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Prions and the Potential Transmissibility of Protein Misfolding Diseases*

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Abstract

Prions, or infectious proteins, represent a major frontier in the study of infectious agents. The prions responsible for mammalian transmissible spongiform encephalopathies (TSEs) are due primarily to infectious self-propagation of misfolded prion proteins. TSE prion structures remain ill-defined, other than being highly structured, self-propagating, and often fibrillar protein multimers with the capacity to seed, or template, the conversion of their normal monomeric precursors into a pathogenic form. Purified TSE prions usually take the form of amyloid fibrils, which are self-seeding ultrastructures common to many serious protein misfolding diseases such as Alzheimer's, Parkinson's, Huntington's and Lou Gehrig's (amytrophic lateral sclerosis). Indeed, recent reports have now provided evidence of prion-like propagation of several misfolded proteins from cell to cell, if not from tissue to tissue or individual to individual. These findings raise concerns that various protein misfolding diseases might have spreading, prion-like etiologies that contribute to pathogenesis or prevalence.

Keywords

prion; TSE; amytrophic lateral sclerosis; Alzheimer's; Parkinson's; Huntington's; transmissibility; protein misfolding

THE PRION CONCEPT

The realm of microbiology continues to be stretched. Despite their lack of nucleic acid genomes, prions have been added over recent decades to the pantheon of pathogens. Now there is a pressing need to consider the possible prion-like credentials of the many misfolded proteins that are central to the pathology of a number of serious diseases, including Alzheimer's and Parkinson's. At issue is the extent to which such misfolded proteins, often in the form of multimeric amyloid fibrils, have the ability to propagate themselves within and between individuals to cause, or at least enhance, disease.

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The prion concept has evolved over time. Well before Stanley B. Prusiner coined the term prion, scientists proposed that the infectious agents of transmissible spongiform encephalopathies (TSEs) were abnormal self-propagating states of host-derived proteins (55). After Prusiner discovered the offending protein of the mammalian TSEs, he defined a prion as “a small proteinaceous infectious particle that is resistant to inactivation by most procedures that modify nucleic acids” (110). Reed Wickner, in discovering yeast prions, extended the prion concept in genetic terms to include protein-based, rather than nucleic acid–based, elements of inheritance (141). In doing so, Wickner explained that the transfer of self-propagating states of certain proteins, i.e., between individual cells, could convey heritable phenotypic changes in the recipient cells.

Both mammalian and fungal prions are derived from the self-propagating modification of endogenous proteins. However, the misfolding or the autocatalytic refolding of proteins alone does not fully embrace the fundamental prion concepts outlined by Prusiner, Wickner, Gadjusek (48), and Jarrett & Lansbury (66), among others. Protein misfolding and the autocatalytic or templated assembly of proteins or other molecules into macromolecular structures occur routinely in biology and does not often involve the replication and propagation between individuals that is inherent to the prion concept.

In practice, several basic biochemical features characterize prions (27). First is the existence of a self-propagating state of a protein (the prion) that is biologically accessible but rarely initiated spontaneously. Second is the ability to replicate by acting on their normal nonprion host-encoded substrate protein. And third is propagation to naïve hosts to find new substrate pools for replication (16).

These features are clearly evident for both mammalian and yeast prions. The self-propagating state of mammalian TSE prions is an oligomeric (122) and often more proteinase-K-resistant conformer of prion protein (PrP), which is often designated PrP^{RES} or PrP^{Sc} (PrP-scrapie) (89). Although PrP^{RES} can accumulate to high levels under physiological conditions, and therefore is biologically accessible, it rarely accumulates spontaneously as exemplified by the low incidence of spontaneously arising TSE diseases. For example, in humans, sporadic Creutzfeldt-Jakob disease has a worldwide incidence of ~1 case per million humans per year. PrP^{RES} propagates by acting on its normal soluble, protease-sensitive cellular substrate PrP^{C}, or PrP^{SEN} (75, 116), and can amplify itself by more than 10^{10}-fold in infected individuals. And finally, although most TSE prions are not highly contagious, they can clearly spread between and within individuals to act on new pools of PrP^{C} substrate.

In the case of yeast prions, the basic biochemical features are exemplified by [URE3], an oligomeric self-propagating form of the protein Ure2p (141). The [URE3] prion state rarely arises spontaneously, but once it does arise, it replicates by incorporating its normal soluble (nonprion) Ure2p substrate as it aggregates in the yeast cell cytoplasm. The [URE3] prion aggregates can be transmitted vertically to daughter cells by cell division or horizontally to other cells through cytoplasmic exchange associated with yeast cell mating.

Although mammalian TSEs and yeast prions share some fundamental characteristics as self-propagating protein isoforms, they also represent extremes of bona fide prions in biology. It seems likely that other types of prions will be found within the diverse organisms that populate the phylogenetic tree. Recent experimental evidence indicates that common protein misfolding diseases might arise from the propagation and transmission of pathogenic misfolded proteins in a manner similar to that for prions. With respect to the many important protein misfolding diseases in mammals, a spectrum of possibilities may exist ranging between fully transmissible TSE diseases and simple protein misfolding events without any self-propagation within or between individuals. Indeed, names such as prionoids (2) have been proposed for misfolded proteins that fall somewhere between these extremes of prion-ness or lack thereof. In any case, it is critical to understand the extent to which the self-propagation, or at least the self-promotion, of the misfolding of various proteins is relevant to disease pathogenesis. Even if epidemiology ultimately determines that diseases such as Alzheimer's and Parkinson's are not contagious or transmissible in any practical sense, it may be critical for diagnostic and therapeutic purposes to know what causes misfolded proteins to accumulate. Accumulation could depend on the initiation of misfolding in a single discrete site, followed by propagation to other sites (a prion-like spreading effect), or on the widespread initiation of protein misfolding and aggregation at many independent sites in affected tissues without spreading (a nonprion etiology). Much effort is rightly being made to discriminate between these possibilities for a number of protein misfolding diseases of humans and animals. In what follows, we review recent advances in the understanding of prion structures and transmission cycles and consider evidence as to whether related prion-like etiologies are at play in various other protein misfolding diseases.

