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Review

The structure of prion: is it enough for interpreting the diverse phenotypes of prion diseases?

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Prion diseases, or transmissible spongiform encephalopathies, are neurodegenerative diseases, which affect human and many species of animals with 100% fatality rate. The most accepted etiology for prion disease is 'prion', which arises from the conversion from cellular PrP^{C} to the pathological PrP^{Sc}. This review discussed the characteristic structure of PrP, including *PRNP* gene, PrP^{C}, PrP^{Sc}, PrP amyloid, and prion strains.

Keywords PrP; PrP^{C}; prion disease; PrP structure

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Introduction

Transmissible spongiform encephalopathies (TSEs), or prion diseases, are neurodegenerative conditions caused by prions, which was characterized by cognitive and motor impairments, neuronal dysfunction, extensive brain damage, and finally death. TSEs may occur in human and various animals, such as Creutzfeldt-Jakob disease in humans [1], scrapie in sheep [2], bovine spongiform encephalopathy (also known as mad cow disease) in cattle, and chronic wasting disease in deer [2]. The infectious agents consist of PrPSc, a misfolded and aggregated form of the cellular prion protein (PrPC). This review will discuss the latest findings about the structure of PrP and its association with the phenotypes of prion diseases.

PRNP Gene

The PrP protein is encoded by a chromosomal gene, *PRNP*. Besides *PRNP*, there are some members of the *Prn* gene family including *Prnd*, the doppel protein encoded gene [3], and *Sprn*, the shadoo protein encoded gene [4]. The full-length open reading frame (ORF) of all known mammalian and avian *PrP* genes locates in a single exon [5–8]. The Syrian hamster PrP (*SHaPrP*) gene has two exons, which are separated by a 10-kb intron. The exon 2 includes the ORF and 3′ untranslated regions, and exon 1 includes a part of the 5′ untranslated leader sequence [6]. The mouse, rat, cattle and sheep *PrP* genes contain three exons and the ORFs for *PrP* locate in exon 3 [9–13]. Additionally, an untranslated 5′ exon was discovered in the genes of *SHaPrP* [14] and human PrP (*HuPrP*) [15]. The *PrP* promoter contains multiple copies of GC-rich repeats, which is a canonical binding site for the transcription factor Sp1 [16], leading to expressing in different tissues, such as brains, muscles, and some immunocytes, and the highest levels of *PrP* mRNA are found in neurons by *in situ* hybridization [17]. The alignment of the ORFs for PrP proteins from more than 40 species shows a conspicuous conservation among the mammals, suggesting an important role of PrP in the evolution progress.

PrP^{C}

PrP is a cell membrane protein. In the process of modification after translation, peptide amino acids (aa) 1–22 is cleaved as signal peptide during trafficking, and peptide from aa 232 to the end is presented as the glycosylphosphatidylinositol (GPI) anchor. The full length of PrP protein is from aa 23 to 231. The nuclear magnetic resonance (NMR) structures for normal PrP of some species, such as mice, humans, Syrian hamsters and cattle, have been successfully illustrated, sharing common features: a long, flexible amino-terminal tail (residues 23–128), three α-helices, and a two-stranded anti-parallel β-sheet that flanks the first α-helix [18]. The second β-sheet and the third α-helix are connected by a large loop with interesting structural properties [19]. Fourier-transform infrared and circular dichroism studies showed that PrP^{C} contains about 40% α-helix and a small amount of β-sheet [20]. The carboxyl terminus of PrP^{C} is stabilized by a disulfide bond that

links helices two and three [21] and exhibits a globular structure. A crystal structure of PrP has been obtained, largely in agreement with the NMR structures [22].

