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Cross Currents in Protein Misfolding Disorders: Interactions and Therapy

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Abstract

Protein Misfolding Disorders (PMDs) are a group of diseases characterized by the accumulation of abnormally folded proteins. Despite the wide range of proteins and tissues involved, PMDs share similar molecular and pathogenic mechanisms. Several epidemiological, clinical and experimental reports have described the co-existence of PMDs, suggesting a possible cross-talk between them. A better knowledge of the molecular basis of PMDs could have important implications for understanding the mechanism by which the diseases appear and progress and ultimately to develop novel strategies for treatment. Due to their similar molecular mechanisms, common therapeutic strategies could be applied for the diseases in this group.

MOLECULAR BASIS OF PROTEIN MISFOLDING DISORDERS

For a protein, the amino acid sequence is the blueprint that dictates the biologically active conformation. However, throughout the life of the protein there are many factors that lead to its unfolding and refolding, opening the door for a misfolding event to occur. This misfolding event can lead to the aggregation of the misfolded protein into amyloids which then wreak havoc on the body, leading to a variety of debilitating diseases. PMDs are a group of diverse, fatal diseases resulting from the failure of a protein to fold into the correct conformation, followed by its subsequent aggregation and deposition in tissues [1,2]. This group of diseases comprises disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), transmissible spongiform encephalopathies (TSEs) and type 2 diabetes among many others [2]. The contribution of these aggregates to pathological decline is better understood in some of these diseases; however their presence is a hallmark in all cases.

Transmissible Spongiform Encephalopathies

TSEs, also known as prion diseases, consist of a group of fatal neurodegenerative disorders that are found in a variety of mammals. TSEs are the only PMDs known to be transmissible by infection and the infectious agent (termed prion) is most likely composed exclusively of the misfolded prion protein. During prion replication, the disease associated isoform of the prion protein (PrP^{Sc}) imposes its three dimensional structure on the normal cellular form of the protein (PrP^C) resulting in the exponential accumulation and aggregation of PrP^{Sc} (Fig. 1) [3,4]. A remarkable feature of these diseases is the prolonged incubation period followed by a very rapid clinical phase that is invariably fatal. TSEs are unique diseases due to the tripartite epidemiological appearance (inherited, sporadic, and acquired), and while rare in humans, with

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a sporadic Creutzfeldt Jacob Disease (sCJD) incidence of 1–2 cases per million persons per year [5], they are more common in animals. TSEs can occur naturally or as a direct result of the consumption of contaminated food. The recently described variant CJD (vCJD) resulted from the consumption of meat infected with the bovine spongiform encephalopathy (BSE) agent [6–8].

Prions, as other more conventional type of infectious agents, have two important properties: prion strains and the species barrier phenomenon [9,10]. Prion strains are characterized classically by their stable pathological and biochemical characteristics, including incubation time in susceptible animals, clinical symptoms, and by their lesion profile in the central nervous system [9]. While it is not entirely clear how strain characteristics are encoded, most data indicates that the folding of PrP^{Sc} is responsible [11–13]. However, recent evidence suggests that quaternary structure also likely has an influence on strain properties [14].

The species barrier phenomenon in TSEs describes the ability of prions from one species to cause disease in another [15,16]. Initial passage of prions from one species to another can be associated with complete resistance or long incubation times with 100% attack rate or less followed by a large drop in the incubation period on subsequent passages with all animals developing disease [17]. A clear example can be seen in the difficulty of hamster prions to cause disease in wild type mice [18]. However, expression of hamster PrP^C in transgenic mice was shown to abolish this resistance [19]. Further experiments then demonstrated that the most important determinant of the species barrier is the degree of homology between PrP^C and PrP^{Sc} [20]. Although this is thought to result in optimal prion transmission, prion strains have been found to overcome the influence of primary structure [21]. One example of this is BSE, which has been found to transmit to multiple species while having a primary structure that is not identical to PrP^C in the host [22–26]. This demonstrates that the factors controlling prion transmission barriers likely work in combination rather than one factor dominating over the others.

Mechanisms of Protein Misfolding and Seeded Aggregation

In each PMD, protein aggregates are composed of a misfolded protein unique to the disease, such as amyloid β (A β peptide in AD or PrP^{Sc} in TSEs). The misfolded protein aggregates in PMDs have similar characteristics, but vary in distribution and composition leading to different deposition profiles and pathologies [2]. In addition, there are no sequence similarities between the various proteins that are implicated in these disorders [2]. Protein aggregation is not a random process, but occurs slowly through an ordered mechanism termed the nucleation-dependent polymerization model [27,28].