PRION STRUCTURES

The typically aggregated and noncrystalline properties of known infectious prions make them poorly suited to atomic-resolution conformational analyses by X-ray diffraction and solution NMR techniques. Thus, the determination of their structures has been challenging. In the case of the prototypic TSEs, the native monomeric prion protein, PrP^{C}, is converted to an abnormal, oligomeric and often amyloid form (PrP^{Sc} or PrP^{RES}) (93, 111, 122, 123). However, after decades of research little is known about PrP^{Sc} structures beyond their aberrant ultrastructures, secondary structure compositions, epitopes, and H/D exchange profiles. In contrast to the largely α-helical and flexibly disordered PrP^{C}, PrP^{RES} has a much higher β-sheet content (20, 102, 117) and appears to lack the native α-helices of PrP^{C} (9, 30, 80, 117, 126). This conversion to high β-sheet conformers has been observed across many PrP^{RES} strains in multiple host species (9, 19, 127). Purified PrP^{RES} usually takes the form of amyloid fibrils (20, 40, 111), but various nonfibrillar ultrastructures have been visualized and some have been shown to be highly infectious (90, 122, 142). Moreover, amyloid fibrils are not always visible within disease-specific PrP deposits in brain tissue (16, 68). Thus, although PrP^{Sc} amyloids are highly infectious and can sometimes be the predominant form deposited in vivo (25, 49), it is likely that nonamyloid or subamyloid forms of PrP^{Sc} are important as well (90, 122, 143). We should emphasize also that many PrP amyloid fibrils, notably most of those formed in vitro from pure recombinant PrP^{C}, are not infectious and can be readily distinguished conformationally from bona fide PrP^{RES} (125, 126, 142). The

cell-free formation of prions from recombinant PrP^{C} that are infectious for wild-type animals requires specific reaction conditions and either seeding with infectious prions (72) or the addition of cofactors (33, 85, 138).

Adding to the complexity of PrP^{RES} structural determinations is the variation between TSE strains (see sidebar, Prion Strains and Amyloid Conformers), even those derived from the same host species expressing a single PrP^{C} sequence (reviewed in 16). Strain differences, as well as the differences observed between various infectious and noninfectious PrP amyloids, provide evidence for the importance of precise templating in PrP^{Sc} propagation (13, 132). Although the details are far from clear, PrP^{Sc} propagation, like amyloid fibril formation in general, occurs via a seeded or templated polymerization reaction in which preexisting PrP^{Sc} oligomers provide a surface that promotes the binding and refolding of incoming PrP monomers (63, 75). Remarkably, the specific geometry and reactivity of the seed somehow dictate that each incoming monomer adopts a similar conformation (13, 132) as has been described for several yeast prions (135).

PRION STRAINS AND AMYLOID CONFORMERS

Historically, distinct TSE strains were distinguished by their biological properties and disease manifestations. Like viruses, individual TSE strains produce distinct and reproducible neuropathological patterns (lesion profiles) and incubation times. Structural and biochemical techniques have indicated that this unique strain pathology may arise from differences at a structural level. Experimental measures of strain-specific PrP amyloid fibril ultrastructure, stability, conformational templating, proteolytic sensitivity, and glycoform pattern indicate that strains have individual structural characteristics that are faithfully propagated through a templated conversion mechanism. Similarly, various fungal prion strains have been associated with distinct biological and structural characteristics that can be maintained as the prions are replicated within a culture. By analogy to prion strains at the structural level, amyloid fibrils composed of other proteins or peptides can also exist in different conformations that can propagate themselves faithfully, at least in vitro. Such amyloid variants of a given protein might be described as amyloid strains, but whether they correlate with distinct biological activities or pathologies in vivo remains to be determined.

Current Models of PrP^{Sc} Structure

As various pieces of the structural puzzle have begun to fall into place, several models for the structure of PrP^{Sc} have been generated based on low-resolution biochemical and biophysical data, computer modeling, and molecular dynamics simulations. These models include a left-handed β-helix model (53, 143), the β-spiral model (34, 35), and a domain-swapped model (57). Although such modeling has helped refine our thinking about PrP^{Sc} structure, recent empirical data seem to be incompatible with these prevailing models (9, 125). Most notably, each of these models proposes that most of the native α-helices of PrP^{C} are retained in PrP^{Sc}, but early circular dichroism data (117) and recent H/D exchange and infrared spectroscopy analyses of brain-derived PrP^{Sc} indicate that this is highly unlikely (6, 9, 125). The data also indicate that most of the protease-resistant C terminus of each

monomeric unit of PrP^{RES} amyloids is refolded into tightly packed intermolecularly hydrogen-bonded β-sheets separated by relatively short turns and/or loops. Moreover, other studies have shown that the C-terminal domain, which contains most of the native α-helical content in PrP^{C}, can readily convert to a structure with high β-sheet content (4, 124), and more specifically parallel in-register intermolecular β-sheets (29, 134), in cell-free amyloid fibrillization reactions starting with recombinant PrP^{C}.

These and other considerations suggest to us that the most plausible type of model would be a parallel, in-register β-sheet, a basic fibril architecture that has already been described for yeast prions (135). The schematic shown in Figure 1 represents an N-terminal extension of a model depicted previously by Cobb & Surewicz (31). In the present model the entire region of residues ~90–220 refolds, eliminating all native α-helices consistent with prior observations (9, 144, 125). PrP^{Sc} monomers stack along the fibril axis, forming stretches of parallel, in-register β-sheets. Detailed rationalization of this model is beyond the scope of this article, but we present it here as a means of visualizing, at least in principle, how the fibril ends could serve as precise templates to guide the folding of additional monomers in the manner described for yeast prions (135). Variations in the location, shape, and length of β-sheets, turns, and loops could define strain-specific conformations that must be propagated faithfully as the fibril grows. In any case, much remains to be done to evaluate the accuracy of this and other models of infectious PrP^{Sc} structures.

