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Neurodegenerative diseases: a decade of discoveries paves the way for therapeutic breakthroughs

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A wide variety of neurodegenerative diseases are characterized by the accumulation of intracellular or extracellular protein aggregates. More recently, the genetic identification of mutations in familial counterparts to the sporadic disorders, leading to the development of in vitro and in vivo model systems, has provided insights into disease pathogenesis. The effect of many of these mutations is the abnormal processing of misfolded proteins that overwhelms the quality-control systems of the cell, resulting in the deposition of protein aggregates in the nucleus, cytosol and/or extracellular space. Further understanding of mechanisms regulating protein processing and aggregation, as well as of the toxic effects of misfolded neurodegenerative disease proteins, will facilitate development of rationally designed therapies to treat and prevent these disorders.

In a brief report in 1907, Alois Alzheimer described senile plaques and neurofibrillary tangles (NFTs) in the neocortex and hippocampus of a middle-aged woman with memory deficits and a progressive loss of cognitive function[1]. This was the first published description of Alzheimer disease. Shortly thereafter, in 1912, Friederich Lewy described the neuropathological hallmark of Parkinson disease, the Lewy body[2]. However, over 80 years elapsed before the principle components that form senile plaques[3], NFTs[4] and Lewy bodies[5] were identified. And just over 20 years ago, new infectious protein particles (designated 'prions') were isolated from the brains of scrapie-infected sheep and identified as the transmissible agent underlying the infectious spread of this neurodegenerative disorder[6]. The recognition that prions are misfolded proteins prone to fibrillize, aggregate and form plaque-like amyloid deposits in human transmissible spongiform encephalopathies, coupled with the characterization of the building block proteins of other filamentous neurodegenerative disease lesions (for example, senile plaques and NFTs), ushered in the modern molecular era of research on these disorders.

The average life expectancy of many populations throughout the world now extends late into the eighth decade, and the prevalence of most neurodegenerative disorders increases dramatically with advancing age[7]. For example, for Alzheimer disease—the most prevalent of these disorders, affecting ~15 million people worldwide today—the number of affected individuals in the United States and Europe is expected to triple by the year 2050 to 13.2 million[8] and 16.2 milllion[9], respectively. Further, the World Health Organization estimates that by 2025 three-quarters of the world's population over 60 years of age will be living in developing countries[7]. Thus, if effective treatments or preventive interventions for Alzheimer disease and other neurodegenerative disorders are not discovered in the near future, the financial, societal and emotional costs of these aging-related brain disorders will be staggering.

This review provides a retrospective assessment of the most significant advances of the explosive increase in research on neurodegenerative diseases over the past decade. The magnitude of this dramatic increase in neurodegenerative disease research is evidenced by a PubMed search of the National Library of Medicine for the keyword 'neurodegenerative disease,' which retrieved more than 65,000 citations for the past ten years, including over 20,000 citations each on Alzheimer disease and tauopathies and approximately 10,000 citations for Parkinson disease. It is clear that the remarkable advances in understanding the genetic basis of neurodegenerative diseases have been a major driving force in all fields of research on these disorders by opening up many new lines of investigation. For example, it now is well established that there is a familial counterpart to each of the major classes of neurodegenerative disease, most of which are inherited in an autosomal dominant pattern (**Table 1**). The identification of genetic mutations that are pathogenic for familial neurodegenerative diseases in many kindreds has facilitated establishment of both *in vitro* and *in vivo* models of these various disorders.

Over the past decade, converging lines of investigation revealed a common pathogenic mechanism underlying many neurodegenerative disorders as seemingly diverse as Alzheimer disease, Parkinson disease and Creutzfeldt-Jakob disease (CJD); that common mechanism is the aggregation and deposition of misfolded proteins leading to progressive central nervous system (CNS) amyloidosis. Thus, highly soluble proteins are gradually converted into insoluble, filamentous polymers with characteristic crossed-β-pleated sheet structures that accumulate in a disease- and protein-specific manner as fibrillar amyloid deposits in the cytosol or nuclei of affected brain cells or in the extracellular space (**Fig. 1**). Enigmatically, despite the fact that many of the amyloidogenic proteins associated with neurodegenerative disease are expressed systemically, the resulting amyloidosis is restricted to the CNS. *In vivo*, these changes develop insidiously over the lifetime of an individual, even though they do not manifest clinically until middle or late life. The cause of this prolonged preclinical phase is poorly understood, but it almost certainly reflects the requirement for progressive

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Table 1 Neurodegenerative diseases characterized by the deposition of aggregated proteins

Toxic protein	Protein deposit	Familial disease	Gene mutated	Sporadic disease	Risk factor
β-amyloid	Senile plaques	FAD	*APP* *PS1* *PS2*	Alzheimer disease	*Apoe4*
Tau	Neuronal and glial inclusions	FTDP-17 inclusions	*MAPT*	AD and tauopathies[a]	*MAPT* haplotype
α-synuclein	Lewy bodies Lewy neurites	Familial PD[b]	*SNCA* (α-synuclein)	Lewy body disease[c]	*SNCA* polymorphism *MAPT* haplotype
	Glial cytoplasmic inclusions	Not identified	Not applicable	Multiple system atrophy	Not identified
Polyglutamine repeat expansion	Nuclear and cytoplasmic inclusions	Huntington disease Kennedy disease DRPLA SCA1 SCA2 SCA3 SCA6 SCA7 SCA17	*HD* (huntingtin) *AR* (androgen receptor) *DRPLA* (atrophin-1) *ATXN1* (ataxin-1) *ATXN2* (ataxin-2) *ATXN3* (ataxin-3) *CACNA1A*[d] *ATXN7* (ataxin-7) *TBP* (TATA binding protein)	Not applicable	Not identified
PrP^{SC}	Protease-resistant PrP^{e}	Familial prion protein disease[f]	*PRNP*	Sporadic prion protein disease[g]	*PRNP* polymorphism
SOD	Hyaline inclusions	Autosomal dominant familial ALS	*SOD1* (Cu/Zn SOD)	Sporadic ALS	Not identified
ABri/ADan	Amyloid plaques and angiopathy	Familial British/Danish dementia	*BRI*	Not identified	Not identified
Neuroserpin	Collins bodies	FENIB[h]	*SERPINI1* (neuroserpin)	Not identified	Not identified

[a]Tauopathies: PiD, corticobasal degeneration and progressive supranuclear palsy. [b]Four additional genes are implicated in familial PD including *PARK2*, *UCHL1*, *DJ1* and *PINK1*. However, it is unclear if these disorders are associated with LB pathology. [c]Lewy body disease: PD and DLB. [d]CACNA1A encodes the α(1A) subunit of voltage-gated calcium channel, type P/Q. [e]Detected by immunohistochemistry or biochemically after digestion with proteinase K. [f]Familial prion protein disease: familial CJD, Gerstmann-Straussler-Scheinker disease and fatal familial insomnia. [g]Sporadic prion protein disease: CJD, variant CJD, iatrogenic CJD and kuru. [h]Familial encephalopathy with neuroserpin inclusion bodies.

damage to specific brain regions or neuroanatomical systems prior to clinical manifestation of the disease, as well as the unfavorable kinetics of protein misfolding, oligomerization and fibrillization that are in turn linked to aging-related metabolic impairments. Furthermore, cells have adapted sophisticated quality-control measures to protect against the accumulation of misfolded and aggregated proteins (**Fig. 1**). For example, molecular chaperones promote proper protein folding and prevent aggregation of non-native proteins[10]. Proteins that remain misfolded are degraded primarily by the ubiquitin-proteasome system (UPS), but also by the phagosome-lysosome system. In many instances, the genetic mutations identified in the familial counterparts to sporadic neurodegenerative diseases result in mutant proteins that fibrillize at an accelerated rate compared to their wild-type counterparts, which may account for the earlier age of disease onset in the familial disorders. Here we review the advances in research of several major classes of these neurodegenerative diseases.

