Cells Expressing Anchorless Prion Protein Are Resistant to Scrapie Infection

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The hallmark of transmissible spongiform encephalopathies (TSEs or prion diseases) is the accumulation of an abnormally folded, partially protease-resistant form (PrP-res) of the normal protease-sensitive prion protein (PrP-sen). PrP-sen is attached to the cell membrane by a glycosylphosphatidylinositol (GPI) anchor. In vitro, the anchor and the local membrane environment are important for the conversion of PrP-sen to PrP-res. In vivo, however, the anchor is not necessary because transgenic mice expressing anchorless PrP-sen accumulate PrP-res and replicate infectivity. To clarify the role of the GPI anchor in TSE infection, cells expressing GPI-anchored PrP-sen, anchorless PrP-sen, or both forms of PrP-sen were exposed to the mouse scrapie strain 22L. Cells expressing anchored PrP-sen produced PrP-res after exposure to 22L. Surprisingly, while cells expressing anchorless PrP-sen made anchorless PrP-res in the first 96 h postinfection, no PrP-res was detected at later passes. In contrast, when cells expressing both forms of PrP-sen were exposed to 22L, both anchored and anchorless PrP-res were detected over multiple passes. Consistent with the in vitro data, scrapie-infected cells expressing anchored PrP-sen transmitted disease to mice whereas cells expressing anchorless PrP-sen alone did not. These results demonstrate that the GPI anchor on PrP-sen is important for the persistent infection of cells in vitro. Our data suggest that cells expressing anchorless PrP-sen are not directly infected with scrapie. Thus, PrP-res formation in transgenic mice expressing anchorless PrP-sen may be occurring extracellularly.

Prion diseases or transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative diseases with no effective treatment or cure. TSEs affect a wide range of species and include scrapie in sheep, bovine spongiform encephalopathy in cattle, chronic wasting disease in deer and elk, and kuru and Creutzfeldt-Jakob disease in humans. Despite the rarity of these diseases, the emergence of a variant form of Creutzfeldt-Jakob disease, linked to the consumption of cattle infected with scrapie, has brought TSEs to the forefront of human health concerns (5, 14, 29).

TSEs are thought to be caused by a protein-only particle called a prion, which is derived from the endogenous and widely expressed host prion protein (PrP). The normal cellular prion protein is a glycoprotein that is attached to the cell surface membrane by a glycosylphosphatidylinositol (GPI) anchor (2, 27). The hallmark of TSE disease is the conversion of this normal, protease sensitive PrP form (PrP-sen) to an abnormally folded, partially protease-resistant form (PrP-res). PrP-res usually correlates with infectivity, although whether the GPI anchor is important for the conversion of PrP-sen to PrP-res (28). Thus, the localization of PrP-sen to rafts via its GPI anchor appears to be important for PrP-res formation.

Unlike GPI-PrP-sen, GPI-PrP-sen is secreted from cells and is not present on the cell surface (16). In addition, flotation gradient experiments have shown that GPI-PrP-sen does not float with raft fractions, indicating its absence from raft environments (10). Thus, the intracellular location of GPI-PrP-sen, its association with membrane domains, and possibly even its structure differ from those of GPI-PrP-sen. Given the importance of PrP-sen membrane localization in PrP-res formation, GPI-PrP-sen would not be expected to form PrP-res very efficiently (28). However, previous studies have shown that GPI-PrP-sen is efficiently converted to GPI-PrP-sen in a cell-free conversion assay (16). In addition, a recent study with transgenic mice expressing GPI-PrP-sen demonstrated that after inoculation with mouse scrapie, these mice could accumulate PrP-res and infectivity although they showed only minimal clinical signs of disease and lived a normal life span (10). Further analysis demonstrated that the GPI-PrP-sen was deposited in amyloid plaques, in contrast to the diffuse PrP-res staining observed in mice expressing GPI-PrP-sen. This striking difference in disease course and pathology could only be attributed to the presence or absence of the GPI anchor on PrP-sen. These results indicate that while the GPI anchor can...
significantly affect TSE disease, it is not essential for PrP-res formation in vivo.