In the unstructured *N*-terminal of PrP molecule, there are two defined and conserved regions. The first region consists of a segment of five repeats of eight amino acids sequence (octapeptide repeat region, OR) [18]. This region is important to bind with divalent metal ions, like copper [23,24] and zinc [25], and could be involved in prion pathogenesis [26,27]. The second region contains a highly hydrophobic and conserved profile, which is proposed to be the transmembrane region of the PrP molecule. In addition, the region around histidine-96 in PrP is believed to be the binding site for manganese [28] and zinc [25], which may also contribute to the pathogenesis of prion diseases. Besides metal ions, unstructured *N*-terminal region also interacts with a broad range of partners. It shows binding activity with small unilamellar vesicles containing phosphatidylserine, particularly at acidic pH, by residues 23–145. Binding lipids may increase the ordered conformation of this normally flexible domain [29]. *N*-terminal of PrP^{C} possesses binding activity with nucleic acid, in which the binding sites are mapped to residues 23–108 or 23–52 [30]. Deletion of the OR (rPrPΔ51–90) abolishes the ability of binding to RNA [31–33]. Recombinant PrP can bind with DNA and RNA molecules *in vitro* and induces the assembly of condensed nucleoprotein structures [30,34]. A cytoplasmic PrP^{C} mutant can even interact with mRNAs [35]. Sulfated glycosaminoglycans (GAGs) were detected in PrP^{Sc} plaques in the brain of Gerstmann-Sträussler-Scheinker syndrome, CJD and scrapie [36]. Several binding domains for GAGs were identified within *N* terminal of PrP^{C}. Residues 23–35 are revealed as a strong binding site for heparin, a highly sulfated GAG [37], and other binding sites include residues 23–52, 53–93, and 110–128 [38]. Accurately, Lys23, Lys24, Lys27, Lys101, and histidine residues in the OR [39] are responsible for the binding. As mentioned previously, although ORs are involved in binding Cu^{2+}, Cu^{2+} enhances heparin binding to PrP^{C} rather than competes with it [37,38,40], which may through a conformational change in the *N*-terminus of PrP [39]. Multiple iron-containing protoporphyrin IX or hemin molecules bind to residues 34–94 of hamster recombinant PrP (rPrP) [41]. However, the role of hemin-PrP^{C} interactions remains unclear. Interestingly, some antiprion compounds that prevent the conversion of PrP^{C} into PrP^{Sc}, like sulfated glycans, sulfonated dyes, and phosphorothioate oligonucleotides, show binding activities to *N* terminal of PrP^{C}. They might competitively interact with PrP^{C} and accordingly block the interaction between PrP^{C} and endogenous GAG that could be required for the conversion to PrP^{Sc} [42,43].

Our lab has identified many protein ligands binding to PrP^{C}. Tubulin interacts with rPrP and the sites were mapped in the *N*-terminus of PrP spanning residues 23–50 and 51–91, in which PrP octapeptide repeats are critical for the binding activity with tubulin [44]. PrP can interact with microtubule associated protein Tau and the octapeptide repeats within PrP, which directly affects the binding activity of PrP with Tau [45]. Another cell skeleton protein, tubulin polymerization promoting protein, interacts with PrP and the binding site of PrP locates at the segment spanning residues 106–126 [46]. Our data highlight a potential role of PrP in regulating the microtubule dynamics in neurons. The recombinant full length PrP interacts with ApoE by the *N*-terminal of PrP (aa 23–90) [47] and may be involved in the conformational change of PrP^{C}. Bioinformatics analysis predicted that a panel of proteins could interact with PrP and some of them have been confirmed experimentally to be able to bind with PrP, even within PrP *N*-terminus [48]. It highlights that the *N*-terminal segment of PrP possesses active biological functions.

Besides the wild-type PrP, there are various mutant PrPs, which can cause familial CJD in human. Some structures of PrP mutants have been unraveled. T188K gCJD, which is the most frequent gCJD in China, almost does not alter the native structure of PrP, but perturbs its stability and makes it accumulate more easily [49]. Fatal familial insomnia is the most frequent genetic prion disease in China and the corresponding mutation is D178N/M129. This point mutation is believed to induce the absence of a salt bridge that causes the instability of the mutant PrP [50]. In addition, the mutants of V180I, T183A, E196K, F198S, E200K, R208H, V210I, and E211Q seem to preserve the native state, but the dynamic changes would perturb the coordination of the α2-α3 hairpin to the rest of the molecule and cause the reorganization of the patches for intermolecular recognition [51].

PrP^{Sc}

The most special features of PrP^{Sc} are its resistance to protease and its insolubility in detergent. So far, neither crystal nor solution-based NMR has been obtained. However, it is well known that when PrP^{C} converts to PrP^{Sc}, the content of β-sheet increases dramatically from about 3%, to roughly 45%, while the content of α-helix reduces slightly from 40% to about 30% [20]. The cleavage site of protease within PrP^{Sc} usually locates around the residue 90 and produces the protease-resistant core of aa 90–231, which has an apparent molecular mass of 27–30 kDa, therefore, referred as PrP27–30 [52]. X-ray crystallographic structure of the prion protein from residues 90–231 is available [53]. It has been demonstrated that the crystal structure of the human prion protein is a dimer, which results from the three-dimensional swapping of the C-terminal helix 3 and rearrangement of the disulfide bond. An interchain links

two stranded antiparallel β-sheet is formed at the dimer interface by residues that are located in helix 2 in the monomeric NMR structures. This result has provided the clue for the details of conversion from PrP^{C} to PrP^{Sc}. The computational modeling revealed that substructure for PrP^{Sc} is the trimeric, left-handed β-helices [54]. Additionally, X-ray diffraction patterns obtained from PrP 27–30 fibers were consistent with this model [55].

