J. Microbiol. Biotechnol. (2007), **17**(7), 1059–1070

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Prions and Prion Diseases: Fundamentals and Mechanistic Details

RYOU, CHONGSUK*

Sanders Brown Center on Aging, Department of Microbiology, Immunology and Molecular Genetics, College of Medicine, University of Kentucky, 800 Rose St. HSRB-326, Lexington, KY 40536, U.S.A.

Received: March 30, 2007 Accepted: May 4, 2007

Abstract Prion diseases, often called transmissible spongiform encephalopathies (TSEs), are infectious diseases that accompany neurological dysfunctions in many mammalian hosts. Prion diseases include Creutzfeldt-Jakob disease (CJD) in humans, bovine spongiform encephalopathy (BSE, "mad cow disease") in cattle, scrapie in sheep, and chronic wasting disease (CWD) in deer and elks. The cause of these fatal diseases is a proteinaceous pathogen termed prion that lacks functional nucleic acids. As demonstrated in the BSE outbreak and its transmission to humans, the onset of disease is not limited to a certain species but can be transmissible from one host species to another. Such a striking nature of prions has generated huge concerns in public health and attracted serious attention in the scientific communities. To date, the potential transmission of prions to humans *via* foodborne infection and iatrogenic routes has not been alleviated. Rather, the possible transmission of human to human or cervids to human aggravates the terrifying situation across the globe. In this review, basic features about prion diseases including clinical and pathological characteristics, etiology, and transmission of diseases are described. Based on recently accumulated evidences, the molecular and biochemical aspects of prions, with an emphasis on the molecular interactions involved in prion conversion that is critical during prion replication and pathogenesis, are also addressed.

Keywords: Prion disease, prion, PrP^{C}, PrP^{Sc}, species barrier, conformational conversion, prion replication, prion pathogenesis

Prion diseases are perhaps the most mysterious and peculiar diseases in nature. These diseases do not rely on the general dogmas of modern biology, seen in other infectious diseases caused by conventional pathogens such as viruses and bacteria. However, after all of the controversial debates, the idea that an infectious agent devoid of genetic material could replicate, the protein-only hypothesis [76], has become the most widely accepted paradigm for prion propagation and represents a novel pathophysiological phenomenon. Search for convincing evidence supporting an infectious agent other than prions has failed, but generation of infectious artificial (synthetic) prions [55] ultimately provides evidence for the protein-only hypothesis.

Kuru, a prion disease of the Fore people in Papua New Guinea who practiced ritualistic cannibalism, and CJD, a classical human prion disease, were first described several decades ago [20, 43, 50]. Scrapie, a prototypic prion disease found in sheep, has been clinically recognized for centuries [9]. However, none has driven global devastation until BSE, a prion disease in cattle, emerged in the United Kingdom [103] and spread to other European countries. Subsequently, BSE has been reported to occur in 25 countries worldwide and spread to non-European countries including Canada, the United States, and Japan (Office International des Epizooties, http://www.oie.int/eng/info/en_esb.htm).

A massive BSE outbreak in the 1980s and 1990s resulted in huge economic, social, and political trouble. During this period in the United Kingdom, BSE was found in hundreds of thousands of cattle yearly, driving economic damage to astronomical figures. In the mid-1990s, subsequent to the summit of the BSE outbreak, the transmission of BSE to humans who consumed contaminated food further exacerbated the panicky situation in Europe and beyond. It became evident that BSE had crossed the species barrier and created a new variety of human prion disease called variant CJD (vCJD) [106]. BSE-contaminated food for animals also caused feline spongiform encephalopathy (FSE) in both domestic and large captive cats, as well as exotic ungulate encephalopathy (EUE) in a number of ungulate species in zoos [104]. These have led to bans on the import and export of beef and bovine products, which have frequently and continuously resulted in conflicts on trading until today.

Concerns about the prevalence of prion disease all over the globe are not limited to the matters with BSE and its transmission to other species. Prions from other sources, in particular, cervids with CWD, might pose a similar risk to

*Corresponding author
Phone: 859-257-4016; Fax: 859-257-8382;
E-mail: cryou2@email.uky.edu

humans [4]. Independent of BSE, CWD is widespread among free-ranging and captive deer and elk populations in North America and is also found in elk in Korea imported from Canada [96, 107]. Although the etiology and transmission mode are not known, CWD transmits laterally at a highly efficient rate that has never been observed in any other prion diseases. Although there is no compelling evidence to suggest that CWD transmits to humans the ease of lateral transmission of CWD has led to concerns of episode similar to the British BSE outbreak.

The catastrophic event does not always begin with prion diseases of animals. Besides the genetic and sporadic CJD (sCJD) cases, CJD transmitted *via* iatrogenic routes in humans threatens public health. Hundreds of patients have been infected by CJD during surgical procedures using prion-contaminated instruments, organ and tissue transplantations, therapies using hormones, and vaccination [105]. More recently, a few cases of CJD transmission *via* blood transfusion have been reported [58, 72]. These alerting reports raised another layer of concern about human public health because blood was believed to be an inefficient reservoir for prion transmission, and thus prion contamination of blood supply had never been suspected.

Little is known about prion diseases despite the terrifying facts. These fatal neurodegenerative diseases have no reliable preclinical screening tests and effective treatments. To comprehend the threat of prion diseases and to develop diagnostics and therapeutics, it is absolutely required to understand about the basics as well as details of diseases. Specifically, it is important to understand how prions emerge, replicate, and transmit. The most fundamental questions behind the etiology, replication, and transmission of prions are how normal prion proteins become the disease-associated isoforms and what the consequences of this event are during prion pathogenesis. This review deals with those questions and discusses the molecular mechanisms of the conformational conversion of prion proteins (PrP) and the role of cellular factors in the process.

PRIONS

Prions, proteinaceous particles devoid of genetic material, are the infectious pathogens that cause prion diseases [77]. Unlike other infectious agents, prion is unusually resistant to the many chemical and physical treatments commonly used to inactivate other conventional pathogens such as bacteria, fungi, and viruses [77]. Despite intensive treatments of the infected tissue homogenates with UV radiation, heat, and nucleases that damage nucleic acids, the infectivity of the prion agents was not reduced [2, 36, 76]. Recently, a synthetic prion driven from recombinant PrP was shown to be infectious when challenged in laboratory transgenic animals [55].

Table 1. Mammalian orthology and chromosomal location of the PrP gene[a].

Species	Locus symbol	Chromosome
Human	PRNP	20p12.17
Chimpanzee	PRNP	20
Cattle	PRNP	13q17
Sheep	PRNP	13q17-18[b]
Goat	PRNP	13q15
River buffalo	PRNP	14q15
Silver fox	PRNP	14
Cat	PRNP	A3
Dog	PRNP	24
Mink	PRNP	11
Rabbit (European)	PRN-P	NR
Rat	Prnp	3q36
Chinese hamster	PRNP	NR
Syrian hamster	PRN-P	NR
Mouse	prnp	2 (75.2 cM)

[a]The data were retrieved on March 2007 from the Mouse Genome Database, Mouse Genome Informatics, The Jackson Laboratory, Bar Harbor, Maine (http://www.informatics.jax.org).
[b]The position of the PRNP locus of sheep was assigned to chromosome 13q15, 13q1.7 or chromosome 10 in other studies and databases.
NR: not reviewed.

Prion Proteins (PrP): PrP^C and PrP^{Sc}

Cellular PrP, designated PrP^C, is encoded on the gene locus PRNP in the genome of hosts [77]. PrP gene orthologs have been revealed in a number of animal species and their chromosomal loci have been determined in some species (Table 1). The human PrP gene includes a single open reading frame encoding a protein composed of 253 amino acid residues [77]. Mature PrP^C is found as a glycosylated protein of 209 amino acids with a disulfide bond [77]. In animals, the PrP gene itself is widely expressed during development and in the adult, with the highest concentration in, but not limited to, neuronal cells of the brain [17, 62, 70]. At the cellular level, PrP^C is synthesized in the cytoplasm, transported through the ER and Golgi, and displayed on the plasma membrane [39]. In neuronal cells, PrP is concentrated in lipid-rich domains of the plasma membrane, known as caveolae-like domains [39, 77]. The protein moiety of PrP^C localizes in the extracellular matrix but is attached to the plasma membrane by the glycosylphosphatidylinositol anchor [39, 77, 98].