Alzheimer disease and β-amyloid

Alzheimer disease is a progressive neurodegenerative disorder characterized clinically by memory and cognitive dysfunction. Although sporadic Alzheimer disease is rare in individuals younger than 60 years of age, the incidence steadily increases with age, affecting up to 40% of those who are more than 85 years old. The neuropathology of Alzheimer disease is characterized by two types of lesions, senile plaques and NFTs, composed respectively of β-amyloid (Aβ), a cleavage product of the amyloid precursor protein (APP), and aberrantly phosphorylated tau, a microtubule-associated protein (**Fig. 2**)[1,3,4]. Although Aβ was identified about 20 years ago as the principle component of the amyloid fibrils that form senile plaques, the subsequent identification of mutations in the genes that encode APP and presenilins 1 and 2, in kindreds with early-onset, autosomal dominant familial Alzheimer disease (FAD), enabled the dissection of the role of Aβ in neurodegeneration[11–15].

The functions of APP remain unknown, although numerous activities have been ascribed to it[16]. In contrast, the metabolism of APP is well characterized, mediated by a series of enzymes termed secretases (α, β and γ)[17,18]. Cleavage of APP by α-secretase occurs in the middle of the Aβ peptide to generate nonamyloidogenic fragments of APP. In contrast, the sequential cleavage of APP by β-secretase followed by γ-secretase generates a variety of potentially amyloidogenic Aβ species. The majority of FAD mutations alter the normal proteolytic processing of APP, thereby promoting the production of longer, more amyloidogenic Aβ species, in particular $Aβ_{42}$[17,18]. Specifically, either FAD mutations in APP increase production of amyloidogenic Aβ peptides by directly altering the processing of APP by the β- or γ-secretases, or they promote Aβ fibrillization. In contrast, the presenilins are the central component of the γ-secretase complex, which is involved in the normal metabolism of a long list of proteins including APP and Notch[19]. Most presenilin mutations alter the specific APP C-terminal cleavage site of the γ-secretase, thereby promoting the generation of $Aβ_{42}$[17,18]. Thus, mutations in the genes encoding both APP and presenilin increase production of Aβ species that more readily fibrillize, thereby leading to neurodegeneration, as delineated in the amyloid cascade hypothesis of Alzheimer disease[20].

There is also a growing body of genetic and epidemiologic evidence associating Aβ with late-onset, sporadic Alzheimer disease[20]. First, the apolipoprotein E4 (*apoE4*) haplotype is a genetic risk factor for Alzheimer disease and, in transgenic mice, apoE modulates Aβ production[21,22]. Although the underlying mechanism accounting for this effect is not completely understood, it is likely to be related to the ability of apoE to interact with Aβ and influence its clearance and aggregation[23]. In addition, apoE affects cholesterol homeostasis, which may be linked to Alzheimer disease. This hypothesis is supported by epidemiologic studies showing a link between elevated cholesterol and Alzheimer disease[24]. Further, cholesterol modulates APP processing and elevated levels of cholesterol promote Aβ deposition in animal models. A genetic locus on chromosome 10 thought to be involved in late-onset alzheimer disease also correlates with elevated Aβ levels, possibly reflecting an influence on Aβ metabolism[25]. Finally, epidemiological studies document a reduced prevalence of Alzheimer disease among individuals who use nonsteroidal anti-inflammatory drugs (NSAIDs), and some of these drugs reduce production of the amyloidogenic $A\beta_{42}$ species in cultured cells and transgenic mice[26,27].

The identification of genes and pathways implicated in the pathogenesis of Alzheimer disease led to the production of transgenic models of Aβ amyloidosis, that recapitulate many of the neuropathological features of Alzheimer disease, including progressive accumulation of senile plaques, synaptic loss and gliosis, associated with learning and memory deficits[28,29]. The molecular basis of the disease phenotype in the majority of these transgenic animals is probably enhanced production of Aβ species. Furthermore, the mechanisms by which additional factors implicated in Aβ metabolism and Alzheimer disease, such as apoE, cholesterol and inflammatory mediators, have been explored[28]. However, most of these model systems lack tau pathology and show very little neuron loss.

More recently, the introduction of Aβ into tau-transgenic mice led to enhanced tau pathology with no change in Aβ deposition, further supporting the amyloid cascade hypothesis[30–32]. Treatment strategies have thus targeted the prevention of Aβ production, aggregation, toxicity or degradation, or a combination[20]. These include γ-secretase inhibitors and NSAIDs that reduce Aβ production, statins to lower cholesterol, neprilysin to promote Aβ degradation and metal chelators that block Aβ aggregation. Further, agents that promote the clearance of Aβ from the brain show promise as therapeutic interventions for Alzheimer disease[33]. For instance, the removal of Aβ by active and passive immunization strategies resulted in clearance of Aβ from brain and improved performance in behavioral tests. This latter finding was quickly translated into a clinical study involving peripheral immunization with an $A\beta_{42}$-containing vaccine. Unfortunately, the phase 2a trial was halted before completion because of complicating meningoencephalitis. Nonetheless, the numerous therapeutic strategies evaluated in animal models suggest that the prevention of Aβ formation or its removal are cause for future optimism about the prospects for developing more effective and meaningful therapies for Alzheimer disease. For instance, in transgenic animals developing tau and β-amyloid pathology, intracerebral Aβ immunotherapy promoted not only clearance of senile plaques, but also tau that was abnormally localized in a somatodendritic compartment[34]. The timing of the treatment was critical because bona fide NFTs were not affected by the therapy.

Tauopathies

Although the role of the microtubule-associated protein tau in the onset and progression of Alzheimer disease is still unresolved, evidence implying a causative role for tau in neurodegeneration is provided by the description of disorders other than Alzheimer disease, which show abundant, filamentous tau pathology and brain degeneration in the absence of extracellular Aβ deposition[35]. These disorders were named 'tauopathies' and encompass a group of clinically heterogeneous neurodegenerative diseases, including progressive supranuclear palsy, Pick disease and corticobasal degeneration. Furthermore, the recent discovery of multiple mutations in *MAPT*, the gene that encodes tau, that are pathogenic for a group of autosomal dominant