To better understand the role of the PrP-sen GPI anchor during TSE infection, we expressed either Gpi-1 PrP-sen or Gpi-2 PrP-sen in cells from a PrP null mouse and exposed them to 22L scrapie. Cells expressing Gpi-1 PrP-sen were infected, as demonstrated by the presence of newly converted PrP-res and their ability to cause disease in mice. In contrast to transgenic mice expressing Gpi-1 PrP-sen, cells expressing Gpi-2 PrP-sen were not able to support a persistent TSE infection. However, when wild-type Gpi-1 PrP-sen was coexpressed in the cells expressing Gpi-1 PrP-sen, the cells became persistently infected and both Gpi-1 and Gpi-2 PrP-res were produced. These results suggest that GPI-anchored PrP-sen is necessary to support a persistent TSE infection in cells and that in transgenic mice expressing Gpi-1 PrP-sen, PrP-res accumulation and/or replication of TSE infectivity may be occurring extracellularly.

MATERIALS AND METHODS

Cell lines and viral transductions. Anchored and anchorless mouse PrP containing the epitope to mouse monoclonal antibody 3F4 (Mo3F4 GPI-PrP-sen and Mo3F4 GPI-PrP-sen, respectively) have been previously described (22). All clones were expressed in the retroviral vector pSFF, and viral transductions were performed with supernatant harvested from transduced retroviral packaging cells as previously described (18, 20). Mouse fibroblast cells (2F3) expressing Mo3F4 GPI-PrP-sen were produced by viral transduction as previously described (21). Vially transduced cells were cloned to isolate a cell clone with high expression of Mo3F4 GPI-PrP-sen (2F3F). Fibroblast cells were maintained in RPMI medium supplemented with 10% fetal bovine serum (Invitrogen), 100 μg of penicillin (Invitrogen), and 100 μg of streptomycin (Invitrogen). PK knockout cells (CF10) were isolated as previously described (13). Knockout cells expressing Mo3F4 GPI-PrP-sen, Mo3F4 GPI-PrP-sen, or wild-type mouse PrP-sen were produced by viral transduction. The CF10 cells expressing Mo3F4 GPI-PrP-sen were cloned to find cells that expressed higher levels of GPI-PrP-sen. The CF10 cells and derivative cell lines were grown in OptiMem (Invitrogen) supplemented with 10% fetal bovine serum, 100 μg of penicillin, and 100 μg of streptomycin.

Infection of cells with 22L scrapie. When passaged into wild-type mice, 22L from a mouse expressing GPI-PrP-sen (10) is indistinguishable from wild-type 22L (Brent Race, personal communication). Based upon the detection of PrP-res at early passages, it also infects cells more efficiently in vitro than 22L scrapie (Brent Race, personal communication). Based upon the detection of PrP-res at early passages, it also infects cells more efficiently in vitro than 22L scrapie (Brent Race, personal communication). Based upon the detection of PrP-res at early passages, it also infects cells more efficiently in vitro than 22L scrapie (Brent Race, personal communication). Based upon the detection of PrP-res at early passages, it also infects cells more efficiently in vitro than 22L scrapie (Brent Race, personal communication).

