



Review

# Virus Infection, Genetic Mutations, and Prion Infection in Prion Protein Conversion

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**Abstract:** Conformational conversion of the cellular isoform of prion protein, PrP<sup>C</sup>, into the abnormally folded, amyloidogenic isoform, PrP<sup>Sc</sup>, is an underlying pathogenic mechanism in prion diseases. The diseases manifest as sporadic, hereditary, and acquired disorders. Etiological mechanisms driving the conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup> are unknown in sporadic prion diseases, while prion infection and specific mutations in the PrP gene are known to cause the conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup> in acquired and hereditary prion diseases, respectively. We recently reported that a neurotropic strain of influenza A virus (IAV) induced the conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup> as well as formation of infectious prions in mouse neuroblastoma cells after infection, suggesting the causative role of the neuronal infection of IAV in sporadic prion diseases. Here, we discuss the conversion mechanism of PrP<sup>C</sup> into PrP<sup>Sc</sup> in different types of prion diseases, by presenting our findings of the IAV infection-induced conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup> and by reviewing the so far reported transgenic animal models of hereditary prion diseases and the reverse genetic studies, which have revealed the structure-function relationship for PrP<sup>C</sup> to convert into PrP<sup>Sc</sup> after prion infection.

**Keywords:** prion; prion protein; prion disease; neurodegenerative disease; virus infection; conformational conversion; influenza virus; protein polymerization



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## 1. Introduction

Prion diseases, or transmissible spongiform encephalopathies, are a group of fatal neurodegenerative disorders in humans and animals, caused by accumulation of proteinaceous infectious particles, termed “prions”, in the brain [1–3]. Characteristic histopathological features, such as spongiform vacuolation, neuronal cell death, and gliosis, are observed in the affected brains [1,2]. Prion diseases manifest as sporadic, hereditary, or acquired disorders. Sporadic Creutzfeldt-Jakob disease (sCJD) is the most common prion disease in humans, accounting for 85–90% of total human cases [4–6]. The annual incidence of sCJD has been estimated to be 1–2 cases per a million people worldwide, although the reported incidence varies from country to country and is influenced by the method and extent of the study conducted [7]. 10–15% of human cases belong to hereditary prion diseases, such as familial CJD (fCJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), and fatal familial insomnia (FFI) [8,9]. They are causatively linked to specific mutations in the gene (*Prnp*) of prion protein (PrP) [8,9]. The remaining cases, accounting for less than 1%, are those of acquired prion diseases, which include iatrogenic CJD (iCJD), variant CJD (vCJD), and kuru. iCJD is a disease caused by human-to-human transmission of prions via medical treatments or procedures [10]. Dura mater, cornea, and growth hormone from the patients, and depth electroencephalogram electrodes and neurosurgical instruments used for the patients have been considered to transmit the disease [11]. vCJD is believed to be transmitted from bovine spongiform encephalopathy (BSE) via oral consumption of BSE prion-contaminated food [10]. As of July 2021, 229 cases of vCJD have been identified worldwide since 1996, with most of them in the U.K. (<http://www.cjd.ed.ac.uk/sites/default/files/worldfigs.pdf>,

accessed on 21 July 2021). Kuru is a disease spread via ritualistic cannibalism among Fore people in Papua New Guinea [11].

BSE is an acquired animal prion disease in cattle spread through contaminated meat-and-bone meals since the mid-1980s [12]. Transmissible mink encephalopathy in minks, chronic wasting disease (CWD) in deer, and feline spongiform encephalopathy in cats also acquired prion diseases [13,14]. Scrapie is a sporadic animal prion disease in sheep [13]. Unlike the case of BSE, epidemiological studies suggest unlikelihood of the zoonotic transmissions of scrapie or CWD to humans [15–19].

Prions are widely believed to consist of the abnormally folded, amyloidogenic isoform of PrP, designated PrP<sup>Sc</sup>, which is produced from the cellular isoform of PrP, PrP<sup>C</sup>, through its conformational conversion [1–3]. We and others have shown that mice devoid of PrP<sup>C</sup> (*Prnp*<sup>0/0</sup>) are resistant to prion infections, neither developing disease nor propagating prions in the brains after intracerebral inoculation with the prions [20–23], confirming that the conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup> eventually leading to accumulation of PrP<sup>Sc</sup> in the brain is a key pathogenic event in prion diseases. In acquired prion diseases, prion infections drive the conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup>. Mutated PrPs in hereditary prion diseases may undergo conformational changes to form PrP<sup>Sc</sup> [8,9]. We have recently reported that neurotrophic influenza A virus (IAV) caused the conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup> in cultured neuronal cells after infection [24], raising the intriguing possibility that IAV infection in neurons might be a cause of or be associated with sporadic prion diseases.

