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Fate of Prions in Soil: A Review

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Abstract

Prions are the etiological agents of transmissible spongiform encephalopathies (TSEs), a class of fatal neurodegenerative diseases affecting humans and other mammals. The pathogenic prion protein is a misfolded form of the host-encoded prion protein and represents the predominant, if not sole, component of the infectious agent. Environmental routes of TSE transmission are implicated in epizootics of sheep scrapie and chronic wasting disease (CWD) of deer, elk, and moose. Soil represents a plausible environmental reservoir of scrapie and CWD agents, which can persist in the environment for years. Attachment to soil particles likely influences the persistence and infectivity of prions in the environment. Effective methods to inactivate TSE agents in soil are currently lacking, and the effects of natural degradation mechanisms on TSE infectivity are largely unknown. An improved understanding of the processes affecting the mobility, persistence, and bioavailability of prions in soil is needed for the management of TSE-contaminated environments.

Transmissible spongiform encephalopathies (TSEs), or prion diseases, comprise a class of fatal, neurodegenerative diseases affecting a variety of mammals. These diseases include bovine spongiform encephalopathy (BSE) in cattle, scrapie in sheep and goats, chronic wasting disease (CWD) in North American members of the deer family (cervids), transmissible mink encephalopathy (TME) in farmed mink, and Creutzfeldt-Jakob disease and kuru in humans. The etiological agents, termed *prions* (proteinaceous infectious particles lacking nucleic acid; Prusiner, 1998), have not been fully characterized, but available evidence indicates that a misfolded form of the host-encoded prion protein (PrP) constitutes the primary, if not sole, component of the prion. The normal, benign cellular form is designated PrP^C; the pathologically misfolded form is denoted PrP^{TSE}. Prion diseases are characterized by long asymptomatic incubation periods, spongiform degeneration in the central nervous system, and accumulation of PrP^{TSE}.

Scrapie and CWD are the only TSEs that appear to be environmentally transmissible, which may reflect disease-specific differences in the levels of infectious agent released into the environment or higher susceptibility of sheep, goats, and cervids to infection by environmental routes (Pedersen and Somerville, 2011). Scrapie has been recognized in

sheep for over 250 yr, and anecdotal evidence for environmental transmission is provided by several reports of naïve sheep contracting scrapie after grazing on fields once occupied by infected animals (Greig, 1940; Palsson, 1979; Georgsson et al., 2006). Chronic wasting disease was first recognized in the late 1960s in mule deer at a wildlife research facility in northern Colorado. The disease has been subsequently found in captive and free-ranging deer, elk and moose in the United States and Canada. A study exposing healthy animals to paddocks containing CWD-infected carcasses decomposed in situ or residual excreta from CWD-infected animals demonstrated environmental transmission of CWD in mule deer (Miller et al., 2004).

The relevant routes of environmental TSE transmission remain to be clarified, yet several lines of evidence suggest that soil may serve as a reservoir of TSE infectivity (Schramm et al., 2006). Prions may enter soil through urinary, salivary, or fecal shedding from infected animals or decomposition of diseased carcasses (Tamgüney et al., 2009; Gregori et al., 2008; Mathiason et al., 2006; Miller et al., 2004). Transmissible spongiform encephalopathy infectivity is known to persist in soil for years (Seidel et al., 2007; Brown and Gajdusek, 1991; Georgsson et al., 2006). Animals may ingest contaminated soils intentionally to supplement mineral intake, incidentally during feeding or grooming, or indirectly (e.g., clearance from lungs) (e.g., Weeks, 1978; Fries, 1996). Together, these studies suggest that soil represents a plausible route of environmental TSE transmission; however, several questions remain regarding the fate and persistence of prions in soil.

In this review, we discuss factors influencing the attachment of prion protein to soil constituents and potential implications for environmental TSE transmission. Association of prions with soil particles may impact TSE infectivity by affecting the bioavailability or persistence of these agents in the environment. To date, the concentration of prions in environmental media in areas where TSEs are endemic is largely unknown due to the limited ability to detect TSE agents in or extracted from environmental samples. Natural biotic and abiotic mechanisms of protein degradation may impact levels of TSE infectivity in the environment. Significant interest exists for evaluating these degradation pathways (e.g., degradation by soil microorganisms, extracellular proteases, or inorganic oxidants) as a means to reduce or eliminate TSE infectivity in contaminated soils.

Structure of the Pathogenic Prion Protein

The infectious agent in prion diseases consists primarily, if not solely, of a misfolded form of the prion protein. Disease propagation appears to occur via a templating mechanism in which PrP^{TSE} catalyzes the misfolding of PrP^C (Prusiner, 1991; Jarrett and Lansbury, 1993). The pathologic conformational isomer possesses the same primary sequence and covalent post-translational modifications as does the innocuous form; the only difference detected to date is the folding of the protein. The mature prion protein (i.e., after post-translational modification) consists of approximately 208 amino acids with two consensus sites for N-linked glycosylation at Asn 180 and Asn 196. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of denatured PrP reveals three bands representing di-, mono-, and unglycosylated protein. The composition of the N-linked glycans is heterogeneous; at least 52 different sets of oligosaccharides have been identified (Stimson et al., 1999; Rudd et al., 1999; Rudd et al., 2001). When both Asn sites are occupied, more than 400 glycoforms are expected based on the diversity of known oligosaccharide structures (Endo et al., 1989). Sialic acid residues impart significant charge heterogeneity with individual PrP molecules exhibiting isoelectric points ranging from 4.6 to 7.9 (Bolton et al., 1985). The prion protein is usually post-translationally modified with a glycosyl-inositol phospholipid (GPI) membrane anchor attached at Ser 231 (Stahl et al., 1987). The GPI anchor can also be sialylated (Stahl et al., 1992).

The majority of PrP^{TSE} in diseased brain tissue is isolated as insoluble amyloid fibrils typically 50 to 300 nm in length and 10 nm in diameter (Fig. 1). Recent studies indicate that smaller subfibrillar aggregates of 14 to 28 PrP^{TSE} molecules possess the highest specific infectivity (i.e., infectivity per unit mass; Silveira et al., 2005). Both the folding and the aggregation states of PrP^{TSE} convey distinct biological and physicochemical properties to the protein. Prions exhibit an extraordinary resistance to inactivation by ultraviolet (UV) and ionizing radiation, exposure to proteases or chemical disinfectants, and heat treatments (Taylor, 1999, 2000; Taylor and Diprose, 1996; Brown et al., 2004). Treatment of PrP^{TSE} with proteinase K (PK) cleaves the N-terminal ~90 amino acid residues, leaving a truncated form of the protein designated PrP²⁷⁻³⁰. Prions also display distinct strains defined by differences in clinical symptoms, incubation times, and pathology (Bruce, 2003; Aguzzi et al., 2007; Morales et al., 2007). Strain differences appear to be enciphered in the conformation of PrP^{TSE} (Telling et al., 1996).

High-resolution structural information on PrP^{TSE} has not been obtained by nuclear magnetic resonance spectroscopy (NMR) or X-ray crystallography due to problems with insolubility, aggregation, and heterogeneity of purified samples. Circular dichroism and infrared spectroscopy data indicate that PrP^{TSE} has dramatically increased β -sheet content relative to PrP^C (Pan et al., 1993). Experimental information on PrP^{TSE} structure is limited to secondary structural analysis, electron crystallography of two-dimensional PrP^{TSE} crystals, antibody epitope mapping studies, H/D exchange experiments, covalent cross-linking studies, and X-ray fiber diffraction data. Two structural models of PrP^{TSE} determined *in silico* and constrained by low-resolution experimental data are consistent with most experimental data (Fig. 2; DeMarco et al., 2006; Govaerts et al., 2004). These models indicate the formation of trimers, which stack to form amyloid fibrils (Govaerts et al., 2004). Asn-linked glycans are predicted to lie at the periphery of the trimers and significantly impact accessibility of the polypeptide surface (DeMarco and Daggett, 2009). A third proposed PrP^{TSE} structure was recently published based on site-directed spin labeling and electron paramagnetic resonance spectroscopy studies of recombinant PrP amyloid fibrils (Cobb et al., 2007). Taken together, structural models provide an initial step toward understanding the conformational changes associated with TSEs, yet further investigation is needed to evaluate the contribution of specific structural elements to infectivity.

Sources of Prion Protein Used in Environmental Fate Studies

Isolation of PrP^{TSE} from Infected Animal Tissue

Discussion of the sources of PrP^{TSE} used in environmental fate studies is warranted before presenting the results of such investigations. Infected brain tissue is the most common source of bona fide PrP^{TSE} since it contains the highest concentrations of TSE agent (Kimberlin and Walker, 1977). Most studies have used rodent-adapted prion strains, which are largely similar to environmentally relevant TSE agents in terms of PrP primary and secondary structure, aggregation, and resistance to common inactivation techniques. However, strain-specific differences in PrP^{TSE} conformation, structural stability (Safar et al., 1998), and degree of glycosylation (Somerville, 1999) have been reported.

Methods for the partial purification of PrP^{TSE} from brain homogenate involve a series of detergent solubilization and centrifugation steps (Bolton et al., 1987; Caughey et al., 1991). The resulting prion preparations lack PrP^C but contain other copurifying biomolecules including carbohydrates, nucleic acids, proteins, and lipids (Stahl et al., 1987; Aiken et al., 1990; Kellings et al., 1993; Stahl et al., 1993; Moore et al., 2010). Purification increases PrP^{TSE} fibrillization producing protein aggregates that are larger than those present in infected brain tissue (McKinley et al., 1991). The effect of aggregation on PrP^{TSE} stability has not been formally evaluated, but changes in aggregation state may influence the extent

to which the protein resists degradation. Experiments analyzing environmental persistence of prions should therefore be modeled with homogenized or intact infected tissues as opposed to purified PrP^{TSE} preparations. In contrast, examining molecular interactions between PrP^{TSE} and soil particle surfaces, such as the extent and rate of adsorption, requires the use of purified PrP^{TSE} to minimize contributions from other sample constituents.

The N-terminus of PrP^{TSE} is susceptible to proteolytic degradation. Pathogenic prion protein released into the environment from decomposing tissues might therefore be expected to exist primarily in the N-terminally truncated form (Saunders et al., 2008). However, species and/or strain differences in N-terminal truncation during tissue decomposition may exist. The N-terminus of PrP^{TSE} is not necessary for TSE infection (Prusiner et al., 1984) but may influence mechanisms of protein attachment to soil particle surfaces (Cooke et al., 2007).

Recombinant Prion Protein

Some studies intending to model the fate of prions in the environment have employed prokaryotically expressed recombinant prion protein (recPrP). Recombinant analogs expressed in prokaryotes lack glycosylation and the GPI anchor, which significantly affect the physicochemical characteristics of the protein. Nonglycosylated ovine recPrP exhibits an isoelectric point (IEP) between 9 and 10 (Vasina et al., 2005), whereas an average IEP of 4.6 was found for PrP^{TSE} aggregates (Ma et al., 2007). The N-linked glycans and GPI anchor of PrP expressed in mammalian systems contain sialic acid residues, resulting in the lower IEP of bona fide PrP^{TSE} (Bolton et al., 1985; Stahl et al., 1992). Under mildly acidic conditions, α -recPrP (proteolytically labile recPrP with a PrP^C-like conformation) folds into PrP^{TSE}-like β -sheet rich oligomers (termed β -recPrP) with increased resistance to PK digestion (Swietnicki et al., 2000). Nonetheless, β -recPrP is more labile than PrP^{TSE} and therefore has limited utility for understanding prion persistence in the environment.

