ANNUAL REVIEWS

Annual Review of Pathology: Mechanisms of Disease

Cellular and Molecular Mechanisms of Prion Disease

Christina J. Sigurdson,[1] Jason C. Bartz,[2] and Markus Glatzel[3]

[1]Departments of Pathology and Medicine, UC San Diego School of Medicine, University of California, San Diego, La Jolla, California 92093, USA; email: csigurdson@ucsd.edu

[2]Department of Medical Microbiology and Immunology, Creighton University, Omaha, Nebraska 68178, USA

[3]Institute of Neuropathology, University Medical Center Hamburg-Eppendorf, 20246 Hamburg, Germany

Annu. Rev. Pathol. Mech. Dis. 2019. 14:497–516

First published as a Review in Advance on October 24, 2018

The *Annual Review of Pathology: Mechanisms of Disease* is online at pathol.annualreviews.org

https://doi.org/10.1146/annurev-pathmechdis-012418-013109

Keywords

neurodegeneration, amyloid, neurotoxicity, strains, prion transmission

Abstract

Prion diseases are rapidly progressive, incurable neurodegenerative disorders caused by misfolded, aggregated proteins known as prions, which are uniquely infectious. Remarkably, these infectious proteins have been responsible for widespread disease epidemics, including kuru in humans, bovine spongiform encephalopathy in cattle, and chronic wasting disease in cervids, the latter of which has spread across North America and recently appeared in Norway and Finland. The hallmark histopathological features include widespread spongiform encephalopathy, neuronal loss, gliosis, and deposits of variably sized aggregated prion protein, ranging from small, soluble oligomers to long, thin, unbranched fibrils, depending on the disease. Here, we explore recent advances in prion disease research, from the function of the cellular prion protein to the dysfunction triggering neurotoxicity, as well as mechanisms underlying prion spread between cells. We also highlight key findings that have revealed new therapeutic targets and consider unanswered questions for future research.

INTRODUCTION

Prion diseases are fatal neurodegenerative disorders of humans and animals and are remarkable due to their infectious nature. The infectious agent causing prion disease, known as PrP^{Sc}, is unusual as it lacks any specific nucleic acid; it is a pathogenic misfolded and aggregated form of the cellular prion protein, PrP^{C} (1, 2). Following transmission to a naive host, prions seed the misfolding of host PrP^{C} in an autocatalytic process, leading to an exponential increase in PrP^{Sc} in the brain and spinal cord that eventually leads to neuronal death (3). The primary amino acid sequence of PrP^{Sc} is determined by host PrP^{C}, which in humans is encoded by the prion gene, *PRNP*, on chromosome 20 (4).

Prions are highly stable and accumulate in the central nervous system over months to years, eventually generating rampant spongiform degeneration and neuronal loss, as well as activated astrocytes and microglia; there is a notable lack of peripheral inflammatory cells (**Figure 1**) (5). Although the incubation period may be years, the clinical phase is typically rapidly progressive (weeks to months) and may include behavioral abnormalities, motor dysfunction, cognitive impairment, and ataxia, depending on the prion and the species affected (6). No therapy is available beyond palliative care.

In humans, prion diseases are categorized as sporadic, genetic, or acquired, with the majority of cases (~85%) being sporadic. Sporadic Creutzfeldt–Jakob disease (sCJD) has no known cause, but it has been hypothesized to be instigated by a somatic mutation in *PRNP* or the spontaneous conversion of PrP^{C} to PrP^{Sc} (7). Genetic prion diseases have been classified by their clinical symptoms and neuropathological features and consist of familial CJD, fatal familial insomnia, and Gerstmann–Sträussler–Scheinker disease. The mutations in *PRNP* are autosomal dominant, highly penetrant, and consist of missense mutations, insertions, and deletions, usually inciting disease onset in the fifth or sixth decade of life (6). The acquired prion diseases have been transmitted between individuals (kuru and iatrogenic CJD) and from cattle to humans [variant

