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## Review: The Prion and Its Potentiality

Dipendra Raj Pandeya  
*Chonbuk National University Medical School (South Korea)*

Nimish Acharya  
*Rowan University School of Osteopathic Medicine*

Seong-Tshool Hong  
*Chonbuk National University Medical School (South Korea)*

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# Review: The Prion and its Potentiality

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## **Review: The Prion and its Potentiality**

**Dipendra Raj Pandeya<sup>1</sup>, Nimish K. Acharya<sup>2</sup> and Seong-Tshool Hong<sup>1\*</sup>**

<sup>1</sup>Laboratory of Genetics, Department of Microbiology and Immunology, Institute for Medical Science, Chonbuk National University Medical School, Chonju, Chonbuk 561-756, South Korea

<sup>2</sup>University of Medicine and Dentistry of New Jersey (UMDNJ) - Graduate School of Biomedical Sciences, 2 Medical Center Drive, Stratford, NJ 08084, USA

### **Abstract**

**A great deal of effort during the past 27 years has been devoted to defining the chemical nature of prions, the infectious agents responsible for transmissible spongiform encephalopathies. Prion diseases are fatal neurodegenerative disorders that can arise spontaneously, be inherited, or be acquired by infection in mammals. They are unique not only in terms of their biological features but also in terms of their impact on public health. It has been hypothesized that in addition to Creutzfeldt - Jakob disease (CJD) in humans and Bovine Spongiform Encephalopathy (BSE) in animals, prions may also play a role in several other neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and frontotemporal dementia; however, the precise mechanism underlying prion-mediated neurodegeneration still remains elusive. In this review, we outline the physico-chemical characteristics of prions and their impact on human and animal health.**

**Keywords:** Prion, prion diseases, bovine spongiform encephalopathy, neurodegeneration

*Accepted*

### **Introduction**

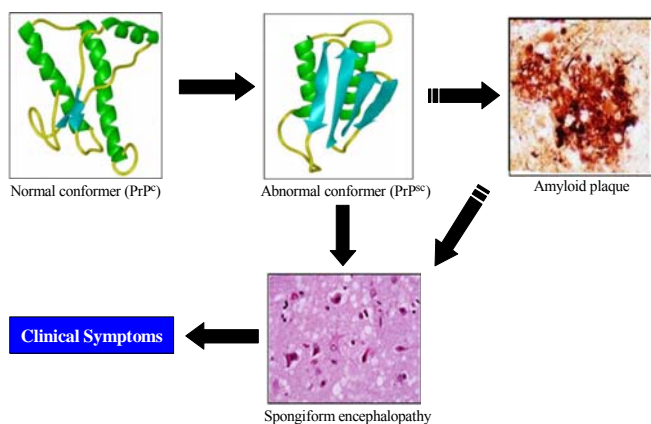
Prion disease (PD) is an untreatable and fatal neurodegenerative disorder that affects both humans and animals. Since various aspects of PD pathogenesis have not been conclusively delineated, PD remains an intriguing puzzle waiting to be solved. Transmissible spongiform encephalopathy (TSE) is the general term assigned to all known prion diseases.

PD is the new designation of a group of spongiform encephalopathies because of the histological appearances of large vacuoles in the cortex and cerebellum and all invariably fatal, which show similar clinical and neuropathological changes. TSEs in sheep and goat is known as Scrapie; in humans, they are known as Kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Straussler-Scheinker syndrome (GSS) and fatal familial insomnia (FFI). Kuru has been described only in the Fore population of New Guinea. For many years after its first recognition in 1957 [1], Kuru was the most common cause of death among women in the affected population, but it is disappearing because of the cessation of ritualistic cannibalism that had facilitated disease transmission [2]. The term chronic wasting disease is used in mules, deer [3] and Rocky Mountain elk [4], bovine spongiform encephalopathy (BSE) or mad cow disease is used in cattle and feline

spongiform encephalopathy in cats, albino tigers, pumas and cheetahs. With the exception of FFI, all of these disorders have been experimentally transmitted to nonhuman primates and laboratory rodents. Severe loss of neurons is a key characteristic for all prion diseases, accompanied by strong astrogliosis and mild microglia activation. This results in a progressive spongiform degeneration of the central nervous system (CNS) which manifests itself in ataxia, behavioral and, in humans, a highly progressive loss of intellectual abilities changes [5]. Though it was initially gestated to explain elusive neurodegenerative diseases in mammals, it has now grown to encompass a number of non-Mendelian traits in fungi [6, 7, 8].

The mode of transmission appears to be novel; a protein agent rather than a particle containing nucleic acid is involved. However, the mechanism and propagation of PD still remain to be conclusively elucidated. Some key players associated with pathogenesis of the disease have been identified. The most important one is the protein agent that induces abnormal refolding of the normal prion protein. Aggregation of these misfolded proteins leads to the formation of dense plaques and fibers known as amyloid. The deposition of amyloid consequently results in cell death and tissue damage in the brain and spinal cord. Spongiform changes are associated with neuronal loss

during amyloid plaque formation; failure to elicit inflammatory responses is a major characteristic of degenerative tissue damage due to prion diseases (Fig.1). Most prions identified so far are self polymerized amyloids that form highly ordered cross- $\beta$  fibrous aggregates. The yeast prion [*PSI*<sup>+</sup>] is a self-perpetuating amyloid of Sup35 [9], an evolutionarily conserved eukaryotic release factor that is required for the termination of translation [10, 11]. This review provides a basic understanding of the nature of prion proteins and highlights the etiology, replication, transmission and other clinical and pathologic features of these debilitating interesting diseases.



