggers α-synuclein pathology and bilateral nigrostriatal degeneration. *Neurobiology of Disease,* **82,** 185-199.

Peden, A. H., Head, M. W., Ritchie, D. L., Bell, J. E. and Ironside, J. W. (2004) Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. *Lancet,* **364,** 527-529.

Peelaerts, W., Bousset, L., Van der Perren, A., Moskalyuk, A., Pulizzi, R., Giugliano, M., Van den Haute, C., Melki, R. and Baekelandt, V. (2015) α-synuclein strains cause distinct synucleinopathies after local and systemic administration. *Nature,* **522,** 340-344.

Pieri, L., Madiona, K., Bousset, L. and Melki, R. (2012) Fibrillar α-synuclein and huntingtin exon 1 assemblies are toxic to the cells. *Biophysical journal,* **102,** 2894-2905.

Powell-Jackson, J., Weller, R. O., Kennedy, P., Preece, M. A., Whitcombe, E. M. and Newsomdavis, J. (1985) Creutzfeldt-Jakob disease after administration of human growth-hormone. *Lancet,* **2,** 244-246.

Prusiner, S. B. (1982) Novel proteinaceous infectious particles cause scrapie. *Science,* **216,** 136-144.

Prusiner, S. B., Woerman, A. L., Mordes, D. A. et al. (2015) Evidence for α-synuclein prions causing multiple system atrophy in humans with parkinsonism. *Proceedings Of The National Academy Of Sciences Of The United States Of America,* **112,** E5308-E5317.

Radde, R., Bolmont, T., Kaeser, S. A. et al. (2006) Aβ42-driven cerebral amyloidosis in transgenic mice reveals early and robust pathology. *EMBO Rep*, **7,** 940-946.

Rodacker, V., Toustrup-Jensen, M. and Vilsen, B. (2006) Mutations Phe785Leu and Thr618Met in Na+,K+-ATPase, associated with familial rapid-onset dystonia parkinsonism, interfere with Na+ interaction by distinct mechanisms. *The Journal Of Biological Chemistry*, **281,** 18539-18548.

Rosen, R. F., Fritz, J. J., Dooyema, J., Cintron, A. F., Hamaguchi, T., Lah, J. J., LeVine, H., Jucker, M. and Walker, L. C. (2012) Exogenous seeding of cerebral β-amyloid deposition in βAPP-transgenic rats. *Journal of Neurochemistry*, **120,** 660-666.

Ruiperez, V., Darios, F. and Davletov, B. (2010) α-synuclein, lipids and Parkinson's disease. *Progress in Lipid Research*, **49,** 420-428.

Rutherford, N. J., Sacino, A. N., Brooks, M., Ceballos-Diaz, C., Ladd, T. B., Howard, J. K., Golde, T. E. and Giasson, B. I. (2015) Studies of lipopolysaccharide effects on the induction of α-synuclein pathology by exogenous fibrils in transgenic mice. *Mol Neurodegener*, **10,** 32.

Saborio, G. P., Permanne, B. and Soto, C. (2001) Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. *Nature*, **411,** 810-813.

Sacino, A. N., Ayers, J. I., Brooks, M. M. T., Chakrabarty, P., Hudson, V. J., 3rd, Howard, J. K., Golde, T. E., Giasson, B. I. and Borchelt, D. R. (2015) Non-prion-type transmission in A53T α-synuclein transgenic mice: a normal component of spinal homogenates from naïve non-transgenic mice induces robust α-synuclein pathology. *Acta Neuropathologica*.

Sacino, A. N., Brooks, M., McGarvey, N. H., McKinney, A. B., Thomas, M. A., Levites, Y., Ran, Y., Golde, T. E. and Giasson, B. I. (2013) Induction of CNS α-synuclein pathology by fibrillar and non-amyloidogenic recombinant α-synuclein. *Acta Neuropathologica Communications*, **1,** 38-38.

Sacino, A. N., Brooks, M., McKinney, A. B., Thomas, M. A., Shaw, G., Golde, T. E. and Giasson, B. I. (2014a) Brain injection of α-synuclein induces multiple proteinopathies, gliosis, and a neuronal injury marker. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **34,** 12368-12378.