Structural Similarities Between PrP^{Sc} and Other Amyloids

The tendency to form high β-sheet amyloid or amyloid-like fibrils is a characteristic that PrP^{Sc} and yeast prions share with many other pathological misfolded protein or peptide aggregates (17, 135). For example, β-amyloid (Aβ) (1, 12, 104, 105), tau (5, 86), and α-synuclein (α-syn) (21, 74) can form parallel, in-register β-sheet fibrils, although antiparallel (112) and β-helical structures (69) have also been observed with fibrillar protein assemblies. Individual polypeptides can assemble into fibrils with different architectures (76, 108) in a manner that is reminiscent of the structural strain variation seen with PrP^{RES} isolates. Aβ, for instance, is capable of following several on- or off-pathway routes in vitro to form amyloids of vastly different structures (44, 108). X-ray crystallography studies have revealed that tight interdigitation of side chains can occur between adjacent β-sheets of numerous short synthetic peptides, forming dehydrated steric zippers (99, 118). These steric zippers can define distinct amyloid structures and help rationalize the propagation of distinct conformers or amyloid strains (see sidebar, Prion Strains and Amyloid Conformers). Steric zipper motifs occur in short stretches of different amyloid proteins (22, 144). They are proposed to stitch together specific sections of the proteins within amyloids to constrain the folding and assembly of newly recruited monomers during seeded/templated polymerization. Thus, from a purely structural perspective it seems possible that many different amyloids could promote their own propagation and do so by conformationally faithful mechanisms such as those of distinct PrP^{Sc} strains.

TRANSMISSIBILITY OF PRIONS

The mammalian prion transmission cycle is perhaps best represented by chronic wasting disease (CWD) because, among TSEs, CWD appears to be the most naturally contagious,

with prevalence rates as high as 50% in free-ranging deer and higher than 90% in captive herds (for review see 50). CWD infects white tail deer, mule deer, Rocky mountain elk, and moose and has been identified in the United States, Canada, and Korea. The zoonotic potential of CWD has still not been fully elucidated. Despite its rampant spread among cervids, natural cross-species transmission of CWD appears to be highly restricted. However, experimentally, transmission has been demonstrated in squirrel monkeys (87), domestic cats (88), voles, golden hamsters, minks, ferrets, goats, and cattle (reviewed in 50). However, macaques have shown resistance to CWD infection (113), implying that CWD is not likely to be readily transmissible to humans. Nonetheless, CWD appears to provide the most robust example of natural prion transmissibility in mammals.

Relative to the simplicity of yeast prion transmission by cellular cytoplasmic exchange, requirements for completing the full CWD transmission cycle between cervids are complex (Figure 2). Horizontal transfer of CWD requires multiple steps. First, PrP^{RES} must be taken up by the host. Exposure to PrP^{RES} will most likely occur at sites peripheral to the central nervous system (CNS) such as the alimentary tract, the skin, and the nasal mucosa. For instance, infections can be initiated via ingestion or exposure to aerosols (36, 37, 121). Second, the development of neurodegenerative disease requires that the infection spread to, and within, the nervous system. At the molecular level, this requires the transport and ultimately the propagation of PrP^{RES} within and between cells and tissues. Finally, propagation of the infectious disease cycle restarts when prions are shed from an infected host into the environment. Conceivably, the successful transmission of any protein misfolding disease might take place via similar mechanisms. As a prelude to considering possible prion-like transmissibility of various protein misfolding diseases, we first discuss spreading mechanisms that are thought to be involved in TSEs.

Cell-to-Cell Spread

Prion infection begins at the protein level, when infectious PrP^{RES} converts endogenous PrP^{C} to more PrP^{RES}. In most cases this occurs on the cell surface or in endocytic vesicles in part because both PrP^{C} and PrP^{Sc} are anchored to the membrane via a glycosylphosphatidylinositol (GPI) anchor. GPI anchoring of PrP distinguishes it from the other misfolded proteins that are proposed to act in a prion-like manner, such as Aβ or tau. Tethering of PrP^{C} to membranes is necessary for its conversion to PrP^{RES} in infected cells (18) and for sustaining their infections (91). When scrapie is inoculated intracerebrally into transgenic mice expressing only PrP^{C} that lacks the GPI anchor, PrP^{Sc} accumulates almost exclusively in extracellular amyloid plaques (24, 25). With peripheral inoculations, PrP^{Sc} amyloid accumulates in peripheral tissues but there is no neuroinvasion, indicating a key role of the GPI anchor in TSE prion spreading into and along neural circuits (73).

Within a particular cell, PrP^{Sc} formation must outpace protein-quality-control mechanisms attempting to degrade or refold the protein. Proteostasis is maintained in a cell through mechanisms that include chaperone proteins, lysosomal proteolysis, proteasomes, or autophagy. Accumulation of PrP^{Sc} must overwhelm the ability of these systems to degrade the misfolded protein. At the same time, growing prion multimers must fragment in order to produce more infectious seeds for propagation to other cells. This principle has been

demonstrated in yeast, in which prion fragmentation mediated by the chaperone protein Hsp104 is necessary for maintaining the prion-infected state (23). There is growing evidence that prions directly impede the function of quality control systems such as autophagy and proteosomal degradation (38, 62). This impediment would impair the cell's ability to deal with misfolded proteins and therefore might increase the susceptibility of the cell to PrP^{Sc} accumulation and the availability of PrP^{Sc} for seeding further propagation.

