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Unravelling the Mysteries of Prions: Insights into the Biochemistry, Pathology and Therapeutics of Devastating Protein Misfolding Diseases

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Abstract: Prion diseases represent an intricate enigma confounding researchers for decades. This comprehensive review illuminates insights into prion biochemistry while charting persistent challenges and future directions in understanding these perplexing disorders. At the core lies the conformational conversion of native prion protein (PrPC) into misfolded isoforms (PrPSc) that polymerize into neurotoxic aggregates. Elucidating the structural mechanisms driving this switch remains pivotal. Prions adopt distinct self-propagating strains exhibiting unique biochemical and pathological properties. Defining the molecular basis of prion infectivity, evolution, and transmission barriers holds promise but remains an ongoing struggle. Prion propagation disrupts neuronal function through toxic protein interactions, synaptic breakdown, apoptosis, and sustained inflammation. Developing treatments has proven exceptionally difficult, though emerging immunotherapies and gene therapies offer optimism. Combining modalities targeting prion replication, toxicity and clearance represents a promising strategy. However, hurdles persist in achieving early diagnosis, overcoming species barriers, and clinical translation. Future priorities include expanding knowledge of prion strains, improving diagnostics by discovering definitive biomarkers, and ushering cutting-edge therapies into clinical trials. Advancing research across disciplines promises breakthroughs in illuminating prion misfolding mechanisms, transmission, and neurotoxicity – critical to combating these devastating protein conformational disorders. Though challenges exist, relentless inquiry and collaborative efforts will unravel remaining mysteries, leading to transformative therapeutic strategies against pathogenic prions.

Keywords: Prions, Misfolded isoforms, Therapeutic, Neurotoxic, Immunotherapies

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Introduction

Prion diseases represent a unique class of fatal neurodegenerative disorders that have a profound impact on human and animal health. Caused by misfolded conformers of cellular prion proteins, these transmissible spongiform encephalopathies (TSEs) result in rapid cognitive and motor decline due to widespread neuronal damage and brain pathology (Imran and Mahmood, 2011; Ritchie and Barria, 2021). As outlined in the attached passage, prion diseases have a long history of

scientific inquiry starting with early investigations of scrapie in sheep (Liberski, 2012). It took pioneering work by Griffith (Griffith, 1967), Alper (Alper *et al.*, 1967) and Prusiner (Prusiner, 1982) to shift the paradigm and demonstrate that proteins, rather than conventional pathogens, were the infectious agent underlying these diseases (Prusiner, 1998).

The key molecule underlying prion pathogenesis is the prion protein (PrP), which exists in two conformations: the normal cellular PrPC and the abnormally folded, aggregated PrPSc (Pan *et al.*, 1993). PrPC is present ubiquitously but is particularly abundant in neural tissue, where it carries out important roles in signaling, neuroprotection and metal homeostasis (Linden *et al.*, 2008; Bonneh-Barkay and Wiley, 2009). However, this protein can misfold into the pathogenic PrPSc isoform, acquiring a higher β-sheet content and partial resistance to proteases (McKinley *et al.*, 1983; Pan *et al.*, 1993). PrPSc can bind to PrPC and induce further misfolding, propagating through a seeded polymerization process akin to crystal growth (Jarrett and Lansbury, 1993). As PrPSc accumulates, it forms neurotoxic aggregates and amyloid plaques that give rise to the spongiform changes and neuronal loss seen in prion disease brains (Hughes and Halliday, 2017).

The unique infectious nature of prions sets them apart from other protein misfolding disorders (Prusiner, 1998). Prion transmission can occur via ingestion of contaminated food products, use of improperly sterilized surgical instruments, or contact with infected cadaveric brain tissue, as during ritual endocannibalism (Collinge and Clarke, 2007). Distinct prion strains exist that exhibit differing biochemical properties of PrPSc and patterns of pathology and disease progression. Understanding prion infectivity and strain diversity remains an intriguing challenge (Bessen and Marsh, 1992).

As outlined, prion diseases have a tremendous impact on public health and agriculture (Aguzzi and Calella, 2009). In humans, these aggressive diseases cause rapid cognitive and motor dysfunction, with prominent symptoms including dementia, ataxia, and hallucinations (Geschwind, 2015). Patients deteriorate over months to years, with prion diseases being universally fatal. In animals, prion infections such as bovine spongiform encephalopathy and chronic wasting disease have resulted in enormous numbers of cattle slaughtered and threats to wildlife (Houston and Andréoletti, 2019). Developing effective therapeutics has proven exceptionally challenging due to the unique underlying etiology. Understanding prion propagation at a molecular level is key to designing targeted therapies and diagnostic modalities for these devastating protein misfolding disorders (Sigurdson *et al.*, 2019). This biochemical complexity of prions will be expanded upon throughout this review.

Prion Protein Misfolding:

The conversion of the normal cellular prion protein (PrPC) into the abnormal disease-associated isoform (PrPSc) is the fundamental event underlying the pathogenesis of prion diseases (Prusiner, 1982; Pan *et al.*, 1993; Colby and Prusiner, 2011). Understanding the structural differences between PrPC and PrPSc and the factors influencing this conformational change provides key insights into disease mechanisms (Eghiaian *et al.*, 2004).

PrPC Structure and Folding:

The mature form of the cellular prion protein consists of 208 amino acids in humans, with a molecular weight of 33-35 kDa. PrPC contains a flexible N-terminal tail, three α-helices, two short β-strand regions, and a disordered C-terminal domain (Basler *et al.*, 1986). It is attached to the outer plasma membrane surface through a glycosylphosphatidylinositol (GPI) anchor at Ser230. PrPC is normally trafficked through the secretory pathway where it acquires two N-linked glycosylations at Asn181 and Asn197, contributing to two di- and mono-glycosylated isoforms.