Prion Amyloid

In the presence of detergent, PrP27–30 polymerizes into amyloid [56]. After formation of amyloid, the PrP can be visualized by Congo red dying. Electron microscopy of negative staining repeatedly demonstrated the irregular rod-shaped particles in the lesions [57]. Unlike conventional viruses, prion rods usually are not uniform [58]. Those special features of prion amyloid have been turned into useful detection tools for prion [59]. Although amyloid plaques exist in some kinds or subtypes of animal and human prion diseases [60–62], they are not the indispensable hallmark of prion diseases. Only about 10% of sporadic Creutzfeldt-Jakob disease cases show amyloid plaques in their brain tissues, in contrary to kuru that 70% cases contain detectable plaques. Interestingly, all variant Creutzfeldt-Jakob disease (vCJD) cases show very special amyloid plaques that are surrounded by a halo of spongiform degeneration, namely florid plaque [63,64], which is used as the definite diagnostic criteria for vCJD. Although partial resistance to protease digestion has been a convenient tool for distinguishing PrP^{Sc} from PrP^{C}, not all PrP^{Sc} molecules are non-proteolysis [65–69]; these protease-sensitive PrP^{Sc} forms are designated as sensitive PrP^{Sc} ($sPrP^{Sc}$). Recently, it has been proved that $sPrP^{Sc}$ is infectious and shares basic structural features with PK-resistant PrP^{Sc} [70].

Topology of PrP Protein

Besides the different secondary structures of PrP^{C} and PrP^{Sc}, PrP^{C} can adopt multiple membrane topologies. As mentioned previously, PrP^{C} is attached to the outer leaflet of the plasma membrane through the GPI anchor, which is referred to SecPrP [71,72]. When expressing the full-length PrP in cultured mammalian cells, two diverse transmembrane orientations may form, which are designated as ^{Ntm}PrP and ^{Ctm}PrP [73–79]. ^{Ntm}PrP and ^{Ctm}PrP span the lipid bilayer once via a highly conserved hydrophobic region (aa 111–134), leaving the *N* or *C* terminus on the extracytoplasmic side of the membrane, respectively. They are generated along with the normal biosynthesis of wild-type PrP in the endoplasmic reticulum. Expressions of the mutations occurred within or near the transmembrane domain, such as A117V mutation linked to GSS, G114V linked to fCJD, as well as several 'artificial' mutations not seen in human patients, and the amount of cellular ^{Ctm}PrP increases [75,80,81]. Moreover, a non-conservative substitution (L9R) within the hydrophobic core of the signal sequence can also enhance the portion of ^{Ctm}PrP [82]. Combining this mutation with a triple substitution (3AV) within the transmembrane domain results in a molecule that is synthesized exclusively as ^{Ctm}PrP. Point mutations (M232R and M232T) in the GPI signal peptide (GPI-SP) of the PrP protein, which segregate with familial CJD, also exhibited a ^{Ctm}PrP topology [83]. ^{Ctm}PrP is neurotoxic and induces neuron apoptosis. Another topological variant of PrP that has been proposed as a neurotoxic intermediate is cytosolic PrP. The artificial form of PrP, which lacks the signal sequence, presumably favors accumulation of PrP in the cytoplasm [84].

Prion Strains

In contrary to conventional viruses, prions are composed only of proteins, and their replication requires merely the conversion of host PrP^{C} to PrP^{Sc}. Hence, differences exhibited by prion strains are hard to be attributed to genetic variability [85]. Prion strains isolated from naturally occurred TSEs may vary largely in many essential events, e.g. incubation periods, clinical manifestations, neuropathological characteristics, patterns of PrP^{Sc} in brains, PrP^{Sc} mobility in electrophoresis, resistances to the detergent and protease, patterns and ratios of three glycosylated PrP^{Sc} [66,86–88]. These traits are often conserved on serial transmissions in natural infections or bioassays [89,90]. More and more evidences have indicated that the prion strains can be created *in vitro*. The artificial prion was first reported in 2004 by Prusiner's group. They have synthesized mouse prion with recombinant PrP protein that causes the wild type and the transgenic mice undergoing neurological dysfunction after inoculation [91]. Subsequently, they have demonstrated that inoculating the mice with the synthetic prions with more labile structure causes experimental TSE with shorter incubation periods. It suggested that except for the factors we have known, the incubation time of TSEs may be affected by the conformations of prions [92]. Furthermore, they have verified that the synthetic protease-sensitive prions are able to cause the transgenic mice Tg9949 (over-expressing *N*-terminal truncated PrP) to be infected [69]. Wang *et al*. [93] have successfully generated the infectious prion with the bacterially expressed recombinant PrP protein. Barria *et al*. [94] also described that *de novo* generated prions induces a new disease phenotype. It seems that prion strains may arise from conformational variability, that is, PrP can assume several different, self-propagating conformations, each of which enciphers a distinct prion strain. However, the exact

molecular and structural mechanisms between conformational variability and pathological phenotype of prions still remain unclear.

Concluding Remark

In conclusion, the conversion from PrP^{C} to PrP^{Sc} is the most important event in pathogenesis of prion diseases, but there are still many gaps, especially the association of the tertiary structure of prion with the diverse pathologies of TSEs, such as, existences of numerous human and animal prion strains and various human genetic prion diseases, need to be filled. Therefore, continuous efforts for understanding the relationship between structure and phenotype of prion may shed light on the mysterious processing and develop the therapy for the disease.

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