Quarterly Reviews of Biophysics **39**, 2 (2006), pp. 167–201. © 2006 Cambridge University Press
doi:10.1017/S0033583506004422 Printed in the United Kingdom
First published online 18 September 2006

Are amyloid diseases caused by protein aggregates that mimic bacterial pore-forming toxins?

Hilal A. Lashuel[1]* and Peter T. Lansbury Jr.[2]*

[1] Laboratory of Molecular Neurobiology and Neuroproteomics, Brain Mind Institute, Ecole Polytechnique Federale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland
[2] Harvard Center for Neurodegeneration and Repair, Center for Neurologic Diseases, Brigham and Women's Hospital and Department of Neurology, Harvard Medical School, 65 Landsdowne St, Cambridge, MA 02139, USA

Abstract. Protein fibrillization is implicated in the pathogenesis of most, if not all, age-associated neurodegenerative diseases, but the mechanism(s) by which it triggers neuronal death is unknown. Reductionist *in vitro* studies suggest that the amyloid protofibril may be the toxic species and that it may amplify itself by inhibiting proteasome-dependent protein degradation. Although its pathogenic target has not been identified, the properties of the protofibril suggest that neurons could be killed by unregulated membrane permeabilization, possibly by a type of protofibril referred to here as the 'amyloid pore'. The purpose of this review is to summarize the existing supportive circumstantial evidence and to stimulate further studies designed to test the validity of this hypothesis.

* Correspondence may be addressed to either author.
Email: hilal.lashuel@epfl.ch *or* plansbury@rics.bwh.harvard.edu

1. Introduction

A compelling circumstantial case can be made that amyloid fibril formation is a primary cause of neurodegeneration in Alzheimer's disease (AD), Parkinson's disease (PD), prion diseases, and related diseases (see Table 1) (Rochet & Lansbury, 2000; Stefani & Dobson, 2003). Supporting evidence for this case, is derived from (1) pathology: fibrillar protein aggregates often colocalize with neuronal loss, (2) genetics: the gene encoding the fibrillar protein is linked to disease (autosomal dominant mutations cause familial disease, polymorphisms can be susceptibility factors, and some haplotypes are linked to risk), (3) animal modeling: overexpression of the fibrillar protein reproduces many features of the human disease, and (4) biophysics: proteins containing disease-associated mutations aggregate more rapidly than wild-type proteins. However, the mechanism by which amyloid fibril formation causes neurodegeneration and the identity of the pathogenic species have not been determined. It is not possible to directly observe the pathogenic event. Therefore, one is left to extrapolate from simplified models in order to produce a working hypothesis, which can then be tested experimentally. The design of a successful therapeutic strategy based on a working hypothesis will be taken as a 'proof' of its veracity.

The pathogenic pathway is likely to be much more complicated than an evolutionarily optimized process like, say, ribosome assembly. Furthermore, there is no *a priori* reason to assume that cell death in neurodegenerative disease occurs by a single mechanism or that, if protein aggregates are responsible, a single species is capable of acting alone as the pathogenic trigger. Despite these caveats, this review will apply Occam's razor and start with the working hypothesis that a single species of protein aggregate may be responsible for initiating a cascade of events that culminate in neurodegeneration. It is our hope that this review will stimulate others to design experiments to test the relevance, or lack of thereof, of this working hypothesis.

The majority of research in the field of neurodegeneration has focused on the cause of neuronal death, rather than on the identity of the neurotoxic species. This review will focus on *in vitro* studies aimed at the characterization of an intermediate on the amyloid pathway as one potential toxic species. This review is based on the premise that understanding the structures of the protein aggregates and the dynamics of their interconversion *in vitro*, can establish potential links between aggregation and toxicity. The key to such an approach is to allow genetic information, especially regarding autosomal dominant (gain of function) mutations, to guide these experiments. Before summarizing these studies, it is important to emphasize that any reductionist approach must ultimately explain essential features of the *in vivo* process that it is seeking to elucidate. In the case of neurodegeneration, these are:

(1) Aging is one of the strongest susceptibility factor for all of the neurodegenerative diseases. Even those that are purely genetic, like Huntington's disease, are primarily adult-onset. This does not necessarily mean that the pathogenic process takes a long time, but only that neurons become more susceptible to occurrence of a pathogenic event as they age.
(2) All of these diseases show selectivity to particular types of neurons (hence the diverse symptoms).
(3) Mutations cause early-onset forms of these diseases that are transmitted in an autosomal dominant manner.

Points (2) and (3) are linked, since different mutations in the same protein can produce distinct clinical entities that affect different neuronal subpopulations (three examples are fatal insomnia, which results from a point mutation in the prion protein (Gambetti *et al.* 1995), hereditary cerebral haemorrhage with amyloidosis of the Dutch type, caused by a mutation in amyloid precursor protein (APP)/Aβ (Maat-Schieman *et al.* 1992), and dementia with Lewy bodies, caused by a mutation in α-synuclein (Zarranz *et al.* 2004)).

Several lines of evidence suggest that *in vitro* amyloid fibril formation mimics the *in vivo* process: (*a*) fibrils formed *in vitro* strongly resemble those in diseased tissues (Sunde *et al.* 1997), (*b*) protofibrillar intermediates, first detected *in vitro*, and later *in vivo* (see Table 1) exhibit strikingly similar structural and neurotoxic properties (Roher *et al.* 1996) and (*c*) the specificity of *in vitro* assembly is reflected *in vivo* (Wetzel, 1994; Helms & Wetzel, 1996; Rajan *et al.* 2001; Kheterpal *et al.* 2003). Taken together, these observations suggest that structural and mechanistic clues derived from *in vitro* studies are relevant to the role of protein fibrillogenesis in neurodegenerative diseases and may provide molecular targets for the design of desperately needed therapeutic agents.

2. What is the significance of the shared structural properties of disease-associated protein fibrils?

Approximately 24 human proteins form amyloid fibrils *in vivo* (Stefani & Dobson, 2003). These proteins are unrelated at the level of primary structure, consistent with the finding that many proteins with no connection to disease can form amyloid fibrils with a common core structure *in vitro*, suggesting that the amyloid fibril is an intrinsically stable structure (Dobson, 2001). Amyloid fibrils are not crystalline, so their structural similarity is based on lower-resolution approaches: X-ray fibril diffraction, electron and atomic force microscopy, and their ability to bind histopathological dyes like Congo Red and Thioflavin T (Sunde *et al.* 1997). The structural convergence among various amyloid fibrils is also corroborated by the findings that antibodies

Table 1. *A summary of studies examining the structure and formation of protofibrils, including amyloid pores by several amyloid-forming priteins* in vitro*, cell cultures and* in vivo *grouped according to the amyloid disease to which they are relevant. Due to space limitations only the most relevant references are cited*

Disease	Amyloid-forming proteins and peptides	Evidence for protofibrils *in vitro*	Evidence for protofibrils In cell culture and in vivo	Evidence for annular structures/amyloid pores	Evidence for channel/pore activity
Alzheimer's disease	Aβ 1-40 (WT)	Goldsbury *et al.* 2005; Harper *et al.* 1997a, b, 1999; Huang *et al.* 2000; Kayed *et al.* 2004; Lambert *et al.* 1998; Stine *et al.* 2003; Walsh *et al.* 1997, 1999; Yong *et al.* 2002)	Funato *et al.* 1999; Gong *et al.* 2003; Kuo *et al.* 1996; Lambert *et al.* 2001; Pitschke *et al.* 1998; Roher *et al.* 1996	Hafner *et al.* 2001; Kayed & Glabe, 2004; Klug *et al.* 2003; Lashuel *et al.* 2002b, 2003	Alarcon *et al.* 2006; Arispe *et al.* 1993a, b, 1994, 1996; Kagan *et al.* 2002; Kawahara & Kuroda, 2000, 2001; Kawahara *et al.* 2000; Kourie *et al.* 2001b; Lin *et al.* 1999; Singer & Dewji, 2006
	Aβ 1-40 (E22G)	Dahlgren *et al.* 2002; Lashuel *et al.* 2002b, 2003; Nilsberth *et al.* 2001	Morishima-Kawashima & Ihara, 1998; Podlisny *et al.* 1995; Walsh *et al.* 2000, 2002	Lashuel *et al.* 2002b, 2003	
	Aβ 1-42 (WT & E22G)	Dahlgren *et al.* 2002; El-Agnaf *et al.* 2000; Parbhu *et al.* 2002; Roher *et al.* 1996; Stine *et al.* 2003; Wang *et al.* 2002a		Chromy *et al.* 2003; Kayed *et al.* 2004; Lashuel *et al.* 2003; Lin *et al.* 2001	Bahadi *et al.* 2003a; Bhatia *et al.* 2000; Hirakura *et al.* 1999; Lin *et al.* 2001; Rhee *et al.* 1998
Parkinson's disease	α-Synuclein (WT, A53T & A30P)	Cappai *et al.* 2005; Conway *et al.* 2000, 2001; Ding *et al.* 2002; Kaylor *et al.* 2005; Lashuel *et al.* 2002a; Rochet *et al.* 2000; Shtilerman *et al.* 2002; Volles & Lansbury, 2003; Zhu & Fink, 2003	El-Agnaf *et al.* 2006; Feany & Bender, 2000; Gosavi *et al.* 2002; Lee & Lee, 2002; Lee *et al.* 2002; Lo Bianco *et al.* 2002; Masliah *et al.* 2000; Sharon *et al.* 2001, 2003a, b.	Lashuel *et al.* 2002a, b; Ding *et al.* 2002; Shtilerman *et al.* 2002; Rochet *et al.* 2000; Zhu & Fink, 2003; Crystal *et al.* 2003; Pountney *et al.* 2004; Zhu *et al.* 2004; Lowe *et al.* 2004; Quist *et al.* 2005	Volles & Lansbury, 2002; Volles *et al.* 2001

Familial British dementia	ABri	El-Agnaf *et al.* 2001a, b; Srinivasan *et al.* 2003	Holton *et al.* 2001	Srinivasan *et al.* 2003	Quist *et al.* 2005
Dialysis-associated amyloidosis	β_2-Microglobulin	Kad *et al.* 2003		Kayed & Glabe, 2004	Hirakura & Kagan, 2001
Secondary systemic amyloidoses	Serum amyloid A	Wang *et al.* 2002b		Wang *et al.* 2002b	Hirakura *et al.* 2002
Prion-related diseases	MHM2 PrP 106 (Δ23-88 and Δ141-176)	Baskakov *et al.* 2000	Riesner *et al.* 1996; Supattapone *et al.* 1999		Kawahara *et al.* 2000; Kourie, 2002; Kourie & Culverson, 2000; Kourie *et al.* 2001a, 2003; Lin *et al.* 1997
	Prion, PrP 106-126 84-146	Kayed & Glabe, 2004 Sokolowski *et al.* 2003		Kayed & Glabe, 2004	
	SHa PrP (90-232) PrP (23-231)	Baskakov *et al.* 2002; Lu & Chang, 2002; Vendrely *et al.* 2005		Sokolowski *et al.* 2003	Hirakura *et al.* 2000b Bahadi *et al.* 2003b, c
Huntington's disease	(polyglutamine)	Poirier *et al.* 2002a		Kayed & Glabe, 2004	Hirakura *et al.* 2000a; Kagan *et al.* 2001; Monoi *et al.* 2000 Demuro *et al.* 2005; Kayed *et al.* 2004
Amyotrophic lateral sclerosis	Superoxide dismutase-1 (WT & A4V, G37R, G85R)	Chung, 2003; Rakhit *et al.* 2002, 2004; Ray *et al.* 2004	Turner *et al.* 2003	Chung, 2003; Ray *et al.* 2004	
Senile & familal amyloidosis	Transthyretin	Cardoso *et al.* 2002a, b; Kayed *et al.* 2003, 2004; Lashuel *et al.* 1998, 1999	Sousa *et al.* 2001, 2002; Teng *et al.* 2001	Kayed & Glabe, 2004	Azimov *et al.* 2001
Type II diabetes	IAPP	Anguiano *et al.* 2002; Green *et al.* 2003; Green, 2004; Kayed & Glabe, 2004; Kayed *et al.* 2003; Porat *et al.* 2003; Rhoades & Gafni, 2003	Butler *et al.* 2003; de Koning *et al.* 1994; Janson *et al.* 1996; O'Brien *et al.* 1994	Janson *et al.* 1999; Anguiano, 2002; Kayed & Glabe, 2004; Porat *et al.* 2003	Anguiano *et al.* 2002; Harroun *et al.* 2001; Hirakura *et al.* 2000b; Kawahara *et al.* 2000; Mirzabekov *et al.* 1996; Porat *et al.* 2003
	P53 Equine lysozyme Insulin	Ishimaru *et al.* 2003 Malisauskas *et al.* 2003 Dzwolak *et al.* 2005		Ishimaru *et al.* 2003 Malisauskas *et al.* 2003 Dzwolak *et al.* 2005	

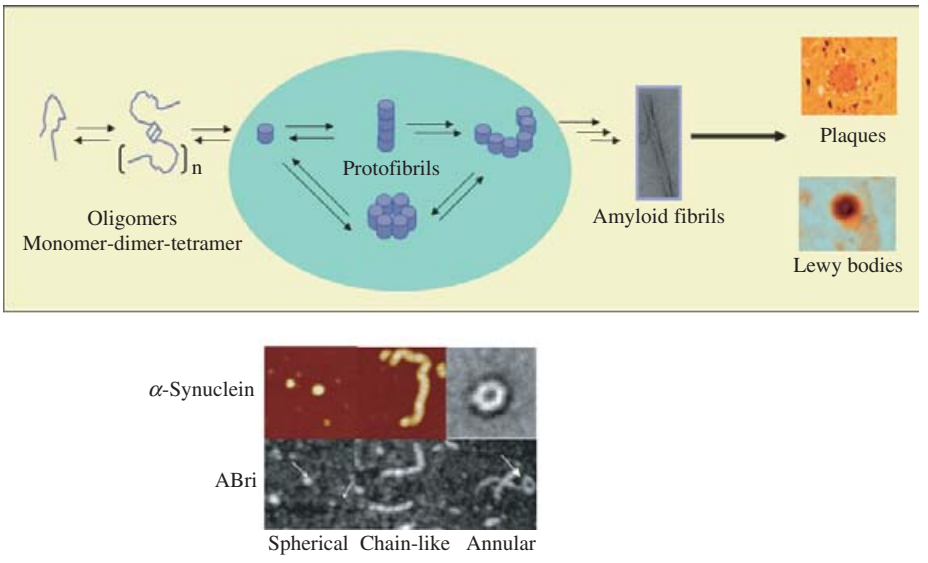

Fig. 1. *In vitro* fibril formation involves transient population of ordered aggregates, or protofibrils. Schematic representation summarizing the quaternary structural species populated during amyloid fibril formation *in vitro* and are thought to form during protein fibrillogenesis and deposition *in vivo*. For simplicity, only the most commonly observed (globular, chain-like and annular structures) protofibrillar structures are shown. (The ABri images were kindly provided by Dr Michael Zagorski.)

raised against the fibrillar form of the amyloidogenic protein amyloid-β (Aβ) recognize amyloid fibrils derived from other amyloid-forming proteins (O'Nuallain & Wetzel, 2002). The shared conformational epitopes have not been identified, but may be involved in pathogenesis. Moreover, the structural similarities suggest a shared mechanism of amyloid fibril formation.

2.1 Mechanism of amyloid fibril formation *in vitro*

2.1.1 *In vitro* fibril formation involves transient population of ordered aggregates of intermediate stability, or protofibrils

In order to form an amyloid fibril, proteins must adopt a β-sheet rich conformation(s). This may involve induction of structure in a disordered monomer, or the partial unfolding of a β-sheet-containing globular protein. The β-sheet-rich species has a high propensity to aggregate/fibrillize (Kelly, 1998). *In vitro* amyloid fibril formation does not proceed via a simple two-state, 'intermediate-less' mechanism akin to microtuble formation from tubulin (these processes have evolved to be efficient and non-toxic), although that model can be instructive (Jarrett & Lansbury, 1993). Rather, discrete β-sheet rich oligomeric intermediates appear before fibrils form and disappear upon fibril formation (Harper *et al.* 1997a; Walsh *et al.* 1997; Lambert *et al.* 1998) (Fig. 1). These are collectively designated protofibrils. Protofibrils are more stable than the monomer, but less stable than the fibrillar product. They appear to be obligate intermediates in fibril formation; the transient formation of protofibrils has been observed in every well-characterized *in vitro* case, suggesting that they are obligate intermediates that are likely to exist, albeit transiently, *in vivo*.

3. Toxic properties of protofibrils

3.1 Protofibrils, rather than fibrils, are likely to be pathogenic

The presence of amyloid fibrils in the post-mortem brains of demented patients led to the first description of AD and resulted in the hypothesis that fibrils themselves are the primary pathogenic species. This hypothesis fails to explain several important pathological and clinical characteristics of AD, raising the possibility that a species other than amyloid fibrils could be the toxic species (Goldberg & Lansbury, 2000). First, there is no correlation between the amounts of fibrillar Aβ deposits at autopsy and the clinical severity of AD (Lemere *et al.* 1996; Terry *et al.* 1991). Such a correlation does exist between 'soluble Aβ' (monomers plus protofibrils) in the brain and early cognitive dysfunction (Lue *et al.* 1999; McLean *et al.* 1999; Naslund *et al.* 2000). Second, transgenic animals that overproduce APP exhibit neuronal and behavioral abnormalities before amyloid plaques can be detected (Chui *et al.* 1999; Hsia *et al.* 1999; Moechars *et al.* 1999; Mucke *et al.* 2000). Third, inhibiting Aβ amyloid fibril formation does not necessarily attenuate Aβ-associated toxicity towards cultured neurons (Aksenova *et al.* 1996; Stege *et al.* 1999). Fourth, one autosomal dominant form of AD results from a mutation [APP (E693G) = Aβ (E22G)] that decreases Aβ production *in vivo* and promotes protofibril formation and toxicity *in vitro* (Nilsberth *et al.* 2001; Lashuel *et al.* 2003; Whalen, 2005). Fifth, in some AD transgenic mice, vaccination with an Aβ-directed antibody prevented or reversed age-dependent memory decline without reducing the amyloid burden (Morgan *et al.* 2000) [in other cases, vaccination has been shown to reduce behavioral and cognitive deficits *and* the amyloid burden (Janus *et al.* 2000; Morgan *et al.* 2000)]. Finally, Aβ aggregates with properties that are indistinguishable from Aβ protofibrils formed *in vitro* have been found in human CSF, human cerebral cortex (McLean *et al.* 1999), and in neuritic amyloid plaques from AD brains (Roher *et al.* 1996), raising the possibility that soluble prefibrillar oligomeric species, rather than the fibrils, could be the pathogenic species in AD and related amyloid diseases. Consistent with this hypothesis, oligomeric (dimer, trimers and tetramers) low-molecular-weight oligomers (Walsh *et al.* 2002; Wang *et al.* 2004; Cleary *et al.* 2005; Townsend *et al.* 2006) and protofibrillar forms of Aβ altered neuronal function and/or caused neuronal death in culture (Lambert *et al.* 1998; Hartley *et al.* 1999; Walsh *et al.* 2002; Whalen *et al.* 2005).