The transfer of PrP^{Sc} between cells appears to be aided by their close proximity (71, 103) and the insertion of GPI-anchored PrP^{RES} into the membrane of the recipient cell (8, 10, 128). Thus, cell-to-cell spread may include the transfer of membrane as well as PrP^{RES}. Consistent with this possibility, PrP^{C}, PrP^{RES}, and scrapie infectivity can be released in cultured cells in association with exosomes (45, 137). GPI anchoring promotes association of proteins with exosomes and might thereby promote prion propagation from cell to cell. Moreover, neuronal cells in culture internalize and transport PrP^{RES} aggregates in acidic vesicles along neurites to points of contact with other cells (84). Spread of PrP^{RES} between cells may also occur through tunneling nanotubes, which are thin membranous bridges formed between cells that are involved in the transfer of organelles, cytoplasmic components, plasma membrane and associated components, and pathogens such as viruses (16, 52). These intercellular conduits have been reported to mediate prion movement from immune cells to neurons as well as between neurons (52).

Tissue-to-Tissue Spread

Because TSE infections must invade the CNS to cause disease, spreading between tissues is required following peripheral infections (Figure 3). For instance, after oral exposure, prions are taken up by the gut mucosa and Peyer's patches and are transferred to the surface of follicular dendritic cells in lymphoid tissue and then to enteric nerves and the CNS (3, 139). With intra-tongue inoculations, prion neuroinvasion occurs via the cranial nerves (11). Alternative routes of prion infection may occur following blood transfusion from infected donors (64, 106). Efficient transmission also requires avoidance of the body's surveillance systems, but how prions avoid the immune system is not fully understood. Most likely, immune responses are restricted because TSE prions are composed of a host protein, albeit a misfolded one, and because most conformational epitopes appear to be obscured by the tight packing and heavy glycosylation of the PrP^{Sc} multimers.

Host-to-Host Spread

To complete the prion transmission cycle, there must be transfer of infectivity between individuals. For CWD, there are multiple potential transmission routes. Prion shedding via skin, feces, urine, milk, nasal secretions, saliva, and placenta from live infected animals has been reported (58, 130; for review see 51), and large amounts of infectivity can also be dispersed from carcasses. CWD persists in the environment, and with common feeding sites and long-range migratory patterns, the spread of CWD can be rapid (50). A classic example of natural human prion transmission is kuru among the Fore people of Papua New Guinea. Cannibalistic rituals led to the spread of kuru from person to person (78). Human Creutzfeldt-Jakob disease (CJD) can be transmitted via iatrogenic routes including transfer of contaminated growth hormone and dura mater grafts derived from human cadavers with

undiagnosed CJD infections, neurosurgical instrument contamination, corneal grafts, gonadotropic hormone injections, and blood transfusions (15, 106). Transmission of prion disease has also occurred between different species. For instance, variant CJD almost certainly resulted from human consumption of cattle infected with bovine spongiform encephalopathy.

PRION-LIKE ACTIVITIES OF OTHER MISFOLDED PROTEINS

Assessing Transmissibility of Prion-Like Amyloids

Multiple lines of evidence have suggested that other misfolded proteins have the capacity to propagate between cells and from tissue to tissue. Ultimately, understanding the extent to which these capacities contribute to the spread of disease between individuals will likely depend on epidemiology, but such studies can be challenging with diseases that can be both slowly progressing and multifactorial. Aside from epidemiology, efforts to evaluate the potential prion-like transmissibility of misfolded proteins can employ experimental approaches ranging from simple in vitro reactions to animal inoculations (Table 1).

Initial indications of prion-like transmissibility may come from demonstrations that a disease-associated, typically amyloid, form of a protein has the ability to induce similar amyloid fibrillization of its normal soluble substrate in cell-free reactions. Because many, if not most, proteins can be induced to form amyloids under partially denaturing conditions (41), it is important to show that this sort of biochemical self-propagation can occur under physiologically compatible conditions, notably in living cell cultures after exposure to the misfolded protein. More relevant are demonstrations that the protein misfolding can be propagated from affected cells to naïve cells in culture or, preferably, from affected/infected cells or tissues into animal models. Usually the most susceptible animal models are transgenic mice overexpressing a mutant familial disease-linked form of the substrate protein. Positive results from artificial mouse models may encourage attempts to induce pathology by inoculation of more natural animal models expressing normal levels of the wild-type substrate protein. However, key additional issues are the extent to which amplification of the disease-inducing agent occurs in inoculated animals and whether the same pathogenic process can be serially propagated to other individuals.

Ultimately, even if serial disease induction is experimentally possible, it remains crucial to determine whether any practical modes of transmission exist in the real world. All these increasingly stringent indices of prion-ness have been satisfied by TSE prions. A number of experimental prion-like characteristics have also been demonstrated to varying degrees for the misfolded proteins/amyloids of other important diseases, but much remains to be done to establish their importance in medicine, agriculture, or wildlife biology.

AA Amyloidosis

Amyloid protein A (AA) amyloidosis can occur after inflammatory stimuli elevate the concentration of the acute-phase reactant serum amyloid A (SAA) protein, potentiating its spontaneous deposition as amyloid. The accumulation of highly ordered amyloid fibrils causes organ damage and subsequent pathology. In vivo seeding of AA was first demonstrated when injection of extracts from the spleen or liver of AA amyloid–laden mice

dramatically shortened the lag phase to AA fibrillogenesis in other mice pretreated with inflammatory stimuli (140). This seeding effect was due to the AA fibril itself, supporting the proposal of an amyloid seeding hypothesis (83). The possibility that AA amyloidosis might be transmitted between captive cheetahs was suggested by demonstrations that AA amyloid excreted in the feces of cheetahs with AA amyloidosis accelerated amyloidosis when inoculated into inflamed mice (146). With demonstrable seeding, nucleation, and a plausible transmission route, AA amyloidosis is reminiscent of prion-like spreading. In addition, AA amyloidosis is currently the only protein misfolding disease other than TSEs with observed host-to-host transmission routes. However, transmission of AA amyloidosis is only known to occur in artificially primed animal models, leaving open the question of whether prion-like spread of misfolded AA ever occurs naturally.

