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Cellular and Molecular Mechanisms of Prion Disease

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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 histopathologic 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, from the function of the cellular prion protein to the dysfunction triggering neurotoxicity, as well as mechanisms underlying prion spread between cells, and the effect of prion conformation on spreading pathways. 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 is encoded by the prion gene, *PRNP,* on chromosome 20 in humans (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, and with a notable lack of peripheral inflammatory cells (5) (Figure 1). Although the incubation period may be years, the clinical phase is typically rapidly progressive (weeks to months) and may include behavior abnormalities, motor dysfunction, cognitive impairment, and ataxia, depending on the prion and species affected (6). No therapy is currently 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 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 clinical symptoms and neuropathological features and consist of familial CJD (fCJD), fatal familial insomnia (FFI), and Gerstmann-Sträussler-Scheinker (GSS) disease. The mutations in *PRNP* are autosomal dominant, highly penetrant, and consist of missense mutations, insertions, and deletions, usually inciting disease onset in the 5^{th} or 6^{th} decade of life (6). Acquired prion diseases have been transmitted between individuals (kuru and iatrogenic CJD) and rarely from cattle to human (variant of 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 from prion-contaminated neurosurgical instruments and electrodes (16).

In addition to iatrogenic prion infection, prions have also precipitated large-scale, multi-species epidemics and even spread as a zoonosis. 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 Great Britain (18). By 1996, vCJD had targeted mainly young people (2^{nd} decade) in the United Kingdom, likely from exposure to BSE-contaminated beef (9, 19), with 229 vCJD cases diagnosed to date (20). No recent cases have occurred, however secondary transmission of vCJD prions arose in transfusion recipients receiving blood or blood products originating from prion-infected donors (10–13).

Prion diseases in animals, including BSE, are largely acquired by ingestion, although 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 or captive deer, elk, reindeer, and moose (family Cervidae) in 25 U.S. states and two Canadian provinces, as well as ranched elk in South Korea (26) and most recently wild reindeer and moose in Norway (27) and Finland (28). Transmissible mink encephalopathy (TME) has been previously identified in farmed mink in the US, Canada, Russia, Finland and East Germany, and was thought to be due to dietary exposure to a prion-infected animal, although the origin of the epidemic remains unclear and no recent cases have been reported (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 subject of this review focused on recent findings in prion pathogenesis. We also highlight new research linking prion conformation to disease phenotype.

Cellular Biology of the prion protein-- function and proteostasis

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 anti-parallel 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 either recycled to the plasma membrane or the Golgi (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 a Disintegrin and Metalloproteinase family) (41) as potential proteases, yet recent data 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, 48).

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, we (MG and colleagues) have recently proposed to list PrP^C among the expanding group of “multifunctional” proteins, in which several functions are attributed to just one protein (36). An incomplete list of PrP^C functions would include its role in neural development (49), cell adhesion (50), axon guidance, synapse formation (51), neuroprotection (52, 53), regulation of circadian rhythm (54), myelin maintenance (55, 56) maintenance of ion homeostasis (57, 58), and signaling (59, 60).

Interestingly, some of the best described functions are not accredited to PrP^C in its membrane-bound, GPI-anchored form but rather to soluble PrP^C fragments, which can only be generated by regulated proteoloysis such as alpha-cleavage and shedding. This is true for the recently described function of soluble PrP in the maintenance of myelin homeostasis (56), or for the role of soluble versions of PrP in inducing neurite outgrowth (61). For myelin maintenance, binding of the flexible N-terminal part of soluble PrP acts as agonistic ligand to a G-protein-coupled receptor (GPCR) expressed on Schwann cells, Adgrg6 (Gpr126) (56), whereas the molecular details for the neurite outgrowth-promoting role of soluble PrP are not understood. Interestingly in this instance, membrane-bound PrP^C itself may act as a receptor via homophilic interactions (61). In both instances, it is obvious that regulated proteolysis would be an elegant mode of functional regulation to transmit information to distant sites. This is reminiscent of functions attributed to proteolytic cleavage fragments from the amyloid precursor protein (APP) (62, 63). 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 function of PrP^{C} vs toxic gain of function of PrP^{Sc}?: 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 devoid of specific nucleic acids (64). Today we know that a pathogenic, conformationally altered version of PrP^{C} is a key component of the infectious agent responsible for transmission of prion diseases. This disease-associated version of PrP^{C} can be partially resistant against protease-digestion and is designated as PrP^{Sc} (where Sc stands for the pathogenic version of the protein found in sheep suffering from "scrapie", a prion disease found only in sheep and goats). 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, referred to here as $sPrP^{Sc}$, and since these versions are just as infectious as PrP^{Sc}, a biochemical definition of protease-resistance is not adequate (65). Thus one has to include protease-sensitive disease associated PrP species into the pool of conformationally-altered versions of PrP able to induce prion disease. Alternatively, the term "PrP^{Sc}" is still widely used to describe disease associated PrP-species and for the sake of clarity, we will 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) (66). 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 tightly interdigitate and effectively stabilize growing fibrils, largely through hydrogen bonds (67, 68). Sequence differences within steric zipper segments have been shown to block prion conversion between species (69, 70).

