The Role of Dimerization in Prion Replication

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ABSTRACT The central theme in prion diseases is the conformational transition of a cellular protein from a physiologic to a pathologic (so-called scrapie) state. Currently, two alternative models exist for the mechanism of this autocatalytic process; in the template assistance model the prion is assumed to be a monomer of the scrapie conformer, whereas in the nucleated polymerization model it is thought to be an amyloid rod. A recent variation on the latter assumes disulfide reshuffling as the mechanism of polymerization. The existence of stable dimers, let alone their mechanistic role, is not taken into account in either of these models. In this paper we review evidence supporting that the dimerization of either the normal or the scrapie state, or both, has a decisive role in prion replication. The contribution of redox changes, i.e., the temporary opening and possible rearrangement of the intramolecular disulfide bridge is also considered. We present a model including these features largely ignored so far and show that it adheres satisfactorily to the observed phenomenology of prion replication.

INTRODUCTION

The transmissible spongiform encephalopathies (TSEs), or prion diseases, are neurodegenerative disorders which can be either transmissible, inherited, or sporadic (Weissmann, 1996; Prusiner, 1998; Horiuchi and Caughey, 1999). Overwhelming evidence supports that all three forms (as stated by the "protein only" hypothesis) are mechanistically united by the conversion of a host-encoded protein (PrP^{c}) to an altered conformation, the scrapie state (PrP^{sc}). In compliance with the genesis of the disease, the initial structural change may be caused by either the transmission of the pathologic prion, a germ-line mutation of its gene, or a chance conformational transition. The subsequent propagation of the scrapie state follows the same path irrespective of the initial event. Currently, there are two basically different models for describing the mechanism of scrapie replication (Bamborough et al., 1996; Cohen and Prusiner, 1998; Horiuchi and Caughey, 1999). In the template assistance model PrP^{sc} is considered more stable than PrP^{c}, and the conformational transition is an autocatalytic process which occurs via the transient interaction of PrP^{c} with PrP^{sc}. In the nucleated polymerization model, PrP^{sc} as a monomer is intrinsically unstable and can only arise because of multiple stabilizing interactions with an amyloid polymer.

A recent variety of the latter is based on the assumption that the prion polymer is linked by intermolecular disulfide bonds (Welker et al., 2001); i.e., disulfide reorganization is essential to PrP^{sc} generation. All three models rely on significant experimental evidence and exhibit the basic temporal aspects of the disease: the long and uniform incubation time and initial exponential growth of infectious titer (Bamborough et al., 1996; Horiuchi and Caughey, 1999). It seems that our current knowledge does not allow an unequivocal decision between them.

This ambiguity in delineating the structural transition largely stems from the uncertainty in defining what the infectious form of PrP is. Various protocols of PrP^{sc} isolation yield different preparations, the relation of which to the infectious agent is not clear. The most noted forms are: 1) fibrillar amyloid prion rods, which are infectious and form from protease-resistant PrP27–30; 2) ordered, nonfibrillar aggregates which are also infectious but are not amyloid by the criterion of Congo Red binding; and 3) amorphous aggregates which show fibrillar structure but are less protease-resistant than the previous ones and are not infectious (Bamborough et al., 1996; Cohen and Prusiner, 1998; Prusiner, 1998). The above polymerization models can only be applied to the rods; as discussed later, significant evidence supports that no higher aggregates are needed for infectivity (Bamborough et al., 1996). A further crucial point is that the ratio of the infectious unit to PrP^{sc} molecules is only ~1:100,000 in prion preparations (Bolton et al., 1991; Weissmann et al., 1996); thus, the structure of the infectious molecule can not be identified, which allows for various models of its structure and propagation.

