

Focus Quality Control

The two faces of protein misfolding: gain- and loss-of-function in neurodegenerative diseases

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The etiologies of neurodegenerative diseases may be diverse; however, a common pathological denominator is the formation of aberrant protein conformers and the occurrence of pathognomonic proteinaceous deposits. Different approaches coming from neuropathology, genetics, animal modeling and biophysics have established a crucial role of protein misfolding in the pathogenic process. However, there is an ongoing debate about the nature of the harmful proteinaceous species and how toxic conformers selectively damage neuronal populations. Increasing evidence indicates that soluble oligomers are associated with early pathological alterations, and strikingly, oligomeric assemblies of different disease-associated proteins may share common structural features. A major step towards the understanding of mechanisms implicated in neuronal degeneration is the identification of genes, which are responsible for familial variants of neurodegenerative diseases. Studies based on these disease-associated genes illuminated the two faces of protein misfolding in neurodegeneration: a gain of toxic function and a loss of physiological function, which can even occur in combination. Here, we summarize how these two faces of protein misfolding contribute to the pathomechanisms of Alzheimer's disease, frontotemporal lobar degeneration, Parkinson's disease and prion diseases.

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Oligomeric protein assemblies in a deadly cascade

The most frequent neurodegenerative disorder is Alzheimer's disease (AD). Major progress has been made in this field due to the identification of the deposited amyloidogenic proteins and genetically linked mutations, which accelerate amyloid formation and disease onset (Haass and Selkoe, 2007). Two major proteins are found within the pathological hallmarks of AD. Amyloid- β peptide ($A\beta$) is deposited in amyloid plaques within the parenchyma and tau in neurofibrillary tangles within neurons. It is now becoming more and more clear that both proteins are required to confer neurotoxicity in a process called the amyloid cascade (Hardy and Selkoe, 2002; Figure 1A). $A\beta$ is derived by proteolytic processing from the β -amyloid precursor protein (β APP, see below) and exists as several species of distinct lengths (Haass, 2004). The most abundant 40 amino-acid species ($A\beta_{40}$) is rather benign, whereas the less abundant 42 amino-acid variant ($A\beta_{42}$) aggregates much faster and is therefore directly related to disease pathology (for a review, see Haass and Selkoe, 2007). However, it is currently unclear how the addition of the two amino acids at the C-terminus of $A\beta$ changes the biophysical properties of the peptide in a way that it aggregates faster. The amyloid cascade is probably initiated by subtle changes in the $A\beta_{42}/A\beta_{40}$ ratio, a total increase of $A\beta$ generation, or reduced clearance of $A\beta$ (Figure 1A). There is strong genetic evidence that specifically the $A\beta_{42}/A\beta_{40}$ ratio is affected by mutations in one of the $A\beta$ -generating enzymes (see below) and the β APP substrate itself (Haass, 2004). In addition, environmental factors and specifically genetic predisposition such as the ApoE status can influence disease onset (Figure 1A; Martins *et al*, 2006). All $A\beta$ species are secreted from healthy neurons throughout life, but specifically the longer $A\beta_{42}$ species tends to form soluble oligomers (Haass and Selkoe, 2007). These *in vivo* generated oligomeric assemblies can be as small as dimers or trimers (Podlisny *et al*, 1995) or as large as dodecamers (Lesne *et al*, 2006). Probably, long before these oligomers are deposited in disease-characterizing pathological plaques, they inhibit the maintenance of long-term potentiation (Walsh *et al*, 2002). This by itself may lead to mild cognitive impairment (MCI) at early stages of AD (Haass and Selkoe, 2007). One should keep in mind that brains of MCI patients may already contain some deposits, so it may be difficult to conclude that exclusively soluble $A\beta$ oligomers are involved in early memory loss. However, the effects of $A\beta$ oligomers on long-term potentiation were observed in rats lacking any amyloid deposits, making it likely that such soluble oligomers could indeed be responsible for the very first symptoms of memory loss in human patients. Inflammatory responses involving microglia and astrocytes follow the deposition of $A\beta$ and may enhance progressive

synaptic and neuronal injury. As a result, ion homeostasis may be affected and oxidative stress occurs. Strikingly, these events then directly can affect tau metabolism (Blurton-Jones and Laferla, 2006). Tau, a microtubule-binding protein, is

required for microtubule stabilization (Mandelkow *et al*, 2007). Misphosphorylation of tau, which is a pathological signature of all AD cases, reduces binding of tau to microtubules, which then detaches. Unbound tau is apparently

