

# Formation of Protease-Resistant Prion Protein in Cell-Free Systems

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## Abstract

**In transmissible spongiform encephalopathies (TSE) or prion diseases, the endogenous protease-sensitive prion protein (PrP<sup>sen</sup>) of the host is converted to an abnormal pathogenic form that has a characteristic partial protease resistance (PrP<sup>res</sup>). Studies with cell-free reactions indicate that the PrP<sup>res</sup> itself can directly induce this conversion of PrP<sup>sen</sup>. This PrP<sup>res</sup> induced conversion reaction is highly specific in ways that might account at the molecular level for TSE species barriers, polymorphism barriers, and strains. Not only has this reaction been observed using mostly purified PrP<sup>sen</sup> and PrP<sup>res</sup> reactants, but also in TSE-infected brain slices. The conversion mechanism appears to involve both the binding of PrP<sup>sen</sup> to polymeric PrP<sup>res</sup> and a conformational change that results in incorporation into the PrP<sup>res</sup> polymer.**

## Introduction

The accumulation of abnormally protease-resistant prion protein (PrP<sup>res</sup>) is common to transmissible spongiform encephalopathies (TSE). The degree of resistance of PrP<sup>res</sup> can vary depending upon the TSE strain and host species (1,2, 75), but the TSE-associated forms of PrP<sup>res</sup> are considerably more resistant to proteinase K than is the corresponding normal PrP isoform (PrP<sup>sen</sup> or PrP<sup>c</sup>). Besides having enhanced protease-resistance, the various abnormal TSE-associated forms of PrP<sup>res</sup> (eg. PrP<sup>Sc</sup>, PrP<sup>CJD</sup> and PrP<sup>BSE</sup>) form insoluble aggregates and have a higher beta sheet content than PrP<sup>sen</sup> (3-6, 75).

Many types of evidence implicate PrP<sup>res</sup> formation as a central process in TSE pathogenesis and TSE agent replication (7-10). In the case of rare familial TSE diseases, it appears that PrP mutations can lead to aberrant behavior of PrP<sup>sen</sup> and its spontaneous conversion to more protease-resistant forms (see other chapters for review). However, spontaneous conversion occurs rarely, if at all, in hosts with wild type PrP<sup>sen</sup> as evidenced by the 1 per million annual incidence of sporadic CJD in humans. Much more common in mammals is the induction of neurotoxic PrP<sup>res</sup> formation from wild type PrP<sup>sen</sup> upon infection of hosts with TSE agents.

Studies with tissue culture cells and animals have revealed a considerable amount of information about the cell biology of PrP<sup>res</sup> formation and its association with TSE pathogenesis; these subjects are reviewed elsewhere (75). Studies in cell-free systems have provided the opportunity to directly investigate PrP<sup>res</sup> formation under

much simpler and more defined conditions. Such *in vitro* studies have provided new insight into the mechanism of PrP<sup>res</sup> formation and the molecular bases of TSE agent replication, strain propagation and species barrier effects. Most notably, these studies have shown that, as has long been predicted (11-15), PrP<sup>res</sup> can itself induce the conversion of PrP<sup>sen</sup> to PrP<sup>res</sup> directly by a mechanism that is so specific that it could conceivably account for most of the biological manifestations of TSE agents *in vivo*. These cell-free studies are reviewed in this chapter.

## Self-Seeded PrP<sup>res</sup> Formation

The central proposal of most protein-only models for the infectious agent of the TSEs is that the putative infectious protein, i.e. PrP<sup>res</sup>, directly interacts with its normal, host encoded homolog, PrP<sup>sen</sup>, to convert it to PrP<sup>res</sup>. In this way, it could propagate itself in the host without mediation by an agent specific nucleic acid (11-15). This fundamental ability of PrP<sup>res</sup> to induce the conversion of PrP<sup>sen</sup> to PrP<sup>res</sup> (converting activity) was first demonstrated by mixing PrP<sup>res</sup> purified from scrapie-infected brain tissue with immunoprecipitated 35S-PrP<sup>sen</sup> and observing that 35S-PrP<sup>sen</sup> was then transformed into 35S-PrP<sup>res</sup> (16). This conversion was not observed in the absence of PrP<sup>res</sup> or in the presence of another type of amyloid (Alzheimer's beta). Furthermore, other labelled proteins were not converted to protease K (PK)- resistant forms in the reaction with PrP<sup>res</sup>. Thus, the conversion reaction is PrP-specific and PrP<sup>res</sup> dependent. The converting activity depends upon the unique conformational structure of PrP<sup>res</sup> because, although partial, **reversible** unfolding of PrP<sup>res</sup> stimulates the conversion efficiency, more complete **irreversible** denaturation eliminates the converting activity (16,17). Further analyses of the effect of denaturants on the converting activity of PrP<sup>res</sup> have indicated that maintenance of the native folding of a C-terminal domain (~16 kDa in the aglycosyl structure) is important to allow refolding and recovery of converting activity upon dilution of the denaturant (17). The denaturation of this critical C-terminal domain coincided with large reductions in both converting activity and scrapie infectivity (18).