Amyloid-β

Aβ seeding in vivo was first reported over a decade ago. Multiple studies observed that injection of brain extracts from Alzheimer's disease patients or APP-transgenic mice resulted in the accumulation of Aβ deposits (70, 94). These studies used transgenic mutant APP (amyloid precursor protein) mouse models that would have eventually accumulated Aβ deposits spontaneously, even without inoculation of Alzheimer's extracts. However, detectable Aβ aggregates were also found in transgenic mice expressing human wild-type APP, which does not aggregate spontaneously, when these mice were injected intracerebrally with Alzheimer's disease brain extracts (95). Following observations of apparent seeding resulting from direct brain inoculation, one study investigated whether, like TSE prions, Aβ propagation could penetrate the CNS from the periphery. Intraperitoneal injection of Aβ-laden brain extract resulted in cerebral β-amyloidosis 7 months post-inoculation (42). Interestingly, the Aβ deposits were predominantly associated with blood vessels as a cerebral β-amyloid angiopathy, indicating that the route of administration influences the location of amyloid deposition. More recently, an Aβ prion was described as part of a study in which injection of either purified Aβ aggregates derived from brain or aggregates composed of synthetic Aβ induced widespread cerebral β-amyloidosis (129). This study was the first to use synthetic Aβ peptides in inducing Aβ deposition. However, we must emphasize that no iatrogenic risk factors or epidemiological evidence of Aβ spread has been associated with Alzheimer's disease in humans to date (reviewed in 119). Aβ seeding thus far has almost exclusively been studied in artificially susceptible animal models, such as mice that overexpress APP. By comparison, it is clear that infection and resulting clinical disease with TSE prions does not require specialized transgenic systems.

α-Synuclein

A hallmark of Parkinson's disease is the accumulation of proteinaceous intraneuronal inclusions known as Lewy bodies, with α-synuclein (α-syn) being the most abundant protein component. Neuropathologically, the appearance of Lewy bodies and the accumulation of α-synuclein in the brain occur in a predictable systematic regional progression (14). This progressive pathology suggests that cell-to-cell spread of misfolded α-syn occurs through neural circuits. Cell-to-cell spread of misfolded α-syn is further indicated by multiple lines of evidence. Lewy bodies, previously identified only in much older neurons, were observed in fetal mesencephalic neurons that had been transplanted into Parkinson's disease patients

(77). This implied that α-syn misfolding had been induced in the naïve transplanted cells due to exposure to the diseased cells in their vicinity. Additionally, the inoculation of synthetic α-syn fibrils into wild-type nontransgenic mice led to the appearance of Lewy body pathology in anatomically interconnected regions of the brain (81). Similar propagation was seen when brain lysate from symptomatic transgenic mice expressing the Parkinson's disease A53T α-syn mutant was injected into young asymptomatic α-syn A53T mice (82). Interestingly, different patterns of pathology were visible following inoculation into the striatum versus the cortex of these mice, with inoculation into both regions manifesting as a cumulative distribution of the individual inoculations.

The above evidence implicates defined routes of misfolded α-syn propagation, reminiscent of the predictable progression of pathology in Parkinson's disease. For prion-like propagation to take place, the misfolded protein (prion) must be able to access its nonprion substrate. Because α-syn lacks a signal sequence and is considered a cytosolic protein, misfolded α-syn aggregates must somehow be released and taken up into the cytoplasm of other cells to initiate new, α-syn misfolding. Recently, α-syn was detected in the brain interstitial fluid in α-syn transgenic mice and human patients with traumatic brain injury, suggesting that its release from cells can occur in vivo (43). In addition, α-syn can be found in the cerebrospinal fluid of Parkinson's patients (133). It is plausible that α-syn might also be released from dying cells instead of being actively secreted. In any case, the release and uptake of α-syn by diseased and naïve cells, respectively, could provide routes for cell-to-cell propagation of α-syn misfolding. However, as is the case with Aβ and AD, there is no epidemiological evidence of host-to-host transmission of α-syn leading to Parkinson's disease.

Tau

Tau is normally a cytosolic protein that modulates microtubule stability. Under pathological conditions such as in Alzheimer's disease, tau is hyperphosphorylated and forms neurofibrillary tangles. Similar to Aβ and α-syn, misfolded tau appears sequentially in anatomically connected neuronal networks in several mouse models of Alzheimer's disease (32, 79). The normal cytosolic localization of tau also means it must be released from cells for the misfolded tau to propagate in a prion-like manner. Secreted tau has been observed in disease models; for example, extracellular tau deposits were found when human tau was overexpressed in lamprey central neurons (60). Extracellular tau aggregates can be taken up by cultured cells and induce the fibrillization of intra-cellular full-length tau (46). There also appears to be experimental evidence for tau transmissibility between regions of the brain. When brain extract from mutant P301S tau-expressing mice was injected into the hippocampus of transgenic mice expressing wild-type human tau, seeded spread of misfolded tau throughout the brain was suggested by the appearance of filamentous tau significant distances from the inoculation site (28). However, similar to observations with overexpressed APP in many of the Aβ transmissibility models, overexpression of human tau may be necessary for the spread of filamentous tau pathology following injection of human P301S tau brain extract. In any case, synthetic tau fibrils inoculated into P301S tau-expressing mice were sufficient to induce the systematic appearance of tau inclusions along interconnected brain regions (65).

ALS-Associated Proteins

Mutations in the gene encoding superoxide dismutase-1 (SOD1) and the RNA/DNA-binding protein TDP-43 gene can result in amytrophic lateral sclerosis (ALS). Ten percent of all ALS cases are dominantly inherited familial cases; the remaining 90% are sporadic and arise from unknown origins (109). Although SOD1 is primarily cytosolic, mutant SOD1 can be secreted (136) and has also been detected in the cerebrospinal fluid in ALS patients, but not in quantities that differed from those of non-ALS neurological controls (145). Propagation potential of SOD1 and TDP-43 was demonstrated when the aggregated form of either protein was observed to seed misfolding of the wild-type protein in vitro (26, 47). Furthermore, misfolded forms of both proteins induce the misfolding and subsequent aggregation of endogenous native proteins in cell culture (47, 54, 97, 107).

