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MINI REVIEW

Prions: structure, function, evolution, and disease

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Abstract
Prions are proteinaceous infectious particles implicated in fatal neurodegenerative disorders known as prion diseases. Herein, we provide an overview of prion biology, emphasizing the structural, functional, and evolutionary aspects of prions, along with their potential applications in protein engineering. Understanding the structure-function relationships of both healthy and disease-associated prion proteins enables a deeper understanding of the mechanisms of prion-induced neurotoxicity. Furthermore, we describe how insights into prion evolution have begun to shed light on their ancient origins and evolutionary resilience, offering deeper insights into the potential roles of prions in primordial chemical processes.

Keywords Prions · Scrapie prion protein · Cellular prion protein · Protein misfolding · Prion structure · Prion evolution · Prion engineering · Transmissible spongiform encephalopathy

Introduction

The term "prion", originating from "proteinaceous infectious particle", was first used in 1982 by Stanley Prusiner, a key figure in the field of prion research (McKinley et al. 1983; Prusiner 1982, 1998). Prion proteins (PrPs) are naturally occurring glycoproteins found in mammals, yeast, bacteria, plants, and fungi (Chakrabortee et al. 2016; Kovač and Čurin Šerbec 2022; Wickner 1994; Wickner et al. 2007). In humans, they are most highly expressed in the brain and Central Nervous System (CNS), typically attached to the lipid bilayer on cell membranes (Linden 2017). Prions have also been found in lower concentrations in other tissues including the spleen (Peden et al. 2004), tonsils (Hill et al. 1997, 1999), lymph nodes (Peden et al. 2004), appendix (Gill et al. 2020), skin (Orrú et al. 2017), rectum (Wadsworth et al. 2001), skeletal muscle (Smith et al. 2011), adrenal gland (Wadsworth et al. 2001) and retina (Wadsworth et al. 2001). However, under pathological conditions, these proteins misfold into disease-associated conformations, forming the neurotoxic and insoluble fibrillary aggregates that characterize prion diseases, also known as transmissible spongiform encephalopathies (TSEs). TSEs are characterized by neuronal loss, gliosis, spongiosis, and abnormal amyloid protein deposition (Tee et al. 2018).

In hedoialthy cells, primarily neurons, prion proteins are referred to as cellular prion protein (PrP^{C}), while the misfolded disease-associated conformation is termed scrapie prion protein (PrP^{Sc}) (Herms et al. 1999; Kovač and Čurin Šerbec 2022). PrP^{Sc}, named after its initial discovery in scrapie-afflicted sheep and goats, initiates a self-templated process upon interaction with PrP^{C} (Griffith 1967; Pattison and Jones 1967). In this process, the abnormal PrP^{Sc} molecule acts as a template that induces the normally folded PrP^{C} to misfold into the pathological PrP^{Sc} conformation (Griffith 1967). This conversion involves a conformational change where the α-helical structure of PrP^{C} is transformed into the β-sheet-rich structure of PrP^{Sc} (Prusiner 1998). The newly formed PrP^{Sc} can then convert additional PrP^{C} molecules, leading to a chain reaction. As more PrP^{Sc} proteins are generated, the conversion process accelerates exponentially, resulting in the accumulation of PrP^{Sc} aggregates in the

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CNS and, ultimately, neurodegeneration (Fig. 1) (Prusiner 1982). Prion diseases can arise from inoculation with PrP^{Sc} (Gajdusek and Zigas 1959), spontaneous misfolding of PrP^{C} (Prusiner 1998), or genetic predisposition (Lugaresi et al. 1986; Monari et al. 1994).

Similar protein misfolding phenomena are observed in neurodegenerative disorders like Alzheimer's Disease (AD), Parkinson's Disease (PD), and Huntington's Disease (HD), where abnormal protein aggregates such as beta-amyloid plaques (AD) (Hardy and Selkoe 2002), tau tangles (AD) (Grundke-Iqbal et al. 1986), Lewy bodies (PD) (Spillantini et al. 1997), and Huntington protein aggregates (HD) (Davies et al. 1997) are prominent. While classic prion diseases are evidently infectious, debates persist as to whether other protein misfolding diseases like AD, PD, and HD exhibit similar transmissibility (Ritchie and Barria 2021). Indeed, some experimental evidence supports the transmission of misfolded proteins associated with these diseases between organisms, akin to prion behavior (Gomez-Gutierrez and Morales 2020). This has sparked significant debate regarding expanding the definition of prions to encompass the misfolded proteins implicated in these neurodegenerative disorders. However, further exploration is needed before a definitive decision is reached (Lim et al. 2015; Mawanda and Wallace 2013).