The toxic protofibril hypothesis also explains features of PD and other neurodegenerative diseases that are inconsistent with the fibril being the pathogenic species (Goldberg & Lansbury, 2000; Caughey & Lansbury, 2003; Silveira *et al.* 2005). In PD, fibrillar intraneuronal inclusions comprising α-synuclein, known as Lewy bodies (LBs), are an invariant feature of sporadic and autosomal dominant forms of PD, but are absent in autosomal recessive juvenile Parkinsonism (AJRP) (Shimura *et al.* 2001). It is likely that AJRP brains have such high levels of protofibrils that complete neurodegeneration occurs before LBs have a chance to form [AJRP results from a deletion in the parkin gene; point mutations produce a later-onset form of PD that is characterized by LBs (Kitada *et al.* 1998)]. Elevated levels of soluble oligomeric forms of α-synulcien have been detected in plasma samples of PD patients compared to controls (El-Agnaf *et al.* 2006). Similarly, transgenic mice that overexpress the human α-synuclein protein become symptomatic (movement disorder plus dopaminergic abnormalities) but do not produce fibrillar deposits (Masliah *et al.* 2000). Furthermore, virus-mediated overexpression of human α-synuclein into the substantia nigra of rats (Kirik *et al.* 2002; Lo Bianco *et al.* 2002) and primates (Kirik *et al.* 2003) results in selective dopaminergic neuronal death with non-fibrillar, α-synuclein-containing inclusions. *In vitro* studies of α-synuclein aggregation provide possible hints as to the underlying

situation: mixtures of human and mouse α-synuclein (as exist in the transgenic mouse brain) accumulate protofibrils, but fibrillize very slowly (Rochet *et al.* 2000). Interestingly, virus-mediated expression of rat α-synuclein in rat brain results in aggregation, but no neurodegeneration (Kirik *et al.* 2002; Lo Bianco *et al.* 2002). Further characterization of the aggregates formed by human and rat α-synuclein could provide important insights into the role of α-synuclein aggregation in the degeneration of dopaminergic neurons.

Other neurodegenerative diseases fail to support the proposal that amyloid fibrils are pathogenic. Prion protein (PrP) fibrils are observed in some prion diseases, but are not an invariant feature (Chiesa & Harris, 2001). In yeast and mouse models of prion disease, toxicity was produced in the absence of the stable, protease-resistant aggregated form of the prion protein (PrP-Sc) (Ma & Lindquist, 2002; Ma *et al.* 2002). A transgenic mouse model of a familial prion disease became symptomatic before PrP-Sc could be detected (Chiesa *et al.* 2003). Recent studies by Caughey and co-workers suggest that the most infectious PrP particles represent protofibrillar particles with molecular masses ranging from 300–600 kDa (corresponding to 14–28 PrP molecules) (Silveira *et al.* 2005). Finally, in systemic amyloid diseases, where amyloid was thought to produce disease by physically interfering with organ function, studies by Reixach *et al.* suggest a primary pathogenic role for protofibrils in initiating cytotoxicity and organ dysfunction (Reixach *et al.* 2004). For example, non-fibrillar aggregates of transthyretin (TTR), a protein associated with systemic amyloidosis and familial amyloid polyneuropathy (FAP), have been detected in transgenic mice expressing wild-type and mutant TTR (Teng *et al.* 2001) and in the nerves of FAP patients (Sousa *et al.* 2001, 2002). These aggregates were linked to significant clinical pathology early in the disease before the fibrillar deposits could be detected.

3.2 The toxic protofibril may be a mixture of related species

In an effort to more precisely identify the neurotoxic protein aggregate, cell culture models of extracellular toxicity have been utilized. Although these simplified models allow more detailed analysis, their relevance to AD is debatable. Given that caveat, as well as the fact that Aβ oligomers added to cell culture media are heterogeneous (Lashuel *et al.* 2003; Goldsbury *et al.* 2005) and are likely to change during the course of an experiment, there is remarkable agreement among these studies that the protofibril, and not the monomer or the fibril, is a toxic entity (Lambert *et al.* 1998; Hartley *et al.* 1999; White *et al.* 2005; Townsend *et al.* 2006). In the case of Aβ, the amyloid protein of AD, globular oligomers (designated ADDLs) were toxic to cultured neurons (Lambert *et al.* 1998) and inhibited hippocampal long-term potentiation (LTP) when introduced to brain slices (Wang *et al.* 2002a). Globular oligomers of similar sizes (based on AFM and EM measurement) have been described by other research groups and were shown to be toxic to cultured neurons (Barghorn *et al.* 2005). In a related study, small spherical and chain-like Aβ protofibrils (but not monomer, dimer, trimer) induced acute electrophysiological changes and progressive neurotoxicity in cortical neurons (Hartley *et al.* 1999). Large spherical aggregates of Aβ (average diameter of $>$10 nm), termed amylospheroids, exhibited significantly higher toxicity than small spherical Aβ oligomers ($<$10 nm) (Hoshi *et al.* 2003). Aβ42 forms spherical aggregates (diameter $>$10 nm) more rapidly and exhibits significantly more ($>$100-fold) toxicity to neuronal cultures than those formed by Aβ40. Similar, apparently spherical structures with diameters ranging from 8–24 nm have also been observed as transient intermediates during the fibrillogenesis of the arctic variant (E22G) of Aβ40 and Aβ42 (Lashuel *et al.* 2003), α-synuclein (Lashuel *et al.* 2002a) and mutant SOD1 (Ray *et al.* 2004). The toxicity of TTR towards cultured

cells is also linked to a non-fibrillar oligomeric species, possibly an octamer (Reixach *et al.* 2004). Finally, it is probable that the protofibril structure, rather than the protein primary sequence, produces toxicity. For example, small spherical protofibrils, formed *in vitro* by an amyloidogenic SH3 domain from bovine phosphatidyl-inositol-3 kinase (which is not linked to any disease) are toxic to cultured cells (Bucciantini *et al.* 2002).

3.3 Morphological similarities of protofibrils suggest a common mechanism of toxicity

The shared morphological and toxic properties of amyloid protofibrils suggest that toxicity depends on shared structural features. Consistent with this hypothesis, antibodies raised against protofibrillar Aβ were reported to recognize protofibrillar species derived from other amyloidogenic proteins (e.g. α-synuclein, polyglutamine, TTR, IAPP, lysozyme, human insulin, and PrP106-126), but not monomeric or fibrillar forms of these proteins (Kayed *et al.* 2003). These antibodies also inhibit the toxicity of these proteins. These findings support detailed structural studies of protofibrils, some of which are summarized below. Furthermore, These observations raise the possibility that these diverse (with respect to sequence, at least) structures may exert their toxicity through common mechanisms. It may be that an intermediate on the assembly pathway that is in equilibrium with the protofibril could be the toxic species. The most likely candidates are low-molecular-weight oligomers (e.g. dimers, trimers or tetramers) or a high-molecular-weight protofibrillar species, most likely the 4 nm ($\pm$1 nm) spherical species. This is consistent with biophysical studies (AFM and EM) demonstrating that these spheres are the precursors to the chain-like and annular protofibrillar structures and appear to exist in equilibrium with the large spherical aggregates (8–70 nm). Several laboratories have demonstrated that these species are capable of self-assembling into pore-like structures on artificial or biological membranes (Lin *et al.* 2001; Ding *et al.* 2002).

3.4 Are the amyloid diseases a subset of a much larger class of previously unrecognized protofibril diseases?

The hypothesis that small protofibrillar aggregates are pathogenic has an interesting corollary: there may be many other diseases that, like ARJP, are not characterized by easily detected protein aggregates. Unlike ARJP, which is genetically and clinically linked to the amyloid disease PD, these diseases may not be recognized as involving protein aggregation at all. In contrast to our expectation that the primary sequence efficiently directs protein folding, a significant portion of newly synthesized proteins ($\sim$35%) are not correctly folded *in vivo* (Schubert *et al.* 2000). We expect that many more protein aggregation-driven pathologies remain to be discovered.

3.5 Fibrils, in the form of aggresomes, may function to sequester toxic protofibrils

According to the toxic protofibril hypothesis, the role of the amyloid fibril is uncertain; it could have some greatly reduced toxicity, it could be inert, or it could protect neurons by sequestering toxic protofibrils and/or by consuming protein monomers, inhibiting continued protofibril formation (Caughey & Lansbury, 2003). There is circumstantial evidence that fibrils may have a protective function. In PD brains, dopaminergic neurons that contain LBs appear to be 'healthier' than neighboring neurons based on morphological and biochemical criteria (Tompkins & Hill, 1997). Several transgenic models of polyglutamine diseases are characterized

by polyglutamine inclusions in healthy neuron populations, but not in vulnerable neurons (Kuemmerle *et al.* 1999). In a transgenic mouse model of one polyglutamine disease, spinocerebellar ataxia-type1 (SCA1), there was a direct correlation between the ability of neurons to sequester ataxin-1 into inclusions and their resistance to cell death (Watase *et al.* 2002). Taken together, these observations suggest that formation of fibrillar inclusions may protect neurons against protofibril-induced neurotoxicity. There is mounting evidence that the sequestration process may be an active one. Expression of any one of the four disease-associated proteins; cystic fibrosis transmembrane conductance regulator (CFTR, associated with cystic fibrosis) (Johnston *et al.* 1998), SOD1 (amyotrophic lateral sclerosis) (Johnston *et al.* 2000), the androgen receptor (AR, X-linked spinobulbar muscular atrophy) (Taylor *et al.* 2003), or Parkin (Junn *et al.* 2002) in cultured cells under conditions of oxidative stress or proteasome impairment leads to sequestration of the mutant protein within multicomponent proteinaceous inclusions known as aggresomes. Sequestration is actively mediated by microtubules (Johnston *et al.* 1998). In the case of Parkin, these inclusions exhibit morphological and immunohistochemical features similar to LBs (Junn *et al.* 2002). In addition to sequestering toxic protein aggregates, aggresomes may play an active role in facilitating the clearance of these toxic species by lysosomal mediated degradation mechanisms (Taylor *et al.* 2003).

4. Amyloid pores, a common structural link among protein aggregation neurodegenerative diseases

4.1 Mechanistic studies of amyloid fibril formation reveal common features, including pore-like protofibrils

The convergent circumstantial evidence implicating protofibrils as pathogens has motivated a more detailed analysis of protofibril structure and formation *in vitro*. These studies are briefly summarized below and in Table 1, grouped according to the amyloid disease to which they are relevant. Similar pathways are followed by other fibrillogenic proteins that are not known to be related to disease (see Table 1 and below).

4.1.1 Amyloid-β (Aβ) (Alzheimer's disease)

Alzheimer's disease (AD) is characterized by the presence of extracellular fibrillar amyloid plaques and intraneuronal neurofibrillary tangles in brain areas associated with memory and learning. The main fibrillar constituent of amyloid plaques is amyloid-β (β(Glenner & Wong, 1984), a 39-42 amino acid peptide that is produced by endoproteolytic processing of the APP (Selkoe, 1994). A central role for Aβ amyloid fibril formation in the etiology of AD is supported by extensive genetic and biochemical evidence (Hardy & Selkoe, 2002). In solution, Aβ is unstructured, but Aβ protofibrils, including spherical (average diameter of 2·4–4·5 nm, containing $\sim$40 monomers) and chain-like protofibrils and are rich in β-sheet structure (Fig. 1) (Harper *et al.* 1997a, b; Walsh *et al.* 1997; Lambert *et al.* 1998; Parbhu *et al.* 2002; Stine *et al.* 2003). Smaller oligomers (e.g. dimers, trimers and tetramers) likely exist, but are not populated in solution (they can only be observed by SDS–PAGE). Once the critical concentration of the spherical and later chain-like species is reached, they are rapidly converted into amyloid fibrils. In addition to the globular and chain-like protofibrils, annular structures with variable diameters (outer diameter 6–9 nm, inner diameter 1·5–2 nm) have also been observed during the

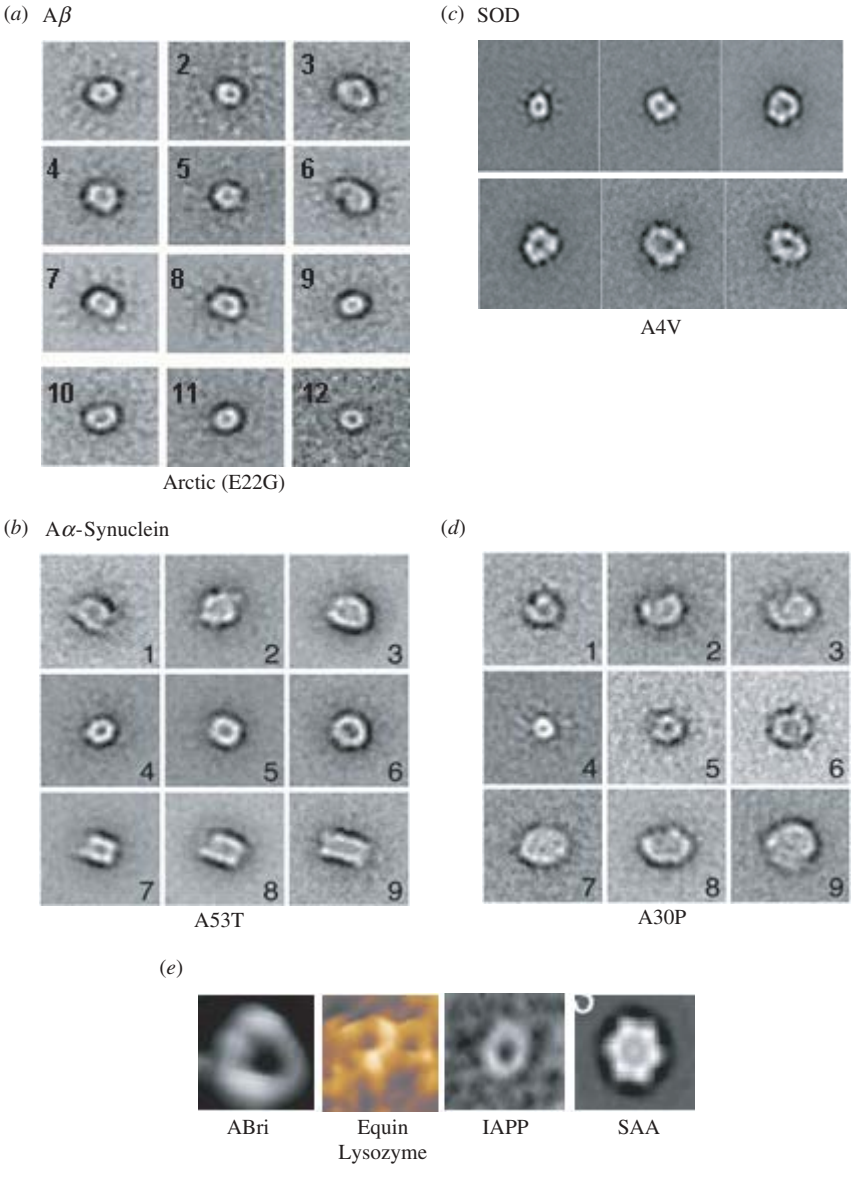

Fig. 2. Many amyloid proteins form annular pore-like protofibrils and have channel- or pore-like properties *in vitro*. Annular pore-like structures with variable diameters form during the *in vitro* fibrillogenesis of disease associated mutants of Aβ [Arctic variant (E22G) of Aβ40] (*a*, *f*), α-synuclein (A53T & A30P) (*b*, *d*), and SOD1 (A4V) (*c*), and other amyloid-forming proteins (*e*).

fibrillogenesis of Aβ *in vitro* (Fig. 2*a*) and during the reconstitution of Aβ in lipid bilayers (Fig. 3*a*) (Hafner *et al.* 2001; Lin *et al.* 2001; Chromy *et al.* 2003; Klug *et al.* 2003; Lashuel *et al.* 2003; Kayed & Glabe, 2004). The similar heights of the spheres, chain-like and annular protofibrils suggests that the spheres are the precursors of the others (Lashuel *et al.* 2003). Formation of annular protofibrils is promoted by a pathogenic mutation [APP (E693G) = Aβ(E22G)] that is linked to familial AD and accelerates the oligomerization of Aβ *in vitro* (Nilsberth *et al.* 2001; Lashuel *et al.* 2002a, b). Annular protofibrils represent only a small fraction of the total population of Aβ

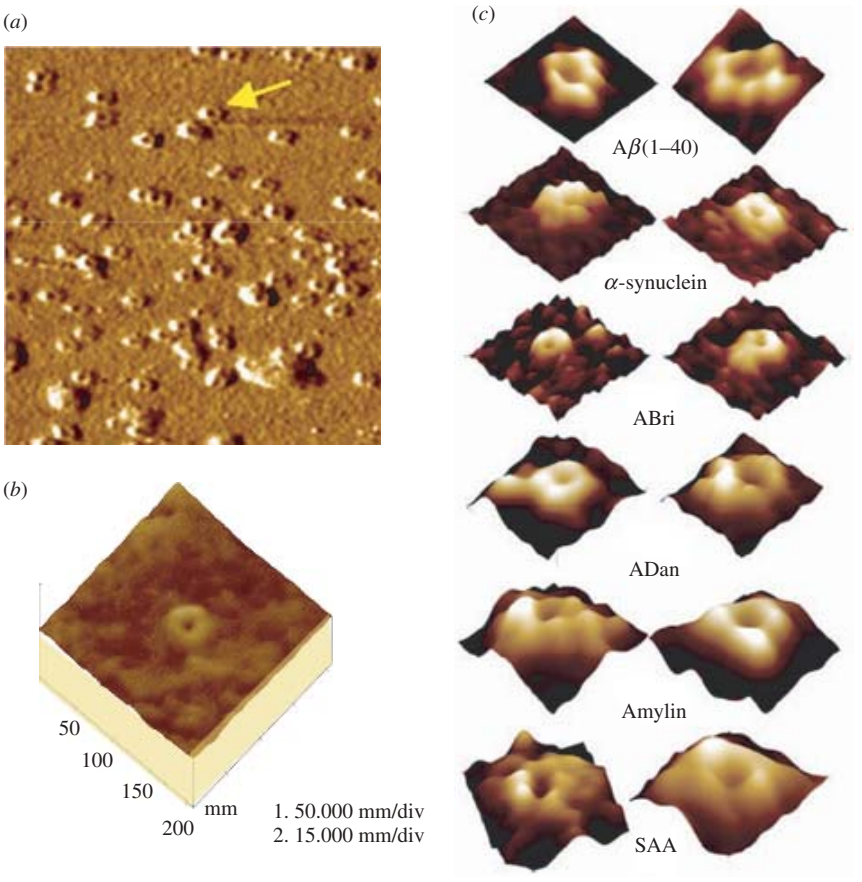

Fig. 3. Amyloid forming proteins form pore-like/channel structures on artificial as well as biological membranes. (*a*) AFM images of pore-like structures formed during the reconstitution of Aβ in lipid bilayers (adapted from Lin *et al.* 2001). (*b*) Pore-like structures that were obtained by incubating predominantly spherical α-synuclein protofibrils (WT and A53T) with brain-derived vesicles (adapted from Ding *et al.* 2002). (*c*) Amyloid-forming proteins/peptides reconstituted in lipid bilayers form pore/channel-like structures (adapted from Quist *et al.* 2005).

protofibrils at any given time, which explains why these structures were missed or ignored in previous studies.

