Chapter 13
Species Barriers in Prion Disease

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Abstract Species barriers in prion diseases are defined by the difficulty that prions from one species have in triggering prion infection in a new species. The fact that bovine spongiform encephalopathy has successfully crossed species barriers to cause disease in human and concerns that chronic wasting disease in deer and elk has the potential to do the same makes understanding the mechanisms underlying species barriers to prion infection critical. The amino acid sequence of the normal host prion protein (PrP^{C}), the conformational diversity of the abnormal and infectious prion protein (PrP^{Sc}), the conformational compatibility between exogenous PrP^{Sc} and the endogenous host PrP^{C}, and the ability to establish a subclinical infection are all important determinants of prion species barriers. However, the potential for host factors and post-translational modifications to PrP^{C} to influence species barriers, and the fact that the critical amino acid residues influencing these barriers differ between species, makes it difficult to predict prion species barriers based upon PrP^{C} sequence alone. Although the recent publication of high-resolution structural information for PrP^{Sc} will be helpful, in vivo or in vitro experiments in relevant models of infection remain the best way to determine species barriers to prion infection.

Keywords Prion · Transmissible spongiform encephalopathy · Species barriers · Prion protein · Scrapie · PrP · TSE · CJD

Abbreviations

PrP	Prion protein
Prnp	Prion protein gene
PrP^{C}	PrP cellular
PrP^{Sc}	PrP scrapie

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TSE	transmissible spongiform encephalopathy
CJD	Creutzfeldt–Jakob disease
sCJD	sporadic Creutzfeldt–Jakob disease
vCJD	variant Creutzfeldt–Jakob disease
TME	transmissible mink encephalopathy
BSE	bovine spongiform encephalopathy
CWD	chronic wasting disease
MBM	meat and bone meal
CNS	central nervous system
Sinc	scrapie incubation time gene
GPI	glycophosphatidyl-inositol
EM	electron microscopy
NMR	nuclear magnetic resonance
PIRIBS	parallel in-register intermolecular β-sheet
NAPA	nonadaptive prion amplification

13.1 Introduction

Prion diseases, also known as transmissible spongiform encephalopathies or TSEs, can be transmitted both within and across species. Intraspecies transmission of prion disease occurs naturally but with variable efficiency. Sheep scrapie, which can be transmitted both vertically and horizontally via placental tissue (Race et al. 1998; Tuo et al. 2001, 2002), can spread to 30–40% of the flock (Hourrigan et al. 1979). Chronic wasting disease (CWD) in deer, where infectivity is present in several tissues (Sigurdson et al. 2001; Spraker et al. 1997, 2002), including saliva, feces, and urine (Haley et al. 2011; Mathiason et al. 2006; Tamguney et al. 2009a), is even more efficient at spreading throughout a herd with up to 100% of the deer becoming infected (Miller and Williams 2003; Sigurdson and Aguzzi 2007). By contrast, bovine spongiform encephalopathy (BSE) in cattle and sporadic Creutzfeldt–Jakob disease (sCJD) in humans, neither of which have detectable infectivity in most tissues outside of the central nervous system (CNS) (Bradley 1996; Brown et al. 1994), do not appear to spread naturally either vertically or horizontally (Brown et al. 1994; Wrathall et al. 2002). Intraspecies transmission of prion infectivity, therefore, correlates with the presence of detectable levels of infectivity in non-CNS tissues.

Regardless of which tissues are positive for infectivity, interspecies transmission of prion infectivity is much more difficult than intraspecies transmission. Species barriers in prion diseases are defined by the difficulty that prion infectivity from one species has in triggering infection in a second species. If low titers are not an issue, a prolonged incubation time upon first passage followed by decreasing disease incubation times in subsequent passages is usually considered indicative of the existence of a prion species barrier. There are no documented instances of naturally occurring prion diseases, such as sheep scrapie, CWD, or sCJD, crossing species barriers

under normal conditions. Thus, natural species barriers to prion infection appear to be very strong.

The only instance in which prion diseases are known to have crossed species barriers outside of a laboratory environment was the result of human intervention. Changes in the rendering of ruminant animal carcasses in the early 1970s allowed material infected either with sheep scrapie (Wilesmith et al. 1988) or a previously unrecognized type of BSE (Beringue et al. 2007) to be processed into meat and bone meal (MBM) which was fed back to cattle. Cattle, which were infected but not clinically ill, were then rendered into MBM and the process repeated until the emergence of clinical BSE was recognized in the late 1980s (Wilesmith et al. 1988; Wells et al. 1987). Although there were concerns at the time that exposure to BSE-contaminated materials could lead to infection of humans, the fact that exposure to sheep scrapie had never been linked to disease suggested that this was unlikely. However, in 1996, a new form of human CJD termed new variant CJD, or more simply variant CJD (vCJD), was identified in young people in the United Kingdom, and it was suggested that this might be the result of exposure to BSE-contaminated materials (Will et al. 1996). Later work confirmed that vCJD was linked both epidemiologically and biologically to exposure to BSE (Bruce et al. 1997; Collinge et al. 1996; Hill et al. 1997). Moreover, it was shown that BSE had crossed species barriers to infect domestic cats, zoo cats, and a variety of exotic ungulates following exposure to BSE-contaminated MBM (Bradley 1996).