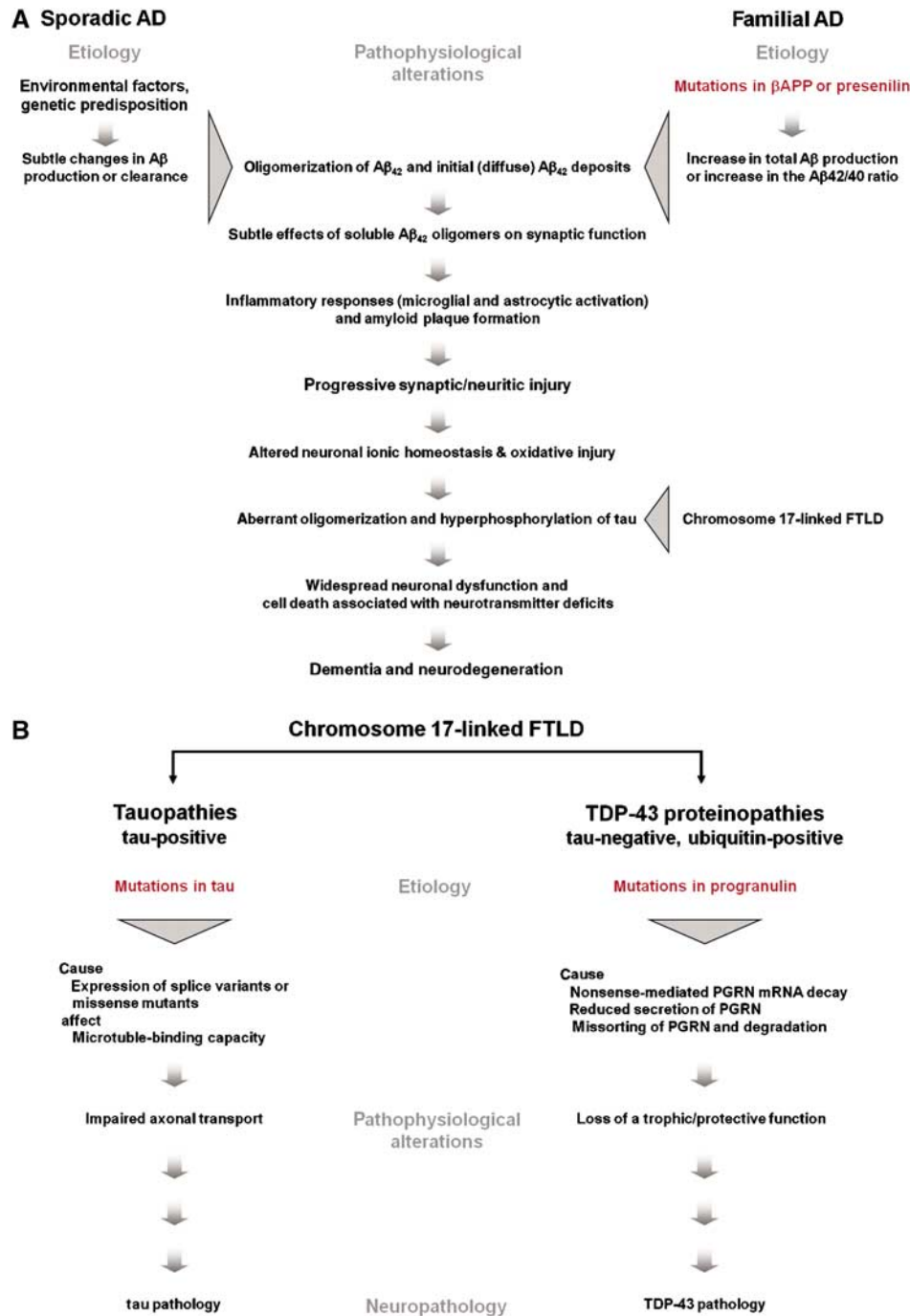


Figure 1 Pathomechanisms in AD (A), FTLD linked to chromosome 17 (B), PD (C) and prion diseases (D). (A) In AD, environmental factors, genetic predisposition and mutations in βAPP and PS can affect the metabolism of Aβ. Initially, small and soluble oligomeric assemblies of Aβ₄₂ are produced, which then cause synaptic dysfunction as well as an induction of the amyloid cascade. Note the ‘shortcut’ to tau pathology and FTLD via chromosome 17-linked tau mutations. (B) The major variants of chromosome 17-linked FTLD. On the left panel, FTLD cases with tau-positive inclusions (tauopathies) are described. On the right panel, the tau-negative, ubiquitin-positive cases are shown. (C) In sporadic PD and familial PD there are common pathophysiological alterations, such as oxidative stress, mitochondrial dysfunction and protein misfolding, which ultimately result in the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta. (D) In the classical form of prion diseases, conversion of PrP^C to PrP^{Sc} leads to a neurodegenerative and infectious disorder. The conformational transition can occur spontaneously (sporadic), or can be induced by invading PrP^{Sc} (acquired) or mutations (inherited). Transgenic mouse models indicated that expression of mutant PrPs can trigger neurodegeneration in the absence of infectious prion propagation; whether such disease entities exist in animals or humans is unknown. PrP^{Sc}: self-propagating isoform, essential component of infectious prions; CtmPrP: a transmembrane form of PrP with the C-terminus facing the cytosol; cytoPrP: cytosolically localized PrP; PG14PrP: mutant PrP containing a nine octarepeat insertion; PrPΔHD: mutant PrP lacking the internal HD.

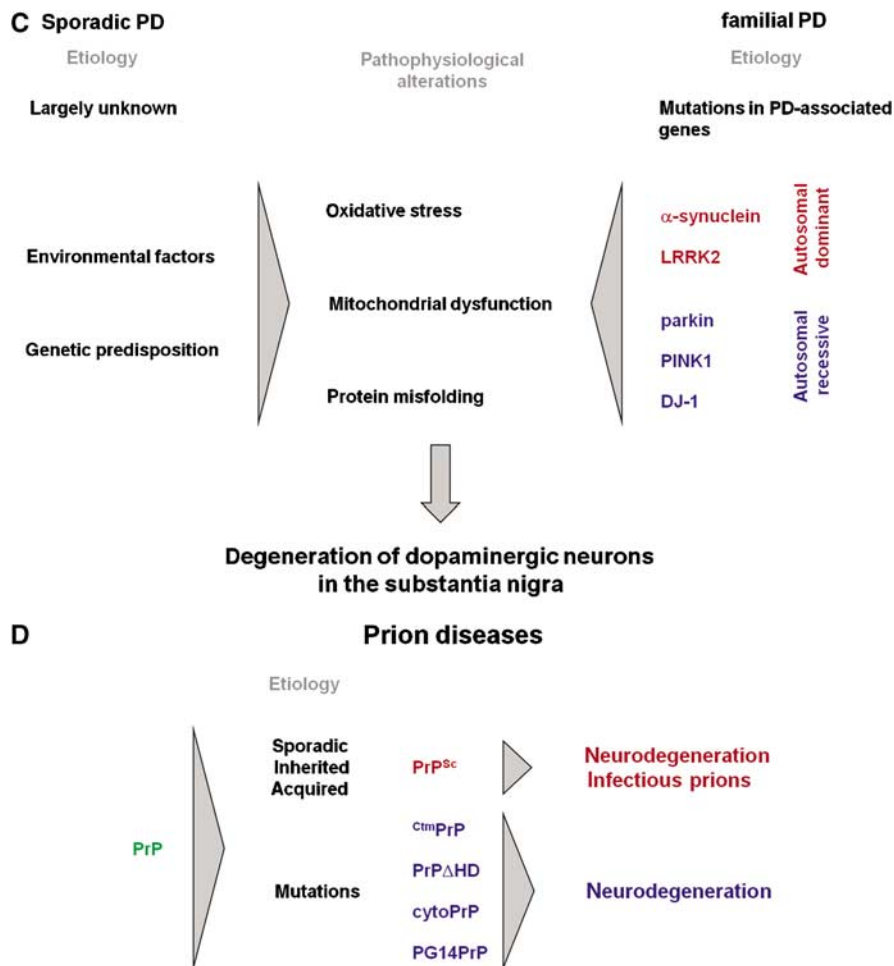


Figure 1 Continued.