Prion diseases rely solely on proteins for disease transmission and progression; a finding which underpins the 'protein-only' theory of infection first proposed in 1967 by Tikvah Alper, John Stanley Griffith and Pattison & Jones (Alper et al. 1967; Griffith 1967; Pattison and Jones 1967). Prion strains exhibit phenotypic diversity, leading to distinct disease manifestations, including fatal familial insomnia (FFI) (Lugaresi et al. 1986), Kuru (Gajdusek and Zigas 1959), familial Creutzfeldt-Jakob disease (CJD) (Creutzfeldt 1920; Jakob 1921), and Gerstmann-Straussler-Sheinker disease (GSS) (Gerstmann et al. 1954). These diseases are classified into acquired, sporadic, and genetic

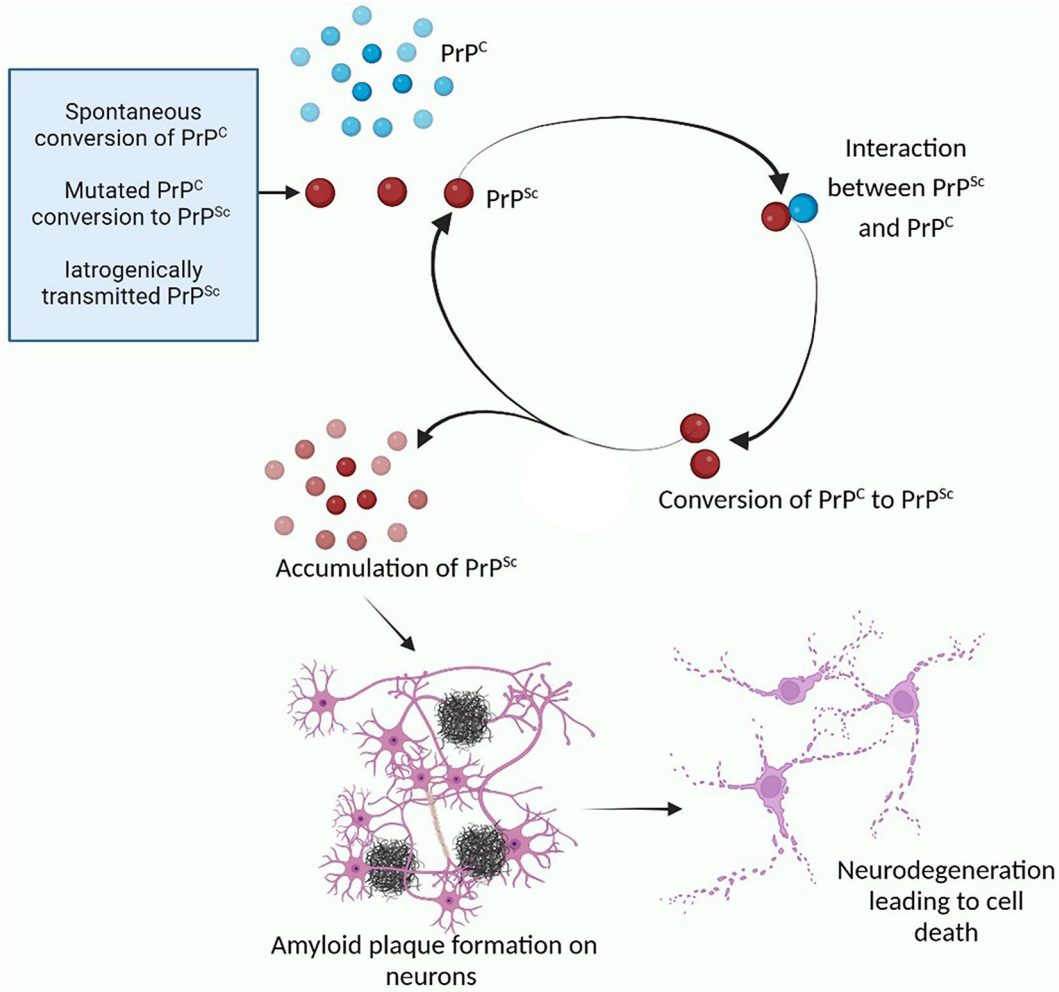

Fig. 1 Schematic of Prion Disease Pathogenesis. PrP^{Sc} occurs spontaneously, genetically, or by transmission. PrP^{Sc} interacts with endogenous PrP^{C}, catalyzing its conversion to PrP^{Sc}. These newly converted pathogenic conformations cause propagation and accumulation of PrP^{Sc}, leading to plaque formation and cell death, primarily in neurons

forms, with sporadic CJD accounting for most cases (Sitammagari and Masood 2024).

Despite ongoing research efforts, effective treatments for prion diseases remain elusive (Zerr et al. 2024). PrP^{Sc} exhibits extraordinary transmissibility, with 10^8 – 10^9 lethal doses per 1 µg of protein (Creutzfeldt 1920). Although prions have the potential to breach species barriers, this zoonotic transmission does not occur universally in all strains. A notable example is bovine spongiform encephalopathy (BSE) or 'mad-cow disease'. During the 1990s, an outbreak of BSE in the United Kingdom had devastating public health and economic consequences. The crisis resulted in 178 human deaths, more than 5 million animals slaughtered, and an estimated cost of $4.6 billion (€4.2 billion) (University of Edinburgh 2024; van Zwanenberg and Millstone 2005).

While the exact mechanism of prion conversion is not yet fully understood, several key mechanistic models have been proposed, based on experimental evidence, including self-templated misfolding (Prusiner 1982), and seeded nucleation for the formation of aggregates of PrP^{Sc} (Eisele 2013). However, significant progress has been made in recent years due to the increasing availability of genomic data, and the development of ever more sophisticated bioinformatics pipelines for proteome-wide analyses (Meisl et al. 2021; Salvi et al. 2023; Tetz and Tetz 2017). Furthermore, new prion disease therapeutics are beginning to come online; including the use of antibodies (White et al. 2003), immunotherapy (Giles et al. 2016; Souan et al. 2001), gene-therapy (Genoud et al. 2008), synthetic small-molecules (Nicoll et al. 2010), and RNA interference (RNAi) for prion gene silencing (Rinaldi and Wood 2018).