Multiple species barriers were, therefore, broken as the result of changes to a common human agricultural process: the possible infection of cattle with sheep scrapie and the infection of humans, felines, and ungulates with BSE. The fact that BSE has successfully and unpredictably crossed species barriers to cause disease in non-ruminant species and concerns that CWD has the potential to do the same makes understanding the mechanisms underlying species barriers to prion infection critical.

13.2 Prion Protein and Prion Species Barriers

Species barriers to prion infection were initially defined based upon the experimental inoculation of different types of TSE agents, as prions were called at the time of these experiments, into multiple mammalian species, including mice, hamsters, ferrets, and mink. For example, transmissible mink encephalopathy (TME) could be transmitted to hamsters but not mice (Marsh et al. 1969). Suffolk sheep scrapie could infect both mice and mink, while Cheviot sheep scrapie could infect mice but not mink (Hanson et al. 1971). Thus, there was a species barrier between mink-derived TME and mice and between some forms of sheep scrapie and mink. Other species, such as rabbits, were found to be resistant to scrapie infection altogether (Gibbs and Gajdusek 1973). Based upon these and multiple other studies, researchers determined that species barriers to TSE infection could be influenced by at least

three different factors: (1) the range of TSE strains in the infectious inoculum; (2) the scrapie incubation time (*Sinc*) gene; and (3) the ability to establish a subclinical infection (Dickinson 1976).

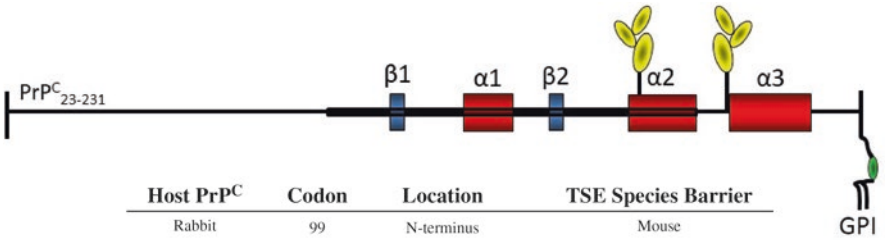
In the early 1980s, it was discovered that an aggregated and protease-resistant mammalian cell-surface glycoprotein-designated prion protein (PrP) was associated with TSE disease (Bolton et al. 1982). As a result, TSE diseases were soon renamed prion diseases and the infectious agent was designated a prion. Soon after its discovery, it was determined that PrP was a normal host protein (Basler et al. 1986; Locht et al. 1986) which was both soluble and protease-sensitive (Bendheim et al. 1988). During prion disease pathogenesis, normal PrP (termed PrP^{C} for PrP cellular) is refolded into an abnormally aggregated, protease-resistant, and infectious form known as PrP^{Sc} (for PrP scrapie) which accumulates, eventually causing disease. Comparison of PrP^{C} molecules from different mammalian species demonstrated that, while the PrP gene *Prnp* is highly conserved, the PrP^{C} amino acid sequence can vary by as much as 20% (Wopfner et al. 1999). This provided a potential molecular basis for prion species barriers: amino acid differences between the incoming infectious PrP^{Sc} and the host PrP^{C} might influence how effectively new PrP^{Sc} could be made and thus determine whether infection and disease could occur.

13.3 Role of PrP Amino Acid Sequence

13.3.1 Region of PrP Involved in Rodent Species Barriers

In order to determine whether or not the sequence of PrP^{C} was a determinant of prion species barriers, researchers took advantage of the strong species barrier to infection that exists between mice and hamsters. In this system, mice are susceptible to infection with mouse scrapie but highly resistant to infection with hamster scrapie. However, when mice were engineered to express hamster PrP^{C}, they became fully susceptible to hamster scrapie, i.e., a prion species barrier had been broken (Scott et al. 1989). Moreover, the incubation time was inversely related to hamster PrP^{C} expression: the higher the expression level of hamster PrP^{C}, the shorter the disease incubation time (Scott et al. 1989). Experiments such as these clearly showed that the amino acid sequence of the host PrP^{C} molecule was a major determinant of species barriers in prion diseases. They also provided an explanation for why earlier studies had implicated the *Sinc* gene in TSE species barriers: the *Prnp* gene and the *Sinc* gene are in fact one and the same (Moore et al. 1998).