misfolded and begins to aggregate due to its enhanced free concentration in the cytoplasm. Finally, hyperphosphorylated tau forms the paired helical filaments found within tangles (Mandelkow and Mandelkow, 1998; Ballatore *et al*, 2007). A strong support of a direct induction of tau pathology by A β came from the observation that pathological phosphorylation of tau is induced by the intracerebral injection of A β 42 fibrils (Gotz *et al*, 2001). Moreover, double transgenic mice expressing a tau mutation, together with mutant APP, showed enhanced tau pathology (Lewis *et al*, 2001). Strikingly, Oddo *et al* (2003) demonstrated that A β accumulation precedes tau pathology by several months in transgenic mouse models of AD. Consistent with these findings, a reduction of A β by an anti-A β vaccination strategy (Haass and Selkoe, 2007) in brains of transgenic mice reduces tau pathology. Importantly, under these conditions, early tau pathology is selectively reduced, whereas late tau pathology is apparently not affected (Oddo *et al*, 2004). Recent evidence further supported the connection between A β and tau within the amyloid cascade by demonstrating that memory deficits can be prevented in a transgenic model for AD pathology upon removal of the tau gene (Roberson *et al*, 2007). Thus, there is culminating evidence that A β is at the beginning of the amyloid cascade and initiates tau mislocalization, misfolding and toxicity. Plaques may serve as reservoirs for a continuing supply of soluble oligomers of A β , which can diffuse and cause neuronal dysfunction and cell

death even far away from amyloid deposits (Haass and Selkoe, 2007). Thus, a direct correlation of the amyloid plaque load with memory loss is not necessarily to be expected. The same may be true for tau, as oligomeric species composed of 8–14 tau molecules have been implicated in neurotoxicity (Wille *et al*, 1992).

Interestingly, in the absence of amyloid toxicity aggregation of tau can result in a different neurodegenerative disorder, named frontotemporal lobar degeneration (FTLD) (Figure 1B). In other words, abnormal tau by itself can induce a ‘shortcut’ within the above-described amyloid cascade (Figure 1A and B). The chromosome 17-linked FTLD cases, which are characterized by tau-positive inclusions (tangles), are caused by mutations within the tau gene (Ballatore *et al*, 2007; Mandelkow *et al*, 2007). These mutations affect tau function by several distinct mechanisms. Some mutations change the splicing pattern of tau and lead to the accumulation of the four repeat variant (Hutton *et al*, 1998). The change in the ratio from four-repeat to three-repeat tau is apparently directly related to its neurotoxic propensity and its ability to cause neurodegeneration, but it is currently not known how it affects neuronal viability. Other mutations within tau do not affect splicing but are missense mutations causing single-amino-acid exchanges. These seem to affect the microtubule-binding capacity of tau and/or increase its aggregation (Mandelkow *et al*, 2007).

Gain of neurotoxic and loss of protective function in combination?

Deposition of aggregated α -synuclein in Lewy bodies and Lewy neurites is a pathological hallmark of Parkinson's disease (PD) (Figure 1C) and some other neurodegenerative entities, collectively termed α -synucleinopathies. The physiological function of α -synuclein, which is abundantly expressed in the central nervous system, is not fully understood. Its enrichment in presynaptic terminals and its association with vesicles suggests a role of α -synuclein in synaptic dynamics. In a manner similar to tau, α -synuclein is a natively unfolded or intrinsically disordered protein with considerable conformational plasticity (for reviews, see Volles and Lansbury, 2003; Beyer, 2007; Uversky, 2007). *In vitro*, different α -synuclein conformers can be populated: monomers, which adopt a N-terminal α -helical structure upon membrane binding, morphologically diverse β -sheet-rich oligomers, called protofibrils, amorphous aggregates and amyloid fibrils with a characteristic cross- β structure. Three α -synuclein missense mutations as well as genomic multiplications promote the propensity of α -synuclein to aggregate and are associated with autosomal dominant PD (Polymeropoulos *et al*, 1997; Kruger *et al*, 1998; Singleton *et al*, 2003; Chartier-Harlin *et al*, 2004; Farrer *et al*, 2004; Ibanez *et al*, 2004; Zarranz *et al*, 2004). As in the case of A β , it is currently discussed that not the final aggregates, but rather oligomeric intermediates might be the toxic species (for review, see Lansbury and Lashuel, 2006; Haass and Selkoe, 2007; Uversky, 2007). Remarkably, dopamine can modify the aggregation pathway of α -synuclein, facilitating the formation of oligomeric intermediates (Conway *et al*, 2001; Li *et al*, 2004; Cappai *et al*, 2005; Norris *et al*, 2005; Mazzulli *et al*, 2006). In support of a role of dopamine in enhancing the toxic potential of α -synuclein, inhibition of dopamine synthesis blocked cell death induced by α -synuclein overexpression in a cell culture model (Xu *et al*, 2002). Various mechanisms have been proposed to explain the toxic effects of α -synuclein based on observations in different model systems (for a review, see Cookson and van der Brug, 2007), including impairment of proteasomal or lysosomal protein degradation (Stefanis *et al*, 2001; Tanaka *et al*, 2001; Petrucelli *et al*, 2002; Snyder *et al*, 2003; Cuervo *et al*, 2004; Lindersson *et al*, 2004), induction of endoplasmic reticulum (ER) stress (Smith *et al*, 2005; Cooper *et al*, 2006), Golgi fragmentation (Gosavi *et al*, 2002), sequestration of antiapoptotic proteins into aggregates (Xu *et al*, 2002) and the formation of pores on cellular membranes (Volles *et al*, 2001; Lashuel *et al*, 2002). Basic insight into early events of α -synuclein toxicity came from a recent study in *Saccharomyces cerevisiae*. Overexpression of wild-type or mutant α -synuclein resulted in defective ER-to-Golgi vesicular transport, and Rab1, a small GTPase identified in a screen for modifiers of α -synuclein toxicity, protected against neuronal loss in some animal models (*Drosophila melanogaster*, *Caenorhabditis elegans*) and in primary cultures of rat midbrain neurons (Cooper *et al*, 2006). Furthermore, Sept4, a presynaptic scaffold protein, has recently been shown to suppress α -synuclein toxicity in a transgenic mouse model (Ihara *et al*, 2007). Clearly, model systems to study the toxicity of α -synuclein have intrinsic limitations, and to discriminate causal events from secondary effects is a difficult task.