High-resolution NMR studies indicate PrPC adopts a globular structure with approx. 40% α-

helical and little β-sheet content (Zahn *et al.*, 2000). The N-terminal half of PrPC forms a flexibly disordered tail while the C-terminal domain contains three α-helices (HI, H2, H3) and two short β-strands (B1, B2), folded into a globular domain stabilized by a disulfide bridge (Riek *et al.*, 1996). This predominance of α-helices is a key feature of the properly folded prion protein.

PrPC folds into its native conformation through a complex pathway influenced by molecular chaperones (Yadav *et al.*, 2019). Folding initiates cotranslationally in the ER lumen and continues through the secretory pathway (Braakman and Hebert, 2013). The flexible N-terminal region remains unstructured while the globular C-terminal domain folds cooperatively to form the three helices. Several chaperones like BiP, calnexin, and Hsp70 facilitate proper folding and prevent misfolding during this process. Only natively conformed PrPC is trafficked to the cell surface (Schilling *et al.*, 2020).

PrPSc Formation and Properties:

In contrast to PrPC, PrPSc has high β-sheet and low α-helical content, conferring increased propensity to aggregate. PrPSc forms insoluble fibrils that are partially resistant to proteolysis, a property used diagnostically. The N-terminus acquires even higher flexibility while parts of the C-terminal helices unwind to form β-strands. This increases the β-sheet content from ~3% in PrPC to ~43% in PrPSc (Prusiner, 1998). However, high-resolution structural characterization remains limited as PrPSc resists crystallization.

PrPSc exhibits diverse biochemical properties depending on the prion strain, including variations in aggregate size, protease resistance, and susceptibility to denaturation (Bessen and Marsh, 1992, 1994). Different PrPSc conformers associated with distinct prion strains likely reflect different misfolded substates. The strain-dependent diversity in PrPSc assemblies provides evidence that specific misfolded protein conformations and aggregations patterns, rather than just protein levels, underlie prion transmission and pathology (Collinge and Clarke, 2007).

Mechanism of PrPSc Formation:

PrPC to PrPSc conversion is initiated when PrPSc oligomers bind to native PrPC and act as a misfolding template (Laganowsky *et al.*, 2012). Binding destabilizes the PrPC structure, causing the α-helices to unwind and reorganize into β-sheets that then grow by recruiting and converting more PrPC (Unterberger *et al.*, 2005; Huang *et al.*, 2013). This self-propagating process allows the aberrant structure to spread exponentially (Stein and True, 2014). Several short protein segments with high β-sheet propensity, such as residues ~90–120 and ~170–220, are implicated as triggers for nucleating the conversion (Sabzekar *et al.*, 2017).

Conversion likely proceeds along parallel pathways forming various misfolded intermediates (Minaki *et al.*, 2009). pH changes, oxidative stress, and interaction of PrP with lipids or glycosaminoglycans can influence these pathways (Wang *et al.*, 2007). Post-translational modifications like cleavages may generate more aggregation-prone species (Hald Albertsen *et al.*, 2022). Co-factors such as RNA and lipids facilitate conversion, providing platforms for nucleation. Ultimately, the misfolded proteins polymerize into highly ordered amyloid fibrils characteristic of PrPSc (Alberti and Hyman, 2021).

PrPC to PrPSc conversion entails a major rearrangement of secondary structure and the overall folding energy landscape. It likely involves partial unfolding of PrPC followed by refolding into the thermodynamically stable PrPSc state. Stabilizing interactions between PrPSc molecules in aggregates drives this conversion towards prion formation both kinetically and thermodynamically (Eghiaian *et al.*, 2004).

Factors Influencing Prion Misfolding:

Certain factors (Fig. 1) *are known to influence PrPC misfolding and the barrier for prion conversion*:

Mutations: Point mutations in the prion gene

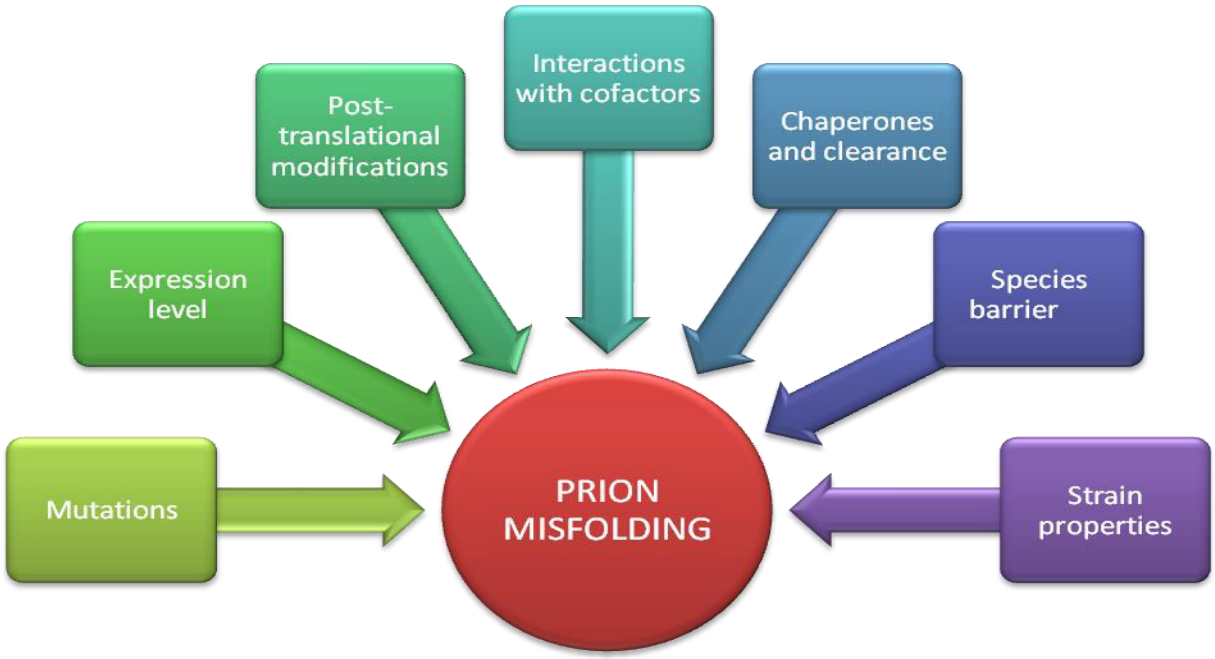

Fig. 1: Factors Influencing Prion Misfolding.

(PRNP) linked to genetic prion diseases lower the kinetic barriers and stability of PrPC, facilitating conversion. Certain prion strains also propagate more readily in individuals with these mutations (Tahiri-Alaoui *et al.*, 2004).

Expression level: Increased PrPC expression enhances prion propagation by providing more substrate for conversion. Polymorphisms regulating Prnp transcription impact susceptibility (Béringue *et al.*, 2020).

Post-translational modifications: Proteolytic cleavages, aberrant glycosylations, and other modifications of PrPC can produce more aggregation-prone species (Ramazi and Zahiri, 2021).

Interactions with cofactors: Polyanions like RNA and glycosaminoglycans, lipids, and metal ions can catalyze conversion by bringing PrPC and PrPSc into proximity (Lawson *et al.*, 2010).

Chaperones and clearance: Reduced activity of chaperones like Hsp70 that mediate proper PrPC folding increases prion formation. Impaired ubiquitin-proteasome system function decreases PrPSc degradation (Unterberger *et al.*, 2005).

Species barrier: Cross-species prion transmission is limited by incompatibility between host PrPC and donor PrPSc structures and sequences (Hashimoto *et al.*, 2020).

Strain properties: Different prion strains have varying propensities to misfold host PrPC due to their distinct PrPSc conformations (Linden, 2017).

Seeded Polymerization:

The conversion of PrPC to PrPSc is widely accepted to occur through a nucleation-polymerization process also seen during the formation of amyloid fibrils. In this seeded polymerization model, PrPSc oligomers act as a template, inducing the conversion of native PrPC by rearranging its structure. Newly converted protein is then incorporated into the growing aggregate. Fragmentation of aggregates exponentially increases the number of growth sites (Chiti and Dobson, 2017).

Prion propagation displays kinetics similar to crystallization processes dependent on seeding, fragmentation, and growth. Thermodynamic barriers need to be overcome to nucleate conversion, but once started, growth rapidly continues in a self-perpetuating manner with kinetics dependent on monomer concentration. This underlies the exponential increase in PrPSc

during prion disease progression (Legname *et al.*, 2006).

In summary, elucidating the conformational switch from the α-helical PrPC to the β-sheet rich PrPSc and factors permitting this conversion provides molecular insights into prion formation, strain diversity, and disease initiation and progression. Understanding prion protein misfolding is crucial for developing therapeutic strategies targeting early conversion events.

Prion Strains, Transmission, and Propagation Mechanisms:

Prions exist as distinct strains that exhibit variation in biochemical and biological properties. The conformational diversity of misfolded prion protein aggregates allows self-propagating strains to emerge, mutate, and transmit between species. Understanding prion strain phenomena and the molecular basis of prion propagation provides key insights into the infectious nature of these aberrant proteins

Description of Prion Strains:

A seminal feature of prions is the existence of multiple strains that display distinct disease phenotypes and PrPSc properties despite absence of any encoded genetic differences (McKintosh *et al.*, 2003; Collinge, 2005). Well-characterized prion strains identified in animal models include scrapie strains such as ME7, 22L, and RML isolated from sheep, and mouse-adapted strains like 301V, 139A, and Chandler from BSE or scrapie sources (Fernández-Borges *et al.*, 2015; Block and Bartz, 2022).

Different strains exhibit characteristic incubation periods and patterns of neuropathology when transmitted to defined mouse lines. For example, upon inoculation into C57BL/6 mice, the 139A strain produces brain lesions in <100 days while ME7 requires >300 days for clinical disease (Carroll *et al.*, 2016). Strains also differ in PrPSc aggregate morphology, protease-resistance, and stability. The 139A strain forms small aggregates and amyloid plaques that are highly PK-resistant, while ME7 produces diffuse synaptic deposits and less PK-resistant PrPSc (Zampieri *et al.*, 2009).

Prion strains appear to be encoded by distinct PrPSc conformations that templated their propagation, analogous to strain phenomena in conventional pathogens. Fourier-transform infrared spectroscopy and quenching of tryptophan fluorescence indicate that PrPSc aggregates associated with individual prion strains have differing degrees of β-sheet versus α-helical structure that imprints strain-specified pathogenic properties (Rhys *et al.*, 2019). The existence of prion strains provides compelling evidence that specific misfolded protein conformations, rather than just PrPSc levels, nucleate disease (Chong *et al.*, 2021).