Expression of PrP-sen in cells which

RESULTS

Expression of Mo3F4 GPI-PrP-sen in CF10 cells. To study the role of the PrP-sen GPI anchor in scrapie infection, we expressed Mo3F4 GPI-1 PrP-sen or Mo3F4 GPI-2 PrP-sen in cells which contained no endogenous PrP-sen (CF10 cells). Western blot analysis was performed on cell lysates and supernatants harvested from each cell type to confirm the expression of the different forms of Mo3F4 PrP-sen. As expected, the CF10 cells alone showed no evidence of PrP-sen expression in either the

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Mo3F4 GPI
lysate (Fig. 2A and data not shown). Both the cells expressing
cells showed no detectable 3F4-positive PrP-res in the cell
Western blotting with the 3F4 antibody. The parental CF10
were harvested and analyzed for newly converted PrP-res by
from a mouse expressing GPI
cells were exposed to a 22L scrapie-infected brain homogenate
three glycoforms of Mo3F4 GPI
/H11001
panel are molecular sizes in kilodaltons.
consistent with lack of the GPI anchor. The values on the left of each
expression and acute PrP-res formation in CF10
cells. (A) Western blot analysis of PrP knockout (CF10) cells expressing
either Mo3F4 GPI\(^{-}\) PrP-sen or Mo3F4 GPI\(^{+}\) PrP-sen. CF10
knockout cells do not express PrP-sen (lanes 1 and 2). CF10 cells
expressing Mo3F4 GPI\(^{-}\) PrP-sen express it in the cell lysate (L, lane 3)
and secrete it into the cell supernatant (S, lane 4). Cells expressing
Mo3F4 GPI\(^{+}\) PrP-sen express it in the cell lysate (L, lane 5), with very little
GPI\(^{-}\) PrP-sen being detected in the supernatant (S, lane 6). The three
glycoforms of Mo3F4 GPI\(^{-}\) PrP-sen are represented by the
letters u (unglycosylated), m (monoglycosylated), and d (diglycosyl-
lated). (B) Western blot analysis of PrP-res from cells exposed to
scrapie for 96 h. Cells expressing Mo3F4 GPI\(^{-}\) PrP-sen or Mo3F4
GPI\(^{+}\) PrP-sen were positive for newly made PrP-res (lanes 1 and 2,
respectively). The Western blots were probed with mouse monoclonal
antibody 3F4. The lower molecular weight of Mo3F4 GPI\(^{-}\) PrP
is consistent with lack of the GPI anchor. The values on the left of each
panel are molecular sizes in kilodaltons.

Acute formation of PrP-res in CF10 cells expressing Mo3F4
PrP-sen. To assess whether CF10 cells expressing Mo3F4
GPI\(^{-}\) or Mo3F4 GPI\(^{+}\) PrP-sen could produce PrP-res, the
cells were exposed to a 22L scrapie-infected brain homogenate
from a mouse expressing GPI\(^{-}\) PrP-sen. After 96 h, cell lysates
were harvested and analyzed for newly converted PrP-res by
Western blotting with the 3F4 antibody. The parental CF10
cells showed no detectable 3F4-positive PrP-res in the cell
lysate (Fig. 1A, lane 1) or the supernatant (Fig. 1A, lane 2).
Lysates from cells expressing Mo3F4 GPI\(^{-}\) PrP-sen showed the
three major glycosylated forms of GPI\(^{-}\) PrP-sen (Fig. 1A, lane 5), with very little detected in the supernatant (Fig. 1A,
lane 6). Cells expressing Mo3F4 GPI\(^{-}\) PrP-sen showed mostly
unglycosylated PrP-sen in the cell lysate (Fig. 1A, lane 3) and
both un- and monoglycosylated forms in the supernatant (Fig.
1A, lane 4). There was no detectable decrease in the expres-
sion levels of either Mo3F4 GPI\(^{-}\) PrP-sen or Mo3F4 GPI\(^{+}\)
PrP-sen after more than 50 passages, indicating that the
proteins were stably expressed (data not shown).

Detection of scrapie infectivity in passaged cells. The reduc-
tion in Mo3F4 GPI\(^{-}\) PrP-res in the passaged cells compared
with the acutely exposed cells suggested that the GPI anchor
was necessary for persistent TSE infection in vitro. However,
previous work has shown that PrP-res is not always detectable
in scrapie-infected cells by Western blotting (24). To test
whether these cells were infected despite the lack of detectable
GPI\(^{-}\) PrP-res, we inoculated the cells into Tga20 mice, which
overexpress mouse PrP-sen and are highly susceptible to

Mo3F4 GPI\(^{-}\) and Mo3F4 GPI\(^{+}\) PrP-sen were converted to
PrP-res acutely within the first 96 h of exposure to 22L scrapie.