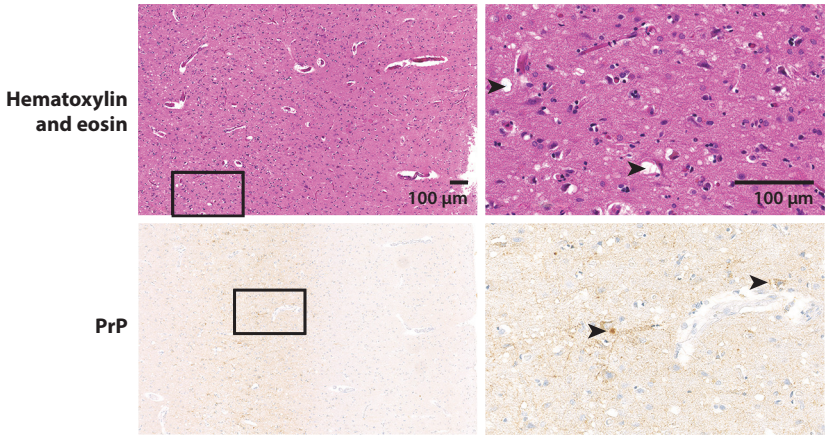

Figure 1

Hematoxylin and eosin and PrP immunostain of brain (frontal cortex) from a patient with sporadic Creutzfeldt–Jakob disease. Hematoxylin and eosin staining shows (*left*) spongiosis in the deep layers of the cortex, and (*right*) the arrows indicate intraneuronal and parenchymal spongiform change. PrP immunostain shows (*left*) synaptic, plaque-like, and perineuronal deposits of pathological prion protein, and (*right*) the arrows indicate the plaque-like and perineuronal deposits. The synaptic deposits of pathological prion protein are pronounced in the deep layers of the cortex. Scale bar = 100 µm.

Creutzfeldt–Jakob disease (vCJD)] (8, 9). Iatrogenic spread has occurred from prion-contaminated corneal and meningeal grafts, blood transfusions (10–13), human growth hormone (14, 15), and prion-contaminated neurosurgical instruments and electrodes (16).

In addition to iatrogenic prion infection, prions have also precipitated large-scale, multispecies epidemics and even spread as a zoonosis. Bovine spongiform encephalopathy (BSE) was first described in 1986 (17), and within a decade, more than 180,000 cases of BSE were diagnosed in cattle, with further expansion to zoo bovids, felids, and primates within the United Kingdom (18). In 1996, vCJD was recognized to affect mainly young people (in their second decade) in the United Kingdom, likely from exposure to BSE-contaminated beef (9, 19) in the United Kingdom and elsewhere; 229 vCJD cases have been diagnosed to date (20). No recent cases have occurred; however, secondary transmission of vCJD prions developed in transfusion recipients receiving blood or blood products originating from prion-infected donors (10–13).

Although prion diseases in animals, including BSE, are largely acquired by ingestion, atypical scrapie and BSE in aged sheep and cattle, respectively, may arise sporadically, similar to sCJD (21–23). Classical scrapie affects sheep and goats nearly worldwide and has been recognized for more than 250 years (24). Chronic wasting disease (CWD) was first discovered in Colorado deer in 1967 (25) and affects free-ranging and captive deer, elk, reindeer, and moose (family, Cervidae) in 25 US states and two Canadian provinces, as well as ranched elk in South Korea (26) and wild reindeer and moose in Norway (27) and Finland (28). Transmissible mink encephalopathy has been previously identified in farmed mink in the United States, Canada, Russia, Finland, and Germany, and it was thought to be due to dietary exposure to a prion-infected animal, although the origin of the epidemic remains unclear and no cases have been reported for more than 30 years (29).

The complicated molecular mechanisms that govern how prions are converted and spread from extraneural entry sites into the brain as well as how prions generate neurotoxic responses are the subjects of this review, which focuses on recent findings in prion pathogenesis. We also highlight new research linking prion conformation to disease phenotype.

CELLULAR BIOLOGY OF THE PRION PROTEIN: SYNTHESIS AND FUNCTION

Prion Protein Synthesis and Modification

The physiological (or cellular) form of the prion protein is glycosylphosphatidylinositol (GPI) anchored and features two variably occupied *N*-linked glycosylation sites (30). Mature PrP^C consists of approximately 210 amino acids, arranged as a disordered N-terminal domain and a globular C-terminal domain composed of three α helices and a short, antiparallel β pleated sheet (31). In its mature form, PrP^C is mainly present as a diglycosylated protein, located at the outer leaflet of the plasma membrane in lipid-enriched microdomains (32). Following internalization, PrP^C is recycled to either the plasma membrane or the Golgi apparatus (the retromer pathway) (33) or is transported to late endosomes, eventually residing in the pinched-off intraluminal vesicles within multivesicular bodies (MVBs) for release as exosomes or for degradation in lysosomes (34, 35).