Here, we present our current findings of the IAV infection-induced conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup>. We also review the so far reported transgenic (Tg) mouse models of hereditary prion diseases, where mutated PrPs are spontaneously converted into PrP<sup>Sc</sup>s, and the reverse genetic studies using *Prnp*<sup>0/0</sup> mice, which have revealed the important regions for PrP<sup>C</sup> to convert into PrP<sup>Sc</sup> after prion infection.

## 2. PrP Conversion

### 2.1. PrP<sup>C</sup> and PrP<sup>Sc</sup>

PrP<sup>C</sup> is a host-encoded glycoprotein tethered to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor moiety and expressed most abundantly in the brain, particularly by neurons, and to lesser extents in other non-neuronal tissues, including heart, lung, pancreas, liver, spleen, testis, and kidney [25–27]. Many physiological functions have been suggested for PrP<sup>C</sup>, including oxidative stress mitigation, neuroprotection, and those associated with cell trafficking, cell adhesion, cell differentiation, cell signaling, and cell survival [28]. We have recently identified that PrP<sup>C</sup> could also function as a protective molecule against IAV infection, mitigating oxidative stress and epithelial cell apoptosis in IAV-infected lungs, and a regulator molecule in macrophage M1/M2 polarization, stimulating the polarization to M2 anti-inflammatory macrophages [29,30].

PrP<sup>C</sup> structurally consists of two domains; the flexible non-structural N-terminal domain including the so-called octapeptide repeat (OR) region in the middle, and the globular C-terminal domain with 2 short  $\beta$ -sheets and 3  $\alpha$ -helices [26,27]. Mouse PrP<sup>C</sup> is translated as a precursor protein comprised of 254 amino acids, with the 22 amino acids of an endoplasmic reticulum (ER) migration signal sequence at the N-terminus and the 23 amino acids of a GPI anchor addition signal sequence at the C-terminus [26,27]. Both signal sequences are removed in the ER as the nascently translated PrP enters the ER [26,27]. The C-terminal domain further undergoes posttranslational modifications, such as disulfide bond formation between cysteine residues at positions 178 and 213 and N-type sugar chain addition at asparagine residues of positions 180 and 196 [26,27].

PrP<sup>Sc</sup> is specifically detectable in the tissues or cells infected with prions, including the central nervous system, particularly neurons, and follicular dendritic cells of the lymphoreticular system [31]. In contrast to PrP<sup>C</sup> being detergent-soluble and sensitive to protease degradation, PrP<sup>Sc</sup> is highly detergent-insoluble, easily assembling to form amyloid fibrils, termed prion rods, with a diameter of 10–20 nm and a length of 100–200 nm, and relatively resistant to protease degradation [32]. These biochemical differences of both molecules

arise from their structural differences. PrP<sup>C</sup> is rich in  $\alpha$ -helix contents while PrP<sup>Sc</sup> abounds with  $\beta$ -sheet contents [33].