PrP^{TSE} Detection

Methods for prion detection can be differentiated in terms of quantitation of prion protein and measurement of TSE infectivity. The amount of protease-resistant prion protein usually correlates with infectivity, although infectious agent may be present in the absence of detectable PrP^{TSE} (Lasmézas et al., 1997; Barron et al., 2007). Direct detection of prions is achieved only by using animal- or cell-based bioassays. Animal bioassays represent the most definitive test for TSE infectivity, and results are commonly expressed in terms of infectious units (IU₅₀). One IU₅₀ represents the amount of infectious agent required to infect half the population of test animals used in a study. Quantitation is achieved by either end-point titration or on the basis of the strong correlation between dose and length of the incubation period (Prusiner et al., 1980). End-point titration is more sensitive with a limit of detection of approximately 0.2 amol of PrP^{TSE} (i.e., 1 IU₅₀) (Lax et al., 1983). Practical limitations of animal bioassays include the long incubation periods required (e.g., 90–200 d for hamsters; 200–500 d for mice) and the expense of maintaining large cohorts of animals.

Prions have been detected using neuroblastoma cell-based culture assays (Klöhn et al., 2003; Mahal et al., 2007). These assays are significantly more rapid and less expensive than animal bioassays and achieve similar sensitivity. However, cell-line resistance to many important prion strains has hampered general application of these assays in TSE research. Cell-based infectivity measurements are compatible with prions bound to surfaces including soil particles (Genovesi et al., 2007) and stainless steel wires (Edgeworth et al., 2009). Measurement of prions in the presence of soil particles or natural organic matter (NOM) is critical for the development of methods to detect prions in or extracted from environmental matrices. Direct quantitation of prions in soil by animal bioassays is complicated by

enhanced disease transmission by prions bound to at least some types of mineral, soil, and dietary particles (Johnson et al., 2007; Johnson et al., 2011).

Standard techniques for protein detection such as Western blotting and enzyme-linked immunosorbent assay (ELISA) are commonly used to measure PrP^{TSE}. Under denaturing conditions, immunodetection cannot discriminate between PrP^{TSE} and PrP^C. Samples are often treated with a protease (e.g., PK, thermolysin) to degrade PrP^C (and the protease-sensitive fraction of PrP^{TSE}) (Tzaban et al., 2002; Owen et al., 2007) before immunodetection. Although immunoassays are rapid and specific for PrP, they suffer from low sensitivity (i.e., detection limits of 2000–20,000 amol and 200 amol for Western blot and ELISA, compared with an estimated 0.2 amol for 1 IU₅₀) and fail to measure TSE infectivity. More sensitive PrP^{TSE} quantitation has been achieved by liquid chromatography–mass spectrometry (LC–MS) (Onisko et al., 2007). This method detects a signature PrP peptide following reduction, purification, and tryptic cleavage of PrP^{TSE}. Despite reported detection limits of 20 to 30 amol for pure standards, LC–MS requires considerable amounts of infectious brain material due to losses during protein purification. Increased sensitivity may be provided with signature peptides lacking methionine that can be easily oxidized and represents a potential source of error in quantitative mass spectrometry (Sturm et al., 2010). Further development is needed for application of LC–MS to complex biological and environmental samples.

Protein misfolding cyclic amplification (PMCA) relies on the structural transition of PrP^C to PrP^{TSE} catalyzed by small quantities of PrP^{TSE} in a manner conceptually similar to polymerase chain reaction (Saborio et al., 2001; Castilla et al., 2005). The amount of PrP^{TSE} is increased by repeated cycles of sonication (to disrupt PrP^{TSE} aggregates) and incubation using PrP^C from healthy brain homogenate as a substrate. Protein misfolding cyclic amplification has the lowest reported PrP^{TSE} detection limits corresponding to approximately 26 molecules or approximately 4×10^{-5} amol (assuming a molecular mass of 30 kDa for PrP^{TSE}) (Saá et al., 2006). Similar techniques have emerged using recPrP as a substrate including the amyloid seeding assay (Colby et al., 2007), quaking induced conversion (Atarashi et al., 2008) and real-time quaking induced conversion (Wilham et al., 2010).

Conversion assays represent a promising approach for sensitive detection of prions in naturally contaminated environmental media. Recently, detection of PrP^{TSE} by PMCA was reported in one of two stream water samples collected from a CWD endemic area (Nichols et al., 2009). Although the report is intriguing, further measurements are needed to substantiate this result due to the propensity for false positives with PMCA and the potential impact of dissolved organic matter on PMCA. Currently, only two studies have used PMCA to detect PrP^{TSE} in soils experimentally spiked with infected brain homogenate. Seidel et al. (2007) extracted hamster PrP^{TSE} from soil with 1% SDS and found that at least a fraction extracted remained catalytically active to seed PMCA. The authors did not report the exact level of amplification, which was presumably low. Nagaoka et al. (2010) used soil-bound PrP^{TSE} to seed PMCA, which provided 10^3 to 10^4 higher sensitivity than methods involving PrP^{TSE} extraction followed by Western blot detection. Effective amplification required small quantities of soil (8 µg), and no amplification was observed in the presence of more soil (80–800 µg). Further development of PMCA is needed for detection of PrP^{TSE} from environmentally relevant prion strains (e.g., CWD, scrapie). The impact of specific soil characteristics (e.g., texture, mineralogy, organic carbon content) on amplification should also be determined.

Protein extraction and sample cleanup complicate application of prion detection methods to environmental samples. Direct detection of prion protein in soil has been proposed to avoid

the effects of inefficiencies in prion protein extraction on detection (Genovesi et al., 2007; Saunders et al., 2009a,b). This approach uses bovine serum albumin to saturate soil particle surfaces not occupied by PrP^{TSE} (or other proteins) and then visualizes soil-bound PrP^{TSE} by immunodetection. Quantitation is limited by accessibility of the antibody binding epitope, a narrow linear range and low sensitivity with PrP^{TSE} detection limits similar to those of Western blotting.

Attachment of PrP^{TSE} to Soil Particle Surfaces

Associations between PrP^{TSE} and soil particle surfaces are expected to impact the fate of pathogenic prion protein and TSE infectivity in the environment. The interaction of proteins with surfaces is commonly described as *adsorption*. We use this term when referring to the interaction of single protein molecules with surfaces. Aggregates of PrP^{TSE} are in the colloidal regime. We therefore use the term *attachment* to describe their interaction with particle surfaces.

Proteins exhibit a pronounced tendency to accumulate at solid–liquid interfaces under most conditions. This is due to the diverse properties of constituent amino acids and the rotational mobility along the polypeptide chain (for reviews, see Norde, 1986, 2003). Factors influencing protein adsorption to well-defined polymer and metallic substrates have been examined extensively in the context of biomaterials research. In contrast, relatively few studies have examined protein adsorption to soil constituents with the exception of investigations on the function of extracellular enzymes in soil and those on the persistence of *Bacillus thuringiensis* toxins (Cry proteins) released from transgenic crops. These studies have found that proteins bind strongly with most soil particle surfaces, and adsorption can induce conformational changes, alter enzyme activity, and protect proteins from degradation (Quiquampoix, 2000). Potential consequences of prion attachment to soil particle surfaces include: limited transport of PrP^{TSE} in porous media; transport with particles entrained in overland flow; protection from enzymatic, chemical, or physical degradation; altered bioavailability or uptake of soil-bound prions by animals; and changes in PrP^{TSE} conformation or aggregation state that may impact TSE infectivity. Below, we discuss the current state of knowledge concerning PrP^{TSE} attachment to soil particle surfaces and its implications for TSE infectivity in the environment.

Extent and Kinetics of PrP^{TSE} Attachment to Soil Particle and Mineral Surfaces

The majority of studies focused on determining the extent of PrP^{TSE} attachment to soil and mineral particles have used solution depletion experiments. Solution conditions including choice of buffer, pH, and ionic strength impact protein adsorption (Docoslis et al., 2001). The ionic strength of the interstitial water in many soils ranges from 0.003 to 0.016 M, and the dominant ions are typically Na⁺, K⁺, Mg²⁺, Ca²⁺, NH₄⁺, NO₃[−], HCO₃[−], Cl[−], and SO₄^{2−} (Helmke, 1999). The soil solution is often approximated with 0.001 to 0.010 M in CaCl₂ or NaHCO₃ solutions. Investigation of the interaction of a protein with soil particles requires discrimination between particle-associated and free protein. The aggregation state of PrP^{TSE} complicates separation of attached from free prion protein because larger, unbound PrP^{TSE} aggregates may be separated with smaller soil particles (thereby overestimating the degree of attachment) if conditions are not found to minimize this. Most solution depletion experiments examining PrP^{TSE} attachment to soil and mineral particles have used centrifugation to separate soil-bound PrP^{TSE} from unbound protein without considering the potential cosedimentation of large, unbound PrP^{TSE} aggregates. Effective separation of clay particle-bound from unbound PrP^{TSE} has been achieved (to the limit of detection system used) by clarifying PrP^{TSE} preparations (to remove larger aggregates) and sucrose cushion centrifugation (Johnson et al., 2006). The attachment of PrP^{TSE} to soil particle surfaces has

also been measured by modeling the transport of PrP^{TSE} in porous media; these experiments are described in the following section.

In light of the difficulty in separating particle-associated and free PrP^{TSE} in solution depletion experiments, interest exists for the application of in situ techniques for measuring PrP^{TSE} attachment. Vasina et al. (2005) used a flow cell and γ -counter to measure attachment of ¹²⁵I-radiolabeled β -recPrP to mica under laminar flow conditions. Disadvantages of this technique include the limited ability to radiolabel PrP^{TSE} aggregates and the potential impact of extrinsic labels on PrP attachment. Label-free, surface-sensitive techniques including optical waveguide lightmode spectroscopy, surface plasmon resonance, ellipsometry, and quartz crystal microbalance with dissipation monitoring have been used extensively to examine protein adsorption. To date, in situ methods have not been applied to measure PrP^{TSE} attachment to representative soil surfaces.

Solution depletion experiments have shown that PrP^{TSE} readily binds to soil minerals (viz. montmorillonite, kaolinite, and quartz) and whole soils varying in composition (Johnson et al., 2006; Leita et al., 2006; Cooke et al., 2007; Ma et al., 2007; Maddison et al., 2010). These studies found that PrP^{TSE} attachment occurs rapidly, with complete attachment reported to occur within 2 h (Johnson et al., 2006). When expressed on a surface area basis, montmorillonite and quartz microparticles exhibited the highest PrP^{TSE} adsorption capacities ($\sim 2\text{--}6 \text{ mg}_{\text{protein}} \text{ m}_{\text{mineral}}^{-2}$), whereas that of kaolinite was smaller by a factor of 25 (Table 1). These results suggest that mineral surface properties contribute to differences in PrP^{TSE} binding. Montmorillonite was also found to have a large sorption capacity for β -recPrP with complete adsorption observed at 1:1 (w/w) ratio of β -recPrP:montmorillonite (Rigou et al., 2006). Similar to PrP^{TSE}, β -recPrP adsorbed rapidly (within 1 h) to montmorillonite and muscovite mica surfaces (Rigou et al., 2006; Vasina et al., 2005).

A recent report of incomplete PrP^{TSE} attachment following protein–soil contact times of several weeks contrasts with all other published studies (Saunders et al., 2009b). This study utilized brain homogenates as the PrP^{TSE} source. Several experimental design problems hinder confident interpretation of the study's results. Specifically, sedimentation conditions appear unlikely to have been sufficient to discriminate particle-associated from “free” PrP^{TSE}; PrP^C and PrP^{TSE} were not differentiated; controls to monitor changes in brain homogenate composition over time were lacking; and solution conditions (phosphate buffered saline [PBS], ionic strength = 0.15 M) deviated considerably from those encountered in most environments (Sposito, 1989). Furthermore, PBS can interfere with protein sorption (Docoslis et al., 2001).