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

The generation and progressive accumulation of PrP^{Sc} is of key importance for the development of clinical prion disease, although there are rare instances, such as subclinical disease in prion-infected mice, where the presence of PrP^{Sc} does not lead to neurodegeneration (71). 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 role in prion disease is that loss of PrP^{C} function in PrP knockout mice does not lead to neuronal death (72). On the other hand, 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 (73). Thus a certainly recurring redundancy in this system may compensate

loss of function phenotypes, which may only become apparent once additional stressors are active (36, 56).

Mechanisms underlying the toxic gain of function of PrP^{Sc}—The evidence for direct or indirect neurotoxicity of PrP^{Sc} is compelling and there is no doubt that cerebral accumulation of misfolded PrP^{Sc} plays a key role in the pathophysiology of prion diseases, but how?

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 (74). Disturbed proteostasis occurs in prion disease at multiple levels. PrP^{Sc} disturbs the ubiquitin/proteasome system already at early disease states leading to impaired function of this protein degradation pathway, enhancing the buildup of PrP^{Sc} (75). There is also mounting evidence that buildup of PrP^{Sc} affects the autophagy–lysosome pathway responsible for 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 (76). Additionally there is evidence that exhaustion of unfolded protein response (UPR) pathways occurs early in prion disease (77–79). UPR is a cellular stress response aiming to protect the endoplasmic reticulum regarding its function in protein synthesis and sorting. PrP^{Sc} stresses the ER 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 (80). Interestingly, restoring the disturbed protein translation has been shown to be neuroprotective (80).

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 (81). Morphological studies have shown the close relationship of PrP^{Sc}-deposits and the neuronal plasma membrane (82). 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 (83). GPI-anchored PrP^{C} is able to efficiently transduce neurotoxicity and prion disease is accelerated in mice where PrP^{C} shedding is impaired, both of which support this line of thought (84, 85). 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, where oligomeric species of Aβ bind membrane PrP^{C} complexed to metabotropic glutamate receptor (mGluR5), activating intracellular Fyn kinase and ultimately leading to synaptotoxicity (86–90).

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 ROS production (91). PrP^{Sc} has been found to cause a similar toxic signaling cascade, again with calpain activation and ROS generation (92). In cultured primary neurons expressing a mutant PrP lacking residues in a central region (105–

125), abnormal ion channel currents occurred, sensitizing neurons to glutamate-induced excitotoxicity. These abnormal currents may represent very early toxic signaling events in affected cells and underlie early neurodegeneration (93). Nevertheless, the sequence of events leading to receptor-mediated neurotoxicity are not yet completely defined and GPI-anchored PrP^{C} would need a co-receptor to enable intraneuronal signal transduction.