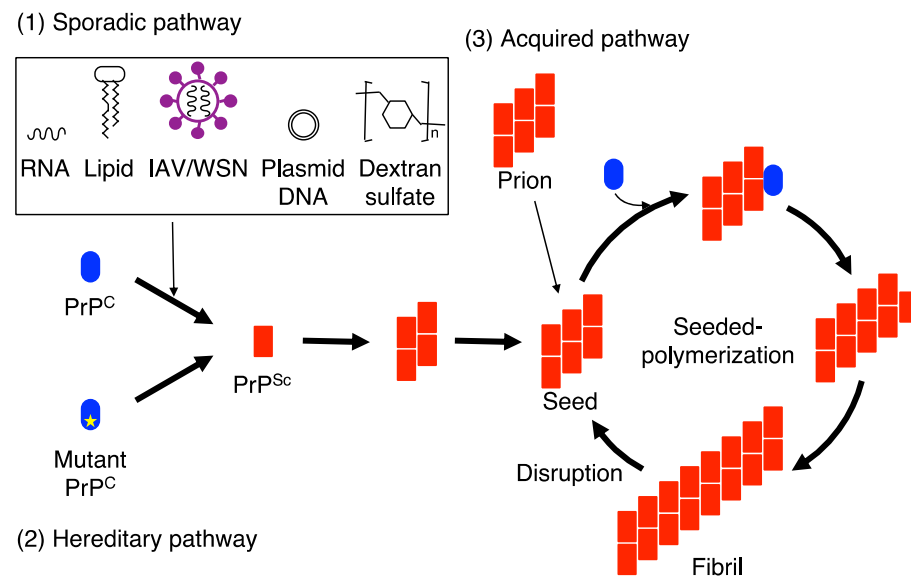
## 2.2. *In Vitro PrP Conversion*

Kocisko et al. succeeded for the first time in producing PrP<sup>Sc</sup>-like proteinase K (PK)-resistant PrP from PrP<sup>C</sup> in vitro, by incubating PrP<sup>C</sup> from prion-uninfected cultured cells with PrP<sup>Sc</sup> partially purified from 263K prion-infected hamster brains in denaturing-renaturing conditions [34]. The protein misfolding cyclic amplification (PMCA) technique was then developed to amplify PrP<sup>Sc</sup>-like PrP in vitro by serially incubating PrP<sup>C</sup> with the diluted PMCA products under sonication, eventually enriching PrP<sup>Sc</sup>-like PrP converted from PrP<sup>C</sup> and contrarily reducing original PrP<sup>Sc</sup> added in the reactions to a negligible level [35]. Subsequently, PrP<sup>Sc</sup>-like PrP generated by PMCA was shown to correlate to prion infectivity, strongly suggesting that PrP<sup>Sc</sup>-like PrP is generated by PMCA is infectious [36]. Prion infectivity was also successfully generated from bacterial recombinant PrP without incubating with PrP<sup>Sc</sup>. The  $\beta$  sheet-rich amyloid fibrils consisting of the N-terminally truncated recombinant mouse PrP were shown to be infectious [37]. PMCA with bacterial recombinant full-length mouse PrP together with the synthetic anionic phospholipid and RNA molecules was also shown to generate PrP<sup>Sc</sup>-like PrP as well as having prion infectivity [38]. Dextran sulfate, mRNA molecules, and plasmid DNA were also reported to function as co-factors to produce infectious prions from bank vole PrP in PMCA, although bank vole PrP was convertible into infectious prions in PMCA without these cofactors [39]. These results confirm that PrP<sup>Sc</sup> is an essential component for prions to be infectious, strongly supporting that prions consist of PrP<sup>Sc</sup> alone. Consistent with this, prion infectivity has been shown to be sensitive to treatments with protein-denaturing agents, but resistant to nucleic acid-degrading treatments with nucleases or UV radiation [40]. No specific nucleic acids have been isolated in the highly purified prion fractions [41].

## 2.3. *Seeded-Polymerization Mechanism in PrP Conversion*

It has been suggested that a seeded-protein polymerization mechanism is an underlying mechanism for PrP<sup>Sc</sup> propagation, or prion propagation [34,42]. Prion-infected brain fractions containing the aggregates of 14–28 PrP<sup>Sc</sup> molecules were shown to be highly infectious compared to those containing large PrP<sup>Sc</sup> fibrils or the aggregates of less than 5 PrP<sup>Sc</sup> molecules [43], suggesting that prions are the multimeric aggregates of PrP<sup>Sc</sup>. In the seeded-protein polymerization mechanism, the aggregates of PrP<sup>Sc</sup> are considered to function as a seed or a scaffold for PrP<sup>C</sup> to undergo conformational conversion into PrP<sup>Sc</sup> (Figure 1). PrP<sup>C</sup> is recruited onto the PrP<sup>Sc</sup> seed and forced to convert into PrP<sup>Sc</sup> on it through structural transition from  $\alpha$ -helix to  $\beta$ -sheet [33], resulting in elongation of the PrP<sup>Sc</sup> seeds, eventually leading to formation of the PrP<sup>Sc</sup> fibrils (Figure 1). Prions, or PrP<sup>Sc</sup> seeds, are postulated to then propagate by cleavage of the PrP<sup>Sc</sup> fibrils into new multiple PrP<sup>Sc</sup> seeds by unknown cellular mechanism (Figure 1) [34,35].

Structural models have been proposed for PrP<sup>Sc</sup> fibrils. In a 4-rung  $\beta$ -solenoid model, a PrP<sup>Sc</sup> molecule adopts a solenoid structure with 4 rungs, each rung consisting of 3  $\beta$ -sheets and perpendicularly stacking with each other to form a PrP<sup>Sc</sup> protofilament [44–46]. Two PrP<sup>Sc</sup> protofilaments are then intertwined, forming a PrP<sup>Sc</sup> fibril [44–46]. The PrP<sup>Sc</sup> protofilaments might function as seeds, recruiting PrP<sup>C</sup> molecules to their upper and lower  $\beta$ -solenoid rungs and inducing the formation of  $\beta$ -solenoid rungs through the structural transition of  $\alpha$ -helices to  $\beta$ -sheet, eventually converting the PrP<sup>C</sup> into PrP<sup>Sc</sup>. In a parallel in-register intermolecular  $\beta$ -sheet model, a PrP<sup>Sc</sup> fibril is formed by stacking PrP<sup>Sc</sup> molecules, each PrP<sup>Sc</sup> comprising the entire cross-section of the fibril with many hairpins, through the intermolecular  $\beta$ -sheet interactions between them [47,48]. The fibrils function as seeds for PrP<sup>C</sup> to convert into PrP<sup>Sc</sup>.