Pertinent to this issue is that under nonphysiologic conditions most globular proteins have a tendency to convert to a high β-sheet form that polymerizes into an insoluble amyloid (Dobson, 1999; Taubes, 1996). This is rarely seen under physiologic conditions but with the prion protein it does occur when its native form contacts the scrapie form. As we have pointed out, this peculiar behavior probably originated in an evolutionary change when PrP, previously an integral membrane protein, got expelled to the extracellular space (Tompa et al., 2001). In our view, this resulted in multiple stable conformations of PrP which, because of functional constraints (Tompa and Friedrich, 1998), has never reached a state where the amino acid sequence encodes a single three-dimensional (3-D) structure. This unique property might manifest itself in the formation of a

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polymer that differs from other amyloids in that it is transmissible.

Neither of the current propagation mechanisms, however, attributes a crucial role to the dimer of either PrP form, although the idea of dimerization appeared very early in the prion literature (Dickinson and Outram, 1979) and relies on considerable experimental data (Cohen and Prusiner, 1998). In this paper we review evidence compatible with the idea that both dimer formation and redox changes play a role in prion replication. Based on these inferences, a model is presented, which incorporates these mechanistic elements. It is shown that this model describes the time course of disease progression satisfactorily and occupies an intermediary position between the two classical alternatives that attribute either strictly kinetic or thermodynamic control to the propagation of the scrapie state.

Evidence for dimer formation

Genetic studies of scrapie pathogenesis led to the original proposal that dimers might be important in scrapie replication (Dickinson and Outram, 1979). Here we survey evidence for dimer formation in three sections: first, it is shown that a large oligomer (amyloid) is not needed for infectivity; second, indirect evidence is compiled; and third, direct evidence under both physiologic and pathologic conditions is discussed.

The first pertinent observation is that although amyloid formation often accompanies prion disease, the pathologic state frequently develops without fibril deposition (Bamborough et al., 1996; Prusiner, 1998). Further, isolated amyloids are not necessary for infectivity, and have been shown to be artifacts generated by proteolytic processing during PrP^{sc} purification (McKinley et al., 1991; Wille et al., 1996). In fact, amyloid prion rods can be dispersed into detergent-lipid-protein complexes and transferred into liposomes with a significant, 10- (Gabizon et al., 1987) to 100-fold (Gabizon et al., 1988) increase in infectivity. In addition, such highly infectious preparations could be made without an intermediate amyloid formation, by direct solubilization of membrane-bound PrP^{sc}. Infectivity can be uncoupled from the amyloid fibril organization of prion rods (Wille et al., 1996), and protease resistance is seen both without low solubility and amyloid formation (Muramoto et al., 1996).

Thus, several observations suggest that the infectious unit is not a polymer. As for its exact size, infectivity has been found to be associated with a wide size range of aggregates, but not with monomers (Prusiner et al., 1993; Hope, 1994; Caughey et al., 1997). To be more specific, several observations indicate that PrP can form dimers, both in its physiologic and pathologic state. First, transgenic studies on the species specificity of prion replication have shown that PrP^{c} transiently interacts with PrP^{sc} in the conversion process (Prusiner et al., 1990). As the interaction requires their sequence similarity or identity, the existence of such a heterodimer possibly reflects the natural tendency of PrP^{c} for homodimerization. Based on such observations, a low-resolution model of the homodimer (Warwicker and Gane, 1996), and later also of the heterodimer based on β-stacking (Warwicker, 1997) has been created. The model was further extended to explain prion propagation by assuming addition of dimers through hairpin stacking (Warwicker, 2000). From structural considerations it was inferred that addition of dimers is much favored over monomers, as these stabilize the β-hairpin core suggested, in accord with the α-helix $\rightarrow$ β-sheet conversion that underlies PrP^{sc} formation (Pan et al., 1993). This theoretical model building has also received support from thermodynamic considerations. A simple lattice model revealed that model proteins, which are less stable in the monomeric state, are susceptible to the formation of alternative native states as homodimers (Harrison et al., 1999). Physical studies have suggested that PrP^{sc} is more stable than PrP^{c} (James et al., 1997; Zhang et al., 1997).