Prions are composed of PrP^{Sc}, a misfolded form of host PrP^C [15, 77]. The primary amino acid sequences and the state of modifications in both isoforms of PrP^{Sc} and PrP^C are identical except for their three-dimensional conformations, which consequently differentiate the biochemical and biophysical properties of both isoforms (Table 2). The secondary structure of PrP^C proposed by nuclear magnetic resonance studies includes three α-helices and two very short β-sheets [44, 83]. Fourier-transformed infrared and circular dichroism spectrometry studies suggest that PrP^C

Table 2. Comparison of PrP^C and PrP^{Sc}.

Properties	PrP^C	PrP^{Sc}
Protein	Normal, cellular protein	Abnormal, disease-associated protein
Infectivity	Not infectious	Infectious; pathogenic
Folding	Dominated by α-helices	β-sheet abundant conformation
Solubility	Easily soluble (hydrophilic)	Insoluble (hydrophobic)
Protease digestion	Sensitive	Partially, but highly resistant
Antibody reactivity	Highly reactive to most anti-PrP antibodies	Not reactive to most anti-PrP antibodies[a]

[a]Availability of PrP^{Sc}-specific antibody is controversial.

is composed of ~40% α-helical and 3% β-sheet conformations, whereas PrP^{Sc} is composed of ~30% α-helical and 40% β-sheet conformations [16, 71]. The increase of β-sheet contents in PrP^{Sc} is due to the major conformational transition of the hydrophobic region (amino acid residues ~90–140) and a portion of the helices in PrP^C molecules. This molecular event makes the molecule hydrophobic and resistant to proteinase K (PK) digestion [65]. The conformationally altered region in PrP^{Sc} is thought to form the repeated stretches of short β-sheets and aggregates to generate multimers of PrP^{Sc}, which can grow further into PrP amyloid fibrils [37]. It is not clear yet if these aggregates are the cause of the cell damage or are simply a byproduct of the underlying disease process.

Roles of PrP in Physiology and Pathogenesis

The expression of PrP^C in a tissue- and time-specific manner along with its subcellular localization on the lipid rafts implicates a certain important biological function of PrP^C. However, independent studies with several different PrP null mouse strains have not found any major impairment as well as distinct physiological and behavioral changes [10, 62, 66, 85, 88]. Nevertheless, many studies using different model systems have proposed several possible roles of PrP^C. Although it remains to confirm the suggested functions of the protein, PrP^C appears to play a role in lymphocyte activation [11], synaptic plasticity [60], neuroprotection [18, 88], signal transduction [67], and metabolic functions related to copper-binding properties [8]. More recently, PrP^C is known to be involved in differentiation and neurogenesis of neural stem cells [99] as well as long-term renewal in hematopoetic stem cells [113].

Although the function of PrP^C in animal physiology is ambiguous, the involvement of PrP^C to the pathogenesis of prion disease is clearly understood. Expression of PrP^C is a prerequisite to demonstrate a susceptibility of prion diseases in the hosts [79]. It was not possible to transmit the disease when prions were inoculated in PrP-deficient mice [10, 62, 88]. These mice did not develop any signs of illness. Furthermore, the incubation time of disease became shortened when prions were transmitted to the transgenic mice, overexpressing PrP^C in the brain at least several times higher than wild-type mice [29, 92].

PRION DISEASES

Prion diseases are also known as TSEs because the diseases are transmissible from one host to another, and manifest a spongiform appearance as a result of the destruction of brain tissue [77]. Prion diseases are caused by the unconventional proteinaceous pathogen, prion. This pathogen manifests the neurological conditions characterized by progressive, but invariably fatal, degeneration in the central nervous system (CNS) during a long incubation period [77]. CJDs in humans, BSE in cattle, scrapie in sheep, and CWD in deer and elks are among the most notable prion diseases (Table 3). Other human prion diseases include kuru, Gerstmann-Straussler-Scheinker (GSS) syndrome, and fatal familial insomnia (FFI). Prion diseases also occur in other animal species such as goat, mink, cats, and exotic ungulates.

Clinical and Pathological Features of Prion Diseases

The unique characteristics of prion diseases are the disease confinement to the CNS, a prolonged incubation time, and a progressive, uniformly fatal course of disease [77]. Human and animal hosts with prion diseases manifest a number of clinical features involved in impaired brain functions [78]. Before the human patients die of prion diseases, the damage in the brain is reflected in such signs as loss of coordination, insomnia, and rapid dementia. Similar symptoms such as irritable demeanor and ataxia are exhibited in animals with prion diseases.

Prion diseases share several neuropathological features including neuronal loss, reactive gliosis, deposition of prion amyloids called plaques, and presence of large vacuoles that generates a spongiform appearance in brain tissue [22]. Unlike the pathology caused by bacterial and viral pathogens, the pathology of TSEs lacks inflammatory responses to prions [1]. Generation of anti-prion antibody is absent in the infected hosts.

Etiology of Prion Diseases

The etiology of prion diseases varies; the source of prions can be either external or internal (Table 3) [77]. The cause of prion diseases is categorized into the spontaneous, inherited, and acquired groups. Acquired prion diseases are caused by ingestion of, or exposure to external prion material

Table 3. The prion disease in humans and animals.

Prion disease[a]	Host	Mechanism of transmission	Year recognized
Kuru	Human (Fore people)	Infectious; Exposure to contaminated human tissues during cannibalistic rituals	1957
sCJD	Human	Spontaneous; Somatic mutations or spontaneous conversion of PrP^{C} to PrP^{Sc}	1920
fCJD	Human	Genetic; Heritable mutations in the PRNP gene	1924
iCJD	Human	Infectious; Exposure to prion-infected surgical equipment, or tissue transplants; blood transfusion; human growth hormone therapy	1974
vCJD	Human	Infectious; Exposure to BSE-infected food including meat	1996
GSS	Human	Genetic; Heritable mutations in the PRNP gene	1936
FFI	Human	Genetic; Heritable mutations in the PRNP gene	1986
sFI	Human	Unknown; Spontaneous; Somatic mutations or spontaneous conversion of PrP^{C} to PrP^{Sc}	1997
Scrapie	Sheep, Goat	Infectious; Ingestion or contact with scrapie-infected animals, tissues and secretions derived from the infected animals, or contaminated environment; possible oral exposure	1732
TME	Mink	Infectious; Ingestion of prion-contaminated feed	1947
BSE	Cattle	Infectious; Ingestion of prion-contaminated feed	1986
FSE	Cat, Ocelot, Asiatic golden cat, Tiger, Lion, Puma, Cheetah	Infectious; Ingestion of BSE-contaminated feed	1990
CWD	Deer, Elk, Moose	Unknown; Infectious; Spontaneous; Contact with or ingestion of prions	1967
EUE	Kudu, Oryx, Nyala, Eland, Gemsbok	Infectious; Foodborne exposure to BSE-infected tissue	1986

[a]sCJD (sporadic Creutzfelt-Jakob disease), fCJD (familial Creutzfelt-Jakob disease), iCJD (iatrogenic Creutzfelt-Jakob disease), vCJD (variant Creutzfelt-Jakob disease), GSS (Gersmann-Straussler-Scheinker syndrome), FFI (fatal familial insomnia), sFI (sporadic fatal insomnia), TME (transmissible mink encephalopathy), BSE (bovine spongiform encephalopathy), FSE (feline spongiform encephalopathy), EUE (exotic ungulate encephalopathy).

derived from either homologous or heterologous sources [78]. Infection of the externally derived prion (PrP^{Sc}) induces conformational changes of the natively expressed normal PrP^{C}, leading to the development of many different prion diseases such as vCJD, kuru, BSE, FSE, EUE, and iatrogenic CJD. Inherited and spontaneous prion diseases are caused by internal factors such as germ-line or somatic mutations on the PRNP gene and a spontaneous change in the conformation of PrP^{C} to PrP^{Sc} [33]. When there are mutations present in the PRNP gene, the mutated normal PrP^{C} is more prone to be refolded into the disease-associated rogue PrP^{Sc}, leading to development of familial CJD, GSS, and FFI. The spontaneous internal change implies a random protein-misfolding event, leading to the development of sCJD. Regardless of their etiology, all prions are infectious and can be transmitted from one individual host to another.