The origins of prion research

Recognition of the existence of prion proteins came only after several manifestations of prion diseases had already been described (Prusiner 1982, 1998). Scrapie, the first documented prion disease in animals, was first observed by Spanish shepherds in 1732, although its causative agent remained unknown due to limited understanding of microorganisms and molecular biology at the time (Leopoldt 1759). Scrapie derives its name from the characteristic behavior of infected animals; compulsively scraping against walls and fences to alleviate intense itching caused by neurological damage (Detwiler and Baylis 2003).

In 1900, Kuru was the first documented human prion disease when it became endemic among the Fore people in Papua New Guinea (Gajdusek and Zigas 1959). Kuru was found to be transmitted through ritualistic cannibalistic funeral practices, primarily affecting women and children who consumed the infectious brain tissues of deceased relatives, while men typically consumed the tougher muscle tissues (Collinge et al. 2006; Gajdusek 1977). The term 'Kuru' means 'to shake from fear' in the Fore language and reflects the primary disease symptoms of 'trembling' or 'shivering' due to neurological damage (Gajdusek 1977). The disease was also known as 'laughing sickness' due to the pathological outbursts of laughter observed in its victims (Lindenbaum 2015). Kuru was first discovered and extensively studied by Carleton Gajdusek, who, along with Baruch Blumberg, was awarded the Nobel Prize in Physiology or Medicine in 1976 for his work on the disease (Blumberg 1965; Gajdusek and Zigas 1959). The eradication of Kuru began in the late 1950s when the Australian government and local authorities intervened to stop the practice of cannibalism among the Fore (Lindenbaum 2015). The disease has an incubation period of 10 to 50 years, so symptoms could appear decades after exposure (Alpers and Gajdusek 1965). The last recorded case of Kuru was reported in 2009 (Collinge 2016).

In 1920, German neurologists Hans Gerhard Creutzfeldt and Alfons Maria Jakob independently reported cases of a different human prion disease, which would later be named Creutzfeldt Jakob Disease (CJD) (Creutzfeldt 1920; Jakob 1921). Creutzfeldt described a 22-year-old woman with a progressive and fatal neurological disorder, while Jakob reported similar cases soon after. Initially, the disease was believed to be a rare form of encephalitis. CJD is characterized by rapidly progressive dementia, myoclonus, and motor dysfunction. The early confusion about its etiology stemmed from the atypical infectious agent involved (Griffith 1967; Pattison and Jones 1967). The identification and study of these diseases were crucial in recognizing prions as a new class of pathogen, leading to groundbreaking research in the field of neurodegenerative diseases.

Preliminary investigations into the etiology of prion diseases suggested 'slow viruses' as potential causative agents, due to their long incubation periods (from months to decades), highly infectious nature, and cytotoxicity (Cho 1976; Rohwer 1984). However, overlooked clues hinted at deviations from typical virus-like behavior; including resistance to formalin inactivation (Gibbs et al. 1968), lack of nucleic acids (Prusiner 1982), and their unique protein-only infectious nature (Prusiner 1998), discussed below.

In 1944, veterinarian William Hadlow's attempt to develop a vaccine for louping-ill virus inadvertently exposed the unique characteristics of prions. Despite using formalin, a potent industrial disinfectant, to neutralize the virus in infected tissues for vaccine development, vaccinated animals succumbed to death from scrapie two years later. This unexpected outcome revealed the remarkable resistance of prions to cross-linking by formalin, a process that

typically neutralizes proteins and nucleic acids by creating covalent bonds between molecules (Fox et al. 1985). While the precise mechanism underlying prion resistance to formalin remains a subject of ongoing research, it is believed to be closely linked to the misfolded structure and aggregation propensity of PrP^{Sc} (Fritschi et al. 2014a; Kamiie et al. 2020). The β-sheet confirmation of PrP^{Sc} is highly stable and inflexible, potentially shielding key amino acids from formalin's cross-linking effects (Fritschi et al. 2014b). Moreover, the dense structure of amyloid fibrils formed by aggregated PrP^{Sc} molecules may offer a protective shield preventing formalin from reaching and inactivating the infectious core (Kamiie et al. 2020). Formalin inactivation is an established and widely used method to inactivate viruses, and resistance to this process thus supported the initial supposition that prions have unique properties when compared to conventional viruses (Amor 2009).

Further experimental evidence eventually led to the recognition of prions as a distinct class of infectious agent (Prusiner 1982; Wickner 1994). Stanley Prusiner, in particular, continued to champion the 'protein-only' hypothesis of prion transmission through the 1980s. Prusiner and his colleagues isolated the infectious agent from tissue of TSE-affected animals, and showed that the agent was a protein fraction that retained its infectious properties, even after the removal of nucleic acids using nucleases (Prusiner 1982). This key finding confirmed that the protein alone was sufficient to transmit the disease, supporting the 'protein-only' hypothesis of prion transmission. Prusiner is also credited with proposing the existence of two conformations of PrP, PrP^{C} and PrP^{Sc}. Initially met with skepticism, this idea gained support after the discovery of yeast prions in 1994, which exhibited similar self-propagation to mammalian prions, validating Prusiner's hypothesis (Wickner 1994). Moreover, the unique features of TSEs, including long incubation periods and immune evasion, further distinguished prion diseases from those caused by other pathogenic agents.