In addition to indirect evidence, models, and calculations, there is also substantial direct evidence for PrP dimerization under physiologic and pathologic conditions. PrP in brain homogenates, but not recombinant PrP, were observed by various techniques to form dimers (Meyer et al., 2000); this behavior was attributed to either glycosylation or an as-yet unidentified accessory protein, known to be involved in the PrP^{c}-PrP^{sc} interaction (Telling et al., 1995; Kaneko et al., 1997). In contrast, recombinant PrP(90–231) under the conditions used for NMR studies forms dimers for a measurable fraction of time (James et al., 1997). In crystallographic studies, human PrP has a domain-swapped dimer structure in which the disulfide bonds are rearranged and occupy an intermolecular position (Knaus et al., 2001). A 54-kDa normal cellular protein, possibly a cross-linked PrP^{c} dimer, was observed in uninfected hamster and mouse brains (Bendheim and Bolton, 1986). In murine neuroblastoma cells expressing hamster PrP, a similar 60-kDa protein was seen and shown to be a covalently cross-linked PrP dimer (Priola et al., 1995). This protein was protease-sensitive, formed larger aggregates, and in a cell-free conversion system, it could be converted to a protease-resistant form (Kocisko et al., 1994); this is thought to be an appropriate in vitro model of scrapie generation. Thus, this dimer displayed both normal and disease-associated attributes, indicating that it might represent a dimeric intermediate state in prion formation. Scrapie dimers have also been reported in infected brain samples. Protease-resistant PrP dimers were observed in hamster brain upon gentle disaggregation (Sklaviadis et al., 1989) or after large-scale purification (Turk et al., 1988). In an earlier work (Bellinger-Kawahara et al., 1988), inactivation of scrapie prions by ionizing radiation exhibited a target size of 55 kDa under various conditions, i.e., in brain homogenates, detergent-extracted microsomes,

or purified amyloid rods. All these data argue for the prevalence of a dimeric PrP form in the infective species.

A final, general comment on dimer formation is that dimerization is the most ancient and common step in the evolution of oligomeric proteins (Monod et al., 1965). In an isologous dimer, the same binding sets on two subunits (protomers) complement one another, for which the two protomers have to be rotated 180° relative to one another. In heterologous dimers two different binding sets on the protomer surface bind one another; this type of association may give rise to closed structures of cyclic symmetry or long, open polymers. Furthermore, domain swapping, observed for human PrP (Knaus et al., 2001) and many other proteins (Bennett et al., 1995; Janowski et al., 2001) is a general mechanism for dimerization and oligomerization, thought to be implicated in both the evolution of oligomeric proteins and fiber formation in amyloidoses.

Disulfide rearrangement in the $PrP^c \rightarrow PrP^{sc}$ conversion

The conversion of PrP^c to PrP^{sc} is thought to occur without the covalent modification of the protein (Pan et al., 1993; Stahl et al., 1993). Recent data, however, suggest one exception: the possible rearrangement of the sole disulfide bond of mature prion protein (Welker et al., 2001).

At first sight the disulfide bridge Cys^{179}-Cys^{214} (hamster numbering) of PrP seems protected from such insults. The 3-D structure of PrP^c determined by NMR (Riek et al., 1997; Lopez Garcia et al., 2000; Zahn et al., 2000) shows that this disulfide is buried within the stable hydrophobic core of the protein. Further, both PrP^c and PrP^{sc} contain an intact, apparently intramolecular disulfide bridge (Turk et al., 1988), i.e., the conversion preserves the disulfide bond. In addition, the conversion process was effectively inhibited by reducing agents such as 2.5 mM dithiothreitol (DTT) in a cell-free system (Herrmann and Caughey, 1998), and the mutation C179A prevented the formation of a protease-resistant, scrapie-like state of ectopic PrP in a scrapie-infected neuroblastoma cell line (Muramoto et al., 1996). Thus, the disulfide bridge seems to be indispensable for the transition into the scrapie state.

