Scrapie Infection of Prion Protein-deficient Cell Line upon Ectopic Expression of Mutant Prion Proteins*

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Expression of the cellular prion protein (PrPC) is crucial for susceptibility to prions. In vivo, ectopic expression of PrPC restores susceptibility to prions and transgenic mice that express heterologous PrP on a PrP knock-out background have been used extensively to study the role of PrP alterations for prion transmission and species barriers. Here we report that prion protein knock-out cells can be rendered permissive to scrapie infection by the ectopic expression of PrP. The system was used to study the influence of sheep PrP-specific residues in mouse PrP on the infection process with mouse adapted scrapie. These studies reveal several critical residues previously not associated with species barriers and demonstrate that amino acid residue alterations at positions known to have an impact on the susceptibility of sheep to sheep scrapie also drastically influence PrPSc formation by mouse-adapted scrapie strain 22L. Furthermore, our data suggest that amino acid polymorphisms located on the outer surfaces of helix 2 and 3 drastically impact conversion efficiency. In conclusion, this system allows for the fast generation of mutant PrPSc that is entirely composed of transgenic PrP and is, thus, ideally suited for testing if artificial PrP molecules can affect prion replication. Transmission of infectivity generated in HpL3-4 cells expressing altered PrP molecules to mice could also help to unravel the potential influence of mutant PrPSc on host cell tropism and strain characteristics in vivo.

Prion diseases or transmissible spongiform encephalopathies (TSEs) are fatal neurological disorders such as Creutzfeldt-Jakob diseases in humans, scrapie in sheep and goats, and bovine spongiform encephalopathy (BSE). A key event in prion diseases is the conversion of the cellular, host-encoded prion protein (PrPc) to its abnormal isoform PrPSc predominantly in the central nervous system of the infected host (1). There is increasing evidence that the major and possibly only component of the infectious agent is PrPSc or a PrP folding intermediate (2). PrPc is a cell surface-anchored glycoprotein of a well characterized function (3). PrPSc is derived from PrPc in a posttranslational process that appears to involve PrPc-PrPSc molecular interactions (4). The crucial role of PrPc expression for prion infection and PrPSc formation has been demonstrated in transgenic mice with an ablated PrP gene (5). Since then transgenic animals have been used extensively to unravel the influence of specific PrP amino acid residues or domains on prion susceptibility (6–11). Although these hallmark experiments added important information to prion biology (12), generation of transgenic animals is laborious and expensive and, thus, not suited for testing broad sets of PrP mutants.

Alternative cell culture models for prion diseases have greatly helped us to understand the molecular mechanism of PrPSc formation (13, 14), the role of the PrP amino acid sequence for the TSE species barrier, and how PrP structural domains affect conversion of PrPc to PrPSc (15–20). However, so far comparative studies on the direct influence of PrP alterations on the prion infection process (as opposed to studies in persistently infected cell cultures) were mainly restricted to transgenic animal models (6, 7, 21–23). One reason for this is that all so-far-identified cell lines susceptible to prions code for endogenous wild-type PrP. Therefore endogenous PrP might be co-expressed with the exogenous PrP of interest during infection experiments (24–27). Thus, successful infection could still be supported by endogenous PrP expression rather than by transgenic PrP. Furthermore, it was unclear whether ectopic PrPSc by itself could initiate the conformational change of subsequent PrPc molecules in the absence of endogenous wild-type PrPSc molecules. Recent progress has been made by the finding that rabbit kidney epithelial cells RK13 expressing low or undetectable levels of endogenous rabbit PrP can be rendered permissive to sheep scrapie by ectopic expression of sheep PrP (26). Although this model elegantly showed that this cell line can be infected by scrapie originating from another species, its usefulness in testing broader sets of PrP mutants was somewhat limited in that PrP-positive clones had to be selected before infection studies could be performed (28). Furthermore,
because of the fact that these cells code for PrP, endogenous PrP expression cannot be absolutely ruled out.

