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Mechanism of aggregation and membrane interactions of mammalian prion protein

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ABSTRACT

The cellular prion protein (PrP^C), which is present ubiquitously in all mammalian neurons, is normally found to be linked to the cell membrane through a glycosylphosphatidylinositol (GPI) anchor. The conformational conversion of PrP^C into misfolded and aggregated forms is associated with transmissible neurodegenerative diseases known as prion diseases. The importance of different misfolded conformations in prion diseases, and the mechanism by which prion aggregates induce neurotoxicity remain poorly understood. Multiple studies have been shown that the toxicity of misfolded prion protein is directly correlated with its ability to interact with and perturb membranes. This review describes the current progress toward understanding prion protein misfolding and aggregation, as well as the interaction of prion protein aggregates with lipid membrane.

1. Protein misfolding diseases and prion diseases

Protein misfolding and amyloid formation are associated with a wide range of diseases known as protein misfolding diseases [1]. Neurodegenerative diseases such as Alzheimer's disease, Huntington disease, Parkinson's disease, and the prion diseases are protein misfolding diseases, and it is believed that the deposition of specific misfolded protein aggregates is responsible for the neurotoxicity [1,2]. Increasing evidence suggests that aggregates formed from different proteins can interact directly with lipid membranes, and several of these aggregates can permeabilize artificial and cellular membranes [3–10]. This could be the primary mechanism for amyloid mediated toxicity, and also for spreading of protein aggregates from cell to cell [11–17]. Self-propagation and cell-to-cell transmission of misfolded and aggregated proteins were first reported half a century ago in the case of prion diseases [18], and observed recently with several other disease-associated proteins [19–22].

Prion diseases are neurodegenerative diseases collectively known as transmissible spongiform encephalopathies (TSEs), and are characterized by spongiform vacuolation throughout the cerebral grey matter, neuronal death, and reactive proliferation of astrocytes and microglia. Prions are fatal pathogenic agents consisting solely of proteins, and the name prion (proteinaceous infectious particle) originates from their ability to cause infectious diseases with no involvement of nucleic acids. Later, it was discovered that the conformational conversion of the native, monomeric, cellular prion protein (PrP^C) into a misfolded and aggregated form (PrP^{Sc}), is associated with prion disease pathology and neurodegeneration [23,24].

2. The prion protein

PrP^C is primarily present in mammalian neuronal cells, and is attached to the cell membrane *via* a glycosylphosphatidylinositol (GPI) anchor [25]. The *Prnp* gene encodes a 253-residue precursor prion protein, in which the first 22 N-terminal signal peptide residues are removed post-translationally during transport to the ER, and the last 23 C-terminal residues are removed after addition of the GPI-anchor [24]. Hence, the final mature prion protein consists of 208 residues. Although the structure of membrane-bound PrP^C remains unknown, it is widely accepted that the structure of recombinant prion protein (PrP) represents that of PrP^C. The three-dimensional structure was determined by NMR spectroscopy using recombinant PrP (23–231) devoid of glycosylation and the GPI-anchor [26] (Fig. 1). The N-terminal region (NTR) (residues 23–120) is largely unstructured, and the C-terminal domain (CTD) of the prion protein is a globular structure containing three α-helices, and a small antiparallel β-sheet. The unstructured NTR and the structured CTD are linked by a highly conserved hydrophobic sequence segment, known as the middle hydrophobic core (HC) region (residues 105–126). This region has been implicated to play a major role in the conformational conversion, and membrane interactions of the prion protein [4,27–29].