**Figure 1.** Seeded protein polymerization model for the conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup> in sporadic, hereditary, and acquired prion diseases. (1) IAV/WSN infection and interaction with RNA, lipid molecules plasmid DNA and dextran sulfate drives PrP<sup>C</sup> to undergo conformational changes to form PrP<sup>Sc</sup> in sporadic prion diseases. (2) In hereditary prion diseases, mutated PrPs are prone to convert into PrP<sup>Sc</sup>. The nascently generated PrP<sup>Sc</sup> molecules in sporadic and hereditary prion diseases assemble to form small oligomeric PrP<sup>Sc</sup> aggregates and then further polymerize to form PrP<sup>Sc</sup> seeds, which are able to recruit and force PrP<sup>C</sup> to convert into PrP<sup>Sc</sup> on them. (3) In acquired prion diseases, exogenously invading prions function as PrP<sup>Sc</sup> seeds for the conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup>. As a result, the PrP<sup>Sc</sup> oligomers are elongated to form fibrils in sporadic, hereditary, and acquired prion diseases. The fibrils are then disrupted into prions, or small PrP<sup>Sc</sup> seeds, which then enter the conversion cycle of PrP<sup>C</sup> into PrP<sup>Sc</sup>.

### 3. Virus Infections in PrP Conversion

#### 3.1. Neurotropic IAV Infection in PrP Conversion

We have recently reported that infection of mouse neuroblastoma N2a cells overexpressing the exogenously transduced mouse PrP<sup>C</sup>, termed N2aC24 cells, with a neurotropic strain of influenza A/WSN/33 (H1N1) virus (hereafter referred to as IAV/WSN) induced spontaneous conformational changes in PrP<sup>C</sup> to form PrP<sup>Sc</sup>-like PK-resistant PrP [24]. These results indicate that, like prion infection, IAV/WSN infection could induce the conformational conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup>. We also detected PrP<sup>Sc</sup>-like PrP in parent N2a cells, which express endogenous mouse PrP<sup>C</sup> alone, after infection with IAV/WSN [24], indicating that overexpression of PrP<sup>C</sup> is not required for the IAV/WSN-induced conversion into PrP<sup>Sc</sup>. We further showed that the anti-IAV agent oseltamivir and anti-IAV mouse antisera blocked IAV/WSN infection of N2aC24 cells and prevented formation of PrP<sup>Sc</sup>-like PrP in the cells [24], not only ruling out the possibility of the contamination of laboratory prions in the cell culture, but also confirming that IAV/WSN infection is essential for the conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup>-like PrP in the cells. We finally demonstrated that wild-type (WT) mice intracerebrally inoculated with cell lysates from PrP<sup>Sc</sup>-like PrP-producing N2aC24 cells developed prion disease, with abundant accumulation of PrP<sup>Sc</sup> and spongiosis in their brains [24], indicating that, like authentic PrP<sup>Sc</sup> produced by prion infection, PrP<sup>Sc</sup>-like PrP produced in IAV/WSN-infected N2aC24 cells could form infectious prions. Taken together, these results suggest that IAV infection in neurons might be a cause of or be associated with sporadic prion diseases, including sCJD.

PrP<sup>Sc</sup>-like PrP (hereafter simply referred to as PrP<sup>Sc</sup>) was constitutively produced in IAV/WSN-infected N2aC24 cells even after IAV/WSN infection was cleared during long culture [24], indicating that persistent IAV/WSN infection is not necessary for the

constitutive conversion PrP<sup>C</sup> into PrP<sup>Sc</sup> in the cells. It is thus possible that IAV/WSN infection could induce the conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup> in a hit-and-run manner, and the nascent PrP<sup>Sc</sup> could polymerize to form the PrP<sup>Sc</sup> seeds, enabling the constitutive conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup> in the seeded-polymerization mechanism. IAVs are negative-stranded, segmented, enveloped RNA viruses [49]. It was reported that RNA molecules were able to convert human recombinant PrP into PK-resistant PrP in vitro [50]. Moreover, RNA molecules were shown to function as a cofactor, together with the synthetic anionic phospholipid, for the conversion of recombinant PrP into PrP<sup>Sc</sup> in PMCA [38]. These results indicate that RNA molecules play an important role in the conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup>. IAV/WSN-derived RNA molecules might play the same role in the conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup> in N2aC24 cells. However, it has been reported that RNA molecules prepared from mammalian tissues but not from invertebrate species including bacteria, yeast, worms, and flies converted PrP<sup>C</sup> into PrP<sup>Sc</sup> in PMCA [51]. For elucidation of the role of IAV/WSN-derived RNA molecules in the conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup>, it would be helpful to investigate if IAV/WSN-derived RNA molecules have an ability to convert PrP<sup>C</sup> into PrP<sup>Sc</sup> in PMCA.