Persistent scrapie infection in CF10 cells expressing Mo3F4
PrP-sen. To determine if CF10 cells expressing GPI\(^{-}\) or GPI
Mo3F4 GPI\(^{-}\) PrP-sen were able to support a persistent TSE infec-
tion, cells that were acutely exposed to 22L scrapie for 96 h
were subsequently passaged. At each passage, cells were lysed
and lysates were analyzed by Western blotting for the presence
of newly formed PrP-res. As expected, the CF10 cells were
negative for PrP-res at all passes (Fig. 2A). The cells expressing
Mo3F4 GPI\(^{-}\) PrP-sen produced PrP-res at all passes, suggest-
ing that these cells were able to support a persistent TSE
infection (Fig. 2B). Despite the early formation of anchorless
PrP-res (Fig. 1B), once the CF10 cells expressing Mo3F4 GPI
PrP-sen were passaged, no PrP-res was detected, suggesting
that these cells were unable to sustain PrP-res formation over
multiple passages (Fig. 2C). The inability of the cells expressing
Mo3F4 GPI\(^{-}\) PrP-sen to support a persistent TSE infec-
tion, in contrast to cells expressing Mo3F4 GPI\(^{+}\) PrP-sen,
emphasizes the importance of the GPI anchor for long-term
PrP-res accumulation in vitro.
mouse scrapie (12). To ensure that the original inoculum was no longer present in the cell culture, all of the cell lines inoculated into the mice were passaged more than 12 times.

None of the uninfected cells caused disease in Tga20 mice (Table 1). As a positive control, brain homogenate from a mouse expressing GPI− PrP-sen infected with 22L was inoculated into Tga20 mice. These mice died of scrapie, with an average incubation time of 87 ± 8 days (Table 1, line 1). Mice inoculated with the parental CF10 cells exposed to the same 22L homogenate have not shown clinical signs of scrapie infection (Table 1, line 2). In contrast, mice inoculated with 22L-exposed CF10 cells expressing Mo3F4 GPI− PrP-sen died of scrapie with an average incubation time of 148 ± 47 days (Table 1, line 4). Supernatant from these cells has not transmitted disease to mice (>278 days, Table 1, line 7), suggesting that any infectivity in the supernatant is present at much lower levels than in the cells. Mice inoculated with the 22L-exposed cells expressing Mo3F4 GPI− PrP-sen have not shown clinical signs of scrapie infection for greater than 574 days (Table 1, line 5). These two glycoforms (u, unglycosylated; m, monoglycosylated; d, diglycosylated) of PrP-sen in the cell lysate (lane 3) but not in the cell supernatant (lane 5). These two glycoforms are also observed in the cell supernatant (lane 6). Cells expressing Mo3F4 GPI− PrP-sen show the characteristic glycoforms (u, unglycosylated; m, monoglycosylated; d, diglycosylated) of PrP-sen in the cell lysate (lane 3) but not in the cell supernatant (lane 4). Cells expressing Mo3F4 GPI− PrP-sen show unglycosylated and monoglycosylated forms of Mo3F4 GPI− PrP-sen in the cell lysate (lane 5). These two glycoforms are also observed in the cell supernatant (lane 6). The lower-molecular-weight products in the supernatant (lane 5). These two glycoforms are also observed in the cell supernatant (lane 6). The lower-molecular-weight products in the supernatant (lane 5). These two glycoforms are also observed in the cell supernatant (lane 6).