PolyQ Proteins

Expanded polyglutamine Q (polyQ) proteins have also been suggested to act in a prion-like manner. There is no evidence to date that polyQ proteins can seed aggregation in animal models, although studies in cell culture models indicate polyQ aggregates can be transmitted cell to cell and internalized into the cytoplasmic compartment (114).

CPEB

The cytoplasmic polyadenylation element-binding protein (CPEB) is an RNA-binding protein involved in synaptic plasticity. *Aplysia* CPEB exists in two conformational states, monomeric and multimeric, with the multimeric conformation thought to be the active conformation (120). Although sometimes called prion-like, CPEB cannot be considered a prion because there is no cell-to-cell transmission or phenotypic inheritance of the multimeric form. Thus, this is an example in which the multimeric organization or even amyloid formation of a protein, though physiologically important, does not necessarily confer prion-like qualities.

Together, these studies suggest that misfolded proteins other than TSE prions can propagate between cells and tissues. Hints to mechanisms of cell-to-cell spread of these prion-like proteins may be implicit in the observation that the appearance of misfolded proteins follows interconnected neural circuitry (e.g., synaptic transfer). The idea that prion-like propagation of misfolded proteins is a common mechanism in disease is enticing; however, other mechanisms may contribute to misfolded protein accumulation and confound interpretations of transmissibility.

Proteostatic Dysfunction and Amyloid Transmissibility

With increasing evidence that TSE prions and other misfolded proteins, including Aβ and α-syn, impair autophagy and other cellular proteostatic systems (38, 62, 92, 96), it is conceivable that transmission of protein misfolding also involves spreading of proteostatic overload and dysfunction. Moreover, aging is a major risk factor for many of the misfolding protein diseases, and proteostatic function declines with age (131). Preexisting deficits in proteostasis could contribute to the mechanisms allowing the effective transmissibility and host susceptibility to misfolded proteins such as Aβ and α-syn. Conceivably, younger hosts

without significant proteostatic dysfunction could be more resistant to exposures to, and propagation of, misfolded proteins than aged hosts.

Exploiting Prion-Like Properties to Detect Pathological Protein Aggregates

An important step in determining the transmissibility of protein misfolding diseases is the evaluation of plausible routes of shedding and uptake. Because the proteinaceous agents responsible for the pathology of these disorders are misfolded isoforms of native proteins, antibody detection is restricted to conformational epitopes. However, detection methods have been developed that exploit the tendency of amyloids and other misfolded protein oligomers to induce seeded polymerization of their normal monomeric isoforms. The first cell-free conversion assay (75), developed for the detection of pathogenic prions, has spawned more sensitive and efficient amplification assays, including the protein misfolding cyclic amplification (PMCA) reaction (115), the real time–quaking induced conversion (RT-QuIC) (7, 101), and the enhanced-QuIC (eQuIC) (100) assays. With the detection limits of the eQuIC and PMCA approaching attogram detection of PrP^{Sc} (100, 115) in various biological samples, it is possible to detect subinfectious levels. Thus far, QuIC-based assays have been capable of detecting seeding activity of prions in brain cells, cerebrospinal fluid, blood plasma and serum, and nasal lavage samples (reviewed in 101). If mechanistically related techniques are applied more broadly to protein misfolding diseases, it may become possible to identify environmental caches of pathogenic protein aggregates and to explore potential transmission routes both into and out of the body. Moreover, such assays may allow for preclinical diagnostic tests that are applicable to many protein misfolding diseases for which early diagnoses can be difficult.

CONCLUSIONS

The prion concept has evolved to include protein-based genetic elements in yeast that are transmitted through simple intercellular cytoplasmic exchange as well as mammalian TSE pathogens that are propagated between hosts through complex multistep transmission cycles to cause devastating neurodegenerative disease. Similarities in structure and self-propagation mechanisms suggest that the misfolded protein amyloids associated with a number of other important human protein misfolding diseases might be capable of spreading in a prion-like manner between cells, tissues, and hosts. These concerns have been fueled to varying extents by results from a battery of experimental approaches. However, the most stringent and significant of such tests, i.e., demonstrations of transmission between natural human or animal hosts in the real world, have not been documented.

Currently there is no epidemiological evidence for the spreading and/or acceleration of non-TSE human protein misfolding diseases due to the transfer of misfolded proteins via natural or iatrogenic routes. Such epidemiological evidence may be difficult to interpret for many of these diseases given their multifactorial etiologies and typically long preclinical and clinical phases. Given the high incidence of diseases such as Alzheimer’s, Parkinson’s, Huntington’s, and Lou Gehrig’s (ALS), it is important to know whether even a small percentage of cases can be initiated by transmission events. Even in the absence of significant human-to-human transmission routes, it is critical to establish whether prion-like

propagation of protein misfolding within individuals can be observed and manipulated to alter the course of disease.