Polymorphisms and Mutations of Prion Strains:

Prion strains are dynamic entities and can evolve mutations that alter their properties. For instance, upon serial passage in congenic mouse lines, prion strains often undergo changes in incubation period and neuropathology, indicative of mutational drift (Bessen and Marsh, 1992). The properties of faster propagating sub-strains become selected for, allowing rapid adaptation. The high mutation rate of prion strains likely reflects occasional mis-templating of PrPC by PrPSc that sends it down an alternative misfolding trajectory (Franz *et al.*, 2012).

Mutational drift provides insights into the conformational basis of prion strains and their evolutionary dynamics. Emergence of new prion strains has implications for disease incubation, transmissibility, and potential inter-species jumps (Franceschini *et al.*, 2017).

Species Barriers in Prion Transmission:

A key feature of prions is their ability to propagate between mammalian species leading to fatal neurodegeneration. However, transmission often displays a significant 'species barrier' that impedes the inter-species jump. This manifests as prolonged incubation periods upon first exposure and incomplete attack rates (Kimberlin *et al.*,

1983). For instance, natural sheep scrapie prions inefficiently transmit to wild-type mice.

Species barriers arise when the incoming PrPSc conformer is mismatched in structure and sequence relative to the host's PrPC, limiting conversion. This conformational incompatibility model is supported by data showing greater barriers when sequence homology between species is low (Aguzzi and Calella, 2009). However, upon repeated passage in the new species, prion strains undergo adaptation through conformational selection of variants better fitting the host PrPC structure. This can allow emergence of novel strains with expanded inter-species transmission (Bartz, 2016).

Molecular Basis of Prion Infectivity:

A central goal in prion biology is defining the molecular basis of prion infectivity – understanding what specific structural features of PrPSc enable transmission between cells and hosts. Research suggests that the size distribution and conformational flexibility of PrPSc aggregates impacts infectivity. For some prion strains, maximal infectivity aligns with particles of ~14-28 PrP monomers, as smaller oligomers efficiently seed monomer addition and avoid clearance (Silveira *et al.*, 2005). However, other strains with infectivity predominantly in larger particles argue against a universal size threshold.

Beyond size, the conformational dynamics of PrPSc may dictate infectious potential. Subsets of PrPSc conformers feature exposed, solvent-accessible hydrophobic regions able to interact with PrPC and template conversion (Caughey and Raymond, 1991). Defining the structural and conformational prerequisites for prion transmission remains key to understanding the profound 'infectiousness' of these protein aggregates (Legname *et al.*, 2006).

Molecular Mechanisms of Prion Replication:

Prions replicate by inducing the conversion of PrPC into PrPSc. This conversion occurs through a template-based mechanism centered around seeding and templating:

PrPSc acts as a template or seed that catalyzes the misfolding of PrPC into the pathological PrPSc conformation. This seeding process begins with PrPSc binding to PrPC, destabilizing its structure, and triggering a conformational change. The newly misfolded PrPC can then convert other PrPC molecules, leading to exponential amplification of the pathological form. Even small quantities of PrPSc can initiate widespread conversion, facilitating prion spread (Collinge, 2001).

Prion Strains and Their Role in Replication:

Prion diseases exhibit remarkable strain diversity, with different strains characterized by distinct biochemical and biological properties attributed to variations in PrPSc conformation and structure:

Biochemical properties: Prion strains display differences in aggregate size, protease resistance, and stability that reflect their distinct misfolded conformations (Legname *et al.*, 2006).

Strain-specific phenotypes: Different prion strains lead to variations in disease progression, symptoms, and affected brain regions based on their particular protein conformations (Colby and Prusiner, 2011).

Cross-species transmission: Prion strains can sometimes infect new species when the PrPSc conformation induces conversion of the host PrPC. This underlies zoonotic spread (Kurt and Sigurdson, 2016).

Prion Propagation in the Brain and Peripheral Tissues:

Prion propagation primarily occurs in the central nervous system (CNS) but can also involve peripheral tissues:

In the brain, PrPSccatalyzes the conversion of PrPC into more PrPSc. Accumulating misfolded proteins disrupt neuronal function, leading to neurodegeneration (Collinge, 2001).

Prions can also replicate in peripheral tissues like lymphoid organs and skeletal muscle. The mechanisms likely involve transport of PrPSc via peripheral nerves or immune cells (Murray and Wynn, 2011).

In summary, prion strain diversity arises from alternative misfolded conformations of PrPSc that template their own propagation. Defining the molecular basis of prion infectivity and the mechanisms of prion replication provides insights into the unique biology of these infectious proteins. Understanding prion conformational templating, seeding, and spread is crucial for elucidating the pathogenesis of these devastating neurodegenerative diseases.

Cellular Pathology of Prion Diseases:

The conversion of the normal prion protein (PrPC) into the misfolded, aggregated isoform (PrPSc) is the key molecular event underlying the pathology of prion diseases (Prusiner, 1998). The accumulation of PrPSc and its deleterious effects on neuronal function, synaptic connectivity, and cell survival pathways ultimately leads to widespread neurodegeneration (Collinge and Clarke, 2007).

Neurotoxicity of Prion Protein Aggregates:

A seminal feature across the prion diseases is the profound neurotoxicity triggered by PrPSc accumulation, resulting in extensive neuronal loss and brain damage. However, the mechanisms by which prion protein aggregates elicit cytotoxicity remain incompletely defined (Aguzzi and Calella, 2009).

Several lines of evidence indicate that smaller PrPSc oligomers, rather than large amyloid fibrils, represent the primary neurotoxic species. Synthetic PrP peptides capable of forming β-sheet rich oligomers induce neuronal apoptosis *in vitro*. In human prion diseases, synapse loss correlates with levels of low-n oligomers rather than large aggregates. The greater membrane permeability and exposed hydrophobic surfaces of oligomers may mediate their toxicity (Sarell *et al.*, 2009; Bieschke *et al.*, 2011).