Sacino, A. N., Brooks, M., Thomas, M. A. et al. (2014b) Intramuscular injection of α-synuclein induces CNS α-synuclein pathology and a rapid-onset motor phenotype in transgenic mice. *Proceedings of the National Academy of Sciences of the United States***,** 10732.

Sacino, A. N., Brooks, M., Thomas, M. A. et al. (2014c) Amyloidogenic α-synuclein seeds do not invariably induce rapid, widespread pathology in mice. *Acta Neuropathologica***,** 645.

Sandberg, M. K., Al-Doujaily, H., Sharps, B., Clarke, A. R. and Collinge, J. (2011) Prion propagation and toxicity in vivo occur in two distinct mechanistic phases. *Nature*, **470,** 540-542.

Sandberg, M. K., Al-Doujaily, H., Sharps, B. et al. (2014) Prion neuropathology follows the accumulation of alternate prion protein isoforms after infective titre has peaked. *Nature Communications,* **5**.

Schapira, A. H. V., Cooper, J. M., Dexter, D., Jenner, P., Clark, J. B. and Marsden, C. D. (1989) Mitochondrial complex I deficiency in Parkinson's disease. *Lancet,* **1,** 1269-1269.

Serrano-Pozo, A., Frosch, M. P., Masliah, E. and Hyman, B. T. (2011) Neuropathological alterations in Alzheimer disease. *Cold Spring Harbor perspectives in medicine,* **1,** a006189.

Shankar, G. M., Bloodgood, B. L., Townsend, M., Walsh, D. M., Selkoe, D. J. and Sabatini, B. L. (2007) Natural oligomers of the Alzheimer amyloid-β protein induce reversible synapse loss by modulating an NMDA-type glutamate receptor-dependent signaling pathway. *Journal of Neuroscience,* **27,** 2866-2875.

Shankar, G. M., Li, S. M., Mehta, T. H. et al. (2008) Amyloid-β protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nature Medicine,* **14,** 837-842.

Shrivastava, A. N., Redeker, V., Fritz, N. et al. (2015) α-synuclein assemblies sequester neuronal α3-Na+/K+-ATPase and impair Na+ gradient. *The EMBO Journal*.

Silveira, J. R., Raymond, G. J., Hughson, A. G., Race, R. E., Sim, V. L., Hayes, S. F. and Caughey, B. (2005) The most infectious prion protein particles. *Nature,* **437,** 257-261.

Simoneau, S., Rezaei, H., Sales, N. et al. (2007) In vitro and in vivo neurotoxicity of prion protein oligomers. *Plos Pathogens,* **3,** 1175-1186.

Stöhr, J., Condello, C., Watts, J. C. et al. (2014) Distinct synthetic Aβ prion strains producing different amyloid deposits in bigenic mice. *Proc Natl Acad Sci U S A,* **111,** 10329-10334.

Stöhr, J., Watts, J. C., Mensinger, Z. L., Oehler, A., Grillo, S. K., DeArmond, S. J., Prusiner, S. B. and Giles, K. (2012) Purified and synthetic Alzheimer's amyloid beta (Aβ) prions. *Proc Natl Acad Sci U S A,* **109,** 11025-11030.

Sturchler-Pierrat, C., Abramowski, D., Duke, M. et al. (1997) Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. *Proc Natl Acad Sci U S A,* **94,** 13287-13292.

Thackray, A. M., Klein, M. A., Aguzzi, A. and Bujdoso, R. (2002) Chronic subclinical prion disease induced by low-dose inoculum. *Journal of Virology,* **76,** 2510-2517.

Thackray, A. M., Klein, M. A. and Bujdoso, R. (2003) Subclinical prion disease induced by oral inoculation. *Journal of Virology,* **77,** 7991-7998.

Thal, D. R., Rub, U., Orantes, M. and Braak, H. (2002) Phases of Aβ-deposition in the human brain and its relevance for the development of AD. *Neurology,* **58,** 1791-1800.

Tompkins, M. M. and Hill, W. D. (1997) Contribution of somal Lewy bodies to neuronal death. *Brain Research,* **775,** 24-29.

Tran, H. T., Chung, C. H. Y., Iba, M., Zhang, B., Trojanowski, J. Q., Luk, K. C. and Lee, V. M. Y. (2014) α-synuclein immunotherapy blocks uptake and templated propagation of misfolded α-synuclein and neurodegeneration. *Cell Reports,* **7,** 2054-2065.

van der Putten, H., Wiederhold, K. H., Probst, A. et al. (2000) Neuropathology in mice expressing human α-synuclein. *Journal of Neuroscience,* **20,** 6021-6029.