Prion Strain

Prions have strains that have been maintained through serial transmissions. Different prion strains are characterized by difference in incubation time, distribution of prion deposits in the brain, and clinical symptoms *in vivo* [93]. Prion strains are also distinguished by different biochemical parameters such as glycosylated patterns, denaturation profiles, and molecular sizes of PK-resistant abnormal PrP^{Sc} [93]. The diversity of prion strains appears to be determined by the conformation of PrP^{Sc}, even though prions lack great diversity in amino acid sequences across the different strains.

Species Barrier in Prion Transmission

An important feature of prion diseases is that prions generally transmit from one species to another much less efficiently than within the same species [93]. In rare cases, prion transmission between different species is possible only after prolonged incubation times. For instance, in a laboratory setting, mouse prions readily infect mice, but not hamsters or transgenic mice expressing hamster PrP [48, 92]. Inefficient transmission to a different species is referred to as the species barrier. Usually, a species barrier prevents the prion transmission in a host species infected by heterologous prions. However, the onset of the disease is not limited to a certain species and can be transmissible from one host species to another by overcoming the species barrier. In the cases of vCJD, humans might become infected with prions after ingesting meat from cattle infected by BSE [106].

Spread of Prion within a Host

Although the CNS is the major target for prions, a small amount of prions are replicated and accumulated in the secondary lymphoid organs and tissues in the periphery

such as Peyer's patches, lymph nods, and spleen, long before the PrP^{Sc} appears in the brain [31, 49]. In natural or artificial exposure in which hosts received prions by oral route ingestion or peritoneal inoculation, the prions are taken up through the gastrointestinal tract, migrate to the lymphoreticular system, and propagate there [1]. A variety of specialized cells including follicular dendritic cells, M cells, and B lymphocytes are known to be involved in the uptake, migration, and propagation of prions. However, propagation of prions in the lymphoreticular system does not appear to be essential for neuroinvasion. Instead, the prion migrates to the brain along peripheral nerves associated with the affected peripheral organs and tissues [80]. Once the prion invades the CNS, it appears to destroy nerve cells and cause neurodegeneration in the brain.

MECHANISM OF PRION PATHOGENESIS, REPLICATION, AND CONVERSION

Prions manifest the diseases through as yet unknown pathogenic mechanisms. However, it appears that prion propagation based on conformational conversion is closely linked with prion pathogenesis.

Prion Pathogenesis

Prion pathogenesis remains enigmatic. Every prion disease shares a common molecular mechanistic feature in that PrP^{C} undergoes conformational conversion to the disease-associated PrP^{Sc} in the host cells during pathogenesis [77]. Prion pathogenesis not only requires expression of PrP^{C} but occurs proportionally to the level of expressed PrP^{C}.

As a result of prion conversion triggered by prion infection, mutations on the PrP gene, or unknown reasons, a misfolded PrP^{Sc} is generated, progressively accumulated, and deposited as amyloids in the brain [23]. Although prion pathogenesis is associated with PrP^{Sc} accumulation, it is not clear whether the gain of toxic PrP^{Sc} function is responsible for the downstream events that cause pathologic phenotypes such as neurotoxicity, cellular stress, cell death, and neurodegeneration. Some recent studies suggest that PrP^{Sc} can cause pathologic effects directly to neuronal cells or indirectly through glial cells by triggering signal transduction cascades for apoptosis [40, 63]. Alternatively, the loss of physiological PrP^{C} function due to the conformational conversion can result in disruption of signal transduction for survival or protection in which PrP^{C} physiologically participates [7, 54]. Prion pathogenesis is also thought to be facilitated not by PrP^{Sc} itself or loss of PrP^{C}, but by an intermediate prion conformer formed during PrP^{C} conversion to PrP^{Sc} [14].

Conversion, Replication, and Aggregation of Prion

During prion pathogenesis, PrP^{C} undergoes conformational alteration and nascent PrP^{Sc} is generated. Two different models have been suggested for prion conversion and aggregation. The nucleated polymerization model suggested by Jarrett and Lansbury [45] represents a hypothetical process of prion aggregation, where multimeric PrP^{Sc} acts as a polymerization nucleus. In this model, the PrP^{Sc} multimer rapidly stabilizes the many monomeric PrP molecules by incorporating into the PrP^{Sc} oligomers. The template-assisted conversion model proposed by Prusiner [75] postulates that the abnormally conformed monomeric PrP^{Sc} serves as a template to convert PrP^{C} to the aberrant conformation by interaction between these two molecules. In this model, prion aggregate forms afterwards from the converted PrP^{Sc}. Both models quite differ in the role of PrP^{Sc} aggregates during prion propagation. PrP^{Sc} aggregates are not considered essential for the prion conversion processes in the template-assisted conversion model, but they are indispensable for prion propagation in the nucleated polymerization model.

Evidence for an Auxiliary Factor in Prion Conversion

Based on the template-assisted conversion model, PrP^{Sc} recruits and converts PrP^{C} to form nascent PrP^{Sc} [77]. Several lines of direct and indirect evidence suggest that a cofactor is involved in the conversion process of PrP^{C} to PrP^{Sc} [19, 55, 87, 100]. In cell-based or animal systems, conversion of PrP^{C} to PrP^{Sc} has been successfully reproduced [79, 81]. PrP^{Sc} was propagated and prion infectivity was maintained by prion replication. To date, molecular conversion in various cell-free systems failed to reproduce the proposed prion conversion process. Conversion of PrP^{C} to PrP^{Sc} seems to be difficult in most cell-free reactions unless it is assessed under the condition that many other molecules besides PrP are also present. Using brain homogenate of normal and scrapie-sick hamsters, the group of scientists independently led by Soto and Supattapone amplified PK-resistant PrP^{Sc} *in vitro* [59, 87]. Recently, Soto's group showed that *in vitro* propagation of PrP^{Sc} resulted in amplification of prion infectivity [12]. This indicates that other molecule(s) such as a host cofactor(s) is crucial for PrP^{C} conversion to occur.

Synthetic prion studies [55] by Prusiner and his colleagues suggested the requirement of a cofactor(s) or a chaperon molecule(s) for the robust generation of infectious artificial prions. Although synthetic prions were infectious in transgenic mice, transmission of disease was not rapid because, presumably, synthetic prions with a proper infectious conformation formed inefficiently, making the infectivity of this artificial prion tremendously low. This argues that generation of synthetic prion in the absence of an auxiliary factor caused the inefficient generation of infectious prions.

Additionally, the studies using transgenic animals also suggested an auxiliary factor that interacts with PrP^{C} and enhances conformational alteration of PrP^{C} to PrP^{Sc} [100]. In prion transmission studies using human prions in transgenic mice expressing human and chimeric mouse-

human PrP, Telling *et al.* [100] found that efficient prion transmission was dependent on homotypic interaction between PrP^C and a hypothetical macromolecular cofactor, provisionally designated protein X. According to the proposed model, the conversion requires protein X that interacts with PrP^C. Thus, it seems that PrP^C and protein X form an initial binary complex where subtle conformational alteration of PrP^C is enhanced, and then the conformational conversion of PrP^C to PrP^{Sc} occurs in a ternary complex composed of PrP^{Sc} and the binary complex [100]. As a result, nascent PrP^{Sc} is generated from PrP^C and may participate in a conversion process to generate additional PrP^{Sc}.

Although the identity of protein X has not been fully revealed, additional lines of evidence such as molecular biological studies of PrP^{Sc} formation in scrapie-infected neuroblastoma cells transfected with chimeric, mutated, and truncated PrP genes have suggested that cofactors play an important role in the conversion process from PrP^C to PrP^{Sc}, and revealed characteristics of the interaction between PrP^C and the hypothetical cofactor [46, 84, 114].

Putative Cofactor Proteins Interacting with PrP

Although no molecule has been found to be a legitimate cofactor of PrP, several proteins have been identified to interact with PrP^C and/or PrP^{Sc}, and the list of molecules that interact with PrP is growing (Table 4) [6, 13, 25, 30, 32, 38, 47, 53, 64, 67–69, 73, 82, 86, 91, 97, 110, 111]. The native localization of proteins identified to interact with PrP is found throughout every cellular compartment. Because prion conversion appears to occur at the caveolae-like domains of the plasma membrane, it is likely that the cofactor involved in prion conversion would be a protein that resides or is readily available in, lipid-rich rafts. Thus, any protein known to interact with PrP but not available in prion conversion sites is thought to be irrelevant to prion conversion. However, it may have relevance to a biological phenomenon other than prion conversion. Some

Table 4. PrP-binding proteins.