Prion durability and clearance

The remarkable durability of prions has posed formidable challenges for conventional sterilization practices, necessitating a re-evaluation of sterilization methods (Rutala and Weber 2010). They can bind to metal and plastic surfaces without losing infectivity (Weissmann et al. 2002). They are also extremely heat resistant, surviving temperatures as high as 140 °C(Taylor 1999), rendering sterilization techniques such as autoclaving unreliable. While alternative physical and chemical methods have been proposed, such as ultraviolet radiation and deprivation of sunlight and oxygen, they also fail to guarantee efficacy (Bellinger-Kawahara et al. 1987). One chemical method that shows promise for reducing prion infectivity is the use of formic acid. Formic acid is widely used in laboratory and clinical settings as it breaks down prion proteins (Kovács et al. 2002), but decontamination is not robust, so more aggressive methods are required for prion clearance (World Health Organization 2000). Indeed, depending on the material to be sterilized, the most effective approaches for reducing prion infectivity include a combination of extreme heat (275 °C) and pressure (over 100,000 psi), alkaline hydrolysis, or incineration (Brown et al. 2003). However, complete neutralization remains elusive.

Prion structure & function

In healthy cells, PrP^{C}, a 27–30 kDa glycoprotein, exists as a monomeric cell-surface protein, predominately found in the CNS in cell membrane rafts and non-membrane-bound forms (Fig. 2) (Westergard et al. 2007). Structural analyses, including by X-ray crystallography and Nuclear Magnetic Resonance (NMR), have provided critical insights into the native structure of PrP^{C} (Riek et al. 1996). PrP^{C} features a globular C-terminal region, with three α-helices, two small antiparallel β-sheets, and two N-glycosylation sites (Fig. 2). The N-terminal domain, intrinsically disordered and flexible, is linked to the C-terminal *via* an evolutionarily conserved hydrophobic core.

PrP^{C} is susceptible to protease digestion by proteinase K (Pan et al. 1993), and soluble in water, facilitating its involvement in essential cellular processes. Its evolutionary conserved core underscores its functional significance in various neurological functions, including cell signaling, neuroprotection, synaptic activity, myelination, and metal ion homeostasis. Ongoing research aims to clarify its precise roles in these processes (Kretzschmar et al. 1986; Acevedo-Morantes and Wille 2014; Cha and Kim 2023).

PrP^{C} acts as the substrate for PrP^{Sc} to induce misfolding by self-templating action (Riesner 2003; Sevillano et al. 2018). Although both are transcribed from the PRNP gene, they possess vastly different secondary and tertiary structures, resulting in distinct characteristics and functions (McKinley et al. 1983; Prusiner 1982; Sigurdson et al. 2019). Notably, PrP^{Sc} exhibits increased β-sheet content in the C-terminal domain and a propensity to form polymeric aggregates, unlike PrP^{C} (Pan et al. 1993). PrP^{Sc} molecules aggregate to form insoluble highly organized polymeric structures with a cross-β architecture, where β-strands align perpendicularly to the amyloid fibril axis and are stabilized by intermolecular hydrogen bonds (McKinley et al. 1983; Prusiner 1982, 1998).

Structural studies using cryogenic electron microscopy (cryo-EM), atomic force microscopy, and