### 3.2. Other Virus Infections in Prion Infection

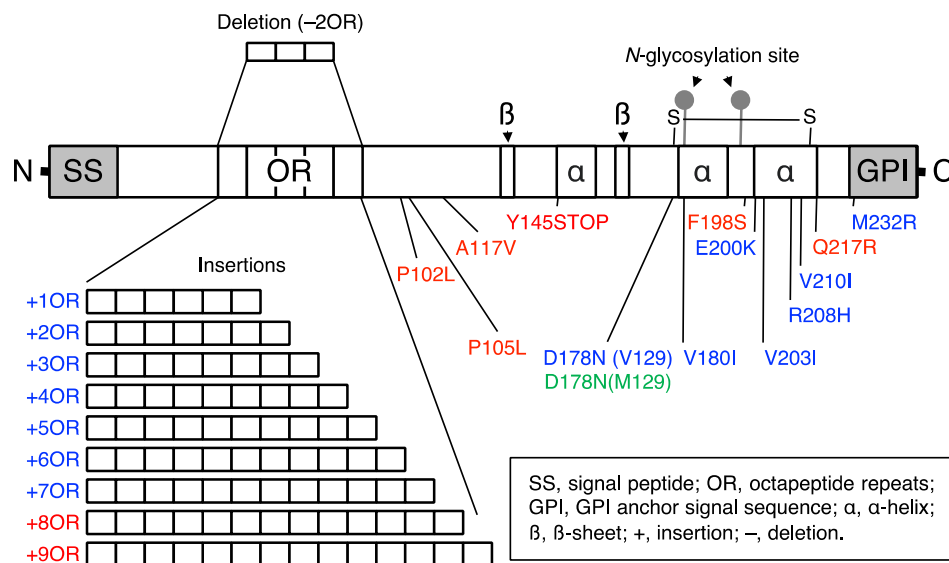
It was shown that mice co-infected with scrapie prions and mouse adenovirus accelerated prion disease compared to those infected with scrapie prions alone, suggesting that mouse adenovirus infection could enhance prion pathogenesis in mice [52]. Infection with the Cork strain of caprine arthritis encephalitis virus, a small-ruminant lentivirus, was also shown to increase PrP<sup>Sc</sup> in scrapie prion-infected primary sheep microglia at levels 2-times higher than in control cells [53]. However, no data were available as to whether these virus infections could cause the de novo conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup> [53]. Mouse A9 fibroblasts infected with murine minute virus, a murine parvovirus, were reported to bind to exogenously added PrP<sup>Sc</sup> molecules more abundantly and internalize them to lysosomal compartments, which are postulated to be one of the cellular sites for the conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup>, more efficiently than control cells [54]. Molony murine leukemia virus infection into 22L scrapie prion-infected NIH3T3 cells was reported to increase the release of PrP<sup>Sc</sup> in culture medium by facilitating the incorporation of PrP<sup>Sc</sup> into virus particles or exosomes [55]. It is thus possible that the increased levels of PrP<sup>Sc</sup> in the prion-infected cells after infection with mouse adenovirus or caprine arthritis encephalitis virus might be due to the increased accessibility of PrP<sup>Sc</sup> to PrP<sup>C</sup> through the enhanced binding of PrP<sup>Sc</sup> molecules on the cells, their stimulated internalization to lysosomal compartments, and the increased release of PrP<sup>Sc</sup> from prion-infected cells.

## 4. Tg Mouse Models of Hereditary Prion Diseases

### 4.1. Genetic Mutations in PrP Conversion

Many point mutations and insertions or a deletion of the OR sequence have been identified in hereditary human prion diseases, including GSS, fCJD, and FFI (Figure 2) [8,9]. To understand the pathogenic mechanism of hereditary prion diseases, many lines of Tg mice expressing the disease-associated mutant PrPs have been reported, uncovering biologically and structurally different forms of PrP<sup>Sc</sup>, or neurotoxic and infectious PrP<sup>Sc</sup>s [56]. Some PrP mutants were shown to convert into neurotoxic forms but not to infectious forms while others converted to neurotoxic and infectious forms [56]. It is thus possible that PrP<sup>C</sup> might undergo conformational changes to convert into PrP<sup>Sc</sup>, then polymerizing to form neurotoxic PrP<sup>Sc</sup> oligomers and to neurotoxic and infectious PrP<sup>Sc</sup> oligomers, or PrP<sup>Sc</sup> fibrils.





**Figure 2.** Schematic illustration of hereditary prion disease-associated mutations in PrP. GSS, fCJD, and FFI mutations are indicated in red, blue, and green, respectively.