4.1.2 α-Synuclein (PD and diffuse Lewy body disease)

PD is associated with the formation of intraneuronal fibrillar inclusions (LBs) (Pollanen *et al.* 1993; Shults, 2006). α-Synuclein is the primary component of LBs in all PD patients (Spillantini *et al.* 1998b) and is strongly implicated as a cause of PD by biochemical (Cookson, 2005) and genetic studies of familial forms of PD (Polymeropoulos *et al.* 1997; Kruger *et al.* 1998; Singleton *et al.* 2003; Zarranz *et al.* 2004) and by mouse (Masliah *et al.* 2000) and *Drosophila* (Feany & Bender, 2000; Chen & Feany, 2005) modeling studies. Three different α-synuclein missense mutations (A30P, A53T and E46K) are associated with rare, autosomal dominant forms of early-onset PD (Polymeropoulos *et al.* 1997; Kruger *et al.* 1998; Zarranz *et al.* 2004). In addition, triplication of the wild-type α-synuclein gene causes autosomal dominant PD in an Iowan

kindred (Singleton *et al.* 2003). Transgenic mice (Masliah *et al.* 2000) expressing human wild-type α-synuclein and *Drosophila* (Feany & Bender, 2000; Chen & Feany, 2005) expressing WT, A30P, or A53T are characterized by α-synuclein non-fibrillar (mice and fly) or fibrillar (Flynn & Theesen, 1999) inclusions and a Parkinsonian phenotype. Like Aβ, α-synuclein is not folded under 'native' conditions (Weinreb *et al.* 1996), but will form β-sheet rich protofibrils and amyloid fibrils *in vitro*. Three mutations (A53T, E46K and A30P) linked to early-onset PD promote the formation of α-synuclein protofibrils, but the A30P mutation was shown to form fibrils more slowly than WT α-synuclein, suggesting that α-synuclein protofibrils may cause neurodegeneration in PD (Conway *et al.* 1998; Conway *et al.* 2000; El-Agnaf *et al.* 1998; Li *et al.* 2001; Pandey *et al.* 2006). Amyloid fibril formation by α-synuclein occurs by a hierarchical assembly mechanism (random coil→spheres→chain-like and annular protofibrils→fibrils) similar to that observed for Aβ in AD (Conway *et al.* 2000; Ding *et al.* 2002). The PD-linked mutations promote formation of annular and tubular protofibrillar structures (wild-type α-synuclein forms annular protofibrils after extended incubation) (Conway *et al.* 2000; Ding *et al.* 2002; Lashuel *et al.* 2002a, b) (Fig. 2*b*, *d*). The diameter of α-synuclein annular protofibrils detected *in vitro* is similar to that of the α-synuclein fibrils. Zhu and colleagues observed the formation of α-synuclein annular protofibrils during drug-induced disaggregation of α-synuclein fibrils *in vitro* (Zhu *et al.* 2004), suggesting that a direct relationship between the two structures may exist. These observations are consistent with cross-sectional analysis of *ex vivo* amyloid fibrils demonstrating that amyloid fibrils have an electron-lucent center, indicative of the presence of a hollow center (Serpell *et al.* 2000).

4.1.3 ABri (familial British dementia)

ABri is the major component of amyloid deposits in the brain of patients with familial British dementia (FBD). FBD is an autosomal dominant neurodegenerative disorder associated with a stop codon mutation in the BRI gene that results in the production of ABri (Vidal *et al.* 1999; Ghiso *et al.* 2000). *In vitro* fibrillogenesis of ABri produced protofibrils and subsequently, amyloid fibrils (El-Agnaf *et al.* 2001a, b; Srinivasan *et al.* 2003, 2004), by a mechanism that is reminiscent of that of Aβ and α-synuclein, including the formation of spherical, chain-like and annular protofibrillar intermediates. Spherical protofibrils exhibited more toxicity to cultured neurons than chain-like protofibrils and fibrils (El-Agnaf *et al.* 2001a). Annular ABri protofibrils have been characterized *in vitro* (see Fig. 2*e*) (Srinivasan *et al.* 2004; O. M. El-Agnaf, personal communication) but their toxicity has not been determined.

4.1.4 Superoxide dismutase-1 (amyotrophic lateral sclerosis)

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease (Rosen, 1978; Julien, 2001), is linked to the gene encoding superoxide dismutase-type 1 (SOD1) (Rosen *et al.* 1993; Brown, 1997). Cytoplasmic inclusions containing mutant SOD1 are present in the motor neurons of familial ALS patients (Shibata *et al.* 1996) and transgenic mouse models (Bruijn *et al.* 1998; Watanabe *et al.* 2001). Significantly, these inclusions do not clearly contain fibrillar sub-structure. SOD1 is a structured homodimer. FALS mutations destabilize the native SOD1 dimer (Nakano *et al.* 1996; Cardoso *et al.* 2002b; Hayward *et al.* 2002; Lindberg *et al.* 2002; Rodriguez *et al.* 2002; Tiwari & Hayward, 2003; Rakhit *et al.* 2004; Ray *et al.* 2004) and promote SOD1 aggregation in *vitro* (Ray *et al.* 2004). Significantly, SOD1 has not been induced to form amyloid

fibrils *in vitro*. Instead, spherical and annular oligomeric structures have been observed (Chung, 2003; Ray *et al.* 2004). As expected, the pathogenic SOD1 mutants aggregate more rapidly than WT SOD1 (Chung, 2003; Ray *et al.* 2004), consistent with what is observed with Aβ and α-synuclein (Lashuel *et al.* 2003; Lashuel *et al.* 2002a, b). Their findings suggest that common pathogenic assemblies may underlie all of these diseases. In addition to pathogenic mutations, conditions that promote metal depletion or oxidative damage of SOD1 accelerate the formation of annular structures by mutants and wild-type SOD (A4V, H46R, G37R, and G85R) (Chung, 2003; Elam *et al.* 2003; Ray *et al.* 2004). The morphology of these oligomeric forms of SOD1 formed *in vitro* resembles that of protofibrillar species of Aβ and α-synuclein, in particular the annular and small spherical species (Fig. 2*c*). Thus, these species have been designated SOD1 protofibrils, yet they do not convert to fibrils over several weeks of incubation (Ray *et al.* 2004).

4.1.5 Prion protein (Creutzfeldt–Jakob disease, bovine spongiform encephalopathy, etc.)

The prion diseases [Creuzfeldt-Jakob disease (CJD), Gertsmann-Straussler syndrome (GSS), and fatal familial insomnia (FFI) in humans, bovine spongiform encephalopathy (BSE) in cattle, scrapie in sheep, and chronic wasting disease in elk] are characterized by the presence of an abnormal form of the PrP in the brain. The ability of these diseases to be transmitted, apparently by an abnormal form of the PrP, distinguishes them from the others discussed here, but there are also sporadic forms of human prion disease as well as forms that are purely genetic; caused by mutations in the gene encoding PrP (CJD, GSS, and FFI). The disease-associated form of the PrP, or PrP-Sc, is resistant to proteolysis and rich in β-sheet structure, relative to the normal form of the PrP, PrP-C (Cohen & Prusiner, 1998). PrP-Sc can also be isolated in fibrous form (Rubenstein *et al.* 1987). However, several lines of evidence suggest that PrP-Sc, like other amyloid fibrils, may not be the neurotoxic form of the protein. Transgenic mice that over-produce a disease-associated form of PrP exhibit disease-like phenotypes before PrP-Sc can be detected (Chiesa & Harris, 2001; Harris *et al.* 2003). During this phase, the protease-resistance of PrP gradually increased, suggesting the stepwise formation of structured oligomers of increasing stability. *In vitro* fibrillization of a fragment of PrP (residues 89–231) involves multiple oligomeric species, some of which appear to be off-pathway β-sheet-rich oligomers (Baskakov *et al.* 2002). Truncated variants of PrP form spherical and elongated protofibril-like structures that share properties with PrP-Sc and are capable of seeding the conversion of PrP^C into amyloid fibrils (Lee & Eisenberg, 2003). Finally, a truncated variant of PrP (SHaPrP 90-232) forms spherical and annular oligomeric structures *in vitro*, similar to those formed by Aβ and α-synuclein (Sokolowski *et al.* 2003). It was estimated that 8 subunits of SHaPrP 90-232 are required to form the β-sheet-rich annular structures (Sokolowski *et al.* 2003).

4.1.6 Huntingtin (Huntington's disease)

Huntington's disease is the most prevalent of a group of purely genetic neurodegenerative movement disorders that are caused by expansions of naturally occurring polyglutamine (polyQ) tracts in several proteins (the normal function of these tracts is not known) (Zoghbi & Orr, 2000). The magnitude of the expansion is related to the age of onset of the disease, with large expansions causing juvenile onset disease. The Huntington's disease brain is characterized by nuclear inclusions comprising the protein huntingtin (htn). Truncated mutant versions of htn with expanded polyQ repeat form spherical (Poirier *et al.* 2002; Wacker *et al.* 2004) chain-like

(Poirier *et al.* 2002), and annular (Wacker *et al.* 2004) protofibrillar structures *in vitro* that closely resemble those discussed above.

4.2 Amyloidogenic proteins that are not linked to disease also from pore-like protofibrils

Several other proteins that are not associated with disease form annular protofibrillar structures during the *in vitro* formation of amyloid fibrils. Conditions required to induce annular protofibrils vary depending on the precursor protein. Under partial denaturing conditions, the calcium-binding protein equine lysozyme (EL) self-assembles into protofibrils and fibrils of various morphologies in a pH and calcium dependent manner (Malisauskas *et al.* 2003). At pH 4·5 and 57° in the presence of 10 mm EDTA, EL forms annular structures with an average diameter of 45 nm ($\pm$5 nm), whereas at pH 2·0 EL forms rings with larger diameters ranging from 70–80 nm. Interestingly, in the presence of calcium at pH 4·5, elongated chain like structure are populated as the major species, but upon removal of calcium, EL forms predominantly annular structures (major species by AFM). Similarly, partial denaturing conditions were sufficient to induce oligomerization and formation of pore-like structures by the core domain of the tumor suppressor protein p53C (Ishimaru *et al.* 2003). Furthermore, Glabe & Kayed reported to have developed conditions for the formation of highly homogeneous preparations of annular protofibrils (outer diameter 8–12 nm, inner diameter 1·8–4·5 nm) from several amyloidogenic proteins, including Aβ, α-synuclein, IAPP, polyglutamine (KKQ40KK), Prion H1 (106–126), TTR and several Aβ variants (Kayed & Glabe, 2004). It has been proposed that the ability of a wide range of proteins to form annular protofibrils suggest that these quaternary structures might represent a second generic form of amyloid structures in addition to amyloid fibrils (Malisauskas *et al.* 2003). Furthermore, the ability to populate pore-like structures as the predominant species *in vitro* by manipulating solution conditions, suggest that *in vivo* conditions that promote the formation of these structure could exist.

4.3 Amyloid proteins form non-fibrillar aggregates that have properties of protein channels or pores

Disruption of Ca^{2+} homeostasis and generation of reactive oxygen species have long been recognized to be key events in the pathogenesis of several neurodegenerative diseases, including AD (Hardy & Higgins, 1992). Cellular factors responsible for initiating these events and the mechanism by which they contribute to the neurodegeneration and cytotoxicity remain poorly understood.

Every protein that forms amyloid fibrils *in vitro* does so via the protofibril. Aggregates comprising many of these proteins have channel-like or pore-like properties *in vitro* (the most relevant studies are summarized below). We propose that the pore-like properties arise from the pore-like protofibrillar structures or the ability of a protofibrillar intermediate to form these structures upon interactions with membranes. We prefer the term pore as opposed to channel, because the latter implies selectivity and regulation and we do not expect that either is involved with amyloid associated diseases. However, the terms used in the sections below reflect literature usage.

4.3.1 Aβ 'channels'

In 1993, Arispe and colleagues demonstrated that Aβ forms calcium channels in lipid bilayers and proposed that channel formation by Aβ is partially or wholly responsible for Aβ-induced

toxicity in AD (Arispe *et al.* 1993a, b). This finding has been reproduced many times, in several different laboratories, using many membrane models (Arispe *et al.* 1993b, 1994; Kawahara *et al.* 1997, 2000; Sanderson *et al.* 1997; Rhee *et al.* 1998; Hirakura *et al.* 1999; Lin *et al.* 1999, 2001; Bhatia *et al.* 2000; Kourie *et al.* 2001b; Kagan *et al.* 2002; Lin & Kagan, 2002; Bahadi *et al.* 2003a; Alarcon *et al.* 2006). The form of Aβ that is closely linked to AD pathogenesis (Aβ42) exhibits higher propensity to form channels, consistent with its increased propensity to aggregate and form annular protofibrils *in vitro* (H. Lashuel & P. T. Lansbury Jr., unpublished observations) as compared to Aβ40, the predominant form (Jarrett *et al.* 1993). β-sheet formation is also linked to Aβ-channel formation (and aggregation) (Sanderson *et al.* 1997). When incorporated into artificial lipid bilayers, Aβ produces uniform pore-like structures with an outer diameter of 8–12 nm and inner diameter of 2 nm (Fig. 3*a*, *c*) (Lin *et al.* 2001; Quist *et al.* 2005). These aggregates, formed at the membrane surface, resemble, pore-like aggregates formed in solution using Aβ42 or the disease-associated mutant form of Aβ40 (E22G) (Lashuel *et al.* 2002b, 2003). Addition of Aβ40 oligomers to hypothalamic GT1-7 neuronal cells results in simultaneous formation of Ca^{2+} channels and increase in intracellular Ca^{2+} levels, suggesting that Aβ40 oligomers is capable of disrupting biological as well as artificial membranes, possibly via pore formation (Kawahara & Kuroda, 2001, 2000; Singer & Dewji, 2006). The nature of the oligomer responsible for this activity was not investigated. AD-derived Aβ oligomers were observed to exhibit high affinity and selective attachment to membranes, suggesting that AD-derived oligomers are either directly integrated into the membranes or bind tightly to other cell surface molecules of cultured hippocampal neurons (Gong *et al.* 2003). Oligomer-specific antibodies were shown to block their interaction with membranes.

4.3.2 α-Synuclein 'pores'

The α-synuclein protofibril (but not the monomer or the fibril) binds very strongly to vesicle membranes and causes leakage of small compounds (less than $\sim$2·5 nm hydrodynamic radius) entrapped within synthetic vesicles (Volles *et al.* 2001; Volles & Lansbury, 2002). This typical pore-like behavior was consistent with the observation that addition of spherical protofibrils of α-synuclein to purified brain-derived vesicle (BDV) fractions resulted in the formation of pore-like structures (Fig. 3*b*) (Ding *et al.* 2002). Additionally, reconstitution of a-synuclein in lipid bilayers also results in the formation of pore-like structures that exhibit channel-like properties (Quist *et al.* 2005). Mutations linked to familial PD promoted the formation of the β-sheet-rich annular, pore-like (d=8–12 nm, 2–2·5 nm inner diameter) structures in solution (Lashuel *et al.* 2002a, b). These structures resembled in morphology and dimension, membrane-spanning pores that are formed by protein toxins (e.g. hemolysin, latrotoxin, and aerolysin) (Valeva *et al.* 1997; Orlova *et al.* 2000; Wallace *et al.* 2000). Vesicle permeabilization was also demonstrated for protofibrils comprising the congener γ-synuclein, but *not* those comprising β-synuclein (Park & Lansbury, 2003). At the same time, γ-synuclein, but not β-synuclein, produced pore-like structures in solution (H. Lashuel, unpublished results), strengthening the case that the pore-like structures are responsible for the pore-like behavior of α-synuclein.

4.3.3 PrP 'channels'

Aguzzi and colleagues have proposed that the pathogenicity of the PrP may be related to abnormal pore formation (Moore *et al.* 1999; Rossi *et al.* 2001; Behrens & Aguzzi, 2002). This proposal, based on studies of a PrP homolog, Doppel, is supported by circumstantial

experimental evidence. First, an abnormal transmembrane (translocation-incompetent) form of PrP has been linked to disease (Hegde *et al.* 1999). Second, a peptide based on the proposed transmembrane domain of PrP produce non-fibrillar aggregates that disrupt membranes (Pillot *et al.* 1997) and induce toxicity in rat cortical neurons (Pillot *et al.* 2000). Third, several PrP-based peptides interacts with lipid membranes and form ion channels (Lin *et al.* 1997; Kourie & Culverson, 2000; Bahadi *et al.* 2003b, c). Fourth, a truncated form of PrP permeabilizes synthetic vesicles (Sanghera & Pinheiro, 2002). Fifth, when disease-associated mutant forms of PrP were expressed in CHO cells, PrP was found to be very tightly associated with plasma membranes (Lehmann & Harris, 1996).

4.3.4 Polyglutamine 'channels'

Peptides with pathological polyglutamine repeats (>35–40 residues) insert into artificial membranes (Hirakura *et al.* 2000a; Monoi *et al.* 2000; Kagan *et al.* 2001) and also affect mitochondrial Ca^{2+} homeostasis (Panov *et al.* 2002). Furthermore a polyglutamine (Gln40) peptide produced homogeneous pore-like protofibrillar structures *in vitro*, while a shortened peptide did not (Monoi *et al.* 2000). Importantly, β-sheet formation, oligomerization, amyloid pore/channel formation, and fibrillogenesis are also very sensitive to polyGln repeat length (Scherzinger *et al.* 1999). Circumstantial evidence suggests that membrane disruption is mediated by direct interaction between the polyglutamine repeats or an aggregated form of these proteins resulting in the formation of ion channels in lipid bilayers and in mitochondrial membranes. Interestingly, only pathological polyglutamine repeat (>35–40) were observed to insert into artificial membranes and exhibited direct effects on mitochondrial Ca^{2+} homeostasis, reproducing the mitochondrial deficit seen in Huntington's disease patients and in transgenic animals expressing these repeats (Hirakura *et al.* 2000a; Monoi *et al.* 2000; Kagan *et al.* 2001; Panov *et al.* 2002). Furthermore, Kayed & Glabe (2004) reported that a polyglutamine protein containing 40 repeat (KKQ40KK) forms homogeneous annular pore-like protofibrillar structures *in vitro*, suggesting that protofibril formation and channel activity are linked.

Monoi and colleagues proposed that a single chain of poly-glutamine polypeptide is capable of forming cylindrical pores by forming a right handed helix (6·2 residues/turn), termed μ-helix, that is further stabilized by backbone side-chain hydrogen bonding interactions between the amide groups and glutmaine side-chain (Monoi, 1995; Monoi *et al.* 2000). What makes this model an attractive one is that it offers an explanation for the requirement of >35 polyglutamine tract to confer pathogenecity in polyglutamine diseases, because 37 residues (each contributing 0·81 Å to the length) would be required to form a μ-helix that spans the hydrophobic region of the bilayer (30 Å). A normal transmembrane domain composed of an α-helix is $\sim$20 amino acids long. However, β-sheet formation, oligomerization and fibrillogenesis by poly-glutamine proteins are highly dependent on the polyQ repeat length with 36 or more glutamines favoring these tertiary and quaternary structure changes (Scherzinger *et al.* 1999). However, further studies are required to elucidate the nature of the membrane active species responsible for polyglutamine membrane disruption properties.