Coexpression of wild-type mouse PrP-sen in the cells expressing Mo3F4 GPI− PrP-sen. The striking difference between the cells expressing Mo3F4 GPI− or Mo3F4 GPI+ PrP-sen in their ability to support a persistent scrapie infection suggested that while the GPI anchor is not required for initial PrP-res formation in cells, it is critically important for the establishment of a long-term infection. To test whether expression of an anchored form of PrP-sen would alter the susceptibility of the cells expressing GPI− PrP-sen to scrapie infection, we expressed wild-type mouse PrP-sen (MoPrP GPI+) in the cells expressing Mo3F4 GPI− PrP-sen. If the anchor is necessary, the presence of a GPI-anchored form of PrP-sen should allow the formation of PrP-res and establishment of a persistent infection.

Cells expressing Mo3F4 GPI− PrP-sen were stably transduced with wild-type mouse PrP-sen. To determine if the cells were expressing both types of mouse PrP-sen, they were labeled with [35S]methionine/cysteine. PrP-sen was immunoprecipitated from the cell lysate and supernatant with the R30 antibody and analyzed by size migration of the 35S-labeled proteins. No PrP-sen was immunoprecipitated from the CF10 cell lysate or supernatant (Fig. 3, lanes 1 and 2, respectively). Immunoprecipitated PrP-sen from cells expressing Mo3F4

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<th>Strain</th>
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<th>No. Sc+/total</th>
<th>Mean no. of days ± SD</th>
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<td>Cells</td>
<td>0/8</td>
<td>&gt;460</td>
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a NA, not applicable.
b Sup, cell supernatant.
c Sc+ (scrapie positive), PrP-res positivity in brain and clinical disease.
d Two mice died at 41 and 71 days postinfection and were negative for PrP-res in their brains.

![FIG. 3. Coexpression of MoPrP GPI− PrP-sen in cells expressing Mo3F4 GPI− PrP-sen. Cells were radiolabeled with [trans-35S]methionine/cysteine, and PrP-sen was immunoprecipitated from cell lysates or supernatants with the R30 antibody and analyzed by size migration of the 35S-labeled proteins. No PrP-sen was immunoprecipitated from the CF10 cell lysate or supernatant (Fig. 3, lanes 1 and 2, respectively). Immunoprecipitated PrP-sen from cells expressing Mo3F4 GPI− PrP-sen were not persistently infected following exposure to 22L scrapie.](http://jvi.asm.org/Downloaded%20from)}
GPI\(^+\) PrP-sen showed the characteristic glycosylation pattern expected for wild-type PrP-sen in the cell lysate (Fig. 3, lane 3), with little or no GPI\(^+\) PrP-sen detected in the cell supernatant (Fig. 3, lane 4). Cells expressing only Mo3F4 GPI\(^+\) PrP-sen showed unglycosylated GPI\(^−\) PrP-sen, as well as a less abundant band representing monoglycosylated GPI\(^+\) PrP-sen, in the cell lysate (Fig. 3, lane 5). Both of these forms of PrP-sen could also be detected in the cell supernatant, indicating that they were secreted (Fig. 3, lane 6). The lower-molecular-weight species in the supernatant are likely GPI\(^+\) PrP-sen degradation products. The cells expressing Mo3F4 GPI\(^+\) PrP-sen that were transduced with wild-type PrP-sen express both Mo3F4 GPI\(^−\) PrP-sen and MoPrP GPI\(^+\) PrP-sen in the cell lysate (Fig. 3, lane 7). However, only Mo3F4 GPI\(^+\) PrP-sen was detected in the cell lysate but not in the supernatant (Fig. 3, lane 8). Thus, these cells express both the Mo3F4 GPI\(^+\) and MoPrP GPI\(^+\) PrP-sen proteins.