PrP^C is subject to proteolytic cleavage, with α cleavage and shedding of PrP^C representing the two most important cleavage events (36). α cleavage occurs in the middle of PrP^C, releasing an unstructured N-terminal protein fragment, while leaving its C-terminal globular part attached to the membrane (37). This cleavage takes place during vesicular trafficking of PrP^C within the secretory pathway (38). Initial reports identified the serine protease plasmin (39, 40) or ADAMs (proteins belonging to the family known as a disintegrin and metalloproteinase) (41) as potential

proteases, yet data within the past 5 years do not support this observation (42–44), and the exact nature of the responsible protease remains unclear (36).

A cleavage event occurring at the distal C terminus of PrP^C and releasing nearly full-length PrP into the extracellular space is referred to as PrP shedding (45, 46). PrP shedding occurs only on the plasma membrane, and ADAM10 is the only relevant PrP sheddase, with diglycosylated PrP^C representing the preferred substrate (47–49).

Prion Protein Function

A detailed explanation of all of the functions attributed to PrP^C would go beyond the scope of this review. In fact, one of our groups (M.G. and colleagues) proposed in 2017 that PrP^C should be listed among the expanding group of multifunctional proteins—that is, those proteins to which several functions are attributed (36). An incomplete list of PrP^C functions would include its role in neural development (50), cell adhesion (51), axon guidance, synapse formation (52), neuroprotection (53, 54), regulation of circadian rhythm (55), myelin maintenance (56, 57), maintenance of ion homeostasis (58, 59), and signaling (60, 61).

Interestingly, some of the best described functions are not credited to PrP^C in its membrane-bound, GPI-anchored form, but rather to soluble PrP^C fragments, which can be generated only by regulated proteolysis, such as α cleavage and shedding. This is true for the described function, reported in 2010, of soluble PrP in maintaining myelin homeostasis (57) or in inducing neurite outgrowth (62). For myelin maintenance, binding of the flexible N-terminal part of soluble PrP acts as an agonistic ligand to a G protein–coupled receptor expressed on Schwann cells, Adgrg6 (Gpr126) (57), whereas the molecular details for the neurite outgrowth–promoting role are not understood. Interestingly, in this instance, membrane-bound PrP^C itself may act as a receptor via homophilic interactions (62). Nevertheless, in both instances, it is obvious that regulated proteolysis would be an elegant mode of functional regulation for transmitting information to distant sites. This is reminiscent of functions attributed to proteolytic cleavage fragments from the amyloid precursor protein (APP) (63, 64). Yet while insights into the processing of APP and its biological and pathogenic consequences are vast, relatively little is known about the physiological roles of PrP^C cleavage fragments.

PRION PROTEIN MALFUNCTION: MECHANISMS OF NEURODEGENERATION IN PRION DISEASE

Loss of PrP^C Function Versus Toxic Gain of Function?

A key event in the pathophysiology of prion diseases is the PrP^{Sc} template-directed misfolding of PrP^C into a pathogenic, conformationally altered, β sheet–rich version of itself. This conversion process lies at the root of the now widely accepted prion hypothesis, which states that the infectious agent for prion diseases (the prion) is entirely made up of proteins and is devoid of specific nucleic acids (65). Today, we know that a pathogenic, conformationally altered version of PrP^C is a key component of the infectious agent responsible for the transmission of prion diseases. This disease-associated version of PrP is designated as PrP^{Sc}. Originally, only highly protease-resistant forms were termed PrP^{Sc}, but it is now accepted that there are also pathogenic PrP conformers that are mildly protease resistant, and since these versions are infectious, a biochemical definition of protease resistance is not adequate (66). Thus, one has to include protease-sensitive disease-associated PrP species in the pool of conformationally altered versions of PrP able to induce prion disease. Currently, the term PrP^{Sc} is widely used to describe disease-associated PrP species, and for

the sake of clarity, we use this term in this review when referring to pathogenic, conformationally altered versions of PrP.

The PrP^{C} to PrP^{Sc} conversion process involves a massive structural rearrangement of the primarily α helical protein into a highly β sheet–rich structure (approximately 47% β sheet) (67). The mechanism that underlies PrP^{C} conversion into PrP^{Sc} remains unknown. One hypothesis is that short segments of PrP^{Sc} interact with PrP^{C} in a steric zipper, in which complementary amino acid side chains from two β sheets are tightly interdigitated and effectively stabilize growing fibrils, largely through hydrogen bonds (68, 69). Sequence differences within steric zipper segments have been shown to block prion conversion between species (70, 71).

PrP^{C} is converted to PrP^{Sc} on the plasma membrane or within the endocytic pathway. A study by Greene and colleagues (34) suggests that prion conversion occurs primarily within MVBs and not on the plasma membrane because preventing MVB maturation sharply reduces PrP^{Sc} production.