4.4 Nature uses β-strand-mediated protein oligomerization to construct pore-forming toxins

A simple working hypothesis emerging from the studies summarized above holds that five properties of disease-associated amyloid proteins are linked: (1) aggregation, (2) β-sheet formation,

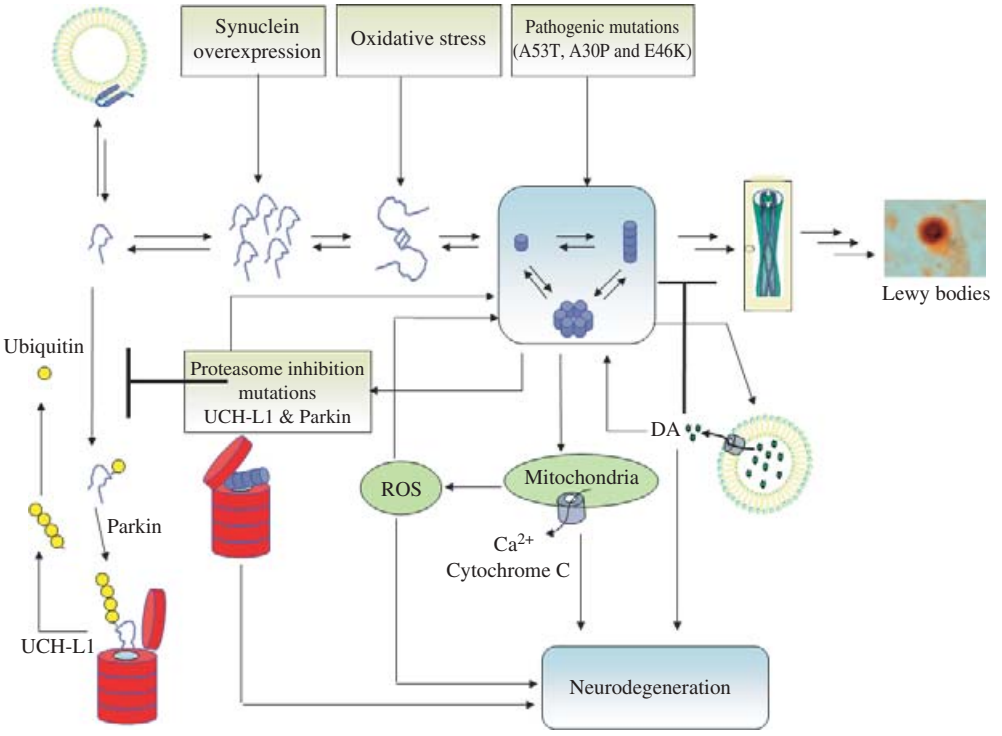

Fig. 4. Schematic depiction of the various steps involved in α-synuclein aggregation and Lewy Body formation and potential mechanisms by which these processes could contribute to the pathogenesis of PD. Several factors have been shown to play a role in initiating and/or accelerating α-synuclein aggregation and fibrillogenesis including, increased protein expression, mutations, oxidative stress induced modifications and crosslinking, and phosphorylation at S129 (Chen & Feany, 2005; Fujiwara *et al.* 2002; Smith *et al.* 2005). The scheme depicts amyloid protofibril as the toxic species and that it may amplify itself by inhibiting proteasome-dependent protein degradation, disrupting dompaniergic vesicles, formation of reactive oxygen species and increased oxidative stress, causing mitochondrial dysfunction (Abou-Sleiman *et al.* 2006), all of which have the affect of increasing levels of α-synuclein and/or accelerating α-synuclien oligomerization and protofibril formation. This hypothesis is supported by the findings that triplication of the α-synuclein gene causes and autosomal dominant PD in Iowan kindred (Singleton *et al.* 2003). Protofibrils may promote their own accumulation and toxicity by inhibiting the proteasome. Two gene products in the protein degradation pathway have been implicated in early onset forms of PD (Lansbury & Brice, 2002). One form is associated with genetic mutations in the gene encoding parkin (a protein with an E3 (ubiquitin ligase)-like activity), where the mutations reduce or abolish parkin's E3-like ligase activity (Kitada *et al.* 1998). A rare point mutation in the gene encoding ubiquitin C-terminal hydrolase (UCH-L1), a neuronal-specific deubiquitinating enzyme, is thought to lead to reduced catalytic activity of UCH-L1, and, thus, indirectly affect the proteasomal function (Leroy *et al.* 1998). Furthermore, reduced expression of UCH-L1 in the substantia nigra of DLB and PD brains suggest that it plays a role in modulating protein ag gregation and LB formation (Barrachina *et al.* 2005). In addition to its beneficial hydrolase activity (hydrolysis of C-terminal ubiquityl esters and amides), UCH-L1 exhibits a concentration dependent ubiquityl ligase activity that is thought to be pathogenic for PD. Taking together, these findings demonstrate that accumulation of α-synuclein either due to increased expression or decreased degradation is sufficient to cause PD, as predicted by the amyloid hypothesis.

(3) amyloid pore formation, (4) membrane permeabilization, and (5) toxicity (see Fig. 4). This proposal is more compelling because natural protein toxins utilize an analogous mechanism. Latrotoxin, α-hemolysin, and aerolysin all form well-ordered, oligomeric membrane-spanning pores characterized by β-sheet structure. It is important to remember that while these toxins are

optimized by evolution to permeabilize target membranes, amyloid pore formation is likely to be an accident and will therefore be very inefficient *in vitro*. Pore formation by β-sheet bacterial toxins involves a series of complex events that include membrane association, oligomerization, and insertion of a β-barrel. The pore-forming protein toxins (PFTs) typically exist as structured, water-soluble monomers; conversion into the membrane-inserted pore requires that these proteins undergo oligomerization and a conformational change. In the case of aerolysin, proteolytic processing by the target is required to initiate the formation of the transmembrane β-barrel domain (Abrami *et al.* 1998). The PFT proteins typically aggregate and/or are activated (Walker *et al.* 1992; Song *et al.* 1996; Sellman *et al.* 1997; Heuck *et al.* 2003) at the membrane surface, a feature that promotes membrane selectivity. Up to 100 β-hairpins can be inserted on activation, to produce the transmembrane β-sheet pore (Hotze *et al.* 2001). β-barrels that span the membrane (Valeva *et al.* 1997; Heuck *et al.* 2001) typically comprise 8–22 β-strands (each 10 13 residues long) and have an average diameter of 1·5–3·5 nm (Heuck *et al.* 2001, 2003). In the α-hemolysin barrel, each monomer contributes an amphipathic hairpin to a 14-stranded heptameric β-barrel (Menestrina *et al.* 2001). PFTs form pores ranging in diameter from 1 to 2 nm for staphylococcal α-hemolysin (Fussle *et al.* 1981) and *Vibrio cholerae* cytolysin (Zitzer *et al.* 1995), and from 15 to 45 nm for streptolysin O (Sekiya *et al.* 1993) and perfringolysin O (Olofsson *et al.* 1993). For some PFTs, some degree of heterogeneity (pore size and oligomeric state) has been reported (Sharpe & London, 1999; Tadjibaeva *et al.* 2000). PFT membrane binding and oligomerization are dependent on the membrane composition and microenvironment and, in some cases, are mediated by interaction with receptor molecules. Cholesterol, for example, can play a critical role in PFT insertion (Cabiaux *et al.* 1997), and may also play a role in amyloid pore toxicity (Arispe & Doh, 2002; Curtain *et al.* 2003; Eckert *et al.* 2003).

5. Mechanisms of protofibril induced toxicity in protein aggregation diseases

5.1 The amyloid pore can explain the age-association and cell-type selectivity of the neurodegenerative diseases

Most cases of AD, PD and ALS are sporadic, that is, they do not involve mutant protein, and are strongly age-associated. Any model for pathogenesis must explain these cases. It is important to emphasize that the wild-type proteins will also form amyloid pores *in vitro*, albeit more reluctantly than the disease-associated mutants (Hafner *et al.* 2001; Ding *et al.* 2002; Lashuel *et al.* 2002a, b, 2003; Shtilerman *et al.* 2002; Chromy *et al.* 2003; Chung, 2003; Klug *et al.* 2003; Kayed & Glabe, 2004). Factors other than mutations could trigger pore formation pathogenesis in sporadic disease (Fig. 4). These factors could include (but are not limited to): (1) increased expression of the amyloid-forming protein (Singleton *et al.* 2004; Uryu *et al.* 2003); (2) impaired degradation of the monomeric protein by the proteasome (Giasson & Lee, 2003); (3) impaired degradation of the protofibrils by a lysosome-mediated autophagic process (Lee *et al.* 2004); (4) changes in chaperone activity and/or expression of homologs that act as specific chaperones (Rochet *et al.* 2000; Conway *et al.* 2001; Hashimoto *et al.* 2001; Masliah & Hashimoto, 2002); (5) changes in levels of post-translational modification, both enzymatic (Shimura *et al.* 2001; Fujiwara *et al.* 2002; Chen & Feany, 2005; Smith *et al.* 2005) and non-enzymatic (Conway *et al.* 2001); (6) changes in the pathogenic microenvironment (pH, membrane composition, increased oxidation). Oxidative damage of amyloid-forming proteins increases their propensity to misfold and aggregate *in vitro* (Rakhit *et al.* 2002; Dauer & Przedborski, 2003). Expression of α-synuclein in

cells, under oxidizing conditions, promotes cytoplasmic aggregation and cell death (Kim *et al.* 2003; Smith *et al.* 2005). Many of the factors listed above are ATP-dependent and would therefore be expected to emerge on aging (Heydari *et al.* 1995; Gaczynska *et al.* 2001; Soti & Csermely, 2002; Ferrington *et al.* 2005; Chondrogianni, 2005), when ATP production becomes less efficient. Aging may also affect the susceptibility of membranes to permeabilization; age-dependent changes in the distribution of cholesterol in neuronal membranes can facilitate oligomerization and accumulation of Aβ in plasma membranes (Wood *et al.* 2002) and cholesterol content influences Aβ toxicity in cell culture (Arispe & Doh, 2002). It must be emphasized that of the factors listed above, all except membrane composition can be easily rationalized by other pathogenic mechanisms.

Another striking feature of the neurodegenerative diseases is that they are selective for certain neuronal populations (hence the clinical diversity). This feature can be explained by an amyloid pore model. It is likely that structural differences among protofibrils formed by the various amyloid-forming proteins would confer some specificity in the way they interact with cellular membranes. In addition, accessory molecules could play a role in modulating pore formation. Finally, conversion of annular protofibrils into amyloid pores may occur at the membrane surface *in vivo*, triggered by specific environmental factors, changes in fluidity of the membrane or interaction with specific proteins. For example, the selective vulnerability of dopaminergic neurons of the substantia nigra to α-synuclein toxicity in PD may be related to (1) the relatively low expression of β-synuclein (Rockenstein *et al.* 2001), a homolog that prevents α-synuclein protofibril formation *in vitro* (Park & Lansbury, 2003), (2) the high concentration of cytoplasmic dopamine (Lotharius & Brundin, 2002; Xu *et al.* 2002) in these cells (some populations of dopaminergic neurons, that have more efficient machinery for removal of cytoplasmic dopamine, are resistant to PD), or (3) highly oxidizing conditions (higher levels of free radical scavengers are expressed in PD-resistant populations of dopaminergic neurons) (Hirsch *et al.* 1997), A combination of the latter two factors may generate dopamine ortho-quinone, which, *in vitro*, covalently modifies α-synuclein and stabilizes protofibrils (Conway *et al.* 2001; Li *et al.* 2004). The extreme sensitivity of dopaminergic neurons to proteasomal inhibition (McNaught *et al.* 2002) may be related to the importance of keeping cytoplasmic α-synuclein levels low in these cells. Second, the differences between cell-types would be expected to influence the specific activity of the amyloid pores in a way that depended on the protein constituent. Microinjection of protofibrillar Aβ42 induces death of human neurons, but not neuroblastoma cells, astrocytes or other non-neuronal cell lines (Zhang *et al.* 2002). Decreased susceptibility to intracellular toxicity could arise from the absence of 'receptor' molecules that mediate Aβ toxicity, the presence of neuroprotective factors, or just the changed composition of target membranes.

5.2 Protofibrils may promote their own accumulation by inhibiting the proteasome

Neurons have developed housekeeping systems to avoid misfolded proteins accumulating in the cytoplasm. These include the chaperone system [some of which is ATP-dependent and would be compromised on aging (Heydari *et al.* 1995; Soti & Csermely, 2002)], which prevents aggregation (Muchowski, 2002; McClellan *et al.* 2005) and the proteasomal system, which degrades misfolded and damaged proteins (Berke & Paulson, 2003; Ross & Pickart, 2004). The importance of ubiquitin-dependent proteasomal degradation in PD is illustrated by the fact that parkin and *UCH-L1*, two PD-linked gene products, are involved in this process (Lansbury & Brice, 2002) (Fig. 4). Once protein aggregation has occurred, several backup systems may take over, including

the HSP90 heat-shock protein, that is capable of disaggregating these species (Ben-Zvi & Goloubinoff, 2001; Ben-Zvi *et al.* 2004), the autophagy system, which engulfs and degrades protein aggregates [including protofibrils (Lee *et al.* 2004)] *via* fusion with the lysosome, and the 'aggresome' system (Kopito, 2000). Complete or partial failure of any of these systems could have detrimental consequences for the cell. Convergent evidence indicates that the protofibrils themselves could compromise proteasomal degradation. First, overexpression of disease-associated proteins (SOD1 or α-synuclein) in non-neuronal cells resulted in inhibition of proteasome activity (Tanaka *et al.* 2001; Urushitani *et al.* 2002). Second, aggregated (protofibrillar and fibrillar) α-synuclein binds directly to the proteasome and inhibits proteasomal activity (Snyder *et al.* 2003; Lindersson *et al.* 2004). Third, inhibition of the neuronal proteasome causes cytosolic inclusion formation (Ma & Lindquist, 2002) and neuronal death (Ma *et al.* 2002). It is not known whether neuronal death is a result of an increase in protein aggregation due to decreased degradation or of another proteasome-mediated event (Bennett *et al.* 2005). The former scenario offers a mechanism whereby toxicity can amplify itself.

6. Testing the amyloid pore hypothesis by attempting to disprove it

In the past 4 years, 18 different amyloid-forming proteins have been reported to form prefibrillar intermediates with pore-like morphologies (Table 1), supporting the notion that formation of the pore and formation of the fibril are tightly linked. However, whether the pore has any relevance to disease, as proposed here, is not at all clear. It must be emphasized that there is a much work to be done to strengthen the case, which must, by definition, be based on circumstantial evidence. A goal of this review is to encourage experiments designed to disprove the hypothesis. In closing, we make three points, each based on different experimental approaches:

(1) *Detection of amyloid pores in tissue from patients or animal models does not support the hypothesis nor does the failure to detect these structures disprove it.* This point is illustrated by the flawed logic that led to the toxic fibril hypothesis. However, one could argue that, in the absence of evidence for the existence of pores in tissue, there is no proof that they are able to form under physiological conditions. Several studies of α-synuclein aggregates extracted from brains affected by rare neurodegenerative diseases related to PD, provide tantalizing evidence that pore-like structures do exist *in vivo*. Several studies of α-synuclein fibrils from diffuse Lewy body disease (DLBD) reveal pore-like structures that co-purify with α-synuclein fibrils (Spillantini *et al.* 1998a; Fujiwara *et al.* 2002). The resemblance of these structures to the amyloid pores produced from α-synuclein *in vitro* (Lashuel *et al.* 2002a) is striking. Direct evidence for the existence of amyloid pore-like structures *in vivo* has been provided by the extraction of annular α-synuclein structures, similar to those seen in *in vitro* preparations, from inclusions from post-mortem brain tissues of a multiple system atrophy (MSA, another α-synuclein aggregation disease) patient (Pountney *et al.* 2004).

(2) *Evidence for membrane abnormalities is consistent with the amyloid pore, but these abnormalities could easily have arisen by another mechanism.* Formation of unregulated pores at the mitochondrial membrane could result in altered Ca^{2+} homeostasis, the release of cytochrome *c* and other proapoptotic molecules, ultimately causing increased oxidative stress and apoptosis. These are features that are clearly seen in the motor neurons of the post-mortem ALS brain, where mitochondrial swelling and cytochrome *c* release are invariant features (Menzies *et al.* 2002). In cellular models of PD, Golgi fragmentation, which is a common feature of human

neurodegenerative diseases, including PD, AD, ALS, CJD, is specifically linked to protofibril formation (Gosavi *et al.* 2002).

(3) *Determination of the toxicity of a mutant protein that efficiently forms very stable pores would be very informative*. According to the amyloid pore hypothesis, mutations in amyloid proteins that stabilized pores to a great extent may have been disfavored by natural selection, since they would have led to juvenile-onset disease. If one could discover a mutation that had this effect *in vitro*, one could then determine its toxicity relative to disease-linked mutants in cellular and/or animal models of disease. If such a mutant lacked *in vivo* toxicity, then the amyloid pore hypothesis would be untenable. If such a mutant was extremely toxic, the circumstantial case would be considerably stronger. We are working hard to find such a mutant. Hopefully, the identification of the pathogenic species will motivate drug discovery efforts aimed at treating the neurodegenerative processes that underlie these devastating diseases.

7. Acknowledgments

We thank members of the Lansbury and Walz laboratories for their contribution to this field and for thoughtful discussions on the subject, Mr Asad Qureshi for reviewing the manuscript and assistance with the references, and Dr Michael Zagorski for providing the protofibril images of ABri.

8. References

Abou-Sleiman, P. M., Muqit, M. M. & Wood, N. W. (2006). Expanding insights of mitochondrial dysfunction in Parkinson's disease. *Nature Reviews Neuroscience* **7**, 207–219.

Abrami, L., Fivaz, M., Decroly, E., Seidah, N. G., Jean, F., Thomas, G., Leppla, S. H., Buckley, J. T. & Van Der Goot, F. G. (1998). The pore-forming toxin proaerolysin is activated by furin. *Journal of Biological Chemistry* **273**, 32656–32661.

Aksenova, M. V., Aksenov, M. Y., Butterfield, D. A. & Carney, J. M. (1996). alpha-1-antichymotrypsin interaction with A beta (1-40) inhibits fibril formation but does not affect the peptide toxicity. *Neuroscience Letters* **211**, 45–48.

Alarcon, J. M., Brito, J. A., Hermosilla, T., Atwater, I., Mears, D. & Rojas, E. (2006). Ion channel formation by Alzheimer's disease amyloid beta-peptide (Abeta40) in unilamellar liposomes is determined by anionic phospholipids. *Peptides* **27**, 95–104.

Anguiano, M., Nowak, R. J. & Lansbury Jr., P. T. (2002). Protofibrillar islet amyloid polypeptide permeabilizes synthetic vesicles by a pore-like mechanism that may be relevant to type II diabetes. *Biochemistry* **41**, 11338–11343.

Arispe, N. & Doh, M. (2002). Plasma membrane cholesterol controls the cytotoxicity of Alzheimer's disease AbetaP (1-40) and (1-42) Peptides. *Faseb Journal* **16**, 1526–1536.

Arispe, N., Pollard, H. B. & Rojas, E. (1993a). Giant multilevel cation channels formed by Alzheimer disease amyloid beta-protein [A beta P-(1-40)] in bilayer membranes. *Proceedings of the National Academy of Sciences USA* **90**, 10573–10577.

Arispe, N., Pollard, H. B. & Rojas, E. (1994). beta-Amyloid Ca $(^{2+})$-channel hypothesis for neuronal death in Alzheimer disease. *Molecular and Cellular Biochemistry* **140**, 119–125.

Arispe, N., Pollard, H. B. & Rojas, E. (1996). Zn^{2+} interaction with Alzheimer amyloid beta protein calcium channels. *Proceedings of the National Academy of Sciences USA* **93**, 1710–1715.

Arispe, N., Rojas, E. & Pollard, H. B. (1993b). Alzheimer disease amyloid beta protein forms calcium channels in bilayer membranes: blockade by tromethamine and aluminum. *Proceedings of the National Academy of Sciences USA* **90**, 567–571.

Azimov, R., Azimova, R., Hirakura, Y. & Kagan, B. L. (2001). Ion channels of different selectivity formed by transthyretin. *Biophysical Journal* **80**, 129a.