Because prions probably enter the soil in biological fluids or tissues containing a mixture of different biomolecules (e.g., lipids, other proteins, nucleic acids), understanding the extent to which matrix components affect PrP^{TSE} adsorption is important for accurately modeling natural systems. Maddison et al. (2010) reported that attachment of PrP^{TSE} to soil was complete within 24 h in experiments using scrapie-infected sheep brain and BSE-infected bovine brain homogenates as prion sources. Similarly, Rigou et al. (2006) found that β -recPrP was completely attached even in the presence of a large excess of serum proteins (500:1 10% fetal calf serum: β -recPrP [w/w]). In contrast, Johnson et al. (2007) noted diminished PrP^{TSE} attachment to montmorillonite in the presence of brain homogenate which may have been attributable to competing macromolecules. The impact of potentially competing adsorbates on PrP^{TSE} attachment to soil constituents under well-controlled conditions warrants further research. However, considering the persistence of PrP^{TSE} and the relative excess of sorption sites in natural soils, competitive adsorption may have little effect on the long-term fate of prions in soil.

PrP^{TSE} Association with Natural Organic Matter in Soil

Natural organic matter (a heterogeneous mixture of organic molecules resulting primarily from the microbial decomposition of plant material) is expected to substantially impact PrP^{TSE} attachment to soil particle surfaces. Protein–NOM complex formation has been suggested on the basis of observations of simultaneous extraction of NOM and soil enzymes including urease, diphenyl oxidases, proteases, and hydrolases (Boyd and Mortland, 1990). These extracts contained enzymatic activity that in some cases displayed increased resistance to proteolytic or thermal degradation. The precise nature of associations between soil enzymes and organic matter remains unclear.

Relatively few studies have examined the interaction of proteins with NOM. Most used humic acid, an operationally defined fraction of NOM molecules that are soluble in base but insoluble under acidic conditions. Tan et al. (2008, 2009) examined the interactions between lysozyme and purified Aldrich humic acid and reported the formation of large protein–humic acid heteroaggregates (average hydrodynamic radius of 2000–3500 nm). Electrostatic attraction may promote this complexation because lysozyme (IEP = 10.4) and humic particles carry opposite net charge at neutral pH. Little information exists on the complexation of net negatively charged proteins with humic substances, yet this interaction may be mediated by positively charged patches on the protein surface or by hydrophobic interactions. Hsu and Hatcher (2005, 2006) used two-dimensional NMR to demonstrate noncovalent interactions as well as covalent bonding between synthetic ¹⁵N-labeled peptides and humic acids varying in aliphatic and aromatic carbon content, suggesting that protein–humic acid interactions may involve the formation of hydrogen or covalent bonds. Natural organic matter can interfere with common protein detection methods including total protein assays (i.e., Bradford assay) and Western blotting (Pedersen et al., 2009; Whiffen et al., 2007), complicating clear interpretation of protein–humic interactions (*vide supra*).

To date, the interaction of PrP^{TSE} with NOM has not been reported; three studies have examined the influence of NOM or NOM surrogates on recPrP adsorption. Pucci et al. (2008) used low temperature ashing to remove layers of NOM from whole soils and found reduced α -recPrP sorption to soils ashed in this manner, suggesting that NOM in soil provides sorption sites for α -recPrP. The amount of soil-bound recPrP was estimated from the intensities of the Amide I and II bands in photoacoustic-Fourier transform infrared spectra, or by measuring solution depletion of α -recPrP by a modified Lowry assay (controls showed no interference in protein signal from soil particles). Polano et al. (2008) used UV-visible spectroscopy to measure α -recPrP adsorption to clay, humic–clay complexes, and humic acid. The humic coating apparently enhanced α -recPrP binding, but the potential for humic substances to interfere with α -recPrP quantification at $\lambda = 280$ nm was not discussed. In a study using polymerized catechol as a surrogate for humic substances, the formation of α -recPrP–polycatechol complexes was suggested on the basis of the loss of characteristic protein peaks in UV-visible and Fourier transform infrared spectra of the supernatant following protein addition (Rao et al., 2007). These complexes were stable against extraction by 0.1 M phosphate pH 7 to 8.5, 1% sarkosyl, and 10% v/v propanol. Overall, studies using α -recPrP have found that organic matter significantly contributes to sorption. Further investigation is needed to assess the influence of NOM on PrP^{TSE} adsorption to soil. Differences may be expected due to the altered conformation, aggregation, glycosylation, glypiation, and lower IEP of the infectious protein.

Interactions between PrP^{TSE} and Soil Particle Surfaces

Prion protein attachment to soil particle surfaces is expected to involve electrostatic, hydrophobic, van der Waals contributions, and possibly conformational changes. Proteins can adsorb to mineral surfaces under globally repulsive electrostatic conditions, indicating

local attractive electrostatic interactions (i.e., interaction with specific amino acid residues on the protein surface bearing charge opposite that of the global protein charge) or the contribution of hydrophobic effects (Arai and Norde, 1990; Norde, 2008). Structural rearrangements can constitute an important driving force for adsorption due to the entropy gain associated with protein unfolding on surfaces. Entropic effects also involve reorganization of water molecules surrounding the protein and surface; adsorption to hydrophobic sorbents may be driven largely by the resulting dehydration of the surface. At present, the mechanisms underlying PrP^{TSE} attachment to soil particle surfaces remain poorly understood. Insight into these mechanisms is expected to aid predictions of prion transport and bioavailability in natural and engineered environments, and the development of efficient extraction methods to recover PrP^{TSE} from soils for detection and decontamination efforts.

The strong attachment of PrP^{TSE} to soil particle surfaces has been evidenced by its limited desorption under a variety of conditions. Extraction of PrP^{TSE} from soil particles typically requires anionic detergents. Reported effective extraction conditions include SDS-PAGE sample buffer (100 mM Tris, 7.5 mM EDTA, 100 mM DTT, 350 mM SDS, pH 8.0) at 100°C (Johnson et al., 2006), 1% N-lauroyl sarcosine in 100 mM phosphate buffer at pH 7.0 with 40 µg mL⁻¹ PK (Cooke et al., 2007), and 1% SDS in water at room temperature (Seidel et al., 2007). Many treatments found effective to extract other proteins from mineral surfaces (e.g., pH extrema, PBS, guanidinium, urea; Docoslis et al., 2001) have been found ineffective in removing PrP^{TSE} from montmorillonite clay surfaces (Johnson et al., 2006). Attachment to particles in fine-textured soils and clay minerals often results in low PrP^{TSE} recoveries. The same is true of recPrP (Cooke et al., 2007; Rigou et al., 2006). Decreased recovery of PrP^{TSE} at longer contact times has been observed (Maddison et al., 2010) and may reflect protein degradation, increase in the strength of interaction with soil particles over time (Hatzinger and Alexander, 1995; van Oss et al., 2001), entrapment in particle-associated NOM, diffusion into small pores in the soil matrix, or the formation of hydrogen or covalent bonds with humic substances (Hsu and Hatcher, 2005, 2006). The extent to which these processes may contribute to the persistence of prions in soil is unclear at present.

Solution pH and ionic strength influence protein adsorption. Proteins typically exhibit maximal adsorption to negatively charged surfaces near the IEP of the protein. At pH values below IEP, decreased adsorption may result from the protein unfolding at the surface, thereby reducing the quantity of protein required for saturation. Above the protein IEP, the mechanism of attachment likely reflects a competition between repulsive electrostatic and attractive hydrophobic interactions between the protein and surface. Additionally, lateral electrostatic repulsion of like-charged protein molecules may reduce adsorption at pH values above and below the protein IEP (Quiquampoix et al., 1993; Norde and Lyklema, 1978; Suzawa and Shirahama, 1991). The influence of solution pH and ionic strength on PrP^{TSE} attachment to quartz sand has been reported (Ma et al., 2007). Quartz surfaces are negatively charged in solutions at environmentally relevant pH (i.e., pH 4–9). Maximal attachment to quartz was found at pH 4, near the average IEP for PrP^{TSE} aggregates. Attachment of PrP^{TSE} to quartz increased with increasing ionic strength, congruent with the shielding of electrostatic repulsion between the protein and quartz surface (Ma et al., 2007). Attachment under globally electrostatically repulsive conditions suggests the importance of oppositely charged patches on the protein surface.

Adsorption of β-recPrP to muscovite mica has similarly been studied as a function of solution pH (Vasina et al., 2005). The β-recPrP (IEP = 9.77 due to the absence of N-linked glycans and GPI anchor, which contain sialic acid residues in mammalian PrP) carried a net positive charge over the pH range examined (pH = 4–9), and its strong attachment to

negatively charged mica surfaces under all solution conditions was attributable to electrostatic interactions. Revault et al. (2005) found that recPrP adsorbed to montmorillonite exhibited a gain in intramolecular β -sheet content and a concomitant loss of α -helix content in deuterated buffers with pD > 4. At pD > 7, the secondary structure of recPrP in solution resembled that of the adsorbed protein. Spectra of adsorbed β -conformers differed from those of soluble β -oligomers (precursors to a fibril state) suggesting adsorption-induced conformational change on montmorillonite surfaces would not lead to a pathogenic (i.e., PrP^{TSE}) conformation.

The N-terminal domain (approximately residues 23–90) of PrP^{TSE} appears important for sorption, at least to some clay mineral surfaces. Both recPrP and PrP^{TSE} desorbed from montmorillonite and clay-rich soils were cleaved at the N-terminus (Johnson et al., 2006; Rigou et al., 2006; Cooke et al., 2007; Maddison et al., 2010). Cooke et al. (2007) found that PK digestion increased PrP^{TSE} desorption from a sandy clay loam but not from the loamy sand soils examined. The N-terminus of PrP^{TSE} and recPrP is flexible and contains 10 basic amino acid residues. These positively charged residues may interact strongly with negatively charged clay surfaces, even when global electrostatics are repulsive. The N-terminus may intercalate into the interlayer spaces of montmorillonite. Although X-ray diffraction failed to provide evidence of penetration of the inter-layer spaces by PrP^{TSE} (Johnson et al., 2006), intercalation of other proteins in the interlayer spaces of smectite clays has been reported (Violante et al., 1995; Harter and Stotzky, 1973).

Predicting the attachment of PrP^{TSE} to soil particles is complicated by the limited information available about its structure and the variable number and composition of oligosaccharides. The N-linked glycans are expected to significantly influence PrP^{TSE} adsorption since they occupy nearly the same volume as the polypeptide and extend toward the exterior of structured aggregates (DeMarco and Daggett, 2009). Few studies have investigated the effect of glycosylation on protein adsorption. A recent study using *3 ermomyces lanuginosus* lipase found no difference in adsorption to hydrophobic surfaces among the unglycosylated, monoglycosylated, and pentaglycosylated protein but adsorption to hydrophilic surfaces of only the pentaglycosylated form (Pinholt et al., 2010). The glycans in this study were composed of uncharged sugar residues. In general, the size and charge of oligosaccharides likely determines their relative contribution to protein adsorption. The GPI anchor at the C terminus of PrP^{TSE} may also influence its adsorption to soil particle surfaces. Because the phosphatidylinositol group is lipophilic, it is expected to interact strongly with hydrophobic surfaces. However, the specific effect of membrane anchors on protein adsorption has not been discussed in the literature.