However, it seems plausible that more than one single mechanism contributes to the complex pathogenesis of α -synucleinopathies, which proceeds over decades in patients. To add another layer of complexity, α -synuclein may also play a neuroprotective role (for a review, see Lee *et al*, 2006). Transgenic expression of α -synuclein has been shown to prevent neurodegeneration caused by the deletion of cysteine-string protein- α , a molecular chaperone that is crucial for folding and refolding of synaptic SNARE proteins (Chandra *et al*, 2005). If and how the physiological activity of α -synuclein is coupled to its pathological potential and which conformers constitute the functional or toxic species is still a challenge for future research.

A deadly and infectious variant of protein misfolding

Protein misfolding can also lead to neurotoxicity and infectivity. The concept that a conformational transition of the cellular prion protein (PrP^C) to the pathological prion protein (PrP^{Sc} for scrapie PrP) implicates the formation of a neurotoxic conformer that in addition is infectious, is unprecedented (Prusiner, 1982; Figure 1D). After it had been proposed that heritable infectious proteins are responsible for mammalian prion diseases, studies in fungi indicated that self-propagating protein conformers might be of broader biological significance (Wickner, 1994). In prion disease research, numerous studies have been focused on the enigmatic composition of the infectious agent. A conclusive answer is still missing partly due to the fact that the purest infectious preparation (prion rods) still contains components in addition to PrP^{Sc}. Nucleic acids longer than 25 nucleotides can definitely be excluded as essential components for infectivity (Safar *et al*, 2005), but a possible role of the polysaccharide scaffold, which accounts for 5–15% of prion rods, remains to be established (Dumpitak *et al*, 2005). The first successful attempts to generate infectivity *in vitro* with recombinantly expressed PrP were reported, but with extremely low infectious titer, and PrP^C-overexpressing mice were used for the bioassays (Legname *et al*, 2004, 2005). Even if infectivity is entirely deciphered in the conformation of PrP^{Sc}, it seems plausible that auxiliary components can significantly modulate various aspects in the pathogenesis of prion diseases (for a review, see Caughey and Baron, 2006).

At present, there is only little understanding of how PrP^{Sc} or neurotoxic PrP mutants cause neurodegeneration (for review, see Hunter, 2006). Interestingly, expression of PrP^C in neuronal cells seems to be required to mediate neurotoxic effects of PrP^{Sc} (Brandner *et al*, 1996; Mallucci *et al*, 2003; Chesebro *et al*, 2005). Neurotoxicity of PrP^{Sc} could be linked to its propagation in neuronal cells, or PrP^{Sc} might elicit a deadly signal through a PrP^C-dependent signaling pathway. Indeed, these observations might provide a link to the physiological function of PrP^C. A stress-protective activity of PrP^C was first observed in cell culture experiments with primary neurons (Kuwahara *et al*, 1999). It was then shown that PrP^C-knockout mice display enlarged infarct volumes after ischemic brain injury and an increased sensitivity to kainate-induced seizures (Walz *et al*, 1999; McLennan *et al*, 2004; Shyu *et al*, 2005; Spudich *et al*, 2005; Weise *et al*, 2006; Mitteregger *et al*, 2007; Rangel *et al*, 2007). In one study, the neuroprotective activity of PrP^C was linked to the octarepeat

region, located within the unstructured N-terminal domain (Mitteregger *et al*, 2007). Based on these and other studies with established cell lines, it is plausible to propose that PrP^C can modulate signaling cascades, in particular stress-protective pathways (for review, see Flechsig and Weissmann, 2004; Roucou and LeBlanc, 2005; Westergard *et al*, 2007). Moreover, a loss of PrP^C function could be implicated in the pathogenesis of prion diseases and PrP^C-dependent pathways might be involved in neurotoxic signaling. For example, *in vivo* crosslinking of PrP^C by antibodies triggered neuronal apoptosis (Solforosi *et al*, 2004) and PrP^C-dependent receptors were postulated to explain neurotoxic effects of a PrP mutant lacking the hydrophobic domain (HD) (see below).

Transgenic mouse models revealed that several aberrant conformers of PrP distinct from PrP^{Sc} can induce neuronal cell death in the absence of infectious prion propagation (Muramoto *et al*, 1997; Chiesa *et al*, 1998; Hegde *et al*, 1998; Shmerling *et al*, 1998; Ma *et al*, 2002; Flechsig *et al*, 2003; Baumann *et al*, 2007; Li *et al*, 2007; Figure 1D). From one class of PrP mutants it emerged that PrP^C can acquire a neurotoxic potential by deleting the internal HD (Shmerling *et al*, 1998; Baumann *et al*, 2007; Li *et al*, 2007). PrP Δ HD is complex glycosylated and linked to the plasma membrane via a GPI anchor, suggesting a similar cellular location to PrP^C (Winklhofer *et al*, 2003b). Two different models were proposed to explain the neurotoxic activity of PrP Δ HD. In one model, it was suggested that PrP Δ HD blocks neurotrophic signaling via binding to a yet unidentified cell-surface receptor (Shmerling *et al*, 1998). In another model, PrP Δ HD competes with PrP^C for binding to a hypothetical signal transducing protein. In this scenario, PrP^C induces neuroprotective signaling, while binding of PrP Δ HD triggers a neurotoxic cascade (Li *et al*, 2007).