Although the structure of PrP^C has been characterized in detail, the

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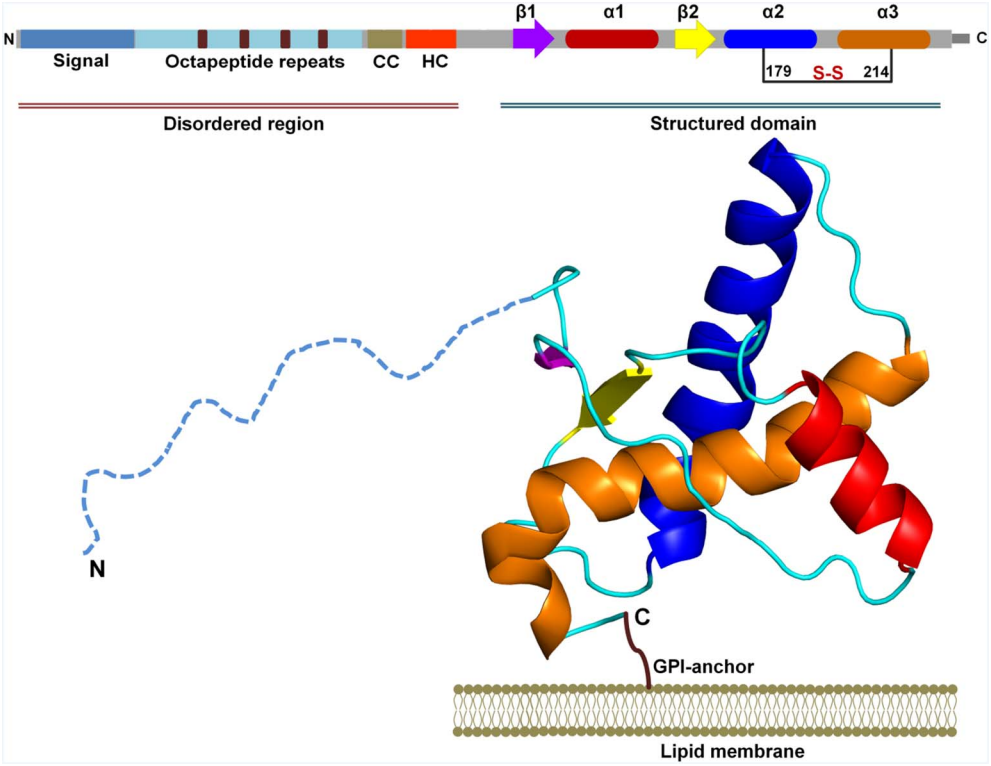

Fig. 1. Structural features of the mammalian prion protein: The prion protein is a 210 amino acid residue protein. The three-dimensional structure of PrP was determined by NMR spectroscopy using recombinant PrP (23–231) devoid of glycosylation and the GPI-anchor. The N-terminal region (NTR) (residues 23–120) is mostly disordered, and contains four or five octapeptide repeats, a charged cluster region (CC), and a hydrophobic core region (HC). The C-terminal domain (CTD) (residues 120–231) consists of a globular core containing three α-helices (α1, residues 144–154; α2, residues 172–193; α3, residues 200–227) and a small anti-parallel β-sheet (residues 129–131 and 161–163). The mature protein is attached to the cell membrane *via* a glycosylphosphatidylinositol (GPI) anchor. The CTD contains a disulfide bond that links α-helices 2 and 3. The structure has been drawn from Protein data bank entry 1AG2, using PyMOL [166].

exact biological function of PrP^{C} remains unclear. Functions that have been attributed to PrP^{C} include copper homeostasis, protection from apoptotic stimuli or induction of apoptosis signal, signal transduction, and immunoregulation [30]. Despite the wide distribution of the prion protein in mammalian neurons, *Prnp* gene knockout mice ($Prnp^{0/0}$) failed to show any significant pathological phenotypes [31]. Moreover, the studies conducted until now have failed to reveal any single essential function of the prion protein, but have suggested that the prion protein might be a pleiotropic protein with multiple functions.

3. Connection between prion diseases: misfolding of the prion protein

Prion diseases can occur spontaneously, by infection, or by hereditary mutations, but the common connection between all prion diseases is the misfolded state of prion [30,32,33]. A large number of prion disease cases appear spontaneously (≈85%), or are transmitted from individuals infected with prion diseases (≈5%). Nevertheless, a significant number (≈10%) of hereditary (familial) forms of prion diseases, due to specific mutations in the *Prnp* gene, have been reported in humans and other mammals [34]. Mutations in the *Prnp* gene that are associated with prion diseases can be broadly divided into two categories: mutations that cause changes in the protein and induce prion diseases, and mutations that prevent prion disease propagation [27,30,35–40]. Despite the fact that pathogenic mutations show different effects on PrP, it is very unlikely that TSEs are induced by the loss of functional PrP^{C} due its misfolding, because mice devoid of PrP^{C} do not show neurodegeneration [41]. Hence, it is possible that TSEs are caused by a gain of toxic function due to the formation of PrP^{Sc}.