Bahadi, R., Farrelly, P. V., Kenna, B. L., Curtain, C. C., Masters, C. L., Cappai, R., Barnham, K. J. & Kourie, J. I. (2003a). Cu^{2+}-induced modification of the kinetics of Aβ[[LB]]1-42[[RB]] channels. *American Journal of Physiology – Cell Physiology* **285**, C873–C880.

Bahadi, R., Farrelly, P. V., Kenna, B. L., Kourie, J. I., Tagliavini, F., Forloni, G. & Salmona, M. (2003b).

Channels formed with a mutant prion protein PrP (82-146) homologous to a 7-kDa fragment in diseased brain of GSS patients. *American Journal of Physiology – Cell Physiology* **285**, C862–872.

Bahadi, R., Farrelly, P. V., Kenna, B. L., Kourie, J. I., Tagliavini, F., Forloni, G. & Salmona, M. (2003c). Ion channels formed with a synthetic mutant prion protein (PrP[[LB]]82-146[[RB]]) homologous to a 7 kDa fragment found in the diseased brain of Gerstmann-Straussler-Scheinker syndrome. *American Journal of Physiology – Cell Physiology* **285**, C862–C872.

Barghorn, S., Nimmrich, V., Striebinger, A., Krantz, C., Keller, P., Janson, B., Bahr, M., Schmidt, M., Bitner, R. S., Harlan, J., Barlow, E., Ebert, U. & Hillen, H. (2005). Globular amyloid beta-peptide oligomer – a homogenous and stable neuropathological protein in Alzheimer's disease. *Journal of Neurochemistry* **95**, 834–847.

Barrachina, M., Castano, E., Dalfo, E., Maes, T., Buesa, C. & Ferrer, I. (2005). Reduced ubiquitin C-terminal hydrolase-1 expression levels in dementia with Lewy bodies. *Neurobiology of Disease Journal* **22**, 265–273.

Baskakov, I. V., Aagaard, C., Mehlhorn, I., Wille, H., Groth, D., Baldwin, M. A., Prusiner, S. B. & Cohen, F. E. (2000). Self-assembly of recombinant prion protein of 106 residues. *Biochemistry* **39**, 2792–2804.

Baskakov, I. V., Legname, G., Baldwin, M. A., Prusiner, S. B. & Cohen, F. E. (2002). Pathway complexity of prion protein assembly into amyloid. *Journal of Biological Chemistry* **277**, 21140–21148.

Behrens, A. & Aguzzi, A. (2002). Small is not beautiful: antagonizing functions for the prion protein PrP(C) and its homologue Dpl. *Trends in Neurosciences* **25**, 150–154.

Ben-Zvi, A., De Los Rios, P., Dietler, G. & Goloubinoff, P. (2004). Active solubilization and refolding of stable protein aggregates by cooperative unfolding action of individual hsp70 chaperones. *Journal of Biological Chemistry* **279**, 37298–37303.

Ben-Zvi, A. P. & Goloubinoff, P. (2001). Review: mechanisms of disaggregation and refolding of stable protein aggregates by molecular chaperones. *Journal of Structural Biology* **135**, 84–93.

Bennett, E. J., Bence, N. F., Jayakumar, R. & Kopito, R. R. (2005). Global impairment of the ubiquitin-proteasome system by nuclear or cytoplasmic protein aggregates precedes inclusion body formation. *Molecular Cell* **17**, 351–365.

Berke, S. J. & Paulson, H. L. (2003). Protein aggregation and the ubiquitin proteasome pathway: gaining the upper hand on neurodegeneration. *Current Opinion in Genetics & Development* **13**, 253–261.

Bhatia, R., Lin, H. & Lal, R. (2000). Fresh and globular amyloid beta protein (1-42) induces rapid cellular degeneration: evidence for AbetaP channel-mediated cellular toxicity. *FASEB Journal* **14**, 1233–1243.

Brown Jr., R. H. (1997). Amyotrophic lateral sclerosis. Insights from genetics. *Archives of Neurology* **54**, 1246–1250.

Bruijn, L. I., Houseweart, M. K., Kato, S., Anderson, K. L., Anderson, S. D., Ohama, E., Reaume, A. G., Scott, R. W. & Cleveland, D. W. (1998). Aggregation and motor neuron toxicity of an ALS-linked SOD1 mutant independent from wild-type SOD1. *Science* **281**, 1851–1854.

Bucciantini, M., Giannoni, E., Chiti, F., Baroni, F., Formigli, L., Zurdo, J., Taddei, N., Ramponi, G., Dobson, C. M. & Stefani, M. (2002). Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. *Nature* **416**, 507–511.

Butler, A. E., Janson, J., Soeller, W. C. & Butler, P. C. (2003). Increased beta-cell apoptosis prevents adaptive increase in beta-cell mass in mouse model of type 2 diabetes: evidence for role of islet amyloid formation rather than direct action of amyloid. *Diabetes* **52**, 2304–2314.

Cabiaux, V., Wolff, C. & Ruysschaert, J. M. (1997). Interaction with a lipid membrane: a key step in bacterial toxins virulence. *International Journal of Biological Macromolecules* **21**, 285–298.

Cappai, R., Leck, S. L., Tew, D. J., Williamson, N. A., Smith, D. P., Galatis, D., Sharples, R. A., Curtain, C. C., Ali, F. E., Cherny, R. A., Culvenor, J. G., Bottomley, S. P., Masters, C. L., Barnham, K. J. & Hill, A. F. (2005). Dopamine promotes alpha-synuclein aggregation into SDS-resistant soluble oligomers via a distinct folding pathway. *FASEB Journal* **19**, 1377–1379.

Cardoso, I., Goldsbury, C. S., Muller, S. A., Olivieri, V., Wirtz, S., Damas, A. M., Aebi, U. & Saraiva, M. J. (2002a). Transthyretin fibrillogenesis entails the assembly of monomers: a molecular model for in vitro assembled transthyretin amyloid-like fibrils. *Journal of Molecular Biology* **317**, 683–695.

Cardoso, R. M., Thayer, M. M., Didonato, M., Lo, T. P., Bruns, C. K., Getzoff, E. D. & Tainer, J. A. (2002b). Insights into Lou Gehrig's disease from the structure and instability of the A4V mutant of human Cu,Zn superoxide dismutase. *Journal of Molecular Biology* **324**, 247–256.

Caughey, B. & Lansbury, P. T. (2003). Protofibrils, pores, fibrils, and neurodegeneration: separating the responsible protein aggregates from the innocent bystanders. *Annual Review of Neuroscience* **26**, 267–298.

Chen, L. & Feany, M. B. (2005). Alpha-synuclein phosphorylation controls neurotoxicity and inclusion formation in a *Drosophila* model of Parkinson disease. *Nature Neuroscience* **8**, 657–663.

Chiesa, R. & Harris, D. A. (2001). Prion diseases: what is the neurotoxic molecule? *Neurobiology of Disease Journal* **8**, 743–763.

Chiesa, R., Piccardo, P., Quaglio, E., Drisaldi, B., Si-Hoe, S. L., Takao, M., Ghetti, B. & Harris, D. A. (2003). Molecular distinction between pathogenic and infectious properties of the prion protein. *Journal of Virology* **77**, 7611–7622.

Chromy, B. A., Nowak, R. J., Lambert, M. P., Viola, K. L., Chang, L., Velasco, P. T., Jones, B. W., Fernandez, S. J., Lacor, P. N., Horowitz, P., Finch, C. E., Krafft, G. A. & Klein, W. L. (2003). Self-assembly of Abeta (1-42) into globular neurotoxins. *Biochemistry* **42**, 12749–12760.

Chui, D. H., Tanahashi, H., Ozawa, K., Ikeda, S., Checler, F., Ueda, O., Suzuki, H., Araki, W., Inoue, H., Shirotani, K., Takahashi, K., Gallyas, F. & Tabira, T. (1999). Transgenic mice with Alzheimer presenilin 1 mutations show accelerated neurodegeneration without amyloid plaque formation. *Nature Medicine* **5**, 560–564.

Chung, J., Yang, H., De Beus, M. D., Ryu, C. Y., Cho, K, Colon, W. (2003). Cu/Zn superoxide dismutase can form pore-like structures. *Biochemical and Biophysical Research Communications* **312**, 873–876.

Cleary, J. P., Walsh, D. M., Hofmeister, J. J., Shankar, G. M., Kuskowski, M. A., Selkoe, D. J. & Ashe, K. H. (2005). Natural oligomers of the amyloid-beta protein specifically disrupt cognitive function. *Nature Neuroscience* **8**, 79–84.

Cohen, F. E. & Prusiner, S. B. (1998). Pathologic conformations of prion proteins. *Annual Review of Biochemistry* **67**, 793–819.

Conway, K. A., Harper, J. D. & Lansbury, P. T. (1998). Accelerated in vitro fibril formation by a mutant alpha-synuclein linked to early-onset Parkinson disease. *Nature Medicine* **4**, 1318–1320.

Conway, K. A., Lee, S. J., Rochet, J. C., Ding, T. T., Williamson, R. E. & Lansbury Jr., P. T. (2000). Acceleration of oligomerization, not fibrillization, is a shared property of both alpha-synuclein mutations linked to early-onset Parkinson's disease: implications for pathogenesis and therapy. *Proceedings of the National Academy of Sciences USA* **97**, 571–576.

Conway, K. A., Rochet, J. C., Bieganski, R. M. & Lansbury Jr., P. T. (2001). Kinetic stabilization of the alpha-synuclein protofibril by a dopamine-alpha-synuclein adduct. *Science* **294**, 1346–1349.

Cookson, M. R. (2005). The biochemistry of Parkinson's disease. *Annual Review of Biochemistry* **74**, 29–52.

Crystal, A. S., Giasson, B. I., Crowe, A., Kung, M. P., Zhuang, Z. P., Trojanowski, J. Q. & Lee, V. M. (2003). A comparison of amyloid fibrillogenesis using the novel fluorescent compound K114. *Journal of Neurochemistry* **86**, 1359–1368.

Curtain, C. C., Ali, F. E., Smith, D. G., Bush, A. I., Masters, C. L. & Barnham, K. J. (2003). Metal ions, pH, and cholesterol regulate the interactions of Alzheimer's disease amyloid-beta peptide with membrane lipid. *Journal of Biological Chemistry* **278**, 2977–2982.

Dahlgren, K. N., Manelli, A. M., Stine Jr., W. B., Baker, L. K., Krafft, G. A. & Ladu, M. J. (2002). Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability. *Journal of Biological Chemistry* **277**, 32046–32053.

Dauer, W. & Przedborski, S. (2003). Parkinson's disease: mechanisms and models. *Neuron* **39**, 889–909.

De Koning, E. J., Hoppener, J. W., Verbeek, J. S., Oosterwijk, C., Van Hulst, K. L., Baker, C. A., Lips, C. J., Morris, J. F. & Clark, A. (1994). Human islet amyloid polypeptide accumulates at similar sites in islets of transgenic mice and humans. *Diabetes* **43**, 640–644.

Demuro, A., Mina, E., Kayed, R., Milton, S. C., Parker, I. & Glabe, C. G. (2005). Calcium dysregulation and membrane disruption as a ubiquitous neurotoxic mechanism of soluble amyloid oligomers. *Journal of Biological Chemistry* **280**, 17294–17300.

Ding, T. T., Lee, S. J., Rochet, J. C. & Lansbury Jr., P. T. (2002). Annular alpha-synuclein protofibrils are produced when spherical protofibrils are incubated in solution or bound to brain-derived membranes. *Biochemistry* **41**, 10209–10217.

Dobson, C. M. (2001). The structural basis of protein folding and its links with human disease. *Philosophical Transactions of the Royal Society B: Biological Sciences* **356**, 133–145.

Dzwolak, W., Grudzielanek, S., Smirnovas, V., Ravindra, R., Nicolini, C., Jansen, R., Loksztejn, A., Porowski, S. & Winter, R. (2005). Ethanol-perturbed amyloidogenic self-assembly of insulin: looking for origins of amyloid strains. *Biochemistry* **44**, 8948–8958.

Eckert, G. P., Kirsch, C., Leutz, S., Wood, W. G. & Muller, W. E. (2003). Cholesterol modulates amyloid beta-peptide's membrane interactions. *Pharmacopsychiatry* **36** (Suppl. 2), S136–S143.

El-Agnaf, O. M., Jakes, R., Curran, M. D. & Wallace, A. (1998). Effects of the mutations Ala30 to Pro and Ala53 to Thr on the physical and morphological properties of alpha-synuclein protein implicated in Parkinson's disease. *FEBS Letters* **440**, 67–70.

El-Agnaf, O. M., Mahil, D. S., Patel, B. P. & Austen, B. M. (2000). Oligomerization and toxicity of beta-amyloid-42 implicated in Alzheimer's disease. *Biochemical and Biophysical Research Communications* **273**, 1003–1007.

El-Agnaf, O. M., Nagala, S., Patel, B. P. & Austen, B. M. (2001a). Non-fibrillar oligomeric species of the amyloid ABri peptide, implicated in familial British dementia, are more potent at inducing apoptotic cell death than protofibrils or mature fibrils. *Journal of Molecular Biology* **310**, 157–168.

El-Agnaf, O. M., Salem, S. A., Paleologou, K. E., Curran, M. D., Gibson, M. J., Court, J. A., Schlossmacher, M. G. & Allsop, D. (2006). Detection

of oligomeric forms of alpha-synuclein protein in human plasma as a potential biomarker for Parkinson's disease. *FASEB Journal* **20**, 419–425.

El-Agnaf, O. M., Sheridan, J. M., Sidera, C., Siligardi, G., Hussain, R., Haris, P. I. & Austen, B. M. (2001b). Effect of the disulfide bridge and the C-terminal extension on the oligomerization of the amyloid peptide ABri implicated in familial British dementia. *Biochemistry* **40**, 3449–3457.

Elam, J. S., Taylor, A. B., Strange, R., Antonyuk, S., Doucette, P. A., Rodriguez, J. A., Hasnain, S. S., Hayward, L. J., Valentine, J. S., Yeates, T. O. & Hart, P. J. (2003). Amyloid-like filaments and water-filled nanotubes formed by SOD1 mutant proteins linked to familial ALS. *Nature Structural Biology* **10**, 461–467.

Falsey, A. R., Walsh, E. E., Francis, C. W., Looney, R. J., Kolassa, J. E., Hall, W. J. & Abraham, G. N. (2001). Response of C-reactive protein and serum amyloid A to influenza A infection in older adults. *Journal of Infectious Diseases* **183**, 995–999.

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

Ferrington, D. A., Husom, A. D. & Thompson, L. V. (2005). Altered proteasome structure, function, and oxidation in aged muscle. *FASEB Journal* **19**, 644–646.

Flynn, B. L. & Theesen, K. A. (1999). Pharmacologic management of Alzheimer disease part III: nonsteroidal antiinflammatory drugs – emerging protective evidence? *Annals of Pharmacotherapy* **33**, 840–849.

Fujiwara, H., Hasegawa, M., Dohmae, N., Kawashima, A., Masliah, E., Goldberg, M. S., Shen, J., Takio, K. & Iwatsubo, T. (2002). alpha-Synuclein is phosphorylated in synucleinopathy lesions. *Nature Cell Biology* **4**, 160–164.

Funato, H., Enya, M., Yoshimura, M., Morishima-Kawashima, M. & Ihara, Y. (1999). Presence of sodium dodecyl sulfate-stable amyloid beta-protein dimers in the hippocampus CA1 not exhibiting neurofibrillary tangle formation. *American Journal of Pathology* **155**, 23–28.

Fussle, R., Bhakdi, S., Sziegoleit, A., Tranum-Jensen, J., Kranz, T. & Wellensiek, H. J. (1981). On the mechanism of membrane damage by Staphylococcus aureus alpha-toxin. *Journal of Cell Biology* **91**, 83–94.

Gaczynska, M., Osmulski, P. A. & Ward, W. F. (2001). Caretaker or undertaker? The role of the proteasome in aging. *Mechanisms of Ageing and Development* **122**, 235–254.

Gambetti, P., Parchi, P., Petersen, R. B., Chen, S. G. & Lugaresi, E. (1995). Fatal familial insomnia and familial Creutzfeldt-Jakob disease: clinical, pathological and molecular features. *Brain Pathology* **5**, 43–51.

Ghiso, J., Vidal, R., Rostagno, A., Miravalle, L., Holton, J. L., Mead, S., Revesz, T., Plant, G. & Frangione, B. (2000). Amyloidogenesis in familial British dementia is associated with a genetic defect on chromosome 13. *Annals of the New York Academy of Sciences* **920**, 84–92.

Giasson, B. I. & Lee, V. M. (2003). Are ubiquitination pathways central to Parkinson's disease? *Cell* **114**, 1–8.

Glenner, G. G. & Wong, C. W. (1984). Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochemical and Biophysical Research Communications* **120**, 885–890.

Goldberg, M. S. & Lansbury Jr., P. T. (2000). Is there a cause-and-effect relationship between alpha-synuclein fibrillization and Parkinson's disease? *Nature Cell Biology* **2**, E115–E119.

Goldsbury, C., Frey, P., Olivieri, V., Aebi, U. & Muller, S. A. (2005). Multiple assembly pathways underlie amyloid-beta fibril polymorphisms. *Journal of Molecular Biology* **352**, 282–298.

Gong, Y., Chang, L., Viola, K. L., Lacor, P. N., Lambert, M. P., Finch, C. E., Krafft, G. A. & Klein, W. L. (2003). Alzheimer's disease-affected brain: presence of oligomeric A{beta} ligands (ADDLs) suggests a molecular basis for reversible memory loss. *Proceedings of the National Academy of Sciences USA* **100**, 10417–10422.

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

Green, J., Goldsbury, C., Mini, T., Sunderji, S., Frey, P., Kistler, J., Cooper, G. & Aebi, U. (2003). Full-length rat amylin forms fibrils following substitution of single residues from human amylin. *Journal of Molecular Biology* **326**, 1147–1156.

Green, J. D., Goldsbury, C., Kistler, J., Cooper, G. S. & Aebi, U. (2004). Human amylin oligomer growth and fibril elongation define two distinct phases in amyloid formation. *Journal of Biological Chemistry* **279**, 12206–12212.

Hafner, J. H., Cheung, C. L., Woolley, A. T. & Lieber, C. M. (2001). Structural and functional imaging with carbon nanotube AFM probes. *Progress in Biophysics & Molecular Biology* **77**, 73–110.

Hardy, J. & Selkoe, D. J. (2002). The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* **297**, 353–356.

Hardy, J. A. & Higgins, G. A. (1992). Alzheimer's disease: the amyloid cascade hypothesis. *Science* **256**, 184–185.

Harper, J. D., Lieber, C. M. & Lansbury Jr., P. T. (1997a). Atomic force microscopic imaging of seeded fibril formation and fibril branching by the Alzheimer's disease amyloid-beta protein. *Chemistry & Biology* **4**, 951–959.

Harper, J. D., Wong, S. S., Lieber, C. M. & Lansbury, P. T. (1997b). Observation of metastable Abeta

amyloid protofibrils by atomic force microscopy. *Chemistry & Biology* **4**, 119–125.

Harper, J. D., Wong, S. S., Lieber, C. M. & Lansbury Jr., P. T. (1999). Assembly of A beta amyloid protofibrils: an in vitro model for a possible early event in Alzheimer's disease. *Biochemistry* **38**, 8972–8980.

Harris, D. A., Chiesa, R., Drisaldi, B., Quaglio, E., Migheli, A., Piccardo, P. & Ghetti, B. (2003). A murine model of a familial prion disease. *Clinical Laboratory Medicine* **23**, 175–186.

Harroun, T. A., Bradshaw, J. P. & Ashley, R. H. (2001). Inhibitors can arrest the membrane activity of human islet amyloid polypeptide independently of amyloid formation. *FEBS Letters* **507**, 200–204.