**Persistent scrapie infection of CF10 cells coexpressing the Mo3F4 GPI\(^+\) and MoPrP GPI\(^+\) PrP-sen proteins.** To test if the CF10 cells expressing both the Mo3F4 GPI\(^+\) and MoPrP GPI\(^+\) forms of mouse PrP-sen were susceptible to persistent scrapie infection, we exposed them to 22L scrapie and analyzed them for the ability to produce PrP-res over multiple passes. In contrast to the cells expressing Mo3F4 GPI\(^−\) PrP-sen alone (Fig. 2C), the cells expressing both forms of PrP-sen became stably infected over time, as indicated by the presence of PrP-res at all passes (Fig. 4A). Moreover, Western blot analysis with the 3F4 antibody clearly demonstrated the presence of Mo3F4 GPI\(^−\) PrP-res at late passages, which was not seen in the cells expressing only Mo3F4 GPI\(^−\) PrP-sen (compare Fig. 4B to Fig. 2C). These results indicate that coexpression of GPI\(^−\)-anchored wild-type PrP-sen enabled the cells expressing anchorless PrP-sen to persistently produce both anchored and anchorless PrP-res and strongly suggest that the cells are persistently infected with 22L scrapie.

To determine if endogenous levels of wild-type GPI\(^+\) PrP-sen would also enable cells expressing anchorless PrP-sen to be infected with scrapie, V2 fibroblast cells, which express endogenous mouse PrP-sen, were virally transduced to express Mo3F4 GPI\(^+\) PrP-sen. Following exposure to 22L scrapie, Mo3F4 GPI\(^+\) PrP-res was detected in the cell lysate (Fig. 5, lane 1) but not in the cell supernatant (Fig. 5, lane 2). Analysis with the R30 antibody showed both GPI\(^−\) and GPI\(^+\) PrP-res in the cell lysate (Fig. 5, lane 3) but not in the supernatant (Fig. 5, lane 4). Inoculation of the fibroblast cells into Tga20 mice caused disease in 110 ± 9 days, confirming that the cells were persistently infected with scrapie (Table 1, line 5). Taken together, these results demonstrate that expression of an anchored form of PrP-sen allows the persistent formation of anchorless PrP-res, even when the anchored form of PrP-sen is expressed at endogenous levels.

**DISCUSSION**

We have shown that cells expressing only GPI\(^−\) PrP-sen are unable to support a persistent TSE infection in vitro. The lack of long-term infection in these cells is not a result of GPI\(^−\) PrP-sen being a nonpermissive template for conversion because (i) it could be converted to GPI\(^+\) PrP-res in the first 96 h after infection and (ii) coexpression of an anchored form of PrP-sen allowed the persistent formation of GPI\(^−\) PrP-res.

Within the first 96 h of scrapie infection, GPI\(^+\) PrP-sen was converted to GPI\(^−\) PrP-res, although detection of acute GPI\(^−\) PrP-res was variable between experiments. It is likely that the level of GPI\(^−\) PrP-res produced within the first 96 h of infection was near the limits of detection for Western blot analysis leading to the observed variability. Interestingly, GPI\(^−\) PrP-res formation decreased to undetectable levels at later passes (Fig. 2C). Therefore, even though GPI\(^−\) PrP-res can be made by cells immediately following infection, GPI\(^−\) PrP-res formation may be inefficient, allowing cell division to outpace PrP-res accumulation and impeding the accumulation of GPI\(^−\) PrP-res over multiple cell passages. However, cell-free studies of PrP-res formation have demonstrated that anchorless forms of PrP-sen are actually converted more efficiently than anchored forms of PrP-sen (16), most likely because anchorless PrP-sen
is less glycosylated (23). Therefore, we consider it unlikely that the replication rate of the cells outpaces the accumulation rate of anchorless PrP-res.