In some, but not all, prion diseases, the formation and accumulation of the pathological form of the prion protein, PrP^{Sc} is seen [30]. The progressive accumulation of PrP^{Sc} in certain prion diseases is known to correlate with the extent of severity of the disease [13,42,43]. It appears that there is a strong correlation between prion protein misfolding and TSEs. However, increasing evidence suggests that misfolding and aggregation of PrP is an important, but not a sufficient factor in prion disease aetiology. Although PrP^{Sc} is well established as the infectious form, it might not be the direct cause of neurodegeneration, at least in some prion diseases. Several disease-linked mutations in animals do not result in any accumulation of such amyloid plaques [29,38,44].

Despite the clear understanding about the presence of these two species, PrP^{C} and misfolded prion protein, the mechanism of conformational conversion, as well as the final structure of the misfolded prion protein remain unclear. Although the structure of PrP^{C} is well known, the structure of PrP^{Sc} remains poorly defined. A detailed structural understanding of the misfolded, aggregated, protease-resistant PrP^{Sc} is therefore essential. *In vitro* studies of prion protein misfolding and aggregation invariably utilize recombinant PrP (PrP).

4. Structure of misfolded prion oligomers and fibrils

Structural studies of prion protein aggregate deposits found *in vivo* suggest that the internal structure of these aggregates is similar to that of the aggregates generated *in vitro* using a PrP [42,45,46]. Amyloid fibrils formed from the unglycosylated anchorless prion protein can be imaged using Transmission Electron Microscopy (TEM) or Atomic Force Microscopy (AFM) (Fig. 2), and these imaging techniques have shown that these fibrillar species are typically long, straight and unbranched polymers having a thickness of 6–10 nm, and a length of a few microns, and are made up of a few proto-filaments [35,47–52]. A high-resolution structural study of natural and synthetic prion protein aggregates using X-ray diffraction methods suggested that the prion protein can form amyloid cross-β structure [53]. Structural characterization of *in vitro* generated amyloid fibrils from full-length prion protein using solid-state NMR (ssNMR) and molecular dynamics simulations indicated that a relatively small fraction of the sequence present at the C-terminal end (173–224) of the prion protein forms the structurally ordered amyloid core [54,55].

Studies with PrP using techniques such as hydrogen-deuterium exchange mass spectrometry (HDX-MS) and electron paramagnetic resonance (EPR) spectroscopy provided evidence that the C-terminal region of the prion protein forms the structural core of the amyloid