Prion spread into the CNS – an update: Similar to rare neurotropic infectious agents such as rabies virus, prions have managed to access the CNS from extraneural entry sites. The initial prion replication site in the CNS can often be linked to the entry site by peripheral nerves, incriminating retrograde axonal transport as a possible mechanism of prion transit. For example, feeding prions to hamsters leads to early prion deposition in enteric and autonomic ganglia as well as vagus and splanchnic nerves, and subsequently in the thoracic spinal cord and dorsal motor nucleus of the vagus in the brainstem, consistent with retrograde prion spread along autonomic PNS pathways into the CNS (94). BSE and CWD prions in cattle and deer, respectively, are also first detected in the CNS within the vagal nucleus, consistent with prion entry through the GI tract and transit via the vagal nerve into the brain (95, 96). Similarly, prion exposure of the mouse eye 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 (97). 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 (98, 99). Interestingly, prion conformation also plays a role in prion neuroinvasion, as fibril-forming prions spread poorly to the brain as compared to oligomeric or subfibrillar prions (100–103). Nevertheless, since prions circulate in blood within minutes post-inoculation (104), additional non-neural 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 that used by other infectious agents exploiting entry portals to invade the host. Transepithelial prions transit through the intestinal epithelium by way of M cells, as M cell depletion reduces oral susceptibility to prion disease (105); additional studies by multiple laboratories support M cells as key players that passage prions across the mucosal barrier (105–109). Enteritis may heighten susceptibility to oral prion infection, potentially by enabling prion passage through the mucosa (110). Once subepithelial, neurotropic prions, such as BSE, are thought to spread by retrograde axonal transport along autonomic PNS pathways into the brainstem (111, 112). Lymphotropic prions, such as sheep scrapie, deer CWD, and likely vCJD, also rapidly spread (within hours) to Peyer's patches and draining lymph nodes, potentially transported by classical dendritic cells (95, 113, 114), as depletion of dendritic cells impedes the early replication of prions in lymph nodes (115, 116). Interestingly, lymphotropic prions also accumulate within inflamed organs harboring lymphoid follicles, such as kidney or mammary gland, leading to prion excretion or secretion into urine or milk, respectively (117–120).

Within 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 (121), where it persists throughout the infection (95). FDCs trap antigens on their plasma membrane for display to B cells (122, 123) and have proven highly capable of replicating prions (124), sustaining lymphoid prion infections for months to years (124). On the surface of FDCs, the CD21/35 receptor is thought to bind a PrP^{Sc} - complement complex, as both soluble C1q and regulatory protein factor H bind PrP^{Sc} (125–128), and CD21/35 receptor knockout mice show low attack rates after a peripheral prion infection (128). Together these studies indicate a crucial role for complement receptors in prion replication and spread to the CNS.

This peripheral phase of prion replication has been exploited for developing prevention strategies to block prion spread to the CNS. FDCs require B cell signalling through tumor necrosis factor and lymphotoxin to develop and maintain a mature state (122), and blocking lymphotoxin signaling induces FDC dedifferentiation and prevents prion accumulation in lymphoid tissue. This prevention strategy has worked very effectively in mice treated with lymphotoxin β-receptor agonists or anti-receptor antibodies (129), abolishing splenic prion replication and prolonging disease following an intraperitoneal challenge (129). Preventing disease by this strategy must begin early, however, as nerve entry occurs quickly after a prion exposure, within 14 days post oral challenge in mice (130).

Lymphoid tissues may also serve as a source of new prion strains. Cross-species prion transmission has generated new prion strains within lymphoid tissues, suggesting that lymphoid tissues may be more promiscuous than CNS in replicating prions having a different PrP sequence (131). The mechanism underlying this reduced threshold for prion replication is unclear, however the PrP^{Sc} glycan sialation levels influence capture by complement receptors and replication in lymphoid tissue, and the glycans on PrP^{Sc} are more sialated in the lymphoid tissue than in brain (132). Highly sialated PrP^{Sc} has been postulated to contribute to the permissiveness of lymphoid tissue to prion replication (132, 133).

Prion conversion within the CNS—Once within the brain and spinal cord, prions are converted by neurons and astrocytes. Astrocytes are highly susceptible to prion infection in vitro and can readily transfer prions to neurons (134, 135). On the other hand, microglia do not have a major role in replication, but are instead critical for prion clearance; depletion of microglia accelerates disease in vivo and increases PrP^{Sc} accumulation in organotypic brain slices (136). In contrast, oligodendrocytes lack any known significant contribution to prion replication or spread through the CNS (124). 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 (137–139) and between neurons and astrocytes (140).

Cell-to-cell prion spread through the CNS—Once in the brain, prions spread through neuroanatomically connected brain regions by poorly understood mechanisms (141–144). *In vitro*, prions spread cell-to-cell via (i) exosomes and (ii) tunneling nanotubes (138, 145–147), with yet-to-be-tested other possible mechanisms including synaptosomes, GPI-painting, microvesicles, or 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 multivesicular bodies (MVB) (147–149)[add Greene]. The extent to which prions are released within exosomes varies depending on the prion strain, as certain strains traffic more extensively into exosomes (145).