Thus, the aim of this study was to establish a novel cell culture system based on PrP<sup>0/0</sup> cells that (a) allows for the generation of transgenic PrP<sup>Sc</sup> in the absence of any endogenous wild-type PrP and (b) enables us to easily test broad sets of PrP mutants for their influence on the prion infection process and PrP<sup>Sc</sup> formation. Here we have identified a permanent hippocampus-derived PrP<sup>0/0</sup> cell line termed Hpl3-4 (29) that becomes susceptible to mouse-adapted scrapie upon ectopic expression of murine PrP<sup>C</sup>. Stable expression of exogenous PrP may be initiated fast and efficiently in a high percentage of cells without selection of PrP<sup>PrPC</sup>-positive clones, and the infection of cells leads to release of prion infectivity into the cell culture supernatant. We have used our cell culture model to study the influence of sheep PrP amino acid residues in mouse PrP on 22L infection and identified several amino acid residue substitutions that drastically influenced PrP<sup>Sc</sup> formation. Interestingly, alterations of amino acid residues known to be absolutely critical for efficient transmission of sheep-derived scrapie to sheep also drastically influenced PrP<sup>Sc</sup> formation by mouse-adapted scrapie strain 22L, suggesting that these PrP<sup>C</sup> positions were critical for the adaptation of scrapie strain 22L from sheep to mice. Furthermore, we demonstrate that certain residues located on the outer surfaces of helix 2 and 3 in PrP<sup>C</sup> critically affect conversion to PrP<sup>Sc</sup>, whereas residues pointing inward do not. These results have mechanistic relevance, as they comply with the predicted structure of PrP<sup>Sc</sup> multimers and suggest that amino acids protruding from the second and third helix might critically affect inter- or intramolecular stacking of PrP<sup>Sc</sup> molecules. Thus, we provide novel insights in how PrP<sup>C</sup> and PrP<sup>Sc</sup> interact and in the surfaces of PrP<sup>Sc</sup> responsible for the formation of the PrP<sup>Sc</sup> aggregate.

**EXPERIMENTAL PROCEDURES**

**Construction of Retroviral Expression Vectors and Transduction of Hpl3-4 Cells with Retroviral Particles**—The coding regions of wild-type and mutated mouse PrP genes were ligated into the retroviral expression vector pSFF (30–32). Single point mutations were introduced into mouse PrP by PCR site-directed mutagenesis, and sequences were verified. The vector pSFF was transfected into a mixed culture of V2 and PA317 cells, and retroviral particles were harvested once cells were positive for PrP. Supernatants were cleared from cell debris (120 × g, 4 °C, 10 min). 3 × 10<sup>5</sup> Hpl3-4 cells were plated in 6-well plates. The next day cells were incubated with 4 μg/ml Polybrene for 2 h before exposure to 1 ml of retroviral particles overnight.

**Phosphatidylinositol-specific Phospholipase C (PI-PLC) Treatments and Flow Cytometry Analysis**—For PI-PLC treatments, cells were grown to confluence on 6-cm dishes. Cells were rinsed twice with PBS, and 1 ml of new medium without FCS was added. Cells were either incubated with 4 μl PI-PLC (Sigma-Aldrich) or without PI-PLC for 8 h. Medium was harvested and cleared twice by centrifugation. Proteins were CH<sub>3</sub>OH-precipitated. The whole sample was loaded. Cells were rinsed in PBS and lysed in lysis buffer according to standard procedures (see above). Cleared cell lysates were CH<sub>3</sub>OH-precipitated, and 1/10 of the sample was loaded. PrP was detected using mouse monoclonal antibody 3F4. For detection of PrP cell surface expression by flow cytometry analysis, cells were detached using PBS, 1 mM EDTA. Cells were fixed in PBS, 2.5% FCS, and 0.05% sodium azide and subsequently incubated with anti-PrP antibody 4H11 (1:100) (33) for 45 min on ice. Cells were rinsed in PBS, 2.5% FCS, and 0.05% sodium azide, and Cy2-conjugated anti-mouse antibody (Dianova) was added (1:100) for 45 min. Rinsed cells were subjected to flow cytometry analysis.