Generation of transgenic mice expressing chimeric mouse/hamster PrP^{C} molecules further demonstrated that the major region of PrP^{C} important in the transmission of hamster scrapie to mice resides within the middle portion of the molecule from amino acid residues 108–189 (Fig. 13.1) (Scott et al. 1992, 1993). When this region was derived from hamster PrP^{C}, the mice were susceptible to hamster scrapie. However, when it was derived from mouse PrP^{C}, the mice were resistant to hamster scrapie infection (Scott et al. 1993). Mouse and hamster PrP^{C} are highly



Host PrP^C	Codon	Location	TSE Species Barrier
Rabbit	99	N-terminus	Mouse
Mouse	101	N-terminus	Human (vCJD), Sheep, Hamster
Rabbit	107	N-terminus	Mouse
Rabbit	108	N-terminus	Mouse
Human	129	β1	Bovine (BSE)
Goat	142	β1-α1 loop	Bovine (BSE)
Mouse	138	β1-α1 loop	Hamster
Hamster	155	α1-β2 loop	Mouse
Vole	154	α1-β2 loop	Bovine (BSE)
Dog	163	α1-β2 loop	Ovine (BSE)
Vole	169	β2-α2 loop	Bovine (BSE)
Sheep	171	β2-α2 loop	Bovine (BSE)
Rabbit	173	β2-α2 loop	Mouse
Ferret	179	β2-α2 loop	Mink (TME)
Rabbit	202	α3	Mouse
Rabbit	214	α3	Mouse
Ferret	224	C-terminus	Mink (TME)

Fig. 13.1 PrP amino acid residues involved in prion disease species barriers. The NMR structure of mouse PrP^C following cleavage of the signal peptide and GPI anchor addition sequence is shown (PrP$^C_{23\text{-}231}$). Glycosylation is indicated by the yellow ovals and the location of the GPI anchor at the C-terminus is indicated. The red boxes represent areas of α-helix, while the blue boxes represent areas of β-strand. Areas of disordered/loop/turn structure are represented by the thin black line, while the thicker line indicates the region of PrP^C where most of the amino acid residues important in prion species barriers reside. The table lists some of these amino acid residues using the PrP^C numbering for the corresponding host species. The structural location of each amino acid is given as is the species barrier with which it is associated. References for each residue listed are given in the main text

homologous (Wopfner et al. 1999) and there are only three amino acid differences, residues 138, 154 and 169, between the two species in the region from codon 108–189, suggesting that one or more of these residues might be contributing to the mouse–hamster prion species barrier (Kocisko et al. 1995).

13.3.2 Influence of Single Amino Acid Residues

The influence of the three amino acid residues at 138, 154, and 169 on the species-specific formation of mouse PrP^{Sc} was analyzed in vitro using mouse neuroblastoma (N2a) cells infected with mouse scrapie (Priola and Chesebro 1995). These cells

express mouse PrP^C and generate both mouse PrP^{Sc} and mouse scrapie infectivity. However, when mouse PrP^C expressing an antibody epitope tag is expressed in scrapie-infected N2a cells, its conversion to PrP^{Sc} can be measured against the background of the endogenous, wild-type mouse PrP^{Sc} which does not have the epitope tag. Thus, the influence of mutations in PrP^C on the species-specific formation of PrP^{Sc} can be studied in cell culture. In the case of the mouse–hamster prion species barrier, it was determined that a single hamster specific amino acid at residue 138 in mouse PrP^C prevented the production of mouse PrP^{Sc} in cells (Priola and Chesebro 1995). Substitution of hamster PrP^C amino acid residues at positions 154 and 169 in mouse PrP^C had no effect (Priola and Chesebro 1995). Thus, a single amino acid difference in the host PrP^C molecule was sufficient to prevent the species-specific formation of PrP^{Sc}, suggesting that prion species barriers to infection could be dependent upon relatively minor differences in sequence between the endogenous host PrP^C and exogenous PrP^{Sc} molecules.

Persistent infection of cells in vitro with some types of prions, including BSE and sCJD, can be extremely difficult and is often not successful. This limits the usefulness of cell-based systems in defining and understanding the mechanisms underlying prion species barriers for many species. Fortunately, in addition to both natural and transgenic models of prion disease, there are cell-free systems that are not restricted by prion species (Kocisko et al. 1995; Bossers et al. 1997; Castilla et al. 2005, 2008; Eiden et al. 2011; Kocisko et al. 1994; Raymond et al. 1997) that can be used to analyze the effect of differences in PrP^C sequence on the species-specific formation of PrP^{Sc}. From these studies, it is now clear that the amino acid residues important in the species-specific formation of PrP^{Sc} and transmission of prion infectivity across species barriers differ depending upon the species (Fig. 13.1). For example, species-specific formation of hamster PrP^{Sc} has been mapped to residue 155 in vitro (Priola et al. 2001) and the same residue has been implicated in species barriers in bank voles in vivo (Agrimi et al. 2008). By contrast, this residue in mice has no effect on the species-specific formation of mouse PrP^{Sc} (Priola and Chesebro 1995). Mutation of amino acid residue 101 in mouse PrP^C has been linked to species barriers to infection of mice with human, sheep, and hamster prions (Barron et al. 2001). In ferrets, resistance to TME infection is linked to residues 179 and 224 (Bartz et al. 1994). For rabbits, a species known to be highly resistant to prion infection (Gibbs and Gajdusek 1973), multiple amino acid residues appear to be important for PrP^{Sc} formation (Fig. 13.1) (Vorberg et al. 2003; Eraña et al. 2017).