Volles, M. J., Lee, S.-J., Rochet, J.-C., Shtilerman, M. D., Ding, T. T., Kessler, J. C. and Lansbury, P. T., Jr. (2001) Vesicle permeabilization by protofibrillar α-synuclein: implications for the pathogenesis and treatment of Parkinson's disease. *Biochemistry***,** 7812.

Volpicelli-Daley, L. A., Luk, K. C., Patel, T. P., Tanik, S. A., Riddle, D. M., Stieber, A., Meaney, D. F., Trojanowski, J. Q. and Lee, V. M. Y. (2011) Exogenous α-synuclein fibrils induce Lewy body pathology leading to synaptic dysfunction and neuron death. *Neuron,* **72,** 57-71.

Wagner, J., Ryazanov, S., Leonov, A. et al. (2013) Anle138b: a novel oligomer modulator for disease-modifying therapy of neurodegenerative diseases such as prion and Parkinson's disease. *Acta Neuropathologica,* **125,** 795-813.

Walker, L. C., Callahan, M. J., Bian, F., Durham, R. A., Roher, A. E. and Lipinski, W. J. (2002) Exogenous induction of cerebral β-amyloidosis in βAPP-transgenic mice. *Peptides,* **23,** 1241-1247.

Walsh, D. M., Klyubin, I., Fadeeva, J. V., Cullen, W. K., Anwyl, R., Wolfe, M. S., Rowan, M. J. and Selkoe, D. J. (2002) Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation in vivo. *Nature,* **416,** 535-539.

Wang, F., Wang, X. H., Yuan, C. G. and Ma, J. Y. (2010) Generating a prion with bacterially expressed recombinant prion protein. *Science,* **327,** 1132-1135.

Wang, F., Yang, F., Hu, Y., Wang, X., Wang, X., Jin, C. and Ma, J. (2007) Lipid interaction converts prion protein to a PrPSc-like proteinase K-resistant conformation under physiological conditions. *Biochemistry,* **46,** 7045-7053.

Watts, J. C., Condello, C., Stohr, J. et al. (2014) Serial propagation of distinct strains of Aβ prions from Alzheimer's disease patients. *Proceedings of the National Academy of Sciences of the United States of America,* **111,** 10323-10328.

Watts, J. C., Giles, K., Oehler, A., Middleton, L., Dexter, D. T., Gentleman, S. M., DeArmond, S. J. and Prusiner, S. B. (2013) Transmission of multiple system atrophy prions to transgenic mice. *Proceedings Of The National Academy Of Sciences Of The United States Of America,* **110,** 19555-19560.

Will, R. G., Ironside, J. W., Zeidler, M. et al. (1996) A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet,* **347,** 921-925.

Willison, H. J., Gale, A. N. and McLaughlin, J. E. (1991) Creutzfeldt-Jakob disease following cadaveric dura-mater graft. *Journal of Neurology Neurosurgery and Psychiatry,* **54,** 940-940.

Winner, B., Jappelli, R., Maji, S. K. et al. (2011) In vivo demonstration that α-synuclein oligomers are toxic. *Proceedings of the National Academy of Sciences of the United States of America,* **108,** 4194-4199.

Woerman, A. L., Stöhr, J., Aoyagi, A. et al. (2015) Propagation of prions causing synucleinopathies in cultured cells. *Proceedings Of The National Academy Of Sciences Of The United States Of America,* **112,** E4949-E4958.

Ye, L., Hamaguchi, T., Fritschi, S. K., Eisele, Y. S., Obermuller, U., Jucker, M. and Walker, L. C. (2015) Progression of seed-induced Aβ deposition within the limbic connectome. *Brain pathology (Zurich, Switzerland),* **25,** 743-752.

Zhou, M. H., Ottenberg, G., Sferrazza, G. F. and Lasmezas, C. I. (2012) Highly neurotoxic monomeric α-helical prion protein. *Proceedings of the National Academy of Sciences of the United States of America,* **109,** 3113-3118.