Proposed mechanisms through which PrPSc oligomers impair neuronal viability include disruption of membrane integrity via pore formation, interference with ion channels and signaling receptors, overload of protein quality control pathways, and induction of oxidative stress through interactions with redox-active metal ions like copper. Ultimately, the neurotoxic species likely comprises a heterogeneous ensemble of soluble PrPSc conformers capable of deleterious interactions with the neuronal membrane and interior (Simoneau *et al.*, 2007).

Synaptic Dysfunction and Neurodegeneration:

Synaptic deficiencies constitute an early pathology in prion diseases that likely contributes to the extensive neurodegeneration. Analysis of brain tissue from infected hosts reveals progressive synaptic loss and damage to dendritic spines preceding neuronal death (Liberski *et al.*, 2001; Liberski, 2004).

In vivo imaging shows prion infection impairs long-term potentiation in mouse hippocampal synapses, indicative of deficits in synaptic plasticity. Electrophysiology recordings demonstrate altered synaptic transmission and defective connectivity. These functional disturbances correlate with synaptic deposition of PrPSc aggregates, which can physically disrupt membranes (Tegenge *et al.*, 2014; Salvadores *et al.*, 2020). Loss of dendritic spines eliminates crucial sites of transmission.

The synaptic dysfunction occurs well prior to significant neuronal loss, suggesting an early insult to neural circuitry connectivity may set the stage for subsequent widespread neurodegeneration. Disruption of axonal transport and deficits in maintenance signaling at nerve terminals also likely contribute to synaptic defects. Restoring synaptic integrity early in disease may represent a promising therapeutic strategy (Gevaert *et al.*, 2015).

Activation of Apoptotic Pathways:

Several lines of evidence indicate that prion infections trigger neuronal apoptosis through destabilization of mitochondrial membranes and activation of caspase cascades (Brown, 2005). Exposure of cultured neurons to PrP peptides leads to changes in membrane permeability, release of apoptogenic factors like cytochrome c

from mitochondria, and activation of pro-apoptotic proteases (Conibear and Davis, 2010; Ladygina *et al.*, 2011).

In mouse models, prion infection causes altered calcium fluxes, resulting in calpain activation and cleavage of apoptosis regulators like Bax. Caspase 3 activation and DNA fragmentation are observed concomitant with synaptotoxicity. Downstream apoptotic processes involving chromatin condensation and DNA fragmentation then occur. Blocking caspase activation in neuronal cultures partially protects against prion peptide toxicity (Mangiarini *et al.*, 1996).

The neurotoxic PrPSc species likely activate apoptotic signaling through direct interactions with neuronal membranes or interference with anti-apoptotic factors. The consequent activation of proteolytic cascades leads to programmed cell death, representing a key pathologic event in prion disease brains (Sun *et al.*, 2006).

Contribution of Inflammatory Responses:

Neuroinflammation triggered by prion infections also contributes to neuronal damage. Reactive gliosis is consistently observed in prion-affected brains, with activated microglia proliferating around PrPSc deposits (Perry and Teeling, 2013). These microglia release cytotoxic mediators like cytokines, chemokines, and reactive oxygen species.

In mouse models, blocking microglial activation slows neurodegeneration and extends survival time, highlighting the contributory effects of inflammation. Complement activation elicited by microglia may also play a role through triggering inflammatory cascades that damage synapses (Block and Hong, 2005).

The role of peripheral immune cells in prion pathology remains less clear. While adaptive immune responses do not appear to limit prion pathogenesis, some reports suggest infiltrating T cells and inflammatory monocytes may exacerbate neurotoxicity in a subset of prion strains (Herber *et al.*, 2007). Ongoing prion replication and neurodegeneration sustains chronic inflammation that likely amplifies neuronal loss (Milovanovic *et al.*, 2012; Chen *et al.*, 2018).

Role of Prion-Like Propagation in Pathology:

The ability of PrPSc aggregates to self-propagate through templating the misfolding of native PrPC appears centrally involved in mediating neurotoxicity and sustaining disease progression (Aguzzi and Lakkaraju, 2016). Studies indicate that blocking PrPC expression to reduce prion replication can rescue neuronal loss and increase survival even after onset of clinical illness (Mallucci *et al.*, 2003).

The kinetics of prion-like spread align with the anatomical pathway of pathology observed in brains. Initial PrPScbuildup in lymphoid follicles is followed by spread along peripheral nerves to the CNS, with subsequent dissemination through neuroanatomically connected brain regions manifesting the progressive symptoms (Béranger *et al.*, 2002).

Biochemical analysis reveals that prion titers and misfolded protein burdens correlate with severity of neuropathology and clinical progression. Thus, the self-propagating nature of prions that allows exponential growth of toxic conformers plays a key role in driving the deleterious neurodegenerative process (Hsiao *et al.*, 1989).

In conclusion, prion pathology involves a multifaceted perturbation of neuronal function. The common denominator is toxic gain-of-function mediated by misfolded prion protein conformers interacting deleteriously with membranes, triggering synaptic breakdown, activating apoptotic cascades, and sustaining inflammation. These disruptive processes are inexorably driven forward through the unique self-replicating nature of pathogenic prions.