The generation and progressive accumulation of PrP^{Sc} are of key importance for the development of clinical prion disease, although there are rare instances, such as subclinical disease in prion-infected mice, in which the presence of PrP^{Sc} does not lead to neurodegeneration (72). It is conceivable that the partial loss of some of the physiological functions of PrP^{C} may contribute to prion-associated neurodegeneration. A key argument against loss of function playing a part in prion disease is that the loss of PrP^{C} function in PrP knockout mice does not lead to neuronal death (73). However, we have only begun to understand how PrP^{C} functions on a molecular level, with PrP^{C} or its proteolytic cleavage products acting as receptor or ligand, or both, most likely in concert with many binding partners (74). Thus, a certainly recurring redundancy in this system may compensate for loss-of-function phenotypes, and these may become apparent only once additional stressors are active (36, 57).

Mechanisms Underlying Prion Toxicity

The evidence for the direct or indirect neurotoxicity of PrP^{Sc} is compelling, and there is no doubt that cerebral accumulation of misfolded PrP^{Sc} has a key role in the pathophysiology of prion diseases, but how does this happen?

Disturbed protein homeostasis in prion disease. Neuronal proteostasis, which is the interplay of protein synthesis (including correct protein folding, trafficking, and processing) and protein degradation, is essential for correct neuronal function (75). Disturbed proteostasis occurs in prion disease at multiple levels. PrP^{Sc} disturbs the ubiquitin/proteasome system at early disease states, leading to impaired function of this protein degradation pathway and thus enhancing the buildup of PrP^{Sc} (76). There is also mounting evidence that the buildup of PrP^{Sc} affects the autophagy/lysosome pathway that is responsible for the degradation of aggregated proteins, although in one study temporal analysis indicates that this is a consequence of protein buildup and not causally involved in disease initiation (77). Additionally, there is evidence that exhaustion of unfolded protein response pathways occurs early in prion disease (78–80). The unfolded protein response is a cellular stress response aiming to protect the endoplasmic reticulum's function in protein synthesis and sorting. PrP^{Sc} stresses the endoplasmic reticulum and sets off a vicious cycle, resulting in disturbed PrP^{C} trafficking, impaired PrP^{C} functions, and translational shutdown that weakens the neurons, causing synaptic loss and, ultimately, inducing cell death pathways (81). Interestingly, restoring the disturbed protein translation has been shown to be neuroprotective (81).

PrP^{Sc}-mediated toxicity at the neuronal membrane. PrP^{Sc} aggregation occurs in a highly ordered fashion, and oligomeric, rather than fibrillar, forms of PrP^{Sc} aggregates are thought to

be more neurotoxic (82). Morphological studies have shown the close relationship between PrP^{Sc} deposits and the neuronal plasma membrane (83). How this translates into neurotoxicity is not fully understood, but two lines of thought have emerged. In the first scenario, PrP^{Sc} aggregates lead to major membrane disturbance, possibly by corrupting the function of neuronal receptors, such as the NMDA receptor, and thus altering plasma membrane permeability (84). GPI-anchored PrP^{C} is able to efficiently transduce neurotoxicity, and prion disease is accelerated in mice in which PrP^{C} shedding is impaired, and both of these support this line of thought (85, 86). In the second scenario, membrane-bound PrP^{C} itself may act as a receptor of prion toxicity. Indeed, a direct interaction between PrP^{Sc} and PrP^{C} induces neurotoxicity similar to a mechanism first described in Alzheimer's disease in which oligomeric species of amyloid β bind membrane PrP^{C} complexed to the metabotropic glutamate receptor mGluR5, activating intracellular Fyn kinase and ultimately leading to synaptotoxicity (87–91).

PrP^{C} has also been incriminated in neurotoxic responses, as antibody binding to the C-terminal globular domain leads to toxic signal generation through the N terminus, inducing calpain activation and reactive oxygen species production (92). PrP^{Sc} has been found to cause a similar toxic signaling cascade, again with calpain activation and reactive oxygen species generation (93). In cultured primary neurons expressing a mutant PrP lacking residues in a central region (Δ105–125), abnormal ion channel currents occurred, sensitizing neurons to glutamate-induced excitotoxicity. These abnormal currents may represent early toxic signaling events in affected cells and underlie early neurodegeneration (94). Nevertheless, the sequence of events leading to receptor-mediated neurotoxicity is not yet completely defined, and GPI-anchored PrP^{C} would need a coreceptor to enable intraneuronal signal transduction.