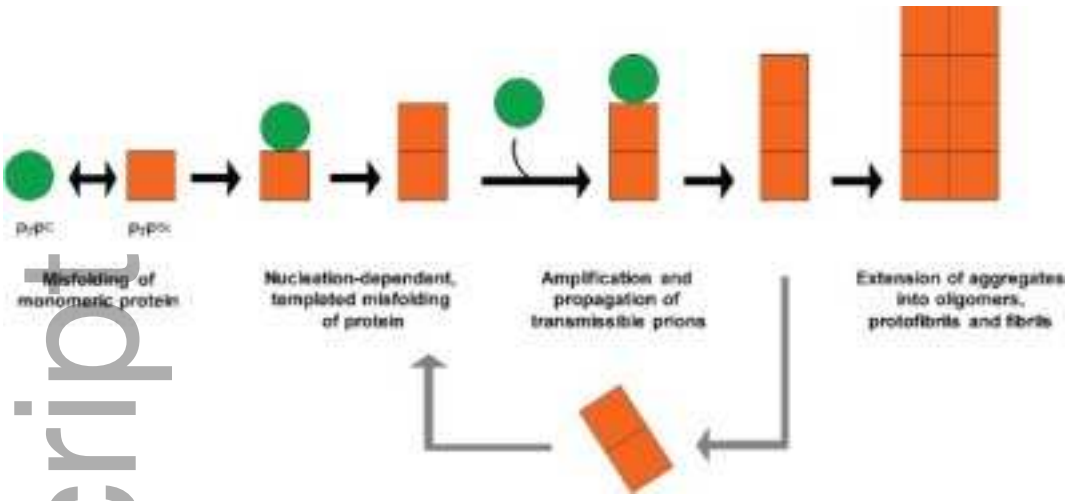
Zobeley, E., Flechsig, E., Cozzio, A., Enari, M. and Weissmann, C. (1999) Infectivity of scrapie prions bound to a stainless steel surface. *Molecular Medicine,* **5,** 240-243.

FIGURE LEGENDS

Figure 1. Nucleation-dependent model of prion replication. Misfolding of normal cellular prion protein, PrP^{C} converts it into its infectious, disease-associated isoform PrP^{Sc}. PrP^{Sc} induces nucleation-dependent misfolding in other PrP^{C} leading to amplification and propagation of transmissible prions. The growing aggregates extend into oligomers, protofibrils and then fibrils that form the protein aggregates characteristic of disease. A natural component of prion propagation is breakage of the growing PrP^{Sc} aggregate, which produces more nucleation sites for templated misfolding.

Figure 2. Disease-associated accumulation of misfolded protein in the central nervous system. The accumulation of PrP^{Sc}, Aβ and αsyn in the central nervous system is associated with prion, Alzheimer's and synucleinopathy disease, respectively. These proteins can accumulate in both intra- and extracellular compartments in neurons or other glial cell types, such as oligodendroglia.

Figure 3. Propagation of misfolded protein in rodent models following intracerebral inoculation of a misfolded seed. Propagation of PrP^{Sc}, Aβ and αsyn has been studied in various mouse models. Images represent the endogenous aggregation of protein in the brain of rodents that occurs over their lifetime (green line) and aggregation that occurs following the injection of a seed (red line) (A) Wild-type mouse models do not spontaneously develop protein aggregation over their lifespan and the induction of a seed induces aggregation of protein (B) Transgenic mouse models that spontaneously develop protein aggregation and the incorporation of the seed accelerates the deposition of protein. (C) Transgenic mouse models that do not spontaneously develop inclusions over the experimental timeframe, and the induction of a seed induces protein aggregation.