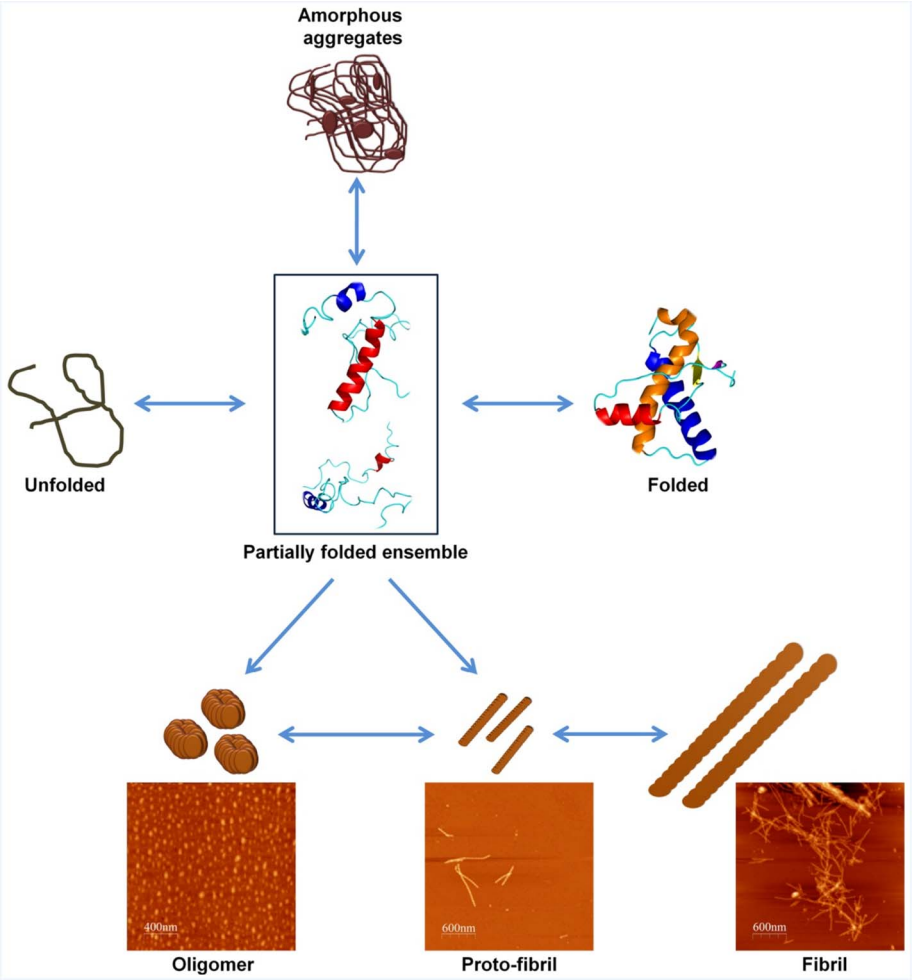

Fig. 2. A schematic representation of some of the many conformations that can be attained by the mammalian prion protein. The transition from an unfolded polypeptide chain to the functionally active native folded form can occur through different intermediate structures. The transition from native folded form to β-sheet rich aggregates could initiate from these partially structured intermediate structures populated during folding or unfolding. The growth of amyloid fibrils can occur by the addition of conformationally converted monomer, or oligomers, or protofibrils.

aggregates [3,46,47,56]. Several studies have also focused on the identification of amyloidogenic sequences in the prion protein. These structural studies of amyloid aggregates formed from different fragments of prion protein suggest that the structural core of the prion aggregates can vary in the sequence stretch 90 to 220 [13,57]. It is widely accepted that the morphology and ultrastructural features of fibrils can vary significantly with different scrapie forms. These features include secondary structures, glycosylation patterns, the stability of final aggregates, as well as conformational templating activities which can contribute to the strain-dependent features of prion protein aggregates [58–60].

Recent studies have challenged the classical theory that conformational conversion of PrP^{C} into a misfolded, insoluble, aggregated, and protease-resistant form, PrP^{Sc}, causes neurodegenerative diseases. These studies have indicated that protease-sensitive, soluble, oligomeric species may be the culprit in several prion diseases [61–63]. The earliest aggregates normally appear as spherical, or rod-like, or curvilinear structures with an approximate diameter of 2.0–6.0 nm (Fig. 2) [3,13,64–66]. Such oligomeric intermediates may be either on-pathway or off-pathway to amyloid fibril formation [64,67].

Structural and kinetic studies using NMR, HDX-MS, EPR, and Fourier-transform infrared spectroscopy (FTIR) show that α2 converts into β-sheet, and that α1 and β1 are unfolded in the oligomers, while the structural status of α3 remains unclear [3,27,46,56,68–70]. FTIR studies indicate a significant loss of helical structure in the oligomers, and an increase in cross-β sheet structure characteristic of amyloid structures [27,65,71].

5. Mechanism of prion protein misfolding and effects of familial mutations

The novel mechanism by which PrP^{Sc} acts like an infectious agent by interacting with the normal PrP^{C} hints that the prion protein misfolding mechanism is very similar to that of nucleation dependent polymerization (NDP) [18,72,73]. Furthermore, a study with Aβ peptide has suggested that the NDP mechanism can be a general mechanism for amyloid-mediated cellular pathology [74]. At physiological pH and in the presence of chemical denaturants, PrP aggregates *via* an NDP mechanism and forms long straight amyloid fibrils [35,52]. PrP^{C} is known to misfold and oligomerize in the endocytic pathway, where it encounters low pH [75]. Moreover, it has been observed that the formation of misfolded oligomers at low pH correlates well with the propensity to get prion disease [76].

In many familial forms of protein misfolding diseases, it has been observed that the destabilization of the native structure of a functional protein is the primary mechanism by which natural pathogenic mutations mediate their pathogenic effects [40,77]. Several disease-linked mutations found in the CTD of the prion protein affect the thermodynamic stability and increase the native state dynamics [39,40,69,70,78–81]. However, some disease-linked mutations found

in the α3 region of CTD do not affect the thermodynamic stability of the prion protein [78]. Pathogenic mutations found in the unstructured NTR do not also affect either the thermodynamic stability or structural dynamics of the prion protein [27,79,82]. Nonetheless, almost all these mutations have been shown to affect both the misfolding and the aggregation of the prion protein [27,38,50]. Moreover, pathogenic mutations perturb the conformational properties of the polypeptide chain, and alter the dynamic equilibrium between the native state and other aggregation-prone intermediate states [83–85].