The nucleation-dependent polymerization model features a slow and thermodynamically unfavorable nucleation phase followed by a rapid elongation phase [28,29]. In the nucleation phase, the rate-determining step is the formation of a stable seed or nucleus of polymerized protein (Fig. 2). *In vivo*, the first seeds in this process can be formed spontaneously (as it is mainly observed in TSEs, AD and PD), favored by mutations (as in Huntington's disease) or exogenously acquired (so far only accepted for TSEs). The seed can then simultaneously bind to and convert multiple molecules of the normal protein, generating a thread of aggregated small oligomers which subsequently will lead to the formation of amyloid fibrils and in some cases, plaques [30] (Fig. 2). The dynamic distribution of these toxic species is characteristic for each disease, but interestingly, it is also dependent on the specific conformation that the proteins can acquire (i.e. as it is observed in different prion strains) [2,9]. Amyloid aggregates are thought to be the triggering factors in various PMDs [30]. Amyloidogenic-specific dyes such as Thioflavin T and Congo Red can be used to observe the kinetics of fibril formation and have demonstrated that the length of the nucleation phase (or lag phase) and extension phase are highly dependent on the protein concentration [2,30].

The infectious nature of prions can be rationalized by the seeding-nucleation model [28]. While the lag phase is normally a slow process, this phase can be reduced or eliminated by the addition of a preformed seed or nucleus to serve as a template [31]. This would mean that in the case of acquired prion diseases, such as vCJD or iatrogenic CJD, a PrP^{Sc} seed is introduced into the body and used as a template to seed the polymerization of PrP^{Sc} aggregates, reducing the lag phase and accelerating the elongation phase (Fig. 2). This provides a plausible explanation for why vCJD appeared in much younger individuals, which is very different from the appearance of disease late in life that occurs with the sporadic and inherited TSEs in which there is no preformed seed introduced [6]. Since *in vitro* and *in vivo* evidence suggest that in all PMDs the process of protein misfolding and aggregation also follow a seeding-nucleation mechanism [29,32], it is possible that other PMDs might be transmissible following a similar route [29].

INTERACTION OF PMDS: FACT OR CHANCE?

The co-existence of various PMDs in the same individual has been extensively described [33–35]. Moreover, the presence of two misfolded proteins has also been described in the same amyloidogenic structure (see below). On this basis, it could be hypothesized that PMDs might interact at the protein level through a process termed heterologous seeding or cross-seeding, suggesting that one PMD could be an important risk factor for the development of a second one.

Cross-Seeding

While it is well established that fibril formation is enhanced by adding preformed homologous or heterologous seeds, it has also been shown *in vitro* that sequence similarity could have an effect on seeding efficiency. By cross-seeding hen lysozyme with a series of proteins, it was demonstrated that differing sequences had a lower efficiency of seeding [36]. *In vivo* studies revealed that non-mammalian protein fibrils can cross-seed amyloid A protein in a murine experimental amyloid A amyloidosis model [37]. In addition, several reports showing the co-existence of different amyloid pathologies in the same tissue further support the existence of cross-seeding as a disease mechanism [34,35,38–42]. This data suggests that one PMD may be capable of influencing the development of another.

The demonstration that seeding with proteins of differing sequences has a lower efficiency is reminiscent of the species barrier described earlier for TSEs. This could be a plausible explanation for the low attack rate and long incubation periods typically observed during the primary transmission of prions from one species to another [9,12,17,18]. However, there are many other factors that could influence this phenomenon, such as the strain of the agent and the genetic background of the host [43,44]. Nevertheless, recent experiments showing that the species barrier phenomenon is reproduced in transgenic mice expressing PrP from different species support the hypothesis that the sequence is the most important factor responsible for this phenomenon [19,45–47].

CO-EXISTENCE OF PMDS

The study of a putative interaction of PMDs at the protein level is not only important from a scientific point of view, but also for public health. A better understanding of this phenomenon could explain the origin and prevalence of several PMDs. On the one hand, several of the proteins related to these diseases are located in different cell compartments or cell types, making a protein-protein interaction unlikely. On the other hand, the fact that some of these proteins can increase their levels under certain pathological conditions enhances the possibility of interaction between them. These events could be caused by several mechanisms which include inflammation, overload of the proteasome and protein-chaperone mechanisms (responsible for the elimination of misfolded proteins) and signaling cascades, among others.

