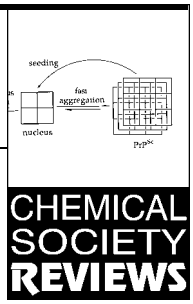
Molecular and chemical basis of prion-related diseases



CHEMICAL SOCIETY REVIEWS

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Prion-related diseases include scrapie in sheep, bovine spongiform encephalopathy in cattle and Creutzfeldt–Jakob disease in humans. The infectious agent for these diseases surprisingly contains no nucleic acid, but is a protein (PrP) which exists in two conformations, PrP^{C} and PrP^{Sc}. The infectious PrP^{Sc} form has a higher β-sheet and lower α-helix content than PrP^{C}. The structures of PrP and models for how PrP^{Sc} is able to replicate by converting PrP^{C} to PrP^{Sc} are discussed.

1 Introduction

The prion-related diseases affect both humans and animals. They are also known as transmissible spongiform encephalopathies because they frequently cause the brain to become riddled with holes. Human prion diseases that have, to date, gained considerable recognition and concern include Creutzfeldt–Jakob disease (CJD), Kuru, Gerstmann–Straussler–Scheinker syndrome (GSS) and fatal familial insomnia (FFI). Scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle, transmissible mink encephalopathy, feline spongiform encephalopathy and chronic wasting disease of captive mule deer and elk are common prion diseases found in animals.[1] All these diseases are characterised by vacuolation in the brain and usually result in ataxia (a condition in which the reflexes are diminished, and the absence of perfect coordination is shown through tremors and jerky movements), motor disturbance, dementia, and progression to a fatal outcome. The presence of abnormal fibrils (linear polymers) or insoluble plaques in the infected brain of all these diseases is another important characteristic and the probable cause (see below).[2] While they may vary in their course and rapidity of development, they all cause brain neuron degeneration and ultimately, death.

In the early 1980s, the name 'prions', an abbreviation for proteinaceous infectious particles,[3–5] was proposed for the infectious agents that cause these devastating diseases. The suggestion that these causative agents consist solely of protein breaks the Central Dogma of Molecular Biology which implies that the conveyers of transmissible diseases require genetic material that is composed of nucleic acid (DNA or RNA) for the establishment of an infection in a host. Prions are defined as 'novel infectious pathogens distinct from bacteria, fungi, parasites, viroids, and viruses, with respect to both their structure and the diseases they cause'.[1]

Table 1 gives the properties of the major prion-related diseases of which the best known is bovine spongiform encephalopathy (BSE), also known as 'Mad Cow Disease'. This is a progressive, lethal central nervous system disease of cattle, characterised by the appearance in neurons in the brain of affected cattle of vacuoles (clear holes), that give the brain the appearance of a sponge (hence the term, spongiform). The condition was first identified in cattle in the UK in 1986 and epidemiology led to the conclusion that the bovine agent had originated from the scrapie agent. The source of the epidemic was soon traced to a food supplement that included sheep and cattle offal and carcasses. The BSE epidemic in cattle in Britain reached a peak in 1993 when more than 1000 cases per week were being reported. More than 160 000 infected cows have been identified to date, involving more than 50% of the dairy herds in the UK, costing about 2 billion pounds sterling in compensation to farmers. The British government banned the use of animal-derived feed supplements in 1988, but it was not until 1991–1992 that the ban was strictly enforced, thus delaying the eradication of the disease. BSE has now been shown to have been transmitted to a number of other species, including mice, domestic cats, pigs and macaques. The epidemic of BSE in the UK and other countries raises the possibility of a significant threat to public health through the consumption of BSE-infected tissues.

2 Identification and properties of prion protein

2.1 Experiments carried out to determine the composition of the disease-causing agent

In search for the cause of these novel illnesses, purification of the infectious material from scrapie-infected brains was an essential first step of investigation. The scrapie agent was first purified from infected Syrian golden hamster brains.[6] The brain

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Table 1 The prion diseases

Disease	Organism	Typical symptoms	Route of acquisition	Distribution	Span of overt illness
Scrapie	Sheep	Loss of coordination and weight, followed by irritability; some develop an intense itch, leading them to scrape off their wool	(1) Infection of pasture with placental tissue scrapie agent followed by ingestion (2) Genetic disorder	Found in many parts of the world, possibly started in Spain. Incidence is related to the breed of sheep	Three to four months
Bovine spongiform encephalopathy	Cow	Loss of coordination and weight, followed by apprehensiveness, agitation and muscle contractions.	Meat and bone meal containing sheep and cow carcasses and offals	Seen in adult cattle of either sex. More than 160 000 cases have been identified	20 months to 18 years
Kuru	Human	Loss of coordination, often followed by dementia	Infection (probably through cannibalism, stopped by 1956)	Known only in highlands of Papua New Guinea	Three months to one year
Creutzfeldt–Jakob disease	Human	Dementia, followed by loss of coordination, although sometimes the sequence is reversed	Spontaneous conversion of PrP (in 'sporadic' disease). Sometimes inheritance of a mutation in the gene coding for the prion protein (PrP). Rarely, *via* an inadvertent consequence of a medical procedure (iatrogenic). Possibly (ipon vCJD), through dietary exposure to bovine offals	Sporadic form: 1 person per million worldwide. Inherited form: some 100 extended families have been identified. Infectious form: about 80 cases have been identified. New variant CJD: Around 20 individuals in UK have been identified (2 cases in France, 1 case in Italy, 1 case in Germany)	Typically about one year; range is one month to more than 10 years
Gerstmann–Straussler–Scheinker disease	Human	Loss of coordination, often followed by dementia	Inheritance of a mutation in the PrP gene	Some 50 extended families have been identified	Typically two to six years
Fatal familial insomnia	Human	Trouble sleeping and disturbance of nervous system, followed by insomnia and dementia	Inheritance of a mutation in the PrP gene	Nine extended families have been identified	Typically about one year