Hartley, D. M., Walsh, D. M., Ye, C. P., Diehl, T., Vasquez, S., Vassilev, P. M., Teplow, D. B. & Selkoe, D. J. (1999). Protofibrillar intermediates of amyloid beta-protein induce acute electrophysiological changes and progressive neurotoxicity in cortical neurons. *Journal of Neuroscience* **19**, 8876–8884.

Hashimoto, M., Rockenstein, E., Mante, M., Mallory, M. & Masliah, E. (2001). beta-Synuclein inhibits alpha-synuclein aggregation: a possible role as an anti-parkinsonian factor. *Neuron* **32**, 213–223.

Hayward, L. J., Rodriguez, J. A., Kim, J. W., Tiwari, A., Goto, J. J., Cabelli, D. E., Valentine, J. S. & Brown Jr., R. H. (2002). Decreased metallation and activity in subsets of mutant superoxide dismutases associated with familial amyotrophic lateral sclerosis. *Journal of Biological Chemistry* **277**, 15923–15931.

Hegde, R. S., Tremblay, P., Groth, D., Dearmond, S. J., Prusiner, S. B. & Lingappa, V. R. (1999). Transmissible and genetic prion diseases share a common pathway of neurodegeneration. *Nature* **402**, 822–826.

Helms, L. R. & Wetzel, R. (1996). Specificity of abnormal assembly in immunoglobulin light chain deposition disease and amyloidosis. *Journal of Molecular Biology* **257**, 77–86.

Heuck, A. P., Tweten, R. K. & Johnson, A. E. (2001). Beta-barrel pore-forming toxins: intriguing dimorphic proteins. *Biochemistry* **40**, 9065–9073.

Heuck, A. P., Tweten, R. K. & Johnson, A. E. (2003). Assembly and topography of the prepore complex in cholesterol-dependent cytolysins. *Journal of Biological Chemistry* **278**, 31218–31225.

Heydari, A. R., Conrad, C. C. & Richardson, A. (1995). Expression of heat shock genes in hepatocytes is affected by age and food restriction in rats. *Journal of Nutrition* **125**, 410–418.

Hirakura, Y., Azimov, R., Azimova, R. & Kagan, B. L. (2000a). Polyglutamine-induced ion channels: a possible mechanism for the neurotoxicity of Huntington and other CAG repeat diseases. *Journal of Neuroscience Research* **60**, 490–494.

Hirakura, Y., Carreras, I., Sipe, J. D. & Kagan, B. L. (2002). Channel formation by serum amyloid A: a potential mechanism for amyloid pathogenesis and host defense. *Amyloid* **9**, 13–23.

Hirakura, Y. & Kagan, B. L. (2001). Pore formation by beta-2-microglobulin: a mechanism for the pathogenesis of dialysis associated amyloidosis. *Amyloid* **8**, 94–100.

Hirakura, Y., Lin, M. C. & Kagan, B. L. (1999). Alzheimer amyloid abeta 1-42 channels: effects of solvent, pH, and Congo Red. *Journal of Neuroscience Research* **57**, 458–466.

Hirakura, Y., Yiu, W. W., Yamamoto, A. & Kagan, B. L. (2000b). Amyloid peptide channels: blockade by zinc and inhibition by Congo red (amyloid channel block). *Amyloid* **7**, 194–199.

Hirsch, E. C., Faucheux, B., Damier, P., Mouatt-Prigent, A. & Agid, Y. (1997). Neuronal vulnerability in Parkinson's disease. *Journal of Neural Transmission* **50** (Suppl.), 79–88.

Holton, J. L., Ghiso, J., Lashley, T., Rostagno, A., Guerin, C. J., Gibb, G., Houlden, H., Ayling, H., Martinian, L., Anderton, B. H., Wood, N. W., Vidal, R., Plant, G., Frangione, B. & Revesz, T. (2001). Regional distribution of amyloid-Bri deposition and its association with neurofibrillary degeneration in familial British dementia. *American Journal of Pathology* **158**, 515–526.

Hoshi, M., Sato, M., Matsumoto, S., Noguchi, A., Yasutake, K., Yoshida, N. & Sato, K. (2003). Spherical aggregates of beta-amyloid (amylospheroid) show high neurotoxicity and activate tau protein kinase I/glycogen synthase kinase-3beta. *Proceedings of the National Academy of Sciences USA* **100**, 6370–6375.

Hotze, E. M., Wilson-Kubalek, E. M., Rossjohn, J., Parker, M. W., Johnson, A. E. & Tweten, R. K. (2001). Arresting pore formation of a cholesterol-dependent cytolysin by disulfide trapping synchronizes the insertion of the transmembrane beta-sheet from a prepore intermediate. *Journal of Biological Chemistry* **276**, 8261–8268.

Hsia, A. Y., Masliah, E., Mcconlogue, L., Yu, G. Q., Tatsuno, G., Hu, K., Kholodenko, D., Malenka, R. C., Nicoll, R. A. & Mucke, L. (1999). Plaque-independent disruption of neural circuits in Alzheimer's disease mouse models. *Proceedings of the National Academy of Sciences USA* **96**, 3228–3233.

Huang, T. H., Yang, D. S., Plaskos, N. P., Go, S., Yip, C. M., Fraser, P. E. & Chakrabartty, A. (2000). Structural studies of soluble oligomers of the Alzheimer beta-amyloid peptide. *Journal of Molecular Biology* **297**, 73–87.

Ishimaru, D., Andrade, L. R., Teixeira, L. S., Quesado, P. A., Maiolino, L. M., Lopez, P. M., Cordeiro, Y., Costa, L. T., Heckl, W. M., Weissmuller, G., Foguel, D. & Silva, J. L. (2003). Fibrillar aggregates of the

tumor suppressor p53 core domain. *Biochemistry* **42**, 9022–9027.

Janson, J., Ashley, R. H., Harrison, D., Mcintyre, S. & Butler, P. C. (1999). The mechanism of islet amyloid polypeptide toxicity is membrane disruption by intermediate-sized toxic amyloid particles. *Diabetes* **48**, 491–498.

Janson, J., Soeller, W. C., Roche, P. C., Nelson, R. T., Torchia, A. J., Kreutter, D. K. & Butler, P. C. (1996). Spontaneous diabetes mellitus in transgenic mice expressing human islet amyloid polypeptide. *Proceedings of the National Academy of Sciences USA* **93**, 7283–7288.

Janus, C., Pearson, J., Mclaurin, J., Mathews, P. M., Jiang, Y., Schmidt, S. D., Chishti, M. A., Horne, P., Heslin, D., French, J., Mount, H. T., Nixon, R. A., Mercken, M., Bergeron, C., Fraser, P. E., St George-Hyslop, P. & Westaway, D. (2000). A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. *Nature* **408**, 979–982.

Jarrett, J. T., Berger, E. P. & Lansbury Jr., P. T. (1993). The carboxy terminus of the beta amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease. *Biochemistry* **32**, 4693–4697.

Jarrett, J. T. & Lansbury Jr., P. T. (1993). Seeding 'one-dimensional crystallization' of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie? *Cell* **73**, 1055–1058.

Johnston, J. A., Dalton, M. J., Gurney, M. E. & Kopito, R. R. (2000). Formation of high molecular weight complexes of mutant Cu, Zn-superoxide dismutase in a mouse model for familial amyotrophic lateral sclerosis. *Proceedings of the National Academy of Sciences USA* **97**, 12571–12576.

Johnston, J. A., Ward, C. L. & Kopito, R. R. (1998). Aggresomes: a cellular response to misfolded proteins. *Journal of Cell Biology* **143**, 1883–1898.

Julien, J. P. (2001). Amyotrophic lateral sclerosis. unfolding the toxicity of the misfolded. *Cell* **104**, 581–591.

Junn, E., Lee, S. S., Suhr, U. T. & Mouradian, M. M. (2002). Parkin accumulation in aggresomes due to proteasome impairment. *Journal of Biological Chemistry* **277**, 47870–47877.

Kad, N. M., Myers, S. L., Smith, D. P., Smith, D. A., Radford, S. E. & Thomson, N. H. (2003). Hierarchical assembly of beta2-microglobulin amyloid in vitro revealed by atomic force microscopy. *Journal of Molecular Biology* **330**, 785–797.

Kagan, B. L., Hirakura, Y., Azimov, R. & Azimova, R. (2001). The channel hypothesis of Huntington's disease. *Brain Research Bulletin* **56**, 281–284.

Kagan, B. L., Hirakura, Y., Azimov, R., Azimova, R. & Lin, M. C. (2002). The channel hypothesis of Alzheimer's disease: current status. *Peptides* **23**, 1311–1315.

Kawahara, M., Arispe, N., Kuroda, Y. & Rojas, E. (1997). Alzheimer's disease amyloid beta-protein forms Zn (2+)-sensitive, cation-selective channels across excised membrane patches from hypothalamic neurons. *Biophysical Journal* **73**, 67–75.

Kawahara, M. & Kuroda, Y. (2000). Molecular mechanism of neurodegeneration induced by Alzheimer's beta-amyloid protein: channel formation and disruption of calcium homeostasis. *Brain Research Bulletin* **53**, 389–397.

Kawahara, M. & Kuroda, Y. (2001). Intracellular calcium changes in neuronal cells induced by Alzheimer's beta-amyloid protein are blocked by estradiol and cholesterol. *Cellular and Molecular Neurobiology* **21**, 1–13.

Kawahara, M., Kuroda, Y., Arispe, N. & Rojas, E. (2000). Alzheimer's beta-amyloid, human islet amylin, and prion protein fragment evoke intracellular free calcium elevations by a common mechanism in a hypothalamic GnRH neuronal cell line. *Journal of Biological Chemistry* **275**, 14077–14083.

Kayed, R. & Glabe, C. G. (2004). Formation, stability and toxicity of annular protofibrils from different amyloid forming proteins. *Neurobiology of Aging* **25** (Suppl. 2), S144.

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

Kayed, R., Sokolov, Y., Edmonds, B., Mcintire, T. M., Milton, S. C., Hall, J. E. & Glabe, C. G. (2004). Permeabilization of lipid bilayers is a common conformation-dependent activity of soluble amyloid oligomers in protein misfolding diseases. *Journal of Biological Chemistry* **279**, 46363–46366.

Kaylor, J., Bodner, N., Edridge, S., Yamin, G., Hong, D. P. & Fink, A. L. (2005). Characterization of oligomeric intermediates in alpha-synuclein fibrillation: FRET studies of Y125W/Y133F/Y136F alpha-synuclein. *Journal of Molecular Biology* **353**, 357–372.

Kelly, J. W. (1998). The alternative conformations of amyloidogenic proteins and their multi-step assembly pathways. *Current Opinion in Structural Biology* **8**, 101–106.

Kheterpal, I., Lashuel, H., Hartley, D., Walz, T., Lansbury Jr., P. & Wetzel, R. (2003). Aß protofibrils possess a stable core structure resistant to hydrogen exchange. *Biochemistry* **42**, 14092–14098.

Kim, H. J., Chae, S. C., Lee, D. K., Chromy, B., Lee, S. C., Park, Y. C., Klein, W. L., Krafft, G. A. & Hong, S. T. (2003). Selective neuronal degeneration induced by soluble oligomeric amyloid beta protein. *FASEB Journal* **17**, 118–120.

Kirik, D., Annett, L. E., Burger, C., Muzyczka, N., Mandel, R. J. & Bjorklund, A. (2003). Nigrostriatal alpha-synucleinopathy induced by viral vector-mediated overexpression of human alpha-synuclein: a new

primate model of Parkinson's disease. *Proceedings of the National Academy of Sciences USA* **100**, 2884–2889.

Kirik, D., Rosenblad, C., Burger, C., Lundberg, C., Johansen, T. E., Muzyczka, N., Mandel, R. J. & Bjorklund, A. (2002). Parkinson-like neurodegeneration induced by targeted overexpression of alpha-synuclein in the nigrostriatal system. *Journal of Neuroscience* **22**, 2780–2791.

Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., Yokochi, M., Mizuno, Y. & Shimizu, N. (1998). Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* **392**, 605–608.

Klug, G. M., Losic, D., Subasinghe, S. S., Aguilar, M. I., Martin, L. L. & Small, D. H. (2003). Beta-amyloid protein oligomers induced by metal ions and acid pH are distinct from those generated by slow spontaneous ageing at neutral pH. *European Journal of Biochemistry* **270**, 4282–4293.

Kopito, R. R. (2000). Aggresomes, inclusion bodies and protein aggregation. *Trends in Cell Biology* **10**, 524–530.

Kourie, J. I. (2002). Prion channel proteins and their role in vacuolation and neurodegenerative diseases. *European Biophysics Journal* **31**, 409–416.

Kourie, J. I. & Culverson, A. (2000). Prion peptide fragment PrP[106-126] forms distinct cation channel types. *Journal of Neuroscience Research* **62**, 120–133.

Kourie, J. I., Farrelly, P. V. & Henry, C. L. (2001a). Channel activity of deamidated isoforms of prion protein fragment 106-126 in planar lipid bilayers. *Journal of Neuroscience Research* **66**, 214–220.

Kourie, J. I., Henry, C. L. & Farrelly, P. (2001b). Diversity of amyloid beta protein fragment [1-40]-formed channels. *Cellular and Molecular Neurobiology* **21**, 255–284.

Kourie, J. I., Kenna, B. L., Tew, D., Jobling, M. F., Curtain, C. C., Masters, C. L., Barnham, K. J. & Cappai, R. (2003). Copper modulation of ion channels of PrP[106-126] mutant prion peptide fragments. *Journal of Membrane Biology* **193**, 35–45.

Kruger, R., Kuhn, W., Muller, T., Woitalla, D., Graeber, M., Kosel, S., Przuntek, H., Epplen, J. T., Schols, L. & Riess, O. (1998). Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. *Nature Genetics* **18**, 106–108.

Kuemmerle, S., Gutekunst, C. A., Klein, A. M., Li, X. J., Li, S. H., Beal, M. F., Hersch, S. M. & Ferrante, R. J. (1999). Huntington aggregates may not predict neuronal death in Huntington's disease. *Annals of Neurology* **46**, 842–849.

Kuo, Y. M., Emmerling, M. R., Vigo-Pelfrey, C., Kasunic, T. C., Kirkpatrick, J. B., Murdoch, G. H., Ball, M. J. & Roher, A. E. (1996). Water-soluble Abeta (N-40, N-42) oligomers in normal and Alzheimer disease brains. *Journal of Biological Chemistry* **271**, 4077–4081.

Lambert, M. P., Barlow, A. K., Chromy, B. A., Edwards, C., Freed, R., Liosatos, M., Morgan, T. E., Rozovsky, I., Trommer, B., Viola, K. L., Wals, P., Zhang, C., Finch, C. E., Krafft, G. A. & Klein, W. L. (1998). Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins. *Proceedings of the National Academy of Sciences USA* **95**, 6448–6453.

Lambert, M. P., Viola, K. L., Chromy, B. A., Chang, L., Morgan, T. E., Yu, J., Venton, D. L., Krafft, G. A., Finch, C. E. & Klein, W. L. (2001). Vaccination with soluble Abeta oligomers generates toxicity-neutralizing antibodies. *Journal of Neurochemistry* **79**, 595–605.

Lansbury Jr., P. T. & Brice, A. (2002). Genetics of Parkinson's disease and biochemical studies of implicated gene products. *Current Opinion in Genetics & Development* **12**, 299–306.

Lashuel, H., Hartley, D., Petre, B., Wall, J., Simon, M., Walz, T. & Lansbury, P. (2003). Mixtures of wild-type and 'Arctic' Abeta40 in vitro accumulate protofibrils, including amyloid pores. *Journal of Molecular Biology* **332**, 795–808.

Lashuel, H., Petre, B., Wall, J., Simon, M., Nowak, R., Walz, T. & Lansbury, P. (2002a). alpha-Synuclein, Especially the Parkinson's Disease-associated Mutants, Forms Pore-like Annular and Tubular Protofibrils. *Journal of Molecular Biology* **322**, 1089.

Lashuel, H. A., Hartley, D., Petre, B. M., Walz, T. & Lansbury Jr., P. T. (2002b). Neurodegenerative disease: amyloid pores from pathogenic mutations. *Nature* **418**, 291.

Lashuel, H. A., Lai, Z. & Kelly, J. W. (1998). Characterization of the transthyretin acid denaturation pathways by analytical ultracentrifugation: implications for wild-type, V30M, and L55P amyloid fibril formation. *Biochemistry* **37**, 17851–17864.

Lashuel, H. A., Wurth, C., Woo, L. & Kelly, J. W. (1999). The most pathogenic transthyretin variant, L55P, forms amyloid fibrils under acidic conditions and protofilaments under physiological conditions. *Biochemistry* **38**, 13560–13573.

Lee, H. J., Khoshaghideh, F., Patel, S. & Lee, S. J. (2004). Clearance of alpha-synuclein oligomeric intermediates via the lysosomal degradation pathway. *Journal of Neuroscience* **24**, 1888–1896.

Lee, H. J. & Lee, S. J. (2002). Characterization of cytoplasmic alpha-synuclein aggregates: Fibril formation is tightly linked to the inclusion forming process in cells. *Journal of Biological Chemistry* **277**, 48976–48983.

Lee, H. J., Shin, S. Y., Choi, C., Lee, Y. H. & Lee, S. J. (2002). Formation and removal of alpha-synuclein aggregates in cells exposed to mitochondrial inhibitors. *Journal of Biological Chemistry* **277**, 5411–5417.

Lee, S. & Eisenberg, D. (2003). Seeded conversion of recombinant prion protein to a disulfide-bonded

oligomer by a reduction-oxidation process. *Nature Structural Biology* **10**, 725–730.

Lehmann, S. & Harris, D. A. (1996). Mutant and infectious prion proteins display common biochemical properties in cultured cells. *Journal of Biological Chemistry* **271**, 1633–1637.

Lemere, C. A., Blusztajn, J. K., Yamaguchi, H., Wisniewski, T., Saido, T. C. & Selkoe, D. J. (1996). Sequence of deposition of heterogeneous amyloid beta-peptides and APO E in Down syndrome: implications for initial events in amyloid plaque formation. *Neurobiology of Disease Journal* **3**, 16–32.

Leroy, E., Boyer, R., Auburger, G., Leube, B., Ulm, G., Mezey, E., Harta, G., Brownstein, M. J., Jonnalagada, S., Chernova, T., Dehejia, A., Lavedan, C., Gasser, T., Steinbach, P. J., Wilkinson, K. D. & Polymeropoulos, M. H. (1998). The ubiquitin pathway in Parkinson's disease. *Nature* **395**, 451–452.

Li, J., Uversky, V. N. & Fink, A. L. (2001). Effect of familial Parkinson's disease point mutations A30P and A53T on the structural properties, aggregation, and fibrillation of human alpha-synuclein. *Biochemistry* **40**, 11604–11613.

Li, J., Zhu, M., Manning-Bog, A. B., Di Monte, D. A. & Fink, A. L. (2004). Dopamine and L-dopa disaggregate amyloid fibrils: implications for Parkinson's and Alzheimer's disease. *FASEB Journal* **18**, 962–964.

Lin, H., Bhatia, R. & Lal, R. (2001). Amyloid beta protein forms ion channels: implications for Alzheimer's disease pathophysiology. *FASEB Journal* **15**, 2433–2444.

Lin, H., Zhu, Y. J. & Lal, R. (1999). Amyloid beta protein (1-40) forms calcium-permeable, Zn^{2+}-sensitive channel in reconstituted lipid vesicles. *Biochemistry* **38**, 11189–11196.