**Figure 1.** A model for the progression of transmissible spongiform encephalopathy (TSE) pathogenesis.

**Prion disease**

Prion diseases are rare and unusual neurodegenerative disorders of the nervous system caused by the accumulation of a misfolded form of the endogenous PrP; these diseases present ongoing threats to humans and animals [12, 13]. Much about TSE diseases remain unknown. The diseases are characterized by certain misshapen protein molecules that appear in brain tissue. Prion diseases result in progressive cognitive and motor impairment and are characterized by the accumulation of proteinaceous brain lesions or plaques [14]. Sheep scrapie was the first of to be recognized, but subsequently a set of human diseases including Kuru and CJD was shown to have similar clinical and pathological features. TSEs have now been identified in a wide range of mammals, including cats, cows, mink, deer and elk [15]. These diseases affect the structure of brain tissue and are all fatal and untreatable. Some of the distinctive features of TSEs include neuronal vacuolation (spongiosis), neuronal death, and glial reactions. In addition, a defining characteristic is the deposition of PrP<sup>sc</sup>, mainly in the brain and lymphoreticular tissues. Also, no adaptive immune responses are elicited upon infection, most likely because the mammalian immune system is largely tolerant to PrP from the same species. This is not surprising, given that many cells in neural and extraneural compartments express PrP<sup>c</sup>. Although TSEs are by definition transmissible, a growing number of

Prnp-associated non-infectious, neurodegenerative proteinopathies are now also being recognized [16]. The only molecules thus far associated with infections are isoforms of PrP. These transmissible agents appear to have a common mechanism of pathogenesis and possibly a common origin. Some have spread across species barriers (transmissible mink encephalopathy and possibly new-variant CJD); some have reached epidemic proportions by entering the food chain (transmissible mink encephalopathy, bovine spongiform encephalopathy, and Kuru); and others have been inherited due to mutations in the PrP gene (familial CJD, GSS and FFI) [17]. Recent evidences suggest a role for the ubiquitin proteasome system (UPS) in prion disease. Both wild-type PrP<sup>c</sup> and disease associated PrP isoforms accumulate in cells after proteasome inhibition leading to increased cell death and abnormal  $\beta$ -sheet-rich PrP isoforms have been shown to inhibit the catalytic activity of the proteasome [18]. The hallmark feature common to all prion diseases, whether sporadic, dominantly inherited or acquired by infection, is that they involve aberrant metabolism of the prion protein.

**The Prion Hypothesis**

The nature of the agents responsible for TSEs has been the focus of intense scrutiny and considerable debate over the last few years. Research on the molecular genetics of PrP protein has contributed greatly to our knowledge of these diseases [19]. The observation that the scrapie agent was resistant to procedures that inactivate or modify nucleic acids, but sensitive to treatments that denature proteins, led to speculation that the agent could be a self-replicating protein devoid of nucleic acids [20]. Based on these events, Stanley Prusiner in 1982 hypothesized the existence of a novel class of infectious agents, which he named prions [21]. Specifically, it was hypothesized that the scrapie agent was a proteinaceous infectious particle because infectivity was dependent on proteins and resistant to methods known to inactivate nucleic acids [22]. A similar proposal was presented more than a decade earlier by Gibbons and Hunter [23] and Griffith and Levine [24], who used irradiation to demonstrate that the scrapie agent was devoid of disease-specific nucleic acid. The alternative virion hypothesis is not a conventional viral hypothesis but rather addresses the diversity of biological properties of TSEs. This hypothesis states that TSEs are caused by a replicable, informational molecule (likely to be a nucleic acid) bound to PrP. Many TSEs, including scrapie and BSE, show strains with specific and distinct biological properties; this feature, according to supporters of the virion hypothesis, is not explained by prions.

No mechanism has yet been proposed that can satisfactorily explain how the PrP protein alone could specify and retain multifactorial TSE strain characteristics. On the other hand, the virino hypothesis [25, 26] proposes the existence of a small, host-independent, informational molecule encoding strain-specific information that is

bound to and protected by a host protein, PrP. This fulfills the requirements of all biological experimental evidence obtained and is compatible with the biophysical and biochemical data [27]. The molecular structure of the agent is still undetermined, but there is now enough evidence to formulate testable hypotheses. For example, *de novo* production of infectivity [28] in a test tube by experimental manipulation of recombinant or synthetic PrP has been clearly demonstrated. The absence of molecular structural data does not invalidate the prion hypothesis but simply underscores the difficulty of extra-cellularly reconstituting this remarkable molecular transformation. However, additional studies are required to substantiate these claims or explain the strain diversity of prions that can lead to variable phenotypic disease expression.

**Prion Protein (PrP<sup>c</sup>- PrP<sup>sc</sup>)**

The prion protein is arguably one of the most extensively studied proteins. Prions are infectious pathogens that differ from bacteria, viruses and virioids in their structure and pathogenesis [29]. They contain information encoded in the shape of the prion protein molecule; this information is transmissible from one molecule to another. Studies of the scrapie agent, and more limited studies on prions in humans, indicate that these agents are resistant to treatments that inactivate nucleic acids and viruses (alcohol, formalin, ionizing radiation, proteases and nucleases) [30], but they are inactivated by treatments that disrupt proteins (autoclaving, phenol, detergents, and extremes of pH) [23]. From a broader view, prions are elements that impart and propagate variability through multiple conformations of a normal cellular protein. The cellular prion protein (PrP<sup>c</sup>) is a cell membrane bound glycoprotein with a molecular weight of 33-35 kDa present in various organs, it is especially abundant in the central nervous sys-

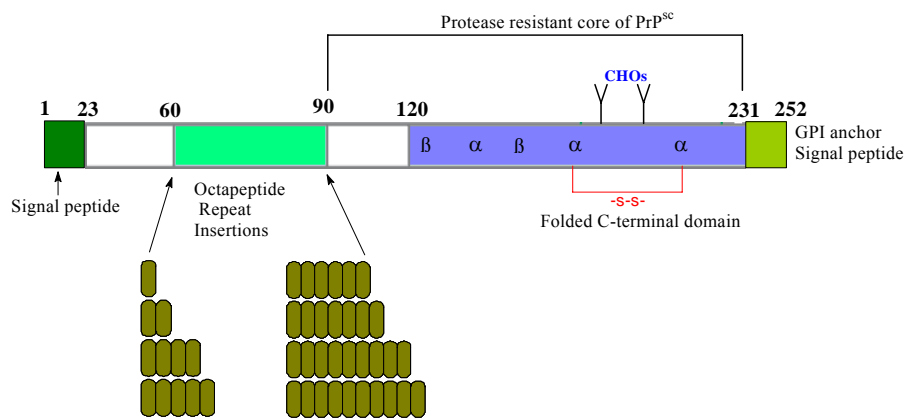
tem (CNS). Little is known about the physiology of PrP<sup>c</sup>. The conformational counterpart of PrP<sup>c</sup>, PrP<sup>sc</sup>, is thought to be the cause of prion diseases, hence, by definition, standing for neurotoxicity and prion infectivity. This assumption has been challenged by several observations during the last decade. The disease-associated isoform of PrP<sup>sc</sup> is post-translationally derived from PrP<sup>c</sup> [31, 32]. PrP<sup>c</sup> is converted into PrP<sup>sc</sup> through a process in which a portion of its  $\alpha$ -helical and coiled structure is refolded into a  $\beta$ -sheet [33]. PrP<sup>c</sup> is linked to the cell membrane by a glycosylphosphatidylinositol (GPI) anchor. It has either one or two sugar chains that are closely linked to the C-terminus; it also exists in an unglycosylated form (Fig. 2).