Acquisition of a neurotoxic activity is not restricted to PrP conformers present in the secretory pathway. Spontaneous neurodegeneration of transgenic mice expressing a PrP mutant without the N-terminal ER-targeting sequence indicated a toxic potential of PrP when located in the cytosolic compartment (cytoPrP) (Ma *et al*, 2002). Toxicity of cytoPrP seems to be dependent on its association with intracellular membranes (Wang *et al*, 2006) and its binding to Bcl-2, an antiapoptotic protein present at the cytosolic side of ER and mitochondrial membranes (Rambold *et al*, 2006). Might the toxic potential of misfolded PrP in the cytosol be relevant to the pathogenesis of prion diseases? First, some pathogenic mutants linked to inherited prion diseases in humans are partially mistargeted to the cytosol (Zanusso *et al*, 1999; Heske *et al*, 2004). Second, access to the cytosol is possible via retrograde translocation of PrP out of the ER (Ma and Lindquist, 2001; Yedidia *et al*, 2001). Third, most recent data revealed an impairment of the ubiquitin-proteasome system (UPS) in prion-infected mice. In conjunction with *in vitro* and cell culture approaches, it was proposed that prion neurotoxicity is linked to PrP^{Sc} oligomers, which translocate to the cytosol and inhibit the UPS (Kristiansen *et al*, 2007).

Loss of a protective function

So far we have discussed how aggregation-prone proteins, such as A β , tau, PrP and α -synuclein can cause neurodegeneration by a gain of toxic function. In the following, we will concentrate on proteins associated with autosomal recessive diseases,

which can lose their function due to misfolding. Mutations in parkin, a gene encoding an E3 ubiquitin ligase (Kitada *et al*, 1998), are responsible for the majority of autosomal recessive PD (Figure 1C). E3 ubiquitin ligases catalyze the covalent attachment of ubiquitin to lysine residues of substrate proteins. Ubiquitin is best known for its role in targeting proteins for proteasomal degradation, therefore, it has been proposed that a loss of parkin function due to pathogenic mutations causes the accumulation of parkin substrates, which ultimately damage dopaminergic neurons. Various putative parkin substrates have been described; however, an accumulation was observed for only two putative substrates in one parkin knockout model, and the pathophysiological relevance of this observation is still unclear (Ko *et al*, 2005, 2006). Notably, degradation-independent functions of ubiquitylation have been implicated in various cellular functions, such as signal transduction, transcriptional regulation, DNA repair, endocytosis and cellular trafficking (for review, see Pickart and Fushman, 2004; Haglund and Dikic, 2005). Recent research from different laboratories revealed that parkin can indeed promote non-degradative ubiquitylation (Doss-Pepe *et al*, 2005; Lim *et al*, 2005; Hampe *et al*, 2006; Matsuda *et al*, 2006; Henn *et al*, 2007) and that a neuroprotective activity of parkin is linked to this mode of ubiquitylation (Fallon *et al*, 2006; Henn *et al*, 2007).

Since its discovery in 1998 (Kitada *et al*, 1998), a large number and a wide spectrum of pathogenic mutations have been identified, including exon deletions and rearrangements, missense, nonsense and frameshift mutations (for a review, see Mata *et al*, 2004). Accumulating evidence indicates that misfolding of parkin is a major mechanism of parkin inactivation. All pathogenic C-terminal deletion mutants spontaneously adopt a misfolded conformation and form aggregates in cell culture models (Winklhofer *et al*, 2003a; Henn *et al*, 2005). Aberrant parkin folding not necessarily induces the accumulation of misfolded conformers, but can also lead to destabilization and rapid proteasomal degradation, exemplified by some missense mutations within the N-terminal ubiquitin-like domain of parkin (Henn *et al*, 2005). Alterations in the detergent solubility or cellular localization of parkin have also been described for various missense mutants (Ardley *et al*, 2003; Cookson *et al*, 2003; Gu *et al*, 2003; Muqit *et al*, 2004; Sriram *et al*, 2005; Wang *et al*, 2005b; Hampe *et al*, 2006). Remarkably, recent publications provide a scientific rationale for the hypothesis that inactivation of parkin by misfolding may also play a role in sporadic PD. Based on the observation that parkin is prone to misfolding and aggregation in the presence of high-level oxidative stress (Winklhofer *et al*, 2003a), LaVoie *et al* (2005) could demonstrate that in the substantia nigra of patients suffering from sporadic PD, detergent-insoluble parkin is present, which is covalently modified by an oxidation product of dopamine. In support of this concept, nitrosative stress has been reported to impair the E3 ligase activity of parkin, and S-nitrosylated parkin was indeed detected in the brains of PD patients (Chung *et al*, 2004; Yao *et al*, 2004). How could the inactivation of parkin promote the demise of dopaminergic neurons? Parkin has the capacity to maintain neuronal integrity under various moderate stress conditions, including mitochondrial stress, excitotoxicity and ER stress. Mechanistic insight into this activity emerged from recent work, showing that parkin can stimulate pro-survival pathways (Fallon *et al*, 2006; Henn *et al*, 2007). Dopaminergic

neurons are characterized by a high oxidative burden and thus require an effective stress-response management, yet parkin is inactivated under severe and dopamine induced stress (Winklhofer *et al*, 2003a; LaVoie *et al*, 2005, 2007; Wang *et al*, 2005a; Wong *et al*, 2007). This inherent imbalance might explain why dopaminergic neurons are particularly vulnerable to a loss of parkin function.

Strikingly, studies in *Drosophila* indicated a genetic link between parkin and another PD-associated gene, namely PINK1 (PTEN-induced kinase 1). Mutations in the PINK1 gene encoding a mitochondrial kinase are the second most common cause of autosomal recessive PD (Valente *et al*, 2004). In *Drosophila*, PINK1 and parkin loss-of-function mutants show a similar phenotype, including mitochondrial defects. Remarkably, parkin can compensate for the loss of PINK1 function, but not vice versa, suggesting that parkin acts downstream of PINK1 (Clark *et al*, 2006; Park *et al*, 2006; Yang *et al*, 2006). Now rescue activity of parkin has also been observed in PINK1-deficient mammalian cells (Exner *et al*, 2007). It will now be an important endeavor to elucidate the underlying mechanism.