Lin, M. C. & Kagan, B. L. (2002). Electrophysiologic properties of channels induced by Abeta25-35 in planar lipid bilayers. *Peptides* **23**, 1215–1228.

Lin, M. C., Mirzabekov, T. & Kagan, B. L. (1997). Channel formation by a neurotoxic prion protein fragment. *Journal of Biological Chemistry* **272**, 44–47.

Lindberg, M. J., Tibell, L. & Oliveberg, M. (2002). Common denominator of Cu/Zn superoxide dismutase mutants associated with amyotrophic lateral sclerosis: decreased stability of the apo state. *Proceedings of the National Academy of Sciences USA* **99**, 16607–16612.

Lindersson, E., Beedholm, R., Hojrup, P., Moos, T., Gai, W., Hendil, K. B. & Jensen, P. H. (2004). Proteasomal inhibition by α-synuclein filaments and oligomers. *Journal of Biological Chemistry* **279**, 12924–12934.

Lo Bianco, C., Ridet, J. L., Schneider, B. L., Deglon, N. & Aebischer, P. (2002). alpha-Synucleinopathy and selective dopaminergic neuron loss in a rat lentiviral-based model of Parkinson's disease. *Proceedings of the National Academy of Sciences USA* **99**, 10813–10818.

Lotharius, J. & Brundin, P. (2002). Pathogenesis of Parkinson's disease: dopamine, vesicles and alpha-synuclein. *Nature Reviews Neuroscience* **3**, 932–942.

Lowe, R., Pountney, D. L., Jensen, P. H., Gai, W. P. & Voelcker, N. H. (2004). Calcium(II) selectively induces alpha-synuclein annular oligomers via interaction with the C-terminal domain. *Protein Science* **13**, 3245–3252.

Lu, B. Y. & Chang, J. Y. (2002). Isolation and characterization of a polymerized prion protein. *Biochemical Journal* **364**, 81–87.

Lue, L. F., Kuo, Y. M., Roher, A. E., Brachova, L., Shen, Y., Sue, L., Beach, T., Kurth, J. H., Rydel, R. E. & Rogers, J. (1999). Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. *American Journal of Pathology* **155**, 853–862.

Ma, J. & Lindquist, S. (2002). Conversion of PrP to a self-perpetuating PrPSc-like conformation in the cytosol. *Science* **298**, 1785–1788.

Ma, J., Wollmann, R. & Lindquist, S. (2002). Neurotoxicity and neurodegeneration when PrP accumulates in the cytosol. *Science* **298**, 1781–1785.

Maat-Schieman, M. L., Van Duinen, S. G., Haan, J. & Roos, R. A. (1992). Morphology of cerebral plaque-like lesions in hereditary cerebral hemorrhage with amyloidosis [in Dutch]. *Acta Neuropathologica* (*Berlin*) **84**, 674–679.

Malisauskas, M., Zamotin, V., Jass, J., Noppe, W., Dobson, C. M. & Morozova-Roche, L. A. (2003). Amyloid protofilaments from the calcium-binding protein equine lysozyme: formation of ring and linear structures depends on pH and metal ion concentration. *Journal of Molecular Biology* **330**, 879–890.

Masliah, E. & Hashimoto, M. (2002). Development of new treatments for Parkinson's disease in transgenic animal models: a role for beta-synuclein. *Neurotoxicology* **23**, 461–468.

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

Mcclellan, A. J., Tam, S., Kaganovich, D. & Frydman, J. (2005). Protein quality control: chaperones culling corrupt conformations. *Nature Cell Biology* **7**, 736–741.

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

Mcnaught, K. S., Mytilineou, C., Jnobaptiste, R., Yabut, J., Shashidharan, P., Jennert, P. & Olanow, C. W. (2002). Impairment of the ubiquitin-proteasome system causes dopaminergic cell death and inclusion body formation in ventral mesencephalic cultures. *Journal of Neurochemistry* **81**, 301–306.

Menestrina, G., Serra, M. D. & Prevost, G. (2001). Mode of action of beta-barrel pore-forming toxins of the staphylococcal alpha-hemolysin family. *Toxicon* **39**, 1661–1672.

Menzies, F. M., Ince, P. G. & Shaw, P. J. (2002). Mitochondrial involvement in amyotrophic lateral sclerosis. *Neurochemistry International* **40**, 543–551.

Mirzabekov, T. A., Lin, M. C. & Kagan, B. L. (1996). Pore formation by the cytotoxic islet amyloid peptide amylin. *Journal of Biological Chemistry* **271**, 1988–1992.

Moechars, D., Dewachter, I., Lorent, K., Reverse, D., Baekelandt, V., Naidu, A., Tesseur, I., Spittaels, K., Haute, C. V., Checler, F., Godaux, E., Cordell, B. & Van Leuven, F. (1999). Early phenotypic changes in transgenic mice that overexpress different mutants of amyloid precursor protein in brain. *Journal of Biological Chemistry* **274**, 6483–6492.

Monoi, H. (1995). New tubular single-stranded helix of poly-L-amino acids suggested by molecular mechanics calculations: I. Homopolypeptides in isolated environments. *Biophysical Journal* **69**, 1130–1141.

Monoi, H., Futaki, S., Kugimiya, S., Minakata, H. & Yoshihara, K. (2000). Poly-L-glutamine forms cation channels: relevance to the pathogenesis of the polyglutamine diseases. *Biophysical Journal* **78**, 2892–2899.

Moore, R. C., Lee, I. Y., Silverman, G. L., Harrison, P. M., Strome, R., Heinrich, C., Karunaratne, A., Pasternak, S. H., Chishti, M. A., Liang, Y., Mastrangelo, P., Wang, K., Smit, A. F., Katamine, S., Carlson, G. A., Cohen, F. E., Prusiner, S. B., Melton, D. W., Tremblay, P., Hood, L. E. & Westaway, D. (1999). Ataxia in prion protein (PrP)-deficient mice is associated with upregulation of the novel PrP-like protein doppel. *Journal of Molecular Biology* **292**, 797–817.

Morgan, D., Diamond, D. M., Gottschall, P. E., Ugen, K. E., Dickey, C., Hardy, J., Duff, K., Jantzen, P., Dicarlo, G., Wilcock, D., Connor, K., Hatcher, J., Hope, C., Gordon, M. & Arendash, G. W. (2000). A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature* **408**, 982–985.

Morishima-Kawashima, M. & Ihara, Y. (1998). The presence of amyloid beta-protein in the detergent-insoluble membrane compartment of human neuroblastoma cells. *Biochemistry* **37**, 15247–15253.

Muchowski, P. J. (2002). Protein misfolding, amyloid formation, and neurodegeneration: a critical role for molecular chaperones? *Neuron* **35**, 9–12.

Mucke, L., Masliah, E., Yu, G. Q., Mallory, M., Rockenstein, E. M., Tatsuno, G., Hu, K., Kholodenko, D., Johnson-Wood, K. & McConlogue, L. (2000). High-level neuronal expression of abeta 1-42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation. *Journal of Neuroscience* **20**, 4050–4058.

Nakano, R., Inuzuka, T., Kikugawa, K., Takahashi, H., Sakimura, K., Fujii, J., Taniguchi, N. & Tsuji, S. (1996). Instability of mutant Cu/Zn superoxide dismutase (Ala4Thr) associated with familial amyotrophic lateral sclerosis. *Neuroscience Letters* **211**, 129–131.

Naslund, J., Haroutunian, V., Mohs, R., Davis, K. L., Davies, P., Greengard, P. & Buxbaum, J. D. (2000). Correlation between elevated levels of amyloid beta-peptide in the brain and cognitive decline. *Journal of the American Medical Association* **283**, 1571–1577.

Nilsberth, C., Westlind-Danielsson, A., Eckman, C. B., Condron, M. M., Axelman, K., Forsell, C., Stenh, C., Luthman, J., Teplow, D. B., Younkin, S. G., Naslund, J. & Lannfelt, L. (2001). The 'Arctic' APP mutation (E693G) causes Alzheimer's disease by enhanced Abeta protofibril formation. *Nature Neuroscience* **4**, 887–893.

O'brien, T. D., Butler, A. E., Roche, P. C., Johnson, K. H. & Butler, P. C. (1994). Islet amyloid polypeptide in human insulinomas. Evidence for intracellular amyloidogenesis. *Diabetes* **43**, 329–336.

O'nuallain, B. & Wetzel, R. (2002). Conformational Abs recognizing a generic amyloid fibril epitope. *Proceedings of the National Academy of Sciences USA* **99**, 1485–1490.

Olofsson, A., Hebert, H. & Thelestam, M. (1993). The projection structure of perfringolysin O (Clostridium perfringens theta-toxin). *FEBS Letters* **319**, 125–127.

Orlova, E. V., Rahman, M. A., Gowen, B., Volynski, K. E., Ashton, A. C., Manser, C., Van Heel, M. & Ushkaryov, Y. A. (2000). Structure of alpha-latrotoxin oligomers reveals that divalent cation-dependent tetramers form membrane pores. *Nature Structural Biology* **7**, 48–53.

Pandey, N., Schmidt, R. E. & Galvin, J. E. (2006). The alpha-synuclein mutation E46K promotes aggregation in cultured cells. *Experimental Neurology* **197**, 515–520.

Panov, A. V., Gutekunst, C. A., Leavitt, B. R., Hayden, M. R., Burke, J. R., Strittmatter, W. J. & Greenamyre, J. T. (2002). Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines. *Nature Neuroscience* **5**, 731–736.

Parbhu, A., Lin, H., Thimm, J. & Lal, R. (2002). Imaging real-time aggregation of amyloid beta protein (1-42) by atomic force microscopy. *Peptides* **23**, 1265–1270.

Park, J. Y. & Lansbury Jr., P. T. (2003). Beta-synuclein inhibits formation of alpha-synuclein protofibrils: a possible therapeutic strategy against Parkinson's disease. *Biochemistry* **42**, 3696–3700.

Pillot, T., Drouet, B., Pincon-Raymond, M., Vandekerckhove, J., Rosseneu, M. & Chambaz, J. (2000). A nonfibrillar form of the fusogenic prion protein fragment [118-135] induces apoptotic cell death in rat cortical neurons. *Journal of Neurochemistry* **75**, 2298–2308.

Pillot, T., Lins, L., Goethals, M., Vanloo, B., Baert, J., Vandekerckhove, J., Rosseneu, M. & Brasseur, R.

(1997). The 118-135 peptide of the human prion protein forms amyloid fibrils and induces liposome fusion. *Journal of Molecular Biology* **274**, 381–393.

Pitschke, M., Prior, R., Haupt, M. & Riesner, D. (1998). Detection of single amyloid beta-protein aggregates in the cerebrospinal fluid of Alzheimer's patients by fluorescence correlation spectroscopy. *Nature Medicine* **4**, 832–834.

Podlisny, M. B., Ostaszewski, B. L., Squazzo, S. L., Koo, E. H., Rydell, R. E., Teplow, D. B. & Selkoe, D. J. (1995). Aggregation of secreted amyloid beta-protein into sodium dodecyl sulfate-stable oligomers in cell culture. *Journal of Biological Chemistry* **270**, 9564–9570.

Poirier, M. A., Li, H., Macosko, J., Cai, S., Amzel, M. & Ross, C. A. (2002). Huntingtin spheroids and protofibrils as precursors in polyglutamine fibrilization. *Journal of Biological Chemistry* **277**, 41032–41037.

Pollanen, M. S., Dickson, D. W. & Bergeron, C. (1993). Pathology and biology of the Lewy body. *Journal of Neuropathology & Experimental Neurology* **52**, 183–191.

Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., Stenroos, E. S., Chandrasekharappa, S., Athanassiadou, A., Papapetropoulos, T., Johnson, W. G., Lazzarini, A. M., Duvoisin, R. C., Di Iorio, G., Golbe, L. I. & Nussbaum, R. L. (1997). Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* **276**, 2045–2047.

Porat, Y., Kolusheva, S., Jelinek, R. & Gazit, E. (2003). The human islet amyloid polypeptide forms transient membrane-active prefibrillar assemblies. *Biochemistry* **42**, 10971–10977.

Pountney, D. L., Lowe, R., Quilty, M., Vickers, J. C., Voelcker, N. H. & Gai, W. P. (2004). Annular alpha-synuclein species from purified multiple system atrophy inclusions. *Journal of Neurochemistry* **90**, 502–512.

Quist, A., Doudevski, I., Lin, H., Azimova, R., Ng, D., Frangione, B., Kagan, B., Ghiso, J. & Lal, R. (2005). Amyloid ion channels: a common structural link for protein-misfolding disease. *Proceedings of the National Academy of Sciences USA* **102**, 10427–10432.

Rajan, R. S., Illing, M. E., Bence, N. F. & Kopito, R. R. (2001). Specificity in intracellular protein aggregation and inclusion body formation. *Proceedings of the National Academy of Sciences USA* **98**, 13060–13065.

Rakhit, R., Crow, J. P., Lepock, J. R., Kondejewski, L. H., Cashman, N. R. & Chakrabartty, A. (2004). Monomeric Cu/Zn superoxide dismutase is a common misfolding intermediate in the oxidation models of sporadic and familial ALS. *Journal of Biological Chemistry* **279**, 15499–15504.

Rakhit, R., Cunningham, P., Furtos-Matei, A., Dahan, S., Qi, X. F., Crow, J. P., Cashman, N. R., Kondejewski, L. H. & Chakrabartty, A. (2002). Oxidation-induced misfolding and aggregation of superoxide dismutase and its implications for amyotrophic lateral sclerosis. *Journal of Biological Chemistry* **277**, 47551–47556.

Ray, S. S., Nowak, R. J., Strokovich, K., Brown Jr., R. H, Walz, T. & Lansbury Jr., P. T. (2004). An intersubunit disulfide bond prevents in vitro aggregation of a superoxide dismutase-1 mutant linked to familial amytrophic lateral sclerosis. *Biochemistry* **43**, 4899–4905.

Reixach, N., Deechongkit, S., Jiang, X., Kelly, J. W. & Buxbaum, J. N. (2004). Tissue damage in the amyloidoses: transthyretin monomers and nonnative oligomers are the major cytotoxic species in tissue culture. *Proceedings of the National Academy of Sciences USA* **101**, 2817–2822.

Rhee, S. K., Quist, A. P. & Lal, R. (1998). Amyloid beta protein-(1-42) forms calcium-permeable, Zn^{2+}-sensitive channel. *Journal of Biological Chemistry* **273**, 13379–13382.

Rhoades, E. & Gafni, A. (2003). Micelle formation by a fragment of human islet amyloid polypeptide. *Biophysical Journal* **84**, 3480–3487.

Riesner, D., Kellings, K., Post, K., Wille, H., Serban, H., Groth, D., Baldwin, M. A. & Prusiner, S. B. (1996). Disruption of prion rods generates 10-nm spherical particles having high alpha-helical content and lacking scrapie infectivity. *Journal of Virology* **70**, 1714–1722.

Rochet, J. C., Conway, K. A. & Lansbury Jr., P. T. (2000). Inhibition of fibrillization and accumulation of prefibrillar oligomers in mixtures of human and mouse alpha-synuclein. *Biochemistry* **39**, 10619–10626.

Rochet, J. C. & Lansbury Jr., P. T. (2000). Amyloid fibrillogenesis: themes and variations. *Current Opinion in Structural Biology* **10**, 60–68.

Rockenstein, E., Hansen, L. A., Mallory, M., Trojanowski, J. Q., Galasko, D. & Masliah, E. (2001). Altered expression of the synuclein family mRNA in Lewy body and Alzheimer's disease. *Brain Research* **914**, 48–56.

Rodriguez, J. A., Valentine, J. S., Eggers, D. K., Roe, J. A., Tiwari, A., Brown Jr., R. H. & Hayward, L. J. (2002). Familial amyotrophic lateral sclerosis-associated mutations decrease the thermal stability of distinctly metallated species of human copper/zinc superoxide dismutase. *Journal of Biological Chemistry* **277**, 15932–15937.

Roher, A. E., Chaney, M. O., Kuo, Y. M., Webster, S. D., Stine, W. B., Haverkamp, L. J., Woods, A. S., Cotter, R. J., Tuohy, J. M., Krafft, G. A., Bonnell, B. S. & Emmerling, M. R. (1996). Morphology and toxicity of Abeta-(1-42) dimer derived from neuritic and vascular amyloid deposits of Alzheimer's disease. *Journal of Biological Chemistry* **271**, 20631–20635.

Rosen, A. D. (1978). Amyotrophic lateral sclerosis. Clinical features and prognosis. *Archives of Neurology* **35**, 638–642.

Rosen, D. R., Siddique, T., Patterson, D., Figlewicz, D. A., Sapp, P., Hentati, A., Donaldson, D., Goto, J., O'regan, J. P., Deng, H. X., *et al.* (1993). Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* **362**, 59–62.

Ross, C. A. & Pickart, C. M. (2004). The ubiquitin-proteasome pathway in Parkinson's disease and other neurodegenerative diseases. *Trends in Cell Biology* **14**, 703–711.

Rossi, D., Cozzio, A., Flechsig, E., Klein, M. A., Rulicke, T., Aguzzi, A. & Weissmann, C. (2001). Onset of ataxia and Purkinje cell loss in PrP null mice inversely correlated with Dpl level in brain. *EMBO Journal* **20**, 694–702.

Rubenstein, R., Merz, P. A., Kascsak, R. J., Carp, R. I., Scalici, C. L., Fama, C. L. & Wisniewski, H. M. (1987). Detection of scrapie-associated fibrils (SAF) and SAF proteins from scrapie-affected sheep. *Journal of Infectious Diseases* **156**, 36–42.

Sanderson, K. L., Butler, L. & Ingram, V. M. (1997). Aggregates of a beta-amyloid peptide are required to induce calcium currents in neuron-like human teratocarcinoma cells: relation to Alzheimer's disease. *Brain Research* **744**, 7–14.

Sanghera, N. & Pinheiro, T. J. (2002). Binding of prion protein to lipid membranes and implications for prion conversion. *Journal of Molecular Biology* **315**, 1241–1256.

Scherzinger, E., Sittler, A., Schweiger, K., Heiser, V., Lurz, R., Hasenbank, R., Bates, G. P., Lehrach, H. & Wanker, E. E. (1999). Self-assembly of polyglutamine-containing huntingtin fragments into amyloid-like fibrils: implications for Huntington's disease pathology. *Proceedings of the National Academy of Sciences USA* **96**, 4604–4609.

Schubert, U., Anton, L. C., Gibbs, J., Norbury, C. C., Yewdell, J. W. & Bennink, J. R. (2000). Rapid degradation of a large fraction of newly synthesized proteins by proteasomes. *Nature* **404**, 770–774.

Sekiya, K., Satoh, R., Danbara, H. & Futaesaku, Y. (1993). A ring-shaped structure with a crown formed by streptolysin O on the erythrocyte membrane. *Journal of Bacteriology* **175**, 5953–5961.

Selkoe, D. J. (1994). Cell biology of the amyloid beta-protein precursor and the mechanism of Alzheimer's disease. *Annual Reviews of Cell Biology* **10**, 373–403.

Sellman, B. R., Kagan, B. L. & Tweten, R. K. (1997). Generation of a membrane-bound, oligomerized prepore complex is necessary for pore formation by *Clostridium septicum* alpha toxin. *Molecular Microbiology* **23**, 551–558.

Serpell, L. C., Sunde, M., Benson, M. D., Tennent, G. A., Pepys, M. B. & Fraser, P. E. (2000). The protofilament substructure of amyloid fibrils. *Journal of Molecular Biology* **300**, 1033–1039.