PrP<sup>c</sup> and PrP<sup>sc</sup> differ in their biochemical properties, with PrP<sup>sc</sup> being protease-resistant and detergent-insoluble (Table 1). Despite intensive investigation, no differences between the primary sequences or covalent modifications of these two isoforms have been found [34]. Rather, they are thought to differ in their three-dimensional conformation, with PrP<sup>sc</sup> having much higher  $\beta$ -sheet content than PrP<sup>c</sup> [35]. Circular dichroism spectrometry and infrared studies suggest that PrP<sup>c</sup> is composed of 42%  $\alpha$ -helical and 3%  $\beta$ -sheet conformations, whereas PrP<sup>sc</sup> is composed of 30%  $\alpha$ -helical and 43%  $\beta$ -sheet conformations [32]. The increased  $\beta$ -sheet content in PrP<sup>sc</sup> is due to the major conformational transition of the hydrophobic region (amino acid residues 90-140) and a portion of the helices in PrP<sup>c</sup> molecules. This molecular event makes the molecule hydrophobic and resistant to proteinase K (PK) digestion. The conformationally altered region in PrP<sup>sc</sup> is thought to form the repeated stretches of short  $\beta$ -sheets, and it can aggregate into multimers of PrP<sup>sc</sup>, which can become PrP amyloid fibrils [36]. A tertiary structure of PrP<sup>c</sup>, based on nuclear magnetic resonance spectroscopic

**Table 1. Comparison of PrP<sup>c</sup> and PrP<sup>sc</sup>**

Properties	PrP <sup>c</sup>	PrP <sup>sc</sup>
Isoform	Normal	Pathogenic
Protease resistance	No	Stable core containing residue 90-231
Location in or on cells	Plasma membrane	Cytoplasmic vesicles
Solubility	Soluble	Insoluble
PK-sensitivity	Sensitive	Partially resistant
Structure	Extended	Globular
$\alpha$ -Helices	45%	30%
$\beta$ -Sheets	3%	45%
Glycoforms	Mixture of un-, mono and di-glycosylated forms	Mixture of un-, mono and di-glycosylated forms
Infectivity	No	Yes
Turnover	Hours	Days
Sedimentation properties	Consistent with monomeric species	Multimeric aggregated species

\*PrP denotes prion protein and PrP<sup>sc</sup> the scrapie isoform of PrP<sup>c</sup>



**Figure 2:** Secondary structure and other features of the prion protein. The N-terminal contain an octapeptide repeat region (OR) (green box). The C-terminal domain contains three  $\alpha$ -helices and two  $\beta$ -strands, one disulphide linkage (-S-S-) between cysteine residues 179 and 214, and sites for N-linked glycosylation (brown fork) at residues 181 and 197. A glycosylphosphatidylinositol (GPI) anchor that tethers the prion to the membrane surface is located at the C-terminal. The resistant core of PrP<sup>Sc</sup> is also shown. [CHOs-Carbohydrates]

analyses of recombinant PrP produced in bacteria, included a long, flexible N-terminal tail (residues 23 to 121), three  $\alpha$ -helices, and two small antiparallel  $\beta$ -sheet strands that flanked the first  $\alpha$ -helix [37, 38]. Molecular modeling studies predicted that PrP<sup>c</sup> is a four helix bundle protein containing four regions of secondary structure denoted by H1, H2, H3 and H4. The secondary structure is dominated by  $\alpha$ -helices. Helices 2 and 3 are joined by a disulfide bond which maintains the original conformation. Although a tertiary structure of PrP<sup>Sc</sup> has not yet been identified, current evidence suggests that generation of this isoform involves primary changes in the N-terminal half of the protein, including folding of a portion of the N-terminal tail from residues 90 to 121 (and possibly part of the first  $\alpha$ -helix) into a  $\beta$ -sheet. A key challenge in the field is now to obtain a complete structure of PrP<sup>Sc</sup> by spectroscopic and crystallographic techniques that would allow atomic level specifications of the PrP<sup>c</sup> conformation.

### Expression and physiological function of Prion

PrP<sup>c</sup> is a glycoprotein that is normally attached to the surface of neurons, especially to synaptic membranes, and glial cells of the brain and spinal cord in all mammals *via* a GPI anchor [39]. The expression pattern of PrP<sup>c</sup> is diverse and developmentally regulated in skeletal muscle, kidney, heart, secondary lymphoid organs and the CNS, suggesting a wide-ranging and conserved function of the protein [40, 41, 42, 43, 44]. Its expression in most tissues, together with its evolutionarily conserved amino acid sequence, supports a fundamental role for PrP<sup>c</sup>. As PrP<sup>c</sup> is most abundantly expressed in the brain, the loss of this protein is expected to result in substantial neurobehavioral modifications even though the specific role of PrP<sup>c</sup> in neural function and behaviour is still elusive [45]. The normal physiological role of PrP<sup>c</sup> remains enigmatic, al-