Mobility of PrP^{TSE} in Soils

The transport of prions in soil has important implications for the disposal of potentially infected materials, management of TSE-contaminated pastures, delivery of PrP^{TSE} to surface waters, and the bioaccessibility of TSE agents in natural environments. In saturated column experiments, the potential for partially purified PrP^{TSE} to migrate through soils and porous landfill materials has been investigated (Jacobson et al., 2009; Jacobson et al., 2010). The migration of PrP^{TSE} through fine quartz sand, shredded municipal solid waste (fresh and aged), and daily cover materials (natural soils and green waste residual) was examined using landfill leachate as the eluant (Jacobson et al., 2009). All detectable PrP^{TSE} was retained near the point of introduction in columns packed with soil or fine quartz sand, but 0.3 to 28% of the added PrP^{TSE} eluted from columns containing green residual waste (i.e., composted materials) and municipal solid waste. A similar study using protease-digested brain homogenate as the prion source assessed the potential for PrP^{TSE} to migrate through natural soils (Jacobson et al., 2010). All detectable PrP^{TSE} was retained in columns of five different soils of high sand or silt content when artificial rainwater was used as the eluant

(Jacobson et al., 2010). A study using α -recPrP also reported limited transport in column experiments monitored for 6 to 9 mo under conditions of normal and reduced microbial activity and varying water content (simulating a fluctuating water table) (Cooke and Shaw, 2007). Overall, limited prion mobility in soil columns is consistent with the strong attachment of PrP^{TSE} to soil particles. Dissolved organic carbon may contribute to PrP^{TSE} mobility in soil and therefore warrants further study. The studies conducted to date suggest that in the absence of preferential flow paths or facilitated transport, prion mobility in most soils and subsurface environments should be quite limited. This suggests maintenance of TSE agents near the soil surface where they would be more accessible to grazing animals. Prions at the soil surface would also be more available for entrainment into overland flow and delivery to water bodies (Nichols et al., 2009).

Infectivity of Soil Particle-Bound PrP^{TSE}

For soil to represent a plausible reservoir of TSE agents in the environment, soil particle-bound prions must remain bioavailable and infectious to susceptible animals. In laboratory experiments, PrP^{TSE} bound to soil and mineral particles retained undiminished infectivity toward hamsters inoculated intracerebrally (Johnson et al., 2006). Oral exposure of hamsters to montmorillonite- and soil-prion complexes demonstrated transmission by this route of uptake (Johnson et al., 2007). Surprisingly, PrP^{TSE} binding to montmorillonite dramatically increased disease penetrance (Fig. 3) with an effective titer higher than that of the unbound agent by a factor of 680. Similarly, two of the three soils tested increased disease transmission, as did quartz microparticles and kaolinite (Johnson et al., 2007; Johnson et al., 2011). Because the digestive physiology of rodents differs significantly from that of animals affected by environmental TSE transmission (deer, elk, goats, sheep), the extent to which soil impacts oral TSE transmission in ruminants warrants research.

Several possible mechanisms may account for increased transmission of soil-bound prions. Attachment to soil particles may alter PrP^{TSE} conformation or aggregation state in a manner that increases infectivity. Surface-induced conformational change is well established for adsorbed proteins, and the extent of this transition depends on surface polarity and the conformational lability of the protein. Several studies have reported the conformational alteration of prteins (including α -recPrP) upon adsorption to clay minerals (Baron et al., 1999; Servagent-Noinville et al., 2000; Revault et al., 2005). As discussed above, conformational alteration of α -recPrP on adsorption to montmorillonite has been reported (Revault et al., 2005). Research examining potential changes to the secondary structure of mineral particle-bound PrP^{TSE} is needed. Slight alteration of PrP^{TSE} structure on binding to soil particles may have a considerable effect on TSE infectivity.

Efficient transmission of soil-bound prions also depends on the effect of particle binding on in vivo mechanisms of uptake. Soil particles may enhance disease transmission by protecting prions from inactivation in the gastrointestinal tract and/or by prolonging PrP^{TSE} residence times. Detachment from mineral particles may not be required for PrP^{TSE} uptake. Peyer's patches (gut-associated lymphatic tissue in the small intestine) are involved in the early stages of CWD and scrapie transmission (Sigurdson et al., 1999; Andréoletti et al., 2000; Miller and Williams, 2002). Peyer's patches sample the gut lumen for microparticles similar in size to montmorillonite, taking them up by endocytotic mechanisms (des Rieux et al., 2006).

Biotic and Abiotic Inactivation of Prions

Efforts to limit the spread of horizontally transmitted TSEs are complicated by the known persistence of prions in soil (Fig. 4; Seidel et al., 2007; Brown and Gajdusek, 1991). Currently, effective methods to remediate naturally contaminated areas are lacking, and the

extent to which natural degradation processes in soil may inactivate prions remains unclear. Potential PrP^{TSE} inactivation pathways include microbial degradation, degradation by extracellular enzymes (proteases and oxidases), oxidation by inorganic oxidants, and denaturation due to repeated soil freeze–thaw and wetting–drying events. Insight into the potential degradation of prions in soil may advance the understanding of environmental TSE transmission and result in practical methods for land decontamination. As discussed above, analytical methods capable of detecting prions in naturally contaminated soils are currently lacking. Studies examining prion degradation have therefore used samples experimentally amended with prions. These experiments often use rodent-adapted prion strains that lack environmental relevance. While useful, inactivation of prions needs to be confirmed for the strain and species of interest. For example, the stability in brain homogenate of rodent-adapted prion strains may vary from those of scrapie and CWD agents (Saunders et al., 2008). Many studies use immunodetection methods to measure declines in PrP^{TSE} immunoreactivity; definitive results on prion inactivation require measurement of TSE infectivity by bioassay. Below we discuss initial research on biotic and abiotic mechanisms of prion degradation in soil with an emphasis on future research needs.

Degradation of PrP^{TSE} by Microorganisms

Considering the diversity of bacterial and fungal organisms in the soil environment, significant interest exists in identifying microorganisms capable of degrading PrP^{TSE}. Several studies have examined PrP^{TSE} degradation in samples with enhanced microbial activity. Scherbel et al. (2006) found that rumen and colonic microorganisms were competent to degrade PrP^{TSE} associated with hamster-adapted scrapie (263K strain) under anaerobic conditions in the presence of soluble carbohydrates. Degradation in this study was evidenced by complete loss of PrP^{TSE} immunoreactivity; however, further analysis by animal bioassay revealed significant residual TSE infectivity (Scherbel et al., 2007). Compost represents a promising matrix for PrP^{TSE} degradation due to the combination of high temperatures (>55°C), pH fluctuations (5.5–8.5), and intense microbial activity. Huang et al. (2007) found that composting scrapie-infected sheep tissue for 3 to 4 mo reduced or eliminated PrP^{TSE} immunoreactivity. The effect of composting on any concomitant reduction in TSE infectivity awaits verification by animal bioassay. The microbial consortia responsible for activated sludge and anaerobic digestion in wastewater treatment plants were also examined for possible PrP^{TSE}-degrading activity. Hinckley et al. (2008) found that PrP^{TSE} (HY strain of hamster-adapted TME) partitioned to activated sludge solids (floc), indicating that the protein will be subject to biosolids treatment processes. PrP^{TSE} was not significantly degraded during incubation with activated sludge consortia or simulated mesophilic anaerobic digestion. Similarly, Kirchmayr et al. (2006) found little reduction in PrP^{TSE} levels following incubation with mesophilic anaerobic digester sludge.

Other microbial consortia that have been studied include bacteria used in food production and earthworm gut microflora (Müller-Hellwig et al., 2006; Nechitaylo et al., 2010). Six strains of cheese-ripening bacteria were found to reduce PrP^{TSE} (263K) levels following 24-h digestion at 30°C. Nechitaylo et al. (2010) found little degradation of α -recPrP by aqueous extracts of casts from two earthworm species and concluded that earthworm digestive enzymes and gut microflora are not expected to significantly degrade PrP^{TSE}.

To our knowledge, only one study to date has investigated soil microorganisms as a means of prion inactivation. Rapp et al. (2006) demonstrated that buried animal carcasses stimulate the proteolytic activity of the surrounding soil and that proteases extracted from the stimulated soil degraded β -recPrP within 8 d. The use of β -recPrP in this study limits the conclusions that can be drawn about PrP^{TSE} degradation under similar conditions.

Prion Degradation by Isolated Enzymes

Proteases (protein-degrading enzymes) are grouped into five distinct classes on the basis of the nature of their catalytic site: serine, cysteine, aspartate, metallo, and threonine proteases. Metallo and aspartate proteases use an activated water molecule to attack the peptide backbone of the protein, whereas serine, cysteine, and threonine proteases are named for the nucleophilic residue in the active site of the enzyme (Puente et al., 2003). A number of proteases have been examined for their ability to degrade PrP^{TSE}. For optimal activity, most require conditions that partially denature the protein such as high temperature, high pH, and/or the presence of detergents.

Subtilisin-like enzymes constitute one of the major subfamilies of serine proteases and have received the most study with respect to potential PrP^{TSE} degradation. In most cases, these subtilisin-like proteases are applied under harsh conditions (temperature $\geq 50^{\circ}\text{C}$, pH ≥ 9). Exposure to the subtilisin enzyme Properase effected a 10^3 -fold reduction in mouse-adapted BSE infectivity at 60°C and pH 12 (McLeod et al., 2004). Amino acid substitutions increased the thermostability of the enzyme, which is now marketed as Prionzyme (Dickinson et al., 2009). Exposure (30-min) to Prionzyme has achieved a $\geq 10^7$ -fold reduction in mouse-adapted BSE infectivity after treatment at 60°C and pH 12. Treatment of PrP^{TSE} with subtilisin enzyme 309-v resulted in a 52% increase in survivorship of mice inoculated with mouse-adapted scrapie agent (Pilon et al., 2009).

Keratinases often exhibit sequence homology with subtilisin enzymes (Macedo et al., 2005; Evans et al., 2000) and have attracted attention because like PrP^{TSE} their natural substrate, keratin, possesses high β -sheet content. The feather-degrading keratinase produced by *Bacillus licheniformis* strain PWD-1 effected the extensive breakdown of PrP^{TSE} (BSE and sheep scrapie), but only in the presence of 2% sarkosyl and at temperatures exceeding 100°C (Langeveld et al., 2003).

Alkaline serine proteases (ASP) are serine proteases that function optimally at an alkaline pH. At high temperature and pH, PrP^{TSE} (263K) was digested by treatment with an ASP (Hui et al., 2004b) and by an ASP-like enzyme isolated from *Streptomyces* strain 99-GP-2D-5 (Hui et al., 2004a). Incubation of PrP^{TSE} (263K) with purified NAPase, a keratinolytic alkaline protease from *Nodocardiopsis* sp. TOA-1, exhibited optimal activity at 60°C and pH 11 (Mitsuiki et al., 2006); however, controls were lacking to demonstrate that the alkali solution (pH 11) used for enzyme purification did not degrade PrP^{TSE}.

Disaggregation and partial denaturation of PrP^{TSE} may be promoted at high pH, elevated temperatures, and in the presence of detergents as inter- and intramolecular hydrogen bonds are broken, access to the peptide backbone is facilitated, and the protein is solubilized. While these conditions enhance proteolytic degradation of PrP^{TSE}, they do not represent conditions prevailing in natural soils and are impractical for treating in-place soils. To date, three studies have reported PrP^{TSE} proteolysis under milder, more environmentally relevant conditions. Prionzyme was shown to degrade PrP^{TSE} (elk CWD and HY strain of TME) at neutral pH and moderate temperatures (Saunders et al., 2010). Proteases produced by the mesophilic soil bacterium *Streptomyces galbus* var. *achromogenes* 695-206 were capable of degrading β -recPrP at 30°C but were less effective at degrading bona fide PrP^{TSE} (mouse-adapted BSE strain 6PB1) (Tsirolnikov et al., 2004). Proteolytically active extracts from whole lichen tissue reduced PrP^{TSE} (Hy, 263K, and CWD) levels at least 100-fold at 37°C (Johnson, personal communication, 2010). The efficacy of the above treatments in reducing TSE infectivity remains to be verified.