**Cell Infections**—The PrP<sup>0/0</sup> hippocampus-derived cell line Hpl3-4 was described previously (29). These cells express a marker of a neuronal-precursor cell lineage, neurofilament NF-68K (29). Scrapie strain 22L was kindly provided by Dr. Suzette A. Priola, Rocky Mountain Laboratories, National Institutes of Health, Hamilton, MT (27). The titer of the 22L strain was 2 × 10<sup>8.5</sup> ID<sub>50</sub>/g of infected brain. 10% brain homogenates were prepared in PBS, and cell infections were performed as previously described (27). Briefly, 5 × 10<sup>4</sup> Hpl3-4 cells were plated in 24-well dishes 1 day before infection. Medium was removed and replaced by 0.2 ml of 1% scrapie brain homogenate diluted into Dulbecco’s modified Eagle’s medium supplemented with 10% FCS. This equals a total dose of infectivity of ~1.26 × 10<sup>8</sup> ID<sub>50</sub> and an estimated multiplicity of infection of 25.2. Four hours later, 0.6 ml medium was added, and the cells incubated for an additional 68 h. Cells were then expanded into 6-cm (passage 1) and 10-cm dishes (passage 2). Passage 3 was tested for its PrP<sup>Sc</sup> content. Cells were subsequently passaged at a ratio of 1:10.

**Transfer of Prion Infectivity by Medium Transfer**—Cell culture supernatant from confluent Hpl3-4 Mo3F4 PrP cells persistently infected with the 22L scrapie strain (cell culture passage number post-scrapie infection: 37) was harvested and transferred through a 0.2-μm Millipore filter to clear the supernatant from any cell contaminants. During the course of the experiment, both 22L scrapie strain donor and acceptor cells were grown on 10-cm dishes supplemented with 10 ml of medium. Subconfluent acceptor cells were incubated with 10 ml of donor cell culture supernatant. The following day 5 ml of the medium was replaced by fresh medium, and the cells were grown for an additional 2 days until confluent. Acceptor cells were split 1:10 and exposed to donor cell culture medium the following days as described above. Treatment was performed for seven passages total. Subsequently, cells were passaged every 3–4 days at a ratio 1:10 in the presence of fresh medium.

**Detection of PrP<sup>Sc</sup> by Western Blot**—PrP<sup>Sc</sup> in cell cultures was detected as described previously (34). PrP was detected by the mouse monoclonal antibodies 3F4 (35) by use of enhanced chemiluminescence (ECL Plus, Amersham Biosciences). For determination of the amount of cells producing PrP<sup>Sc</sup>, cells were cloned as described previously (36).

**Bioassays**—For preparation of inocula, cells were exposed to 1.26 × 10<sup>6</sup> ID<sub>50</sub> of 22L and passaged 14 (Hpl3-4 Mo3F4 PrP) or 11 times (Hpl3-3), respectively. Prion-infected cells were passaged 12 of 14 times at a ratio 1:10 (see “Cell Infections” above). Cells of passage 14 or 11 were counted (1.9 × 10<sup>7</sup> Hpl3-4 Mo3F4 PrP infected, 1.5 × 10<sup>7</sup> Hpl3-3 Mo3F4 PrP uninfected per 10-cm dish). 1/3 of the infected cells equaling 3 × 10<sup>5</sup> cells
**PrP Knock-out Cells Rendered Permissive to Scrapie**

![Table](image)

