Chapter 9
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 amino acid sequence of the normal host prion protein (PrP^{C}), the available pool of exogenous abnormal and infectious prion protein (PrP^{Sc}), and the ability to establish a subclinical infection are all important determinants of prion species barriers. Mechanistically, maintenance of species barriers to prion infection is likely dependent upon the conformational diversity of the PrP^{Sc} molecules in an infectious inoculum and the potential for conformational compatibility between the exogenous PrP^{Sc} and endogenous host PrP^{C}. However, the lack of high resolution structural information for PrP^{Sc}, the potential for host factors and posttranslational modifications to PrP^{C} to influence species barriers, and the fact that the amino acids important in prion species barriers differ between species, makes it difficult to predict prion species barriers based on PrP^{C} sequence alone. In vivo or in vitro experimentation in relevant models of infection remains the only way to determine species barriers to prion infection.

Keywords Prion • Transmissible spongiform encephalopathy • Species barriers • Prion protein • Scrapie • PrP

Abbreviations

BSE	Bovine spongiform encephalopathy
CJD	Creutzfeldt–Jakob disease
CNS	Central nervous system

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GPI	Glycophosphatidylinositol
MBM	Meat and bone meal
NMR	Nuclear magnetic resonance
Prnp	Prion protein gene
PrP	Prion protein
PrP^{C}	PrP cellular
PrP^{Sc}	PrP scrapie
sCJD	Sporadic Creutzfeldt–Jakob disease
Sinc	Scrapie incubation time gene
TME	Transmissible mink encephalopathy
TSE	Transmissible spongiform encephalopathy
vCJD	Variant Creutzfeldt–Jakob disease

9.1 Introduction

Prion diseases, also known as transmissible spongiform encephalopathies or TSEs, can be transmitted both within and across species. Intraspecies transmission of TSE diseases 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. 2009b), 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 TSE infectivity therefore correlates with the presence of detectable levels of infectivity in non-CNS tissues.

Regardless of which tissues are positive for TSE infectivity, interspecies transmission of prions is much more difficult than intraspecies transmission. Species barriers in prion diseases are defined by the difficulty that prions from one species have in triggering TSE infection in a second species. As long as low prion 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 1986 (Wells et al. 1987; Wilesmith et al. 1988). 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 variant CJD (vCJD) was identified in young people in the UK 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 (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 prion diseases in nonruminant species and concerns that CWD has the potential to do the same, makes understanding the mechanisms underlying species barriers to TSE infection critical.

9.2 Prion Protein and TSE Species Barriers

Species barriers to TSE infection were initially defined based primarily upon the experimental inoculation of different types of TSE agent into multiple mammalian species including mice, hamsters, ferrets, and mink. For example, transmissible mink encephalopathy (TME) can be transmitted to hamsters but not mice (Marsh et al. 1969). Suffolk sheep scrapie can infect both mice and mink, while Cheviot sheep scrapie infects 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

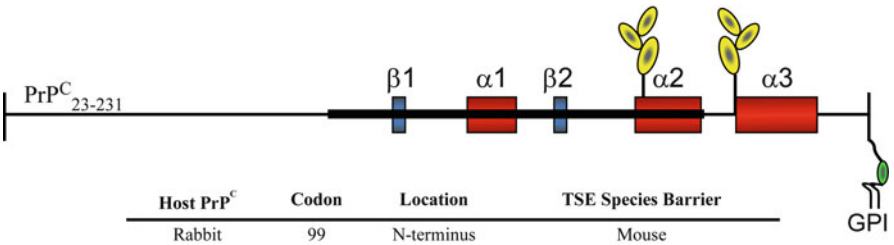
with TSE disease (Bolton et al. 1982). Soon after its discovery, it was determined that PrP was a normal host protein (Basler et al. 1986; Locht et al. 1986) which was both soluble and protease sensitive (Bendheim et al. 1988). During prion disease pathogenesis, normal PrP (termed PrP^{C} for PrP cellular) is refolded into an abnormally aggregated, protease-resistant, and infectious form known as PrP^{Sc} (for PrP scrapie) which accumulates, eventually causing a TSE disease. Comparison of PrP^{C} molecules from different mammalian species demonstrated that, while the *Prnp* gene is highly conserved, its amino acid sequence can vary by as much as 20% (Wopfner et al. 1999). This provided a potential molecular basis for TSE 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 or not infection and disease could occur.