In addition to parkin and PINK1, DJ-1 has been associated with autosomal recessive PD (Bonifati *et al*, 2003). While PINK1 and parkin genetically interact as described above, DJ-1 is not part of this signaling cascade, as recent evidence demonstrated that DJ-1 fails to rescue the pathological phenotype caused by PINK1 reduction (Exner *et al*, 2007; Yang *et al*, 2006). Mutations in the DJ-1 gene are extremely rare, accounting for about 1% of early onset PD. Diverse cellular functions have been attributed to DJ-1; the most relevant function linked to the pathogenesis of PD might be a role of DJ-1 in the response to oxidative stress. DJ-1 has been shown to protect cells against oxidative stress-induced cell death in various cell culture and animal models. Different mechanisms have been proposed to explain the protective activity of DJ-1. It could serve as a sensor of oxidative stress via modification of a cysteine residue to sulfenic acid, and/or it might have an intrinsic antioxidative or chaperone activity (for a review, see Moore *et al*, 2005). A recent study has provided evidence for an atypical peroxiredoxin-like peroxidase activity of DJ-1 implicated in the scavenging of mitochondrial H₂O₂ (Andres-Mateos *et al*, 2007). Furthermore, DJ-1 has been shown to influence signaling pathways implicated in the regulation of cell death; it stimulates the pro-survival PI3K/Akt pathway and inhibits the pro-apoptotic ASK1 pathway (Junn *et al*, 2005; Yang *et al*, 2005; Gorner *et al*, 2007). There is reason to assume that the generation of a non-native conformation also plays a role in the inactivation of DJ-1. The L166P mutant impairs the formation of functional DJ-1 dimers, resulting in a highly unstable protein (Macedo *et al*, 2003; Miller *et al*, 2003; Moore *et al*, 2003; Gorner *et al*, 2004, 2007; Olzmann *et al*, 2004). Whether misfolding of other PD-associated gene products, such as PINK1 or LRRK2 (leucine-rich repeat kinase 2, dardarin), might contribute to the pathogenesis of PD, has not been reported so far. Mutations in the LRRK2 gene are regarded as the most common cause of genetic PD; they are responsible for the majority of autosomal dominant PD typically associated with late-onset and are also found in some cases which would have been classified as sporadic PD (Paisan-Ruiz *et al*, 2004; Zimprich *et al*, 2004). The LRRK2 gene encodes a large multidomain protein, including a kinase domain related to

the mixed lineage kinase family, a Rho/Ras-like GTPase domain, a WD40-repeat domain and leucine-rich repeats. Some pathogenic mutations seem to increase the kinase activity of LRRK2 *in vitro*, assessed by autophosphorylation or phosphorylation of generic substrates, which may suggest a toxic gain-of-function mechanism (West *et al*, 2005; Gloeckner *et al*, 2006; Greggio *et al*, 2006; Smith *et al*, 2006). However, we still do not know the physiological and pathological function of LRRK2 *in vivo*.

Lack of a neuroprotective factor may also play a role in a different neurodegenerative disorder. In addition to the tau-positive FTLD cases, a significant number of FTLD patients carried tau- and α -synuclein-negative but ubiquitin-positive neuronal inclusions (Rademakers *et al*, 2002; Figure 1B). These inclusions define a novel type of FTLD, called frontotemporal lobar degeneration with ubiquitin-positive inclusions, FTLD-U (Pickering-Brown, 2007). The 43 kDa TAR DNA-binding protein (TDP-43) has been shown to be a major component of these inclusions (Neumann *et al*, 2006). Strikingly, TDP-43-positive inclusions were also found in sporadic and familial non-SOD1 amyotrophic lateral sclerosis (Neumann *et al*, 2006; Dickson *et al*, 2007; Mackenzie *et al*, 2007; Tan *et al*, 2007). TDP-43 is a nuclear protein, which may be involved in RNA binding (Buratti *et al*, 2001; Zuccato *et al*, 2004; Ayala *et al*, 2006) or DNA binding (Ou *et al*, 1995), and accumulates frequently in cytoplasmic and sometimes also in nuclear deposits in FTLD-U cases (Neumann *et al*, 2006, 2007; Cairns *et al*, 2007; Davidson *et al*, 2007; Seelaar *et al*, 2007). These neuronal deposits contain insoluble hyperphosphorylated, proteolytically generated C-terminal fragments of the full-length protein (Neumann *et al*, 2006). Currently, it is unclear if these deposits are toxic entities of the disease and/or if they result in a loss-of-function of TDP-43 due to its reduced concentrations within the nucleus, where it is normally located and expected to be functional, for example as an mRNA-stabilizing factor (Strong *et al*, 2007). While familial cases of FTLD with tau pathology often carry mutations in the tau gene on chromosome 17, three genes and an uncharacterized locus on chromosome 9p have been linked to familial FTLD-U: the valosin-containing protein (VCP) gene on chromosome 9 (Watts *et al*, 2004), the charged multivesicular body protein 2B gene (CHMP2B) on chromosome 3 (Skibinski *et al*, 2005) and the progranulin gene (PGRN) on chromosome 17 in close vicinity of the tau locus (Baker *et al*, 2006; Cruts *et al*, 2006). VCP/p97 is a multifunctional AAA (ATPases associated with a variety of activities) ATPase, which has been implicated in the ubiquitin-proteasome pathway, ER-associated protein degradation (ERAD), membrane fusion and cell-cycle control (for review, see Wang *et al*, 2004; Halawani and Latterich, 2006). It has been reported that some pathogenic VCP mutants impair the ubiquitin-proteasome and ERAD pathway (Weihl *et al*, 2006). CHMP2B constitutes a subunit of the endosomal sorting complex required for transport III (ESCRTIII), involved in the trafficking of ubiquitylated proteins along the endosomal pathway via multivesicular bodies to lysosomes (for a review, see Williams and Urbe, 2007). Expression of mutant CHMP2B has recently been associated with ESCRTIII dysfunction, resulting in the accumulation of autophagosomes (Lee *et al*, 2007). PGRN is a secreted protein, which has properties of a growth factor or wound-healing factor (Zanocco-Marani *et al*, 1999; He *et al*, 2003). Numerous nonsense, frameshift and