**FIGURE 1. Efficient transduction of HpL3-4 cells with retroviral particles coding for 3F4 antibody epitope-tagged PrP.** A, HpL3-4 cells were transduced with retroviral particles coding for 3F4 antibody epitope-tagged PrP (Mo3F4 PrP) and passaged 10 times, and cell lysates were tested for PrPSc expression by Western blot using the antibody 3F4. As a control, lysate of HpL3-4 cells not transduced with retrovirus was loaded. B, single cell clones were isolated and 69 passages post-transduction and tested for the expression of 3F4 epitope-tagged PrPSc.

were injected per mouse. Thus, each mouse was theoretically injected with $2 \times 10^{-8}$ ID$_{50}$ of original inoculum. Cells were suspended in Dulbecco’s modified Eagle’s medium supplemented with 10% FCS. After subsequent freeze-thaw cycles, cell suspensions were passed through 20- and 24-gauge needles. Lysates and control brain homogenates were incubated at 70 °C for 10 min, and 30 µl of cell lysate was injected intracranially into 6–8-week-old tga20 mice. These mice overexpress wild-type mouse PrP$^{C}$ and have previously been shown to succumb to scrapie with substantially shortened incubation times compared with wild-type mice (22). Mice were regularly checked for the onset of neurological symptoms, and mice showing signs of disease were sacrificed.

**RESULTS**

**Efficient Transduction of Hippocampus-derived PrP-deficient Cell Line HpL3-4 with Retrovirus Coding for 3F4 Epitope-tagged PrP—**Several cell lines have been generated from PrP$^{0/0}$ mice; however, susceptibility to prion infection upon ectopic expression of prion protein has not been demonstrated. For fast, efficient, and stable expression of cellular prion protein in the PrP knock-out cells, a retroviral expression system was utilized (36). The hippocampal cell line HpL3-4 derived from PrP$^{0/0}$ mice (29) was transduced with retroviral particles coding for murine PrP tagged with the epitope for the monoclonal antibody 3F4 (Mo3F4 PrP). This epitope can be introduced into murine PrP by the substitution of two amino acid residues at positions L108M and V111M (37) and allows for the discrimination of de novo formed PrPSc from PrPSc present in the inoculum (36). Western blot analysis of cells harvested 10 passages after transduction revealed that transduced cells expressed high amounts of Mo3F4 PrP (Fig. 1A). To determine the percentage of cells that were transduced by the retroviral particles and expressed Mo3F4 PrP persistently, transduced cells were passaged 14 or 69 times, respectively, before single cell clones were isolated and tested for Mo3F4 PrP expression (Fig. 1B). Western blot analysis of cell lysates from transduced clones isolated after 14 or 69 passages demonstrated that 96.3 and 97.6% of the cells expressed epitope-tagged PrP$^{C}$, respectively. Thus, transduction of HpL3-4 cells with retrovirus yields a nearly homogenous population of cells stably expressing recombinant PrP over multiple cell culture passages.

**Mo3F4 PrP Expression Renders HpL3-4 Cells Susceptible to Mouse-adapted Scrapie—**Prion infection and disease pathology in vivo are strictly dependent on the expression of cellular prion protein (5). However, in vitro, PrP$^{C}$ expression is not the only prerequisite for prion infection (36), and susceptible cell lines can only be determined empirically (27). To test if hippocampal HpL3-4 cells can be rendered permissive to mouse-adapted scrapie by ectopic expression of epitope-tagged PrP, cells were exposed to scrapie strain 22L (Fig. 2), a strain that has been shown to be capable of infecting a variety of different cell lines (27, 38, 39). As a control, HpL3-4 cells not expressing PrP were also incubated with 22L scrapie brain homogenate (Fig. 2A, lane 2). Wild-type and transduced HpL3-4 cells not exposed to scrapie brain homogenate were concomitantly cultured to demonstrate that cells do not per se accumulate PrPSc (Fig. 2A, lanes 1 and 3). Western blot analysis demonstrated that a faint 3F4 antibody-positive PrPSc signal was already apparent after ~3 passages post-scrapie inoculation in HpL3-4 cells stably expressing 3F4 epitope-tagged PrP that were exposed to scrapie brain homogenate (data not shown). Ten passages post-inoculation, cells still tested positive for PrPSc.
TABLE 1  
Production of scrapie infectivity in HpL3-4 Mo3F4 PrP cells exposed to scrapie strain 22L 