The low glycosylation of GPI− PrP-sen suggests altered trafficking through the secretory pathway. Thus, it is possible that GPI− PrP-sen may not be in an appropriate cellular compartment for efficient conversion to occur. For example, GPI− PrP-sen may not be in the raft environment believed to be important for the conversion of PrP-res to PrP-res. One possible implication of this is that the cells are unable to spread infectivity to neighboring cells. Many GPI-anchored proteins have been shown to transfer spontaneously from one cell membrane to another, a process termed cell surface painting (19). In fact, PrP-sen has been shown to transfer from one cell type to another in vitro and this transfer required the GPI anchor (17). Whether this occurs with PrP-res is unknown, but if it does, cell surface painting could be one mechanism for the spread of PrP-res to uninfected cells. The lack of a GPI anchor on PrP-res would prevent such a process from occurring and could explain why the cells expressing GPI− PrP-sen were unable to sustain a scrapie infection over time.

Another possibility is that secretion of GPI− PrP-sen from the cells (Fig. 1A) leads to depletion of the substrate required for the template-dependent conversion of PrP-res to PrP-res. Alternatively, GPI− PrP-sen could be secreted from the cells, thereby preventing a persistent infection from occurring. However, we were unable to detect GPI− PrP-res in the supernatant of the scrapie-exposed cells (data not shown). While we cannot rule out the possibility that it is being secreted at levels undetectable by Western blot analysis, our data demonstrating that the supernatant from these cells has not transmitted disease to mice for over 490 days is strong evidence that GPI− PrP-res is not being secreted into the supernatant (Table 1).

The importance of the PrP-sen GPI anchor for the persistent infection of cells is demonstrated by our results showing that coexpression of GPI− PrP-sen in cells expressing GPI− PrP-sen “rescues” the susceptibility of the cells to TSE infection. These results suggest that GPI− and GPI− PrP are interacting at some point during the process of PrP-res formation. This interpretation is supported by a recent study by Schiff et al. which found that coexpression of anchored PrP-sen and a mutant PrP-sen changed the subcellular localization of the anchored form of PrP-sen (26). While it is possible that GPI− and GPI− PrP-sen proteins are interacting in our cells, we have been unable to confirm a direct interaction between anchored and anchorless PrP-sen by coimmunoprecipitation (data not shown). The most likely possibility is that GPI− PrP-res is directly interacting with GPI− PrP-sen, leading to the persistent formation of GPI− PrP-res. Studies with transgenic mice support the hypothesis that complex interactions between different PrP-sen and PrP-res proteins can occur in vivo since coexpression of GPI− and GPI− PrP molecules significantly affected the incubation time and pathology of scrapie infection compared to scrapie-infected mice expressing only GPI− PrP-sen (10).

Interestingly, our in vitro results do not fully correlate with previously published in vivo data obtained with transgenic mice expressing GPI− PrP-sen (10). While these mice had minimal clinical symptoms after exposure to scrapie, they did accumulate GPI− PrP-res and infectivity in their brains, indicating their susceptibility to TSE infection. Thus, in contrast to the cells expressing GPI− PrP-sen, which were unable to support a TSE infection, mice expressing GPI− PrP-sen are fully capable of becoming infected with scrapie.

We speculate that the formation and accumulation of GPI− PrP-res in vivo may require components of the extracellular space that are not present in our tissue culture model. The fact that GPI− PrP-res is localized primarily in amyloid plaques adjacent to blood vessels in vivo is consistent with GPI− PrP-res formation occurring in the extracellular space. We suggest that in the transgenic mice, GPI− PrP-sen is secreted into the extracellular space, where it comes into contact with residual PrP-res from the inoculum. Once a critical concentration of GPI− PrP-sen is present, conversion occurs and GPI− PrP-res accumulates over time, leading to the long incubation periods characteristic of these transgenic mice. In vitro, the extracellular environment required to support the interaction and accumulation of anchorless PrP molecules is not present. Therefore, once secreted, GPI− PrP-sen becomes diluted in the medium and GPI− PrP-res formation does not persist. Thus, our in vitro data are consistent with the idea that in transgenic mice expressing GPI− PrP-sen, PrP-res formation and accumulation of PrP amyloid are occurring primarily extracellularly.

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