Depending upon the species, resistance to BSE is associated with different amino acids in PrP^C (Fig. 13.1). In goats, amino acid residue 142, which is analogous to amino acid 138 in mouse PrP, is associated with resistance to BSE (Goldmann et al. 1996). In sheep, it is residue 171 that is associated with susceptibility to BSE (Raymond et al. 1997; Goldmann et al. 1994), while in dogs, which are highly resistant to infection with multiple species of prions, susceptibility to sheep-derived BSE is dependent upon amino acid residue 163 (Vidal et al. 2020). In humans, all clinically positive cases of vCJD have been homozygous for methionine at codon 129 (Mackay et al. 2011), suggesting that susceptibility to BSE correlates with the methionine/valine polymorphism at this residue (Raymond et al. 1997; Wadsworth

et al. 2004). When overlayed onto the structure of PrP^C, it is clear that the amino acid residues important in prion species barriers reside within different regions of PrP^C (Fig. 13.1).

13.3.3 Effect of Prnp Heterozygosity

Heterozygosity at the *Prnp* gene may also influence prion species barriers. In vivo, transgenic mice expressing both mouse and hamster PrP^C are susceptible to infection with mouse and hamster scrapie, but mouse scrapie incubation times are significantly increased when hamster PrP^C is present (Scott et al. 1989). In vitro, expression of hamster PrP^C in mouse scrapie-infected cells can completely abolish PrP^{Sc} formation (Priola et al. 1994). This phenomenon, known either as interference (Priola et al. 1994) or dominant negative inhibition (Zulianello et al. 2000), is seen when heterologous PrP^C and PrP^{Sc} molecules bind, but PrP^C is not subsequently converted to PrP^{Sc}. Interference may explain why all clinical cases of vCJD in humans are homozygous for methionine at codon 129 and why heterozygosity at codon 129 might be protective. A valine at codon 129 would block vCJD PrP^{Sc} formation from the susceptible PrP^C methionine 129 allele in a dominant-negative fashion, slowing down or preventing clinical disease. In this manner, heterozygosity at the PrP^C allele may contribute to the maintenance of prion species barriers.

13.4 Influence of PrP Post-translational Modifications

13.4.1 Glycosylation

Post-translational modifications to PrP^C also appear to impact the species-specific formation of PrP^{Sc}. PrP^C is post-translationally modified by glycosylation at two N-linked glycosylation sites as well as by the addition of a C-terminal glycophosphatidyl-inositol (GPI) membrane anchor (Caughey et al. 1989; Haraguchi et al. 1989; Stahl et al. 1987). In vitro, PrP^C glycosylation can influence PrP^{Sc} formation and the binding between heterologous PrP^C and PrP^{Sc} molecules in a species-specific manner (Priola and Lawson 2001; Burke et al. 2020), and removal of the sialic acids on the ends of the N-linked sugars can lower the barrier to cross species formation of PrP^{Sc} (Katorcha et al. 2014). At a molecular level, less efficient binding of heterologous PrP^C and PrP^{Sc} molecules could result in the production of less PrP^{Sc}, while the negative charge of the sialic acids may affect the stability of PrP^{Sc} aggregates. Finally, abrogation of the second N-linked glycosylation site in mouse PrP^C makes mice more susceptible to infection with some strains of human CJD (Wiseman et al. 2015), suggesting that the second glycosylation site helps to protect against transmission across a species barrier. Thus, PrP^C glycosylation appears to

contribute mechanistically in several ways to the maintenance of prion species barriers in vivo.

13.4.2 GPI Anchor

In vitro, the GPI anchor appeared to have little or no effect on the species-specific formation of abnormal prion protein (Priola and Lawson 2001). However, a recent study has shown that mouse prions without a GPI anchor infect transgenic mice over-expressing human PrP^{C}, which are normally resistant to infection with mouse prions, much more efficiently than mouse prions with a GPI anchor (Race et al. 2015). Anchorless PrP molecules are primarily mono- or un-glycosylated (Kocisko et al. 1994), suggesting again that complex glycosylation may be a protective factor in cross-species transmission of prions, possibly by interfering with the binding of PrP^{C} and PrP^{Sc} (Priola and Lawson 2001). In addition, removal of the GPI anchor would remove negatively charged sialic acids attached to the GPI moiety, which could potentially increase the efficiency of conversion of PrP^{C} to PrP^{Sc} (Baskakov and Katorcha 2016). Indeed, anchorless PrP^{C} is known to be converted into PrP^{Sc} more efficiently in vitro (Kocisko et al. 1994). Thus, the ability of anchorless PrP^{Sc} to cross a species barrier may be related more to its glycosylation state than to the GPI anchor itself.