| Scrape strain | Inoculum (brain homogenate or cell lysate) | Clinical scrapie/total | Incubation time, p.i. |
|---------------|------------------------------------------|-----------------------|----------------------|
| None          | HpL3-4 Mo34 PrP cells                     | 0/5*                  | >280                 |
| 22L           | Brain homogenate (1%)                     | 6/6                   | 80.3 ± 2.6           |
| 22L           | HpL3-4 Mo3F4 PrP cells                    | 6/6                   | 116 ± 4.5            |

* One mouse died of pneumonia 201 days post-infection and was excluded from the analysis.

(Fig. 2A, right panel). Thus, ectopic expression of murine PrP rendered HpL3-4 cells susceptible to the mouse-adapted scrapie strain 22L.

The fact that HpL3-4 Mo3F4 PrP cells generated proteinase K-resistant PrP over multiple passages strongly argues that the cells are chronically infected with mouse-adapted prion strain 22L. To confirm production of infectivity, lysates of cells harvested 14 passages post-infection were inoculated intracranially into tg20 mice. A late passage number was chosen to guarantee dilution of potential residual inoculum present in the cell culture. Passaging of cells resulted in a theoretical dilution of at least 6.3 × 10^13 of the original inoculum (see “Experimental Procedures”). Because a total of 1.26 × 10^17 ID50 were used for infection, inoculum should be completely diluted out. As controls, 1% brain homogenate of a terminally sick mouse infected with 22L scrapie as well as HpL3-4 Mo3F4 PrP cells exposed to brain homogenate from a healthy mouse were inoculated (passage 11 post-exposure) (Table 1). Mice inoculated with 22L scrapie brain homogenate or lysates of cells inoculated with 22L showed signs of scrapie disease ~80 days and 116 days post-infection, respectively, and were sacrificed. Thus, we have shown for the first time that brain-derived PrP<sup>0/0</sup> cells can be rendered susceptible to scrapie infection by ectopic expression of mouse PrP and produce infectivity over multiple passages. Furthermore, our data are consistent with the finding that ectopic expression of PrP in transgenic mice with an ablated PrP gene can be rendered susceptible to prion infection.

**Gradual Increase in PrP<sup>Sc</sup>-positive Cells upon Continuous Passage Suggests Spread of Infection in Cell Culture—**To estimate if continuous passage of cells leads to an increase in PrP<sup>Sc</sup>-producing cells, persistently infected cells were passaged 14 and 69 times and subsequently cloned for isolation of single cell clones. Testing of cell clones revealed that 15.5% of the clones expressing Mo3F4 PrP tested positive for PrP<sup>Sc</sup> production upon 14 passages (Fig. 2B). Interestingly, 60% of the 3F4-positive clones isolated after 69 passages post-infection accumulated PrP<sup>Sc</sup> (Fig. 2, B and C), suggesting that the scrapie strain 22L is capable of spreading in HpL3-4 cell cultures ectopically expressing PrP.