Further supporting the importance of EVs in prion transport, Saa and colleagues showed that vCJD prion-infected mice harbored EVs containing infectious prions in plasma starting at preclinical disease stages (150), which suggests that vesicles may transport prions long-range. Nevertheless, whether exosomes or other EVs are the most relevant mechanism for prion spread through the CNS is not yet clear. Recent methodological advances for isolating exosomes and other EVs from the brain is expected to shed light on the role of exosomes for prion spread *in vivo* (151).

Another possible route for direct cell-to-cell spread of prions is through tunneling nanotubes. Tunnelling 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 (139, 152). In addition to organelles, PrP^{Sc} was transferred to naïve cells via nanotubes, including transfer from primary dendritic cells to neurons as well as from neuron-to-neuron (138). Tunnelling nanotubes may be induced by cell stress.

Lysosomes may also be involved in the cell-to-cell transport of prions, either through transfer within tunnelling nanotubes or through lysosomal exocytosis, as observed for amyloid-β and α-synuclein (153, 154).

PrP^{Sc} conformation impacts disease phenotype—In experimental prion disease of rodents, a wide range of incubation periods and brain targets have been reported (155), depending on the prion conformation, or strain. Much work has been done to examine the relationship between the biochemical properties of PrP^{Sc} and the survival time. Studies of yeast prions (Sup35) indicated that the rate of prion propagation is inversely proportional to the aggregate stability, and suggested that more fibril fragmentation, or a higher “frangibility”, would produce new free ends for prion formation and accelerate prion propagation (156). Consistent with this notion, murine prion strains with shorter incubation periods typically have a lower PrP^{Sc} stability compared to that of longer incubation period strains (157–159). In contrast however, hamster prion strains with short incubation periods had a relatively high PrP^{Sc} stability compared to long incubation period strains (160, 161). Similar to the hamster prion model, patients with sporadic CJD accumulating stable PrP^{Sc} had a shorter, more rapidly progressive clinical disease, potentially due instead to faster PrP^{C} conversion (162, 163).

Protease-sensitive forms of PrP^{Sc}, $sPrP^{Sc}$, have been implicated in disease pathogenesis (164), and factoring in these species may also help explain the above discrepancies in PrP^{Sc} stability and incubation period. The relative ratio of $sPrP^{Sc}$ to PK-resistant PrP^{Sc} is strain-specific, and evidence suggests that these small $sPrP^{Sc}$ oligomers can influence the prion conversion rate (165, 166). However, some groups have suggested that the abundance of $sPrP^{Sc}$ does not exceed 10% of the total amount of PrP^{Sc} and, therefore, downplay the

relative contributions of these species to disease (167). 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* (168), 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 (169–172). Conversely, increasing PrP^{C} expression results in a reduction in the incubation period (173, 174). Consistent with these *in vivo* studies, *in vitro* experiments have shown that the abundance of PrP^{C} positively correlates to conversion efficiency (175). Interestingly, recent work has shown that as the prion disease progresses, the PrP^{C} level is reduced (44). The reduction in PrP^{C} levels may contribute to a decline in the rate of prion conversion and/or slow the onset of neurodegeneration (176). Additional PrP^{C} factors that influence conversion include the post-translational modifications of PrP^{C}. Specifically, the sialylation status of the N-links glycans impacts prion conversion in a strain dependent manner (177). Consistent with this observation, removal of sialylation can increase the efficiency of prion formation (133, 178).