#### 4.2. Tg Mouse Models of GSS

The first reported Tg mouse model of hereditary prion diseases is Tg(PrP-P101L) mice, which express mouse PrP-P101L, the analogous mutation to human PrP-P102L in GSS (Table 1) [57]. They expressed the mutant PrP at levels at least 3 times higher than PrP<sup>C</sup> in WT mice and spontaneously developed prion disease-like disease at 150–600 days after birth, with the disease-characteristic pathologies of amyloid plaques, spongiform degeneration, and gliosis in their brains [57]. However, in contrast to authentic PrP<sup>Sc</sup>, which is highly resistant to PK, PrP<sup>Sc</sup>-P101L accumulated in the brains of ill Tg(PrP-P101L) mice was easily digested by a high dose of PK [57]. In addition, intracerebral inoculation with brain homogenates from ill Tg(PrP-P101L) mice transmitted the disease to 40% of Tg(PrP-P101L) mice, which express low levels of the mutant protein, and 10% of hamsters, but not to WT mice [58]. These results suggest that PrP-P101L could convert into neurotoxic and infectious PrP<sup>Sc</sup>-P101L. However, the infectivity of PrP<sup>Sc</sup>-P101L was dependent on recipient animals. Tg(PrP-A116V) mice, which express mouse PrP-A116V (the human homologue of PrP-A117V in GSS) at levels 4–6 times higher than PrP<sup>C</sup> in WT mice, also spontaneously developed neurological disease around 150 days after birth, with spongiosis and PrP amyloid plaques in their brains (Table 1) [59]. PrP<sup>Sc</sup>-A117V in the brains of ill Tg(PrP-A116V) mice was partially detergent-insoluble and weakly resistant to PK [59]. No data are available as to whether PrP<sup>Sc</sup>-A117V is infectious [59]. Tg(PG14)/*Prnp*<sup>0/0</sup> mice are another GSS mouse model expressing a mutant PrP with 9 extra OR sequences in the OR region on the genetic background of *Prnp*<sup>0/0</sup> (Table 1) [60,61]. These mice developed spontaneous cerebellar neurodegeneration, with granule cell death and very slight but substantial accumulation of PrP<sup>Sc</sup>-PG14 in their brains [60,61]. PrP<sup>Sc</sup>-PG14 showed weak resistance to PK and had no prion infectivity in animals [62]. These results indicate that PrP-PG14 could convert into neurotoxic PrP<sup>Sc</sup>-PG14 but not into infectious PrP<sup>Sc</sup>-PG14.

**Table 1.** Hereditary prion disease-associated mutations in PrP conversion.

Hereditary Prion Diseases	Mutant PrPs	Conversion to Neurotoxic PrP <sup>Sc</sup> /Infectious PrP <sup>Sc</sup>	References
GSS	Mo-PrP-P101L	Yes/Yes	[57,58]
	Mo-PrP-A116V	Yes/N.D.	[59]
	Mo-PrP-PG14	Yes/No	[60–62]
fCJD	Mo/Hu-PrP-E199K	Yes/Yes	[63]
	Hu-PrP-E200K	No/No	[64]
	Mo-PrP-E199K(3F4)	Yes/Yes	[65]
	Mo-PrP-D177N(V128)	Yes/No	[66]
FFI	Mo-PrP-D177N(M128)	Yes/No	[67]
	Mo-PrP-D177N(M128)(3F4)	Yes/Yes	[68]

Mo, mouse; Hu, human; N.D., not determined.

#### 4.3. Tg Mouse Models of fCJD

Tg(Mo/Hu-PrP-E199K) mice, which express mouse-human chimeric PrP, or Mo/Hu-PrP, with the E199K mutation (the human homologue of E200K in fCJD) 2 times higher than PrP<sup>C</sup> in WT mice, were reported to develop neurological disease around 200 days of age, with hind-limb paralysis and kyphosis (Table 1) [63]. Mo/Hu-PrP<sup>Sc</sup>-E199K in the brains of sick Tg(Mo/Hu-PrP-E199K) mice showed atypical PK-resistant bands on Western blotting [63]. Inoculation of brain homogenates from sick Tg(Mo/Hu-PrP-E199K) mice transmitted the disease into non-Tg mice with highly variable efficiency [63]. These results indicate that Mo/Hu-PrP-E199K could convert into neurotoxic and infectious Mo/Hu-PrP<sup>Sc</sup>-E199K. However, interestingly, Tg mice overexpressing human PrP with the E200K mutation in the brains at levels 2 to 3 times higher than PrP<sup>C</sup> in human brains developed no neurological diseases (Table 1) [64]. In contrast, knock-in mice expressing mouse PrP-E199K with the 3F4 epitope, which derives from human PrP, exhibited abnormal behaviors, such as reduced performance on the rotarod test and decreased burrowing behavior, with spongiosis and punctuate deposition of PrP<sup>Sc</sup>-E199K(3F4) in their brains (Table 1) [65]. PrP<sup>Sc</sup>-E199K(3F4) was weakly resistant to PK [65]. Inoculation of brain homogenates from sick Tg[PrP-E199K(3F4)] mice transmitted the disease in Tg mice overexpressing Mo-PrP as well as in non-Tg mice [65]. It is thus possible that the human PrP-derived 3F4 sequence might affect mouse PrP to spontaneously convert into neurotoxic and infectious PrP<sup>Sc</sup>.