Additional Factors Affecting Prion Degradation in Soil

The attachment of PrP^{TSE} to soil particles may confer partial protection from denaturation and proteolysis (Johnson et al., 2006). Mineral-bound Cry proteins are protected from proteolysis by soil microorganisms (Koskella and Stotzky, 1997; Chevallier et al., 2003). Glomalins, glycoproteins associated with arbuscular mycorrhizal fungi, represent a significant fraction of the carbon immobilized in soil due to their resistance to microbial hydrolysis (Wright and Upadhyaya, 1996, 1998). This persistence is probably due to their association with soil minerals including iron and aluminum oxyhydroxides (Quiquampoix and Burns, 2007). The extraordinary persistence of prions in the environment may likewise be due, at least in part, to their strong attachment to soil particles.

Physical processes that prions may be exposed to in the environment such as freeze–thaw cycles or wetting and drying events may also contribute to the degradation of prions. These processes disrupt soil structure (Hillel, 1998) and may partially denature PrP^{TSE} to the extent that enzymes gain access to the peptide backbone or infectivity is lost. To date, effects of freeze–thaw and wetting–drying cycles on PrP^{TSE} and prion infectivity have not been formally investigated.

Abiotic Degradation of Prions

Transition metal oxides found in soils (viz. manganese, iron and aluminum oxides) can mediate the transformation of organic compounds including aromatic amines (Laha and Luthy, 1990), phenolic compounds (McBride, 1989), estrogens (Xu et al., 2008), herbicides (Barrett and McBride, 2005; Cheney et al., 1998), and antibiotics (Zhang et al., 2008; Rubert and Pedersen, 2006; Zhang and Huang, 2007; Chen and Huang, 2010). Aqueous suspensions of a synthetic manganese oxide similar to vernadite (δ -MnO₂) were found to effect PrP^{TSE} degradation (HY strain of hamster-adapted TME) at pH $\leq$ 5 (Russo et al., 2009). At pH 4 and the highest MnO₂ concentration used, PrP^C-to-PrP^{TSE} converting ability (as measured by PMCA) was reduced by $\geq$ 4 orders of magnitude.

Lignolytic fungi produce reactive oxygen species in their initial assault on woody plant debris (Cohen et al., 2002), and a methoxyhydroquinone-driven Fenton reaction has been used to simulate the extracellular chemistry of such fungi in vitro (Metz et al., 2009). The Fenton reaction can induce radical-mediated damage to both the peptide backbone and side chains of proteins through hydroxylation or direct oxidation of amino acid residues (Stadtman and Berlett, 1997) and may represent a pathway of PrP^{TSE} inactivation in the environment. Fenton reagent induced degradation of PrP^{TSE} (263K, Sc237) in vitro, significantly delayed onset of clinical symptoms (Park et al., 2008) and appeared to effect a 10⁶-fold reduction in PrP^C-to-PrP^{TSE} converting ability (based on PMCA) (Suyama et al., 2007). In the latter study, untreated positive controls also failed to efficiently amplify, limiting confidence in the PMCA results. Overall, these studies were conducted at hydroxyl radical concentrations considerably higher than those expected in soil environments.

Conclusions

The pathogenic prion protein readily attaches to soil particle surfaces, and the interaction between PrP^{TSE} and some clay mineral surfaces is particularly avid. Prions exhibit limited mobility in soil columns, suggesting that in the absence of preferential flow paths or facilitated transport, TSE agents released into soil would remain near the soil surface where they are more accessible to grazing animals. Particle-associated PrP^{TSE} molecules may migrate from locations of deposition via transport processes affecting soil particles, including entrainment in and movement with air and overland flow. The mechanisms of PrP^{TSE} attachment to soil particle surfaces remain to be elucidated. The extent of PrP^{TSE}

interaction with natural organic matter and the influence of N-linked glycosylation and glypiation on PrP^{TSE} attachment to soil particle surfaces warrant research. Soil and mineral particle-bound prions exhibit increased disease transmission by the oral route relative to that of the unbound agent. Enhanced disease transmission by particle-bound PrP^{TSE} may result from altered bioavailability of soil-bound prions by animals, changes in PrP^{TSE} conformation or aggregation state that impact TSE infectivity, or both. Research is needed to elucidate these mechanisms and evaluate the extent to which soil impacts oral TSE transmission in ruminants.

Several reports have shown that prions can persist in soil for several years. Significant interest remains in developing methods that could be applied to degrade PrP^{TSE} in naturally contaminated soils. Preliminary research suggests that serine proteases and the microbial consortia in stimulated soils and compost may partially degrade PrP^{TSE}. Transition metal oxides in soil (viz. manganese oxide) may also mediate prion inactivation. Overall, the effect of prion attachment to soil particles on its persistence in the environment is not well understood, and additional study is needed to determine its implications on the environmental transmission of scrapie and CWD.

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Abbreviations

α-recPrP	proteolytically labile recombinant prion protein
β-recPrP	recombinant prion protein folded into β -sheet rich oligomers
ASP	alkaline serine protease
BSE	bovine spongiform encephalopathy
CWD	chronic wasting disease
ELISA	enzyme-linked immunosorbent assay
GPI	glycosylinositol phospholipid
IEP	isoelectric point
IU	infectious unit
LC-MS	liquid chromatography–mass spectrometry
NAP	keratinolytic alkaline protease from <i>Nodocardiopsis</i> sp. TOA-1
NMR	nuclear magnetic resonance spectroscopy
NOM	natural organic matter
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
pD	negative logarithmic value of the deuterium ion concentration
PK	proteinase K
PMCA	protein misfolding cyclic amplification
PrP	prion protein

PrP^C	normal, benign cellular form of the prion protein
PrP^{TSE}	pathologically misfolded form of prion protein
recPrP	recombinant prion protein
SDS	sodium dodecyl sulfate
TME	transmissible mink encephalopathy
TSE	transmissible spongiform encephalopathy
UV	ultraviolet

References

- Aguzzi A, Heikenwalder M, Polymenidou M. Insights into prion strains and neurotoxicity. *Nat Rev Mol Cell Biol.* 2007; 8:552–561. [PubMed: 17585315]
- Aiken JM, Williamson JL, Borchardt LM, Marsh RF. Presence of mitochondrial d-loop DNA in scrapie-infected brain preparations enriched for the prion protein. *J Virol.* 1990; 64:3265–3268. [PubMed: 1972202]
- Andréoletti O, Berthon P, Marc D, Sarradin P, Grosclaude J, van Keulen L, Schelcher F, Elsen JM, Lantier F. Early accumulation of PrP^{Sc} in gut-associated lymphoid and nervous tissues of susceptible sheep from a romanov flock with natural scrapie. *J Gen Virol.* 2000; 81:3115–3126. [PubMed: 11086143]
- Arai T, Norde W. The behavior of some model proteins at solid-liquid interfaces: 1. Adsorption from single protein solutions. *Coll Surf.* 1990; 51:1–15.
- Atarashi R, Wilham JM, Christensen L, Hughson AG, Moore RA, Johnson LM, Onwubiko HA, Priola SA, Caughey B. Simplified ultrasensitive prion detection by recombinant PrP conversion with shaking. *Nat Methods.* 2008; 5:211–212. [PubMed: 18309304]
- Baron MH, Revault M, Servagent-Noinville S, Abadie J, Quiquampoix H. Chymotrypsin adsorption on montmorillonite: Enzymatic activity and kinetic FTIR structural analysis. *J Colloid Interface Sci.* 1999; 214:319–332. [PubMed: 10339371]
- Barrett KA, McBride MB. Oxidative degradation of glyphosate and aminomethylphosphonate by manganese oxide. *Environ Sci Technol.* 2005; 39:9223–9228. [PubMed: 16382946]
- Barron RM, Campbell SL, King D, Bellon A, Chapman KE, Williamson RA, Manson JC. High titers of transmissible spongiform encephalopathy infectivity associated with extremely low levels of PrP^{Sc} in vivo. *J Biol Chem.* 2007; 282:35878–35886. [PubMed: 17923484]
- Bolton DC, Bendheim PE, Marmorstein AD, Potempska A. Isolation and structural studies of the intact scrapie agent protein. *Arch Biochem Biophys.* 1987; 258:579–590. [PubMed: 2890330]
- Bolton DC, Meyer RK, Prusiner SB. Scrapie PrP 27-30 is a sialoglycoprotein. *J Virol.* 1985; 53:596–606. [PubMed: 3918176]
- Boyd, SA.; Mortland, MM. Enzyme interactions with clays and clay-organic matter complexes. In: Bollag, J-M.; Stotzky, G., editors. *Soil biochemistry.* Vol. 6. Marcel Dekker; New York: 1990. p. 1-28.
- Brown P, Gajdusek DC. Survival of scrapie virus after 3 years' interment. *Lancet.* 1991; 337:269–270. [PubMed: 1671114]
- Brown P, Rau EH, Lemieux P, Johnson BK, Bacote AE, Gajdusek DC. Infectivity studies of both ash and air emissions from simulated incineration of scrapie-contaminated tissues. *Environ Sci Technol.* 2004; 38:6155–6160. [PubMed: 15575075]
- Bruce ME. TSE strain variation. *Br Med Bull.* 2003; 66:99–108. [PubMed: 14522852]
- Castilla J, Saa P, Hetz C, Soto C. In vitro generation of infectious scrapie prions. *Cell.* 2005; 121:195–206. [PubMed: 15851027]
- Caughey BW, Dong A, Bhat KS, Ernst D, Hayes SF, Caughey WS. Secondary structure analysis of the scrapie-associated protein PrP 27-30 in water by infrared spectroscopy. *Biochemistry.* 1991; 30:7672–7680. [PubMed: 1678278]