**Persistently Infected HpL3-4 Mo3F4 PrP Cells Release Infectivity into the Cell Culture Medium—**It has previously been shown that spread of scrapie infectivity can occur by release of infectivity into the cell culture supernatant (25, 26), possibly by exosomes (40). However, other studies report that spread of infectivity is dependent on cell to cell contact (41). Thus, it is possible that the mechanism of prion transmission between cells can vary depending on the prion strain or the cell line (42). To test if infectivity could spread to uninfected cells in a HpL3-4 Mo3F4 PrP cell culture via cell culture supernatant, uninfected HpL3-4 Mo3F4 PrP cells were exposed to cell culture medium from scrapie-infected HpL3-4 Mo3F4 PrP donor cells. Treatment was performed for seven passages followed by subsequent cell culture passages in fresh medium. In parallel, uninfected HpL3-4 Mo3F4 PrP cells were also subsequently passaged in the absence of donor cell culture medium. Interestingly, very faint PrP<sup>Sc</sup> signals were detectable by Western blot upon treatment of cells for three passages (data not shown). However, because this signal could also be due to epitope-tagged PrP<sup>Sc</sup> present in the donor cell culture medium, cells were tested again for epitope-tagged PrP<sup>Sc</sup> 7–11 passages after treatment with donor cell culture medium was terminated (total passage numbers 14–18) (Fig. 3). Strong PrP<sup>Sc</sup> signals could readily be detected in HpL3-4 Mo3F4 PrP cells seven passages post donor cell culture medium treatment (total passage number was 14). PrP<sup>Sc</sup> signals even increased in subsequent passages (Fig. 3, right panel), whereas total PrP remained constant (Fig. 3, left panel). Thus, these results strongly argue that scrapie infectivity was released into the cell culture medium.

**PrP Sequence Alterations Influence PrP<sup>Sc</sup> Formation in HpL3-4 Cells—**Transmission of prions from one species to another is usually associated with prolonged incubation times, a phenomenon termed species barrier (6). It is now widely accepted that the PrP amino acid sequence of donor and recipient is a major determinant of the species barrier. Scrapie strain 22L had originally been isolated from sheep and subsequently been passaged in mice (43), suggesting that it might have adapted to the murine PrP sequence. To test if sheep-specific amino acid residues in murine PrP can abrogate transmission of mouse-adapted scrapie strain 22L to HpL3-4 cells, 3F4 epitope-tagged mouse PrP constructs were created harboring single amino acid substitutions (Fig. 4A). We chose to focus on several amino acid differences in the central core of the PrP molecule, and mouse PrP mutants were generated introducing sheep PrP-specific amino acid residues at positions 96, 132, 150, 167, 183, 202, and 204 (corresponding to sheep PrP specific amino acid residues 100, 136, 154, 171, 187, 206, and 208, respectively) (Fig. 4A).
PrP Knock-out Cells Rendered Permissive to Scrapie

4A). The structural positions of the amino acid residue within mouse PrP are depicted in Fig. 4B.

Flow cytometry analysis revealed correct cell surface expression of mutant PrP. All constructs were expressed in at least 70% of the uncloned cell populations (Fig. 5A). PI-PLC treatments demonstrated glycosylphosphatidylinositol anchorage of Mo3F4 PrP mutants (Fig. 5, A and B). For all mutants, the amount of PrP(C) associated with the cells decreased upon treatment with PI-PLC, whereas the amount of PrP(C) in the medium fraction increased (Fig. 5B). Some PrP was also secreted into the medium (44). Expression of transgenes was further verified by Western blot, demonstrating expression of 3F4 epitope-tagged PrP in all populations except untransduced control cells (Fig. 6A, upper panel). Uncloned cell populations were exposed to 22L scrapie brain homogenate and tested for de novo-generated, 3F4-positive PrP(SC) (as defined by partial resistance to 20 μg/ml proteinase K). Upon exposure of HpL3-4 cells stably expressing Mo3F4 PrP N96S to 22L mouse scrapie, very faint 3F4-positive PrP(SC) signals were detected in some but not all infection experiments performed (compare Fig. 6, A and B, right panel). Faint PrP(SC) signals persisted over several passages tested (data not shown), indicating that such a substitution had a strong influence on PrP(SC) formation but did not completely prevent initial infection. Interestingly, expression of Mo3F4 PrPs harboring sheep-specific residues at positions 132, 150, 167, or 204 did not lead to PrP(SC) formation in our cell culture system (Fig. 6A), suggesting that these mutations did not support effective prion transmission. Infection experiments were repeated at least three times, but PrP(SC) formation was not detected for any of these mutants.