Collectively, the studies with disease-linked mutant variants of the prion protein suggest that the mutations affect the conformational conversion processes and induce prion disease pathology, with or without affecting the thermodynamic stability of the prion protein. Studies with deletion variants and pathogenic mutant variants of prion protein have shown that the unstructured NTR, especially the middle hydrophobic region, plays a critical role in the conformational conversion, and membrane interaction of the prion protein [4,27,35,86,87].

6. Prion protein pre-fibrillar aggregates and channel hypothesis

In prion diseases, the disease pathology is associated mostly with the gain of toxic function, rather than with the loss of normal functional protein [21,30,88]. The toxic oligomer hypothesis of amyloid disease suggests that the oligomeric protein assemblies, formed from non-toxic proteins, are responsible for the toxic gain of function [13,14,89–91]. In many neurodegenerative diseases, including prion diseases, pre-fibrillar aggregates, as opposed to mature amyloid fibrils, are considered responsible for the neurotoxicity [14,92]. Moreover, it has been shown that the most infectious structures for the prion protein are small oligomers [91]. Pre-fibrillar aggregates made *in vitro* from both PrP^{C} and PrP have been shown to be cytotoxic, and to spread disease in healthy animals [51,93–96]. However, it is important to point out that these *in vitro* generated aggregates are not as infectious as the *in vivo* generated PrP^{Sc}.

Although many studies have shown the significance of amyloid conformations of proteins in neurodegenerative disorders, the molecular properties of the pathogenic species, and the mechanism by which protein aggregates induce cell damage remain unclear [14,97]. Increasing evidence indicates that the toxicity of misfolded prion protein is directly correlated with the ability to interact with, and to disrupt, lipid membranes, thereby perturbing the ionic homeostasis of a cell [3,4,13,70,98]. Indeed, amyloid oligomers formed from several different proteins can permeabilize lipid bilayers and cellular membranes, without any post-translational modifications, suggesting that this might be the primary toxic mechanism of amyloid pathogenesis [6,7,14,99–101].

In the case of the prion protein, it has been shown that peptides derived from sequence segments 82–146, 105–126, and 185–206, have the capability to form ion channels [7,86,102–104]. Recent studies have shown that pre-fibrillar aggregates of PrP can disrupt lipid membrane structures [3,70], suggesting that such disruption may be the mechanism of prion protein mediated neurotoxicity (Fig. 3). The prion protein can also assemble in the membrane and form specific channels [4]. Similarly, in eukaryotes, it has been shown that pro-apoptotic members of the Bcl-2 family assemble into oligomers that form non-specific channels in the target mitochondrial membrane [105]. In many cases, protein insertion into the plasma membrane of the target cell requires a conformational change leading to changes in its secondary structural elements. It is not clear, however, if the structural changes in the Bcl-2 family proteins [106], as well as the pore-forming toxins [107] and are equivalent to those in the case of pre-fibrillar aggregates of PrP [108] (Fig. 4).

7. Neurotoxicity of prion oligomers

Many protein misfolding diseases share a significant number of common pathological features such as evidence of membrane interaction and damage, oxidative stress, elevated levels of cytosolic calcium, metal and ion dyshomeostasis, mitochondrial dysfunction, up-regulation of autophagy, and apoptosis [109–115]. The well-studied apoptotic regulatory pathways connected with neurodegenerative disorders include those involving caspases and ER stress [116–119]. Caspase-dependent apoptotic pathways can be triggered by the activation of death receptors, and also by mitochondrial stress [115,119]. ER stress-induced apoptosis can be activated by problems associated with calcium homeostasis, or by the accumulation of misfolded proteins above a certain threshold inside the cell. The connection between calcium homeostasis and cell death is supported by many studies that show the formation of non-specific ion channels by protein oligomers [4,99,120,121]. Many cell-based studies have suggested that dysregulation in cellular machinery is associated with protein misfolding and aggregation [122]. However, none of the studies has shown the involvement of a specific pathway in the apoptosis induced by protein aggregation [123]. Elucidation of the regulatory pathways involved is important for identifying novel targets for therapeutic intervention.

8. Molecular features of misfolded prion protein-mediated toxicity

Several studies have provided insights into the formation, biophysical and biochemical characteristics, structural conversion, as well as the mechanism of neurotoxicity of prion oligomers [124–126]. PrP oligomers exhibit neurotoxicity and induce neuronal apoptosis under *in vivo* and *in vitro* conditions [14,65,127]. Consequently, the focus of research on pathological effects associated with protein aggregates has shifted to pre-fibrillar intermediates such as oligomers. It has been observed that the cytotoxic effects of *in vitro* generated aggregates depend on the supramolecular assembly of the amyloid aggregates, and the oligomers formed by different proteins share a common structure, as well as mechanism of toxicity [13,15,89,90].