## Binding vs Conversion

The PrP<sup>res</sup>-induced conversion of PrP<sup>sen</sup> to the PK-resistant form has now been observed in several laboratories (19-22). However, in one of the resulting papers, the authors preferred to describe it as merely a binding phenomenon rather than conversion (19). Indeed there is binding of the PrP<sup>sen</sup> precursor to the PrP<sup>res</sup> aggregate in the conversion process which we believe is an integral part of the conversion mechanism (see below). However, not all binding or aggregation of PrP<sup>sen</sup> results in conversion to this PK-resistant state that is characteristic of PrP<sup>res</sup> (22-25,76,77). Although PK completely digests PrP<sup>sen</sup>, it characteristically removes only ~67 residues

from the N-terminus of each monomeric unit of the PrP-res aggregate, resulting in 6-7 kDa downward shift in their apparent molecular weight in SDS-PAGE gels. Since nearly all of the PrP-res molecules are equivalently exposed to PK and similarly truncated, this type of PK resistance is not due to the nonspecific sequestration of whole PrP molecules within aggregates that are not penetrated by PK. If the latter had been the case, virtually full-length PrP molecules would have remained after PK treatment as has been observed when 35S-PrP-sen is incubated with large molar excesses of a synthetic PrP peptide fragment (19). The similar partial exposure of the N-terminal residues of both PrP-res molecules and the 35S-PrP-res products of the conversion reaction provide evidence for the incorporation of the monomers units into highly ordered polymeric structures such as amyloid fibrils. Hence, PrP-sen not only binds to PrP-res, but also can convert from a PK-sensitive state to the partially PK-resistant state that is characteristic of TSE brain-derived PrP-res (76,77).

Riesner and colleagues observed that acetonitrile treatment of SDS-solubilized, alpha helical and PK-sensitive PrP27-30 results in aggregation of PrP and an enhancement of its total PK-resistance and beta sheet content without recovery of scrapie infectivity or fibrils (25). As was noted, this may be an example of an aggregated and PK-resistant form of PrP that is not PrP<sup>Sc</sup>. On the basis of this observation, they argue that a similar apparently nonspecific aggregation process could explain our PrP-res-induced conversion of 35S-PrP-sen to 35S-PrP-res. However, an important distinction must be made between their observation and ours. As described above, the 6-7 kDa size shift showed that the 35-PrP-res conversion products were exposed to PK but had the characteristic **partial** PK-resistance of brain-derived PrP-res (e.g., PrP<sup>Sc</sup>) (16,26). However, one cannot conclude the same about the acetonitrile-induced PK-resistant PrP27-30 because there was no control in the experiments reported to indicate actual exposure of the "PK-resistant" PrP molecules to PK during the digestion procedure. Thus, the acetonitrile-induced total PK-resistance could have been due to the nonspecific isolation of PrP from PK within poorly penetrable aggregates that may be quite distinct from native TSE-associated PrP-res and the products of our cell-free conversion reaction.

As mentioned briefly above, incubation of PrP-sen with 5000-fold molar excesses of certain synthetic peptide fragments of PrP (eg., PrP90-145) can induce the formation of PK-resistant complexes that polymerize into fibrils (19,27). These complexes are unstable and the associated PrP molecules do not display the same partial PK-resistance of brain-derived PrP-res or the 35S-PrP-res generated in the conversion reaction induced by brain-derived PrP-res. Nonetheless, these results provide another line of evidence that complex formation can lead to the formation of PK-resistant PrP polymers.