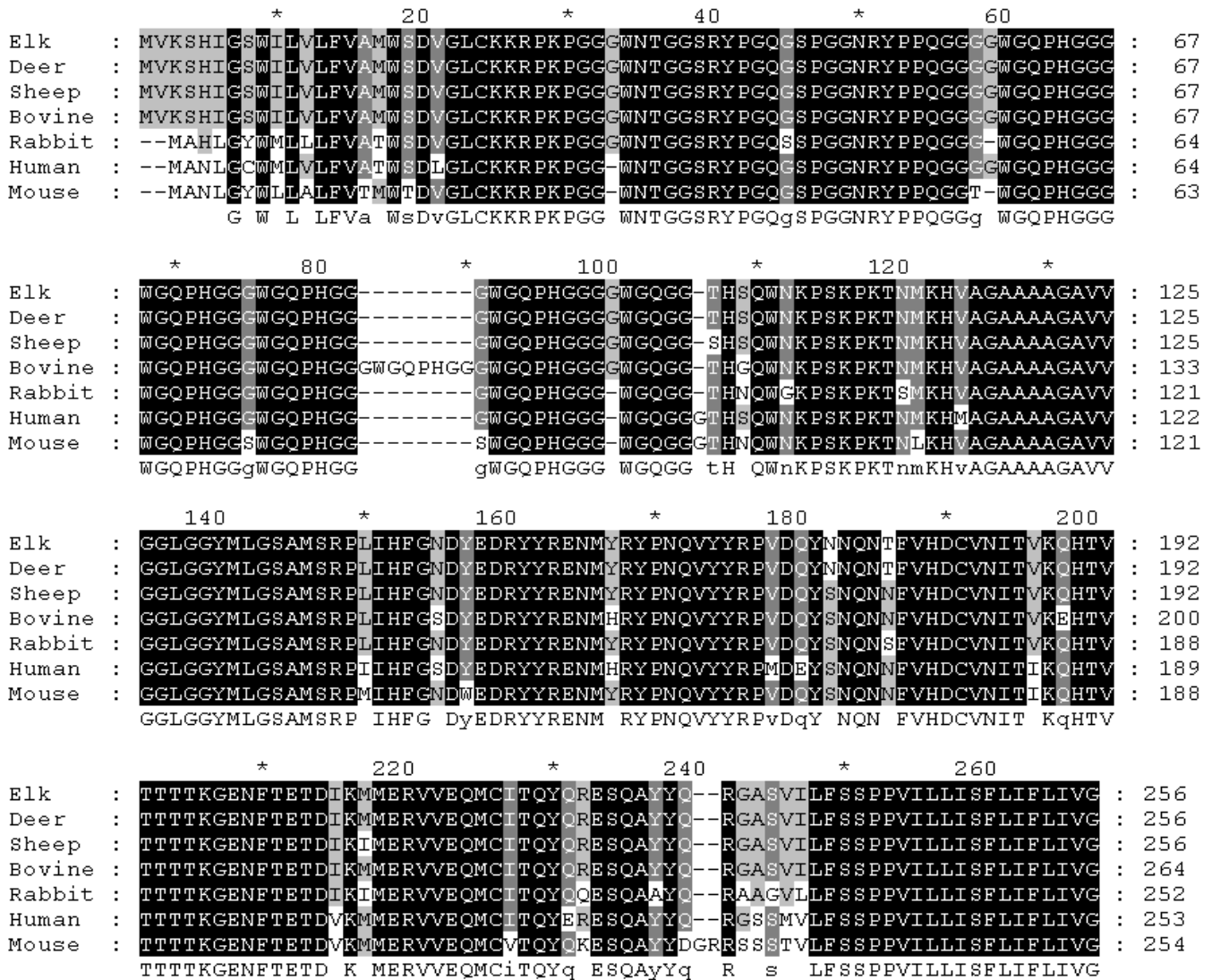
though a number of roles have been proposed due to its remarkable conservation across species. However, studies of PrP-null mice have shown that it is not essential for viability, and various investigators have suggested that it may have a function in sleep regulation [46], cell adhesion [47], or Purkinje cell viability [48]. *In vitro* studies showing that PrP<sup>-/-</sup> neurons are extremely vulnerable to oxidative stress, and that PrP<sup>c</sup> has superoxide dismutase-1 (SOD-1)-like activity [49], have led to the proposal that PrP<sup>c</sup> might have a role in cellular oxidative responses. It has been suggested that ablation of this antioxidant function of PrP<sup>c</sup> might be associated with neurodegeneration in prion disease. There is evidence that the protein binds copper [50, 51] and may play roles in the trafficking of copper ions [52, 53] or protection from oxidative damage in the nervous system [54]. On the other hand, compelling evidence now suggests that PrP<sup>c</sup> has neuroprotective properties. It is upregulated upon ischemic brain damage [55], and in PrP-deficient mice the infarct size is drastically increased [56]. In addition, PrP<sup>c</sup> is able to protect against several pro-apoptotic stimuli [57], as well as long-term renewal in hematopoietic stem cells [58]. Besides its various functions, the amino acid sequences of human, bovine, sheep, deer, elk, rabbit and mouse prion proteins and found a great deal of similarity among them (Fig. 3). This homology across all mammalian prion protein sequences could facilitate the transmission of TSEs between species.

### Prion Replication

The mechanism by which prion infectivity increases is still unknown, since the infectious agents do not contain nucleic acids. Information appears to be stored in the structure of the protein aggregates. Prion aggregates can grow by incorporating new prion protein and inducing a



refolding into the pathological prion form. Growth of prion aggregates, however, is not enough for replication.



**Figure 3:** Amino acid alignment of PrP from seven species. Protein accession numbers in the NCBI database (<http://www.ncbi.nlm.nih.gov/>) are X55882 (Bovine), NM-001009481 (sheep), AAT77255 (European elk), AY639093 (Reindeer), P04156 (Human), NP- 035300 (Mouse), AAC48697 (Rabbit). Amino acid numbering is according to boPrP (60R). The alignment was done using ClustalW and the figure was generated in BioEdit (v.7.0.5).

At some point, one prion must become two prions. The *in vivo* kinetics of elongation and breakage are exponential over time [59] and quite different from the *in vitro* kinetics of nucleation and growth (Fig. 4). Nucleation is a very rare process and can generally be ignored *in vivo*, since disease usually follows introduction of the infectious agent. Even if the disease arises spontaneously, intervention will always be too late to interfere with nucleation. Instead, we have focused on the exponential rate of growth. Since the process appears to be exponential, the post-translational conversion of PrP<sup>c</sup>, or a precursor of PrP<sup>sc</sup>, may be obligatory [31]. A PrP<sup>sc</sup> molecule might combine with a PrP<sup>c</sup> molecule to produce a heterodimer that is subsequently transformed into other PrP<sup>sc</sup> mole-

cules. In the next cycle, two PrP<sup>sc</sup> molecules combine with two PrP<sup>c</sup> molecules, giving rise to four PrP<sup>sc</sup> molecules, which then combine with four more PrP<sup>c</sup> molecules, creating an exponential process. Regarding the thermodynamic and kinetic analysis of prion replication and the replication cycle for the inherited, sporadic, and infectious scenarios, several inferences can be made about the biophysical properties of the normal cellular and disease-causing PrP isoforms.

PrP<sup>sc</sup> replication requires the presence of the gene in the host cell to direct PrP<sup>c</sup> synthesis. Although PrP<sup>sc</sup> replication requires a PrP gene in the host cell, this gene does not need to be carried by the infectious pathogen. Therefore,

PrP<sup>sc</sup> must be more stable than PrP<sup>c</sup>, and a plausible origin for this distinction may be the extensive network of intramolecular interactions between PrP monomers in the

PrP<sup>sc</sup> multimer. Protease resistance could be a corollary of this increased stability and not necessarily the cause of the increased metabolic stability of PrP<sup>sc</sup> [21].