The toxicity induced by pre-fibrillar aggregates and oligomers is likely to arise from the exposure of hydrophobic side chains, and other regions in the polypeptide chains [13,127–130]. These sites are more accessible in the pre-fibrillar aggregates and oligomers than in either the monomer or the fully formed amyloid fibrils. It is possible that the surface exposure of amino acid residues enables these species to interact with a wide range of cellular components such as the cell membrane, cellular signaling components, and protein homeostasis machineries. Such abnormal interactions might be the responsible for oligomer toxicity [13,15,110,126]. The PrP^{C} present on cells has a role in mediating neurotoxic signaling *via* interaction with different toxic conformers of other proteins [131]. It has been shown that the NTR of PrP^{C} binds to the toxic amyloid β oligomer, and modulates its toxic effects through an unknown mechanism [43,132,133].

Although pre-fibrillar species are considered as the major toxic entity, it has been shown that fibrils and fibril fragments of some disease-linked proteins are also equally toxic to both cultured neuronal cell lines and organisms [93,134]. Despite the significant advancement in the understanding of oligomer structure, conformational conversion and mechanism of toxicity, the transmissibility and self-propagating features have not been shown for these *in vitro* generated PrP oligomers. It appears that these oligomers may be a dead-end product, off-pathway to the amyloid fibril formation reaction [64,70]. Therefore, the formation of amyloid deposits, which is the characteristic feature of many protein misfolding diseases, is now considered as a pathway for the sequestration of the toxic pre-fibrillar species [67,135–137]. A detailed understanding of the size, structure, conformation, and toxicity of protein aggregates involved in protein misfolding diseases is critical to the elucidation of the molecular mechanisms behind the amyloid mediated pathology.

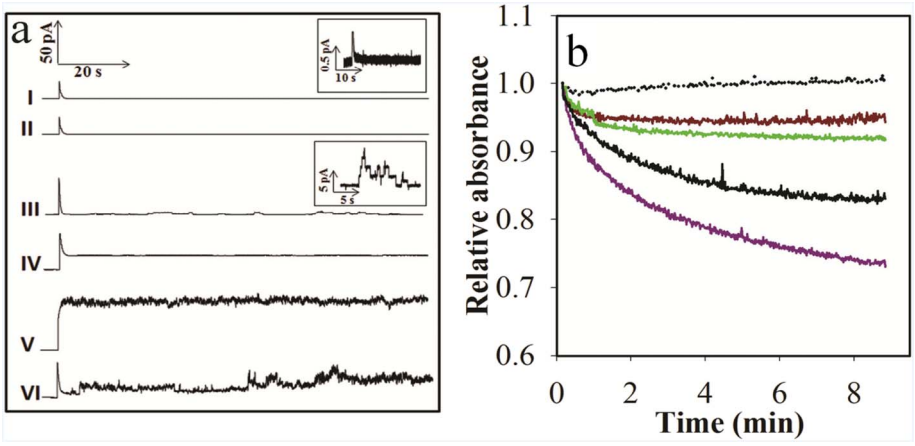

Fig. 3. Comparison of the abilities of mouse prion protein (moPrP) amyloid aggregates to perturb lipid membrane structures. Traces of current flow through the membrane after adding (I) 50 μL of aggregation buffer, (II) 100 nM monomeric moPrP, (III) 250 nM monomeric moPrP, (IV) 100 nM worm-like fibrils, (V) 100 nM oligomer L, and (VI) 100 nM oligomer S (Panel a). The insets in I and III show expanded sections of the recordings. Single channel opening and closing events are seen when 250 nM monomeric moPrP is added to the BLM. Panel b shows liposome swelling monitored by decrease in absorbance at 520 nm. Unilamellar liposomes were made from 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhPC) and cholesterol (1:1 v/v), and with Dextran (10 kDa) encapsulated. Swelling of liposomes pre-incubated with different aggregates was monitored upon dilution into isosmotic buffer containing PEG200. Liposomes were pre-incubated with buffer (black dotted line); 5 μM monomeric moPrP (brown line); 100 nM oligomer L (purple line); 100 nM oligomer S (black solid line) and 100 nM worm-like fibrils (green line) (Figure is adapted with permission and modified from [3]).(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