Proteins[a]	Identification method	Cellular localization	References
GFAP	Ligand blot	Cytosol	[69]
Glyscosaminoglycan (heparin sulfate)	*In vitro* affinity binding	Extracellular matrix	[13, 32]
Bcl-2	Yeast two-hybrid system	Membranes of endoplasmic reticulum and mitochondria	[53]
Hsp60	Yeast two-hybrid system	Mitochondrial matrix, cytosol	[25]
LRP/LR	Yeast two-hybrid system	Plasma membrane	[82]
p66/STI1	Immunoprotein chemistry, 2-D gel proteomics	Cytosol, nucleus, plasma membrane	[64, 111]
Nrf2, APLP1	Expression cloning using lambda phage	Nucleus, unknown	[110]
Dystroglycan complex	CoIP[b]	Plasma membrane, endosomal compartments	[47]
Caveolin-1, Fyn	Antibody-mediated cross-linking and CoIP[b]	Plasma membrane (lipid rafts), cytoplasm	[67]
PLG	CoIP[b]	Secreted, extracellular matrix, lipid rafts	[30, 95]
Laminin	*In vitro* binding assay	Secreted, extracellular matrix	[38]
NCAM	Chemical crosslinking, CoIP[b], and LC-MS/MS[c]	Plasma membrane	[91]
Synapsin 1b, Grb2, Pint1	Yeast two-hybrid system	Synaptic vesicle, cytosol, unknown	[97]
PLG & tPA	Enzymatic assay	Secreted, extracellular matrix, lipid rafts	[26]
PLG & plasmin	*In vitro* cleavage	Secreted, extracellular matrix, lipid rafts	[52]
Angiostatin & PLG	Screening of a cDNA expression phage display library	Secreted, extracellular matrix, lipid rafts	[86]
NRAGE	Yeast two-hybrid system	Cytosol	[6]
Tubulin	Crosslinking, cosedimentation	Cytosol	[68]
Protein complex[d]	Crosslinking, CoIP[b], LC-MS/MS[c]	Cytosol	[73]

[a]GFAP (glial fibrillary acidic protein), LRP/LR (laminin receptor precursor/laminin receptor), STI1 (stress-inducible protein 1), Nrf2 (NF-E2-related factor 2), APLP1 (amyloid precursor-like protein 1), PLG (plasminogen), NCAM (neural cell adhesion molecule), Pint1 (PrP interactor 1, uncharacterized), tPA (tissue type plasminogen activator), NRAGE (neurotrophin receptor interacting MAGE homolog).

[b]CoIP: coimmunoprecipitation.

[c]LC-MS/MS: liquid chromatography-mass spec/mass spec.

[d]The protein complex composed of ribosomal protein P0, β-actin, CNPase (2'-3'-cyclic nuclotide 3'-phosphodiesterase), creatine kinase B, neuron-specific enolase, clathrin heavy-chain 1, α-spectrin, Na+/K+ ATPase α3 subunit, GFAP, PLP (proteolipid protein), STXBP1 (syntaxin-binding protein 1), ζ-14-3-3, and BASP1 (brain abundant signal protein 1).

putative proteins investigated toward their functions in prion conversion are selected and further discussed below.

Neural Cell Adhesion Molecule

Using an *in situ* formaldehyde crosslinking method in mouse neuroblastoma (N2a) and prion-infected N2a (ScN2a) cells, the neural cell adhesion molecule (NCAM) was identified as a PrP^C interacting protein [91]. Although *in vitro* binding studies demonstrated specific interaction and determined binding regions in both PrP^C and NCAM molecules, prion challenge in NCAM-deficient mice did not yield changes in the onset and pathology of disease, suggesting that NCAM is not involved in PrP^{Sc} replication [91]. Instead, interaction between PrP^C and NCAM appear to facilitate neurite outgrowth during development of the nervous system by transducing signals through a pathway associated with fyn kinase [90].

Laminin Receptor Precursor Protein and Glycoaminoglycan

Among many putative cofactors, laminin receptor precursor (LRP/LR) and glycosaminoglycan (heparan sulfate) were studied most extensively [5, 13, 24, 32, 35, 41, 42, 56, 94, 101, 102, 108]. Employing the yeast two-hybrid system, LRP/LR was identified as an interacting protein for PrP^C [82]. Cell binding and internalization studies demonstrated that LRP/LR directly interacted with PrP^C *in vitro* [35] and mediated PrP^{Sc} uptake to the cells [34]. Moreover, the studies employing RNA interference and antibody against LRP/LR in the cultured cell lines infected with prions revealed that PrP^{Sc} propagation required LRP/LR, suggesting that LRP/LR participates in PrP^{Sc} formation [56]. Similar studies with heparin sulfate, which has long been known to interact with both PrP^C and PrP^{Sc}, demonstrated that this glycosaminoglycan served as a cell-surface receptor for prions and participated in PrP^{Sc} biosynthesis, suggesting a role in PrP^{Sc} propagation [5, 13, 24, 32, 41, 94, 102, 108]. However, the role of both LRP/LR and glycosaminoglycan in prion pathogenesis has not been evaluated in animal model systems. Development of knock-out or transgenic animals of these molecules and prion bioassay in these animals will help to address the functional relevance of these molecules to prion pathogenesis [57].

Kringle Domain-Containing Proteins

Among numerous PrP-interacting molecules, a group of molecules that harbor kringle domains are notable. This group of proteins includes plasminogen (Plg) and its internal proteolytic fragments such as plasmin (Pln) and angiostatins (AS), hepatocyte growth factor, lipoprotein (a), plasminogen activators (PA), and others. To date, Plg, Pln, AS K1-3 (AS composed of the first three kringle domains), and tissue type PA (tPA), but not urokinase type PA (uPA), are known to interact with PrP [21, 26–28, 30, 51, 52, 61, 74, 86, 95]. All these interactions seem to be mediated by lysine residues in PrP and their binding site in kringle domains of proteins.

Although Plg was found to interact exclusively with PrP^{Sc} in blood and brain tissue homogenates of prion-infected humans and animals [30, 61], many studies suggested that it interacted with both PrP^{Sc} and PrP^C depending on binding conditions, or more likely interacted with PrP^C [21, 26–28, 51, 52, 74, 86, 95]. Independently, screening of a phage display cDNA expression library for identification of a PrP^C ligand revealed that α-helical recombinant mouse PrP, which represents the folded state of PrP^C, interacted with kringle domains of Plg [86].

High-affinity interaction between PrP^C and Plg was shown in several *in vitro* studies where binding property, binding affinity, biophysical alteration, and biochemical consequence of the interaction were assessed [21, 26–28, 51, 52, 74, 86]. Because Pln and AS are internal proteolytic fragments of Plg, which contain kringle domains, the mechanism of their interaction with PrP^C appeared to be virtually identical to that of Plg. AS K1-3 interacted with PrP^C in a positive cooperative manner [86]. Interaction of PrP^C with Pln resulted in cleavage of PrP^C at lysine residue 110, generating N-terminally truncated PrP molecules (termed C1) *in vitro* [52, 74]. However, generation of the C1 fragment of PrP^C by Pln was not confirmed in Plg null mice [3]. In pathological conditions, the specific activity of Pln was lower, but the Plg concentration was higher in CJD patients than in control groups [112].

Interestingly, *in vitro* activation of Plg to Pln was stimulated by interaction of PrP^C with another kringle domain-containing protein, tPA [26, 28]. Activity of tPA was greatly increased in the presence of PrP^C, and cleavage of PrP^C by Pln occurred in a tPA-dependent manner [26, 28]. The N1 fragment of PrP^C (PrP23-110), liberated by Pln, showed interaction with tPA and stimulated Plg activation [27, 28, 74]. *In vitro* binding and chromogenic assays demonstrated that lysine clusters exposed at both ends of the N1 fragment of PrP^C played an essential role in Plg activation. Interaction between PrP^C and tPA occurred through the independent binding sites in PrP^C and the second kringle domain in tPA [26].