Other observations, however, suggest that a metastable intermediate with its disulfide bridge temporarily broken can not be excluded. The C179A mutant expressed in uninfected CHO cells suffered from severe subcellular trafficking abnormalities which probably resulted from its aggregation in the early secretory pathway (Yanai et al., 1999). Thus, its inability to produce PrP^{sc} in scrapie-infected cells could be attributable to improper cellular processing and not to the lack of a disulfide bond per se. The inhibition of PrP^{sc} formation in vitro is also revealing. As noted, the intramolecular disulfide bridge in PrP is very rigid (Hosszu et al., 1999) and is not accessible to reducing agents (Maiti and Surewicz, 2001). At pH 8.0, its reduction requires denaturing conditions such as 6.0 M GuHCl and high concentration (100 mM) of DTT (Jackson et al., 1999; Maiti and Surewicz, 2001). At lower pH (6.0), known to favor structural transitions of PrP toward the scrapie state (Taraboulos et al., 1992; Borchelt et al., 1992; Jackson et al., 1999; Maiti and Surewicz, 2001), a lower DTT concentration (1–2 mM) under milder conditions (1.0 M GuHCl) is sufficient to reduce it and inhibit its conversion to a scrapie-like protease-resistant state (PrPres). Possibly, under conditions which favor structural transition (i.e., lower pH, slight denaturation, the presence of PrPres) the disulfide bridge is sensitive to reduction and is more accessible than otherwise. A plausible explanation is to assume a metastable intermediate with its structure partially unfolded. For example, reduced and mildly acidified PrP was found to switch between its native conformation and a partially protease-resistant, β-rich amyloidogenic state (Jackson et al., 1999) under conditions probably encountered in vivo when PrP^{sc} formation occurs in endosomes or lysosomes (Taraboulos et al., 1992; Borchelt et al., 1992; Aguzzi and Weissmann, 1997). In refolding studies with recombinant hamster PrP, the native α-helical structure appeared only after formation of the intramolecular disulfide bond, whereas a scrapie-like, β-rich form was accessible both with and without the disulfide bond (Mehlhorn et al., 1996); in thermal denaturation studies the α-helical form rapidly converted into the thermodynamically more stable β-sheet form (Zhang et al., 1997). In a mutagenesis study the C179A mutant folded into a stable monomeric form only under mildly acidic conditions; at a slightly higher ionic strength, these structures underwent a transition to a β-rich state and oligomerization (Maiti and Surewicz, 2001). As a final note, the human PrP dimer has its disulfides rearranged into intermolecular bonds (Hosszu et al., 1999), which shows that disulfide reshuffling may be involved in dimerization/polymerization leading to PrP^{sc} formation.

Thus, redox changes during the transition to the scrapie state can not be discounted. The imbalance of either of the multiple redox sytems within the cell might also contribute by upsetting the redox state of PrP. For example, the thioredoxin/thioredoxin reductase system could reduce PrP with an immediate increase in β-sheet content and a parallel diminution in solubility (Requena and Levine, 2001). Another candidate is homocysteine; as in Alzheimer disease, its level correlates with the progress of the disease (Clarke et al., 1998). This was interpreted in terms of homocysteine contributing the free thiol needed for thiol-disulfide interchange involved in amyloid formation (Schweers et al., 1995).

Such observations have led to the proposal that disulfide rearrangement, i.e., a transiently reduced intermediate, plays a role in the structural transition to the scrapie

state (Mehlhorn et al., 1996). In one mechanistic scheme, the disulfide bridge may break down temporarily because of thiol-disulfide rearrangement catalyzed by a free thiol group (Feughelman and Willis, 2000). Thus, the α-helical cluster becomes unstable and rearranges into a β-hairpin structure, which is further stabilized by the disulfide bond that reforms in the reversal of the exchange reaction. In a possible alternative model, a related mechanism is assumed, but the catalytic thiol is thought to be provided by the terminal free Cys of a PrP^{sc} polymer (Welker et al., 2001). In this model the initial thiolate attack is facilitated by the association of a PrP^{c} monomer and the PrP^{sc} polymer, which ensures high effective concentration of the thiol group and destabilizes the tertiary fold of PrP^{c}. The novel disulfide bond thus created brings together the respective parts of the two molecules, initiating the structural transition to the β-fold characteristic of the scrapie state. This disulfide reshuffling can also proceed the other way, which may explain the dissociation of PrP monomers with an intact disulfide bond from PrP^{sc} aggregates upon denaturation (Welker et al., 2001).