13.5 Non-PrP Host Factors

There are several examples in transgenic mice where, even though the host PrP^{C} amino acid sequence is identical to the incoming PrP^{Sc} amino acid sequence, species barriers to infection are maintained. For example, transgenic mice expressing human PrP^{C} can be more resistant to infection with vCJD than wild-type mice while simultaneously being more susceptible to infection with sCJD (Hill et al. 1997; Bishop et al. 2006). Substitution of leucine for proline at position 101 in mouse PrP^{C} can modulate the susceptibility to prions from different mouse strains as well as to prions from different species (Barron et al. 2001). While prion strain-dependent differences in PrP^{Sc} conformation may account for some of these observations, these experiments suggest that host factors other than PrP might play a role in species barriers to prion infection. Consistent with this idea, in vitro studies have identified the phospholipid phosphatidylethanolamine as a cofactor in mouse PrP^{Sc} formation (Deleault et al. 2012) and RNA as a cofactor in hamster PrP^{Sc} formation (Deleault et al. 2003). However, whether these cofactors are important in the transmission of prions across species barriers in vivo remains unclear.

13.6 Prion Protein Structure and Prion Species Barriers

13.6.1 Structural Regions of PrP^C Implicated in Species Barriers

The structure of PrP^C has been determined by both NMR (Donne et al. 1997; Liu et al. 1999; Riek et al. 1996, 1997) and X-ray crystallography (Knaus et al. 2001). For all mammalian species, PrP^C has a disordered N-terminal region starting from the signal peptide cleavage site at residue 23 through to approximately reside 121 [see (Wuthrich and Riek 2001) for review]. This is followed by a folded C-terminal domain which spans residues 122–231 and is composed of two β strands that form a short region of β-sheet and three α-helices. The three α-helices and two β strands are connected by generally poorly defined regions of disordered loop/turn structure (Fig. 13.1).

The NMR structure of PrP^C can be used to provide some insight into the structural components of PrP^C which help to control species-specific formation of PrP^{Sc}. The region of PrP which has been most often implicated in controlling prion disease species barriers extends from approximately residue 100 to residue 190 and includes two of the three α-helices, both β strands and multiple regions of disordered loop/turn structure (Fig. 13.1). The N-terminal region of PrP^C encompassing residues 23–90 does not appear to be involved (Davenport et al. 2016). When amino acid residues that have been experimentally shown to have a strong influence on species-specific PrP^{Sc} formation are superimposed onto the structure of PrP^C, the majority of them reside within the disordered loop/turn regions (Fig. 13.1). This suggests that conformational variability within the loop structures of different species of PrP molecules may influence prion species barriers (Moore et al. 2009).

Polymorphisms within the loop/turn structure that connects the second β-strand to the second α-helix (β2–α2 loop) have been associated with reduced PrP^{Sc} formation and/or resistance to prion infection in sheep (Bossers et al. 1997; Eiden et al. 2011; Goldmann et al. 1994), mice (Striebel et al. 2011), and bank voles (Agrimi et al. 2008; Piening et al. 2006). In PrP^C from mice (Riek et al. 1996), sheep (Lysek et al. 2005), cattle (Lopez Garcia et al. 2000), and humans (Zahn et al. 2000), the β2–α2 loop is disordered. However, in other species such as elk (Gossert et al. 2005), hamsters (Donne et al. 1997), and bank voles (Christen et al. 2008), the β2–α2 loop adopts a well-defined structure called the rigid loop. It has been hypothesized that rigidity within the β2–α2 region may determine susceptibility to prion disease (Gossert et al. 2005), and transgenic mice expressing mouse PrP^C genetically engineered to have the rigid loop appear to be more susceptible to scrapie infection (Sigurdson et al. 2010). However, species that are highly resistant to prion infection such as rabbits (Wen et al. 2010), pigs (Lysek et al. 2005), and horses (Perez et al. 2010) also have the β2–α2 rigid loop. Furthermore, there are multiple polymorphisms outside of this region that clearly influence prion species barriers (Fig. 13.1). Thus, it is unlikely that the presence of a rigid loop structure in the β2–α2 region of PrP^C is by itself sufficient to determine species barriers to prion infection in every case.