splice-site mutations have now been identified, which all lead to haploinsufficiency (for review, see Kumar-Singh and Van Broeckhoven, 2007; Mackenzie and Rademakers, 2007; van der Zee *et al*, 2007a). Apparently, all these mutations result in the degradation of the mutant mRNA by nonsense-mediated mRNA decay (Baker *et al*, 2006; Cruts *et al*, 2006). Thus, these mutations cause a loss-of-function by neutralizing the mRNA derived from one mutant allele. Together with the fact that PGRN has many properties of wound healing and growth factors, this suggests that the loss-of-function of PGRN probably results in reduced neuroprotection. This is supported by the observation that PGRN is upregulated in activated microglia surrounding amyloid plaques of AD patients (Baker *et al*, 2006). If that indicates a general function of PGRN in neuroprotection is currently unknown. Interestingly, three exceptional PGRN mutations have been observed, which all lead to the exchange of only one amino acid within the mature protein (PGRN P248L and PGRN R432C; Schymick *et al*, 2007; van der Zee *et al*, 2007b) or the signal sequence (PGRN A9D; Mukherjee *et al*, 2006). The two mutations occurring C-terminal to the signal sequence allow the synthesis of the immature protein and its translocation into the ER (Shankaran *et al*, 2007). However, possibly due to misfolding (van der Zee *et al*, 2007b) these proteins fail to be efficiently transported through the secretory pathway, which leads to a significant reduction in PGRN secretion. In contrast, the mutation within the signal sequence is hardly expressed at all. This protein is mislocated to the cytoplasm, where it is rapidly degraded by the proteasome (Shankaran *et al*, 2007). Thus, three independent mechanisms, nonsense-mediated mRNA decay, reduced secretion and degradation upon mislocalisation all lead to the same result, that is a loss-of-function of a putative neuroprotective factor (Figure 1B). It is currently unclear if and how the loss-of-function of PGRN is associated with the cytoplasmic deposition of TDP-43. However, very recently it has been suggested that a reduction of PGRN leads to caspase-mediated generation and subsequent accumulation of insoluble TDP-43 fragments, similar to those observed in human patients (Zhang *et al*, 2007).

A loss-of-function, which results in a gain-of-function?

We have now described a number of pathological consequences, which occur upon a loss or a gain-of-function; however, there is still one rather surprising and very challenging variant to be described, namely a loss-of-function that may result in a gain of (toxic) function. This occurs in at least some autosomal dominant AD-associated presenilin (PS1 and PS2) mutations. PSs are directly involved in the proteolytic generation of A β from its precursor, the β APP. A β is produced from APP by proteolytic processing mediated by secretases. First, β -secretase (BACE1) has to cleave at the N-terminus of the A β domain to generate a membrane-retained C-terminal stub. This is the immediate substrate for the subsequent γ -secretase cleavage, which liberates A β (Haass, 2004). The intramembrane cleavage mediated by γ -secretase is heterogeneous and can take place at several positions within the transmembrane domain. Familial AD (FAD)-associated mutations shift the cleavage from position 40 to position 42 of the

A β domain to produce a more aggregation prone A β species (A β 42) or at least increase the ratio of A β 42/A β 40 (Haass and Selkoe, 2007). This is clearly a toxic gain-of-function, which is directly related to the disease, as disease onset and the amount of A β 42 generated roughly correlate (Duering *et al*, 2005; Page *et al*, 2007).

PSs harbor the catalytically active center of the γ -secretase complex. More than 150 missense mutations are spread throughout the entire amino-acid sequence with no obvious hot spots observed (Haass, 2004). However, all of the mutations investigated so far specifically affect the precision of the γ -secretase cut. This can only be explained by slight structural changes, which all affect the catalytically active center in a similar manner. In fact, studies using fluorescence resonance energy transfer suggested that PS mutations can cause alterations in its conformation (Berezovska *et al*, 2005). Moreover, certain drugs, such as the nonsteroidal anti-inflammatory drugs (NSAIDs) modulate the γ -secretase cleavage by shifting the cut from amino acid 42 to amino acid 38, thereby reducing the generation of the neurotoxic A β 42 (Weggen *et al*, 2001). Strikingly, this shift in cleavage precision is accompanied by a structural change of the domains containing the catalytic center within PS (Lleo *et al*, 2004). These findings suggest that FAD-associated PS mutations result in a gain of (toxic) function due to structural changes affecting the catalytic center of the protease. However, there is also strong evidence that at least some PS mutations are associated with a loss-of-function (Bentahir *et al*, 2006; De Strooper, 2007; Wolfe, 2007). Apparently, these PS mutants, if expressed in cells lacking endogenous PS1 and PS2, show reduced A β production. As A β 40 is more severely reduced than A β 42, these mutations still affect the A β 42/A β 40 ratio (Bentahir *et al*, 2006). How can such an apparent paradox be explained? The γ -secretase complex is physiologically required for Notch signaling, a process which requires the liberation of the cytoplasmic domain and its subsequent nuclear translocation (Haass, 2004). Already very early evidence suggested that FAD-associated PS mutations do not efficiently rescue a Notch-related loss-of-function of the *C. elegans* PS homolog sel-12 (Levitan *et al*, 1996; Baumeister *et al*, 1997; De Strooper, 2007; Wolfe, 2007). Moreover, some FAD-associated mutations reduce intramembrane proteolysis of Notch and thus its nuclear signaling capacity (Moehlmann *et al*, 2002; Bentahir *et al*, 2006). However, in mice the knock-in of certain FAD-associated mutations rescued the PS1-knockout phenotype (Guo *et al*, 1999; Nakano *et al*, 1999; Siman *et al*, 2000). But which model is true? We suggest that a somewhat reduced function of at least selected PS mutants is associated with a favored production of A β 42. This may be explained by a packman-like processing model starting from the cytoplasmic end of the transmembrane domain. If in line with the data in *C. elegans*, PS mutations reduce the catalytic activity of the γ -secretase, one may assume that the enzyme simply cleaves slower and slower until it reaches position 42. If the substrate is then released from the complex before further cleavage at the subsequent sites (positions 40 and 38) occurs, the A β 42/A β 40 ratio may be changed by an apparent loss-of-function. However, one should keep in mind that this may not be a general mechanism of all FAD-associated mutations, but rather of individual mutations, which in most cases are extremely aggressive and cause an unusually strong increase of A β 42. Although mutations may generally