Tg mice overexpressing Mo-PrP with the D177N mutation (the human homologue of D178N in fCJD) along with the polymorphic amino acid substitution of M128V (the human homologue of M129V) 2 times higher than PrP<sup>C</sup> in WT mice were also reported to develop neurological disease around 150 days of age, with ataxia, kyphosis, and foot claspings (Table 1) [66]. PrP<sup>Sc</sup>-D177N(V128) was detergent-insoluble and mildly resistant to PK [66]. Inoculation of brain homogenates from sick Tg[PrP-D177N(V128)] mice failed to transmit the disease to non-Tg mice as well as Tg mice overexpressing mouse PrP and Tg mice expressing lower levels of PrP-D177N(V128) [67]. These results suggest that PrP-D177N(V128) could convert into neurotoxic PrP<sup>Sc</sup>-D177N(V128) but not to infectious PrP<sup>Sc</sup>-D177N(V128).

#### 4.4. Tg Mouse Models of FFI

Tg(FFI)/Prnp<sup>0/0</sup> mice, which express mouse PrP-D177N(M128) corresponding to the human homologue of PrP-D178N(M129) in FFI in their brains 2 times higher than PrP<sup>C</sup> in WT mice, were reported to develop fatal neurological disease with motor and cognitive deficits (Table 1) [67]. Circadian rhythms of sleep and motor activity were also disturbed in Tg(FFI)/Prnp<sup>0/0</sup> mice, as observed in FFI patients [67]. Sick Tg(FFI)/Prnp<sup>0/0</sup> mice accumulated weakly PK-resistant PrP<sup>Sc</sup>-D177N(M128) in their brains [67]. Inoculation of brain homogenates from sick Tg(FFI)/Prnp<sup>0/0</sup> mice failed to transmit the disease to WT mice and Tg mice overexpressing mouse PrP [67]. These results suggest that PrP-D177N(M128) could convert into neurotoxic PrP<sup>Sc</sup>-D177N(M128) but not to infectious PrP<sup>Sc</sup>-D177N(M128). However, knock-in mice expressing PrP-D178N(M129) with the 3F4 epitope sequence were shown to develop neurological disease with neuronal loss and

gliosis in the thalamus and transmit the disease to Tg mice overexpressing mouse PrP after inoculation with their brain homogenates (Table 1) [68]. It is thus suggested that the 3F4 sequence could force PrP-D178N(M129) to undergo conformational changes to form neurotoxic PrP<sup>Sc</sup>-D177N(M128) and then to neurotoxic and infectious PrP<sup>Sc</sup>-D177N(M128).

## 5. Reverse Genetic Studies for Acquired Prion Diseases

### 5.1. The N-Terminal Polybasic Residues of PrP<sup>C</sup> in Prion Infection

Successful restoration of the susceptibility to prion infection in *Prnp*<sup>0/0</sup> mice by transgenic expression of full-length mouse PrP<sup>C</sup> has enabled reverse genetic studies to identify the regions or amino acids in PrP<sup>C</sup> important for the conversion into PrP<sup>Sc</sup> after prion infection [69]. Tg(PrPΔ23–31)/*Prnp*<sup>0/0</sup> mice, which express PrP with a deletion of the so-called N-terminal polybasic region consisting of residues 23–31 on the background of *Prnp*<sup>0/0</sup>, were shown to develop disease after longer incubation times with a slower accumulation of PrP<sup>Sc</sup>Δ23–31 in their brains after infection with RML scrapie prions, indicating that the polybasic region is important for PrP<sup>C</sup> to convert into PrP<sup>Sc</sup> after prion infection (Table 2) [70]. We generated Tg(PrP3K3A)/*Prnp*<sup>0/0</sup> mice, which express mouse PrP<sup>C</sup> with lysine residues at positions 23, 24 and 27 mutated to alanine residues in the polybasic region, and intracerebrally inoculated them with RML and 22L scrapie prions (Table 2) [71]. They were highly resistant to the prions, developing disease with longer incubation times [71], suggesting that lysine residues at 23, 24, and 27 are important for the polybasic region to support prion infection. Recombinant PrPΔ23–31 was weakly interacted with PrP<sup>Sc</sup> compared to full-length PrP [70]. The polybasic region might thus be involved in the intermolecular interaction with PrP<sup>C</sup> and PrP<sup>Sc</sup> through the positively charged lysine residues, thereby supporting prion infection through driving the conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup>.