Such processes could interact, become synergic and enhance disease hallmarks (as misfolded protein deposition) which could increase the chance of misfolded protein-protein interactions. Next, we will discuss epidemiological and experimental data related to misfolded protein cross-seeding and its putative role in the pathological progression.

Epidemiological Evidence

Probably the clearest evidence for the interaction of PMDs comes from the link between AD and diabetes type-2, characterized by A β and amylin deposition, respectively. Clinical studies had shown that a high percentage of patients affected with AD are also positive for diabetes type-2 [48]. In 2004, Janson and co-workers showed that in a cohort of AD patients, 81% of them had either diabetes type-2 or Impaired Fasting Glucose (IFG) [48]. In addition, and comparing with age matched non-demented individuals, AD patients have a higher incidence of islet amyloidosis than healthy individuals. However, this study described that A β deposition is not increased in patients affected with diabetes type-2, suggesting that this phenomenon could be unidirectional. Other reports have been published supporting or contradicting this conclusion [49–54]. However, the location of amyloid proteins in different tissues and the lack of reports showing the co-existence of both misfolded aggregates in the same organ question the idea that the interaction between AD and diabetes type 2 is due to cross-seeding mechanisms.

Other clinical examples are found in patients affected with PD and AD where the co-existence of A β and α -synuclein has been described [33,55,56]. In this case, pathological features of both diseases co-exist in the brain, increasing the probabilities that misfolded protein aggregates can interact. Recently, Tsigelini *et al.* showed that α -synuclein and A β directly interact in the brain of patients with Lewy body disease [55]. This report also describes patients clinically diagnosed with AD presenting higher accumulations of α -synuclein compared to healthy individuals. In addition, co-immunoprecipitation experiments using anti-A β antibodies showed that brains from Lewy body disease patients presented strong anti-A β immunoreactivity after analysis by Western blot [55].

The phenomenon of amyloids' co-existence has been described for several other amyloidogenic proteins. Among them, the simultaneous presence of A β and prions has been extensively documented in patients affected with sCJD, Gerstmann-Sträussler-Scheinker (GSS) disease and AD [34,35,39,57–60]. In some cases of AD, accumulation of PrP within A β deposits occurs as diffuse plaques [57,60]. These aggregates are easily eliminated after proteinase K treatment on brain slides, suggesting that prion aggregates are composed in part or totally by PrP^C or by protease-sensitive PrP^{Sc}. These findings are similar to what is observed for double transgenic mice over-expressing amyloid precursor protein with Swedish and Indiana mutations (associated with strong A β deposition) and hamster PrP [61]. However, due to the high density of PrP in these areas, the possibility of *de novo* PrP^{Sc} generation should be considered. In addition, it is important to mention that many cases of TSEs are associated with proteinase K sensitive PrP^{Sc} [62–64], which could be mainly composed of oligomeric protease-sensitive species.

Several cases of sCJD and GSS patients with plaques composed of misfolded PrP and A β have been reported [34,35,39,58,59]. A recent study shows that a subgroup of sCJD patients show higher levels of A β ₄₂ in their brains [58]. As a consequence, A β plaques were found in the brain of these sCJD affected individuals. As expected, little or no senile plaques were identified in sCJD patients harboring lower levels of A β . Interestingly, sCJD patients harboring higher amounts of A β have lower levels of PrP^{Sc} accumulation. This data can be interpreted in two different ways. The first explanation is that the presence of A β inhibits misfolding and accumulation of PrP^{Sc}. The second one is that part of the PrP^{Sc} molecules are used to induce misfolding and aggregation of A β instead of further accumulation of PrP. The presence of both

amyloidogenic species could have enhanced toxic effects, triggering clinical disease without substantial accumulation of PrP^{Sc}. Due to the specific characteristics of these diseases, it is possible that some clinical and pathological features of AD may camouflage sCJD [58,65]. In a more extreme case, it could be possible to imagine that PrP^{Sc} formation, produced for example by an infectious exposure may trigger A β misfolding and accumulation and lead to AD clinical disease, instead of CJD. Thus, it could be interesting to study whether in countries with high exposure to BSE (such as UK or France) there is an increased number of cases of AD or other neurodegenerative diseases associated to protein misfolding and aggregation. It would also be important to carry out more experiments in animal models to properly assess this important issue.