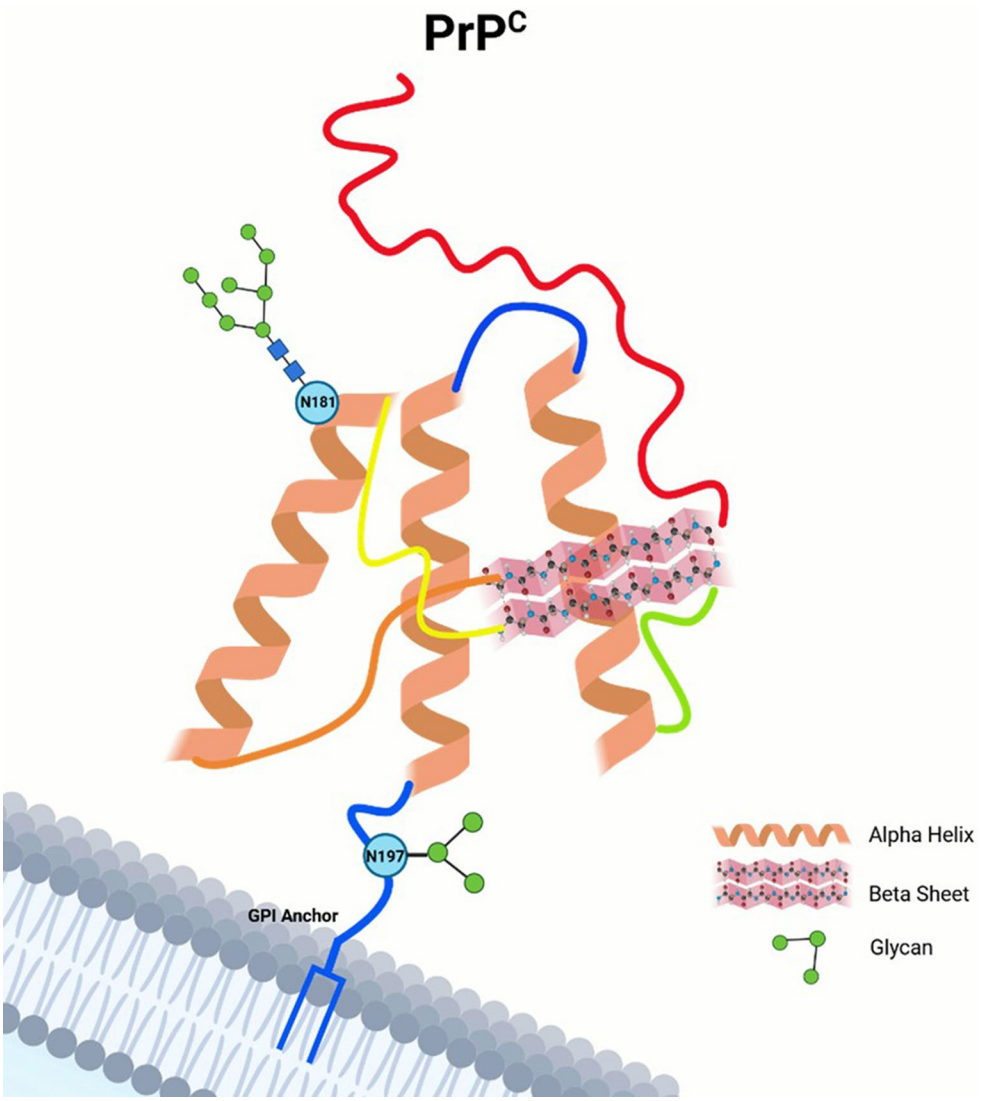

Fig. 2 Proposed structure of cellular prion protein (PrP^C). PrP^C is a flexible, protease-sensitive glycoprotein containing three α-helices and two small β-sheets (PDB: 1QLX). Its glycans are on arginine (N) residues 181 and 197, and a C-terminal glycophosphatidyl-inositol anchor facilitates membrane interactions

X-ray-crystallography have contributed to our understanding of PrP^{Sc} fibril structure (Baskakov et al. 2019; Spagnolli et al. 2019; Vázquez-Fernández et al. 2016; Wille et al. 2009). The 4-rung β-solenoid model proposes a twisted, elongated structure for PrP^{Sc} fibrils with multiple β-strands arranged like the rungs of a ladder (Fig. 3) (Vázquez-Fernández et al. 2016). In contrast, the parallel in-register intermolecular β-sheet (PIRIBS) model suggests beta-strands run parallel to each other and are aligned in-register with strong intermolecular interactions (Fig. 4). A recent cryo-EM study which supports the PIRIBS model compared infectious, ex vivo, prion fibrils from hamster and mice (Manka et al. 2023). Their highly detailed structural resolution showed that in both models, PrP monomers form protofilaments, subunits of amyloid fibrils. Slight structural differences between the models were observed in the folding of their C-terminal lobes, possibly attributed to variations in amino acid sequence or prion strains. They also showed that specific amino acid residues 94–225 on PrP form the core of these protofilaments. This research provides crucial insights into prion amyloid fibril assembly and propagation, potentially guiding the development of therapies.

Despite efforts to elucidate the true molecular composition of membrane bound PrP^{Sc}, challenges persist due to its biochemical complexity and lethal infectivity, hindering research progress (Yim et al. 2015). However, recent AI based protein structure prediction tools, such as AlphaFold, hold some promise for prion research (Jumper et al. 2021). For instance, these tools could contribute to our understanding of the structural transitions that lead to PrP^{Sc} formation. This process remains largely unresolved due to the infectivity, insolubility, and aggregation properties of PrP^{Sc}, which significantly complicate wet-lab experimentation (Wille and Requena 2018). Additionally, they can aid in identifying small molecule binding sites that could stabilize PrP^C or prevent PrP^{Sc} aggregation (Wozniak et al. 2024). However, at