The pathophysiological relevance of PrP^C interaction with Plg and tPA was not clearly understood, even after prion challenge in Plg- and tPA-deficient mice [89, 109]. When these mice were intracerebrally infected with prions, anticipated alterations in survival time, PrP^{Sc} accumulation, and pathology were not obviously different from the controls [89, 109]. The average incubation time was either shortened or unchanged. Instead, Plg-deficient mice intraperitoneally infected with prions demonstrated minimal prolongation of incubation time, little accumulation of PrP^{Sc}, and less severe pathology [89]. Thus, it seems that Plg may play a role in peripheral prion pathogenesis. More comprehensive

and detailed studies are required to understand the role of kringle domain-containing proteins in prion pathogenesis.

During the last few decades, great deals of prion research have been made toward our understanding about prions and prion diseases. A class of devastating diseases believed to be caused by viruses is now known to be caused by proteinaceous prions. Unusual properties of prions were characterized and the biochemical composition of prions was revealed. The generation of infectious artificial prions and propagation of prions *in vitro* provided the ultimate evidence for a protein-only hypothesis. However, unclearness in mechanistic details about the prion conversion, replication, and pathogenesis still remains as a major obstacle for the better understanding of prion diseases.

Although many lines of evidence strongly argue that cellular cofactors besides PrP molecules are necessary for prion conversion, the identity of the cofactors has resisted to be revealed. Further studies on the identification of PrP-interacting proteins and functional ascertainment of the identified PrP-interacting proteins should continue. Those efforts will ultimately provide evidence to explain many phenomena involved in prion diseases. In addition to the perspectives related to pathophysiology, investigations dealing with protein-protein interactions between PrP and its interacting proteins will be important to elucidate a fundamental molecular event required for a role of PrP^C under physiological states. Because the physiological function of PrP^C may also be involved with prion pathogenesis by the loss of function mechanism, it is of great interest to elucidate the cellular response and corresponding signaling pathways mediated by the interaction. This may provide an important lead for developing diagnostic markers for prion diseases. Lastly, understanding of the interactions of PrP with its interacting proteins in both physiological and disease states will result in a major impact on identifying a new target for prion therapy.

Acknowledgments

The author thanks Charles Mays and Younghwan Kim for assistance in preparation of the manuscript. The author's group is supported by the University of Kentucky. Because of the growing number of literatures, the author has primarily cited more recent references in the present review. The author had to use previous review articles and book chapters for citation of many important discoveries that could not be cited in the text and references.

REFERENCES

1. Aguzzi, A. and M. Heikenwalder. 2005. Prions, cytokines, and chemokines: A meeting in lymphoid organs. *Immunity* **22:** 145–154.
2. Alper, T., W. A. Cramp, D. A. Haig, and M. C. Clarke. 1967. Does the agent of scrapie replicate without nucleic acid? *Nature* **214:** 764–766.
3. Barnewitz, K., M. Maringer, G. Mitteregger, A. Giese, U. Bertsch, and H. A. Kretzschmar. 2006. Unaltered prion protein cleavage in plasminogen-deficient mice. *Neuroreport* **17:** 527–530.
4. Belay, E., R. Maddox, E. Williams, M. Miller, P. Gambetti, and L. Schonberger. 2004. Chronic wasting disease and potential transmission to humans. *Emerg. Infect. Dis.* **10:** 977–984.
5. Ben-Zaken, O., S. Tzaban, Y. Tal, L. Horonchik, J. D. Esko, I. Vlodavsky, and A. Taraboulos. 2003. Cellular heparan sulfate participates in the metabolism of prions. *J. Biol. Chem.* **278:** 40041–40049.
6. Bragason, B. T. and A. Palsdottir. 2005. Interaction of PrP with NRAGE, a protein involved in neuronal apoptosis. *Mol. Cell. Neurosci.* **29:** 232–244.
7. Brandner, S., S. Isenmann, A. Raeber, M. Fischer, A. Sailer, Y. Kobayashi, S. Marino, C. Weissmann, and A. Aguzzi. 1996. Normal host prion protein necessary for scrapie-induced neurotoxicity. *Nature* **379:** 339–343.
8. Brown, L. R. and D. A. Harris. 2002. The prion protein and copper: What is the connection?, pp. 103–113. *In* E. J. Massaro (ed.), *Handbook of Copper Pharmacology and Toxicology.* Humana Press, Totowa, NJ.
9. Brown, P. and R. Bradley. 1998. 1755 and all that: A historical primer of transmissible spongiform encephalopathy. *Br. Med. J.* **317:** 1688–1692.
10. Bueler, H., M. Fisher, Y. Lang, H. Bluethmann, H.-P. Lipp, S. J. DeArmond, S. B. Prusiner, M. Aguet, and C. Weissmann. 1992. Normal development and behavior of mice lacking the neuronal cell-surface PrP protein. *Nature* **356:** 577–582.
11. Cashman, N. R., R. Loertscher, J. Nalbantoglu, I. Shaw, R. J. Kascsak, D. C. Bolton, and P. E. Bendheim. 1990. Cellular isoform of the scrapie agent protein participates in lymphocyte activation. *Cell* **61:** 185–192.
12. Castilla, J., P. Saa, C. Hetz, and C. Soto. 2005. *In vitro* generation of infectious scrapie prions. *Cell* **121:** 195–206.
13. Caughey, B., K. Brown, G. J. Raymond, G. E. Katzenstein, and W. Thresher. 1994. Binding of the protease-sensitive form of PrP (prion protein) to sulfated glycosaminoglycan and Congo red. *J. Virol.* **68:** 2135–2141.
14. Caughey, B. and P. T. Lansbury Jr. 2003. Protofibrils, pores, fibrils, and neurodegeneration: Separating the responsible protein aggregates from the innocent bystanders. *Annu. Rev. Neurosci.* **26:** 267–298.
15. Caughey, B. and G. J. Raymond. 1991. The scrapie-associated form of PrP is made from a cell surface precursor that is both protease- and phospholipase-sensitive. *J. Biol. Chem.* **266:** 18217–18223.
16. Caughey, B. W., A. Dong, K. S. Bhat, D. Ernst, S. F. Hayes, and W. S. Caughey. 1991. Secondary structure analysis of the scrapie-associated protein PrP 27-30 in water by infrared spectroscopy. *Biochemistry* **30:** 7672–7680.
17. Chesebro, B., R. Race, K. Wehrly, J. Nishio, M. Bloom, D. Lechner, S. Bergstrom, K. Robbins, L. Mayer, J. M. Keith,

C. Garon, and A. Haase. 1985. Identification of scrapie prion protein-specific mRNA in scrapie-infected and uninfected brain. *Nature* **315:** 331–333.