Prion Diseases in Humans and Animals:

Prion diseases are a group of fatal neurodegenerative disorders that affect humans and animals worldwide. They are caused by the

accumulation and aggregation of misfolded prion proteins in the brain, leading to progressive neurological symptoms and inevitable death (Prusiner, 1996; Zhu and Aguzzi, 2021; Liberski *et al.*, 2023). Major prion diseases include Creutzfeldt-Jakob disease (CJD), bovine spongiform encephalopathy (BSE), scrapie in sheep, and chronic wasting disease (CWD) in cervids.

In humans, the most prevalent prion disease is CJD, occurring sporadically, genetically, or acquired through exposure to prion-contaminated tissues (Hill *et al.*, 2003). The sporadic form arises spontaneously at a rate of 1-2 cases per million people annually, typically affecting older adults (Parchi *et al.*, 2009). It manifests as a rapidly progressive dementia accompanied by abnormalities of gait, vision, and motor control (Poser *et al.*, 2000). Genetic CJD stems from mutations in the PRNP gene and accounts for 10-15% of cases, arising at an earlier age (Baiardi *et al.*, 2021). Iatrogenic CJD develops after transmission from surgical instruments or tissue grafts, as tragically seen in growth hormone recipients (Kobayashi *et al.*, 2018).

Variant CJD, linked to ingestion of BSE contaminated beef, highlights the zoonotic transmission potential of animal prion diseases (Ghani *et al.*, 2003; Houston and Andréoletti, 2018). The BSE epidemic that peaked in the UK during the 1990s was fueled by feeding cattle meat and bone meal containing scrapie prions (Tan *et al.*, 1999; Brown, 2001). Over 180,000 cattle cases necessitated the slaughter of millions of animals. Stringent control measures have since curbed BSE's prevalence (Kingdom, 1993; Alarcon *et al.*, 2023).

Scrapie, identified first in sheep over 250 years ago, persists endemically in many regions. Occurring sporadically or through horizontal transmission, it manifests as behavioral changes, abnormal gait, and compulsive itching (Andréoletti *et al.*, 2002). Scrapie prion isolates exhibit significant strain variability affecting incubation times and pathology patterns (Castilla *et al.*, 2005). This diversity has provided insights into prion conformational templating.

CWD in cervids like deer, elk, and moose is concerning due to its facile animal-to-animal spread. Its distribution continues to expand across North America and globally (Jennelle *et al.*, 2014). As infected animals shed prions through saliva, urine, and feces, CWD poses challenges for containment and threatens valued wildlife populations (Peluso *et al.*, 1985). The potential for zoonotic transmission remains unclear.

In summary, prion diseases have a profound impact on human health, agriculture, and ecosystems. Preventing transmission and developing effective therapeutics is extremely challenging given the distinct biology of infectious proteins. Understanding shared molecular underpinnings as well as unique strain properties will aid in devising broad-spectrum anti-prion interventions. Ongoing surveillance and research into prion pathogenesis are crucial to manage these devastating protein misfolding disorders.

Biochemical Assays and Detection Methods for Prion Diseases:

Detecting (Fig. 2) and diagnosing prion diseases poses substantial challenges due to the unique biochemical properties of the infectious prion protein (PrPSc) and its variable distribution throughout the body. A combination of laboratory techniques is employed to identify PrPSc, but significant limitations remain in achieving sensitive, early diagnosis.

Western Blotting:

Western blotting detects PrPSc by exploiting its partial resistance to protease digestion and electrophoretic mobility differences from normal prion protein (PrPC)(Kübler *et al.*, 2003; Soto, 2004). After protease treatment, brain homogenate samples are electrophoresed and the proteins transferred to a membrane for probing with PrP-specific antibodies (Hur *et al.*, 2002; Aguzzi and Heikenwalder, 2006). The three characteristic glycoforms of PrPSc generate a distinct banding pattern allowing discrimination

Fig. 2: Detection methods of Prion disease.

from PrPC. However, Western blot lacks sensitivity when PrPSc levels are very low.

Enzyme-Linked Immunosorbent Assay:

ELISA allows higher throughput prion detection by utilizing PrP-specific capture and detection antibodies (Shan *et al.*, 2016; Hnasko*et al.*, 2018). When prions are present in a sample, they bind to the capture antibody on the assay plate. Subsequent enzymatic reactions with the detection antibodies produce quantifiable colorimetric or fluorescent signals indicative of prion infection (Tattum *et al.*, 2010; Connor *et al.*, 2019). While quick and scalable, ELISA can have limited sensitivity and specificity depending on the antibody pairs used.

Protein Misfolding Cyclic Amplification:

PMCA exploits the capacity of PrPSc to template the misfolding of PrPC by cycling incubation and ultrasonication steps (Hnasko *et al.*, 2018). This mimics prion replication, amplifying minute amounts of PrPSc for ultrasensitive detection(Schmitz *et al.*, 2016a). PMCA can detect prions at femtogram levels in tissues where PrPSc has very limited distribution. However, careful protocol standardization is needed to avoid artifacts.

Real-Time Quaking-Induced Conversion:

RT-QuIC offers the highest analytical sensitivity for prions by using recombinant PrP in a fluorescence readout assay (Cheng *et al.*, 2017). Trace prion seeds induce exponential templated misfolding of substrate PrP, detected in real-time by thioflavin T fluorescence (Atarashi, Sano, *et al.*, 2011). RT-QuIC can detect prions in bodily fluids at presymptomatic stages. Adaptations for high-throughput automated analysis have been implemented.

Challenges and Limitations:

Prion detection faces several inherent challenges:

Sensitivity and Specificity: Achieving both high analytical sensitivity and clinical specificity is difficult, with issues of false positives and negatives. Rigorous cutoff establishment and protocol optimization can help to improve reliability (Quadrio *et al.*, 2011).