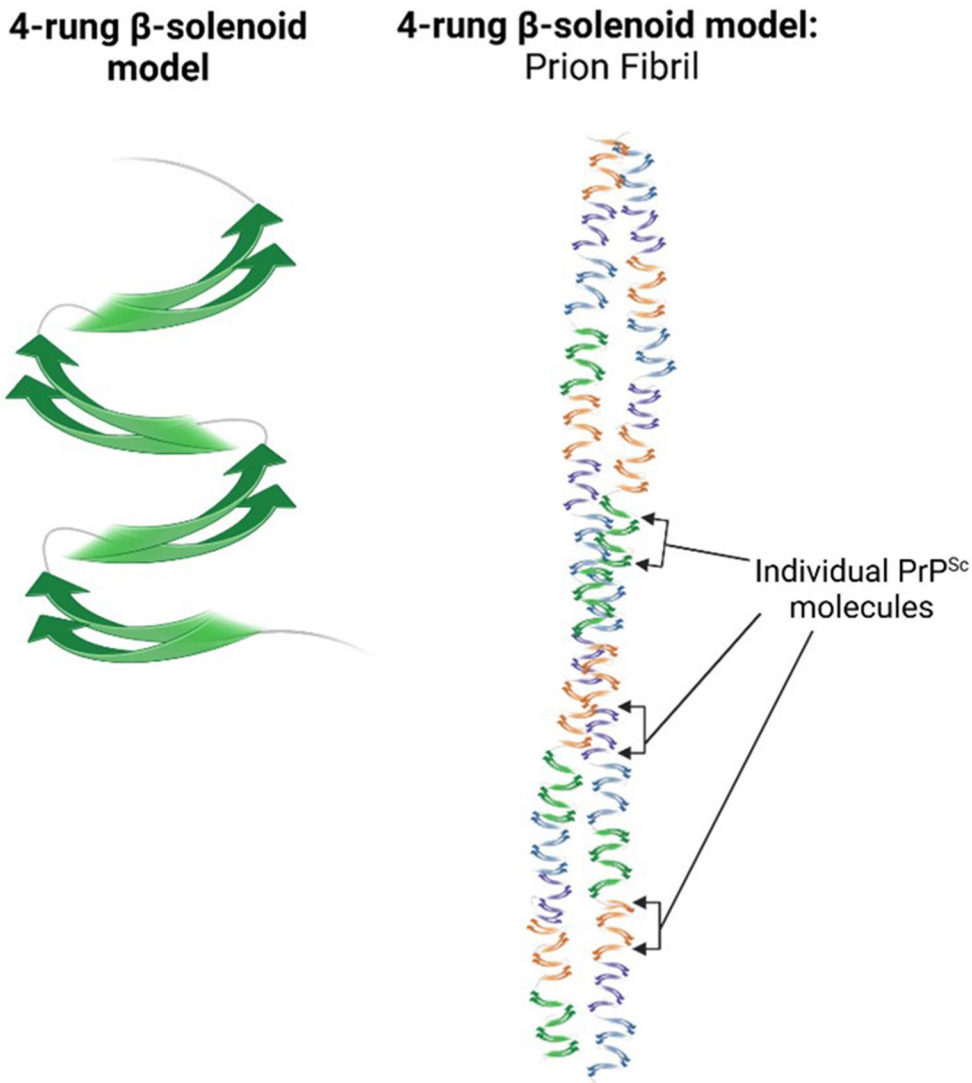

Fig. 3 Proposed structure of the 4-rung β-solenoid model of PrP^{Sc}. The left image shows a single PrP^{Sc} molecule in the 4-rung β-solenoid model, with 4 stacked beta sheets or 'rungs'. The right image illustrations the aggregation of PrP^{Sc} molecules to form the elongated stable fibrillar structure (Vázquez-Fernández et al. 2016)

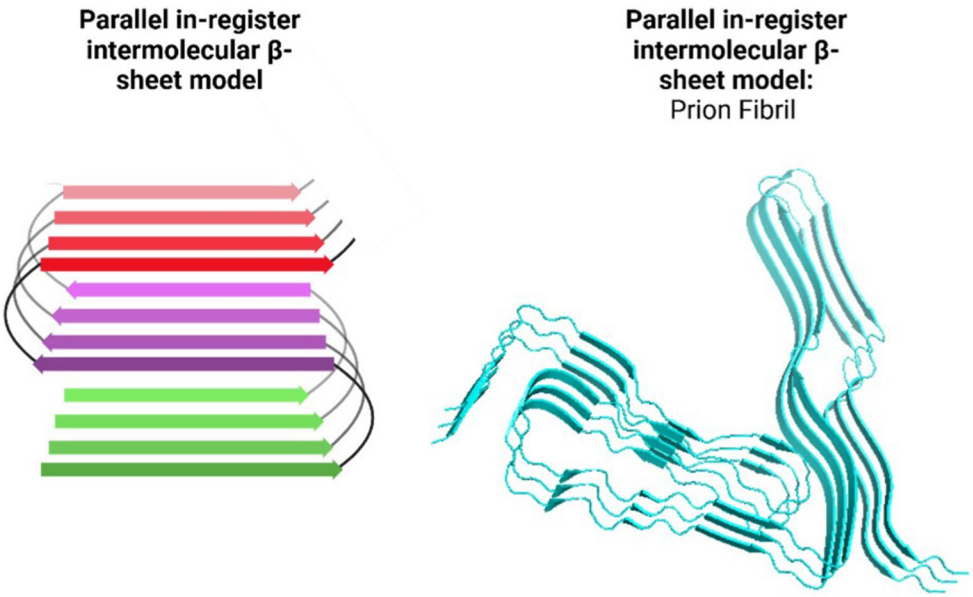

Fig. 4 Proposed structure of the parallel in-register intermolecular β-sheet model of PrP^{Sc}. The left image displays three PrP^{Sc} molecules in the parallel in-register intermolecular β-sheet model. The -strands are indicated by arrows, and run in the same direction in each molecule. In this model, the β -sheets in PrP^{Sc} molecules line up and form an extended -sheet structure within the fibril, as shown in the structure on the right, which is derived from ME7 mouse PrP^{Sc} fibrils (PDB: 8A00) (Manka et al. 2023)

the time of writing, the per-residue model confidence score (pLDDT) of the human prion remains at low to very low confidence (Jumper et al. 2021). This indicates that there is a considerable degree of uncertainty regarding the predicted structure, and it should be interpreted with caution. Nonetheless, further improvements in this fast-growing field will likely advance our understanding of prions and provide a safer alternative to wet lab-based methods (Pritzkow et al. 2021). By reducing the necessity for high-risk experiments involving infectious prions, these in silico approaches can mitigate infection risks and enhance lab safety.