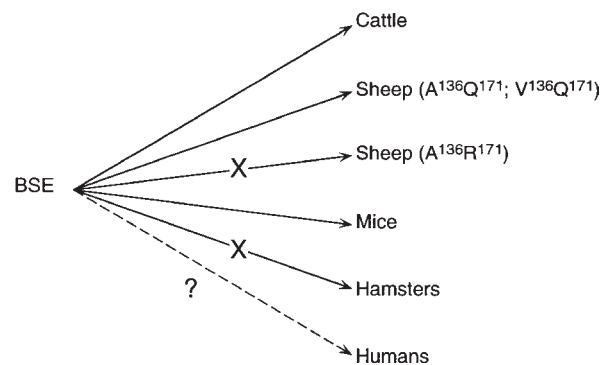
#### Sequence Specificity in the PrP Conversion Reaction: a Basis for TSE Species and Polymorphism Barriers?

A striking aspect of the PrP conversion reaction is its sequence specificity (20,21,28,77,78). Studies in transgenic mice and scrapie-infected tissue culture cells (29-31) suggested that interactions between homologous PrP-res and PrP-sen molecules were required for efficient

PrP-res formation and host susceptibility to TSE disease. *In vitro* studies with small synthetic PrP peptide fragments also highlighted the effects of PrP sequence mismatches in the polymerization process (32-34). More recently, the sequence specificity of interactions of the full-length PrP protein in the conversion process has been investigated directly with the cell-free system (20,21,28,77,78). These studies have provided insight into molecular basis for barriers to the transmission of TSEs between species (species barriers) and same-species hosts with different PrP genotypes (polymorphism barriers).

Combinations of PrP-res and PrP-sen from several different species (mice, hamsters, sheep, cattle and human)

#### A. *In vivo* transmission



#### B. *In vitro* conversion

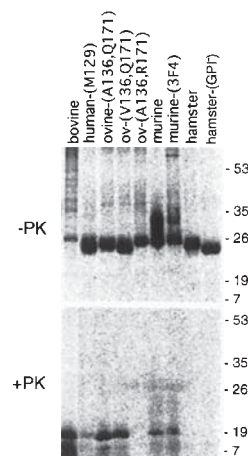


Figure 1. Correlation of *in vivo* transmissibilities of BSE infectivity with the *in vitro* conversions induced by PrP<sup>BSE</sup>. A; Known experimental or natural transmissibilities of BSE to various host species or genotypes. Although it now is apparent that BSE has transmitted to humans to give rise to new variant CJD (73,74), the efficiency and extent of this transmission remains unclear. B; PrP<sup>BSE</sup>-induced *in vitro* conversions of different radiolabelled PrP-sen molecules as described in text and (21). Top panel shows the phosphor autoradiographic analysis of [<sup>35</sup>S]PrP-sen molecules used in the conversion reaction without proteinase K treatment. The bottom panel shows the labelled PK-resistant [<sup>35</sup>S]-PrP products resulting from the incubation of the [<sup>35</sup>S]PrP-sen molecules with (unlabelled) PrP<sup>BSE</sup>. The major [<sup>35</sup>S]PrP-res products of interest are the expected 6-7 kDa lower in molecular weight than the [<sup>35</sup>S]PrP-sen precursor and comigrate approximately with the 19 kDa molecular mass marker shown on the right. Adapted from (21).

have been tested (20,21,28). To a remarkable extent, the efficiency of the cell-free conversion reactions correlated with *in vivo* transmissibilities of the corresponding agents within or between species. The homologous conversion reactions between PrP-res and PrP-sen molecules of the same sequence were most efficient, just as TSE agents are usually most efficiently transmitted to hosts of the same PrP genotype. In contrast, little or no conversion was observed with PrP-res-PrP-sen combinations associated with a lack of transmissibility *in vivo*. For example, PrP<sup>BSE</sup> readily converted bovine, murine, and two different ovine PrP-sen molecules to PrP-res and this correlates with the fact that BSE is transmissible to hosts expressing those types of PrP-sen (Fig.1). However, PrP<sup>BSE</sup> failed to convert the PrP-sen of two BSE-resistant hosts, Syrian hamsters and sheep of the A<sup>136</sup>R<sup>171</sup> PrP genotype. Sheep PrP-res with the V<sup>136</sup>Q<sup>171</sup> sequence efficiently converted the PrP-sen of V<sup>136</sup>Q<sup>171</sup> and A<sup>136</sup>Q<sup>171</sup> sheep, which are susceptible to scrapie agent from V<sup>136</sup>Q<sup>171</sup> sheep, but not the A<sup>136</sup>R<sup>171</sup> PrP-sen of a scrapie-resistant strain of sheep (20). Results from these and other PrP-res-PrP-sen conversions have provided strong support for the concept that the sequence specificity in the conversion of PrP-sen to protease-resistant forms modulates the interspecies or intraspecies transmissibilities of TSE agents *in vivo*.