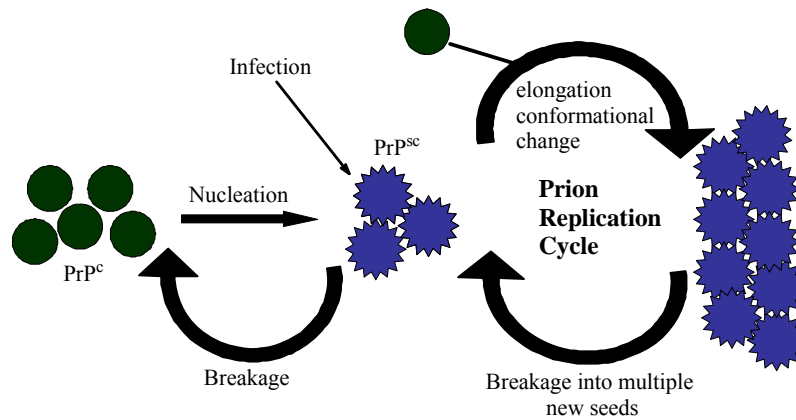


Figure 4. Prion Replication Cycle. Elongation and breakage are exponential overtime.

**Pathogenesis of prion disease**

The pathogenesis of prion disease is also poorly understood. In peripheral infection, prions silently accumulate and replicate in peripheral organs or reservoirs and transit through at least one PrP-positive (PrP<sup>+</sup>) tissue before reaching the CNS. Prions indeed replicate in lymphoid organs during the early stages of infection [60]. Within the lymphoreticular system, follicular dendritic cells (FDCs) are a prominent site of PrP<sup>sc</sup> deposition [61], both in wild-type and nude mice (defective in T-cell responses).

Thus, neuroinvasion typically begins upon ingestion of the TSE agent (Fig. 5). The pathogen must first cross the intestinal epithelium in a process that still remains elusive

amid some data suggesting a mechanism involving transcytosis by microfold (M) cells [62]. Migratory dendritic cells are also known to directly capture antigens within the intestinal lumen and could also be responsible for initial uptake of the TSE agent. Once past the epithelial wall, PrP<sup>sc</sup> appears to be phagocytosed by antigen-displaying cells such as macrophages and dendritic cells. While macrophages appear to serve a more protective role [62], some experimental evidence suggests that dendritic cells deliver the TSE agent to follicular dendritic cells located in the germinal centers of B cell-rich follicles present in Peyer’s patches and other gut-associated lymphoid tissue (GALT) underlying the intestinal epithelium. After incubation in lymphoid tissue such as the GALT and spleen,

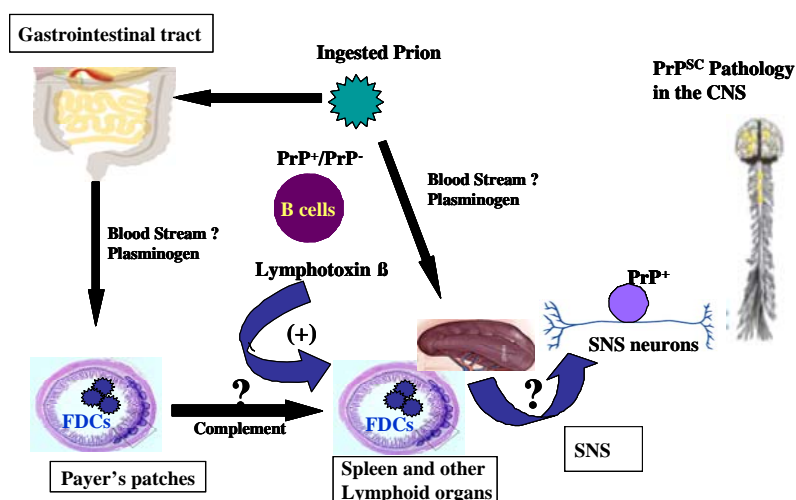


Figure 5. The Route of Prion Neuroinvasion

After absorption through the intestinal epithelium, prion reach the peyer’s patches, via blood constituents (Plasminogen that bind PrPsc). FDCs are infected in the patches and in other lymphoid organs, including the spleen. The prions reach the spleen by a B-cell independent route involving complement factors. Other factors that are required for spreading infection to the CNS are lymphotoxin (stimulus for FDCs), and at least one interposed PrP<sup>+</sup> tissue.



**Table 2. Prion protein interaction with other protein**

Proteins	Functions	Possible binding sites	Identification methods	Reference
GFAP	Cell repair	Cytosol	Ligand blot	[91]
GAG	Biomolecular transport	Cell surface	<i>In vitro</i> affinity binding	[102]
Synapsin 1b	Regulation of neurotransmitter	Synaptic vesicle	Yeast two-hybrid system	[97]
Grb2	Adapter protein	Cytosol	Double hybrid in yeast and co-immunoprecipitation	[97]
Pint 1	Unknown	Unknown	Double hybrid in yeast and co-immunoprecipitation	[97]
Caveolin -1	Aggregation PrP-cav 1 includes signaling cascades	Plasma membrane	<i>In vitro</i> interaction	[105]
Bcl-2	Apoptosis	Membrane of endoplasmic reticulum and mitochondria	Yeast two-hybrid system	[94]
STII	Signal transduction, Activation, neuritogenesis and neuroprotection	Cell surface	<i>In vivo</i> , antibodies	[103]
Laminin	Neuronal plasticity	Cell surface	<i>In vitro</i> binding assay	[101]
Hsp60	Chaperone	Mitochondrial matrix, Cytosol	Yeast two-hybrid system	[95]
APLP1	Regulation of neurite outgrowth	Cell surface	Expression cloning using lambda phage	[93]
NCAM	Adhesion	Caveolae-like domain, Plasma membrane	Chemical cross linking and LC-MS	[100]
Laminin Receptor	Laminin binding	Cell surface	Double hybrid in yeast	[96]
Pli45, Pli110	Unknown	Unknown	Cell binding	
Fyn	Signal transduction via PrP receptor	Cytoplasm	Ligand blot	[91]
			Cross-linking with antibodies	[105]
Plasminogen	Specific binding of PrP <sup>c</sup>	Extracellular matrix, lipid raft	Binding, co-precipitation	[106]

*GFAP* (glial fibrillary acidic protein), *STII* (stress-inducible protein 1), *GAG* (glycosaminoglycans), *APLP1* (amyloid precursor-like protein), *NCAM* (neural cell adhesion molecule), *Bcl-2* (B-cell lymphoma-2), *Hsp60* (Heat shock protein 60), *Pint 1* (PrP interactor 1, uncharacterized), *Grb2* (Growth factor receptor-bound protein 2)

the TSE agent spreads to the central nervous system (CNS) via the enteric nervous system. A new study finds that tunneling nanotubes are important for the intracellular transfer of prion during neuroinvasion [63]. Prions gain access into and between neurons by hijacking tunneling nanotubes. Whereas previous studies have shown that prions can spread by other mechanisms, these are far less efficient. For example, transportation by exosomes requires 5 days of co-culture, compared with 12 hours by nanotubes [64].