Sharon, R., Bar-Joseph, I., Frosch, M. P., Walsh, D. M., Hamilton, J. A. & Selkoe, D. J. (2003a). The formation of highly soluble oligomers of alpha-synuclein is regulated by fatty acids and enhanced in Parkinson's disease. *Neuron* **37**, 583–595.

Sharon, R., Bar-Joseph, I., Mirick, G. E., Serhan, C. N. & Selkoe, D. J. (2003b). Altered fatty acid composition of dopaminergic neurons expressing alpha-synuclein and human brains with alpha-synucleinopathies. *Journal of Biological Chemistry* **278**, 49874–49881.

Sharon, R., Goldberg, M. S., Bar-Josef, I., Betensky, R. A., Shen, J. & Selkoe, D. J. (2001). alpha-Synuclein occurs in lipid-rich high molecular weight complexes, binds fatty acids, and shows homology to the fatty acid-binding proteins. *Proceedings of the National Academy of Sciences USA* **98**, 9110–9115.

Sharpe, J. C. & London, E. (1999). Diphtheria toxin forms pores of different sizes depending on its concentration in membranes: probable relationship to oligomerization. *Journal of Membrane Biology* **171**, 209–221.

Shibata, N., Hirano, A., Kobayashi, M., Siddique, T., Deng, H. X., Hung, W. Y., Kato, T. & Asayama, K. (1996). Intense superoxide dismutase-1 immunoreactivity in intracytoplasmic hyaline inclusions of familial amyotrophic lateral sclerosis with posterior column involvement. *Journal of Neuropathology & Experimental Neurology* **55**, 481–490.

Shimura, H., Schlossmacher, M. G., Hattori, N., Frosch, M. P., Trockenbacher, A., Schneider, R., Mizuno, Y., Kosik, K. S. & Selkoe, D. J. (2001). Ubiquitination of a new form of alpha-synuclein by parkin from human brain: implications for Parkinson's disease. *Science* **293**, 263–269.

Shtilerman, M. D., Ding, T. T. & Lansbury Jr., P. T. (2002). Molecular crowding accelerates fibrillization of alpha-synuclein: could an increase in the cytoplasmic protein concentration induce Parkinson's disease? *Biochemistry* **41**, 3855–3860.

Shults, C. W. (2006). Lewy bodies. *Proceedings of the National Academy of Sciences USA* **103**, 1661–1668.

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

Singer, S. J. & Dewji, N. N. (2006). Evidence that Perutz's double-beta-stranded subunit structure for beta-amyloids also applies to their channel-forming structures in membranes. *Proceedings of the National Academy of Sciences USA* **103**, 1546–1550.

Singleton, A., Myers, A. & Hardy, J. (2004). The law of mass action applied to neurodegenerative disease: a hypothesis concerning the aetiology and pathogenesis of complex diseases. *Human Molecular Genetics* **13**, R123–R126.

Singleton, A. B., Farrer, M., Johnson, J., Singleton, A., Hague, S., Kachergus, J., Hulihan, M., Peuralinna,

T., Dutra, A., Nussbaum, R., Lincoln, S., Crawley, A., Hanson, M., Maraganore, D., Adler, C., Cookson, M. R., Muenter, M., Baptista, M., Miller, D., Blancato, J., Hardy, J. & Gwinn-Hardy, K. (2003). alpha-Synuclein locus triplication causes Parkinson's disease. *Science* **302**, 841.

Smith, W. W., Margolis, R. L., Li, X., Troncoso, J. C., Lee, M. K., Dawson, V. L., Dawson, T. M., Iwatsubo, T. & Ross, C. A. (2005). Alpha-synuclein phosphorylation enhances eosinophilic cytoplasmic inclusion formation in SH-SY5Y cells. *Journal of Neuroscience* **25**, 5544–5552.

Snyder, H., Mensah, K., Theisler, C., Lee, J., Matouschek, A. & Wolozin, B. (2003). Aggregated and monomeric alpha-synuclein bind to the S6′ proteasomal protein and inhibit proteasomal function. *Journal of Biological Chemistry* **278**, 11753–11759.

Sokolowski, F., Modler, A. J., Masuch, R., Zirwer, D., Baier, M., Lutsch, G., Moss, D. A., Gast, K. & Naumann, D. (2003). Formation of critical oligomers is a key event during conformational transition of recombinant syrian hamster prion protein. *Journal of Biological Chemistry* **278**, 40481–40492.

Song, L., Hobaugh, M. R., Shustak, C., Cheley, S., Bayley, H. & Gouaux, J. E. (1996). Structure of staphylococcal alpha-hemolysin, a heptameric transmembrane pore. *Science* **274**, 1859–1866.

Soti, C. & Csermely, P. (2002). Chaperones and aging: role in neurodegeneration and in other civilizational diseases. *Neurochemistry International* **41**, 383–389.

Sousa, M. M., Cardoso, I., Fernandes, R., Guimaraes, A. & Saraiva, M. J. (2001). Deposition of transthyretin in early stages of familial amyloidotic polyneuropathy: evidence for toxicity of nonfibrillar aggregates. *American Journal of Pathology* **159**, 1993–2000.

Sousa, M. M., Fernandes, R., Palha, J. A., Taboada, A., Vieira, P. & Saraiva, M. J. (2002). Evidence for early cytotoxic aggregates in transgenic mice for human transthyretin Leu55Pro. *American Journal of Pathology* **161**, 1935–1948.

Spillantini, M. G., Crowther, R. A., Jakes, R., Cairns, N. J., Lantos, P. L. & Goedert, M. (1998a). Filamentous alpha-synuclein inclusions link multiple system atrophy with Parkinson's disease and dementia with Lewy bodies. *Neuroscience Letters* **251**, 205–208.

Spillantini, M. G., Crowther, R. A., Jakes, R., Hasegawa, M. & Goedert, M. (1998b). alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. *Proceedings of the National Academy of Sciences USA* **95**, 6469–6473.

Srinivasan, R., Jones, E. M., Liu, K., Ghiso, J., Marchant, R. E. & Zagorski, M. G. (2003). pH-dependent amyloid and protofibril formation by the ABri peptide of familial British dementia. *Journal of Molecular Biology* **333**, 1003–1023.

Srinivasan, R., Marchant, R. & Zagorski, M. (2004). ABri peptide associated with familial British dementia forms pore-like protofibrillar structures. *Amyloid* **11**, 10–13.

Stefani, M. & Dobson, C. M. (2003). Protein aggregation and aggregate toxicity: new insights into protein folding, misfolding diseases and biological evolution. *Journal of Molecular Medicine* **81**, 678–699.

Stege, G. J., Renkawek, K., Overkamp, P. S., Verschuure, P., Van Rijk, A. F., Reijnen-Aalbers, A., Boelens, W. C., Bosman, G. J. & De Jong, W. W. (1999). The molecular chaperone alphaB-crystallin enhances amyloid beta neurotoxicity. *Biochemical and Biophysical Research Communications* **262**, 152–156.

Stine Jr., W. B., Dahlgren, K. N., Krafft, G. A. & Ladu, M. J. (2003). In vitro characterization of conditions for amyloid-beta peptide oligomerization and fibrillogenesis. *Journal of Biological Chemistry* **278**, 11612–11622.

Sunde, M., Serpell, L. C., Bartlam, M., Fraser, P. E., Pepys, M. B. & Blake, C. C. (1997). Common core structure of amyloid fibrils by synchrotron X-ray diffraction. *Journal of Molecular Biology* **273**, 729–739.

Supattapone, S., Bosque, P., Muramoto, T., Wille, H., Aagaard, C., Peretz, D., Nguyen, H. O., Heinrich, C., Torchia, M., Safar, J., Cohen, F. E., Dearmond, S. J., Prusiner, S. B. & Scott, M. (1999). Prion protein of 106 residues creates an artifical transmission barrier for prion replication in transgenic mice. *Cell* **96**, 869–878.

Tadjibaeva, G., Sabirov, R. & Tomita, T. (2000). Flammutoxin, a cytolysin from the edible mushroom Flammulina velutipes, forms two different types of voltage-gated channels in lipid bilayer membranes. *Biochimica et Biophysica Acta* **1467**, 431–443.

Tanaka, Y., Engelender, S., Igarashi, S., Rao, R. K., Wanner, T., Tanzi, R. E., Sawa, A., Dawson, V. L., Dawson, T. M. & Ross, C. A. (2001). Inducible expression of mutant alpha-synuclein decreases proteasome activity and increases sensitivity to mitochondria-dependent apoptosis. *Human Molecular Genetics* **10**, 919–926.

Taylor, J. P., Tanaka, F., Robitschek, J., Sandoval, C. M., Taye, A., Markovic-Plese, S. & Fischbeck, K. H. (2003). Aggresomes protect cells by enhancing the degradation of toxic polyglutamine-containing protein. *Human Molecular Genetics* **12**, 749–757.

Teng, M. H., Yin, J. Y., Vidal, R., Ghiso, J., Kumar, A., Rabenou, R., Shah, A., Jacobson, D. R., Tagoe, C., Gallo, G. & Buxbaum, J. (2001). Amyloid and nonfibrillar deposits in mice transgenic for wild-type human transthyretin: a possible model for senile systemic amyloidosis. *Laboratory Investigation* **81**, 385–396.

Terry, R. D., Masliah, E., Salmon, D. P., Butters, N., Deteresa, R., Hill, R., Hansen, L. A. & Katzman, R. (1991). Physical basis of cognitive alterations in

Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Annals of Neurology* **30**, 572–580.

Tiwari, A. & Hayward, L. J. (2003). Familial amyotrophic lateral sclerosis mutants of copper/zinc superoxide dismutase are susceptible to disulfide reduction. *Journal of Biological Chemistry* **278**, 5984–5992.

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

Townsend, M., Shankar, G. M., Mehta, T., Walsh, D. M. & Selkoe, D. J. (2006). Effects of secreted oligomers of amyloid β-protein on hippocampal synaptic plasticity: a potent role for trimers. *Journal of Physiology* **572**, 477–492.

Turner, B. J., Lopes, E. C. & Cheema, S. S. (2003). Neuromuscular accumulation of mutant superoxide dismutase 1 aggregates in a transgenic mouse model of familial amyotrophic lateral sclerosis. *Neuroscience Letters* **350**, 132–136.

Urushitani, M., Kurisu, J., Tsukita, K. & Takahashi, R. (2002). Proteasomal inhibition by misfolded mutant superoxide dismutase 1 induces selective motor neuron death in familial amyotrophic lateral sclerosis. *Journal of Neurochemistry* **83**, 1030–1042.

Uryu, K., Giasson, B. I., Longhi, L., Martinez, D., Murray, I., Conte, V., Nakamura, M., Saatman, K., Talbot, K., Horiguchi, T., Mcintosh, T., Lee, V. M. & Trojanowski, J. Q. (2003). Age-dependent synuclein pathology following traumatic brain injury in mice. *Experimental Neurology* **184**, 214–224.

Valeva, A., Palmer, M. & Bhakdi, S. (1997). Staphylococcal alpha-toxin: formation of the heptameric pore is partially cooperative and proceeds through multiple intermediate stages. *Biochemistry* **36**, 13298–13304.

Vendrely, C., Valadie, H., Bednarova, L., Cardin, L., Pasdeloup, M., Cappadoro, J., Bednar, J., Rinaudo, M. & Jamin, M. (2005). Assembly of the full-length recombinant mouse prion protein I. Formation of soluble oligomers. *Biochimica et Biophysica Acta* **1724**, 355–366.

Vidal, R., Frangione, B., Rostagno, A., Mead, S., Revesz, T., Plant, G. & Ghiso, J. (1999). A stop-codon mutation in the BRI gene associated with familial British dementia. *Nature* **399**, 776–781.

Volles, M. J. & Lansbury Jr., P. T. (2002). Vesicle permeabilization by protofibrillar alpha-synuclein is sensitive to Parkinson's disease-linked mutations and occurs by a pore-like mechanism. *Biochemistry* **41**, 4595–4602.

Volles, M. J. & Lansbury Jr., P. T. (2003). Zeroing in on the pathogenic form of alpha-synuclein and its mechanism of neurotoxicity in Parkinson's disease. *Biochemistry* **42**, 7871–7878.

Volles, M. J., Lee, S. J., Rochet, J. C., Shtilerman, M. D., Ding, T. T., Kessler, J. C. & Lansbury Jr., P. T. (2001). Vesicle permeabilization by protofibrillar alpha-synuclein: implications for the pathogenesis and treatment of Parkinson's disease. *Biochemistry* **40**, 7812–7819.

Wacker, J. L., Zareie, M. H., Fong, H., Sarikaya, M. & Muchowski, P. J. (2004). Hsp70 and Hsp40 attenuate formation of spherical and annular polyglutamine oligomers by partitioning monomer. *Nature Structural & Molecular Biology* **11**, 1215–1222.

Walker, B., Krishnasastry, M., Zorn, L. & Bayley, H. (1992). Assembly of the oligomeric membrane pore formed by Staphylococcal alpha-hemolysin examined by truncation mutagenesis. *Journal of Biological Chemistry* **267**, 21782–21786.

Wallace, A. J., Stillman, T. J., Atkins, A., Jamieson, S. J., Bullough, P. A., Green, J. & Artymiuk, P. J. (2000). E. coli hemolysin E (HlyE, ClyA, SheA): X-ray crystal structure of the toxin and observation of membrane pores by electron microscopy. *Cell* **100**, 265–276.

Walsh, D. M., Hartley, D. M., Kusumoto, Y., Fezoui, Y., Condron, M. M., Lomakin, A., Benedek, G. B., Selkoe, D. J. & Teplow, D. B. (1999). Amyloid beta-protein fibrillogenesis. Structure and biological activity of protofibrillar intermediates. *Journal of Biological Chemistry* **274**, 25945–25952.

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

Walsh, D. M., Lomakin, A., Benedek, G. B., Condron, M. M. & Teplow, D. B. (1997). Amyloid beta-protein fibrillogenesis. Detection of a protofibrillar intermediate. *Journal of Biological Chemistry* **272**, 22364–22372.

Walsh, D. M., Tseng, B. P., Rydel, R. E., Podlisny, M. B. & Selkoe, D. J. (2000). The oligomerization of amyloid beta-protein begins intracellularly in cells derived from human brain. *Biochemistry* **39**, 10831–10839.

Wang, H. W., Pasternak, J. F., Kuo, H., Ristic, H., Lambert, M. P., Chromy, B., Viola, K. L., Klein, W. L., Stine, W. B., Krafft, G. A. & Trommer, B. L. (2002a). Soluble oligomers of beta amyloid (1-42) inhibit long-term potentiation but not long-term depression in rat dentate gyrus. *Brain Research* **924**, 133–140.

Wang, L., Lashuel, H. A., Walz, T. & Colon, W. (2002b). Murine apolipoprotein serum amyloid A in solution forms a hexamer containing a central channel. *Proceedings of the National Academy of Sciences USA* **99**, 15947–15952.

Wang, Q., Walsh, D. M., Rowan, M. J., Selkoe, D. J. & Anwyl, R. (2004). Block of long-term potentiation by naturally secreted and synthetic amyloid beta-peptide in hippocampal slices is mediated via activation of the kinases c-Jun N-terminal kinase, cyclin-dependent kinase 5, and p38 mitogen-activated protein kinase as well as

metabotropic glutamate receptor type 5. *Journal of Neuroscience* **24**, 3370–3378.

Watanabe, M., Dykes-Hoberg, M., Culotta, V. C., Price, D. L., Wong, P. C. & Rothstein, J. D. (2001). Histological evidence of protein aggregation in mutant SOD1 transgenic mice and in amyotrophic lateral sclerosis neural tissues. *Neurobiology of Disease Journal* **8**, 933–941.

Watase, K., Weeber, E. J., Xu, B., Antalffy, B., Yuva-Paylor, L., Hashimoto, K., Kano, M., Atkinson, R., Sun, Y., Armstrong, D. L., Sweatt, J. D., Orr, H. T., Paylor, R. & Zoghbi, H. Y. (2002). A long CAG repeat in the mouse Sca1 locus replicates SCA1 features and reveals the impact of protein solubility on selective neurodegeneration. *Neuron* **34**, 905–919.

Weinreb, P. H., Zhen, W., Poon, A. W., Conway, K. A. & Lansbury Jr., P. T. (1996). NACP, a protein implicated in Alzheimer's disease and learning, is natively unfolded. *Biochemistry* **35**, 13709–13715.

Wetzel, R. (1994). Mutations and off-pathway aggregation of proteins. *Trends in Biotechnology* **12**, 193–198.

Whalen, B. M., Selkoe, D. J. & Hartley, D. M. (2005). Small non-fibrillar assemblies of amyloid beta-protein bearing the Arctic mutation induce rapid neuritic degeneration. *Neurobiology of Disease Journal* **20**, 254–266.

White, J. A., Manelli, A. M., Holmberg, K. H., Van Eldik, L. J. & Ladu, M. J. (2005). Differential effects of oligomeric and fibrillar amyloid-beta 1-42 on astrocyte-mediated inflammation. *Neurobiology of Disease Journal* **18**, 459–465.

Wood, W. G., Schroeder, F., Igbavboa, U., Avdulov, N. A. & Chochina, S. V. (2002). Brain membrane cholesterol domains, aging and amyloid beta-peptides. *Neurobiology of Aging* **23**, 685–694.

Xu, J., Kao, S. Y., Lee, F. J., Song, W., Jin, L. W. & Yankner, B. A. (2002). Dopamine-dependent neurotoxicity of alpha-synuclein: a mechanism for selective neurodegeneration in Parkinson disease. *Nature Medicine* **8**, 600–606.

Yong, W., Lomakin, A., Kirkitadze, M. D., Teplow, D. B., Chen, S. H. & Benedek, G. B. (2002). Structure determination of micelle-like intermediates in amyloid beta -protein fibril assembly by using small angle neutron scattering. *Proceedings of the National Academy of Sciences USA* **99**, 150–154.

Zarranz, J. J., Alegre, J., Gomez-Esteban, J. C., Lezcano, E., Ros, R., Ampuero, I., Vidal, L., Hoenicka, J., Rodriguez, O., Atares, B., Llorens, V., Tortosa, E. G., Del Ser, T., Munoz, D. G. & De Yebenes, J. G. (2004). The new mutation, E46K, of alpha-synuclein causes parkinson and Lewy body dementia. *Annals of Neurology* **55**, 164–173.

Zhang, Y., McLaughlin, R., Goodyer, C. & Leblanc, A. (2002). Selective cytotoxicity of intracellular amyloid beta peptide 1-42 through p53 and Bax in cultured primary human neurons. *Journal of Cell Biology* **156**, 519–529.

Zhu, M. & Fink, A. L. (2003). Abstract Viewer/Itinerary Planner. Washington, DC: Society for Neuroscience, Program No. 132.12.

Zhu, M., Rajmani, S., Kaylor, J., Han, S., Zhou, F. & Fink, A. L. (2004). The flavonoid baicalein inhibits fibrillation of alpha-synuclein and disaggregates existing fibrils. *Journal of Biological Chemistry* **279**, 26846–26857.

Zitzer, A., Walev, I., Palmer, M. & Bhakdi, S. (1995). Characterization of *Vibrio cholerae* El Tor cytolysin as an oligomerizing pore-forming toxin. *Medical Microbiology and Immunology* (*Berlin*) **184**, 37–44.

Zoghbi, H. Y. & Orr, H. T. (2000). Glutamine repeats and neurodegeneration. *Annual Review of Neuroscience* **23**, 217–247.