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Visions & Reflections (Minireview)

Prion: the chameleon protein

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Abstract. From Creutzfeldt-Jakob disease (CJD) to variant CJD through Gerstmann-Sträussler-Scheinker syndrome, kuru and fatal familial insomnia, the journey leading to current understanding of the basic aspects of human prion diseases has been full of unexpected, but often dramatic and always fascinating twists. Recent progress in modeling prion diseases and characterization of the various prion protein forms reveal that such a wide spectrum of the diseases is associated with the chameleon-like conformational features of prions.

Keywords. Prion, prion protein, prion diseases, Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker syndrome, Alzheimer's disease, neurodegenerative disorders.

The most common human neurodegenerative disorder is Alzheimer's disease, the first case having been described by Alois Alzheimer in 1907 [1]. Alzheimer's accurate description established diagnostic landmarks that remain reliable to this day. In contrast to the relative clarity of this diagnosis, human prion diseases revealed their complexity from the beginning of their history. In his first report on the condition now known as Creutzfeldt-Jakob disease (CJD), published in 1921, Alfons Maria Jakob incorrectly included a case that had previously been described by Hans Gerhard Creutzfeldt in 1920 [2–4]. Although Creutzfeldt's case eventually turned out to bear little resemblance to the five cases that Jakob had described, Jakob's report has resulted in Creutzfeldt's name being connected erroneously and forever with this disease [4, 5]. The characterization of this new disease was so challenging that only two of Jakob's original five cases (the third and fifth cases) satisfy the present diagnostic criteria for spongiform degeneration in the brain [4, 5].

The discovery and detailed characterization of the abnormal, sickness-causing prion protein (PrP^{Sc}) have provided enormous critical insights into the nature of prion diseases, their pathogenic mechanism, and above all, the most striking feature of these neurodegenerative diseases, namely, their propensity to spread by infection [6]. The infectious pathogens associated with all prion diseases are widely believed to be nothing more than the abnormal prion protein. Therefore, in contrast with viruses, the prion infectious agent consists of amino acids and lacks nucleic acids. The heterogeneity conferred to viruses by the presence of genetic material is reproduced in prions by the broad variability of PrP conformations.

Knowledge of PrP^{Sc} has made it possible to diagnose prion diseases much more accurately, not only at the pathological level but also at the molecular level. In turn, this advance has greatly facilitated molecular identification of new prion diseases [7, 8]. In the 86 years since the original description of CJD, new

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disease forms, phenotypes and sub-phenotypes have joined the original CJD class, resulting in the now much larger family of human prion diseases. Etiologically, human prion diseases are distinguished as being sporadic, inherited, or acquired by infection. In addition to the diseases subsumed under CJD, major phenotypes include, Gerstmann-Sträussler-Scheinker (GSS) syndrome, kuru, and fatal insomnia (FI). Furthermore, based on clinical and neuropathological manifestations as well as gene and prion protein typing, sporadic CJD (sCJD), the most common form of human prion diseases, can be classified into six molecular or five clinical subtypes [9]. This heterogeneity thus creates an ever-growing family of CJD [10].

This pronounced phenotypic heterogeneity of human prion diseases likely arises as the effect of the number and variety of conformations that PrP^{Sc} can adopt. PrP^{Sc} is derived from the cellular prion protein (PrP^{C}), normally expressed in most tissues. Most of PrP^{C} is full length, diglycosylated and attached to the cell surface *via* a glycosylphosphatidylinisotol anchor; but small amounts of PrP^{C} may be N-terminally truncated [11–13], monoglycosylated or unglycosylated, anchorless, and cytosolic. PrP^{C} and PrP^{Sc} share the primary structure. The critical difference between the two lies in the conformation because PrP^{C} converts into PrP^{Sc} through an alpha-helix to beta-sheet structural transition [6], which results in profound differences in the physicochemical and biological properties of the two conformers. PrP^{Sc} is rich in beta-sheets and is detergent-insoluble, multimeric, resistant to proteinase K (PK) treatment, and infectious, whereas PrP^{C} is rich in alpha-helix, detergent-soluble, monomeric, sensitive to PK and noninfectious [6, 14–18] (Fig. 1). Studies using recombinant PrP *in vitro* indicated that PrP possesses a highly flexible conformation [19]. Similar to strains of conventional bacteria and viruses, but characterized by a different mechanism, PrP^{Sc} exists in multiple conformations which function as strains. This property translates into the formation of a variety of PK-resistant PrP species upon digestion with PK. In human prion diseases, in addition to the most common PrP27-30 consisting of PrP^{Sc} type 1 (21 kDa) and PrP^{Sc} type 2 (19 kDa), a variety of other PK-resistant PrP species has also been identified including PrP11, PrP7-8, PrP14, PrP-CTF12/13, PrP16-17, and PrP17.5-18 [9, 20–25] (Fig. 1). These fragments can be readily detected by immunoblotting using anti-PrP antibodies. 3F4, the most commonly used monoclonal antibody (mAb) for the diagnosis of human prion diseases and directed to residues 109–112 [26], can efficiently demonstrate several of these fragments, such as PrP^{Sc} type 1, PrP^{Sc} type 2, PrP11, PrP14 and PrP7-8. However, the other PK-resistant