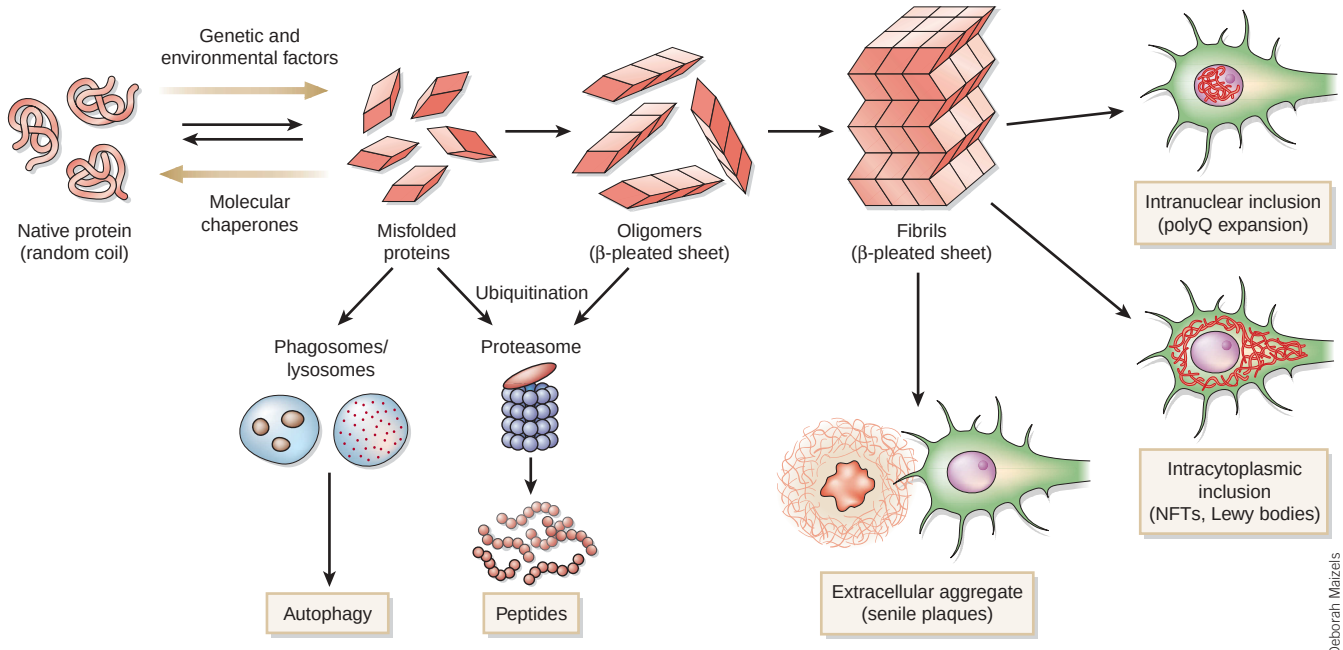

Figure 1 Model of protein misfolding and fibrillization, leading to the deposition of aggregated protein in the nucleus, cytoplasm and extracellular space. Genetic and environmental factors may accelerate this process, whereas cellular quality-control systems including molecular chaperones, the ubiquitin-proteasome system and the phagosome-lysosome system limit the accumulation of misfolded proteins.

multisystem neurodegenerative disorders, collectively referred to as frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17), provided unequivocal evidence of the role of tau abnormalities in the onset and progression of neurodegenerative disease[36–38]. In addition, specific *MAPT* haplotypes are linked to several of the sporadic tauopathies. But in contrast to fibrillar Aβ plaques in Alzheimer disease, the majority of tau amyloid fibrils are composed of the entire tau protein and form inclusions in the cytoplasm of both neurons and glia (**Fig. 2**).

Tau is a low-molecular-weight microtubule-associated protein that binds and stabilizes microtubules, promoting microtubule assembly and thereby influencing axonal transport[39]. To date, more than 30 distinct *MAPT* mutations have been identified in a large number of families with FTDP-17[35]. These mutations are located in exons throughout *MAPT* as well as in the intron following exon 10 that regulates the alternative splicing of the second (of four) microtubule-binding repeats. The intronic mutations as well as several exonic mutations affect the alternative splicing of exon 10, causing abnormal patterns of tau isoform expression. Other *MAPT* mutations compromise the microtubule-binding function of tau, thereby reducing microtubule stability leading to impairment in axonal transport. This function of tau is negatively regulated by phosphorylation and the tau protein in inclusions is abnormally phosphorylated, implicating tau aggregation in both a loss of function (the disruption of axonal transport) and a gain of toxic function (formation of tau aggregates). Indeed, several *MAPT* mutations alter the biophysical properties of tau, leading to enhanced fibrillization *in vitro*, further supporting the gain-of-toxic-function hypothesis. Moreover, unlike Aβ, tau does not readily fibrillize *in vitro* without the addition of cofactors[40]. The ability of many cofactors to induce tau fibrillization may explain in part the occurrence of tau pathology in many different neurodegenerative diseases including Alzheimer disease, Niemann-Pick type C and prion diseases, as well as the tauopathies.

Numerous genetic models of tau pathology were generated in a variety of species ranging from nematodes to mice by overexpressing wild-type or mutant tau proteins or expressing proteins thought to be involved in the regulation of tau phosphorylation[28]. A subset of these models recapitulates many of the features of tauopathies, including the age-dependent accumulation of filamentous tau pathology associated with behavioral abnormalities, variable neuron loss and defects in axonal transport. Interestingly, in Drosophila, neurodegeneration was linked to the overexpression of tau, but in the absence of neurofibrillary pathology[41]. In contrast, the use of combinatorial genetic models with APP or α-synuclein accelerates the formation of tau pathology, suggesting that tau may be part of a final common pathway for neurodegeneration[30–32,42]. Unfortunately, the development of therapeutic agents that reverse or block the tau pathology observed in these models is at a very early stage.

Lewy body disease and α-synucleinopathies

Parkinson disease, the most common neurodegenerative movement disorder, is characterized clinically by L-dopa–responsive parkinsonism and pathologically by Lewy bodies. Much as with Alzheimer disease, the number of people afflicted with the disease increases steadily by age group, affecting ~1% of the population at age 65 and up to 5% of the population by age 85. Although Lewy bodies are regarded as the hallmark lesion of Parkinson disease, they also occur in dementing disorders including dementia with Lewy bodies (DLB) and a subset of patients with Alzheimer disease pathology[43]. Little was known about the molecular composition of the filamentous Lewy body inclusions until 1997, when a point mutation was identified in *SNCA*, the gene that encodes α-synuclein, in two kindreds with autosomal dominant Parkinson disease[44]. Subsequently, two additional point mutations and triplication of *SNCA* were identified in kindreds with familial Parkinson disease and DLB[45–47]. Subsequent studies showed that α-synuclein is the primary building block of the 10-nm fibrils that form the neuronal intracytoplasmic Lewy bodies of both Parkinson disease and DLB (**Fig. 2**), as well as the glial cytoplasmic inclusions typical of multiple-system atrophy, thus redefining the molecular neuropathology of these disorders and delineating a new class of neurodegenerative diseases termed 'α-synucleinopathies'[43].

α-synuclein is a small, presynaptic protein without a well-defined function[43]. Similar to tau, the entire α-synuclein molecule undergoes a biophysical conformational change in α-synucleinopathies, acquiring a predominantly β-pleated sheet structure that facilitates polymerization of α-synuclein into amyloid fibrils. Although it is known that one of the mutations in *SNCA* increases the propensity of mutant α-synuclein to fibrillize, the mechanism(s) leading to the fibrillization of α-synuclein in sporadic Lewy body diseases are unknown. Environmental factors such as rotenone exposure may promote the formation of Lewy bodies, because rotenone-treated rats develop Lewy body–like α-synuclein inclusions[48]. Furthermore, oxidative injury may also facilitate the fibrillization of α-synuclein by either promoting fibril formation or stabilizing formed fibrils or their precursors, or both[49].