Evidence *In Vitro*

The easiest way to test the hypothesis of cross-seeding is by *in vitro* aggregation assays. Classical seeding assays show that the addition of a preformed seed reduced the extent of the lag phase of aggregation in amyloidogenic proteins [27,29,66]. In addition, several reports show that the extent of the nucleation phase of a specific protein could be reduced after the addition of a heterologous seed [55,67]. This cross-seeding effect can be different according to the protein/protein pair studied.

Subsequent to the clinical evidence described earlier in diabetes type-2 and AD patients [48], *in vitro* seeding assays using amylin and A β were performed [67]. Interestingly, there is some degree of sequence similarity between amylin and A β . If we add this information to the previously mentioned reports that link AD and diabetes type-2 [48], it would be rational to look for a putative interaction between both proteins. Seeding assays show that A β amyloids are good seeds for amylin aggregation. However, the same report described that globular and fibrillar aggregates of amylin have inert or very low effects on A β aggregation [67]. These results are interesting if we consider the clinical data reported by Janson *et al.*, where the interaction between diabetes Type-2 in AD seems to work only in one direction [48].

Another important example of *in vitro* cross-seeding comes from studies involving α -synuclein and A β [55,68]. The evidence in this case goes further, explaining a possible association between different oligomeric aggregates [55]. Additionally, it is suggested that both proteins could form annular structures able to generate pores in the cell membrane [55]. It is hypothesized that these structures could produce cationic imbalance between intracellular and extracellular spaces, leading to cell death events observed in this disorders.

By coupling seeding assays with other *in vitro* and *in silico* techniques, we could enormously improve our knowledge regarding this important issue. However, in order to understand the contribution of other events such as inflammation, signaling cascades and protein clearance mechanisms in the progression of the disease, experimentation in animal models is absolutely necessary.

Studies in Animal Models

The best way to analyze if two PMDs can cross-talk is by using animal models. Animal models allow us to measure many pathological features in a controlled way and at different stages of the disease. Several reports studying PMDs interactions in mouse models are available. Again, the A β / α -synuclein pair is one of the best studied cases [55,69]. The results indicate that A β enhances α -synuclein accumulation and neuronal deficits in a double transgenic mouse model of AD and PD [69]. Considering their cellular distribution, interaction between A β extra-cellular and α -synuclein (intra-cellular) seems unlikely. Nevertheless, immunoprecipitation assays demonstrated the co-existence of both proteins in the mouse brain [55]. These data open

new questions regarding the mechanisms of interaction for these particular proteins and their consequences in pathological events.

The presence of A β aggregates and Tau neurofibrillary tangles is the major hallmark in AD. It has been proposed for a long time that a putative interaction between both proteins could enhance the clinical decline observed in AD patients. Two interesting pieces of evidence were supplied in 2001 by Lewis *et al.* [70] and Gotz *et al.* [71]. These studies show that the simultaneous presence of A β and Tau enhanced neurofibrillary tangle formation in mouse models. In the first approach, researchers crossed Tg2576 mice with transgenic mice expressing a mutant form of Tau protein. They observed that Tg2576 and double transgenic mice develop A β deposit at the same age, however neurofibrillary tangle deposition was significantly enhanced compared to the single mutant Tau transgenic [70]. Interestingly, the increase in Tau deposition was not correlated with areas rich in A β deposits. The second study consisted in the inoculation of pre-formed A β ₄₂ fibrils in the brain of P301L Tau transgenic mice. As in the previous report, the presence of misfolded A β in the brain of these mice increased Tau deposition [71].

Considering the findings suggesting the co-existence of AD and sCJD, the study of a possible link between both diseases is important. As suggested by Debatin *et al.* [58], the fact that the AD phenotype could mask some sCJD cases makes this issue even more relevant. Since A β and PrP^{Sc} have similar aggregation mechanisms and are located in the same tissue (brain) and subcellular location (extracellular space), it is likely that these misfolded proteins may interact. However, two recent reports suggest that PrP^C biological function may be related to A β . First, it was shown that PrP^C could be a protective molecule in AD *via* β -secretase inhibition [72]. Second, it was shown that PrP^C may be a receptor for A β oligomers, contributing to AD neurodegeneration [73]. These somehow contradictory findings need to be further explored. The stable characteristic of A β accumulation in transgenic mice [74–76] and the constant incubation periods for prions [13] should make these studies easy to follow.

STRATEGIES FOR PMDs TREATMENT

It is well accepted that the misfolding process is the key event in the development of PMDs. Therefore, therapeutic strategies targeted to the prevention of misfolding are today the most promising for delaying the disease progression.