By contrast, expression of Mo3F4 PrP 1183V and Mo3F4 PrP V202I did not drastically impair PrP(SC) formation and, thus, allowed for 22L infection (Fig. 6A). The fact that mutant V202I was expressed in only 70% of the cells (Fig. 5A) but still led to high PrP(SC) accumulation (Fig. 6A) strongly argues that inefficient conversion of PrPs A132V, R150H, Q167R, and M204I was not due to fewer cells expressing the mutants compared with cells transduced with Mo3F4 PrP. Analysis of the structural position of PrP mutations in the second and third α-helices revealed that the substitution that critically affected conversion (M204I) was located on the surface of the third α-helix, whereas substituting residues 183 and 202 that face inwards did not. We, therefore, tested another PrP polymorphism that is present in mice located on the outside of the second α-helix for its effect on PrP(SC) formation by 22L (Fig. 4B). PrP polymorphism T189V has previously been associated with differences in disease incubation times depending on the prion strain. Mutant Mo3F4 PrP T189V was correctly located on the surface of the third α-helix, whereas substituting residues 183 and 202 that face inwards did not. We, therefore, tested another PrP polymorphism that is present in mice located on the outside of the second α-helix for its effect on PrP(SC) formation by 22L (Fig. 4B). PrP polymorphism T189V has previously been associated with differences in disease incubation times depending on the prion strain. Mutant Mo3F4 PrP T189V was correctly located on the surface of the third α-helix, whereas substituting residues 183 and 202 that face inwards did not.
suggest that PrP polymorphisms in helix 2 and 3 that are located on the surface of both helices may be involved in inter- and intraspecies transmissions of prions.

In conclusion, we have identified several amino acid residues in mouse PrP that critically affect PrPSc formation in HpL3-4 cells exposed to mouse-adapted scrapie strain 22L. Our data suggest that the adaptation of originally sheep-derived scrapie to mice is accompanied by a preference of the agent to a mouse-specific PrP sequence. Furthermore, our data reveal that amino acid polymorphisms on the outer surfaces of helices 2 and 3 of PrPC can drastically impact conversion efficiency.

**DISCUSSION**

In this study we have demonstrated for the first time that a PrP-deficient cell line can be rendered permissive to prion infection by ectopic expression of PrP. Regions outside of the open reading frame of PrP played little or no role in conferring susceptibility of HpL3-4 cells to prion infection. Multiple cell culture studies have demonstrated that PrPPrSc is not the only cellular factor needed for efficient infection, and relatively few cell culture lines have been shown to be susceptible to infection with scrapie agent (45). Thus, the finding that the PrP-deficient cell line HpL3-4 can be rendered susceptible to mouse-adapted scrapie by ectopic PrP expression was surprising, and our data reveal that amino acid polymorphisms on the outer surfaces of helices 2 and 3 of PrP can drastically impact conversion efficiency.
populations stably and exclusively expressing ectopic PrP (Fig. 7). Such an approach could also be interesting for generating artificial PrPSc molecules with amino acid changes that might be associated with altered scrapie strain characteristics in mice (8, 46). Thus, this system may also help to better understand the role of PrP sequence alterations on the emergence of TSE strains with new disease phenotypes.

Transgenic animals and TSE cell culture models have been extensively used and demonstrated that PrP polymorphisms affect PrP conversion efficiency. Previous studies demonstrate that co-expression of both mutant and endogenous PrP in cell culture clearly influence PrPSc formation as not only the PrP of interest but also the endogenous PrP is potentially converted to its abnormal isoform (17, 19, 47). Thus, it is possible that wild-type PrPSc molecules may act as “helper” molecules that drive PrPSc formation of mutant PrP. Absolute evidence that there is no influence of endogenous PrP on prion transmission and PrPSc formation can only come from studies with prion-susceptible cells that exclusively express the PrP mutant of interest without the context of normal wild-type PrP, as described here.