Before α-synuclein was identified as the predominant component of Lewy bodies, most models of Parkinson disease involved

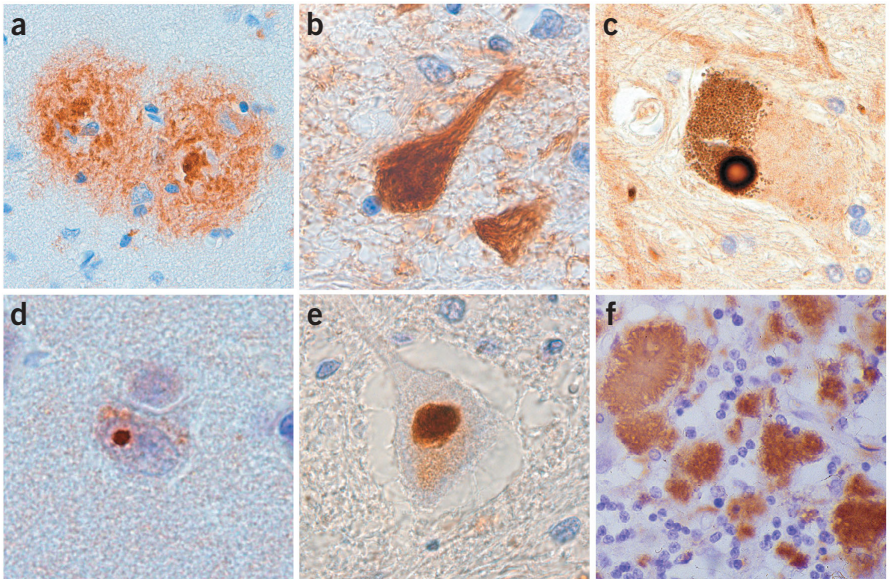

Figure 2 Protein aggregates in neurodegenerative disease. (**a**) Senile plaques in neocortex of Alzheimer disease. (**b**) NFTs in hippocampus of FTDP-17 (R406W mutation). (**c**) Lewy body in substantia nigra of Parkinson disease. (**d**) Intranuclear polyglutamine inclusion in neocortex of Huntington disease. (**e**) Ubiquitinylated inclusion in spinal cord motor neuron of ALS. (**f**) Protease-resistant PrP in cerebellum of CJD (panel **f** courtesy of Nigel Cairns).

either chemical or mechanical disruption of dopaminergic (or striatonigral) pathways[50]. Most of these models fail to recapitulate the Lewy body pathology of Parkinson disease, and, importantly, it is now known that Lewy body pathology and neurodegeneration are widespread throughout the brain rather than restricted to the striatonigral system. Numerous genetic models of Lewy body disease that involve overexpression of wild-type and mutant α-synuclein in both neurons and oligodendrocytes have been developed[50]. Although they are imperfect replicas of either Parkinson disease or DLB, transgenic mice expressing mutant α-synuclein recapitulate many of the features of Lewy body disease, including α-synuclein fibrillization and aggregation in association with neurodegeneration and progressive motor dysfunction. Furthermore, the expression of α-synuclein in Drosophilia leads to an age-dependent loss of a subset of dopaminergic neurons with L-dopa–responsive progressive motor dysfunction[29]. The overexpression or induction of the molecular chaperone hsp70 in α-synuclein transgenic flies prevents this neuron loss, implicating the misfolding or aggregation of α-synuclein in disease pathogenesis[51,52].

Although α-synuclein was the first molecule causally linked to familial Parkinson disease, numerous additional genetic loci were subsequently identified. For example, autosomal recessive mutations in *PARK2*, the gene encoding parkin, a putative E3 ligase, are responsible for up to 50% of juvenile and early-onset parkinsonism[53]. In addition, polymorphisms and possibly mutations in *UHCL1*, the gene that encodes ubiquitin carboxy-terminal hydrolase L1 (UCH-L1), are linked to Parkinson disease[54]. Both parkin and UCH-L1 function in the ubiquitination of proteins, possibly including α-synuclein, thus suggesting a general defect in the UPS in Parkinson disease[55]. Mutations were also identified in *PARK7*, the gene encoding DJ-1, a ubiquitous protein of unknown function, in autosomal recessive early-onset Parkinson disease, leading to aberrant cytoplasmic localization of DJ-1[56]. More recently, autosomal recessive mutations were identified in *PINK1* (the gene encoding PTEN-induced kinase 1), implicating mitochondrial dysfunction in Parkinson disease[57]. On autopsy, the majority of patients with *PARK2* mutations lack Lewy body pathology, and autopsy information is not available on patients with mutations in the genes encoding UCH-L1, DJ-1 and PTEN-induced kinase 1 (ref. 55). Thus, it is unclear whether these patients represent Lewy body disease or simply an overlapping clinical phenotype of genetically distinct disorders. Finally, some FTDP-17 patients present with parkinsonism, thereby implicating *MAPT* mutations in the pathogenesis of Parkinson disease–like disorders[35].

Polyglutamine repeat diseases

The polyglutamine repeat (polyQ) diseases encompass at least nine inherited neurodegenerative diseases, including Huntington disease, Kennedy disease (spinobulbar muscular atrophy or SBMA), dentatorubro-pallidoluysian atrophy and six forms of spinocerebellar ataxia (SCA1, 2, 3, 6, 7 and 17)[58]. In each of these autosomal dominant diseases (SBMA is X-linked), an overlapping but distinct topographic distribution of pathology is observed. But, in contrast to the tauopathies and α-synucleinopathies, no disease-specific brain lesions were recognized in the polyQ diseases before the identification of the candidate genes and their pathogenic expansions. In the early 1990s, a trinucleotide (CAG) repeat expansion that codes for long stretches of polyglutamine was identified in the gene encoding the androgen receptor of patients with SBMA[59]. Subsequently, similar expansions were identified in the coding region of seemingly unrelated genes, including those encoding huntingtin, atrophin-1 and ataxin-1, -2, -3 and -7 (ref. 58) (**Table 1**). Thus, trinucleotide expansions represent a new mechanism of neurodegenerative disease. The length of the normal polyglutamine tract is polymorphic, typically ranging from 10 to 36 glutamine residues, whereas in each of the polyQ diseases there is an unstable expansion beyond the normal range, with longer expansions correlating with an earlier onset and more severe disease.

The identification of the CAG expansion was soon followed by the demonstration that long polyglutamine tracts fibrillize *in vitro* to form aggregates with extensive β-pleated sheet structure similar to that observed in fibrillar Aβ and other types of amyloids[60]. This was closely followed by the generation of transgenic models that overexpressed either expanded polyglutamine tracts alone or in the context of full-length protein[61]. Although the expression of expanded polyglutamine alone was toxic, causing profound neuron death, the placement of the expanded glutamines in the appropriate protein context leads to slowly progressive cell dysfunction that more closely resembles the human diseases. Furthermore, the expanded polyglutamine stretches form ubiquitinylated intraneuronal nuclear inclusions[62]. These findings led to the identification of similar intranuclear inclusions in affected neurons of patients with a variety of polyQ diseases (**Fig. 2**)[63]. Thus, the animal models facilitated the identification of disease-defining pathological lesions. Notably, in both animal models and cell culture systems, the overexpression of specific heat-shock proteins reversed polyglutamine-induced toxicity similar to that observed in the a-synucleinopathies (**Fig. 1**)[64].

The role of the nuclear aggregates of mutant proteins in neurodegeneration remains controversial[65]. Thus, in some experimental systems, the toxicity of the expanded polyglutamine tracts can be dissociated from that of the nuclear aggregates. Moreover, whereas several studies indicated a requirement for nuclear localization of the mutant protein, others suggested cytoplasmic toxicity, including the induction of apoptotic pathways[66]. Within the nucleus, the expanded glutamine repeats may lead to a toxic gain of function such as impairment of the UPS[67]. Conversely, the polyglutamine repeats may affect normal protein function. For example, huntingtin and the androgen receptor are transcription factors. Thus, it is plausible that the expanded polyglutamine stretches perturb the interaction of these transcription factors with other DNA-binding proteins, and alter patterns of gene transcription, leading to the suggestion that these diseases are 'transcriptionopathies'[68]. Furthermore, disease-specific features and cell-type specificity could be explained by the retained functional capacity of a portion of the molecule. For instance, in SBMA the androgen receptor with the expanded polyglutamine tract can still bind its ligand. Despite these unresolved issues, the development of cell culture models that recapitulate polyglutamine-mediated toxicity distinguishes the polyQ diseases and facilitates the development of rational therapeutic strategies, many of which have already gone on to be tested in animal models.