Currently, therapies for most PMDs are just palliative. It is possible that attacking the central event in the disease (the misfolding and aggregation of the protein) could lead to more effective disease-modifying therapies. In order to attack misfolding processes, different approaches have been followed. One approach is attack specifically abnormally folded proteins that can later on act as aggregation nuclei. Another strategy consists of inhibiting the misfolding process itself. The former may allow depletion of putative seeds, whereas the latter might inhibit the formation of newly converted units. Both approaches could help the endogenous protein clearance systems, resulting in the total depletion of disease associated structures.

β -Sheet Breakers and Small Molecule Amyloid Inhibitors

One approach that has been used to discover small chemical inhibitors of protein misfolding and aggregation has been the screening of large libraries of compounds using simple *in vitro* assays [77–79]. The development of high throughput screening requires a robust, simple and relevant *in vitro* assay to monitor compound activity. In the case of the search for compounds able to prevent or reverse the protein misfolding cascade, the development of a screening assay with these characteristics has been challenging. Nevertheless, there have been many small chemical compounds that have been reported to inhibit protein misfolding and aggregation. Some of these compounds came from screening, but several others were identified

serendipitously or based on epidemiological studies which have suggested they may be active. Some small molecules that have been reported to prevent the misfolding and aggregation of proteins involved in PMDs include Congo red and derivatives, curcumin and quinacrine, among others (a more comprehensive list of compounds, including the specific references, can be found in [77–80]).

Even though many molecules have been identified as strong inhibitors, the usefulness of these small molecules is compromised by their lack of specificity, toxicity and their unclear mechanism of action in most of the cases.

A strategy that has been more successful in identifying potential hit compounds is the rational development of specific inhibitors based on the use of short peptides targeting the protein region needed for protein-protein interaction [80,81]. The approach consists of synthesizing short peptides combining a self-recognition motif with a β -sheet disrupting element. The self-recognition domain is typically the region of the protein implicated in early misfolding and protein-protein interaction. As disrupting elements, different groups have used distinct strategies, including: the use of a bulky group (e.g. choly) that sterically inhibit protein aggregation; N-methylations (or N-alkylations) to generate peptides having a blocking face; β -sheet breaker amino acids to disrupt beta-sheet conformation; and addition of charged residues to reduce the hydrophobic interaction which trigger protein aggregation [80,81]. The use of peptides as a therapy struggles with three main problems, namely transport across biological membranes (i.e. the blood brain barrier and intestinal barrier), their rapid degradation in the body and generation of an immune response. However, these problems can be minimized by shortening the length of the peptide or by introducing chemical modifications [82]. The usefulness of these types of molecules has been clearly shown by β -sheet breaker peptides in models of AD and TSEs. In these studies it was shown that β -sheet breaker peptides decrease the burden of misfolded protein aggregates in brain [83–85] and reduce the toxicity associated to these proteins in cell cultures [83].

However, the recent identification of oligomers as the most toxic species in both diseases [86–88] opens a new window to consider in the exploration and use of these therapeutic agents. Following these findings it could be possible that the disruption of large aggregates into small oligomeric units by these molecules could even increase the problem by releasing new toxic species. These possibilities need to be experimentally evaluated in order to choose the right therapeutic concentrations for the chosen molecule.

Vaccines

Several attempts for immunization against PMDs have been tried. Probably the best known is the one tried by Elan pharmaceuticals against A β deposition [89]. The experimental strategy included synthetic A β_{42} as immunogen in PDAPP transgenic mice, which spontaneously generate AD plaques with age. Results showed an almost complete prevention of A β aggregates and significant reduction of astrogliosis in mice immunized before plaque generation. Animals treated with the A β vaccine at late stages of A β deposition showed a significant reduction in amyloid burden compared to PBS treated controls. As expected, high levels of antibodies against A β were found in the blood of A β_{42} treated mice. Unfortunately, clinical trials using this vaccine in humans were not successful since some of the patients involved in the trial developed meningoencephalitis, leading in some of the cases to the death of the individuals [90,91]. Currently, several approaches looking to reduce these adverse effects are under development. They include, passive immunization or vaccination with non-fibrillary A β derivatives and DNA immunization, among others [92–94].

Similar strategies have been tried for α -synuclein in Parkinson's disease [95] and PrP in TSEs [96]. For the latter we can mention the studies of Sigurdsson *et al.* [97] and Magri *et al.* [98],

where the treatment with recombinant PrP or synthetic prion derived peptides increase the incubation periods of mice and hamsters inoculated intraperitoneally with the 139A and 263K prion strains, respectively. These results expand the concept applied for AD and confirm that immunization using sequence specific peptides can decrease clinical features of PMDs. Recently, an interesting approach using an attenuated *Salmonella* vaccine expressing mouse PrP showed promising results in mice infected with 139A prions [99].