9.3 Role of PrP Amino Acid Sequence

9.3.1 Region of PrP Involved in Species Barriers

In order to determine whether or not the sequence of PrP^{C} was a determinant of TSE 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 TSE 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). These experiments clearly showed that the amino acid sequence of the host PrP^{C} molecule was a major determinant of species barriers in TSE diseases. They also provided an explanation for why earlier studies had implicated the *Sinc* gene in TSE species barriers: the gene for PrP (*Prnp*) 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 to 189 (Fig. 9.1) (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 homologous (Wopfner et al. 1999) and there are only three amino acid differences between the two species in the region from codon 108 to 189, suggesting that one or more of these residues were contributing to the mouse–hamster TSE species barrier (Kocisko et al. 1995).



Host PrP^C	Codon	Location	TSE Species Barrier
Rabbit	99	N-terminus	Mouse
Mouse	101	N-terminus	Human (vCJD), Sheep, Hamster
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)
Vole	169	β2-α2 loop	Bovine (BSE)
Sheep	171	β2-α2 loop	Bovine (BSE)
Rabbit	173	α2	Mouse
Ferret	179	α2	Mink (TME)
Rabbit	214	α3	Mouse
Ferret	224	C-terminus	Mink (TME)

Fig. 9.1 *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 black line* indicates the region of PrP^C where most of the amino acid residues important in TSE 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

9.3.2 Influence of Single Amino Acid Residues

The influence of these three amino acid residue differences at codons 138, 154, and 169 on the species-specific formation of mouse PrP^{Sc} was analyzed in vitro using mouse neuroblastoma cells infected with the mouse scrapie strain RML (Priola and Chesebro 1995). These cells express mouse PrP^C and generate both mouse PrP^{Sc} and mouse scrapie infectivity. When mouse PrP^C expressing a unique antibody epitope tag is expressed in scrapie-infected 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 TSE species barrier, it was determined that a single hamster-specific residue at codon 138 in mouse PrP^C prevented the production of mouse PrP^{Sc} in cells (Priola and Chesebro 1995). The other mutations at codons 154 and 169 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 TSE species barriers to infection could be dependent upon relatively minor differences in sequence between the endogenous host PrP^{C} and exogenous PrP^{Sc} molecules.

Some types of prions, including BSE and sCJD, have never been successfully used to persistently infect cells in vitro. This limits the usefulness of cell-based systems in defining and understanding the mechanisms underlying TSE species barriers. Fortunately, in addition to both natural and transgenic models of prion disease, there are cell-free systems that are not restricted by prion species (Bossers et al. 1997; Castilla et al. 2005, 2008; Eiden et al. 2011; Kocisko et al. 1994, 1995; Raymond et al. 1997) which 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 TSE infectivity across species barriers differ depending upon the species (Fig. 9.1). For example, species-specific formation of hamster PrP^{Sc} has been mapped to codon 155 in vitro (Priola et al. 2001) and the same residue has been implicated in species barriers in 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). 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 TSE infection (Gibbs and Gajdusek 1973), multiple amino acid residues appear to be important for PrP^{Sc} formation (Vorberg et al. 2003).

Depending upon the species, resistance to BSE is associated with different amino acids in PrP^{C} (Fig. 9.1). 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 (Goldmann et al. 1994; Raymond et al. 1997). 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 TSE species barriers reside in different regions of PrP^{C} (Fig. 9.1). Thus, it would appear that there is no single structural component of PrP^{C} which is absolutely associated with species barriers to TSE infection.

9.3.3 Effect of **Prnp** *Heterozygosity*

Heterozygosity at the *Prnp* gene may also influence TSE 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 molecules in a dominant-negative fashion, slowing down or preventing clinical disease. In this manner, heterozygosity at the *Prnp* allele may contribute to the maintenance of TSE species barriers.