PRION SPREAD INTO AND THROUGH THE CENTRAL NERVOUS SYSTEM: AN UPDATE

Similar to neurotropic infectious agents such as rabies virus, prions have managed to access the central nervous system (CNS) from extraneural entry sites. In experimental models, prions appear to spread from site of entry to the CNS via peripheral nerves. For example, feeding prions to hamsters leads to early prion deposition in enteric and autonomic ganglia, vagus and splanchnic nerves, and, subsequently, in the thoracic spinal cord and dorsal motor nucleus of the vagus in the brain stem, consistent with retrograde prion spread along autonomic peripheral nervous system pathways into the CNS (95). Oral BSE prion infection in cattle and CWD in deer are first detected in the CNS within the vagal nucleus, consistent with prion entry through the gastrointestinal tract and transit via the vagal nerve into the brain (96, 97). Similarly, exposure of the mouse eye to prions induces prion deposition along the optic nerve and tract, followed by the contralateral superior colliculus to which it projects, further suggesting prion spread via neural circuitry (98). Additional support for prion transit in nerves was provided by studies manipulating sympathetic innervation to the prion-infected spleen, which markedly affected prion entry into the CNS (99, 100). Interestingly, prion conformation also plays a part in prion neuroinvasion, as fibril-forming prions spread poorly to the brain compared with oligomeric or subfibrillar prions (101–104). Since prions circulate in blood within minutes postinoculation (105), additional nonneural pathways of prion entry into the CNS, such as passage across the blood–brain barrier, cannot be excluded.

Prion Spread from the Gastrointestinal Tract to the Brain

Prion spread following ingestion is similar to the pathway used by other infectious agents exploiting entry portals to invade the host. Upon contact with the intestinal epithelium, prions transit

via M cells, as M cell depletion reduces oral susceptibility to prion disease (106); additional studies by multiple laboratories support M cells as key players that facilitate the passage of prions across the mucosal barrier (106–110). Enteritis may heighten susceptibility to oral prion infection, potentially by enabling prion passage through the mucosa (111). Once in the subepithelial region, neurotropic prions, such as BSE, are thought to spread by retrograde axonal transport along autonomic peripheral nervous system pathways into the brain stem (112, 113). Lymphotropic prions, such as sheep scrapie, deer CWD, and potentially vCJD, rapidly spread (within hours) to Peyer's patches and draining lymph nodes, potentially transported by classical dendritic cells (96, 114, 115), as depletion of dendritic cells impedes the early replication of prions in lymph nodes (116, 117). Lymphotropic prions also spread to inflamed organs harboring lymphoid follicles, such as kidney or mammary gland, leading to prion excretion or secretion into, respectively, urine or milk (118–121).

In the lymphoid tissue, PrP^{Sc} accumulates within the germinal centers of lymphoid follicles, both on the plasma membrane of follicular dendritic cells (FDCs) and within tingible body macrophages (122), where it persists throughout the infection (96). FDCs trap antigens on their plasma membrane for display to B cells (123, 124) and have proven highly capable of replicating prions (125), sustaining lymphoid prion infections for months to years (125). On the surface of FDCs, CD21/35 receptors are thought to bind a PrP^{Sc} complement complex, as both soluble C1q and regulatory protein factor H bind PrP^{Sc} (126–129), and CD21/35 receptor knockout mice show low attack rates after a peripheral prion infection (129). Together these studies indicate a crucial role for complement receptors in lymphoid prion replication.

This peripheral phase of prion replication has been exploited to block prion spread to the CNS. FDCs require B cell signaling through tumor necrosis factor and lymphotoxin to develop and maintain a mature state (123), and blocking lymphotoxin signaling induces FDC dedifferentiation and prevents prion accumulation in lymphoid tissue. This prevention strategy has worked effectively in mice treated with lymphotoxin β receptor agonists or antireceptor antibodies (130), abolishing splenic prion replication and prolonging survival following an intraperitoneal challenge (130). Preventing disease by this strategy must begin early, however, as nerve entry occurs quickly after prion exposure, within 14 days after oral challenge in mice (131).

Lymphoid tissues may serve as sources of new prion strains. Cross-species prion transmission has generated new prion strains within lymphoid tissues, suggesting that these tissues may be more promiscuous than the CNS in replicating prions having a different PrP sequence (132). The mechanism underlying this reduced threshold for prion replication is unclear; however, PrP^{Sc} glycan sialylation levels influence capture by complement receptors in lymphoid tissue, and the glycans on PrP^{Sc} are more sialylated in lymphoid tissue than in brain (133), potentially contributing to the permissiveness of lymphoid tissue to prion replication (133, 134).