Amyotrophic lateral sclerosis and superoxide dismutase

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease predominantly affecting upper and lower motor neurons, leading to muscle weakness, spasticity and atrophy. The disease prevalence is ~5 people per 100,000 and the risk of ALS increases by one order of magnitude after age 60. Although ALS was originally defined as a pure motor neuron disease, it is now clear that up to 50% of ALS patients manifest cognitive deficits, particularly in executive function, including many who meet clinical criteria for frontotemporal dementia[69]. Neuropathologically, ALS is characterized by motor neuron loss and gliosis, associated with ubiquitin-positive inclusions in a subset of residual neurons (**Fig. 2**). Similar inclusions are also observed in neurons of the frontal and temporal cortex, including the dentate gyrus of patients who clinically manifest frontotemporal dementia. But the

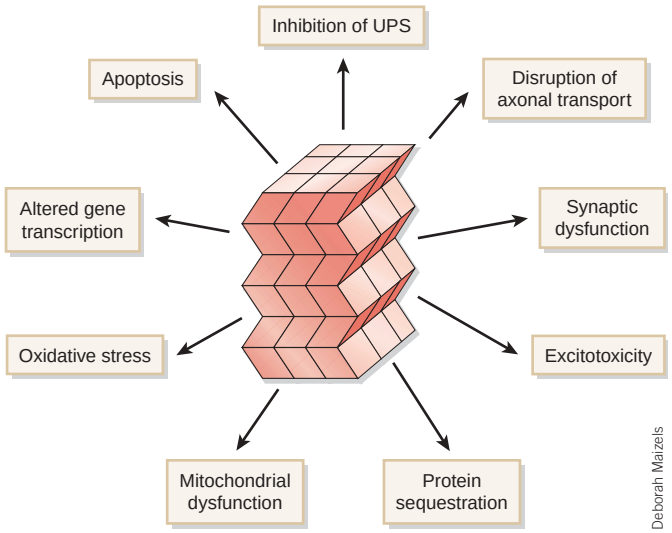

Figure 3 Potential mechanisms of toxicity of misfolded or aggregated proteins (depicted schematically in center).

major protein constituent of these inclusions remains unknown. And, even though ALS was initially described almost 150 years ago, a variety of hypotheses of disease pathogenesis are still actively pursued, including glutaminergic excitotoxicity, oxidative damage, defects in axonal transport resulting from disruption of the neurofilament network and toxicity due to intracellular protein aggregation[70]. This latter hypothesis is based on the detection of filamentous ubiquitinylated inclusions in surviving spinal motor neurons, as well as analysis of familial ALS patients. Thus, approximately 10% of ALS cases are familial, manifesting a variety of inheritance patterns with linkage to multiple independent chromosomal loci[70]. In the past 10 years, mutations in three genes have been identified. One gene is *ALS2*, encoding a guanine nucleotide exchange factor; mutations in this gene were identified in rare cases of autosomal recessive juvenile ALS[71,72]. Subsequently, mutations in the p150 subunit of the transporter protein dynactin were identified in a family with autosomal dominant lower motor neuron disease[73]. Mutations in dynein were also identified in a mouse model of motor neuron degeneration, and dynein interacts with dynactin in the regulation of retrograde transport, thus implicating the disruption of axonal transport in disease pathogenesis[74]. In addition, more than 90 mutations were identified in *SOD1*, the gene that encodes SOD1, a copper-zinc superoxide dismutase, and these account for ~20% of familial ALS[75].

SOD1 is a copper-dependent enzyme that catalyzes the conversion of superoxide radicals to hydrogen peroxide and water. Early data suggested that mutations might impair the anti-oxidant activity of SOD1. However, neurodegeneration was not observed in *SOD1* knockout animals. Rather, the generation of transgenic animals expressing mutant *SOD1* leads to motor neuron degeneration without reduction in *SOD1* activity[70,76]. Furthermore, in the spinal cords of ALS patients with mutations in *SOD1*, hyalinized inclusions are present that are immunopositive for SOD1[77]. Similar SOD1-containing aggregates are also detected in motor neurons and, in some cases, astrocytes of *SOD1* transgenic mice before the onset of clinical symptoms[77]. Interestingly, in chimeric mutant *SOD1* transgenic animals, protein expression in motor neurons was not sufficient for toxicity, but rather was dependent on expression of mutant protein in the surrounding glia, implicating the non-neuronal cells in the pathogenesis of ALS[78]. Similar to many neurodegenerative diseases, the SOD1 aggregates may be toxic through a variety of mechanisms, including induction of caspase-dependent apoptotic pathways and proteasomal inhibition[70,76]. The aggregates are associated with heat-shock proteins and the further upregulation of these heat-shock proteins can reduce aggregate formation and ameliorate toxicity. But in contrast to the diseases described above, SOD1 does not readily fibrillize *in vitro* and is not detected in the ubiquitinylated aggregates of sporadic ALS. Thus, the relationship of familial ALS to its sporadic counterpart remains unclear.

Creutzfeldt-Jakob and prion protein diseases

Prion protein (PrP) diseases are a heterogeneous group of disorders that manifest a variety of clinical features including dementia, psychiatric disturbances, myoclonus, insomnia and ataxia. Pathologically these disorders are linked by the accumulation of protease-resistant PrP in affected brain regions (**Fig. 2**)[79]. There are both sporadic PrP diseases, such as CJD, as well as familial forms resulting from a variety of mutations in the *PRNP* gene, including familial CJD, Gerstmann-Straussler-Scheinker disease and fatal familial insomnia[79]. These diseases are distinct from other neurodegenerative diseases by virtue of their transmissibility, as observed in iatrogenic CJD, kuru and, more recently, new-variant CJD, as well as nonhuman PrP disease variants affecting sheep (scrapie), goats and cattle (bovine spongiform encephalopathy). Of note, new-variant CJD has caused public health concerns owing to the zoonotic linkage to bovine spongiform encephalopathy through the introduction of contaminated meat into the human food chain[80].

A large body of data supports the hypothesis that the protease-resistant PrP (PrP^{SC} or PrP^{res}) is an abnormal structural conformation of the normal PrP (PrP^{C} or PrP^{Sen})[79,81]. PrP^{C} is a small, glycosylphosphatidylinositol-linked protein with ill-defined function, composed predominantly of α-helix and random coil structure. In contrast, PrP^{SC} is highly aggregated, with extensive β-sheet structure that results in a CNS-specific amyloidosis. Moreover, PrP^{SC} fibrils are resistant to proteolytic digestion. *In vitro*, PrP^{C} does not readily convert to PrP^{SC}; however, the conversion of PrP^{C} to PrP^{SC} may be induced by the addition of small quantities of PrP^{SC} (ref. 82). Similarly, in PrP-transgenic animal models, PrP^{C} rarely converts into PrP^{SC}. But the inoculation of PrP^{SC} into animals expressing species-matched PrP results in animals accumulating PrP^{SC} with associated spongiform change in the CNS[83]. But, with the exception of PrP^{P102L}, the generation of transgenic animals expressing familial mutations does not recapitulate the pathology of PrP disease, and PrP^{P102L} transgenic animals do not accumulate PrP^{SC} (ref. 83). Thus, the low rate of transmission with inoculation led to the proposal that a second protein, termed 'protein X,' is necessary for PrP^{SC} formation. Others still argue for alternative, non-PrP modes of disease pathogenesis[84,85]. Recently, *in vitro* production of recombinant mouse PrP^{SC} capable of disease transmission provided compelling evidence that prions are the infectious protein[86]. The precise mechanism of disease notwithstanding, the use of both *in vitro*, cell-free PrP conversion assays and chronically infected neuroblastoma cells has facilitated the screening of therapeutic agents that block conversion of PrP^{C} to PrP^{SC}, promote clearance and diminish neurotoxicity of PrP^{SC} (ref. 87).