Nevertheless, it is clear that species-specific polymorphisms which are outside of the more thermodynamically stable α-helical and β-sheet structures of PrP^{C} have a major impact on the species-specific formation of PrP^{Sc} and prion species barriers. Since detailed mechanistic and structural information on how PrP^{C} refolds into PrP^{Sc} is lacking, it is difficult to determine how these loop structures contribute to species-specific PrP^{Sc} formation. One possible explanation is that these regions have a lower free energy barrier for refolding into β-sheet structures (Rezaei et al. 2002). Another is that certain polymorphisms in PrP^{Sc} may favor the formation of β-oligomers (Sweeting et al. 2010), small ordered aggregates that are believed to be important in the conversion of PrP^{C} to PrP^{Sc}. Structural studies using small peptides derived from regions of PrP^{C} associated with species barriers, including residue 138 in mouse PrP^{C} (Priola and Chesebro 1995), have shown that single amino acid differences can lead to very different β-sheet structures (Apostol et al. 2010, 2011). Thus, a third hypothesis is that these short segments of β-sheet structure may help abnormal PrP stack to form different types of parallel or anti-parallel steric zippers, the stability of which may determine prion species barriers (Apostol et al. 2011). Support for this latter hypothesis comes from studies on the β2–α2 loop which suggest that this region may form a tightly packed steric zipper in PrP^{Sc}, the disruption of which may be important in prion species barriers (Zink 2020; Kurt et al. 2015). All of these hypotheses accommodate the idea that even minor differences in conformation between different PrP species can have outsized effects on PrP^{Sc} production and susceptibility to disease.

13.6.2 Effect of Variable PrP^{Sc} Conformation

Differences in PrP^{Sc} conformation may also help to explain the early observation that the range of prion strains in an infectious inoculum is one determinant of whether or not a prion species barrier is crossed. Conformational differences within a pool of PrP^{Sc} molecules are thought to be the basis of prion strains (Caughey et al. 1998; Safar et al. 1998), which are defined as PrP^{Sc} molecules with the same primary sequence but with different biochemical properties in vitro and different biological phenotypes in vivo [for review, see (Bruce 1996)]. If the conformation of a particular strain of PrP^{Sc} was not compatible with the conformation of the host PrP^{C} molecule then, regardless of the PrP primary sequence, a species barrier to infection would exist. This would explain why a single amino acid change in mouse PrP^{C} can control multiple species barriers and restrict infection with different mouse scrapie strains (Barron et al. 2001) and why mink are susceptible to Suffolk, but not Cheviot, sheep scrapie (Hanson et al. 1971). Thus, differences in PrP^{Sc} conformation could also influence prion species barriers, likely by modulating the effect of species-specific differences in the primary sequence of PrP^{C} (Torres et al. 2014).

It would be very informative to have structural information from multiple prion strains and species of PrP^{Sc} to better understand how differences in its structure could impact prion species barriers. While multiple PrP^{Sc} structures have been

proposed over the years (Moore et al. 2009), the two most prominent are the 4-rung β-solenoid model (Vazquez-Fernandez et al. 2016; Spagnolli et al. 2019) and the parallel in-register intermolecular β-sheet (PIRIBS) model (Groveman et al. 2014). Of the two, atomic-level resolution of hamster PrP^{Sc} using high-resolution Cryo-EM analysis supports the PIRIBS model (Kraus et al. 2021). Analysis of the hamster PrP^{Sc} PIRIBS structure suggests that the asparagine at residue 155, which is critical in maintaining the species barrier between hamsters and mice (Priola et al. 2001), resides in an area of PrP^{Sc} where substitution with the corresponding tyrosine from mouse PrP would lead to steric clashes that could negatively impact conversion (Kraus et al. 2021). In vitro PrP^{Sc} formation studies have also suggested that asparagine residues are important in the formation of PrP^{Sc} across species, because they may help to stabilize intermolecular interactions within the PrP^{Sc} aggregate (Kurt et al. 2017). Future high-resolution PrP^{Sc} structures from different species should help to resolve why, depending upon the species, different amino acid residues impact transmission of prions across species barriers.

13.7 Molecular Model of Prion Species Barriers

13.7.1 Initial Prion Infection and Species Barriers

The fact that critical amino acid residues in the species-specific formation of PrP^{Sc} differ between species as well as the observation that PrP^{C} glycosylation can influence species barriers suggests that it is the tertiary structure of PrP, and not its primary structure, that may ultimately be most important in determining whether or not there are species-specific barriers to PrP^{Sc} formation and prion infection. This in turn suggests a molecular mechanism by which species barriers to prion infection are controlled at the level of PrP conformation (Fig. 13.2).

In intraspecies transmission of prions, where the host PrP^{C} and the exogenous infectious PrP^{Sc} are homologous, both the binding of PrP^{C} to PrP^{Sc} and its subsequent conversion to PrP^{Sc} occur as efficiently as possible, because they are conformationally compatible. Thus, there is no barrier to infection (Fig. 13.2a). By contrast, interspecies transmission of prions can occur when the host PrP^{C} and the exogenous infectious PrP^{Sc} are heterologous, but only if the amino acid residue differences are not within critical regions of the PrP molecule. In this instance, either the amino acid differences do not significantly change the conformation of PrP^{C} or the new conformation is still compatible with the incoming PrP^{Sc}. In either case, the binding of PrP^{C} to PrP^{Sc} and its subsequent conversion to PrP^{Sc} occurs efficiently enough that PrP^{Sc} can "replicate" to pathogenic levels (Fig. 13.2b). Thus, the differences in PrP^{C} conformation are insufficient to cause a species barrier to infection.