We have used PrP-deficient Hpl3-4 cells to experimentally test the influence of species specific amino acid replacements on the conversion efficiency of mouse PrP and infection by mouse adapted prion strain 22L. The mechanism of PrPSc formation is still unresolved, yet it is evident that PrPSc and PrPSc differ in their three-dimensional structures. PrPSc molecules from various mammals share a common structure, with the amino-terminal region remaining flexible, a three α-helix bundle and a short antiparallel β-sheet (A and B). Recent electron crystallography of two-dimensional crystals and homology modeling suggest that the amino-termin al part is refolded into trimers of left-handed β-helix composed of 2 or 4 β-helical rungs in PrPSc (51–53). β-Helical rungs appear to assemble to stacks with β-helical rungs of different PrPSc molecules through polar backbone interactions (52), with stacked monomers being organized as trimers (52, 53). In PrPSc oligomers, α-helices 2 and 3 appear to be located toward the outside of the PrPSc and remain relatively unaltered. This model has, however, recently been challenged by Lu and colleagues (54).

In vitro studies indicate that the amino acid sequence can dictate the conformational spectrum that a given PrPSc can adopt upon conversion to its abnormal isoform (55). Transmission of prions may, however, only occur if this spectrum includes the donor PrPSc conformation. In light of this, substitutions of residues 96, 183, and 202 led to a murine PrPSc molecule that could adopt the conformation of 22L PrPSc, whereas replacement of residues 132, 150, 167, 189, and 204 led to a molecule incompatible with the 22L PrPSc conformation. In sheep, polymorphisms at the sites 136, 154, and 171 (mouse PrP residues 132, 150, and 167, respectively) critically affect susceptibility to sheep scrapie isolates. According to the modeling of Langedijk and colleagues (53), alanine at position 132 is one of the amino acid residues in the first rung of PrPSc important for folding with side chains pointing toward the center of the left-handed β-helix. Substitution of this residue by valine may lead to an alternative alignment of the rungs, resulting in different surface properties of the side of the β-helical trimer that might negatively affect packing of the remainder polypeptide (53). According to the three-dimensional model of PrPSc multimers, the bulky and charged residues from sequence 142 to 159 protrude from the β-helix in a loop structure that may come into contact with adjacent PrPSc molecules (52, 56). Thus, a change from aspartate to histidine at position 150 might critically affect initial binding of PrPSc to PrPSc and/or intermolecular interactions in PrPSc aggregates. Amino acid residue 167 is located in the loop region between the second β-strand and the second α-helix in PrPSc, an area for which a role of surface charge distribution for TSE susceptibility has been suggested (57). Substitution of amino acid residue 167 with arginine also interfered with PrPSc formation in neuroblastoma cells chronically infected with the RML scrapie strain (15), indicating that changes at this position might be generally critical to PrPSc formation driven by diverse scrapie strains.

Recent structural data argue that helices 2 and 3 are located toward the outside of the PrPSc oligomers (51). Helix 2 and 3 are linked by a disulfide bridge between Cys-178 and Cys-213, potentially stabilizing the constant, structural core that is present in both PrPSc and PrPSc (58). An interesting finding of this study was that the influence of amino acid changes in the second and third helix appeared to depend on their respective
locations on the outer or inner surfaces of the helices. Exchanges of amino acid residues in helix 2 and 3 either abrogated the conversion process (T189V, M204I) or left it relatively unaltered (I83V, V202I). Interestingly, side chains of residues 183 and 202 are buried in the interior of the globular domain of PrPSc formed by helix 2 and 3. Hydrophobic exchanges at these positions are unlikely to drastically perturb overall architecture of helix 2 and 3. By contrast, residues 189 and 204 are located on the outer surfaces of helix 2 and 3 (Fig. 4B), respectively, with their side chains being water-exposed. Thus, although potentially not involved in initial binding of the two PrP conformers (59), changes on the outer surfaces of helix 2 and 3 might sterically hinder stacking of α-helices 2 and 3 in PrPSc multimers.

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