- Chen W, Huang C. Adsorption and transformation of tetracycline antibiotics with aluminum oxide. *Chemosphere*. 2010; 79:779–785. [PubMed: 20378149]
- Cheney MA, Shin JY, Crowley DE, Alvey S, Malengreau N, Sposito G. Atrazine dealkylation on a manganese oxide surface. *Colloids Surf A Physicochem Eng Asp*. 1998; 137:267–273.
- Chevallier T, Muchaonyerwa P, Chenu C. Microbial utilisation of two proteins adsorbed to a vertisol clay fraction: Toxin from *Bacillus thuringiensis* subsp. *Tenebrionis* and bovine serum albumin. *Soil Biol Biochem*. 2003; 35:1211–1218.
- Cobb NJ, Sonnichsen FD, McHaourab H, Surewicz WK. Molecular architecture of human prion protein amyloid: A parallel, inregister beta-structure. *Proc Natl Acad Sci USA*. 2007; 104:18946–18951. [PubMed: 18025469]
- Cohen R, Jensen KA, Houtman CJ, Hammel KE. Significant levels of extracellular reactive oxygen species produced by brown rot basidiomycetes on cellulose. *FEBS Lett*. 2002; 531:483–488. [PubMed: 12435597]
- Colby DW, Zhang Q, Wang S, Groth D, Legname G, Riesner D, Prusiner SB. Prion detection by an amyloid seeding assay. *Proc Natl Acad Sci USA*. 2007; 104:20914–20919. [PubMed: 18096717]
- Cooke CM, Rodger J, Smith A, Fernie K, Shaw G, Somerville RA. Fate of prions in soil: Detergent extraction of PrP from soils. *Environ Sci Technol*. 2007; 41:811–817. [PubMed: 17328187]
- Cooke CM, Shaw G. Fate of prions in soil: Longevity and migration of recPrP in soil columns. *Soil Biol Biochem*. 2007; 39:1181–1191.
- DeMarco ML, Daggett V. Characterization of cell-surface prion protein relative to its recombinant analogue: Insights from molecular dynamics simulations of diglycosylated, membrane-bound human prion protein. *J Neurochem*. 2009; 109:60–73. [PubMed: 19226372]
- DeMarco ML, Silveira J, Caughey B, Daggett V. Structural properties of prion protein protofibrils and fibrils: An experimental assessment of atomic models. *Biochemistry*. 2006; 45:15573–15582. [PubMed: 17176078]
- des Rieux A, Fievez V, Garinot M, Schneider YJ, Prat V. Nanoparticles as potential oral delivery systems of proteins and vaccines: A mechanistic approach. *J Control Release*. 2006; 116:1–27. [PubMed: 17050027]
- Dickinson J, Murdoch H, Dennis MJ, Hall GA, Bott R, Crabb WD, Penet C, Sutton JM, Raven NDH. Decontamination of prion protein (BSE301v) using a genetically engineered protease. *J Hosp Infect*. 2009; 72:65–70. [PubMed: 19201054]
- Docoslis A, Rusinski LA, Giese RF, van Oss CJ. Kinetics and interaction constants of protein adsorption onto mineral microparticles-measurement of the constants at the onset of hysteresis. *Colloids Surf B Biointerfaces*. 2001; 22:267–283.
- Edgeworth JA, Jackson GS, Clarke AR, Weissmann C, Collinge J. Highly sensitive, quantitative cell-based assay for prions adsorbed to solid surfaces. *Proc Natl Acad Sci USA*. 2009; 106:3479–3483. [PubMed: 19204279]
- Endo T, Groth D, Prusiner SB, Kobata A. Diversity of oligosaccharide structures linked to asparagines of the scrapie prion protein. *Biochemistry*. 1989; 28:8380–8388. [PubMed: 2574992]
- Evans KL, Crowder J, Miller ES. Subtilisins of *Bacillus* spp. hydrolyze keratin and allow growth on feathers. *Can J Microbiol*. 2000; 46:1004–1011. [PubMed: 11109488]
- Fries GF. Ingestion of sludge applied organic chemicals by animals. *Sci Total Environ*. 1996; 185:93–108. [PubMed: 8643962]
- Genovesi S, Leita L, Sequi P, Andrighetto I, Sorgato MC, Bertoli A. Direct detection of soil-bound prions. *PLoS One*. 2007; 2:e1069.10.1371/journal.pone.0001069 [PubMed: 17957252]
- Georgsson G, Sigurdarson S, Brown P. Infectious agent of sheep scrapie may persist in the environment for at least 16 years. *J Gen Virol*. 2006; 87:3737–3740. [PubMed: 17098992]
- Govaerts C, Wille H, Prusiner SB, Cohen FE. Evidence for assembly of prions with left-handed beta-helices into trimers. *Proc Natl Acad Sci USA*. 2004; 101:8342–8347. [PubMed: 15155909]
- Gregori L, Kovacs GG, Alexeeva I, Budka H, Rohwer RG. Excretion of transmissible spongiform encephalopathy infectivity in urine. *Emerg Infect Dis*. 2008; 14:1406–1412. [PubMed: 18760007]
- Greig JR. Scrapie: Observations on the transmission of the disease by mediate contact. *Vet J*. 1940; 96:203–206.

- Harter RD, Stotzky G. X-ray diffraction, electron microscopy, electrophoretic mobility, and pH of some stable smectite-protein complexes. *Soil Sci Soc Am J.* 1973; 37:116–123.
- Hatzinger PB, Alexander M. Effect of aging of chemicals in soil on their biodegradability and extractability. *Environ Sci Technol.* 1995; 29:537–545.
- Helmke, PA. Chemistry of cadmium in solution. In: McLaughlin, MJ.; Singh, BR., editors. *Cadmium in soils and plants.* Kluwer Academic; Norwell, MA: 1999. p. 39-63.
- Hillel, D. *Environmental soil physics.* Academic Press; San Diego, CA: 1998.
- Hinckley GT, Johnson CJ, Jacobson KH, Bartholomay C, McMahon KD, McKenzie D, Aiken JM, Pedersen JA. Persistence of pathogenic prion protein during simulated wastewater treatment processes. *Environ Sci Technol.* 2008; 42:5254–5259. [PubMed: 18754377]
- Hsu PH, Hatcher PG. New evidence for covalent coupling of peptides to humic acids based on 2D NMR spectroscopy: A means for preservation. *Geochim Cosmochim Acta.* 2005; 69:4521–4533.
- Hsu PH, Hatcher PG. Covalent coupling of peptides to humic acids: Structural effects investigated using 2D NMR spectroscopy. *Org Geochem.* 2006; 37:1694–1704.
- Huang H, Spencer JL, Soutyrine A, Guan J, Rendulich J, Balachandran A. Evidence for degradation of abnormal prion protein in tissues from sheep with scrapie during composting. *Can J Vet Res.* 2007; 71:34–40. [PubMed: 17193880]
- Hui Z, Doi H, Kanouchi H, Matsuura Y, Mohri S, Nonomura Y, Oka T. Alkaline serine protease produced by streptomyces sp. degrades PrP^{Sc}. *Biochem Biophys Res Commun.* 2004a; 321:45–50. [PubMed: 15358213]
- Hui Z, Minamiguchi K, Doi H, Kinoshita N, Kanouchi H, Oka T. Recombinant alkaline serine protease II degrades scrapie isoform of prion protein. *In Vitro Cell Dev Bio Anim.* 2004b; 40:293–296. [PubMed: 15723565]
- Jacobson KH, Lee S, McKenzie D, Benson CH, Pedersen JA. Transport of the pathogenic prion protein through landfill materials. *Environ Sci Technol.* 2009; 43:2022–2028. [PubMed: 19368208]
- Jacobson KH, Lee S, Somerville RA, McKenzie D, Benson CH, Pedersen JA. Transport of pathogenic prion protein through soils. *J Environ Qual.* 2010; 39:1145–1152. [PubMed: 20830901]
- Jarrett JT, Lansbury PT. Seeing “one-dimensional crystallization” of amyloid: A pathogenic mechanism in Alzheimer’s disease and scrapie? *Cell.* 1993; 73:1055–1058. [PubMed: 8513491]
- Johnson CJ, Pedersen JA, Chappell RJ, McKenzie D, Aiken JM. Oral transmissibility of prion disease is enhanced by binding to soil particles. *PLoS Pathog.* 2007; 3:E93.10.1371/journal.ppat.0030093 [PubMed: 17616973]
- Johnson CJ, Pedersen JA, McKenzie D, Aiken JM. Meat and bone meal and mineral feed additives increase the risk of oral prion disease transmission. *J Toxicol Environ Health A.* 2011; 74:161–166. [PubMed: 21218345]
- Johnson CJ, Phillips KE, Schramm PT, McKenzie D, Aiken JM, Pedersen JA. Prions adhere to soil minerals and remain infectious. *PLoS Pathog.* 2006; 2:E32.10.1371/journal.ppat.0020032 [PubMed: 16617377]
- Kellings K, Meyer N, Mirenda C, Prusiner SB, Riesner D. Analysis of nucleic acids in purified scrapie prion preparations. *Arch Virol Suppl.* 1993; 7:215–225. [PubMed: 8219805]
- Kimberlin RH, Walker C. Characteristics of a short incubation model of scrapie in the golden hamster. *J Gen Virol.* 1977; 34:295–304. [PubMed: 402439]
- Kirchmayr R, Reichl HE, Schildorfer H, Braun R, Somerville RA. Prion protein: Detection in “spiked” anaerobic sludge and degradation experiments under anaerobic conditions. *Water Sci Technol.* 2006; 53:91–98. [PubMed: 16784193]
- Klöhn PC, Stoltze L, Flehsig E, Enari M, Weissmann C. A quantitative, highly sensitive cell-based infectivity assay for mouse scrapie prions. *Proc Natl Acad Sci USA.* 2003; 100:11666–11671. [PubMed: 14504404]
- Koskella J, Stotzky G. Microbial utilization of free and clay-bound insecticidal toxins from *Bacillus thuringiensis* and their retention of insecticidal activity after incubation with microbes. *Appl Environ Microbiol.* 1997; 63:3561–3568. [PubMed: 16535692]
- Laha S, Luthy RG. Oxidation of aniline and other primary aromatic amines by manganese dioxide. *Environ Sci Technol.* 1990; 24:363–373.

- Langeveld JP, Wang JJ, Van de Wiel DF, Shih GC, Garssen GJ, Bossers A, Shih JC. Enzymatic degradation of prion protein in brain stem from infected cattle and sheep. *J Infect Dis.* 2003; 188:1782–1789. [PubMed: 14639552]
- Lasmez C, Deslys JP, Robain O, Jaegly A, Beringue V, Peyrin JM, Fournier JG, Hauw JJ, Rossier J, Dormont D. Transmission of the BSE agent to mice in the absence of detectable abnormal prion protein. *Science.* 1997; 275:402–405. [PubMed: 8994041]
- Lax AJ, Millson GC, Manning EJ. Involvement of protein in scrapie agent infectivity. *Res Vet Sci.* 1983; 34:155–158. [PubMed: 6407080]
- Leita L, Fornasier F, De Nobili M, Bertoli A, Genovesi S, Sequi P. Interactions of prion proteins with soil. *Soil Biol Biochem.* 2006; 38:1638–1644.
- Ma X, Benson CH, McKenzie D, Aiken JM, Pedersen JA. Adsorption of pathogenic prion protein to quartz sand. *Environ Sci Technol.* 2007; 41:2324–2330. [PubMed: 17438782]
- Macedo AJ, da Silva WO, Gava R, Driemeier D, Henriques JA, Termignoni C. Novel keratinase from *Bacillus subtilis* s14 exhibiting remarkable dehairing capabilities. *Appl Environ Microbiol.* 2005; 71:594–596. [PubMed: 15640244]
- Maddison BC, Owen JP, Bishop K, Shaw G, Rees HC, Gough KC. The interaction of ruminant PrP^{Sc} with soils is influenced by prion source and soil type. *Environ Sci Technol.* 2010; 44:8503–8508. [PubMed: 20968294]
- Mahal SP, Baker CA, Demczyk CA, Smith EW, Julius C, Weissmann C. Prion strain discrimination in cell culture: The cell panel assay. *Proc Natl Acad Sci USA.* 2007; 104:20908–20913. [PubMed: 18077360]
- Mathiason CK, Powers JG, Dahmes SJ, Osborn DA, Miller KV. Infectious prions in the saliva and blood of deer with chronic wasting disease. *Science.* 2006; 314:133–136. [PubMed: 17023660]
- McBride MB. Oxidation of dihydroxybenzenes in aerated aqueous suspensions of birnessite. *Clays Clay Miner.* 1989; 37:341–347.
- McKinley MP, Meyer RK, Kenaga L, Rahbar F, Cotter R, Serban A, Prusiner SB. Scrapie prion rod formation in vitro requires both detergent extraction and limited proteolysis. *J Virol.* 1991; 65:1340–1351. [PubMed: 1704926]
- McLeod AH, Murdoch H, Dickinson J, Dennis MJ, Hall GA, Buswell CM, Carr J, Taylor DM, Sutton JM, Raven NDH. Proteolytic inactivation of the bovine spongiform encephalopathy agent. *Biochem Biophys Res Commun.* 2004; 317:1165–1170. [PubMed: 15094392]
- Metz KM, Mangham AN, Bierman MJ, Jin S, Hamers RJ, Pedersen JA. Engineered nanomaterial transformation under oxidative environmental conditions: Development of an in vitro biomimetic assay. *Environ Sci Technol.* 2009; 43:1598–1604. [PubMed: 19350941]
- Miller MW, Williams ES. Detection of PrP^{CWD} in mule deer by immunohistochemistry of lymphoid tissues. *Vet Rec.* 2002; 151:610–612. [PubMed: 12463538]
- Miller MW, Williams ES, Hobbs NT, Wolfe LL. Environmental sources of prion transmission in mule deer. *Emerg Infect Dis.* 2004; 10:1003–1006. [PubMed: 15207049]
- Mitsuiki S, Hui Z, Matsumoto D, Sakai M, Moriyama Y, Furukawa K, Kanouchi H, Oka T. Degradation of PrP^{Sc} by keratinolytic protease from *Nocardia* sp. TOA-1. *Biosci Biotechnol Biochem.* 2006; 70:1246–1248. [PubMed: 16717429]
- Moore RA, Timmes A, Wilmarth PA, Priola SA. Comparative profiling of highly enriched 22L and Chandler mouse scrapie prion protein preparations. *Proteomics.* 2010; 10:2858–2869. [PubMed: 20518029]
- Morales R, Abid K, Soto C. The prion strain phenomenon: Molecular basis and unprecedented features. *Biochim Biophys Acta.* 2007; 1772:681–691. [PubMed: 17254754]
- Müller-Hellwig S, Groschup MH, Pichner R, Gareis M, Martlbauer E, Scherer S, Loessner MJ. Biochemical evidence for the proteolytic degradation of infectious prion protein PrP^{Sc} in hamster brain homogenates by foodborne bacteria. *Syst Appl Microbiol.* 2006; 29:165–171. [PubMed: 16464698]
- Nagaoka K, Yoshioka M, Shimozaki N, Yamamura T, Murayama Y, Yokoyama T, Mohri S. Sensitive detection of scrapie prion protein in soil. *Biochem Biophys Res Commun.* 2010; 397:626–630. [PubMed: 20570651]