Host cellular co-factors 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 (179). Interestingly, the extent of reduction induced by RNA depletion was strain dependent as was the composition of nucleic acid that restored PrP^{Sc} formation (180). Phosphatidylethanolamine (PE) also supported the formation of both mouse and hamster PrP^{Sc} *in vitro* (181). Importantly, PMCA conversion of three separate prion strains with PE as a co-factor resulted in the three strains converging into a single strain (181). Recent evidence suggests that strain specific co-factors may not be the only mechanism responsible for prion tissue tropism. For example, if the relative rate of PrP^{Sc} clearance exceeds PrP^{Sc} formation, infection is not established (182). Overall, the distribution of convertible PrP^{C} and host cellular co-factors, 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—A co-existence of prion subtypes is commonly found in sCJD-affected patients (183), and interestingly, the subtypes have different rates of PrP^{Sc} formation *in vitro* (184). The relative percentage of sCJD cases that contain both PrP^{Sc} subtypes is not agreed upon (185–187). Differences in estimates of the co-occurrence of PrP^{Sc} subtypes may be explained by incomplete PK digestion of PrP^{Sc} that may allow for an overestimation (188), or sampling 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 effect of these subtype mixtures on disease development and transmission in natural cases of prion disease are unclear.

Prion strains can interfere with conversion when present in mixtures. Prion strain interference occurs when a slowly replicating (long incubation period) strain interferes with the replication of a relatively quickly replicating (shorter incubation period) prion strain. The relative onset of replication of the blocking and superinfecting strain dictates the outcome of strain emergence (189). Consistent with this observation, replication of the blocking strain is required for strain interference to occur (190, 191). Interestingly, in animals infected with two strains under conditions where strain interference does not occur, PrP^{Sc} levels of both strains are altered (192). This is consistent with the hypothesis that prions have properties of a quasispecies, hypothesized to be populations of similar, but not identical, conformations of PrP^{Sc} (193). Altering the prion conversion environment *in vitro* can also alter the strain properties (194–197), and the selection of drug resistant prions that revert to a drug sensitive phenotype once the drug is removed is consistent with this hypothesis (194). Overall, prion strains are highly dynamic mixtures regardless of incubation period or clinical outcome of disease, and must be considered in the development of therapies that may target specific prion conformations.

Therapeutic Implications—Prions cause toxicity in the central nervous system and yet the underlying mechanisms remain incompletely defined. Neuronal PrP^{C} is part of a key pathway inciting neurodegeneration, as an elegant study from Mallucci and colleagues showed that depleting neuronal PrP^{C} in transgenic mice 8 weeks post-inoculation reverses early spongiform degeneration and the progression to clinical scrapie (198). Such remarkable findings, together with a rich body of research that indicates the requirement of 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 associated with synaptic failure and neuronal loss in prion-diseased mice (80), and restoring protein translation is neuroprotective (80). Thus as a second possible therapy, pharmacologic restoration of protein translation may aid neuronal survival (199). 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. Taking these studies t(85)ogether, the essential role of PrP^{C} in mediating neuronal toxicity is clear, and much has been learned in recent years about the mechanisms of toxicity, yet a complete understanding of how neurodegeneration develops remains to be elucidated.

Future Directions

Although much has been discovered in recent years on the mechanisms of prion conversion, transmission, and pathogenesis, basic structural and mechanistic questions in the prion disease field 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 widely do PrP^{Sc} molecules from different strains vary in structure? How does the structure of PrP^{Sc} impact neural cell targeting and influence 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 in recent years and answers to questions raised above are within reach. Additionally, answers to these basic questions will enable the rationale design of new therapeutic strategies, which may also help to restore lost confidence regarding novel therapeutic approaches in the wider field of dementia research.

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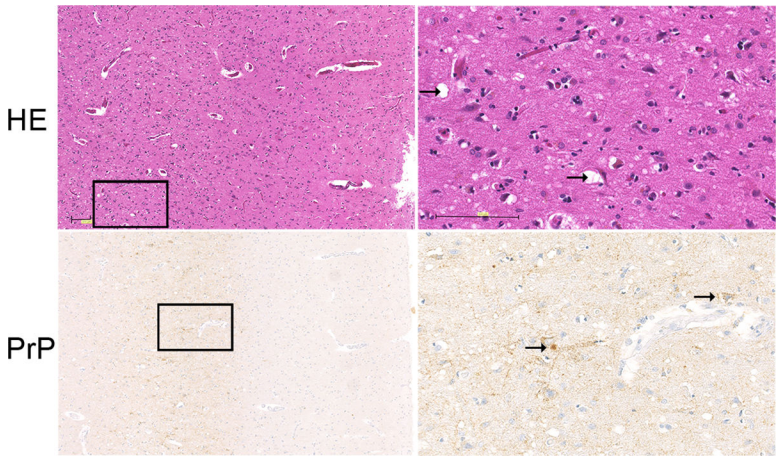

Figure 1.