As it appears from all the foregoing considerations, the disulfide bond and hydrophobic packing are tightly linked in maintaining the native fold of PrP; for the structural transition probably both have to break down. This scenario can be supported by two further considerations. First, mutations involved in inherited forms of prion diseases are often seen to destabilize PrP structure (Swietnicki et al., 1998; Liemann and Glockshuber, 1999), thus increasing the relative abundance of a putative metastable intermediate. Second, the preferred oxidation state and the ability of a cysteine for disulfide formation can be estimated on the basis of its conservation in homologous proteins and sequential environment. As calculated according to Fiser et al. (1992), both Cys^{179} and Cys^{214} are highly conserved, which indicates their tendency to exist in an oxidized form. Predicting their disulfide-forming ability according to Fiser and Simon (2000), however, suggests only an intermediate potential for Cys^{179} (0.49) and a low potential for Cys^{214} (0.049). This latter value indicates $\sim$10% probability for Cys^{214} to be involved in a disulfide bond. One reason for this low value is the lack of a Gly in its vicinity; this residue would make the chain more flexible and enable it to adopt the correct conformation demanded by the covalent bond between the two cysteines. Although the two Cys residues are separated by $>$30 amino acids, this intervening segment is highly structured, which may cause structural constraints. As the disulfide bond in PrP^{c} is located within an extremely stable region of the hydrophobic core (Hosszu et al., 1999), probably a range of very favorable interactions compensate for the lack of glycines; this gives an overall stability to the disulfide bond despite its non-ideal sequential environment. Nevertheless, for an intermolecular disulfide bond within a β-structure, segmental flexibility is less important because of the higher degree of freedom of the system of two separate molecules. Consequently, the strain of the disulfide bond in the native α-helical structure may be relieved, providing increased stability to the proposed intermolecular bond.

Dimerization model of PrP^{sc} replication

Taken together, all the above data and considerations underline that PrP dimerization and disulfide rearrangement may play a significant role in the propagation of the scrapie state. So far, these details only implicitly appeared in mechanistic models; based on the evidence presented here, we propose a model of scrapie replication in which these elements play a fundamental role.

The basic element of our model is that PrP^{sc} is a dimer with stabilizing intermolecular disulfide bridges (Fig. 1). Replication of this scrapie state begins with recruiting a normal PrP^{c} dimer, with native intramolecular disulfide bonds. The binding energy within this dimer of dimers loosens the native fold of PrP^{c} dimer and destabilizes its intramolecular disulfide bonds. Within this transient, partially unfolded structure, disulfide rearrangement occurs and results in novel, intermolecular disulfide bridges. Because of these bonds, the transient structure relaxes into the β-rich scrapie conformation which draws its stability from the mutual reinforcement of the β-sheet structure and the covalent bond. The newly formed scrapie dimer then diffuses away, enabling a new catalytic cycle to commence. This model mixes features of kinetic and thermodynamic control prominent in previous models, in that the scrapie dimer is a catalytic unit resembling the monomer in the template assistance model, whereas the binding energy and intermolecular disulfide bridges within a dimer add to stabilization of the scrapie conformer, just as an amyloid in the nucleated polymerization model would. The concentration of prion (PrP^{sc}) and all other species in the model obeys the kinetic equations formulated in Scheme 1. Solution of this differential equation system yields the time course of scrapie replication. As seen in Fig. 2, our model describes the kinetics of scrapie replication adequately as it accounts for the long incubation time and the exponential growth of infectivity (*trace A*). Species barrier is also easily demonstrated: a slight decrease in the rate constant of PrP^{sc}-PrP^{c} interaction delays appearance of the scrapie state significantly (Fig. 2, *trace B*).