Prion Conversion Within the Central Nervous System

Once within the brain and spinal cord, prions are further amplified by neurons and astrocytes. Astrocytes are highly susceptible to prion infection in vitro and can readily transfer prions to neurons (135, 136). However, microglia do not have a major role in replication, but instead are critical for prion clearance: The depletion of microglia accelerates disease in vivo and increases PrP^{Sc} accumulation in organotypic brain slices (137). In contrast, oligodendrocytes lack any known significant contribution to prion replication or spread through the CNS (125). Although much is known about the cells that replicate prions in the brain, a pressing research need is to better understand how protein aggregates spread through the brain, from neuron to neuron (138–140) and between neurons and astrocytes (141).

Cell-to-Cell Prion Spread Through the Central Nervous System

Once in the brain, prions spread through neuroanatomically connected brain regions by poorly understood mechanisms (142–145). In vitro, prions spread from cell to cell via (*i*) exosomes and (*ii*) tunneling nanotubes (139, 146–148), with other as-yet-to-be-tested possible mechanisms, including through synaptosomes, GPI painting, microvesicles, and PrP^{Sc} cleavage from the plasma membrane (**Figure 2**).

PrP^{C} and PrP^{Sc} were both shown to sort into MVBs for release within exosomes, 40–100 nm extracellular vesicles (EVs) that arise within MVBs (34, 148–150). The extent to which prions are released within exosomes varies depending on the prion strain, as certain strains traffic more extensively with exosomes (146).

Further supporting the importance of EVs in prion transport, Saa and colleagues (151) showed that vCJD prion-infected mice harbored EVs containing infectious prions in plasma starting at preclinical disease stages, which suggests that EVs may transport prions across a long range. Nevertheless, it is not yet clear whether exosomes or other EVs are the most relevant mechanism for prion spread through the CNS. Methodological advances within the past 3 years in isolating exosomes and other EVs from the brain are expected to shed light on the role of exosomes in prion spread in vivo (152).

Another possible route for the direct cell-to-cell spread of prions is through tunneling nanotubes. Tunneling nanotubes are thin, membranous tubes that connect cells and serve as a mechanism for cell-to-cell communication, as organelles including lysosomes and mitochondria can be transported in nanotubes (140, 153). In addition to organelles, PrP^{Sc} was transferred to naive cells via nanotubes, including transfers from primary dendritic cells to neurons, as well as from neuron to neuron (139). Tunneling nanotubes may be induced by cell stress.

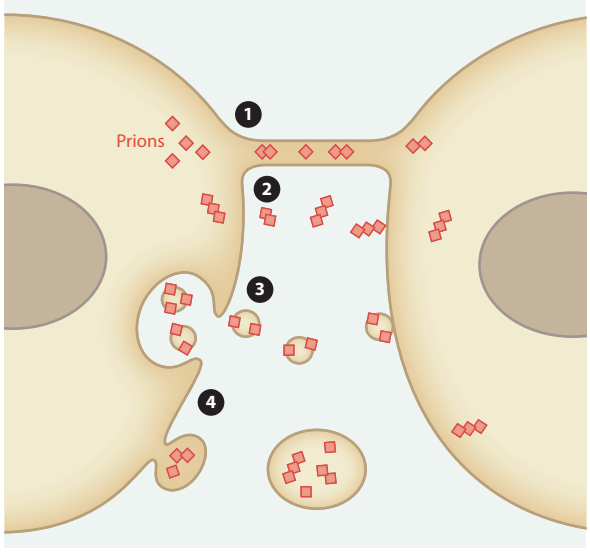

Figure 2

Possible pathways of prion spread from cell to cell. Prion aggregates may spread through ❶ transport in tunneling nanotubes; ❷ glycosylphosphatidylinositol (GPI) painting, by which GPI-anchored proteins transfer from one cell surface to a neighboring cell surface; ❸ trafficking within exosomes; or ❹ from membrane budding and transport within vesicles.

Lysosomes may also be involved in the cell-to-cell transport of prions, either through transfer within tunneling nanotubes or through lysosomal exocytosis, as observed for amyloid β and α synuclein (154, 155).

PrP^{Sc} CONFORMATION IMPACTS DISEASE PHENOTYPE

In experimental prion disease of rodents, a wide range of incubation periods and brain targets has been reported (156), depending on the prion conformation, or strain. Much work has been done to examine the relationship between the biochemical properties of PrP^{Sc} and survival time. Studies of yeast prions (Sup35) indicated that the rate of prion propagation is inversely proportional to aggregate stability and suggested that more fibril fragmentation, or higher frangibility, would produce new free ends for prion formation and accelerate prion propagation (157). Consistent with this notion, murine prion strains with shorter incubation periods typically have lower PrP^{Sc} stability compared with that of strains with longer incubation periods (158–160). In contrast, hamster prion strains with short incubation periods have relatively high PrP^{Sc} stability compared with strains with long incubation periods (161, 162). Similar to the hamster prion model, patients with sCJD who are accumulating stable PrP^{Sc} had a shorter, more rapidly progressive clinical disease, potentially due to faster PrP^{C} conversion (163, 164).