The 'prion' concept has also been extended to yeast and fungal genetic elements[88]. These proteins are unrelated to PrP but can undergo a structural change to self-sustaining conformations that alter protein function and the cell phenotype. Moreover, the altered phenotypes are transferable from mother to daughter cell without any underlying nucleic acid change and, similar to the animal models of prion diseases, the abnormal protein can be isolated and generated *in*

vitro[89,90]. The subsequent propagation of the altered phenotype in yeast provides strong evidence for the 'protein-only' transmission of yeast prions. Because the yeast prions show no homology with the vertebrate PrP and do not cause pathology, the relevance of yeast prions to human prion diseases is unclear.

Protein misfolding and neurodegeneration—unanswered questions

There still remain many unanswered questions about the pathogenesis of neurodegeneration, including: how do misfolded or aggregated proteins lead to neurodegeneration? What is the toxic protein species and are the aggregates themselves toxic? What underlies the selective vulnerability of proteins that are often ubiquitously expressed throughout the brain?

Since their initial description almost 100 years ago, scientists have assumed that the protein aggregates and inclusions were the toxic species. The density of lesions formed by these aggregates is high in the brains of patients with many of these diseases such as Alzheimer disease, Lewy body disease and the tauopathies. Thus, it is difficult to imagine that this pathology, particularly when located in the cellular processes, would not affect biological functions such as cell trafficking and synaptic transmission. Furthermore, most of the familial mutations promote protein aggregation in both *in vitro* and *in vivo* model systems. But the density of pathological aggregates is relatively low in some of these disorders, including the polyQ diseases and ALS. And both senile plaques and NFTs are detected in many cognitively intact elderly individuals[91]. Furthermore, several animal models show cognitive deficits in the absence of aggregate formation[92]. Thus, it was proposed that the formation of aggregates may be neuroprotective[93,94]. For instance, in model systems of polyQ diseases, the formation of the intranuclear inclusions can be dissociated from the neuronal toxicity[95]. Preventing the formation of nuclear inclusions also caused enhanced polyglutamine toxicity, suggesting that aggregation facilitates protein clearance. In prion disease, recent data suggest that the PrP^{SC} can be distinguished from the neurotoxic species[96], whereas in the tauopathies, the overexpression of soluble tau species can impede axonal transport without protein aggregation, and defects in axonal transport are observed in several transgenic mouse models[28,97]. Others have proposed that small oligomers are the neurotoxic species leading to neuron dysfunction and degeneration[93]. Thus, oligomers of Aβ can inhibit long-term potentiation in adult rats[98]. In addition, the A30P mutation in α-synuclein promotes the formation of protofibrils, but slows their conversion to filaments *in vitro*[99].

Whether or not monomers, protofibrils or mature filaments formed by the disease protein are the important toxic moieties, the question of how misfolded species perturb cellular homeostasis must be addressed. Experimental evidence implicates a variety of mechanisms of toxicity, most of which are not mutually exclusive (**Fig. 3**)[100–102]. Whereas the UPS may have a role in limiting the formation and accumulation of misfolded proteins, disease-related protein aggregates may be toxic to the proteasome. The protein aggregates found in many neurodegenerative diseases may sequester normal cellular proteins, thus directly altering cellular physiology. This may be a nonspecific process because many of these protein aggregates are inherently sticky. But, as in the case of polyQ diseases, this may represent the sequestration of specific DNA-binding proteins, consequently altering the patterns of gene transcription. Conversely, the misfolded protein may produce a toxic gain of function by inducing excitotoxicity or promoting apoptotic pathways such as the induction of caspase 3, as demonstrated in models of polyQ disease, Alzheimer disease and ALS. In addition, the pathogenic proteins may have effects on neuron-specific processes, including alterations of synaptic function and disruption of axonal transport, particularly if aggregates form within cellular processes. Lastly, the toxic species may promote oxidative stress, mitochondrial dysfunction and inflammatory responses. These effects may in turn enhance the rate of protein aggregation, thus creating a self-propagating cycle of protein misfolding followed by cell toxicity leading to further protein aggregation. These mechanisms of toxicity do not explain the selective vulnerability of distinct cell types and brain regions observed in the various diseases. Recently, two groups demonstrated selective accumulation of mutant SOD1 in mitochondria of spinal cord, suggesting a molecular mechanism for the selective vulnerability observed in familial ALS[103,104]. These exciting findings notwithstanding, the complexity of selective vulnerability is highlighted by the *MAPT* mutations in FTDP-17, whereby the same mutation can lead to divergent topographic distributions of pathology, even in affected members of the same kindred[105]. But, although there are still many unanswered questions, these insights into the role of toxic proteins in neurodegeneration have provided a starting point for the rational approaches to drug development.

The next decade

The past 10 years were an extraordinary decade in neurodegenerative disease research, and for many of these diseases, the field has moved from neuropathologic descriptions to the generation of animal models to the elucidation of disease mechanisms, all by way of advances in the genetics of these disorders. And, in the process, a new mechanism of disease pathogenesis was defined. Specifically, it is increasingly evident that misfolded and aggregated disease proteins are not simply neuropathologic markers of neurodegenerative disorders but, instead, they almost certainly contribute to disease pathogenesis, thereby paving the way for the identification of rational therapeutic targets. Thus, there is a palpable sense of optimism among neurodegenerative disease investigators about near-term prospects for therapeutic advances in this field.

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COMPETING INTEREST STATEMENT

The authors declare that they have no competing financial interests.

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1. Alzheimer, A. Über eine eigenartige Eskrankung der Nirnrinde. *Allg. Z. Psychiatr. Psych.-Gerichtl.* **64**, 146–148 (1907).
2. Lewy, F. in *Handbuch der Neurologie*, **3**, 920–933. Springer Vertag, Berlin (1912).
3. Glenner, G.G. & Wong, C.W. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem. Biophys. Res. Commun.* **120**, 885–890 (1984).
4. Lee, V.M.-Y., Balin, B.J., Otvos, L., Jr. & Trojanowski, J.Q. A68: a major subunit of paired helical filaments and derivatized forms of normal Tau. *Science* **251**, 675–678 (1991).
5. Spillantini, M.G. *et al.* α-synuclein in Lewy bodies. *Nature* **388**, 839–840 (1997).
6. Prusiner, S.B. Novel proteinaceous infectious particles cause scrapie. *Science* **216**, 136–144 (1982).
7. World Health Organization. Active ageing: a policy framework. Second United Nations World Assembly on Aging, Madrid, Spain (World Health Organization,