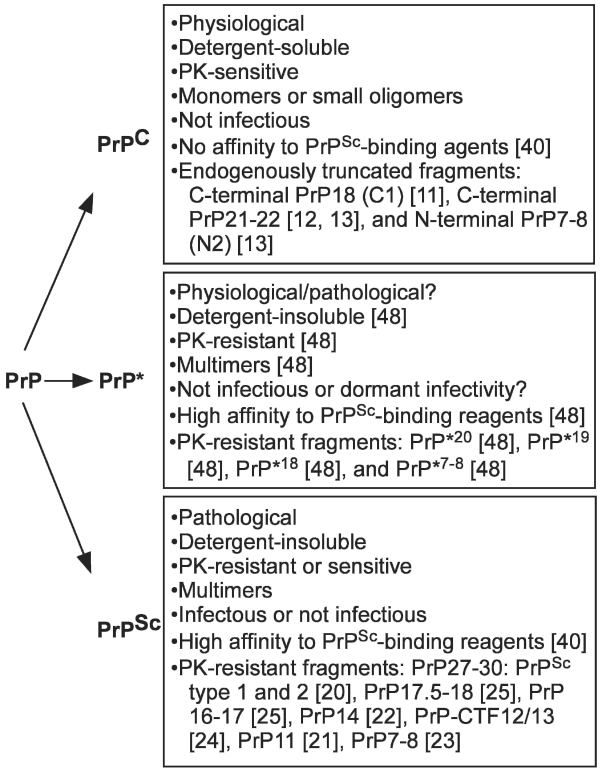

Figure 1. Summary of physicochemical and biological properties of different human PrP species identified so far. The human PrP molecule is classified into three isoforms based on their distinct physiochemical and biological features including PrP^{C}, PrP* and PrP^{Sc}. PrP* refers to several novel PK-resistant PrP species identified recently in normal human brains including PrP^{*20}, PrP^{*19}, PrP^{*18}, and PrP^{*7-8} [48]. In addition to the resistance to PK digestion, PrP* is also detergent insoluble and prone to form multimers. It also possesses a high affinity to PrP^{Sc}-binding reagents [48]. However, whether PrP* is physiological or pathological remains to be determined.

PrP fragments do not include the 3F4 epitopes and cannot be detected by this antibody. Detection of these PK-resistant fragments associated with varied PrP conformations is important not only in establishing a definite diagnosis but also in distinguishing phenotypes of the diseases. For instance, the 19-kDa and 21-kDa fragments are associated with sCJD type 2 and type 1, respectively, whereas PrP7-8, PrP11 and PrP14 are characteristic of GSS [9]. The unglycosylated PrP16-17 is associated with sCJD type 2 carrying methionine/methionine homozygosity at PrP codon 129 and all sCJD type 1, but the glycosylated PrP17.5-18 is usually observed in sCJD type 2 carrying valine/valine homozygosity or methionine/valine heterozygosity at codon 129 [10, 25]. Furthermore, in GSS linked to PrP proline to leucine mutation at residue 102 (P102L), it has been reported that the accumulation of PrP7-8 is associated with the deposition of multicentric amyloid plaques, but the accumulation of a PK-resistant 21-kDa PrP fragment similar to PrP^{Sc}

type 1 in gel mobility is associated with the formation of spongiform degeneration [27, 28].