Interspecies transmission of prions would not occur if the host PrP^{C} and the exogenous infectious PrP^{Sc} are heterologous, but the amino acid differences reside within critical regions of the PrP molecule. In this case, the amino acid differences

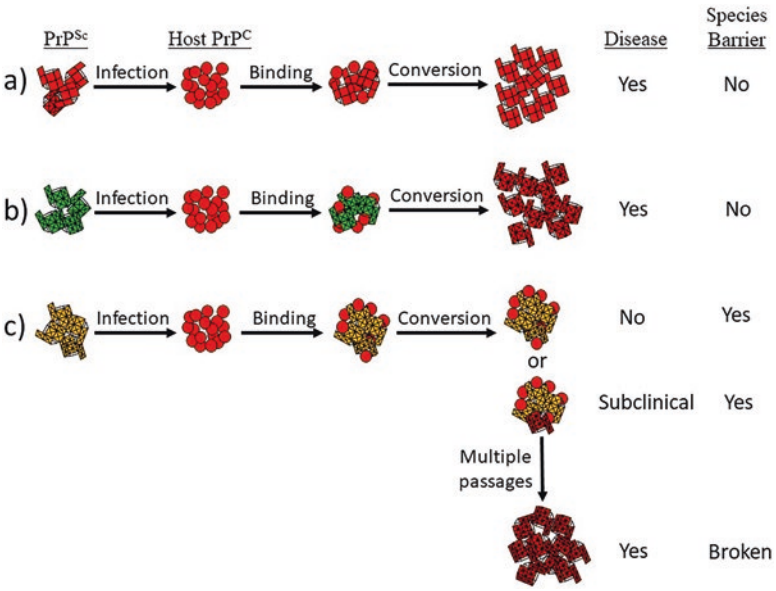


Fig. 13.2 Molecular model of prion disease species barriers. Red indicates PrP molecules derived from the same host species with aggregates of squares representing PrP^{Sc} and circles representing PrP^{C}. The degree of hatching within the squares represents different PrP^{Sc} conformations. Aggregates of green or yellow squares represent PrP^{Sc} molecules from different species. (**a**) Incoming PrP^{Sc} has the same sequence as the host PrP^{C}. Binding of PrP^{Sc} and PrP^{C} occurs, and since there is no conformational incompatibility, new PrP^{Sc} is formed. There is no species barrier and infection leads to disease. (**b**) Incoming PrP^{Sc} has a different primary sequence than the host PrP^{C}. Any resulting conformational differences are still compatible and binding of PrP^{Sc} and PrP^{C} occurs leading to new PrP^{Sc} formation. Despite both amino acid sequence and conformational differences between PrP^{Sc} and PrP^{C}, there is no species barrier and infection leads to disease. (**c**) Incoming PrP^{Sc} has a different primary sequence than the host PrP^{C} and the molecules are conformationally incompatible. Binding of PrP^{Sc} and PrP^{C} still occurs, but no new PrP^{Sc} is formed. Thus, there is a species barrier to infection unless a small, conformationally divergent fraction of PrP^{Sc} is present that can trigger new PrP^{Sc} formation. A subclinical infection would then be established that, given continued passage through the same host species, could eventually lead to clinical disease and a species barrier to prion infection being broken

change the conformation of PrP^{C}, such that it is incompatible with the incoming PrP^{Sc}. As a result, the binding of PrP^{C} to PrP^{Sc} and/or its subsequent conversion to PrP^{Sc} are significantly impaired (Fig. 13.2c). PrP^{Sc} would be unable to "replicate" itself very efficiently and would not accumulate to sufficient levels to trigger disease in the new host, i.e., a prion disease species barrier would exist. Thus, newly formed PrP^{Sc} would not be permanently altered by replication in the new host species, would likely retain its original properties, and would fail to adapt to the new host. This process has recently been termed nonadaptive prion amplification or NAPA (Bian et al. 2017), and suggests that selective pressures on PrP^{Sc} may ultimately dictate its host range (Bian et al. 2017; Duque Velásquez et al. 2020).