Although this is the most likely route for neuroinvasion, it has been suggested that blood may also play a role in the pathogenesis of prion diseases [65]. For example, a number of studies in animals have produced prion infections from inoculation of buffy coats and other blood components

[66, 67]. These observations set the stage for a model of neuroimmune invasion that comprises two phases. The first phase is characterized by the widespread colonization of lymphoreticular organs by a mechanism that is dependent on B lymphocytes, follicular dendritic cells and additional factors such as complement [68]. The second phase requires expression of PrP<sup>c</sup> in peripheral sympathetic nervous system (SNS) nerves and results in prion dissemination in the CNS. Recently, the effectiveness of standard leukoreduction for removing TSE infectivity from whole blood was investigated [69]. The removal of all white cells reduced infectivity by only 42%, suggesting that other blood components, cells or plasma, could be infectious. These data, when considered together with animal studies of prion infectivity in blood, have

highlighted both the significance of PrP<sup>c</sup> and the role of individual blood components in the pathogenesis of TSEs.

### Etiology of prion diseases

Despite a lack of mechanistic knowledge, prion diseases are emerging as one of the best understood neurodegenerative disorders with respect to etiology and pathogenesis. The etiologic agent of TSEs was hypothesized to be a “slow virus” by Sigurdsson [70], in an effort to explain the transmissible nature and prolonged incubation period observed during experimental transmission studies [71]. The “slow virus” was indeed unconventional because it provoked no immune response in the host and was resistant to formalin, UV light and ionizing radiation treatments that normally destroy viruses [72]. Stanley Prusiner pursued this finding and eventually showed that a single protein, dubbed the prion protein, was consistently present in the infectious fraction and that surprisingly, this protein was encoded by a normal chromosomal gene of the host [73]. Sequencing of the prion protein gene (PRNP) was pivotal to understanding prion disease because it showed that mutations in this gene could give rise to CJD, as well as to two other conditions (GSS and FFI) not previously considered to be prion diseases. Subsequent studies by Prusiner et al. [74] demonstrated that a hydrophobic protein was an essential component of the scrapie agent, but no specific polypeptide was identified. To underscore the requirement of a protein for scrapie infectivity, Prusiner [21] introduced the term “prion” in 1982 to describe the proteinaceous infectious particle. In the same year, Prusiner et al. [75] and Bolton et al. [73] reported the purification of scrapie prion and demonstrated its relatively high resistance to PK treatment. Soon after the discovery of prions, a similarity with a normal cellular protein that is a structural component of cell membranes was identified. The neurotoxic form of PrP could represent distinct proteinaceous species, which is intermediate or byproduct of PrP<sup>c</sup>→PrP<sup>sc</sup> conversion pathway. The current understanding suggests a number of possible mechanisms to cell death in TSE diseases, including an increased level of NMDA receptor mediated excitation [76] and activation of Erk1/2 pathway [77]. The generation of TSEs by prions in different species, or in some instances different disease phenotypes in the same species, suggested the existence of different prion “strains” [78]. Although neither the viral nor the protein-only theory has been proven, evidence in favor of the protein-only hypothesis has overwhelmed the virus camp. Hence for better understanding of identity and structural characteristics of the neurotoxic form of PrP, more rigorous efforts are mandatory.

### The Species Barrier

Passage of transmissible spongiform encephalopathies between species is often a stochastic process and may be limited by a species barrier. This barrier represents the decreased efficiency with which TSEs are passed from

one animal to another animal of a different species, as compared to the efficiency of transmission among animals of the same species. In spite of centuries of exposure, sheep scrapie is not known to have been transmitted to humans, but bovine spongiform encephalopathy (BSE) has (fortunately only rarely) done so. The primary determinants of the species barrier are the sequences of the potential prion proteins of the two species. The transmission of prions from one species to another requires prolonged incubation times, compared to intra-species transmission; in some instances, the species barrier seems to confer complete resistance to transmission. Although transmission of a TSE from one species to another might be less efficient than transmission within the same species, once it occurs the TSE may become adapted to the new host. Following adaptation, it can be transmitted more efficiently among members of the new species, and the incubation period becomes shorter and less variable. For example, when scrapie is transmitted experimentally from one species to another, the incubation period is usually longer during the first passage than for subsequent passages within the new species [79]. This is an important topic that is relevant to the debate over the possible transmission of BSE to man.

Species barriers in TSE diseases have been studied experimentally in several laboratory species including mice, rat, hamsters and non-human primates. From studies with transgenic mice, three factors have been identified that contribute to the species barrier: 1) the difference in PrP sequences between the prion donor and recipient, 2) the strain of prion, and 3) the species specificity of protein X, a factor that facilitates PrP<sup>sc</sup> formation by binding to PrP<sup>c</sup>. This factor is probably a protein, hence the provisional designation protein X [80, 81]. Even a single amino acid change in the PrP of the recipient can bring about a radical change in incubation times [82] or even result in resistance to disease. Since these classic studies were performed, several transgenic experiments have confirmed the intimate relationship between the sequence of the prion protein and specificity of transmission [83, 84]. Nonetheless, other studies established that in some contexts, it may not be the sole determinant.

### Prion strains

One of the most puzzling phenomena in prion biology is the existence of prion “strains”. The prion “strain” concept originates from the multiple but distinct transmissible prion diseases that can be passed in the same inbred mouse lines despite their identical PrP-encoding genes. A remarkable feature of prion biology is the strain phenomenon, where prion particles apparently composed of the same protein lead to phenotypically distinct transmissible states. These strains have distinct neuropathologies, and differential rates of disease progression have provided evidence of discrete subtypes of TSEs [85]. The existence of prion strain was first discovered during the transmis-