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Glossary

Prion	an infectious, misfolded, self-propagating protein or protein aggregate with the capability of spreading between hosts
Amyloid	protein fibrils exhibiting a cross-β core structure
TSE	transmissible spongiform encephalopathy
PrP	prion protein
PrP^{RES}	abnormal, partially protease-resistant, prion disease-associated isoform of PrP; largely synonymous with PrP^{Sc}
PrP^{C}	normal cellular isoform of PrP
Protein misfolding disease	disease associated with the accumulation of a misfolded host protein
β-amyloid (Aβ)	amyloid-forming peptide associated with Alzheimer's disease
α-syn	α-synuclein
Steric zipper	structural unit of amyloid fibrils where amino acid side chains of neighboring β-sheet layers are tightly interdigitated
Seeded/templated polymerization	a mechanism by which a misfolded protein multimer grows by acting as a template to induce the binding and conformational conversion of natively folded substrate proteins
Transmissibility	ability of an infectious agent to propagate between individual host organisms
GPI	glyco-phosphatidylinositol
Autophagy	a process by which intracellular organelles or proteins are sequestered into double-membrane vacuoles and degraded following fusion with lysosomes
APP	amyloid precursor protein
Proteostatic dysfunction	imbalance in degradation or clearance of misfolded proteins; disruption in protein synthesis, folding, or transport
PMCA	protein misfolding cyclic amplification
QuIC	quaking-induced conversion

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SUMMARY POINTS

1. Prions are altered transmissible states of host proteins exemplified by epigenetic elements in fungi and TSE agents in mammals. Their transmission routes range from simple cytoplasmic exchange between single yeast cells to a complex multistep process of initial TSE infection, evasion of clearance mechanisms and immune responses, neuroinvasive propagation between cell types and tissues, dissemination within the CNS, and shedding into the environment.
2. Prion structures and propagation mechanisms determine their capacity to act as infectious agents. Although definitive models of TSE prions remain elusive, most prions can be isolated as multimeric fibrillar amyloids with the ability to seed or template their own formation from their normal nonprion isoform.
3. The precedents of known prions provide a basis for examining the potential transmissibility of misfolded, pathological forms of other mammalian proteins.
4. Prion-like propagation between cells and tissues has been observed experimentally with a number of important human-disease-associated misfolded proteins. However, so far there is no definitive evidence for transmission of misfolded proteins between individuals except in the case of TSE prions.
5. Other mechanisms, such as proteostatic dysfunction, may confound interpretations of amyloid transmissibility as both can result in the detectable accumulation of misfolded proteins.
6. Extraordinarily sensitive prion seeding assays could be adapted for other amyloid diseases to aid early diagnoses and investigations of potential routes of prion-like propagation or transmission of major protein misfolding diseases.

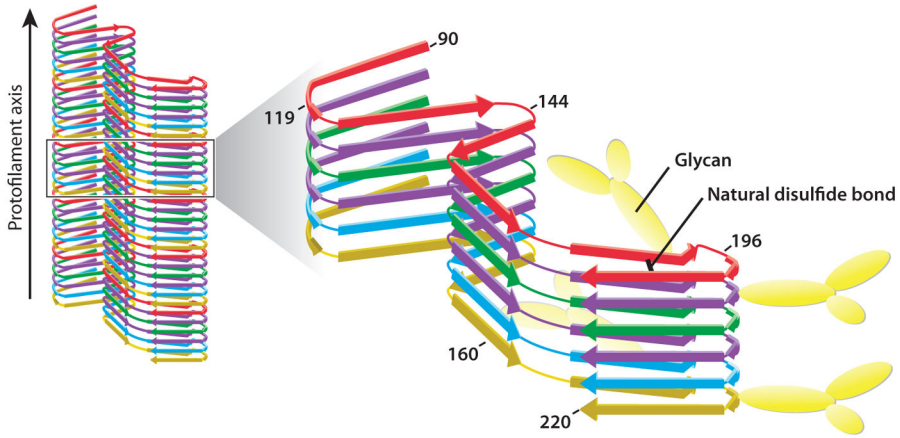

Figure 1.

Potential structural model of PrP^{RES}. An N-terminal extension of a model depicted previously by Cobb, Surewicz, and colleagues (30). PrP^{Sc} monomers stack along the protofilament axis, forming stretches of parallel, in-register β-sheets. Each colored strand shows a PrP monomer. Ribbons indicate β-sheets and lines indicate loops and turns. The natural disulfide bond and glycans (*yellow structures*) are as indicated.

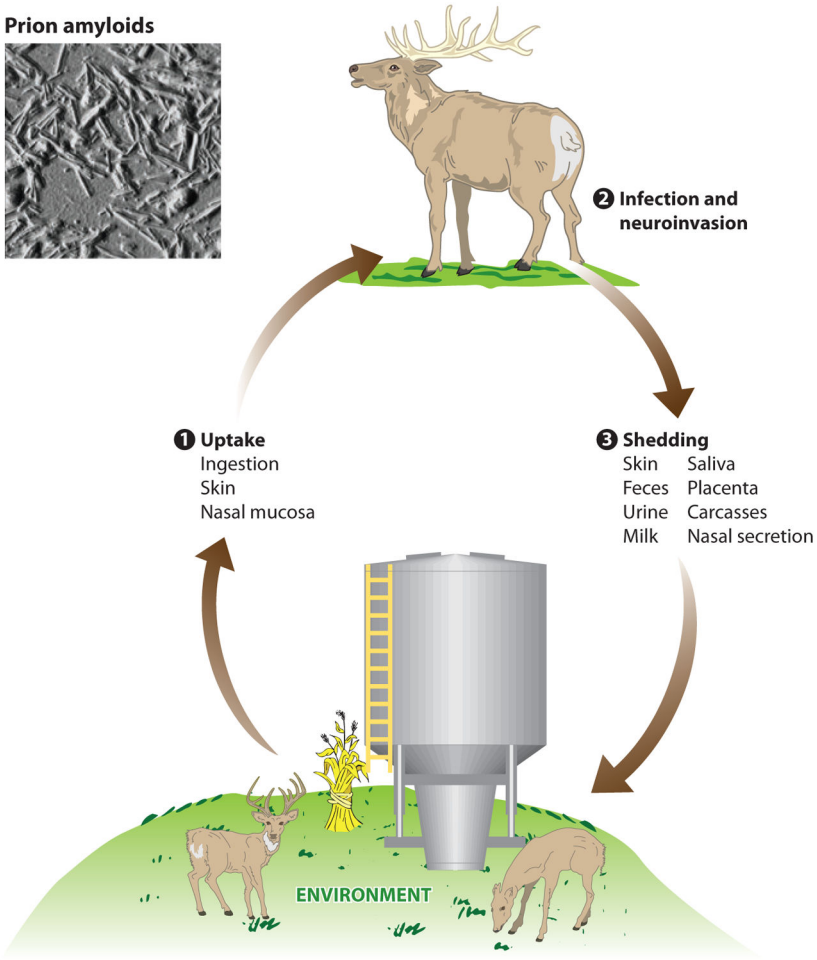

Figure 2.