Conformational Antibodies

Current evidence supports the hypothesis that smaller and soluble oligomeric aggregates along the pathway to form large fibrillar deposits could be the molecules mostly responsible for the toxic effects observed in these maladies [86–88]. In this scenario, fibers could be acting as a protective mechanism in order to trap these particles and encapsulate them in tissue. Recent studies pioneered by the group of Charles Glabe showed the generation of antibodies specific to diverse misfolded aggregated forms of amyloidogenic proteins, including oligomers and fibrils [100]. Surprisingly, these antibodies are able to recognize oligomeric or fibrillar species regardless of the sequence. The use of such antibodies may represent a universal treatment for all amyloidogenic proteins and has the additional advantage that it targets exclusively the structure that is thought to be the toxic one.

One of the main problems with using antibodies for treatment is their stability and delivery into the CNS. Improvements in their delivery and stability could increase the use of these molecules for the treatment of PMDs affecting the brain. However, this approach could be very interesting in treating peripheral amyloidoses such as diabetes type-2. Additionally, the use of these antibodies may contribute to increase our understanding of the amyloid biology and the nature of the toxic species.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

This article describes common mechanisms associated with a variety of human diseases, collectively called PMDs. Compelling evidence accumulated over the years indicates that the key event in these diseases is the misfolding, aggregation and tissue accumulation of a protein. The available evidence suggests that these proteins misfold and aggregate by a seeding - nucleation mechanism. The formation of a stable seed, corresponding to an oligomer of the protein adopting the misfolded structure, is the key event in the process. The oligomeric seed is not only the most likely candidate for the toxic species, but also is able to accelerate the polymerization of the aggregates. As such, seeds have the intrinsic ability to propagate the misfolding and aggregation process. This provides a plausible model to explain the infectious nature of TSEs, which is so far the only PMD considered transmissible. Importantly, the ability of seeds composed of one protein to nucleate the aggregation of a different protein, through a phenomenon termed cross-seeding, provides a mechanistic explanation for the relatively common co-existence of more than one PMD in the same patient. In this article we also discussed epidemiological and experimental evidence for the interaction of PMDs and their implications for public health.

These topics open new issues in public health. The interaction between PMDs and the effect that some of them can have as risk factors for more prevalent PMDs need to be considered. In addition, the information gathered so far suggests that in all PMDs amyloidogenic structures could be transmissible. Considering the high incidence of sporadic cases in some PMDs, it is possible that a fraction of sporadic PMDs are originally generated by seeding or cross-seeding mechanisms. Therefore, disease propagation by seeding and cross-seeding is an important topic that needs to be further addressed in the years to come. A better understanding of these mechanisms could help for rationale design of novel therapeutic and diagnostic methodologies targeting these disorders.

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ABBREVIATIONS

AD	Alzheimer's disease
A β	Amyloid β
BSE	Bovine spongiform encephalopathy
GSS	Gerstmann-Sträussler-Scheinker
PMDs	Protein misfolding disorders
PD	Parkinson's disease
PrP ^C	Cellular form of the prion protein
PrP ^{Sc}	Disease-associated form of the prion protein
sCJD	sporadic Creutzfeldt Jacob Disease
TSEs	Transmissible spongiform encephalopathies
vCJD	Variant Creutzfeldt Jacob Disease

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Fig. 1. Generation of misfolded prions in TSEs

PrP^{Sc}, the disease-associated form of the prion protein, is able to impose its structure to a normally produced protein, termed PrP^C. As result, an exponential generation of misfolded prions is obtained. PrP^{Sc} has been associated with several cytotoxic events *in vitro* and *in vivo*. The accumulation of the abnormal protein in the brain leads to clinical decline, which invariably ends in the death of the affected individuals.

Fig. 2. Seeding and cross-seeding mechanisms in Protein Misfolding Disorders

A variety of factors, including mutations, abnormal biosynthesis, changes in cellular environment or impairments on the quality control machinery, induce the initial misfolding of normally folded proteins. Misfolded proteins become stabilized upon oligomerization and formation of long fibrillar amyloid aggregates. Preformed seeds have been described to speed this process *in vitro* and *in vivo*. In addition, the presence of misfolded nuclei could enhance the aggregation of other amyloids, a phenomenon known as cross-seeding.