9.4 Influence of PrP Posttsranslational Modifications

PrP^C is posttranslationally modified by glycosylation at two N-linked glycosylation sites as well as by the addition of a glycophosphatidylinositol (GPI) membrane anchor (Caughey et al. 1989; Haraguchi et al. 1989; Stahl et al. 1987). The GPI anchor appears to have little or no effect on the species-specific formation of abnormal prion protein (Priola and Lawson 2001). However, PrP^C glycosylation can influence the binding between heterologous PrP^C and PrP^{Sc} molecules in a species-specific manner (Priola and Lawson 2001). At a molecular level, less efficient binding of heterologous PrP^C and PrP^{Sc} molecules would result in the production of less PrP^{Sc}. In vivo, this would likely contribute to the prolonged disease incubation times which are the hallmark of prion disease species barriers.

9.5 Non-PrP Host Factors

There are several examples in transgenic mice where, despite the fact that the host PrP^C amino acid sequence is identical to the incoming PrP^{Sc} amino acid sequence, species barriers to infection were maintained. For example, transgenic mice expressing human PrP^C can be more resistant to infection with vCJD than wild-type mice but more susceptible to infection with sporadic CJD (Bishop et al. 2006; Hill et al. 1997). 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 still suggest that host factors other than PrP might play a role in species barriers to prion infection. However, no such factor has yet been identified and thus the role of non-PrP host factors in transmission of prions across species remains unclear.

9.6 Molecular Mechanisms of TSE Species Barriers

9.6.1 Mechanism of PrP^{Sc} Formation Across Species

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 also influence this process suggests that it is the tertiary structure of PrP, and not its primary structure, which is ultimately 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 TSE infection are controlled at the level of PrP conformation. 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. 9.2a).

Interspecies transmission of prions can occur when the host PrP^{C} and the exogenous infectious PrP^{Sc} are heterologous, but the amino acid 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/or its subsequent conversion to PrP^{Sc} occurs efficiently enough that PrP^{Sc} can "replicate" to pathogenic levels (Fig. 9.2b). Thus, the differences in PrP^{C} conformation are insufficient to cause a species barrier to infection.

Interspecies transmission of prions would not occur when the host PrP^{C} and the exogenous infectious PrP^{Sc} are heterologous, but the amino acid differences do reside within critical regions of the PrP molecule. In this case, the amino acid differences 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. 9.2c). Thus, PrP^{Sc} would be unable to "replicate" itself very efficiently (if at all) and would not accumulate to sufficient levels to trigger disease in the new host, i.e., a prion disease species barrier would exist.

9.6.2 Structural Regions of PrP^{C} Implicated in Species Barriers

If PrP conformation is the driving force behind species-specific formation of PrP^{Sc}, species barriers to prion infection cannot be predicted based upon the primary sequence of PrP^{C} alone. Unfortunately, PrP^{Sc} conformation cannot be used to predict prion species barriers either as there are no high resolution PrP^{Sc} structures available (Moore et al. 2009). However, 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