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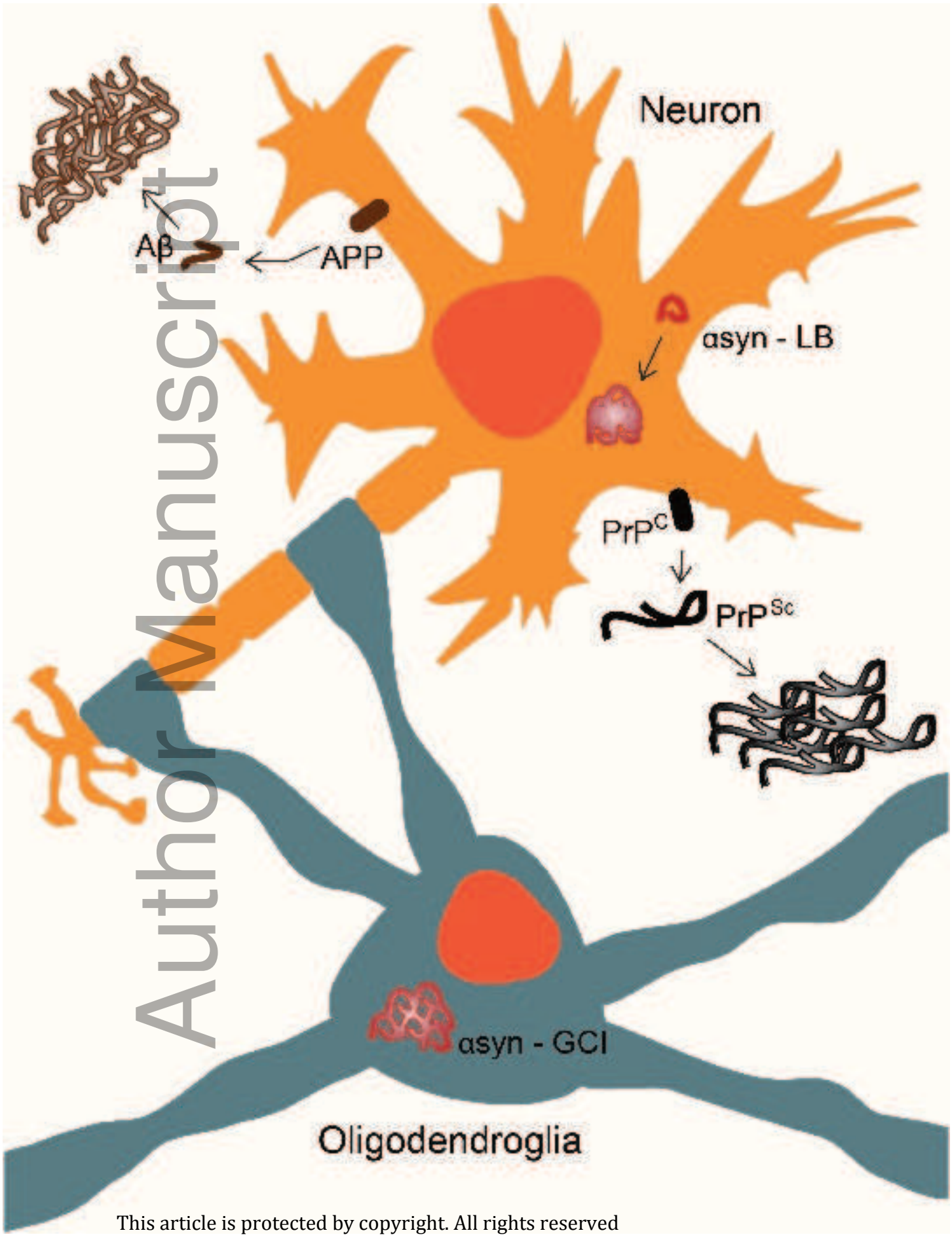