Both conformations of PrP contain the evolutionary conserved hydrophobic core and a C-terminal glycophosphatidyl-inositol (GPI) membrane anchor (Govaerts et al. 2004; Riek et al. 1997). In the disease-associated conformation, the hydrophobic core is responsible for forming and stabilizing the misfolded structure, and mutations in this region can influence its disease-causing ability (Biasini et al. 2010). The core provides a stable scaffold for the formation and stabilization of the β-sheet structures that are characteristic of PrP^{Sc} (Hannaoui et al. 2017). Once conversion is complete, the core also stabilizes the interactions between neighboring PrP^{Sc} molecules, facilitating the elongation of amyloid fibrils and formation of misfolded protein aggregates (Wasmer et al. 2008).

In essence, the interplay between the structures and functions of cellular and scrapie prions underscores their dual roles in normal cellular physiology and pathology. While PrP^{C} has a soluble monomeric structure with roles in essential neurological functions, such as neuronal development (Benvegnù et al. 2010) and synaptic plasticity (Ondrejcak et al. 2018; Sakaguchi et al. 1996), PrP^{Sc} forms insoluble polymeric aggregates with distinct β-sheet-rich architecture, contributing to prion propagation and pathology (Prusiner 1982, 1998). Further elucidation of the structural differences, and functional consequences, between PrP^{C} and PrP^{Sc} is crucial to our understanding of prion propagation, aggregation, and toxicity, paving the way for potential therapeutic interventions.

Prion evolution

Prions, potentially among the oldest molecules on Earth, have origins dating back to the emergence of life (Jheeta et al. 2021; Zajkowski et al. 2021). Their remarkable resilience to extreme conditions like radiation, heat, crosslinking, and enzymatic digestion suggests their existence in the harsh environments of early Earth (Das and Zou 2016; Jheeta et al. 2021). Additionally, the auto-catalytic self-replication and assembly mechanisms of prions suggest their potential role as catalysts for early chemical reactions and the formation of biological structures, contributing to chemical evolutionary processes (Lupi et al. 2006). Prions exhibit diverse conformations with distinct biochemical and pathological properties, enabling them to adapt and thrive in various environments (Stein and True 2014). While prions are proteins encoded by DNA, the prion-first hypothesis speculates that prion-like mechanisms could have played a role in early biochemical evolution before the full establishment of DNA and protein-based life (Jheeta et al. 2021). Supporting evidence, in favour of this hypothesis, includes discoveries linking small RNA viruses to prions, fueling speculation about their ancient origins (Harrison and Shorter 2017).

Structural conservation

Phylogenetic analysis has provided crucial insights into prions, revealing some prion variants that are exclusive to tetrapods and primates (Comoy et al. 2023). Several studies have highlighted significant structural conservation among prion proteins across vertebrates, suggesting functional similarities and evolutionary conservation (Premzl & Gamulin, 2007). This conservation of prion-like properties across diverse taxa suggests that prions offer evolutionary advantages or serve essential functions.

Indeed, phylogenetic analysis has exemplified prion evolutionary resilience, with their highly conserved hydrophobic core enduring billions of years of evolutionary changes (Jheeta et al. 2021). The core is a product of positive selection in response to environmental pressures and an example of the evolutionary resilience of prions. The conservation of this core, which is characterized by an alanine-rich amino acid sequence that is preserved across mammalian species (Abskharon et al. 2019), underscores its functional importance.

Strain variability

Despite the observed structural conservation, prions exist in distinct variants called prion strains. These strains are characterized by phenotypic traits such as length of incubation time, specific disease manifestation, and the structure of the misfolded protein. Prions exhibit remarkable strain variability; resulting in distinct neuropathological properties across prion diseases (Collinge et al. 1996; Dickinson et al. 1968). While all strains share a common ancestor, evolutionary divergence, often through single amino acid substitutions, has led to the emergence of diverse prion variants (Collinge and Clarke 2007). In organisms like yeast, for example, multiple prion variants with unique biochemical properties have been identified (Wickner et al. 2023; Xu et al. 1995).

This variability not only contributes to the diversity of prion strains but also influences an individual's risk of prion disease by either conferring protection or increasing susceptibility (Arshad et al. 2023; Golanska et al. 2013). Comparative genomics has identified various polymorphisms that illustrate the intricate interplay between prions, genetics, and evolution. For instance, specific nucleotide substitutions in the PRNP gene of raccoon dogs reduce amyloid propensity, offering resistance against prion diseases (Jo et al. 2022). Similarly, protective polymorphisms in deer populations mitigate chronic wasting disease (CWD) (Arifin et al. 2021), while in humans, amino acid substitutions at codons 127 and 129 alter susceptibility to prion disease (Parchi et al. 2012). Notably, among the Fore people of Papua New Guinea, a single Gly-Val substitution at codon 127 provides complete resistance to Kuru (Asante et al. 2015): highlighting the impact of natural variation and positive selection on prion genetics and evolution.