Analogies in other diseases

As a final corroboration of the view elaborated in this paper, we shortly discuss four analogous cases of related phenomena or diseases. These examples constitute a good precedent for the feasibility of the model proposed for prion replication in TSEs.

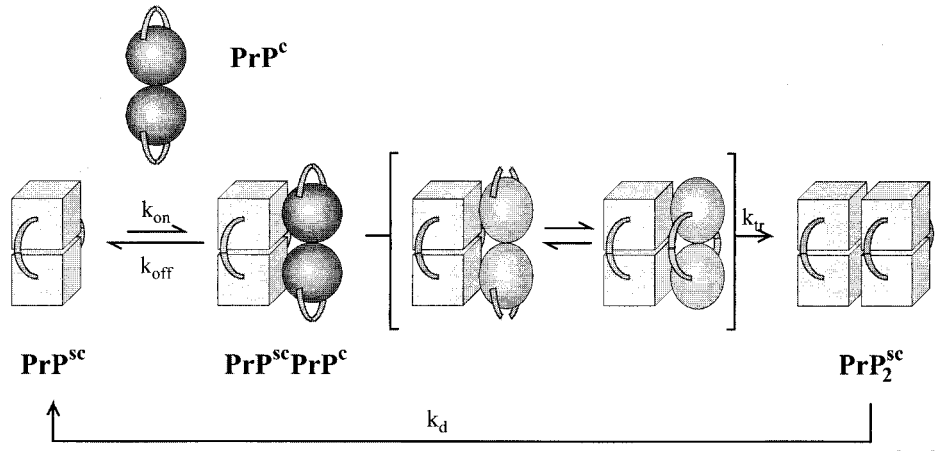

FIGURE 1 Replication of the scrapie state based on dimer formation and disulfide rearrangement. The figure is a schematic rendering of a $PrP^{c} \rightarrow PrP^{sc}$ conversion model based on both dimer formation and disulfide rearrangement. It is assumed that PrP^{sc} is a dimer of predominantly β-structure, stabilized by two intermolecular disulfide bridges. The critical step in replication is the recruitment of a PrP^{c} dimer with an α-helical structure. Upon binding, the structure of PrP^{c} dimer unfolds to a large extent; within this transient structure the disulfide bonds open up and reform in an intermolecular fashion. This initiates the structures to collapse into the more stable scrapie state with prevailing β-sheet(s). The newly formed scrapie dimer either diffuses away or remains in place, serving as a seed for amyloid.

The first example is the tendency of a yeast prion for dimerization. In yeast, inheritance of certain non-Mendelian genetic elements is associated with the prion-like propagation of the altered conformation of normal cytosolic proteins (Wickner et al., 1999; True and Lindquist, 2000). One of these, Ure2, is involved in the regulation of nitrogen metabolism; its altered conformation results in a stable phenotype that can be passed on to the progeny. Recently, the recombinant protein was shown to be a dimer both in solution (Perrett et al., 1999) and in crystals (Bousset et al., 2001). Incidentally, Ure2 has a domain organization similar to mammalian PrP as it can be separated into globular and unstructured halves (Lopez Garcia et al., 2000; Zahn et al., 2000).