Early Detection: Preclinical diagnosis is critical but complicated by low initial PrPSc levels. Combining

ultrasensitive techniques like RT-QuIC with accessible sampling from peripheral tissues may enable early detection (Orrú *et al.*, 2014).

Sample Variability: PrPSc distribution in tissues and body fluids varies among prion strains and host factors, necessitating broad sampling approaches (Uro-Coste *et al.*, 2008).

Standardization: Variability in protocols, instrumentation, and data interpretation across laboratories hinders broad implementation of diagnostic assays. International collaborative efforts to establish robust standards could enable consistent, reliable prion detection (Schmitz *et al.*, 2016a).

In summary, a diverse toolbox of prion detection methods now exists spanning conventional techniques like Western blotting to modern amplification assays and immunoassays. While recent advances have dramatically boosted analytical sensitivity, challenges remain in achieving clinical sensitivity and specificity during pre-symptomatic stages. Ongoing refinement and standardization of prion diagnostic protocols will aid in implementing sensitive, reliable testing to prevent further spread of these devastating diseases.

Prion Evolution and Adaptation:

Prions possess a remarkable ability to adapt and evolve, resulting in the emergence of distinct strains that can propagate within and between mammalian species. The conformational diversity of misfolded prion proteins allows selection of 'fit' variants that can overcome barriers to spread both within hosts and across species (Collinge, 2016).

Conformational Adaptability Underlies Prion Evolution:

The ability of prions to adapt resides in the conformational variability of the misfolded, disease-associated prion protein (PrPSc). PrPSc exists not as a single entity, but rather a heterogeneous ensemble of distinct self-propagating conformers. When prions cross the 'species barrier' into a new host, they encounter a native prion protein (PrPC) with slightly varied amino acid sequence and structure. Most incoming PrPSc conformations will be poorly converted by the new host PrPC. However, the diverse PrPSc cloud likely contains minority variant conformers that can more readily seed misfolding of the new host's PrPC. These fitter prion conformers then get selectively amplified, allowing adaptation to the new species (*et al.*, 1996a; Collinge and Clarke, 2007; Collinge, 2016).

The concept that select PrPSc conformers are differentially amplified underpins the evolution of distinct prion strains. As certain misfolded conformers are preferentially propagated, they can become fixed, exhibiting consistent biological properties (Makarava and Baskakov, 2013; Bartz, 2016). The diversity of possible PrPSc misfolded states provides a substrate for evolutionary selection of self-perpetuating prion strains.

Biological Implications of Prion Strain Diversity:

The propagation of distinct prion conformers manifests in the phenomenon of prion strains. Different strains adapted to particular host environments display unique characteristics:

Biochemical properties: Prion strains vary in size distribution, protease-resistance, and structural stability of PrPSc aggregates. These properties reflect the underlying conformational differences (Fiorini *et al.*, 2017).

Pathology: Strains induce differing patterns of pathology and disease progression based on the specific interactions of their PrPSc conformers with the host brain environment (Zafar *et al.*, 2018).

Transmission: Strains can differ in transmissibility profiles both within and between species based on conversion efficiency and interaction compatibility (Ghazi *et al.*, 2022).

Mutation: Prion strains can undergo mutation and drift upon repeated passage, indicative of the conformational flexibility and evolutionary capacity (Ghaemmaghami, 2017).

Risks of Cross-Species Transmission:

While species barriers exist, prions on rare occasion achieve inter-species jumps, most concerningly from animals to humans as seen with bovine spongiform encephalopathy (BSE) and variant Creutzfeldt-Jakob disease (vCJD). The heterogeneous PrPSc ensemble likely contains 'promiscuous' conformers that can convert a new host PrPC and spawn new strains with unpredictable effects (Cassard *et al.*, 2014).

Ongoing prion evolution within reservoir species also poses risks by generating novel strains that may breach barriers. Active surveillance is therefore crucial. Understanding the molecular determinants and evolutionary trajectories of prion cross-species transmission will help in assessing and mitigating risks to both animal and human health.

In summary, the remarkable adaptability of prions arising from the diverse ensemble of self-replicating PrPSc conformers leads to ongoing evolution and generation of new strains with complex biological implications. Defining the variables that facilitate or impede prion adaptation will reveal key insights into prion infectivity, pathogenesis, and emergence (Angers *et al.*, 2010).

Therapeutic Approaches and Challenges:

Prion diseases remain among the most challenging neurodegenerative conditions to treat effectively due to the unique underlying pathogenesis of misfolded prion protein propagation and aggregation. Despite extensive efforts, no curative therapies exist, underscoring the need for enhanced understanding of prion conformational conversion and strain-specific mechanisms. Promisingly, emerging approaches provide renewed optimism for counteracting prion protein misfolding and toxicity (Trevitt and Collinge, 2006; Giles *et al.*, 2017).

Blocking prion conversion and aggregation represents a major therapeutic aim. Compounds like pentosan polysulfate and quinacrine that directly bind prion protein show some promise in stabilizing native structure and inhibiting conversion in animal models (Kocisko *et al.*, 2006; Takatsuki *et al.*, 2022). Other agents such as DAPH help maintain normal prion protein conformation while molecules like Congo red derivatives block pathological aggregation (Kocisko *et al.*, 2003). However, issues with limited efficacy post-symptomatically, variable strain-specific responses, and toxicity have challenged clinical translation. Efforts continue to identify safe compounds that target distinct misfolded prion strain conformers.