Hematoxylin and eosin and PrP immunostain of brain (frontal cortex) from a sCJD patient. Spongiosis is visible in the deep layers of the cortex (HE) (arrow indicates intaneuronal and parenchymal spongiform change, arrow) and synaptic, plaque-like, and perineuronal deposits of pathological prion protein (arrow indicates 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.

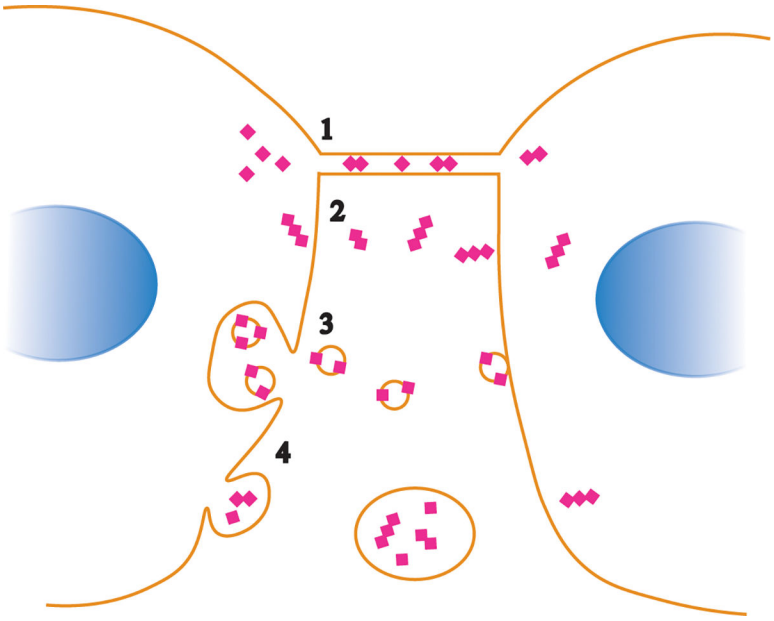

Figure 2.

Possible pathways of prion spread from cell-to-cell. Prion aggregates may spread through transport in tunneling nanotubes (1), GPI painting, by which GPI-anchored proteins transfer from one cell surface to a neighboring cell surface (2), trafficking within exosomes (3), or from membrane budding and transport within vesicles (4).

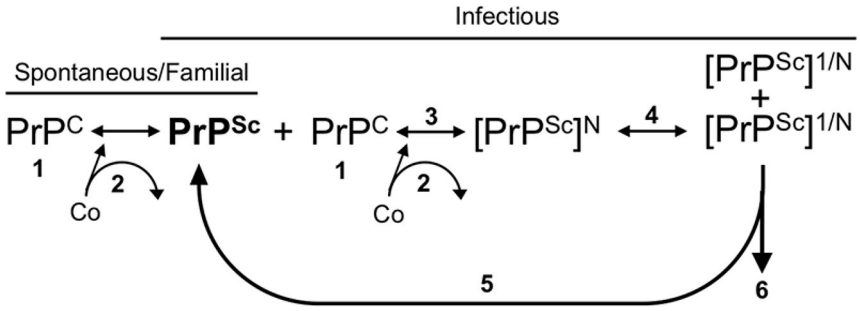

Figure 3.

Strain-specific factors in prion formation. Prion formation is dependent on the presence of PrP^{C} (1). For the conversion of PrP^{C} to PrP^{Sc} in spontaneous, familial, or infectious etiologies, cofactors (Co) may participate in the formation of PrP^{Sc}, although it is unknown if they are incorporated into the growing polymer or simply used as a structural scaffold (2). The rate of PrP^{Sc} formation (3) is dictated by the incoming prion strain (PrP^{Sc}), the level of PrP^{C} (1), and the cofactors present (2). PrP^{Sc} fragmentation can result in newly fragmented PrP^{Sc} serving as a seed for conversion (5) or PrP^{Sc} clearance from the cell (6). The rate of prion formation (3) must be greater than the rate of clearance (6) to establish a productive infection. Strain-specific PrP^{Sc} conformations may utilize specific subpopulations of PrP^{C}, cofactors, and clearance mechanisms that may all contribute to strain-specific cellular and tissue tropism.