The protease-sensitive forms of PrP^{Sc}, $sPrP^{Sc}$, have been implicated in disease pathogenesis (165), and factoring in these species may also help explain the above discrepancies in PrP^{Sc} stability and incubation periods. The relative ratio of $sPrP^{Sc}$ to proteinase K–resistant PrP^{Sc} is strain specific, and evidence suggests that these small $sPrP^{Sc}$ oligomers can influence the prion conversion rate (166, 167). However, some groups have suggested that the abundance of $sPrP^{Sc}$ does not exceed 10% of the total amount of PrP^{Sc} and, therefore, they downplay the relative contributions of these species to disease (168). Overall, the relationship between the biochemical properties of PrP^{Sc} and the outcome of disease is still poorly understood. This may be due, in part, to the many other factors that contribute to the incubation period of disease in vivo, including the various clearance mechanisms. The use of protein misfolding cyclic amplification (PMCA), which recapitulates prion conversion in vitro (169), continues to provide useful information on factors that influence the rate of PrP^{Sc} formation.

PrP^{C} is the major host factor that controls the tempo of prion formation. Genetic ablation of *Prnp* renders animals resistant to prion infection and agent replication (170–173). Conversely, increasing PrP^{C} expression results in a reduction in the incubation period (174, 175). Consistent with these in vivo studies, in vitro experiments have shown that the abundance of PrP^{C} positively correlates with conversion efficiency (176). Interestingly, in 2014, Mays and colleagues (44) reported that as prion disease progresses, the PrP^{C} level is reduced. The reduction in PrP^{C} levels may contribute to a decline in the rate of prion conversion or slow the onset of neurodegeneration, or both (177). Additional PrP^{C} factors that influence conversion include the posttranslational modifications of PrP^{C}. Specifically, the sialylation status of the *N*-linked glycans impacts prion conversion in a strain-dependent manner (178). Consistent with this observation, removal of sialylation can increase the efficiency of prion formation (134, 179).

Host cellular cofactors also influence the rate of prion formation. Removal of RNA significantly reduced PrP^{Sc} formation, whereas RNA supplementation restored PrP^{Sc} formation in a PMCA reaction (180). Interestingly, the extent of reduction induced by RNA depletion was strain dependent, as was the composition of nucleic acid that restored PrP^{Sc} formation (181). Phosphatidylethanolamine (PE) also supported the formation of both mouse and hamster PrP^{Sc} in vitro (182). Importantly, PMCA conversion of three separate prion strains with PE as a cofactor resulted in the three strains converging into a single strain (182). Recent evidence suggests that

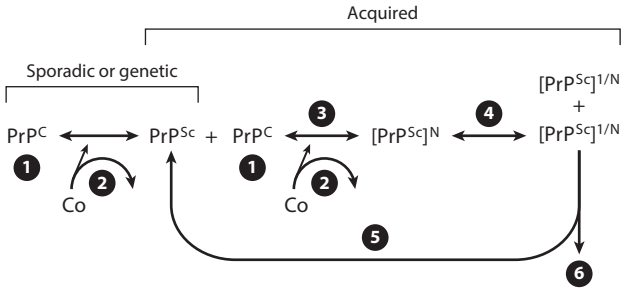

Figure 3

Strain-specific factors in prion formation. ❶ Prion formation depends on the presence of PrP^C. ❷ For the conversion of PrP^C to PrP^{Sc} in sporadic, genetic, or acquired etiologies, cofactors (Co) may participate in the formation of PrP^{Sc}, although it is unknown whether they are incorporated into the growing polymer or simply used as a structural scaffold. ❸ The rate of PrP^{Sc} formation is dictated by the incoming prion strain (PrP^{Sc}), the level of PrP^C (❶), and the cofactors present (❷). ❹ PrP^{Sc} fragmentation can result in newly fragmented PrP^{Sc} serving as ❺ a seed for conversion or ❻ PrP^{Sc} clearance from the cell. The rate of prion formation (❸) must be greater than the rate of clearance (❻) to establish a productive infection. Strain-specific PrP^{Sc} conformations may utilize specific subpopulations of PrP^C, cofactors, and clearance mechanisms, and all of these may contribute to strain-specific cellular and tissue tropism.

strain-specific cofactors may not be the only mechanisms responsible for prion tissue tropism. For example, if the relative rate of PrP^{Sc} clearance exceeds PrP^{Sc} formation, infection is not established (183). Overall, the distribution of convertible PrP^C and host cellular cofactors, in combination with the relative rates of prion formation and clearance, may influence the strain-specific pace and tropism of disease (**Figure 3**).