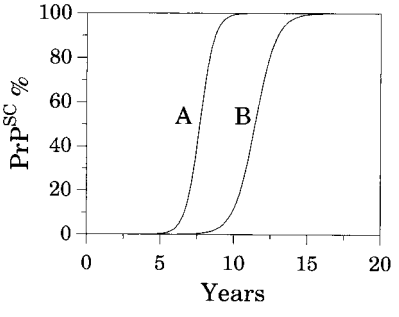

FIGURE 2 Time course of scrapie replication by dimerization-disulfide rearrangement. Calculations based on the kinetic scheme (Scheme 1) demonstrate that scrapie replication based on the dominant role of dimer formation and disulfide rearrangement adequately describes the observed time course of the increase of infectivity in prion diseases. This includes a significant incubation period (lag phase) in the infectious unit reaching an appreciable level and an exponential growth in the initial phase of the disease. The initial concentrations and rate constants used in solving the kinetic equations were as follows: $[PrP^{sc}]$, 10^{-7} M; $[PrP^{c}]$, 1 M; k_{on}, $6 \times 10^{-3}\ M^{-1}day^{-1}$ (*A*) or $4 \times 10^{-3}\ M^{-1}day^{-1}$ (*B*); k_{off}, $2 \times 10^{-2}\ day^{-1}$; k_{tr}, $1\ day^{-1}$; k_{d}, $1\ day^{-1}$. As seen, a slight weakening of the PrP^{sc}-PrP^{c} interaction causes a significant delay in the conversion to the scrapie state.

As a second example, human cystatin C is cited. This potent inhibitor of cysteine proteases contributes to amyloid formation in amyloid angiopathy of elderly people; its point mutation causes massive amyloidosis, cerebral hemorrhage, and death in young adults. The crystal structure of this protein reveals dimers which form via 3-D domain swapping; it is suggested that a similar mechanism may account for amyloid formation in the disease (Janowski et al., 2001).

The third and fourth examples are related to both dimerization and disulfide reorganization. Prion diseases show significant analogy to Alzheimer disease, a neurodegenerative disorder with similar lesions in the central nervous system (Iqbal and Grundke-Iqbal, 1996). One hallmark of Alzheimer's disease is the pathologic aggregation of τ, a neuron-specific microtubule-associated protein, into paired helical filaments within degenerating neurones. Studies with single-Cys τ constructs have shown that the formation of τ dimers linked by intermolecular disulfide bonds is essential for amyloid formation (Schweers et al., 1995); two-Cys constructs formed compact monomers with intramolecular disulfide bridges and could not nucleate paired helical filaments formation. Familial British dementia, in contrast, is also a neurodegenerative disorder which shares some features, most notably the deposition of amyloid, with TSEs. In this disease amyloids arise from a peptide fragment of a larger precursor protein (El-Agnaf et al., 2001). It has been demonstrated that the formation of a disulfide bridge is essential for dimerization of the peptide; dimer-

$$\frac{d[PrP^{sc}]}{dt} = -k_{on}[PrP^{sc}][PrP^{c}] + k_{off}[PrP^{sc}PrP^{c}] + 2k_{d}[PrP_{2}^{sc}]$$

$$\frac{d[PrP^{c}]}{dt} = -k_{on}[PrP^{sc}][PrP^{c}] + k_{off}[PrP^{sc}PrP^{c}]$$

$$\frac{d[PrP^{sc}PrP^{c}]}{dt} = k_{on}[PrP^{sc}][PrP^{c}] - k_{off}[PrP^{sc}PrP^{c}] - k_{tr}[PrP^{sc}PrP^{c}]$$

$$\frac{d[PrP_{2}^{sc}]}{dt} = k_{tr}[PrP^{sc}PrP^{c}] - k_{d}[PrP_{2}^{sc}]$$

SCHEME 1 Kinetic scheme of the prion dimerization-disulfide rearrangement model. The concentration of various species in the prion propagation model (Fig. 1) obey the kinetic equations formulated in this scheme. The time course of scrapie replication can be calculated by solving this differential equation system. Please note that this scheme is based on the condition that prion replication follows simple solution kinetics; a lot of data shows that the actual situation is more complex.

ization, in turn, was found necessary for the elongation of oligomers and formation of fibrils.

CONCLUSION

The model presented in this paper attempts to reconcile dimerization of PrP and disulfide reshuffling with other aspects of scrapie replication; its details are consistent with a range of observations not incorporated into mechanistic models so far. Its inferences are testable by carefully planned experiments and, we hope, will deepen our understanding of the unorthodox phenomenon of propagation of an altered protein state. This bears the promise of conceiving novel therapeutic strategies against the so far fatal prion diseases.

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