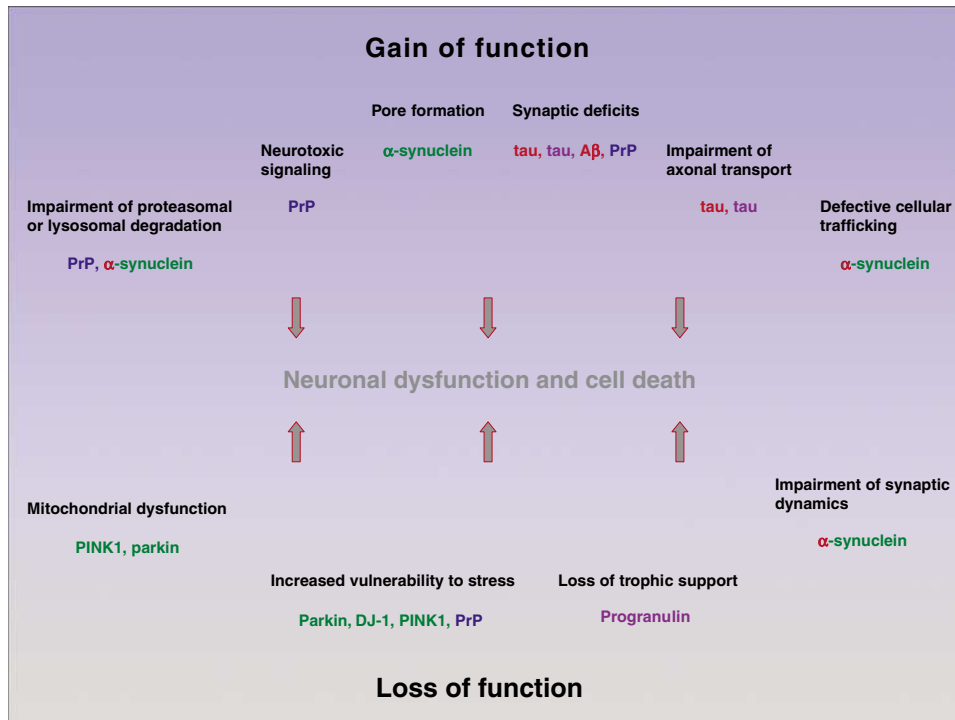


Figure 2 Examples for gain- and loss-of-function mechanisms leading to neuronal dysfunction and cell death. red: AD; blue: prion diseases; green: PD; purple: FTLD.

affect the structure of the active site of PS within the γ -secretase complex (Berezovska *et al*, 2005), individual mutations may affect the PS structure more severely and thus cause a measurable loss-of-function.

Perspective

Aberrations in protein folding, processing and/or degradation are common features of neurodegenerative diseases, resulting in the accumulation of misfolded conformers. The identification of genes, which are responsible for rare familial variants of neurodegenerative diseases, provided important insights into common as well as specific features of different disease entities. Clearly, amyloidogenic proteins, such as A β , tau, PrP and α -synuclein, which accumulate in sporadic and autosomal dominant forms of the respective diseases, can acquire a toxic gain-of-function. Considerable evidence from recent research indicates that soluble oligomeric assemblies and not amyloid fibers or the final aggregates mediate the toxic effects. However, a disease-relevant impact of large deposits cannot be excluded: on one hand they can sequester and thereby inactivate toxic oligomers, on the other hand they may serve as a dynamic reservoir for the liberation of soluble oligomers. Different mechanisms have been proposed to explain the toxic potential of misfolded protein conformers as summarized in Figure 2. Although some aspects of toxicity may be specific for a distinct entity, certain common mechanisms have emerged. Strikingly, prion diseases illustrated that misfolding can induce not only a toxic but also a self-propagating protein species. The transmissible nature of prion diseases brings up the question how this feature can be explained and how it can be demarcated from seeding effects observed after the experimental inoculation of misfolded conformers. Whether transmissibility is associated

with a specific pathological conformation and/or with structural dynamics of the physiological isoform remains to be seen. In some neurodegenerative diseases, protein misfolding is implicated in the pathogenesis via a loss-of-function mechanism, exemplified by autosomal recessive PD (parkin, DJ-1) and FTLD-U with PGRN mutations. In these diseases, the deficiency of a neuroprotective factor may be associated with an increased neuronal vulnerability. In addition, there is experimental evidence suggesting that PrP^C as well as α -synuclein may have a neuroprotective capacity, which is lost upon the formation of misfolded conformers. Finally, a rather puzzling case occurs with the FAD-associated PS mutations, where a loss-of-function can surprisingly result in a gain-of-function, in at least some cases.

How can our current understanding of neurodegenerative diseases be translated into the development of therapeutic strategies? Remarkably, different oligomeric assemblies, formed by either A β , α -synuclein or PrP^{Sc}, share structural features, suggesting a common harmful potential of these species (Kayed *et al*, 2003). Defining the toxic signature of these oligomers might pave the way for immunization approaches. Strikingly, anti-A β vaccination has been shown to prevent cognitive deficits and disease progression in mouse models of AD (Roberson and Mucke, 2006; Haass and Selkoe, 2007). Another therapeutic target may be the modulation of the protein quality control machinery, above all molecular chaperones, which are the first line of defence to encounter misfolded conformers.

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