- Nechitaylo TY, Timmis KN, Byzov BA, Kurakov AV, Belogolova E, Jones DL, Ferrer M, Golyshin PN. Fate of prions in soil: Degradation of recombinant prion in aqueous extracts from soil and casts of two earthworm species. *Soil Biol Biochem.* 2010; 42:1168–1171.
- Nichols TA, Pulford B, Wyckoff AC, Meyerett C, Michel B, Gertig K, Hoover EA, Jewell JE, Telling GC, Zabel MD. Detection of protease-resistant cervid prion protein in water from a CWD-endemic area. *Prion.* 2009; 3:171–183. [PubMed: 19823039]
- Norde W. Adsorption of proteins from solution at the solid-liquid interface. *Adv Colloid Interface Sci.* 1986; 25:267–340. [PubMed: 3333131]
- Norde, W. *Colloids and interfaces in life sciences.* Marcel Dekker; New York: 2003.
- Norde W. My voyage of discovery to proteins in flatland .. And beyond. *Colloids Surf B Biointerfaces.* 2008; 61:1–9. [PubMed: 18023976]
- Norde W, Lyklema J. The adsorption of human plasma albumin and bovine pancreas ribonuclease at negatively charged polystyrene surfaces: I. Adsorption isotherms. Effects of charge, ionic strength, and temperature. *J Colloid Interface Sci.* 1978; 66:257–265.
- Onisko B, Dynin I, Requena JR, Silva CJ, Erickson M, Carter JM. Mass spectrometric detection of attomole amounts of the prion protein by nanoLC/MS/MS. *J Am Soc Mass Spectrom.* 2007; 18:1070–1079. [PubMed: 17446085]
- Owen JP, Maddison BC, Whitlam GC, Gough KC. Use of thermolysin in the diagnosis of prion diseases. *Mol Biotechnol.* 2007; 35:161–170. [PubMed: 17435282]
- Palsson, PA. Rida (scrapie) in Iceland and its epidemiology. In: Prusiner, SB.; Hadlow, WJ., editors. *Slow transmissible diseases of the nervous system.* Vol. 1. Academic Press; New York: 1979. p. 357–366.
- Pan KM, Baldwin M, Nguyen J, Gasset M, Serban A, Groth D, Mehlhorn I, Huang Z, Fletterick RJ, Cohen FE, Prusiner SB. Conversion of alpha-helices into beta-sheets features in the formation of the scrapie prion proteins. *Proc Natl Acad Sci USA.* 1993; 90:10962–10966. [PubMed: 7902575]
- Park SJ, Kim NH, Jeong BH, Jin JK, Choi JK, Park YJ, Kim JI, Carp RI, Kim YS. The effect of fenton reaction on protease-resistant prion protein (PrP^{Sc}) degradation and scrapie infectivity. *Brain Res.* 2008; 1238:172–180. [PubMed: 18771660]
- Pedersen, JA.; Johnson, CJ.; Bell, CM.; Jacobson, KH.; Benson, CH.; McKenzie, D.; Aiken, JM. Goldschmidt. Davos; Switzerland: 2009 June 21–26. Soil and the transmission of prion diseases.
- Pedersen, JA.; Somerville, R. Why and how are TSEs sometimes spread via environmental routes?. In: Deslys, JP.; Pocchiari, M.; Reisner, D.; Somerville, R., editors. *Decontamination of prions.* Düsseldorf Univ. Press; Düsseldorf, Germany: 2011. (in press)
- Pilon JL, Nash PB, Arver T, Hoglund D, Vercauteren KC. Feasibility of infectious prion digestion using mild conditions and commercial subtilisin. *J Virol Methods.* 2009; 161:168–172. [PubMed: 19467265]
- Pinholt C, Fanø M, Wiberg C, Hostrup S, Bukrinsky JT, Frokjaer S, Norde W, Jorgensen L. Influence of glycosylation on the adsorption of *3 ermomyces lanuginosus* lipase to hydrophobic and hydrophilic surfaces. *Eur J Pharm Sci.* 2010; 40:273–281. [PubMed: 20380877]
- Polano M, Anselmi C, Leita L, Negro A, De Nobili M. Organic polyanions act as complexants of prion protein in soil. *Biochem Biophys Res Commun.* 2008; 367:323–329. [PubMed: 18174023]
- Prusiner SB. Molecular biology of prion diseases. *Science.* 1991; 252:1515–1522. [PubMed: 1675487]
- Prusiner SB. Prions. *Proc Natl Acad Sci USA.* 1998; 95:13363–13383. [PubMed: 9811807]
- Prusiner SB, Groth DF, Bolton DC, Kent SB, Hood LE. Purification and structural studies of a major scrapie prion protein. *Cell.* 1984; 38:127–134. [PubMed: 6432339]
- Prusiner SB, Groth DF, Cochran SP, Masiarz FR, McKinley MP, Martinez HM. Molecular properties, partial purification, and assay by incubation period measurements of the hamster scrapie agent. *Biochemistry.* 1980; 19:4883–4891. [PubMed: 6775697]
- Pucci A, D'Acqui LP, Calamai L. Fate of prions in soil: Interactions of recPrP with organic matter of soil aggregates as revealed by Itapas. *Environ Sci Technol.* 2008; 42:728–733. [PubMed: 18323094]
- Puente XS, Sanchez LM, Overall CM, Lopez-Otin C. Human and mouse proteases: A comparative genomic approach. *Natl Rev.* 2003; 4:544–558.

- Quiquampoix H, Staunton S, Baron MH, Ratcliffe RG. Interpretation of the pH dependence of protein adsorption on clay mineral surfaces and its relevance to the understanding of extracellular enzyme activity in soil. *Colloids Surf A Physicochem Eng Aspects*. 1993; 75:85–93.
- Quiquampoix, H. Mechanisms of protein adsorption on surfaces and consequences for extracellular enzyme activity in soil. In: Bollag, JM.; Stotzky, G., editors. *Soil biochemistry*. Vol. 10. Marcel Dekker; New York: 2000. p. 171–206.
- Quiquampoix H, Burns RG. Interactions between proteins and soil mineral surfaces: Environmental and health consequences. *Elements*. 2007; 3:401–406.
- Rao MA, Russo F, Granata V, Berisio R, Zagari A, Gianfreda L. Fate of prions in soil: Interaction of a recombinant ovine prion protein with synthetic humic-like mineral complexes. *Soil Biol Biochem*. 2007; 39:493–504.
- Rapp D, Potier P, Jocteur-Monrozier L, Richaume A. Prion degradation in soil: Possible role of microbial enzymes stimulated by the decomposition of buried carcasses. *Environ Sci Technol*. 2006; 40:6324–6329. [PubMed: 17120560]
- Revault M, Quiquampoix H, Baron MH, Noinville S. Fate of prions in soil: Trapped conformation of full-length ovine prion protein induced by adsorption on clays. *Biochim Biophys Acta*. 2005; 1724:367–374. [PubMed: 15950385]
- Rigou P, Rezaei H, Grosclaude J, Staunton S, Quiquampoix H. Fate of prions in soil: Adsorption and extraction by electroelution of recombinant ovine prion protein from montmorillonite and natural soils. *Environ Sci Technol*. 2006; 40:1497–1503. [PubMed: 16568762]
- Rubert KF, Pedersen JA. Kinetics of oxytetracycline reaction with a hydrous manganese oxide. *Environ Sci Technol*. 2006; 40:7216–7221. [PubMed: 17180969]
- Rudd PM, Endo T, Colominas C, Groth D, Wheeler SF, Harvey DJ, Wormald MR, Serban H, Prusiner SB, Kobata A, Dwek RA. Glycosylation differences between the normal and pathogenic prion protein isoforms. *Proc Natl Acad Sci USA*. 1999; 96:13044–13049. [PubMed: 10557270]
- Rudd PM, Wormald MR, Wing DR, Prusiner SB, Dwek RA. Prion glycoprotein: Structure, dynamics, and roles for the sugars. *Biochemistry*. 2001; 40:3759–3766. [PubMed: 11300755]
- Russo F, Johnson CJ, McKenzie D, Aiken JM, Pedersen JA. Pathogenic prion protein is degraded by a manganese oxide mineral found in soils. *J Gen Virol*. 2009; 90:275–280. [PubMed: 19088299]
- Saá P, Castilla J, Soto C. Ultra-efficient replication of infectious prions by automated protein misfolding cyclic amplification. *J Biol Chem*. 2006; 281:35245–35252. [PubMed: 16982620]
- Saborio GP, Permanne B, Soto C. Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. *Nature*. 2001; 411:810–813. [PubMed: 11459061]
- Safar J, Wille H, Itri V, Groth D, Serban H, Torchia M, Cohen FE, Prusiner SB. Eight prion strains have PrP^{Sc} molecules with different conformations. *Nat Med*. 1998; 4:1157–1165. [PubMed: 9771749]
- Saunders SE, Bartz JC, Bartelt-Hunt SL. Influence of prion strain on prion protein adsorption to soil in a competitive matrix. *Environ Sci Technol*. 2009a; 43:5242–5248. [PubMed: 19708348]
- Saunders SE, Bartz JC, Bartelt-Hunt SL. Prion protein adsorption to soil in a competitive matrix is slow and reduced. *Environ Sci Technol*. 2009b; 43:7728–7733. [PubMed: 19921886]
- Saunders SE, Bartz JC, Telling GC, Bartelt-Hunt SL. Environmentally-relevant forms of the prion protein. *Environ Sci Technol*. 2008; 42:6573–6579. [PubMed: 18800532]
- Saunders SE, Bartz JC, Vercauteren KC, Bartelt-Hunt SL. Enzymatic digestion of chronic wasting disease prions bound to soil. *Environ Sci Technol*. 2010; 44:4129–4135. [PubMed: 20450190]
- Scherbel C, Pichner R, Groschup MH, Mueller-Hellwig S, Scherer S, Dietrich R, Maertlbauer E, Gareis M. Degradation of scrapie associated prion protein (PrP^{Sc}) by the gastrointestinal microbiota of cattle. *Vet Res*. 2006; 37:695–703. [PubMed: 16820134]
- Scherbel C, Pichner R, Groschup MH, Mueller-Hellwig S, Scherer S, Dietrich R, Maertlbauer E, Gareis M. Infectivity of scrapie prion protein (PrP^{Sc}) digestion with bovine gastrointestinal microbiota. *Zoonoses Public Health*. 2007; 54:185–190. [PubMed: 17542960]
- Schramm PT, Johnson CJ, Mathews NE, McKenzie D, Aiken JM, Pedersen JA. Potential role of soil in the transmission of prion disease. *Rev Mineral Geochem*. 2006; 64:135–152.