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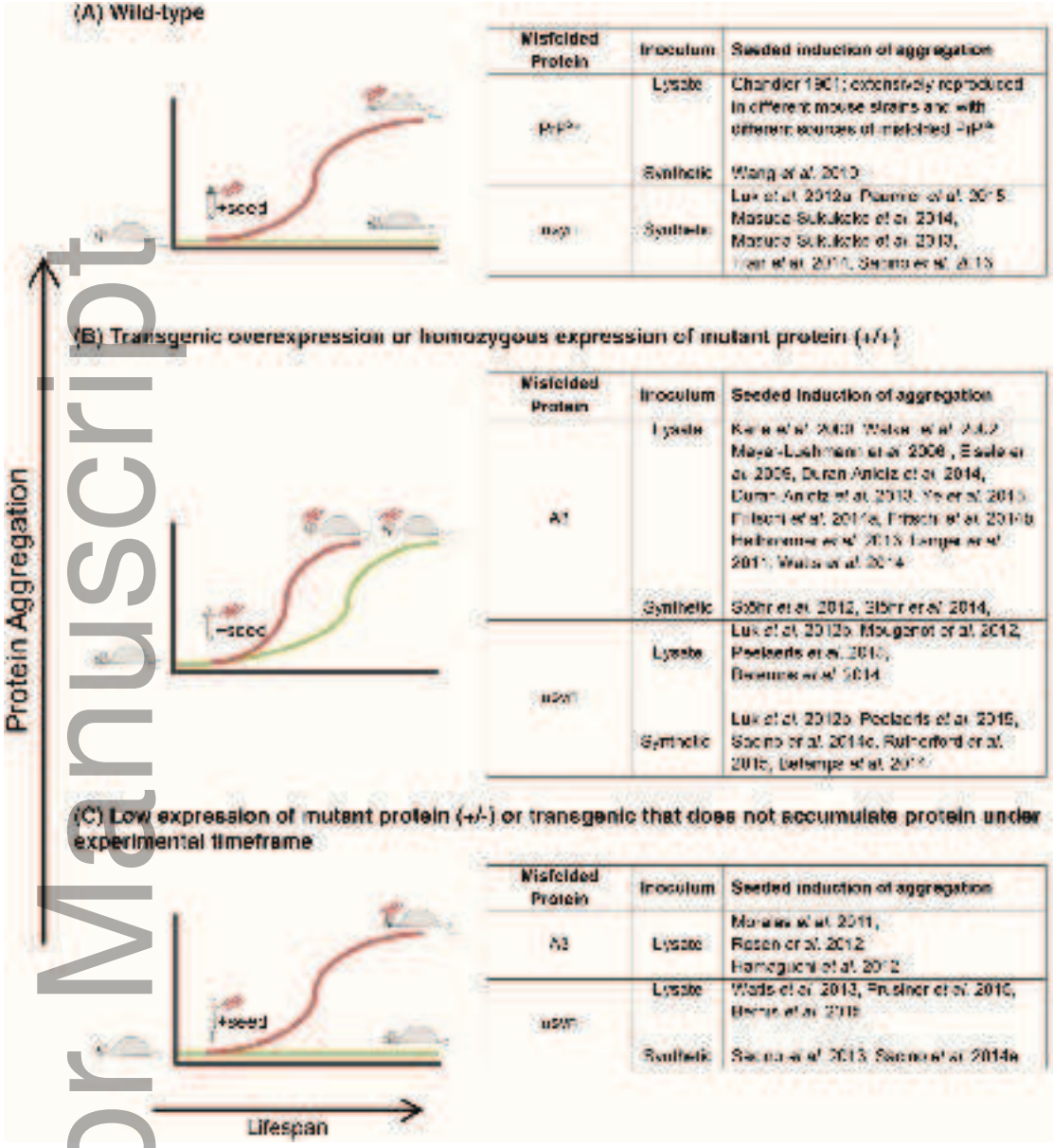

Misfolded Protein	Inoculum	Seeded induction of aggregation
PrP^{Sc}	Lysate	Chandler 1961; extensively reproduced in different mouse strains and with different sources of misfolded PrP^{Sc}
	Synthetic	Wang et al. 2010
mAβ	Synthetic	Lee et al. 2012a, Peeraer et al. 2015, Masuda-Suzukake et al. 2014, Masuda-Suzukake et al. 2013, Meyer et al. 2014, Sandberg et al. 2015

Misfolded Protein	Inoculum	Seeded induction of aggregation
Aβ	Lysate	Kane et al. 2000, Weber et al. 2002, Meyer-Luehmann et al. 2006, Eisele et al. 2009, Duran-Aniotz et al. 2014, Duran-Aniotz et al. 2013, Ye et al. 2015, Fritschi et al. 2014a, Fritschi et al. 2014b, Heilbronner et al. 2013, Lampe et al. 2014, Watts et al. 2014
	Synthetic	Stöhr et al. 2012, Stöhr et al. 2014,
mTau	Lysate	Lee et al. 2012b, Mougenot et al. 2012, Peeraer et al. 2015, Boluda et al. 2014
	Synthetic	Lee et al. 2012b, Peeraer et al. 2015, Sacino et al. 2014c, Rutherford et al. 2015, Delenpe et al. 2014

Misfolded Protein	Inoculum	Seeded induction of aggregation
Aβ	Lysate	Morales et al. 2011, Rosen et al. 2012, Hamaguchi et al. 2012
mTau	Lysate	Wolfe et al. 2013, Prudlner et al. 2010, Boluda et al. 2015
	Synthetic	Sacino et al. 2013, Sacino et al. 2014a

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