Plausible transmission cycle of chronic wasting disease (CWD). CWD appears to be the most efficient model of natural transmissible spongiform encephalopathy prion spread. ❶ The cycle may begin with uptake of the infectious prion amyloids through routes such as ingestion, inhalation, or contact with the skin or nasal mucosa. ❷ After prion uptake, replication and neuroinvasion occur. ❸ An infected cervid can then shed prions back into the environment through skin, feces, urine, milk, nasal secretions, saliva, placenta, or carcasses, restarting the infectious cycle. (Atomic force micrograph of infectious prion amyloid fibrils reproduced with permission from Reference 123).

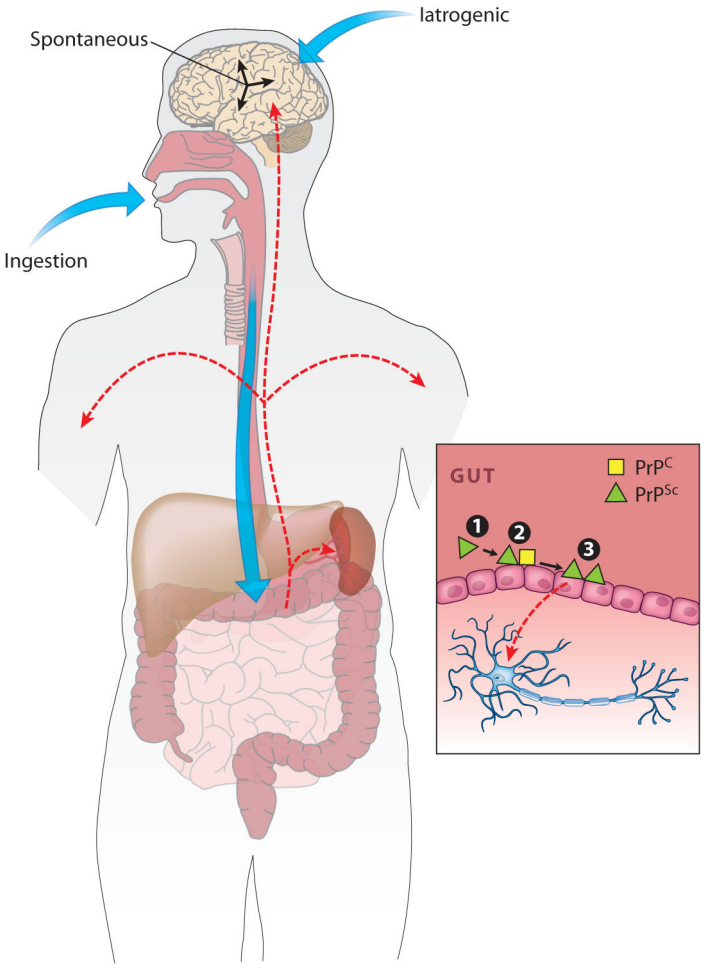

Figure 3.

Sources of prion infection in humans. Prion diseases in humans can arise spontaneously (*black arrows*) or following oral or iatrogenic exposure to infectious materials (*solid blue arrows*). Following oral challenge, infectious prions travel to the gut, where, in animal models at least, they are taken up by the gut mucosa and Peyer's patches. (*Inset*) ❶ Incoming infectious prions presumably interact with ❷ native PrP^{C} (*yellow square*) and ❸ convert the PrP^{C} into PrP^{Sc} (*green triangles*). PrP^{Sc} is then transferred to enteric nerves, where it can then spread to the central nervous system. Presumed routes of tissue-to-tissue spread are indicated by dashed red arrows.

Table 1

Prion-like characteristics of misfolded mammalian proteins

Disease(s)	Misfolded protein	Self-propagating state in vitro	"Infection" of naïve cells in vitro	Cell-to-cell spread in vitro	Inducible by inoculation into abnormally primed animal models	Cell-to-cell spread in vivo	Transmissible into normal wild-type animal models	Naturally transmissible	Reference(s)[a]
TSE (prion)	PrP^{Sc}	Yes	Yes	Yes	Yes	Yes	Yes	Yes	16, 67
AA amyloidosis	SAA	Yes	Unknown	Unknown	Yes	Unknown	No	Unknown	83, 146
Alzheimer's; cerebral β-amyloidosis	Aβ	Yes	Yes	Yes	Yes	Yes	Unknown	No	42, 70, 94, 95, 98, 119, 129
Parkinson's; synuclein-opathies	α-synuclein (Lewy bodies)	Yes	Yes	Yes	Yes	Yes	Yes	Unknown	39, 61, 77, 81, 82
Alzheimer's; tauopathies	Tau	Yes	Yes	Yes	Yes	Yes	Unknown	Unknown	28, 32, 46, 56, 59, 60, 79
ALS	SOD1	Yes	Yes	Yes	Unknown	Unknown	Unknown	Unknown	26, 54, 97, 109
Huntington's	Huntingtin, polyQ expansions	Yes	Yes	Yes	Unknown	Unknown	Unknown	Unknown	114

[a] Because of space constraints, only select references are indicated.

Abbreviations: Aβ, β-amyloid; ALS, amytrophic lateral sclerosis; PrP, prion protein; SAA, serum amyloid A; SOD, superoxide dismutase; TSE, transmissible spongiform encephalopathy.