Based upon the correlation between *in vitro* conversion efficiencies and known transmissibilities, we used the cell-free conversion system to gauge the potential transmissibility of BSE to humans (21). We found limited conversion of human PrP-sen to PrP-res driven by PrP<sup>BSE</sup> (Fig.2). Interestingly, the Met-129 human PrP-sen was more efficiently converted than the Val-129 PrP and this correlates with the fact that all of the human new variant CJD patients identified to date are homozygous for the Met-129 allele. The efficiencies of both of these heterologous bovine PrP<sup>BSE</sup> human PrP-sen conversions was much lower than those of relevant homologous conversions, yet similar to that of the conversion of human PrP-sen by sheep PrP-res. This *in vitro* analysis suggests

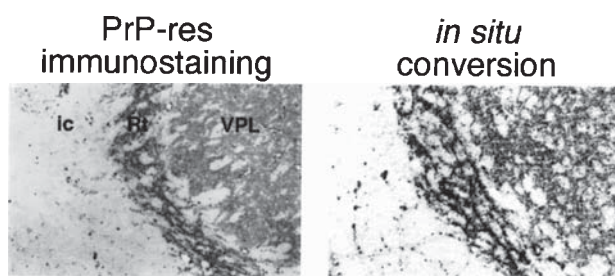


Figure 2. *In situ* conversion in TSE-infected brain slices. Brain slices from transmissible mink encephalopathy-infected hamsters either immunocytochemically stained for PrP-res or processed through the *in situ* conversion procedure(24). For the latter procedure, the slice was incubated with hamster [<sup>35</sup>S]PrP-sen, treated with PK to remove any labelled PrP that had not converted to a PK resistant form and then dipped in photographic emulsion to expose sites of [<sup>35</sup>S]PrP-res deposition. A close correspondence was observed between the patterns of staining pre-existing PrP-res and the *in situ* conversion product, providing evidence that the PrP-res in the slice causes the conversion of [<sup>35</sup>S]PrP-sen to [<sup>35</sup>S]PrP-res *in situ*. Regions shown: ic, internal capsule; Rt, reticular thalamic nucleus; VPL, ventral postlateral thalamic nucleus. Adapted from (24).

that the inherent ability of these infectious agents of BSE and scrapie to affect humans following equivalent exposure may be finite but similarly low.

### Self-Propagating PrP-res Conformations as a Possible Basis of TSE Strains

Strains of TSE agents can be distinguished on the basis of species tropism, incubation period, clinical disease, neuropathological manifestations and PrP-res distribution in brain tissue [for review (35)]. Numerous TSE strains have been documented even within isogenic hosts. This fact poses an interesting challenge for the protein-only hypothesis for the infectious agent: It requires that the "inheritance" or propagation of the agent strain differences must be mediated by stable variations in PrP-res structure rather than mutations in an agent-specific nucleic acid. Structural differences in PrP-res have been correlated with different strains of TSEs (2,36-38,79,80,81). Particularly notable are the different forms of PrP-res associated with the hyper (HY) and drowsy (DY) strains of hamster-adapted transmissible mink encephalopathy (TME). Although these PrP-res forms are both derived from Syrian hamster PrP, they are cleaved differently by PK. This suggests that they differ in conformation rather than covalent structure (2) and this has been confirmed by FTIR analysis (79). Furthermore, when incubated with hamster PrP-sen molecules, HY and DY PrP-res faithfully induce the formation of strain-specific PrP-res conversion products and thereby propagate themselves by a direct, nongenetic mechanism (26). These data provided the first direct evidence that strain-specific PrP-res polymers with the same amino acid sequence but different 3-D structures or conformations are capable of self-propagation. This is consistent with the possibility that the self-propagation of PrP-res polymers is a molecular basis for scrapie strains. Also consistent with this notion is a recent study showing that the passage of agent derived from different types of familial CJD into mice caused the accumulation of PrP-res with apparently distinct conformations (39).