Understanding prion polymorphisms, coupled with the ability to synthesize proteins, holds potential for designing prion proteins that mimic genetic variants. This approach could aid in further investigating the disease and developing novel therapeutics to modulate disease progression (Makarava et al. 2016).

Prion engineering – directed evolution

Protein engineering strategies aim to either mitigate prion propagation or harness their unique properties for biotechnological applications (Chiesa et al. 2020). Sophisticated techniques such as rational (*de novo*) design (Dahiyat and Mayo 1997), semi-rational design (Lutz 2010), and directed evolution (Arnold 1998) enable us to precisely engineer proteins with desired properties. The recent emergence of innovative protein engineering tools such as RoseTTAFold (Baek et al. 2021) and AlphaFold (Jumper et al. 2021) have facilitated significant recent advancements in this domain (Lian et al. 2022; Williams et al. 2023). RoseTTAFold, in particular, harnesses deep learning algorithms to generate synthetic proteins with tailored properties, promising significant therapeutic potential (Krishna et al. 2024).

Charles Weissmann (2004), following his discovery of the prion protein encoding PRNP gene, proposed a physiological method for prion clearance. This method leverages cellular mechanisms by passing prions through the brains of PRNP knockout mice, which remain free from infection, suggesting that the absence of a binding substrate for PrP^{Sc} eliminates infectivity (Safar et al. 2005). Phagocytosis has also emerged as a prominent prion clearance mechanism, as demonstrated by spleen macrophages and myeloid dendrites degrading prions in vitro and in vivo (Beringue et al. 2000; Luhr et al. 2004). While the precise mechanism remains unclear, phagocytosis represents an efficient means of neutralizing prions, laying the groundwork for future therapeutic interventions.

Indeed, continued exploration of the heightened phagocytic abilities of PRNP-knockout mice presents a unique opportunity for protein engineering, with beneficial applications in medicine, biotechnology, and biosafety (Arshad and Watts 2023; Biasini 2019). The increased macrophage activity exhibited by these mice holds promise for developing engineered proteins for prion clearance in industrial and biological contexts (Kuhlman 2019). By studying the specific mechanisms that lead to this heightened phagocytic activity, researchers can identify key receptors, signaling molecules, and regulatory proteins involved in macrophage activation (Aguzzi et al. 2013). This knowledge can inform the development of synthetic proteins or antibodies that mimic or boost this process, potentially facilitating the clearance of prions. This research is far-reaching, with prion-related discoveries benefiting other amyloid-beta plaque disorders like AD and other neurodegenerative conditions involving misfolded proteins (Gomez-Gutierrez and Morales 2020; Wells et al. 2021).

Amyloids are aggregated forms of proteins that adopt a specific misfolded structure, characterized by stable and insoluble fibrils that are rich in β-sheets. This unique conformation enables them to resist proteolytic degradation and promotes their accumulation in various pathological conditions. As such, the inherent ability of amyloid proteins to form robust, highly organized fibrillar structures has found practical applications in drug delivery (Silva et al. 2013), cell culture scaffolds (Onur et al. 2018), biomimetic tissues (Ruan et al. 2019), and biosensors (Kaushik et al. 2016). Prions exhibit unique amyloidogenic properties characterized by slower and adjustable aggregation kinetics compared to conventional amyloids, making them appealing for precisely controlled assembly in biomaterial design (Díaz-Caballero et al. 2018). Recent studies have demonstrated the potential of prion-like materials in nanowires, enzyme immobilization, and redox biofilms (Díaz-Caballero et al. 2021; Men et al. 2010; Xu et al. 2023; Zhou et al. 2014). However, the utilization of prion-based nanomaterials remains limited compared to their amyloid-based counterparts due to the complexity of prion structure, risk of transmission, and our current incomplete understanding of their functional mechanisms (Díaz-Caballero et al. 2018). The various conformational states of prions render them unpredictable for applications in controlled environments such as nanomaterial production (Walsh et al. 2023). Furthermore, the propensity of PrP^{Sc} to propagate its misfolded state to other proteins represents safety concerns in the research and application of prion-based nanomaterials (Prusiner 1982; Weissmann et al. 2002).

Conclusion and future directions

In conclusion, this review offers a comprehensive exploration of prions, with a focus on structural complexities and functional properties of the normal and disease-associated prion protein. By tracing prion research from the early discovery of scrapie to the evolving knowledge surrounding prion diseases, we show how prions challenge established paradigms in protein folding and disease propagation. Prion evasion of conventional sterilization techniques is discussed, highlighting their novel mechanisms of infectivity. Understanding the precise mechanism of propagation could unlock new therapeutic routes aimed at targeting prion pathogenesis at a molecular level. This review underscores the critical need for continued research to fully decipher prion biology.

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Author contributions C.C. wrote the main manuscript text and prepared the figures. R.D.S. supervised the work, reviewed and revised the manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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