Geneva, 2002). www.who.int/hpr/ageing/ActiveAgingPolicyFrame.pdf
8. Hebert, L.E., Scherr, P.A., Bienias, J.L., Bennett, D.A. & Evans, D.A. Alzheimer disease in the US population: prevalence estimates using the 2000 census. *Arch. Neurol.* **60**, 1119–1122 (2003).
9. Wancata, J., Musalek, M., Alexandrowicz, R. & Krautgartner, M. Number of dementia sufferers in Europe between the years 2000 and 2050. *Eur. Psychiatry* **18**, 306–313 (2003).
10. Hartl, F.U. & Hayer-Hartl, M. Molecular chaperones in the cytosol: from nascent chain to folded protein. *Science* **295**, 1852–1858 (2002).
11. Chartier-Harlin, M.C. *et al.* Early-onset alzheimer's disease caused by mutations at codon 717 of the β-amyloid precursor protein gene. *Nature* **353**, 844–846 (1991).
12. Rogaev, E.I. *et al.* Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. *Nature* **376**, 775–778 (1995).
13. Levy-Lahad, E. *et al.* Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science* **269**, 973–977 (1995).
14. Sherrington, R. *et al.* Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature* **375**, 754–760 (1995).
15. Goate, A. *et al.* Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature* **349**, 704–706 (1991).
16. De Strooper, B. & Annaert, W. Proteolytic processing and cell biological functions of the amyloid precursor protein. *J. Cell Sci.* **113**, 1857–1870 (2000).
17. Haass, C. & De Strooper, B. The presenilins in Alzheimer's disease—proteolysis holds the key. *Science* **286**, 916–919 (1999).
18. Price, D.L. & Sisodia, S.S. Mutant genes in familial Alzheimer's disease and transgenic models. *Annu. Rev. Neurosci.* **21**, 479–505 (1998).
19. De Strooper, B. Aph-1, Pen-2, and nicastrin with presenilin generate an active γ-secretase complex. *Neuron* **38**, 9–12 (2003).
20. Hardy, J. & Selkoe, D.J. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* **297**, 353–356 (2002).
21. Corder, E.H. *et al.* Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* **261**, 921–923 (1993).
22. Bales, K.R. *et al.* Lack of apolipoprotein E dramatically reduces amyloid β-peptide deposition. *Nat. Genet.* **17**, 263–264 (1997).
23. Poirier, J. Apolipoprotein E and cholesterol metabolism in the pathogenesis and treatment of Alzheimer's disease. *Trends Mol. Med.* **9**, 94–101 (2003).
24. Puglielli, L., Tanzi, R.E. & Kovacs, D.M. Alzheimer's disease: the cholesterol connection. *Nat. Neurosci.* **6**, 345–351 (2003).
25. Tanzi, R.E. & Bertram, L. New frontiers in Alzheimer's disease genetics. *Neuron* **32**, 181–184 (2001).
26. Weggen, S. *et al.* A subset of NSAIDs lower amyloidogenic Aβ42 independently of cyclooxygenase activity. *Nature* **414**, 212–216 (2001).
27. Launer, L. Nonsteroidal anti-inflammatory drug use and the risk for Alzheimer's disease: dissecting the epidemiological evidence. *Drugs* **63**, 731–739 (2003).
28. Götz, J. *et al.* Transgenic animal models of Alzheimer's disease and related disorders: histopathology, behavior and therapy. *Mol. Psychiatry* **9**, 664–683 (2004).
29. Bonini, N.M. & Fortini, M.E. Human neurodegenerative disease modeling using Drosophila. *Annu. Rev. Neurosci.* **26**, 627–656 (2003).
30. Götz, J., Chen, F., Van Dorpe, J. & Nitsch, R.M. Formation of neurofibrillary tangles in P301L tau transgenic mice induced by Aβ42 fibrils. *Science* **293**, 1491–1495 (2001).
31. Lewis, J. *et al.* Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. *Science* **293**, 1487–1491 (2001).
32. Oddo, S., Billings, L., Kesslak, J.P., Cribbs, D.H. & LaFerla, F.M. Aβ immunotherapy leads to clearance of early, but not late, hyperphosphorylated tau aggregates via the proteasome. *Neuron* **43**, 321–332 (2004).
33. Monsonego, A. & Weiner, H.L. Immunotherapeutic approaches to Alzheimer's disease. *Science* **302**, 834–838 (2003).
34. Oddo, S., Billings, L., Kesslak, J.P., Cribbs, D.H. & LaFerla, F.M. Aβ immunotherapy leads to clearance of early, but not late, hyperphosphorylated tau aggregates via the proteasome. *Neuron* **43**, 321–332 (2004).
35. Lee, V.M.-Y., Goedert, M. & Trojanowski, J.Q. Neurodegenerative tauopathies. *Ann. Rev. Neurosci.* **24**, 1121–1159 (2001).
36. Hutton, M. *et al.* Association of missense and 5′-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature* **393**, 702–705 (1998).
37. Poorkaj, P. *et al.* Tau is a candidate gene for chromosome 17 frontotemporal dementia. *Ann. Neurol.* **43**, 815–825 (1998).
38. Spillantini, M.G. *et al.* Mutation in the tau gene in familial multiple system tauopathy with presenile dementia. *Proc. Natl. Acad. Sci. USA* **95**, 7737–7741 (1998).
39. Billingsley, M.L. & Kincaid, R.L. Regulated phosphorylation and dephosphorylation of tau protein: effects on microtubule interaction, intracellular trafficking and neurodegeneration. *Biochem. J.* **323**, 577–591 (1997).
40. Yen, S.H., Hutton, M., DeTure, M., Ko, L.W. & Nacharaju, P. Fibrillogenesis of tau: insights from tau missense mutations in FTDP-17. *Brain Pathol.* **9**, 695–705 (1999).
41. Wittmann, C.W. *et al.* Tauopathy in Drosophila: neurodegeneration without neurofibrillary tangles. *Science* **293**, 711–714 (2001).
42. Giasson, B.I. *et al.* Initiation and synergistic fibrillization of tau and α-synuclein. *Science* **300**, 636–640 (2003).
43. Goedert, M. α-synuclein and neurodegenerative diseases. *Nat. Rev. Neurosci.* **2**, 492–501 (2001).
44. Polymeropoulos, M.H. *et al.* Mutation in the α-synuclein gene identified in families with Parkinson's disease. *Science* **276**, 2045–2047 (1997).
45. Kruger, R. *et al.* Ala30Pro mutation in the gene encoding α-synuclein in Parkinson's disease. *Nat. Genet.* **18**, 106–108 (1998).
46. Singleton, A.B. *et al.* α-Synuclein locus triplication causes Parkinson's disease. *Science* **302**, 841 (2003).
47. Zarranz, J.J. *et al.* The new mutation, E46K, of α-synuclein causes Parkinson and Lewy body dementia. *Ann. Neurol.* **55**, 164–173 (2004).
48. Betarbet, R. *et al.* Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat. Neurosci.* **3**, 1301–1306 (2000).
49. Ischiropoulos, H. Oxidative modifications of α-synuclein. *Ann. N.Y. Acad. Sci.* **991**, 93–100 (2003).
50. Maries, E., Dass, B., Collier, T.J., Kordower, J.H. & Steece-Collier, K. The role of α-synuclein in Parkinson's disease: insights from animal models. *Nat. Rev. Neurosci.* **4**, 727–738 (2003).
51. Auluck, P.K. & Bonini, N.M. Pharmacological prevention of Parkinson disease in Drosophila. *Nat. Med.* **8**, 1185–1186 (2002).
52. Auluck, P.K., Chan, H.Y., Trojanowski, J.Q., Lee, V.M.-Y. & Bonini, N.M. Chaperone suppression of α-synuclein toxicity in a Drosophila model for Parkinson's disease. *Science* **295**, 865–868 (2002).
53. Kitada, T. *et al.* Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* **392**, 605–608 (1998).
54. Leroy, E. *et al.* The ubiquitin pathway in Parkinson's disease. *Nature* **395**, 451–452 (1998).
55. Giasson, B.I. & Lee, V.M.-Y. Are ubiquitination pathways central to Parkinson's disease? *Cell* **114**, 1–8 (2003).
56. Bonifati, V. *et al.* Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. *Science* **299**, 256–259 (2003).
57. Valente, E.M. *et al.* Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science* **304**, 1158–1160 (2004).
58. Zoghbi, H.Y. & Orr, H.T. Glutamine repeats and neurodegeneration. *Annu. Rev. Neurosci.* **23**, 217–247 (2000).
59. La Spada, A.R., Wilson, E.M., Lubahn, D.B., Harding, A.E. & Fischbeck, K.H. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature* **352**, 77–79 (1991).
60. Scherzinger, E. *et al.* Huntingtin-encoded polyglutamine expansions form amyloid-like protein aggregates *in vitro* and *in vivo*. *Cell* **90**, 549–558 (1997).
61. Lin, X., Cummings, C.J. & Zoghbi, H.Y. Expanding our understanding of polyglutamine diseases through mouse models. *Neuron* **24**, 499–502 (1999).
62. Skinner, P.J. *et al.* Ataxin-1 with an expanded glutamine tract alters nuclear matrix-associated structures. *Nature* **389**, 971–974 (1997).
63. Ross, C.A. Intranuclear neuronal inclusions: a common pathogenic mechanism for glutamine-repeat neurodegenerative diseases? *Neuron* **19**, 1147–1150 (1997).
64. Bonini, N.M. Chaperoning brain degeneration. *Proc. Natl. Acad. Sci. USA* **99**, 16407–16411 (2002).
65. Ross, C.A. Polyglutamine pathogenesis: emergence of unifying mechanisms for Huntington's disease and related disorders. *Neuron* **35**, 819–822 (2002).
66. Lipinski, M.M. & Yuan, J. Mechanisms of cell death in polyglutamine expansion diseases. *Curr. Opin. Pharmacol.* **4**, 85–90 (2004).
67. Bence, N.F., Sampat, R.M. & Kopito, R.R. Impairment of the ubiquitin-proteasome system by protein aggregation. *Science* **292**, 1552–1555 (2001).
68. La Spada, A.R. & Taylor, J.P. Polyglutamines placed into context. *Neuron* **38**, 681–684 (2003).
69. Lomen-Hoerth, C. *et al.* Are amyotrophic lateral sclerosis patients cognitively normal? *Neurology* **60**, 1094–1097 (2003).
70. Bruijn, L.I., Miller, T.M. & Cleveland, D.W. Unraveling the mechanisms involved in motor neuron degeneration in ALS. *Annu. Rev. Neurosci.* **27**, 723–749 (2004).
71. Hadano, S. *et al.* A gene encoding a putative GTPase regulator is mutated in familial amyotrophic lateral sclerosis 2. *Nat. Genet.* **29**, 166–173 (2001).
72. Yang, Y. *et al.* The gene encoding alsin, a protein with three guanine-nucleotide exchange factor domains, is mutated in a form of recessive amyotrophic lateral sclerosis. *Nat. Genet.* **29**, 160–165 (2001).
73. Puls, I. *et al.* Mutant dynactin in motor neuron disease. *Nat. Genet.* **33**, 455–456 (2003).
74. Hafezparast, M. *et al.* Mutations in dynein link motor neuron degeneration to defects in retrograde transport. *Science* **300**, 808–812 (2003).
75. Rosen, D.R. *et al.* Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* **362**, 59–62 (1993).
76. Julien, J.P. Amyotrophic lateral sclerosis. unfolding the toxicity of the misfolded. *Cell* **104**, 581–591 (2001).
77. Bruijn, L.I. *et al.* Aggregation and motor neuron toxicity of an ALS-linked SOD1 mutant independent from wild-type SOD1. *Science* **281**, 1851–1854 (1998).
78. Clement, A.M. *et al.* Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. *Science* **302**, 113–117 (2003).
79. Prusiner, S.B., Scott, M.R., DeArmond, S.J. & Cohen, F.E. Prion protein biology. *Cell* **93**, 337–348 (1998).
80. Collinge, J. Variant Creutzfeldt-Jakob disease. *Lancet* **354**, 317–323 (1999).
81. Collins, S.J., Lawson, V.A. & Masters, C.L. Transmissible spongiform encephalopathies. *Lancet* **363**, 51–61 (2004).
82. Kocisko, D.A. *et al.* Cell-free formation of protease-resistant prion protein. *Nature* **370**, 471–474 (1994).
83. Weissmann, C. & Flechsig, E. PrP knock-out and PrP transgenic mice in prion research. *Br. Med. Bull.* **66**, 43–60 (2003).
84. Telling, G.C. *et al.* Prion propagation in mice expressing human and chimeric PrP transgenes implicates the interaction of cellular PrP with another protein. *Cell* **83**, 79–90 (1995).