18. Chiarini, L. B., A. R. Freitas, S. M. Zanata, R. R. Brentani, V. R. Martins, and R. Linden. 2002. Cellular prion protein transduces neuroprotective signals. *EMBO J.* **21:** 3317–3326.
19. Cohen, F. E., K.-M. Pan, Z. Huang, M. Baldwin, R. J. Fletterick, and S. B. Prusiner. 1994. Structural clues to prion replication. *Science* **264:** 530–531.
20. Creutzfeldt, H. G. 1920. Uber eine eigenartige herdformige Erkrankung des Zentralnervensystems. *Z. Gesamte Neurol. Psychiatrie* **57:** 1–18.
21. Cuccioloni, M., M. Amici, A. M. Eleuteri, M. Biagetti, S. Barocci, and M. Angeletti. 2005. Binding of recombinant PrP^C to human plasminogen: Kinetic and thermodynamic study using a resonant mirror biosensor. *Proteins* **58:** 728–734.
22. DeArmond, S. J., J. W. Ironside, E. Bouzamondo-Bernstein, D. Peretz, and J. R. Fraser. 2004. Neuropathology of prion diseases, pp. 777–856. *In* S. B. Prusiner (ed.), *Prion Biology and Diseases*, 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
23. DeArmond, S. J., M. P. McKinley, R. A. Barry, M. B. Braunfeld, J. R. McColloch, and S. B. Prusiner. 1985. Identification of prion amyloid filaments in scrapie-infected brain. *Cell* **41:** 221–235.
24. Deleault, N. R., J. C. Geoghegan, K. Nishina, R. Kascsak, R. A. Williamson, and S. Supattapone. 2005. Protease-resistant prion protein amplification reconstituted with partially purified substrates and synthetic polyanions. *J. Biol. Chem.* **280:** 26873–26879.
25. Edenhofer, F., R. Rieger, M. Famulok, W. Wendler, S. Weiss, and E.-L. Winnacker. 1996. Prion protein PrP^C interacts with molecular chaperones of the Hsp60 family. *J. Virol.* **70:** 4724–4728.
26. Ellis, V., M. Daniels, R. Misra, and D. R. Brown. 2002. Plasminogen activation is stimulated by prion protein and regulated in a copper-dependent manner. *Biochemistry* **41:** 6891–6896.
27. Epple, G., K. Langfeld, M. Baier, H.-G. Holzhütter, W.-D. Schleuning, E. Köttgen, R. Gessner, and M. Praus. 2004. Both lysine-clusters of the NH_2-terminal prion-protein fragment PrP23-110 are essential for t-PA mediated plasminogen activation. *Thromb. Haemost.* **91:** 465–472.
28. Epple, G., W.-D. Schleuning, G. Kettelgerdes, E. Köttgen, R. Gessner, and M. Praus. 2004. Prion protein stimulates tissue-type plasminogen activator-mediated plasmin generation *via* a lysine-binding site on kringle 2. *J. Thromb. Haemost.* **2:** 962–968.
29. Fischer, M., T. Rulicke, A. Raeber, A. Sailer, M. Moser, B. Oesch, S. Brandner, A. Aguzzi, and C. Weissmann. 1996. Prion protein (PrP) with amino-proximal deletions restoring susceptibility of PrP knockout mice to scrapie. *EMBO J.* **15:** 1255–1264.
30. Fischer, M. B., C. Roeckl, P. Parizek, H. P. Schwarz, and A. Aguzzi. 2000. Binding of disease-associated prion protein to plasminogen. *Nature* **408:** 479–483.
31. Fraser, H. and A. G. Dickinson. 1970. Pathogenesis of scrapie in the mouse: The role of the spleen. *Nature* **226:** 462–463.
32. Gabizon, R., Z. Meiner, M. Halimi, and S. A. Ben-Sasson. 1993. Heparin-like molecules bind differentially to prion-proteins and change their intracellular metabolic fate. *J. Cell. Physiol.* **157:** 319–325.
33. Gambetti, P., Q. Kong, W. Zou, P. Parchi, and S. G. Chen. 2003. Sporadic and familial CJD: Classification and characterisation. *Br. Med. Bull.* **66:** 213–239.
34. Gauczynski, S., D. Nikles, S. El-Gogo, D. Papy-Garcia, C. Rey, S. Alban, D. Barritault, C. I. Lasmezas, and S. Weiss. 2006. The 37-kDa/67-kDa laminin receptor acts as a receptor for infectious prions and is inhibited by polysulfated glycanes. *J. Inf. Dis.* **194:** 702–709.
35. Gauczynski, S., J. M. Peyrin, S. Haik, C. Leucht, C. Hundt, R. Rieger, S. Krasemann, J. P. Deslys, D. Dormont, C. I. Lasmezas, and S. Weiss. 2001. The 37-kDa/67-kDa laminin receptor acts as the cell-surface receptor for the cellular prion protein. *EMBO J.* **20:** 5863–5875.
36. Gibbs, C. J. Jr., D. C. Gajdusek, and R. Latarjet. 1978. Unusual resistance to ionizing radiation of the viruses of kuru, Creutzfeldt-Jakob disease. *Proc. Natl. Acad. Sci. USA* **75:** 6268–6270.
37. Govaerts, C., H. Wille, S. B. Prusiner, and F. E. Cohen. 2004. Evidence for assembly of prions with left-handed β-helices into trimers. *Proc. Natl. Acad. Sci. USA* **101:** 8342–8347.
38. Graner, E., A. F. Mercadante, S. M. Zanata, O. V. Forlenza, A. L. B. Cabral, S. S. Veiga, M. A. Juliano, R. Roesler, R. Walz, A. Mineti, I. Izquierdo, V. R. Martins, and R. R. Brentani. 2000. Cellular prion protein binds laminin and mediates neuritogenesis. *Mol. Brain Res.* **76:** 85–92.
39. Harris, D. A., P. J. Peters, A. Taraboulos, V. Lingappa, S. J. DeArmond, and S. B. Prusiner. 2004. Cell biology of prions, pp. 483–544. *In* S. B. Prusiner (ed.), *Prion Biology and Diseases*, 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
40. Hetz, C., M. Russelakis-Carneiro, K. Maundrell, J. Castilla, and C. Soto. 2003. Caspase-12 and endoplasmic reticulum stress mediate neurotoxicity of pathological prion protein. *EMBO J.* **22:** 5435–5445.
41. Horonchik, L., S. Tzaban, O. Ben-Zaken, Y. Yedidia, A. Rouvinski, D. Papy-Garcia, D. Barritault, I. Vlodavsky, and A. Taraboulos. 2005. Heparan sulfate is a cellular receptor for purified infectious prions. *J. Biol. Chem.* **280:** 17062–17067.
42. Hundt, C., J. M. Peyrin, S. Haik, S. Gauczynski, C. Leucht, R. Rieger, M. L. Riley, J. P. Deslys, D. Dormont, C. I. Lasmezas, and S. Weiss. 2001. Identification of interaction domains of the prion protein with its 37-kDa/67-kDa laminin receptor. *EMBO J.* **20:** 5876–5886.
43. Jakob, A. 1921. Uber eigenartige Erkrankungen des Zentralnervensystems mit bemerkenswertem anatomischen Befunde (spastische Pseudosklerose-Encephalomyelopathie mit disseminierten Degenerationsherden). *Z. Gesamte Neurol. Psychiatrie* **64:** 147–228.