**Table 2.** PrP deletional and point mutants in prion infections.

PrPs	Susceptibility to Prions	References
PrPΔ23–31	Reduced to RML prions	[70]
PrP3K3A	Reduced to RML and 22L prions	[71]
PrPΔ32–80	Not reduced to RML prions	[72]
PrPΔ32–93	Reduced to RML prions	[73]
PrPΔ32–106	Resistant to RML prions	[73]
PrPΔOR	Not reduced to RML and 22L prions Reduced to BSE prions	[74]
PrPΔ91–106	Resistant to RML and 22L prions Reduced to BSE prions	[75]

### 5.2. The Central Residues of PrP<sup>C</sup> in Prion Infection

Tg(PrPΔ32–80)/*Prnp*<sup>0/0</sup> mice, which express mouse PrP lacking residues 32–80 including most of the OR region, developed disease without prolonged incubation times after infection with RML prions and accumulated PrP<sup>Sc</sup>Δ32–80 in their brains (Table 2) [69]. However, Tg(PrPΔ32–93)/*Prnp*<sup>0/0</sup> mice expressing PrP with the deletion of residues 32–93 encompassing the entire OR region exhibited longer incubation times and reduced PrP<sup>Sc</sup>Δ32–93 in their brains after infection with RML prions (Table 2) [72]. Furthermore, Tg(PrPΔ32–106)/*Prnp*<sup>0/0</sup> mice expressing a mutant PrP with a deletion extended to residue 106 neither developed disease nor accumulated PrP<sup>Sc</sup>Δ32–106 in their brains even after intracerebral inoculation with RML prions (Table 2) [73]. These results suggest that the deletion of residues 91–106 not the OR region (residues 51–90) renders PrPΔ32–106 unsusceptible to RML prions. Consistent with this, we demonstrated that, while Tg(PrPΔOR)/*Prnp*<sup>0/0</sup> mice expressing PrP lacking the OR region alone were susceptible to RML and 22L prions (Table 2) [74], Tg(PrPΔ91–106)/*Prnp*<sup>0/0</sup> mice expressing PrP lacking residues 91–106 were resistant to RML and 22L prions, neither developing disease nor accumulating PrP<sup>Sc</sup>Δ91–106 in their brains (Table 2) [75]. Using RML prion-infected N2a cells, ScN2a, it was shown that



residues 100–104 are important for PrP<sup>C</sup> to convert into PrP<sup>Sc</sup> in the cells [76]. Residues 100–104 form a loop between the  $\beta$ -sheets in the PrP<sup>Sc</sup> structural model [77–79]. It is thus possible that the loop region might play an important role in the conversion of PrP<sup>C</sup> to PrP<sup>Sc</sup> after infection with RML and 22L prions. Intriguingly, in contrast to the case for RML and 22L prions, Tg(PrP $\Delta$ 91–106)/Prnp<sup>0/0</sup> mice were susceptible to BSE prions and Tg(PrP $\Delta$ OR)/Prnp<sup>0/0</sup> mice had reduced their susceptibility to BSE prions (Table 2) [74,75]. These results indicate that, in contrast to RML and 22L prions, BSE prions might require the OR region partially but not residues 91–106 to induce the conversion of PrP<sup>C</sup> to PrP<sup>Sc</sup>, suggesting that the OR region and residues 91–106 of PrP<sup>C</sup> are involved in prion infection in a prion strain-dependent manner.

## 6. Perspectives

The etiologies of sporadic prion diseases including sCJD remain unknown. Our findings that IAV/WSN infection induced the conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup> and infectious prions in N2a cells has raised the possibility that IAV infection in neurons might be an etiological mechanism of sporadic prion diseases [24]. Elucidation of (1) the mechanism of how IAV/WSN infection could induce the conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup>, (2) whether or not IAV/WSN infection in neurons could induce prion disease in animals, and (3) whether or not IAV infection in humans could be epidemiologically linked to sCJD are crucial for further understanding of the causative role of IAV infection in sporadic prion diseases. Also, addressing (1) the mechanism of the conversion of the hereditary prion disease-associated mutated PrPs into PrP<sup>Sc</sup>, (2) the molecular nature of neurotoxic and infectious PrP<sup>Sc</sup> molecules, and (3) the mechanism of how the N-terminal polybasic region and the central residues 91–106 are involved in the conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup> is important not only for further understanding of the pathogenic mechanisms of prion diseases, but also for the development of effective treatment and prevention measures for prion diseases.

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