85. Chesebro, B. BSE and prions: uncertainties about the agent. *Science* **279**, 42–43 (1998).
86. Legname, G. *et al.* Synthetic mammalian prions. *Science* **305**, 673–676 (2004).
87. Brown, P. Drug therapy in human and experimental transmissible spongiform encephalopathy. *Neurology* **58**, 1720–1725 (2002).
88. Uptain, S.M. & Lindquist, S. Prions as protein-based genetic elements. *Annu. Rev. Microbiol.* **56**, 703–741 (2002).
89. King, C.Y. & Diaz-Avalos, R. Protein-only transmission of three yeast prion strains. *Nature* **428**, 319–323 (2004).
90. Tanaka, M., Chien, P., Naber, N., Cooke, R. & Weissman, J.S. Conformational variations in an infectious protein determine prion strain differences. *Nature* **428**, 323–328 (2004).
91. Knopman, D.S. *et al.* Neuropathology of cognitively normal elderly. *J. Neuropathol. Exp. Neurol.* **62**, 1087–1095 (2003).
92. Koistinaho, M. *et al.* Specific spatial learning deficits become severe with age in β-amyloid precursor protein transgenic mice that harbor diffuse β-amyloid deposits but do not form plaques. *Proc. Natl. Acad. Sci. USA* **98**, 14675–14680 (2001).
93. Caughey, B. & Lansbury, P.T. Protofibrils, pores, fibrils, and neurodegeneration: separating the responsible protein aggregates from the innocent bystanders. *Annu. Rev. Neurosci.* **26**, 267–298 (2003).
94. Sisodia, S.S. Nuclear inclusions in glutamine repeat disorders: are they pernicious, coincidental, or beneficial? *Cell* **95**, 1–4 (1998).
95. Klement, I.A. *et al.* Ataxin-1 nuclear localization and aggregation: role in polyglutamine-induced disease in SCA1 transgenic mice. *Cell* **95**, 41–53 (1998).
96. Ma, J., Wollmann, R. & Lindquist, S. Neurotoxicity and neurodegeneration when PrP accumulates in the cytosol. *Science* **298**, 1781–1785 (2002).
97. Stamer, K., Vogel, R., Thies, E., Mandelkow, E. & Mandelkow, E.M. Tau blocks traffic of organelles, neurofilaments, and APP vesicles in neurons and enhances oxidative stress. *J. Cell Biol.* **156**, 1051–1063 (2002).
98. Walsh, D.M. *et al.* Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation *in vivo*. *Nature* **416**, 535–539 (2002).
99. Conway, K.A., Harper, J.D. & Lansbury, P.T. Accelerated *in vitro* fibril formation by a mutant α-synuclein linked to early-onset Parkinson disease. *Nat. Med.* **4**, 1318–1320 (1998).
100. Taylor, J.P., Hardy, J. & Fischbeck, K.H. Toxic proteins in neurodegenerative disease. *Science* **296**, 1991–1995 (2002).
101. Selkoe, D.J. Folding proteins in fatal ways. *Nature* **426**, 900–904 (2003).
102. Wolozin, B. & Behl, C. Mechanisms of neurodegenerative disorders: Part 1: protein aggregates. *Arch. Neurol.* **57**, 793–796 (2000).
103. Liu, J. *et al.* Toxicity of familial ALS-linked SOD1 mutants from selective recruitment to spinal mitochondria. *Neuron* **43**, 5–17 (2004).
104. Pasinelli, P. *et al.* Amyotrophic lateral sclerosis-associated SOD1 mutant proteins bind and aggregate with Bcl-2 in spinal cord mitochondria. *Neuron* **43**, 19–30 (2004).
105. Forman, M.S. Genotype-phenotype correlations in FTDP-17: does form follow function? *Exp. Neurol.* **187**, 229–234 (2004).