9. GPI-anchored prion protein and its effects on membrane interaction

Although PrP^{C} is known to be present as a GPI-anchored extracellular protein, the GPI-anchor status of misfolded prion protein remains unclear [138]. Moreover, the role of the GPI-anchor in prion protein misfolding, and in aggregates mediating neurotoxicity remains unclear. It should be pointed out that most of the *in vitro* toxicity and infectivity experiments are carried out with prion protein lacking a GPI anchor. Cytosolic PrP has also been shown to cause neurodegenerative features, without any significant accumulation of PrP^{Sc} [139,140]. It appears that alternative forms of PrP, both soluble and membrane-attached, which are different from PrP^{Sc} in both structural and biochemical properties, may have important roles in prion-mediated neurodegeneration [141]. Anchorless prion protein expression has been shown to cause brain damage in transgenic mice [142]. Moreover, PrP can be “shed” from the cell surface upon cleavage, either within the protein or at the linkage to the GPI anchor, under physiological conditions [143–145].

Many studies have utilized PrP^{C} to study the importance of the GPI-anchor in prion protein misfolding and aggregation, and prion diseases. Several pathogenic mutations are found in the middle hydrophobic region, and most of these mutations cause the Gerstmann-Straussler-Scheinker (GSS) syndrome [34]. Several studies have reported the effects of these NTR mutations. Several studies of have shown that the expression of deletion variants of PrP^{C} or of pathogenic mutations present in the NTR induces spontaneous inward currents at negative potentials [87,146]. An *in vivo* study of both the G113V and A116V mutant variants has reported that these mutations lead to the formation of a highly neurotoxic protease-sensitive PrP conformation [38]. Recently, the A116V mutation in mouse PrP without the GPI-anchor has been shown to enhance the membrane interaction ability of the prion protein, leading to the formation of ion-selective channels [4].

10. Prion-like behavior of protein aggregates in neurodegenerative diseases

The self-propagating nature of prion amyloid aggregates, similar to seeding under *in vitro* conditions, is also shown by aggregates of the proteins in many age-dependent neurodegenerative disorders including Alzheimer's disease, Parkinson's disease and the tauopathies [22,147–152]. These aggregates have the ability to propagate across neuronal cells; however, the molecular mechanism behind the prion-like behavior remains unclear [12,22,147]. Hence, in many neurodegenerative disorders, it appears that the prion-like behavior of misfolded and aggregated proteins can account not only for the disease progression but also for the propagation of these diseases [151]. However, it remains unclear as to what kind of protein aggregates form the most infectious material [32,91,153].

It is now widely accepted that virtually all proteins can aggregate under specific conditions, and form amyloid fibril-like structures [154]. Whether the protein misfolding and aggregation propensity has any connection with disease or not, many protein aggregates have been shown to have seeding abilities, at least, under *in vitro* conditions. The