- Seidel B, Thomzig A, Buschmann A, Groschup MH, Peters R, Beekes M, Terytze K. Scrapie agent (strain 263k) can transmit disease via the oral route after persistence in soil over years. *PLoS One*. 2007; 2:E435.10.1371/journal.pone.0000435 [PubMed: 17502917]
- Servagent-Noinville S, Revault M, Quiquampoix H, Baron M. Conformational changes of bovine serum albumin induced by adsorption on different clay surfaces: FTIR analysis. *J Colloid Interface Sci*. 2000; 221:273–283. [PubMed: 10631031]
- Sigurdson CJ, Williams ES, Miller MW, Spraker TR, O'Rourke KI, Hoover EA. Oral transmission and early lymphoid tropism of chronic wasting disease PrP^{res} in mule deer fawns (*Odocoileus hemionus*). *J Gen Virol*. 1999; 80:2757–2764. [PubMed: 10573172]
- Silveira JR, Raymond GJ, Hughson AG, Race RE, Sim VL, Hayes SF, Caughey B. The most infectious prion protein particles. *Nature*. 2005; 437:257–261. [PubMed: 16148934]
- Somerville RA. Host and transmissible spongiform encephalopathy agent strain control glycosylation of PrP. *J Gen Virol*. 1999; 80:1865–1872. [PubMed: 10423157]
- Sposito G. Surface reactions in natural aqueous colloidal systems. *Chimia (Aarau)*. 1989; 43:169–176.
- Stadtman ER, Berlett BS. Reactive oxygen-mediated protein oxidation in aging and disease. *Chem Res Toxicol*. 1997; 10:485–494. [PubMed: 9168245]
- Stahl N, Borchelt DR, Hsiao K, Prusiner SB. Scrapie prion protein contains a phosphatidylinositol glycolipid. *Cell*. 1987; 51:229–240. [PubMed: 2444340]
- Stahl N, Baldwin M, Hecker R, Pan KM, Burlingame A, Prusiner SB. Glycosylinositol phospholipid anchors of the scrapie and cellular prion proteins contain sialic acid. *Biochemistry*. 1992; 31:5043–5053. [PubMed: 1350920]
- Stahl N, Baldwin MA, Teplow DB, Hood L, Gibson BW, Burlingame AL, Prusiner SB. Structural studies of the scrapie prion protein using mass spectrometry and amino acid sequencing. *Biochemistry*. 1993; 32:1991–2002. [PubMed: 8448158]
- Stimson E, Hope J, Chong A, Burlingame AL. Site-specific characterization of the n-linked glycans of murine prion protein by high-performance liquid chromatography/electrospray mass spectrometry and exoglycosidase digestions. *Biochemistry*. 1999; 38:4885–4895. [PubMed: 10200178]
- Sturm, R.; Booth, C.; Smith, C.; Pedersen, J.; Li, L. Protease evaluation for production of methionine-deficient peptides for MRM quantitation of the infectious prion protein. Abstract 3116. 58th ASMS Conference on Mass Spectrometry; Salt Lake City, UT. 23–27 May 2010;
- Suyama K, Yoshioka M, Akagawa M, Murayama Y, Horii H, Takata M, Yokoyama T, Mohri S. Assessment of prion inactivation by fenton reaction using protein misfolding cyclic amplification and bioassay. *Biosci Biotechnol Biochem*. 2007; 71:2069–2071. [PubMed: 17690456]
- Suzawa T, Shirahama H. Adsorption of plasma proteins onto polymer latices. *Adv Colloid Interface Sci*. 1991; 35:139–172. [PubMed: 2043288]
- Swietnicki W, Morillas M, Chen SG, Gambetti P, Surewicz WK. Aggregation and fibrillization of the recombinant human prion protein huPrP 90-231. *Biochemistry*. 2000; 39:424–431. [PubMed: 10631004]
- Tamgüney G, Miller MW, Wolfe LL, Sirochman TM, Glidden DV, Palmer C, Lemus A, DeArmond SJ, Prusiner SB. Asymptomatic deer excrete infectious prions in faeces. *Nature*. 2009; 461:529–532. [PubMed: 19741608]
- Tan WF, Koopal LK, Norde W. Interaction between humic acid and lysozyme, studied by dynamic light scattering and isothermal titration calorimetry. *Environ Sci Technol*. 2009; 43:591–596. [PubMed: 19244988]
- Tan WF, Koopal LK, Weng LP, van Riemsdijk WH, Norde W. Humic acid protein complexation. *Geochim Cosmochim Acta*. 2008; 72:2090–2099.
- Taylor DM. Inactivation of prions by physical and chemical means. *J Hosp Infect*. 1999; 43(Suppl):S69–S76. [PubMed: 10658760]
- Taylor DM. Inactivation of transmissible degenerative encephalopathy agents: A review. *Vet J*. 2000; 159:10–17. [PubMed: 10640408]
- Taylor DM, Diprose MF. The response of the 22a strain of scrapie agent to microwave irradiation compared with boiling. *Neuropathol Appl Neurobiol*. 1996; 22:256–258. [PubMed: 8804028]

- Telling GC, Parchi P, DeArmond SJ, Cortelli P, Montagna P, Gabizon R, Mastrianni J, Lugaresi E, Gambetti P, Prusiner SB. Evidence for the conformation of the pathologic isoform of the prion protein enciphering and propagating prion diversity. *Science*. 1996; 274:2079–2082. [PubMed: 8953038]
- Tsiroulnikov K, Rezai H, Bonch-Osmolovskaya E, Nedkov P, Gousterova A, Cuff V, Godfroy A, Barbier G, Metro F, Chobert JM, Clayette P, Dormont D, Grosclaude J, Haertle T. Hydrolysis of the amyloid prion protein and nonpathogenic meat and bone meal by anaerobic thermophilic prokaryotes and streptomyces subspecies. *J Agric Food Chem*. 2004; 52:6353–6360. [PubMed: 15453713]
- Tzaban S, Friedlander G, Schonberger O, Horonchik L, Yedidia Y, Shaked G, Gabizon R, Taraboulos A. Protease-sensitive scrapie prion protein in aggregates of heterogeneous sizes. *Biochemistry*. 2002; 41:12868–12875. [PubMed: 12379130]
- van Oss CJ, Docoslis A, Giese RF. Free energies of protein adsorption onto mineral particles: From the initial encounter to the onset of hysteresis. *Colloids Surf B Biointerfaces*. 2001; 22:285–300.
- Vasina EN, Dejardin P, Rezaei H, Grosclaude J, Quiquampoix H. Fate of prions in soil: Adsorption kinetics of recombinant unglycosylated ovine prion protein onto mica in laminar flow conditions and subsequent desorption. *Biomacromolecules*. 2005; 6:3425–3432. [PubMed: 16283775]
- Violante A, de Cristofaro A, Rao MA, Gianfreda L. Physicochemical properties of protein-smectite and protein-Al(OH) x-smectite complexes. *Clay Miner*. 1995; 30:325–336.
- Weeks HP. Characteristics of mineral licks and behavior of visiting white-tailed deer in southern Indiana. *Am Midl Nat*. 1978; 100:384–395.
- Whiffen LK, Midgley DJ, McGee PA. Polyphenolic compounds interfere with quantification of protein in soil extracts using the Bradford method. *Soil Biol Biochem*. 2007; 39:691–694.
- Wilham JM, Orrú CD, Bessen RA, Atarashi R, Sano K, Race B, Meade-White KD, Taubner LM, Timmes A, Caughey B. Rapid end-point quantitation of prion seeding activity with sensitivity comparable to bioassays. *PLoS Pathog*. 2010; 6:E1001217. [PubMed: 21152012]
- Wright SF, Upadhyaya A. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. *Soil Sci*. 1996; 161:575–586.
- Wright SF, Upadhyaya A. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant Soil*. 1998; 198:97–107.
- Xu L, Xu C, Zhao M, Qiu Y, Sheng GD. Oxidative removal of aqueous steroid estrogens by manganese oxides. *Water Res*. 2008; 42:5038–5044. [PubMed: 18929389]
- Zhang H, Huang CH. Adsorption and oxidation of fluoroquinolone antibacterial agents and structurally related amines with goethite. *Chemosphere*. 2007; 66:1502–1512. [PubMed: 17083963]
- Zhang H, Chen WR, Huang CH. Kinetic modeling of oxidation of antibacterial agents by manganese oxide. *Environ Sci Technol*. 2008; 42:5548–5554. [PubMed: 18754474]

Fig. 1. Transmission electron microscopy (TEM) image of PrP^{TSE} amyloid fibrils (HY strain of hamster-adapted transmissible mink encephalopathy) negatively stained with phosphotungstic acid. (Image kindly provided by Kurt H. Jacobson and Randy Massey.)

Fig. 2. Model structures of PrP^{TSE} monomers and trimers. (A) Spiral model, derived from molecular dynamic simulations at low pH. (B) β-Helix model, derived by threading the PrP sequence through a known β-helix fold. Trimeric representations of the (C) spiral and (D) β-helix models. The (E) spiral and (F) β-helix model with the protein-only portion highlighted (gray, space-filling) and biologically relevant glycans (cyan, space-filling) attached to the appropriate glycosylation sites. Images C–F are on the same scale (bar = 50 Å). (Used by permission of the American Chemical Society, from DeMarco et al. [2006] *Biochem.* 45, Fig. 1, p. 15574.)

Fig. 3.

Oral inoculation of hamster with PrP^{TSE} (HY strain of hamster-adapted transmissible mink encephalopathy) and montmorillonite (Mte) dramatically increases disease penetrance and shortens incubation period relative to that of PrP^{TSE} alone. Hamsters dosed with Mte alone remained healthy throughout the course of the experiment. (From Johnson et al. [2007] PLoS Pathog. 3, Fig. 3B, p. 877.)

Fig. 4. PrP^{TSE} persists in soil for at least 29 mo. Western blot detection of PrP^{TSE} extracted from experimentally contaminated soils after different time periods. Lane 1: proteinase K–digested hamster brain homogenate (positive control); lanes 2–10: PrP^{TSE} extracted at time point 0 (lane 2), after 1 mo (lane 3), after 3 mo (lane 4), after 6 mo (lane 5), after 12 mo (lane 6), after 18 mo (lane 7), after 21 mo (lane 8), after 26 mo (lane 9), and after 29 mo (lane 10). (From Seidel et al. [2007] PLoS ONE 2, Fig. 2A, p. 435.)

Table 1

Reported PrP^{TSE} adsorption capacities for common soil minerals (from Johnson et al. [2006] PLoS Pathog. 2, Table 1).

Mineral [†]	Estimated binding (sorbent mass basis) [‡] capacity	Estimated binding capacity (sorbent surface area basis) [‡]
	$\mu\text{g protein mg}_{\text{mineral}}^{-1}$	$\text{mg}_{\text{protein}} \text{m}_{\text{mineral}}^{-2}$
Mte	87–174	2.8–5.7
Kte	1.7–2.6	0.15–0.22
Qtz	13.6–27.1	2.7–5.4

[†] Kte, kaolinite; Mte, montmorillonite; Qtz, quartz.

[‡] Protein concentration determined by Bradford assay; PrP^{TSE} concentration was taken as 87% of total protein (Silveira et al., 2005). Reported adsorption capacities represent upper estimates, as the fraction of PrP^{TSE} in clarified preparations may have been lower. For Mte, binding capacity was based on the external (N₂-accessible) surface area (see Johnson et al. [2006] for discussion).