Enhancing clearance of prion aggregates via cellular protein quality control pathways is also being explored. Activation of the ubiquitin-proteasome system and autophagy-lysosome pathways with compounds like carfilzomib, bortezomib, rapamycin and trehalose has displayed some ability to degrade pathological prion proteins in model systems (McKinnon *et al.*, 2016; Wang *et al.*, 2017). However, again, human trials have yet to demonstrate major clinical improvements, potentially due to intervention at later disease stages with already substantial prion deposition.

Other strategies aim to prevent intercellular prion transmission and mitigate neurotoxicity. Inhibiting endocytic pathways blocks cell uptake of prion aggregates while anti-inflammatory approaches may counteract prion-induced neuroinflammation (Aguzzi and Glatzel, 2006; Westergard *et al.*, 2007). However, these methods only provide symptom relief without tackling root misfolding causes.

Excitingly, emerging immunotherapies and gene therapies offer more targeted strategies. Antibodies clearing prion aggregates and siRNAs suppressing prion protein expression show particular promise in preclinical studies (White *et al.*, 2003; Pfeifer *et al.*, 2006). Prion-directed nanoparticles, vaccines, and structure-specific antibodies also offer fresh approaches (Re *et al.*, 2012; Rossi *et al.*, 2019). Such innovative techniques that directly target fundamental prion replication mechanisms may finally offer a path to

effective treatments.

A key challenge going forward is translating promising preclinical findings into clinically viable therapies while also confirming safety and efficacy across prion strains, stages, and species (Collinge *et al.*, 1996; Wadsworth *et al.*, 2008). Combining modalities that combat prion propagation, enhance clearance, and mitigate neurotoxicity may provide synergistic benefits. Advancing diagnostic capabilities for early pre-symptomatic detection will also expand treatment opportunities (Atarashi, Satoh, *et al.*, 2011; Orrú *et al.*, 2014). Ultimately, solving the complex puzzle of prion misfolding and aggregation through enhanced basic, translational and clinical research promises new hope for individuals impacted by these devastating protein conformational disorders.

Ethical and Societal Implications of Prion Diseases:

The unique biology and transmission properties of prions raise multifaceted ethical and societal challenges. Responsible practices are imperative in prion research and policymaking to uphold public trust and safety.

Conducting studies with infectious prion samples risks accidental exposures. Stringent containment protocols and oversight bodies are vital to minimize hazards (Mead and Evans, 2021). Safely disposing of prion waste also presents difficulties given the resistance of prions to standard decontamination methods. Developing innovative but prudent decontamination approaches is key.

The zoonotic transmission potential of animal prions generates dilemmas in studying cross-species spread (Angers *et al.*, 2010). While understanding interspecies jumps is crucial for preparedness, this requires judiciously working with prions capable of infecting humans. Oversight on such research must weigh risks versus benefits to global health.

Prion animal models are invaluable for developing treatments and elucidating disease mechanisms (Trevitt and Collinge, 2006). However, ethical obligations exist to minimize animal usage and suffering. Adhering to replacement, reduction and refinement principles in animal research is imperative.

From a public health perspective, policies enacted to safeguard blood supplies and food systems from prion contamination, while burdensome, are warranted to prevent new epidemics (Bradley and Wilesmith, 1993). However, care must be taken to avoid unduly restricting individual liberties out of prion transmission fears.

Prion diseases evoke public misconceptions fueled by sensationalized media portrayals (Farrugia, 2009). Stigma can isolate patients and families, highlighting the need for greater awareness. Scientists have an ethical responsibility to communicate responsibly with the public about prion risks and realities.

Allocating resources for combating rare prion diseases presents difficulties, especially in countries with inadequate healthcare access (Hermann *et al.*, 2020; Choudhury and Chaube, 2022). Fair and consistent funding frameworks are needed that balance prion research investments against competing priorities.

To build public trust, the prion research community must demonstrate commitment to transparency, safety and ethical practices (Jankovska *et al.*, 2021). This includes communicating risks honestly, undergoing ethical reviews of protocols, disclosing conflicts of interest, and adhering to biosafety regulations.

Engaging affected communities in a participatory, culturally sensitive manner should inform prion science policies (Oyebode *et al.*, 2013). Researchers and policymakers have an ethical duty to consider input from patients, families, indigenous groups and other stakeholders.

In summary, analyzing prion diseases through both the scientific and ethical lenses reveals complex challenges for researchers and policymakers but also opportunities to uphold the highest standards of responsibility, safety and

compassion. By embracing transparency, accountability and awareness, the prion research community can build public trust while advancing knowledge to tackle these confounding diseases.

Conclusion

This comprehensive exploration of prion diseases has illuminated key insights while underscoring persistent knowledge gaps. Fundamental understanding of prion protein misfolding and strain-specific propagation mechanisms has expanded, enabled by advances in structural biology and development of amplification techniques for ultrasensitive prion detection. Animal models continue to provide insights into species barriers and zoonotic potential. However, early diagnosis remains challenging due to the lack of reliable biomarkers. Therapeutic progress is hindered by the multifaceted nature of prion pathogenesis and the difficulties of clinical translation. Combination therapies and emerging approaches like immunotherapies and gene editing offer optimism but require further optimization informed by a deeper grasp of prion strain diversity. Formidable challenges also persist in upholding rigorous safety and ethical standards in prion research to maintain public trust. Overall, the complex questions surrounding prion structure, evolution, and transmission warrant extensive ongoing research efforts. While many obstacles exist on the path to understanding prion disease etiology and developing effective diagnostics and treatments, continued vigorous investigation promises breakthroughs in illuminating these perplexing protein misfolding disorders. Creative, collaborative approaches coupling basic, translational and clinical research will unravel remaining mysteries and ultimately conquer the threats posed by pathogenic prions.

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