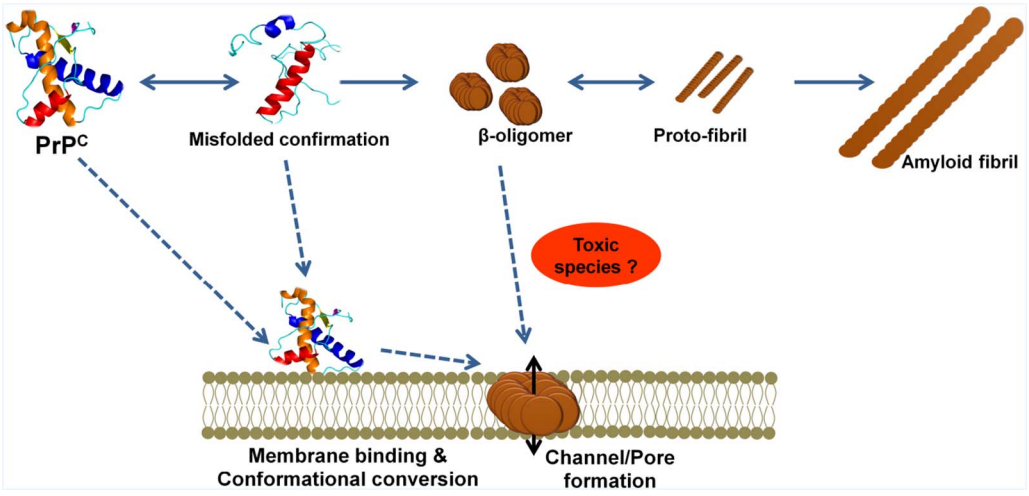

Fig. 4. Prion protein amyloid fibril formation and amyloid membrane interaction: Amyloid fibril formation starts from the misfolding of partially structured folding intermediates into β-sheet rich oligomers and proto-fibrils, and these structures further associate and form amyloid fibrils. Native PrP and/or misfolded intermediates, which expose the hydrophobic core (HC) region could interact with lipid membrane [4], and undergo conformational conversion on the membrane. A recent study has shown PrP oligomers with central cavity [167], and it is possible that such oligomers could interact with lipid membrane and form pores/channels in the membrane, resulting in the leakage of membrane contents.

seeding ability of protein aggregates is the ability to abolish the lag phase (nucleation phase), upon the addition of the preformed aggregates formed under similar/different conditions. Increasing concentrations of the seed increase the rate constant of polymerization, and completely abolishes the lag phase by bypassing the nucleation step [155–158]. The seeding ability appears to be an intrinsic feature of amyloid fibrils. Nevertheless, it is not yet clear whether this seeding ability is a necessary criterion, in the case of disease-causing proteins, for their cell-to-cell transmission and prion-like behavior in disease conditions [159,160]. If seeding ability is a salient criterion for prion-like behavior, then virtually all proteins, at least under *in vitro* conditions, possess prion-like self-propagation behavior. Hence, the seeding ability might be the primary mechanism by which an infectious agent propagates to another cell or individual.

11. Inhibitors of amyloid aggregate-membrane interactions can be potential drug molecules against protein misfolding diseases

Current therapeutic strategies against prion diseases are based on the assumption that PrP^{C} converts into PrP^{Sc}, and consequently accumulates in the central and peripheral nervous system. Based on this assumption, recent therapeutic strategies include direct inhibition of prion conversion by either stabilizing the monomer or blocking monomer-monomer interactions, degradation or the clearance of PrP^{Sc} or by altering the expression and localization of PrP^{C} [161,162]. Many studies have attempted to develop immunotherapies against prion diseases, as antibodies can prevent prion conformational conversion under *in vitro* conditions [163]. Although immunotherapeutic approaches can affect the accumulation of PrP^{Sc} in various parts of the peripheral nervous system [164], the ability to prevent the progression of the diseases in the central nervous system is very limited. The channel hypothesis has been proposed as a mechanism for amyloid-induced cell toxicity [7,102]. Insights into the mechanism of membrane poration caused by amyloid proteins will have implications in the design of novel therapeutic molecules. Such therapeutic molecules can be used for inhibiting pore formation, or for blocking the pore forming activity. Since the middle hydrophobic region of the prion protein is known to play an important role both in the productive association of PrP^{C} with PrP^{Sc} [28], and in the prion protein-membrane interaction [4,29,86,165], this region might be a potential drug target for preventing prion mediated neurotoxicity. The possibility of generating monoclonal antibodies against different regions of the prion protein can be used for understanding the pathophysiology of prion diseases, and also for obtaining passive immunization against prion diseases.

12. Conclusion

The misfolding and aggregation of the prion protein is a feature of the neurodegenerative diseases known as transmissible spongiform encephalopathies, or prion diseases. Mechanistic studies have shown that the prion protein forms oligomers and proto-fibrils, and amyloid fibrils under different aggregation conditions. Understanding the mechanism of misfolding and aggregation is necessary from the perspective of designing therapeutics that will abrogate the formation and propagation of protein aggregates. Studies with different pathogenic mutant variants of the prion protein have revealed how different pathogenic mutations affect the process of misfolding and aggregation. Early aggregate species like oligomers and proto-fibrils are in general known to be the species responsible for neurotoxicity in prion diseases. There is growing evidence that these aggregates act by perturbing lipid membrane structure. Thus, mechanistic and structural characterization of these aggregate species, and their interactions with the cell membrane, may help explain the correlation between amyloid deposits and neuronal death. Although high-resolution structures of the final misfolded, disease-causing species of the prion protein are lacking, significant progress has been made toward understanding the molecular mechanism of misfolding and finding out the critical regions important for both the misfolding and unusual prion-membrane interactions. This knowledge can be used as the starting point for the search for therapeutic molecules that prevent the conformational conversion of the prion protein.

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