44. James, T. L., H. Liu, N. B. Ulyanov, S. Farr-Jones, H. Zhang, D. G. Donne, K. Kaneko, D. Groth, I. Mehlhorn, S. B. Prusiner, and F. E. Cohen. 1997. Solution structure of a 142-residue recombinant prion protein corresponding to the infectious fragment of the scrapie isoform. *Proc. Natl. Acad. Sci. USA* **94:** 10086–10091.
45. Jarrett, J. T. and P. T. Lansbury Jr. 1993. Seeding "one-dimensional crystallization" of amyloid: A pathogenic mechanism in Alzheimer's disease and scrapie? *Cell* **73:** 1055–1058.
46. Kaneko, K., L. Zulianello, M. Scott, C. M. Cooper, A. C. Wallace, T. L. James, F. E. Cohen, and S. B. Prusiner. 1997. Evidence for protein X binding to a discontinuous epitope on the cellular prion protein during scrapie prion propagation. *Proc. Natl. Acad. Sci. USA* **94:** 10069–10074.
47. Keshet, G. I., O. Bar-Peled, D. Yaffe, U. Nudel, and R. Gabizon. 2000. The cellular prion protein colocalizes with the dystroglycan complex in the brain. *J. Neurochem.* **75:** 1889–1897.
48. Kimberlin, R. H., S. Cole, and C. A. Walker. 1987. Temporary and permanent modifications to a single strain of mouse scrapie on transmission to rats and hamsters. *J. Gen. Virol.* **68:** 1875–1881.
49. Kimberlin, R. H. and C. A. Walker. 1979. Pathogenesis of mouse scrapie: Dynamics of agent replication in spleen, spinal cord and brain after infection by different routes. *J. Comp. Pathol.* **89:** 551–562.
50. Klatzo, I., D. C. Gajdusek, and V. Zigas. 1959. Pathology of kuru. *Lab. Invest.* **8:** 799–847.
51. Kornblatt, J. A., S. Marchal, H. Razaei, and C. Balny. 2004. Characterization of a complex formed between human plasminogen and recombinant sheep prion: Pressure and thermal sensitivity of complex formation. *Cell. Mol. Biol. (Noisy-le-grand)* **50:** 387–396.
52. Kornblatt, J. A., S. Marchal, H. Rezaei, M. J. Kornblatt, C. Balny, R. Lange, M.-P. Debey, G. Hui Bon Hoa, M. C. Marden, and J. Grosclaude. 2003. The fate of the prion protein in the prion/plasminogen complex. *Biochem. Biophys. Res. Commun.* **305:** 518–522.
53. Kurschner, C. and J. I. Morgan. 1995. The cellular prion protein (PrP) selectively binds to Bcl-2 in the yeast two-hybrid system. *Mol. Brain Res.* **30:** 165–168.
54. Kuwahara, C., A. M. Takeuchi, T. Nishimura, K. Haraguchi, A. Kubosaki, Y. Matsumoto, K. Saeki, Y. Matsumoto, T. Yokoyama, S. Itohara, and T. Onodera. 1999. Prions prevent neuronal cell-line death. *Nature* **400:** 225–226.
55. Legname, G., I. V. Baskakov, H.-O. B. Nguyen, D. Riesner, F. E. Cohen, S. J. DeArmond, and S. B. Prusiner. 2004. Synthetic mammalian prions. *Science* **305:** 673–676.
56. Leucht, C., S. Simoneau, C. Rey, K. Vana, R. Rieger, C. I. Lasmezas, and S. Weiss. 2003. The 37 kDa/67 kDa laminin receptor is required for PrP^{Sc} propagation in scrapie-infected neuronal cells. *EMBO Rep.* **4:** 290–295.
57. Leucht, C., K. Vana, I. Renner-Müller, D. Dormont, C. I. Lasmézas, E. Wolf, and S. Weiss. 2004. Knock-down of the 37-kDa/67-kDa laminin receptor in mouse brain by transgenic expression of specific antisense LRP RNA. *Transgenic Res.* **13:** 81–85.
58. Llewelyn, C. A., P. E. Hewitt, R. S. Knight, K. Amar, S. Cousens, J. Mackenzie, and R. G. Will. 2004. Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. *Lancet* **363:** 417–421.
59. Lucassen, R., K. Nishina, and S. Supattapone. 2003. *In vitro* amplification of protease-resistant prion protein requires free sulfhydryl groups. *Biochemistry* **42:** 4127–4135.
60. Maglio, L. E., M. F. Perez, V. R. Martins, R. R. Brentani, and O. A. Ramirez. 2004. Hippocampal synaptic plasticity in mice devoid of cellular prion protein. *Mol. Brain Res.* **131:** 58–64.
61. Maissen, M., C. Roeckl, M. Glatzel, W. Goldmann, and A. Aguzzi. 2001. Plasminogen binds to disease-associated prion protein of multiple species. *Lancet* **357:** 2026–2028.
62. Manson, J. C., A. R. Clarke, M. L. Hooper, L. Aitchison, I. McConnell, and J. Hope. 1994. 129/Ola mice carrying a null mutation in PrP that abolishes mRNA production are developmentally normal. *Mol. Neurobiol.* **8:** 121–127.
63. Marella, M., C. Gaggioli, M. Batoz, M. Deckert, S. Tartare-Deckert, and J. Chabry. 2005. Pathological prion protein exposure switches on neuronal mitogen-activated protein kinase pathway resulting in microglia recruitment. *J. Biol. Chem.* **280:** 1529–1534.
64. Martins, V. R., E. Graner, J. Garcia-Abreu, S. J. de Souza, A. F. Mercadante, S. S. Veiga, S. M. Zanata, V. M. Neto, and R. R. Brentani. 1997. Complementary hydropathy identifies a cellular prion protein receptor. *Nat. Med.* **3:** 1376–1382.
65. McKinley, M. P., R. K. Meyer, L. Kenaga, F. Rahbar, R. Cotter, A. Serban, and S. B. Prusiner. 1991. Scrapie prion rod formation *in vitro* requires both detergent extraction and limited proteolysis. *J. Virol.* **65:** 1340–1351.
66. Moore, R. C., I. Y. Lee, G. L. Silverman, P. M. Harrison, R. Strome, C. Heinrich, A. Karunaratne, S. H. Pasternak, M. A. Chishti, Y. Liang, P. Mastrangelo, K. Wang, A. F. A. Smit, S. Katamine, G. A. Carlson, F. E. Cohen, S. B. Prusiner, D. W. Melton, P. Tremblay, L. E. Hood, and D. Westaway. 1999. Ataxia in prion protein (PrP)-deficient mice is associated with upregulation of the novel PrP-like protein doppel. *J. Mol. Biol.* **292:** 797–817.
67. Mouillet-Richard, S., M. Ermonval, C. Chebassier, J.-L. Laplanche, S. Lehmann, J. M. Launay, and O. Kellermann. 2000. Signal transduction through prion protein. *Science* **289:** 1925–1928.
68. Nieznanski, K., H. Nieznanska, K. J. Skowronek, K. M. Osiecka, and D. Stepkowski. 2005. Direct interaction between prion protein and tubulin. *Biochem. Biophys. Res. Commun.* **334:** 403–411.
69. Oesch, B., D. B. Teplow, N. Stahl, D. Serban, L. E. Hood, and S. B. Prusiner. 1990. Identification of cellular proteins binding to the scrapie prion protein. *Biochemistry* **29:** 5848–5855.
70. Oesch, B., D. Westaway, M. Walchli, M. P. McKinley, S. B. H. Kent, R. Aebersold, R. A. Barry, P. Tempst, D. B. Teplow, L. E. Hood, S. B. Prusiner, and C. Weissmann. 1985. A cellular gene encodes scrapie PrP 27-30 protein. *Cell* **40:** 735–746.
71. Pan, K.-M., M. Baldwin, J. Nguyen, M. Gasset, A. Serban, D. Groth, I. Mehlhorn, Z. Huang, R. J. Fletterick, F. E.