MULTIPLE PRION STRAINS CAN COEXIST IN A HOST

Multiple prion subtypes are commonly found to coexist in patients with sCJD (184), and interestingly, the subtypes have different rates of PrP^{Sc} formation in vitro (185). The relative percentage of sCJD cases that contain both PrP^{Sc} subtypes has not been agreed upon (186–188). Differences in estimates of the co-occurrence of PrP^{Sc} subtypes may be explained by incomplete proteinase K digestion of PrP^{Sc} that allows for an overestimation (189) or by the sampling of a limited number of brain regions or employing a limited number of anti-PrP antibodies, which may lead to an underestimation. Overall, it is clear that in human prion disease, mixtures of prion subtypes occur. The effects of these subtype mixtures on disease development and transmission in natural cases of prion disease are unclear.

Prion strains can interfere with conversion when mixtures of them are present. Prion strain interference occurs when a slowly replicating (long incubation period) strain interferes with the replication of a relatively quickly replicating (shorter incubation period) strain. The relative onset of replication of the blocking and superinfecting strains dictates the outcome of strain emergence (190). Consistent with this observation, replication of the blocking strain is required for strain interference to occur (191, 192). Interestingly, in animals infected with two strains under conditions in which strain interference does not occur, PrP^{Sc} levels of both strains are altered (193). This is consistent with the hypothesis that prions have the properties of quasispecies, which are considered to be populations of similar, but not identical, conformations of PrP^{Sc} (194). Altering the prion conversion environment in vitro can also alter the strain's properties (195–198), and the selection of drug-resistant prions that revert to a drug-sensitive phenotype once the drug is

removed is consistent with this hypothesis (195). Overall, prion strains are highly dynamic mixtures regardless of their incubation periods or the clinical outcome of the disease, and this must be considered when developing therapies that may target specific prion conformations.

THERAPEUTIC IMPLICATIONS

Prions cause toxicity in the CNS, and yet the underlying mechanisms remain incompletely defined. Neuronal PrP^C is part of a key pathway inciting neurodegeneration, as demonstrated by an elegant study from Mallucci and colleagues (199) showing that depleting neuronal PrP^C in transgenic mice 8 weeks postinoculation reverses early spongiform degeneration and the progression to clinical scrapie. Such remarkable findings, together with a rich body of research that indicates a requirement for PrP^C in prion-induced neurodegeneration, indicate that reducing PrP^C expression may be a key therapeutic intervention.

Prion activation of the unfolded protein response leads to a decrease in protein translation that is associated with synaptic failure and neuronal loss in mice with prion disease (81), and restoring protein translation is neuroprotective (81). Thus, as a second possible therapy, pharmacological restoration of protein translation may aid neuronal survival (200). Additional potential therapeutic strategies may rely on increasing the clearance of prion aggregates, blocking the cell-to-cell spread of prions, and directly inhibiting prion conversion using mutated full-length or peptide fragments of PrP^C that bind PrP^{Sc} and block fibril growth.

FUTURE DIRECTIONS

Although much has been discovered in recent years about the mechanisms of prion conversion, transmission, and pathogenesis, basic structural and mechanistic questions about prion diseases remain unresolved. How are the multiple functions of PrP^C executed, and how do PrP^C proteolytic cleavage products contribute to the purported functions? What is the structure of PrP^{Sc}, and how do PrP^{Sc} molecules from different strains vary in structure? How does the structure of PrP^{Sc} impact neural cell targeting and neuronal toxicity? What are the pathways of prion-induced neuronal toxicity? How do prions spread through the brain? What are the major prion clearance pathways? Prion disease investigation has led the way in dementia research, and answers to the questions raised here are within reach. Answers to these basic questions will enable the rational design of new therapeutic strategies.

DISCLOSURE STATEMENT

M.G. is funded by the Federal Ministry of Education and Research, the German Research Foundation, and the Herz Foundation (UndoAD, SFB877, Infectophysics). C.J.S. is supported in part by grants from the US National Institutes of Health (NS069566, NS0756896, and NS105498).

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Annual Review of Pathology: Mechanisms of Disease

Volume 14, 2019

Contents

Errata

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