sion of scrapie among goats [86]. Different prion strains are characterized by length of incubation period of disease, the distribution of CNS vacuolation that they produced, and whether or not prion deposits formed. Different strains frequently associated with PrP<sup>Sc</sup> species show distinct physical features such as susceptibility to PK digestion, stability toward denaturing agents, proportions of di-, mono-, and unglycosylated forms and differential electrophoretic mobility following PK treatment; this reflects diversity at the amino-terminus that results in multiple cleavage sites. These observations taken together begin to build an argument for PrP<sup>Sc</sup> as the information molecule in which prion strain-specific information is encrypted. Deciphering the mechanisms by which PrP<sup>Sc</sup> carries information for prion diversity and passes it on to the nascent prions is a challenge. Whether PrP<sup>Sc</sup> can adopt multiple conformations, each with a distinct incubation time and pattern of PrP<sup>Sc</sup> deposition, remains to be determined [87]. The existence of different prion strains casts a shadow on the protein-only hypothesis. The prion strains and species barriers in prion transmissibility appear to be intricately related, representing two sides of the same coin. While cross-species transmission often results in faithful propagation of the inoculating strain, in some cases it can result in strain switching, as observed in animal studies [88], yeast prion systems [16], and other in vitro experiments [89]. The exact mechanism by which strain switching occurs is still not clear. The existence of the strain phenomenon is not only a scientific challenge, but it also represents a serious risk for public health. The dynamic nature and inter-relations between strains and the potential for the generation of many new prion strains depending on the polymorphisms and the crossing of species barrier is the perfect recipe for the emergence of extremely dangerous new infectious agents [90].

### Interaction of PrP<sup>C</sup> with other proteins

PrP<sup>C</sup> interacts with a large number of proteins. In order to investigate the transformation of PrP<sup>C</sup> into PrP<sup>Sc</sup>, possible interactions with other protein candidates were identified (Table 2). The first interacting proteins identified were a pair of prion protein ligands, Pli45 and Pli110 [91]; the former is a glial fibrillary acidic protein (GFAP), a marker for astrocytes that proliferate in response to TSE infections [92]. Later on, other prion protein ligands, including Pli3, Pli4, Pli5, Pli6, Pli7 and Pli8, were identified using the PrP-alkaline phosphatase screening method [93]. Subsequently, a number of PrP<sup>C</sup>-interacting proteins have been identified using a yeast two-hybrid system: these include the anti-apoptotic protein Bcl-2 [94], the cellular chaperone heat shock protein 60 (Hsp60) [95], the 37 KDa laminin receptor precursor [96], the synaptic vesicle marker synapsin1b, the adaptor protein Grb2 and the prion interaction protein, for which no function has been determined [97]. In addition to Hsp60, other chaperones such as Hsp73 and GroEL can interfere with  $\alpha$  to  $\beta$  conversion of prion, while chaperones such as Hsp70 have no

role in the PrP conversion [98]. PrP<sup>C</sup> also binds to laminin in PC12 cells and rodent primary neurons, and this interaction promotes neurite outgrowth in these cells [99]. A number of additional cell surface proteins interact with PrP<sup>C</sup> including neuronal cell adhesion molecules (NCAMs) [100], apolipoprotein 1 (an amyloid precursor protein that has been implicated in Alzheimer's disease), the 67 KDa laminin receptor [101] and Glycosaminoglycans (GAGS) [102]. The complementary hydropathy, a technique in which cDNA is used to generate a complementary mirror image of the target protein, identified that the 66 KDa stress inducible protein STI-1 binds to PrP<sup>C</sup> and might be involved in neuroprotection [103].

The list of putative PrP<sup>C</sup> binding partners is equally long; some of these cellular cofactors have been suggested to contribute not only to normal PrP<sup>C</sup> function but also to the conformational conversion process [104].

### Relationship to other diseases

Researchers of prion diseases believe that PrP may play important roles in other brain disorders. Ongoing studies may also help determine whether prions consisting of other proteins may play a part in more common neurodegenerative conditions, including Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis (ALS). Clinical studies also showed a striking similarity between TSE and common age-related conditions such as Alzheimer's or Parkinson's disease, both of which show symptoms of progressive dementia and loss of motor control, respectively. Both of these diseases are spontaneous, but they can sometimes be inherited. With regard to CJD and Alzheimer's disease, DeArmond et al. [107] stated that "although there are obvious differences in the etiology and pathogenesis of both sets of disorders, a remarkable number of similarities exist." In both cases, pathogenesis involves an abnormal form of a neuronal membrane protein. One feature that distinguishes the TSE diseases from other neurodegenerative diseases is the glycosphosphatidylinositol membrane anchor on prion protein, the molecule that is corrupted in TSE diseases. The presence of this anchor profoundly affects TSE pathogenesis, which involves major membrane distortions in the brain, and may be a key reason for the greater neurovirulence of TSE prions relative to many other autocatalytic protein aggregates [108]. The abnormal build-up of amyloid- $\beta$  (A $\beta$ ) peptides in the brain is regarded as the causes of Alzheimer's disease. Lauren *et al.* [109] show that the prion protein might mediate the pathogenic effects of A $\beta$  oligomers. Their groups find that, within PrP<sup>C</sup>, aminoacid residues 95–110 are crucial for A $\beta$  binding. Interestingly, the enzyme  $\alpha$ -secretase - which precludes A $\beta$  production by cleaving the A $\beta$  precursor protein APP within the A $\beta$  domain - also cleaves PrP<sup>C</sup> between residues 111 and 112 [110], thus releasing from the membrane the portion of PrP<sup>C</sup> to which A $\beta$  would otherwise bind. For instance,

does PrP<sup>c</sup> mediate the effects of A $\beta$  dimers isolated from brains of people with Alzheimer's disease [111, 112], or of the A $\beta$ \*56 oligomer, which causes memory deficits in mouse models of this disease [113, 114]? Notwithstanding these unresolved questions, the discovery that PrP<sup>c</sup> may be a mediator in the development of Alzheimer's disease is fascinating, not least from a therapeutic perspective. There are still many researches are on the way to elucidate the relationship of prion protein with other neurodegenerative disorders.

### Therapeutic approach to prion disease

Prion diseases are always fatal, often not until months after the outbreak of the disease. According to studies conducted on mice suffering with scrapie, PrP<sup>c</sup> seems to have a protective effect in certain conditions such as stroke. Interestingly, mice that do not produce PrP<sup>c</sup> appear to be completely healthy. This property provided a start-

ing point for a new therapeutic approach that has recently become a focal point: can the production of healthy PrP<sup>c</sup> be switched off in infected animals, thereby depriving the diseased PrP<sup>sc</sup> of its ability to spread? In this way, the chain reaction would be interrupted. Insights into prion research and techniques might also prove to be useful in later-stage treatment regimes for other diseases. Newly designed proteins might be able to convert viral or bacterial proteins into a disabled state. The unique biological features of the prion protein have encouraged investigation of new prophylactic strategies and therapeutics with multiple compounds aimed at a single target, i.e., PrP (Table 3) [115, 116, 117, 118, 119, 120, 121, 122] Over the past three decades, number of drugs has been isolated as active against mammalian prion [123]. These include polysulfate anions, dextrans, heparins, oligonucleotides, cyclic tetrapyrroles, anthrocyclines, porphyrins and diazo dyes.