13.7.2 Prion Adaptation and Species Barriers

Even if a species barrier is not crossed during primary passage into a new host species, the presence of multiple prion strains in the infectious inoculum coupled with the potential for sub-clinical infection (i.e., prion replication but no disease) might eventually lead to a breach of the barrier to infection. For example, wild-type mice inoculated with hamster scrapie survive 1–2 years with no clinical signs of illness but, after 4–5 serial passages, prions that are mouse tropic, hamster tropic, or dually mouse and hamster tropic can be isolated (Race et al. 2002). In this instance, a species barrier is likely broken, because a minor fraction of the exogenous PrP^{Sc} is conformationally compatible with the endogenous host PrP^{C}, resulting in the generation of low levels of infectious PrP^{Sc} which now have the sequence of the host PrP^{C} molecule. This host compatible PrP^{Sc} begins to accumulate over the lifetime of the infected host but does not reach levels sufficient to cause disease (subclinical disease in Fig. 13.2c). However, when the infectious material is then transferred from the first infected host into a second host, from the second host into a third host, and so on, at each passage, more and more of the incoming PrP^{Sc} is homologous to the host PrP^{C} decreasing the time it takes for PrP^{Sc} to reach pathogenic levels until eventually it causes disease within the lifetime of the host (Fig. 13.2c). In essence, multiple passages in the new host species have allowed time for the prions to adapt and cause disease, a process that may explain the emergence of BSE in cattle. Thus, as long as prions can be transmitted between animals, it is likely that any prion species barrier can be crossed if there are prion strains in the inoculum capable of establishing a subclinical infection in the new host.

13.8 Intermediate Species and Prion Species Barriers

13.8.1 Altered Properties of BSE After Passage into New Species

The fact that hamster prions passaged through mice can acquire a new host range (Race et al. 2002) raises concerns that for both BSE, which has infected humans to cause vCJD (Bruce et al. 1997; Collinge et al. 1996; Hill et al. 1997) and CWD, which is circulating unchecked in wild populations of cervids (Miller and Williams 2003; Sigurdson and Aguzzi 2007), infection of an intermediate species could generate prions that could infect humans. In fact, the species tropism of BSE can be changed by passage through different hosts. While BSE prions derived from cattle cannot infect transgenic mice expressing cervid PrP^{C}, BSE prions passaged through red-tailed deer can (Vickery et al. 2014). Sheep-passaged BSE can infect transgenic mice expressing elk PrP^{C} (Tamguney et al. 2009b) and establish a subclinical infection in transgenic mice expressing human PrP^{C} (Plinston et al. 2011), while BSE from cattle cannot (Tamguney et al. 2009b; Plinston et al. 2011). Similarly, when

different strains of BSE are passaged through transgenic mice expressing different sheep *Prnp* genotypes, some can acquire the ability to infect transgenic mice expressing human PrP^{C} (Marín-Moreno et al. 2020). Mechanistically, in vitro studies suggest that the increased host range of BSE prions following passage through sheep may be because the prions that emerge appear to be more efficient at converting PrP^{C} from other species to PrP^{Sc} (Priem et al. 2014). All these experiments show that crossing species barriers can change the properties of the infectious prion, leading to the unpredictable emergence of prions with distinct species tropisms which can differ from that of the original inoculum.

13.8.2 CWD Host Range and Species Barriers

To date, there are no known cases of human prion disease related to exposure to CWD. Multiple studies have examined whether or not CWD can cross species barriers to cause disease in humans. Non-human primate models of CWD have shown that CWD can be transmitted to squirrel monkeys (Marsh et al. 2005; Race et al. 2014) but not to cynomologous macaques (Race et al. 2018), which are more closely related to humans. Transgenic mice expressing human PrP^{C} are also highly resistant to CWD infection (Sandberg et al. 2010; Wilson et al. 2012), with only one study able to detect potentially low levels of infectivity in approximately 4% of the mice infected (Race et al. 2019). PrP^{Sc} derived from CWD isolates in general does not convert human PrP^{C} very efficiently (Davenport et al. 2015), a resistance that has been mapped to differences in amino acids in the β2–α2 loop of PrP^{C} (Kurt et al. 2015), although using CWD prions stabilized by multiple passages in vivo or in vitro can lead to more efficient conversion (Barria et al. 2011). Indeed, a recent study has shown that elk PrP^{Sc} produced in vitro was able to convert human PrP^{C} into PrP^{Sc} which was then infectious for transgenic mice expressing human PrP^{C} (Wang et al. 2021).

These studies suggest that, while a robust species barrier to infection of humans with CWD prions exists, under the right circumstances, it may be broken. The concern is that, as with BSE, passage of CWD through intermediate species could alter its tropism. CWD can infect several species, including sheep (Cassmann et al. 2021), cats (Mathiason et al. 2013), pigs (Moore et al. 2017), and ferrets (Bartz et al. 1998). Following passage in ferrets, the tropism of mule deer CWD changed, enabling it to infect hamsters (Bartz et al. 1998) that normally have some resistance to initial infection with CWD (Bartz et al. 1998; Raymond et al. 2007). Thus, there is evidence for CWD prions being able to breach a species barrier following passage through a non-cervid species. When combined with the fact that CWD is circulating uncontrolled among wild cervid populations in North America, concerns remain that CWD may one day emerge as a threat to human health.

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