### Correlating Scrapie Infectivity With Converting Activity, Protease-Resistance and Aggregation of PrP-res

Given that PrP-res might be the TSE agent which depends on the converting activity for its propagation in the host, we tested whether the effects of GdnHCl on the converting activity, PK-resistance and aggregation of PrP<sup>Sc</sup> might coincide with effects on scrapie infectivity (18). Large GdnHCl-induced reductions in infectivity were associated with the irreversible elimination of both the proteinase K-resistance and apparent self-propagating converting activity of PrP<sup>Sc</sup>. In intermediate GdnHCl concentrations that stimulate converting activity and partially disaggregate PrP<sup>Sc</sup>, both scrapie infectivity and converting activity were associated with residual partially protease-resistant multimers of PrP<sup>Sc</sup>. These results are consistent with scrapie infectivity being related to converting activity.

### *In situ* PrP Conversion in TSE-Infected Brain Tissue

After observing the PrP-sen to PrP-res conversion reaction in a cell-free system using largely purified and concentrated reactants, efforts were made to recapitulate the conversion under conditions more like those of intact brain tissue. This resulted in the development of the *in situ* conversion assay (24). Infected brain tissue slices mounted on slides were

incubated with radiolabelled PrP-sen and then treated with PK to digest any PrP-sen that was not converted to the PK-resistant form. Biochemical analysis of the tissue indicated that the expected labelled PrP-res conversion products were formed in, and bound to, infected, but not uninfected brain slices. Autoradiographic analysis of the pattern of newly formed PrP-res *in situ* conversion product in the infected brain slice closely matched the distribution of the pre-existing PrP-res. Punctate *in situ* PrP conversion was observed in brain regions containing PrP-res amyloid plaques and a more dispersed conversion product was detected in areas containing diffuse PrP-res deposits. These studies provided direct evidence that PrP-res formation involves the incorporation of soluble PrP-sen into both amyloid plaque and sub-plaque PrP-res deposits in TSE-infected brain tissue.

### **Mechanism of PrP-res Formation: Seeded Polymerization vs Heterodimer**

The first visualizations and biochemical characterizations of PrP-res polymers (scrapie associated fibrils or prion rods) were reminiscent of amyloid fibrils (40-42). Amyloids can be composed of a number of proteins, depending upon the disease (43). Amyloid deposits are composed of linear fibrils that result from the polymerization of a usually soluble precursor protein or peptide. Seeding solutions of the precursor with pre-existing amyloid fibril fragments can greatly accelerate the polymerization of amyloidogenic proteins (13,15). Amyloid polymerization often involves an increase in the beta sheet content of the constituent protein. The similarities between PrP-res and other amyloids suggested that the mechanism of PrP-res formation is like that of other amyloids (13,15,44). Early support for this idea came from observations that that small synthetic peptide fragments of the PrP sequence can form amyloid fibrils (32,33,45-48) and that this occurs by a seeded polymerization mechanism (32).

Recent studies with the full-length PrP-res protein have provided evidence that only ordered multimers of PrP-res, albeit widely variable in size, can induce the conversion of PrP-sen to the protease-resistant form in the cell-free system (49). The polymerized state of PrP-res also correlates with its PK-resistance, its ability to renature to full proteinase K resistance after partial denaturation, and with the presence of scrapie infectivity (17,18). Furthermore, the *in situ* conversion reaction in brain slices shows that the conversion product is bound to those deposits and not released into the medium (24). These observations are also consistent with a seeded polymerization mechanism for PrP-res formation.

Because not all deposits of PrP-res *in vivo* show birefringent staining with Congo red or have readily visible amyloid fibril structures by electron microscopy [e.g. (50)], some investigators have argued that polymerization/aggregation of PrP is not required for PrP-res formation (51,52). For instance, the heterodimer model posits that a PrP-res exists as a monomer and that the PrP-res monomer binds to a monomer of PrP-sen to form a heterodimer (11,14,51). The PrP-sen in the heterodimer then spontaneously converts to PrP-res, making a homodimer which splits into two PrP-res monomers. The fact that no proteinase K-resistant and/or high beta sheet monomer of PrP has been identified is inconsistent with this model. There have been reports of scrapie infectivity

that cofractionates with monomeric forms of PrP (53,54), but these studies have not been confirmed (55,56). Studies showing that ordered aggregates of PrP-res are active in converting PrP-sen to PrP-res demonstrate that there is, at least, no obligate requirement for a free PrP-res monomer, if one should exist, in the conversion mechanism (49).