Cohen, and S. B. Prusiner. 1993. Conversion of α-helices into β-sheets features in the formation of the scrapie prion proteins. *Proc. Natl. Acad. Sci. USA* **90:** 10962–10966.
72. Peden, A. H., M. W. Head, D. L. Ritchie, J. E. Bell, and J. W. Ironside. 2004. Preclinical vCJD after blood transfusion in a *PRNP* codon 129 heterozygous patient. *Lancet* **364:** 527–529.
73. Petrakis, S. and T. Sklaviadis. 2006. Identification of proteins with high affinity for refolded and native PrP^C. *Proteomics* **6:** 6476–6484.
74. Praus, M., G. Kettelgerdes, M. Baier, H.-G. Holzhütter, P. R. Jungblut, M. Maissen, G. Epple, W.-D. Schleuning, E. Köttgen, A. Aguzzi, and R. Gessner. 2003. Stimulation of plasminogen activation by recombinant cellular prion protein is conserved in the NH2-terminal fragment PrP23-110. *Thromb. Haemost.* **89:** 812–819.
75. Prusiner, S. B. 1991. Molecular biology of prion diseases. *Science* **252:** 1515–1522.
76. Prusiner, S. B. 1982. Novel proteinaceous infectious particles cause scrapie. *Science* **216:** 136–144.
77. Prusiner, S. B. 1998. Prions. *Proc. Natl. Acad. Sci. USA* **95:** 13363–13383.
78. Prusiner, S. B. 2001. Shattuck Lecture: Neurodegenerative diseases and prions. *N. Engl. J. Med.* **344:** 1516–1526.
79. Prusiner, S. B., M. Scott, D. Foster, K.-M. Pan, D. Groth, C. Mirenda, M. Torchia, S.-L. Yang, D. Serban, G. A. Carlson, P. C. Hoppe, D. Westaway, and S. J. DeArmond. 1990. Transgenetic studies implicate interactions between homologous PrP isoforms in scrapie prion replication. *Cell* **63:** 673–686.
80. Race, R., M. Oldstone, and B. Chesebro. 2000. Entry versus blockade of brain infection following oral or intraperitoneal scrapie administration: Role of prion protein expression in peripheral nerves and spleen. *J. Virol.* **74:** 828–833.
81. Race, R. E., L. H. Fadness, and B. Chesebro. 1987. Characterization of scrapie infection in mouse neuroblastoma cells. *J. Gen. Virol.* **68:** 1391–1399.
82. Rieger, R., F. Edenhofer, C. I. Lasmezas, and S. Weiss. 1997. The human 37-kDa laminin receptor precursor interacts with the prion protein in eukaryotic cells. *Nat. Med.* **3:** 1383–1388.
83. Riek, R., S. Hornemann, G. Wider, M. Billeter, R. Glockshuber, and K. Wuthrich. 1996. NMR structure of the mouse prion protein domain PrP(121-231). *Nature* **382:** 180–182.
84. Rogers, M., F. Yehiely, M. Scott, and S. B. Prusiner. 1993. Conversion of truncated and elongated prion proteins into the scrapie isoform in cultured cells. *Proc. Natl. Acad. Sci. USA* **90:** 3182–3186.
85. Rossi, D., A. Cozzio, E. Flechsig, M. A. Klein, T. Rulicke, A. Aguzzi, and C. Weissmann. 2001. Onset of ataxia and Purkinje cell loss in PrP null mice inversely correlated with Dpl level in brain. *EMBO J.* **20:** 694–702.
86. Ryou, C., S. B. Prusiner, and G. Legname. 2003. Cooperative binding of dominant-negative prion protein to kringle domains. *J. Mol. Biol.* **329:** 323–333.
87. Saborio, G. P., B. Permanne, and C. Soto. 2001. Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. *Nature* **411:** 810–813.
88. Sakaguchi, S., S. Katamine, N. Nishida, R. Moriuchi, K. Shigematsu, T. Sugimoto, A. Nakatani, Y. Kataoka, T. Houtani, S. Shirabe, H. Okada, S. Hasegawa, T. Miyamoto, and T. Noda. 1996. Loss of cerebellar Purkinje cells in aged mice homozygous for a disrupted PrP gene. *Nature* **380:** 528–531.
89. Salmona, M., R. Capobianco, L. Colombo, A. De Luigi, G. Rossi, M. Mangieri, G. Giaccone, E. Quaglio, R. Chiesa, M. B. Donati, F. Tagliavini, and G. Forloni. 2005. Role of plasminogen in propagation of scrapie. *J. Virol.* **79:** 11225–11230.
90. Santuccione, A., V. Sytnyk, I. Leshchyns'ka, and M. Schachner. 2005. Prion protein recruits its neuronal receptor NCAM to lipid rafts to activate p59fyn and to enhance neurite outgrowth. *J. Cell Sci.* **169**: 341–354.
91. Schmitt-Ulms, G., G. Legname, M. A. Baldwin, H. L. Ball, N. Bradon, P. J. Bosque, K. L. Crossin, G. M. Edelman, S. J. DeArmond, F. E. Cohen, and S. B. Prusiner. 2001. Binding of neural cell adhesion molecules (N-CAMs) to the cellular prion protein. *J. Mol. Biol.* **314:** 1209–1225.
92. Scott, M., D. Foster, C. Mirenda, D. Serban, F. Coufal, M. Walchli, M. Torchia, D. Groth, G. Carlson, S. J. DeArmond, D. Westaway, and S. B. Prusiner. 1989. Transgenic mice expressing hamster prion protein produce species-specific scrapie infectivity and amyloid plaques. *Cell* **59:** 847–857.
93. Scott, M., D. Peretz, R. M. Ridley, H. F. Baker, S. J. DeArmond, and S. B. Prusiner. 2004. Transgenetic investigations of the species barrier and prion strains, pp. 435–482. *In* S. B. Prusiner (ed.), *Prion Biology and Diseases*, 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
94. Shaked, G. M., Z. Meiner, I. Avraham, A. Taraboulos, and R. Gabizon. 2001. Reconstitution of prion infectivity from solubilized protease-resistant PrP and nonprotein components of prion rods. *J. Biol. Chem.* **276:** 14324–14328.
95. Shaked, Y., R. Engelstein, and R. Gabizon. 2002. The binding of prion proteins to serum components is affected by detergent extraction conditions. *J. Neurochem.* **82:** 1–5.
96. Sohn, H. J., J. H. Kim, K. S. Choi, J. J. Nah, Y. S. Joo, Y. H. Jean, S. W. Ahn, O. K. Kim, D. Y. Kim, and A. Balachandran. 2002. A case of chronic wasting disease in an elk imported to Korea from Canada. *J. Vet. Med. Sci.* **64:** 855–858.
97. Spielhaupter, C. and H. M. Schatzl. 2001. PrP^C directly interacts with proteins involved in signaling pathways. *J. Biol. Chem.* **276:** 44604–44612.
98. Stahl, N., D. R. Borchelt, K. Hsiao, and S. B. Prusiner. 1987. Scrapie prion protein contains a phosphatidylinositol glycolipid. *Cell* **51:** 229–240.
99. Steele, A. D., J. G. Emsley, P. H. Ozdinler, S. Lindquist, and J. D. Macklis. 2006. Prion protein (PrP^C) positively regulates neural precursor proliferation during developmental and adult mammalian neurogenesis. *Proc. Natl. Acad. Sci. USA* **103:** 3416–3421.
100. Telling, G. C., M. Scott, J. Mastrianni, R. Gabizon, M. Torchia, F. E. Cohen, S. J. DeArmond, and S. B. Prusiner. 1995. Prion propagation in mice expressing human and

chimeric PrP transgenes implicates the interaction of cellular PrP with another protein. *Cell* **83:** 79–90.

101. Vana, K. and S. Weiss. 2006. A trans-dominant negative V37 kDa/67 kDa laminin receptor mutant impairs PrP^{Sc} propagation in scrapie-infected neuronal cells. *J. Mol. Biol.* **358:** 57–66.
102. Warner, R. G., C. Hundt, S. Weiss, and J. E. Turnbull. 2002. Identification of the heparan sulfate binding sites in the cellular prion protein. *J. Biol. Chem.* **277:** 18421–18430.
103. Wells, G. A. H., A. C. Scott, C. T. Johnson, R. F. Gunning, R. D. Hancock, M. Jeffrey, M. Dawson, and R. Bradley. 1987. A novel progressive spongiform encephalopathy in cattle. *Vet. Rec.* **121:** 419–420.
104. Wells, G. A. H. and J. W. Wilesmith. 2004. Bovine spongiform encephalopathy and related diseases, pp. 595–628. *In* S. B. Prusiner (ed.), *Prion Biology and Diseases*, 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
105. Will, R. G., M. P. Alpers, D. Dormont, and L. B. Schonberger. 2004. Infectious and sporadic prion diseases, pp. 629–671. *In* S. B. Prusiner (ed.), *Prion Biology and Diseases*, 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
106. Will, R. G., J. W. Ironside, M. Zeidler, S. N. Cousens, K. Estibeiro, A. Alperovitch, S. Poser, M. Pocchiari, A. Hofman, and P. G. Smith. 1996. A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet* **347:** 921–925.
107. Williams, E. S. 2005. Chronic wasting disease. *Vet. Pathol.* **42:** 530–549.
108. Wong, C. N., L.-W. Xiong, M. Horiuchi, L. Raymond, K. Wehrly, B. Chesebro, and B. Caughey. 2001. Sulfated glycans and elevated temperature stimulate PrP^{Sc}-dependent cell-free formation of protease-resistant prion protein. *EMBO J.* **20:** 377–386.
109. Xanthopoulos, K., I. Paspaltsis, V. Apostolidou, S. Petrakis, C. J. Siao, A. Kalpatsanidis, N. Grigoriadis, A. Tsaftaris, S. E. Tsirka, and T. Sklaviadis. 2005. Tissue plasminogen activator in brain tissues infected with transmissible spongiform encephalopathies. *Neurobiol. Dis.* **20:** 519–527.
110. Yehiely, F., P. Bamborough, M. D. Costa, B. J. Perry, G. Thinakaran, F. E. Cohen, G. A. Carlson, and S. B. Prusiner. 1997. Identification of candidate proteins binding to prion protein. *Neurobiol. Dis.* **3:** 339–355.
111. Zanata, S. M., M. H. Lopes, A. F. Mercadante, G. N. Hajj, L. B. Chiarini, R. Nomizo, A. R. Freitas, A. L. Cabral, K. S. Lee, M. A. Juliano, E. de Oliveira, S. G. Jachieri, A. Burlingame, L. Huang, R. Linden, R. R. Brentani, and V. R. Martins. 2002. Stress-inducible protein 1 is a cell surface ligand for cellular prion that triggers neuroprotection. *EMBO J.* **21:** 3307–3316.
112. Zerr, I., M. Bodemer, U. Kaboth, H. Kretzschmar, M. Oellerich, and V. W. Armstrong. 2004. Plasminogen activities and concentrations in patients with sporadic Creutzfeldt-Jakob disease. *Neurosci. Lett.* **371:** 163–166.
113. Zhang, C. C., A. D. Steele, S. Lindquist, and H. F. Lodish. 2006. Prion protein is expressed on long-term repopulating hematopoietic stem cells and is important for their self-renewal. *Proc. Natl. Acad. Sci. USA* **103:** 2184–2189.
114. Zulianello, L., K. Kaneko, M. Scott, S. Erpel, D. Han, F. E. Cohen, and S. B. Prusiner. 2000. Dominant-negative inhibition of prion formation diminished by deletion mutagenesis of the prion protein. *J. Virol.* **74:** 4351–4360.