However, the PK-resistant PrP fragments that are detected in prion-infected brains are surprisingly not neurotoxic and PrP-knockout mice are resistant to prion infection [29, 30]. Moreover, subclinical forms of prion diseases have been observed in experimentally or naturally infected animals that harbor high levels of infectivity and PrP^{Sc} but are asymptomatic during a normal life-span [31, 32]. Conversely, wild-type mice inoculated with PrP^{Sc} of bovine spongiform encephalopathy showed no detectable PK-resistant PrP in the brain despite the presence of neurological symptoms and neuronal death [33]. These conditions were observed not only in animals but also in humans. Familial FI or GSS with substitution of valine for alanine at residue 117 (A117V) revealed striking clinical manifestations but little or undetectable PK-resistant PrP [34, 35]. Therefore, the molecular features of the neurotoxic forms of PrP remain to be determined.

Several potentially toxic PrP isoforms have been studied in prion-infected transgenic mice, rodents and humans including transmembrane, cytosolic and PK-sensitive forms of abnormal PrP [36–40]. Based on the "refolding" or "seeding" models, PrP^{C} may unfold to an intermediate before it refolds under the influence of PrP^{Sc} or the conversion of PrP^{C} into PrP^{Sc} requires a PrP^{Sc}-like form (PrP*) [41, 42]. The intermediates have been widely observed in cell-based and cell-free models [43–46]. These intermediates generated in the process of conversion of PrP^{C} to PrP^{Sc} could be the neurotoxic PrP species. It cannot be ruled out that the PK-sensitive PrP^{Sc} may be an intermediate in the formation of PK-resistant PrP^{Sc}. Moreover, the most infectious PrP species has been found to be of median molecular sizes ranging between 300 and 600 kDa, whereas infectivity is virtually absent in oligomers of ≤ 5 PrP molecules or in large aggregates [47], indicating that prion infectivity is closely associated with the oligomeric state of PrP^{Sc} which, in turn, is likely to be affected by the PrP conformation.

Several novel PrP species have recently been identified and characterized in normal human and animal brains, which are assumed to be intermediate forms between PrP^{C} and PrP^{Sc} or silent prions [48]. They possess PrP^{Sc}-like properties such as detergent-insolubility, resistance to PK digestion and a greater tendency to form aggregates (Fig. 1). One of them, called PrP^{*20}, exhibits a striking affinity for mAb 1E4 to human PrP 97–108 but poor affinity for mAb 3F4 to human PrP 109–112 [48]. We have observed a group of subjects that showed a significant increase in the level of PrP^{*20} compared with normal controls in brain samples obtained at biopsy (data not shown). Clinically, all these subjects manifested prion disease-related symptoms and signs. However, no detectable neurohistopathological changes associated with prion disease were observed. Moreover, PrP27-30, the molecular hallmark of prion disease, was undetectable with 3F4 by both immunoblotting and immunohistochemistry. In one of these subjects the presence of sporadic prion disease was confirmed at autopsy 1.5 years after biopsy, while others are being followed up. It is possible that in at least some cases, an increased level of PrP^{*20} characterizes the early stage of prion diseases.

All evidence so far leaves no doubt that the co-existence of pathological PrP^{Sc} and physiological PrP^{C} in the brain is a prerequisite for prion diseases. The highly heterogeneous phenotypes of human prion diseases could be associated with varied PrP conformations emerging during the conversion of PrP^{C} into PrP^{Sc}. In contrast with the clarity of Alzheimer's disease, 86 years after the original report, human prion diseases identified initially with CJD have turned out to be a clinical chameleon. It is conceivable that new forms of human prion diseases will continue to be identified, principally because of the chameleon-like conformational features of the single protein. It has been proposed that prion diseases should now be redefined and they should include a group of disorders characterized by the accumulation of abnormal PrP including protease-sensitive and protease-resistant forms in the brain and/or other organs, regardless of the presence of transmissibility and spongiform degeneration [49]. One can therefore sympathize with Alfons Maria Jakob and the difficulties he encountered in the description and classification of the first cases of human prion diseases.

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