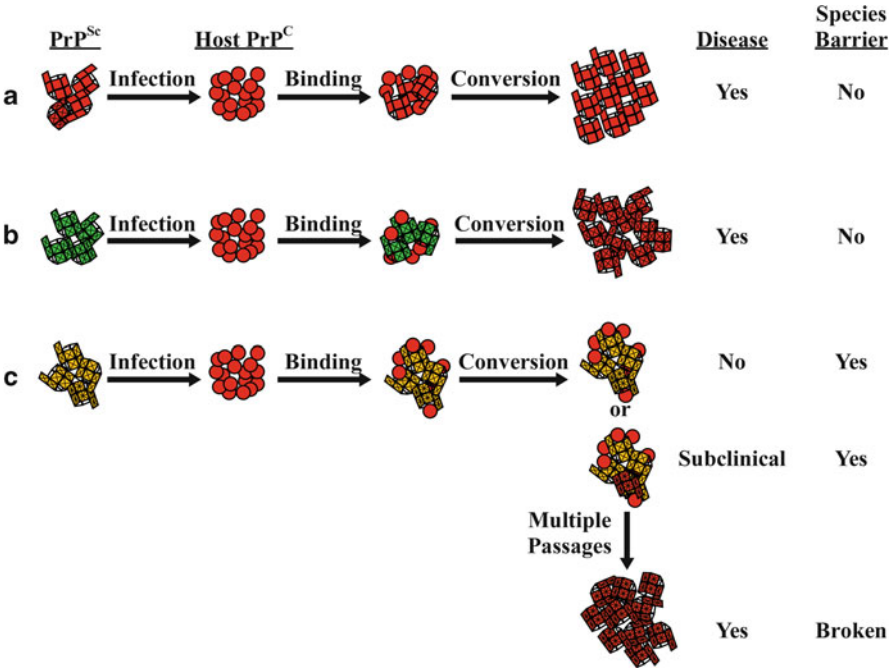


Fig. 9.2 *Molecular mechanism of prion disease species barriers*. *Red* indicates PrP molecules derived from the host species with aggregates of *squares* representing PrP^{Sc} and *circles* representing PrP^{C}. The degree of hatching within the *squares* represents different PrP^{Sc} conformations. 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, i.e., there is a species barrier to infection unless there is a small, conformationally divergent fraction of PrP^{Sc} which can trigger new PrP^{Sc} formation. A subclinical infection would then be established which, given continued passage through the host species, could eventually lead to clinical disease and a species barrier to prion infection being broken

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. 9.1).

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 is important 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. 9.1). When amino acid residues that have been experimentally shown to have a major influence on species-specific PrP^{Sc} formation are superimposed onto the structure of PrP^C, the vast majority of them reside within the disordered loop/turn regions (Fig. 9.1) suggesting that these are the critical structures. Thus, conformational variability within these loop regions between different species of PrP molecules may influence prion species barriers (Moore et al. 2009).

Polymorphisms within the disordered loop/turn structure which 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), bovine (Lopez et al. 2000), and human (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. 9.1). 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 TSE 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) (Fig. 9.1), 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 antiparallel steric zippers, the stability of which may determine TSE species barriers (Apostol et al.

2011). 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.

9.6.3 Effect of Variable PrP^{Sc} Conformation

Differences in PrP^{Sc} conformation may also help to explain the early observation that the range of TSE strains in the infectious inoculum is one determinant of whether or not a TSE 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). Prion strains are defined by PrP^{Sc} molecules with the same 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 would effectively have the same influence on species barriers as differences in the primary sequence of PrP^{C}.

9.7 Breaching TSE Species Barriers

Even if a species barrier is not crossed during primary passage into a new host species, the presence of multiple TSE strains in the infectious inoculum coupled with the potential for subclinical infection (i.e., prion replication but no disease) might eventually lead to a breach of the barrier to infection. 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). Similarly, the species tropism of BSE can be changed by passage through sheep. Sheep-passaged BSE can infect transgenic mice expressing human PrP^{C} (Plinston et al. 2011) while BSE from cattle cannot (Plinston et al. 2011; Tamguney et al. 2009a). All of these experiments show that crossing species barriers can change the tropism of the infectious agent leading to the unpredictable emergence of prions with distinct species tropisms which can differ from that of the original inoculum.

The existence of PrP^{Sc} molecules with the same PrP sequence but different conformations can also help to explain adaptation across even a strong species barrier. A minor fraction of the exogenous PrP^{Sc} could be conformationally compatible with the endogenous host PrP^{C} resulting in the generation of low levels of infectious PrP^{Sc} which now has the sequence of the host PrP^{C} molecule (Fig. 9.2c). This new

PrP^{Sc} begins to accumulate over the lifetime of the infected host but does not reach levels sufficient to cause disease (i.e., subclinical infection). 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}. This effectively decreases the time it takes for PrP^{Sc} to reach pathogenic levels until eventually it causes disease within the lifetime of the host (Fig. 9.2c). The tropism of the final prion agent would likely reflect the minor fraction of PrP^{Sc} that was eventually able to amplify efficiently enough over multiple passages to cause disease. Thus, as long as infectivity 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.

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