The lack of visible fibrils in some tissue and membrane fractions containing PrP-res might readily be explained by a prevalence of short PrP-res polymers or the association of the PrP-res polymer with PrP-sen or other factors that obscure its ultrastructure and affect its birefringent staining with Congo red. In addition, there may often be small ordered, but ultrastructurally amorphous, subfibrillar structures or protofilaments which, under certain circumstances, fuse into, or seed the formation of, fibrils and amyloid plaques. Similar structures have been reported as early diabetes-associated deposits of islet amyloid polypeptide *in vivo* (57) and as intermediates or initiation sites for amyloid formation by synthetic Alzheimer's beta peptide (58) and PrP peptide fragments (59). Immunoelectron microscopy studies of PrP accumulation in scrapie-infected brain tissue have provided evidence for the accumulation of PrP in apparently amorphous subfibrillar forms prior to obvious amyloid fibril formation (60-62). Both the diffuse deposits and amyloid plaque of PrP-res are capable of inducing PrP conversion as shown with the *in situ* conversion reaction in brain slices (24).

The nucleated polymerization model is consistent with all of these observations because it predicts that PrP-res polymers ranging in size from huge amyloid plaques down to stable oligomers containing only several PrP monomers could seed the polymerization reaction. Theoretical consideration of the likely volume of a PrP monomer compared to the dimensions of classic scrapie associated fibrils suggests that fibrils containing 60 PrP molecules might be no longer than they are wide and, therefore, would not be visible as fibrils ultrastructurally. Furthermore, unless the PrP-res polymers are long and aligned in large oriented bundles or radiating amyloid plaques that are visible by light microscopy (>~0.5  $\mu\text{m}$ ), staining by Congo red would not appear birefringent under polarized light. Thus, the absence of readily visible, congophilic fibrils containing hundreds or thousands of PrP-res molecules is *not* persuasive evidence that PrP-res is usually monomeric.

### **The Role of Conformational Change in PrP-res Formation**

Since the available evidence suggests that there is a difference in conformation, as well as aggregation state, between PrP-res and PrP-sen (3-5), it is important to consider whether the conformational change and polymerization occur simultaneously or separately and, if separately, which event is the rate determining step in the conversion process? One possibility is that monomeric PrP(-sen) rapidly interchanges between high alpha helical and high beta sheet conformations on a rapid time scale (but presumably with a strong bias toward the high alpha helical conformation) and that the PrP-res-induced polymerization traps PrP in the high beta sheet conformation. In this case, the polymerization would be the rate determining step that stabilizes the high beta sheet conformer to allow the accumulation of PrP-res. A second possibility (heterodimer model) is that

the conformational change in nonpolymerized PrP<sup>sen</sup> is the rate-determining step which is catalyzed by nonpolymeric PrP of the high beta conformation (if it exists) and that polymerization may occur subsequently with little consequence to the rate of PrP<sup>res</sup> formation and accumulation. A third possibility is that the conformational change and polymerization occur together and both contribute to the rate determining step. Finally, it is possible that PrP<sup>sen</sup> molecules may first bind to PrP<sup>res</sup> polymers in the high alpha helix conformation (or an intermediate conformation) and then more slowly convert to the high beta sheet conformation to extend the PrP<sup>res</sup> polymer. As noted above, current evidence favors a important role for nucleated polymerization in the rate determining step of PrP<sup>res</sup> formation and would argue against the second (heterodimer) model. Consistent with the fourth possibility are recent experiments with the cell-free conversion system which provide evidence that the binding of PrP<sup>sen</sup> to PrP<sup>res</sup> polymers precedes its conversion to PrP<sup>res</sup> (22,24,76,77). Otherwise, it remains to be determined which of these scenarios most accurately describes the mechanism of PrP<sup>res</sup>-induced conversion of wild type PrP<sup>sen</sup> to PrP<sup>res</sup>.

#### Potential Cofactors

The conversion of PrP<sup>sen</sup> to PrP<sup>res</sup> can be induced by mostly purified, PK-treated PrP<sup>res</sup> in the cell-free conversion reaction (26,49), suggesting that these PrP reactants are all that is required under certain conditions. However, it is possible that under other conditions, such as *in vivo*, other factors are required for efficient conversion to occur. For instance, the binding of glycosaminoglycans to both PrP<sup>sen</sup> and PrP<sup>res</sup> and the potent inhibition of PrP<sup>res</sup> formation by these same compounds and their analogs suggests that they may play an important role *in vivo* (63-66).