**Table 3. Targets and potential therapeutic compounds for prion diseases**

Compounds	Examples	Advantage and Disadvantage	Reference
Anionic dyes (amyloid stain)	Congo red	<ul style="list-style-type: none"> <li>•Modest prophylactic activity against scrapie in rodents.</li> <li>•Potential toxicity</li> </ul>	[128]
Sulphated glycans	Pentosan polysulphate Dextran sulphate 500	<ul style="list-style-type: none"> <li>•Effective in protecting rodents against scrapie infection by inhibiting conversion of PrP<sup>c</sup> to PrP<sup>sc</sup>.</li> <li>•Unproven efficacy</li> </ul>	[115]
Polyene antibiotics	Amphotericin B MS-8209 & Filipin	<ul style="list-style-type: none"> <li>•Inhibit membrane PrP<sup>sc</sup> formation.</li> <li>•Toxic</li> </ul>	[122]
Statins	Lovastatin and Squalestan	<ul style="list-style-type: none"> <li>•<i>In vitro</i> activity showing inhibition of PrP<sup>c</sup> to PrP<sup>sc</sup> conversion.</li> </ul>	[121]
Quinacrine, line ,acridines, phenathiazines and related molecules	Quinacrine, quinine, biquinoline, and chlorpromazine	<ul style="list-style-type: none"> <li>•<i>In vitro</i> activity by binding with PrP<sup>c</sup> and blocking conversion into PrP<sup>sc</sup>.</li> <li>•Epatotoxic</li> </ul>	[118]
Cyclic tetrapyrroles	Porphyrins and phthalocyanines	<ul style="list-style-type: none"> <li>•PrP<sup>sc</sup> inhibitor by directly block Cell-free PrP conversion reaction.</li> </ul>	[129]
Growth factors	Basic fibroblast growth factor	<ul style="list-style-type: none"> <li>•Raise the possibility of using neurotrophin therapy to intervene at a relatively late stage to delay neurodegeneration and the development of clinical disease in TSEs.</li> </ul>	[116]

However, none of these has proven to be an effective therapy for sick animals or patients, although there was some success using quinacrine and chlorpromazine *in vitro*. For most of these compounds, the mode of action and targets remain largely unknown. Mostly, two main modes of action for antiprion drugs can be imagined: either in *cis*, or in *trans*. Some compounds are thought to bind directly to PrP<sup>c</sup> or PrP<sup>sc</sup> like Congo Red (CR), Pento-

san Polysulfate (PPS) or Glycosaminoglycans (GAGs) have *cis* action. Other compounds are thought to act in *trans* by affecting PrP<sup>c</sup> or PrP<sup>sc</sup> indirectly. Among these molecules are various lysosomotropic factors including the antimalarial drugs Quinacrine (QC) and Chloroquine. Indeed, the lysosome is a potential site of conversion of PrP<sup>c</sup> to PrP<sup>sc</sup> [124]. In addition, a recent report [28], proposes that QC's antiprion activity is related to its ability

to redistribute cholesterol from the plasma membrane to intracellular compartments, thereby destabilizing membrane domains. Other compounds like Curcumin and Dimethyl sulphoxide (DMSO) have been used for the therapeutic purposes. Curcumin is an efficient inhibitor of PrP<sup>Sc</sup> propagation in RML-infected N2a cells (IC<sub>50</sub> 10 nM), and causes a decrease in detectable protease-resistant PrP in cell free conversion studies (40% decrease with 10 nM curcumin), but it has no effect on disease progression after i.c. prion infection in hamsters regardless of the treatment regime [125]. DMSO treatment decreases the amount of detectable PrP<sup>Sc</sup> in ScN2a culture [126] and reduces the infectivity titre of scrapie-infected brain material [127] in prion propagation systems. It is uncertain whether these findings can be extrapolated to the clinical realm. Furthermore, results suggested that such drugs could adversely impact the health of the patients.

Most described therapeutic strategies target the infectious prion particles; some researchers are seeking methods of repairing the disease-related structural damage to the brain. Others have reported that stem or fetal cell transplants can colonize damaged areas and restore some of the lost tissue in experimental animals. Intercepting disease progression in advance of debilitation and irreversible brain damage, however, could increase treatment options. Many researchers have emphasized the dire need for diagnostic tools that would permit widespread screening for carriers of the infectious agent. Such tools could indicate potential candidates for early treatment with therapeutic compounds that might prevent continued infection.

### Closing remarks

Our understanding of prion diseases has advanced dramatically over the past half-century. It is now clear that these diseases, once thought to be medical and veterinary curiosities, exemplify novel principles of protein structure and transfer of biological information. Some of these principles may have applicability to other neurodegenerative disorders such as Alzheimer's, Parkinson's, and Amyotrophic lateral sclerosis (ALS) diseases, which all involve accumulation of conformationally altered proteins. In addition to their intrinsic scientific and medical significance, prion diseases have also assumed increasing public health importance. The emergence of BSE and variant CJD emphasizes the need for designing more sensitive procedures for detecting prions in food, blood products and donor organs. Although prion diseases currently affect a relatively small number of individuals, it is wise to take steps to prevent potential increases in their incidence. The knowledge gained from the study of prion diseases may provide effective strategies geared toward defining disease etiology and dissecting molecular pathogenesis of more common neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and ALS. Since the risk from inherited disease is present decades before neu-

rologic dysfunction is evident, development of effective therapies is imperative.

A critical issue is whether the phenomenon of propagation of biological information through transmission of protein conformation is exclusively associated with a small group of proteins, like PrP, or a more general process in biology. The discovery of proteins with prion-like behavior in yeast and fungi has provided some insight [130, 131]. Understanding prion multiplication and disease processes will certainly open up new vistas in biochemistry and genetics. As PDs are incurable and fatal, a continuous vigilance is needed to pre-empt outbreaks of prion-induced diseases.

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**Correspondence:**

Seong-Tshool Hong  
Laboratory of Genetics  
Department of Microbiology and Immunology,  
Institute for Medical Science, Chonbuk National University Medical School,  
Chonju, Chonbuk 561-756, South Korea  
Phone/Fax: (+) 82-063-270-3105  
E-mail: [seonghong@chonbuk.ac.kr](mailto:seonghong@chonbuk.ac.kr)