The likelihood that refolding of PrP<sup>sen</sup> occurs during conversion has led to suggestions that chaperone proteins might be involved (67-70) as been documented for yeast prion phenomena (71). As noted above, the PrP conversion reaction is much more efficient if the PrP<sup>res</sup> seed is pretreated with a mild denaturant that partially and reversibly unfolds the polypeptide chain. Recent experiments have shown that certain chaperone proteins can substitute for this partial denaturation step to promote the PrP<sup>res</sup>-induced conversion reaction (22). On the other hand, none of the chaperone proteins tested induced the conversion of PrP<sup>sen</sup> to PrP<sup>res</sup> in the absence of a PrP<sup>res</sup> seed. These results emphasize the importance of seeding by PrP<sup>res</sup> and are consistent with the idea that PrP<sup>sen</sup> is refolded during the conversion process. The stimulatory activity of certain chaperone proteins *in vitro* suggests that chaperones may be cofactors in the PrP conversion process *in vivo*.

#### Concluding Remarks

At this point, one might reasonably ask several important questions: Is the acquisition of protease-resistance in PrP due to the conformational change, polymerization, or both? And which attribute of the abnormal PrP is most important in TSE pathogenesis and transmission? Is PrP<sup>res</sup> itself the transmissible agent? Are the most pathogenic and

neurotoxic forms of PrP necessarily the most transmissible (assuming for the moment that any form of PrP is by itself transmissible)? Finally, is the sequence of events and rate limiting steps in the presumably spontaneous PrP<sup>res</sup> formation from mutant PrP<sup>sen</sup> in familial TSE diseases necessarily the same as the induced formation of wild type PrP<sup>res</sup> following TSE infection?

The ultimate answers to these questions are not yet clear, but several relevant points can be made. Although the characteristic partial PK-resistance of PrP<sup>res</sup> appears to faithfully reflect a specific abnormal conformational and/or aggregation state that differs from PrP<sup>sen</sup> in most cases, this characteristic is not an equally hard and fast attribute of all disease-associated forms of PrP. Because there is no PK in mammals, there is no reason to expect that all types of pathogenic PrP need to be resistant to PK specifically to cause disease. However, some sort of general resistance to proteolysis would likely promote both the survival of PrP<sup>res</sup> as a potential transmissible agent and its accumulation as a pathogenic substance in the host. Nonetheless, even overexpression of wild type PrP<sup>sen</sup> can cause neurological disease (72), so pathological (but not necessarily transmissible) accumulations of PrP can be achieved without PK-resistance under some circumstances. Since no one has clearly documented the existence of a **monomeric** form of PrP that is high in beta sheet and PK-resistant, one can conclude only that, to date, all sufficiently well characterized forms of PrP<sup>res</sup> are both high in beta sheet and **multimeric**. Since conformational change and polymerization have not been convincingly separated temporally, one cannot yet determine which parameter might be most relevant in TSE pathogenesis and transmission. It must be noted that not **all** aggregates of PrP are PK-resistant, high in beta sheet, and associated with infectivity (23), nor are all PK-resistant, high beta sheet aggregates the same or associated with infectivity (25). Indeed, not all binding of PrP<sup>sen</sup> to preformed PrP<sup>res</sup> aggregates results in conversion to PrP<sup>res</sup>, though binding appears to be a prerequisite for PrP-induced conversion (22,24,76,77). These points emphasize that only a specific mode of PrP polymerization/aggregation correlates with the characteristic partial PK-resistance of PrP<sup>res</sup> and existence of TSE infectivity. Since this is a correlation, it remains of great importance to determine directly whether the formation of PrP<sup>res</sup> (alone) generates new TSE infectivity. Faced with these variables and complexities, it is tempting to search for a grand unifying pathological mechanism that would account for all possible disease states related to perturbations and accidents of PrP conformation, aggregation state and sequence. However, the mechanisms and attributes of PrP that might account for TSE transmissibility may differ somewhat from those accounting for pathogenesis. Furthermore, the apparently spontaneous conversion of mutant PrP<sup>sen</sup> molecules to PrP<sup>res</sup> in familial TSE diseases may differ mechanistically and cell biologically from the PrP<sup>res</sup> induced conversion of wild type PrP<sup>sen</sup> in TSEs of infectious origin.

#### Further Reading

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