extracts of the infected hamsters were subjected to a range of tests, designed to reveal the nature of the disease-causing component. Surprisingly, procedures which modify nucleic acids did not lead to a decrease in scrapie infectivity.[7] In contrast, the scrapie agent was inactivated by protein denaturants, indicating that an essential component of the infectious agent was protein because experiments involved procedures that denature (unfold) or degrade proteins reduced scrapie infectivity. Nevertheless, it is still very resistant to degradation for a protein.

2.2 Identification of prion protein isoforms

Researchers identified a protease-resistant protein of about 27 to 30 kDa in fractions purified from Syrian hamster brains, designated PrP 27–30. *N*-Terminal amino acid sequencing of PrP 27–30 allowed the selection of a clone from a hamster brain chromosomal DNA library and the identification of the PrP gene.[8] The PrP gene was found to be encoded within a single exon as a protein of 254 amino acids.[9] The same PrP gene was also found in other mammals, including humans. Knowledge of the gene sequence led to the identification of the normal PrP gene product, a protein of 33 to 35 kDa, designated PrP^{C}. PrP^{C} is protease-sensitive and the cellular isoform of the prion protein PrP (hence the superscript C), whereas the abnormal, infectious, scrapie-form, designated PrP^{Sc}, is protease-resistant. PrP^{Sc} is now used to refer to the protein molecules that constitute the prions causing all scrapie-like diseases (hence the superscript Sc) of animals and humans.

2.3 Primary structure of prion protein

A signal peptide of 22 amino acids at the NH_2-terminus is cleaved from full-length PrP^{C} in the rough endoplasmic reticulum while 23 amino acids are removed from the C-terminus on addition of glycosylphosphatidylinositol (GPI). PrP is attached to cell surface membranes by its GPI. Most of the PrP molecules carry bi-, tri-, and quater-nary neutral and asparagine-linked oligosaccharides at two sites. A disulfide bond links the only two cysteine residues in the mature prion protein.

2.4 Detection of plaques in some prion diseases

The protease resistant fragments of PrP^{Sc}, PrP 27–30, accumulate as insoluble deposits of protein, or amyloid plaques, in the brains of some patients, implying that amyloid formation is essential for the formation of PrP^{Sc}.[10,11] Similar plaques are also seen in Alzheimer's disease, although Alzheimer's amyloid consists of a different protein.

2.5 Normal function of PrP

Mice devoid of PrP^{C} can develop and reproduce normally, though abnormalities in the action of the neurotransmitter GABA (γ-aminobutyric acid) suggest a role for PrP in nerve signalling.[12] PrP may also be involved in the regulation of sleep, as well as circadian activity rhythms.[13]

2.6 The prion hypotheses, virino *vs*. protein

We have come to expect that all life forms (from viruses to bacteria to plants to humans) hand down the blueprints to all their progeny *via* their DNA (or RNA for some viruses). This concept, however, is not adopted by the various scrapie-related prion diseases discussed here. Two different views have dominated this field of research. The first view argues that the infectious agent consists of, or contains, an informational molecule (independent of the affected animal) that is capable of

Table 2 Properties of the prion protein, PrP

Properties	PrP^{C}	PrP^{Sc}
Primary sequence	Same	Same
Molar mass/kDa	33–35	33–35
Position where instructions specifying amino acid sequence reside	Chromosomal gene	Chromosomal gene
Proteolytic resistance	Destroyed completely by proteolytic enzymes	Reduced to a protease-resistant core of about 27–30 kDa, termed PrP 27–30
Solubility	Soluble in presence of detergent	Forms insoluble aggregates
Turnover rate	Rapid	Very slow
Presence	Found in all cells, especially on suface of neurons	Found only in the brain and to a lesser extent, other tissues of animals/humans with prion disease
Content levels	Constant throughout disease	Levels in brain rise during disease progression in cases of prion disease
Aggregate characteristics	Does not aggregate in the form of insoluble polymers	Aggregates have a fibrillar structure with similar properties as amyloid plaques
Infectivity	Non-infectious	Infectious

mutation. The agent is given the name, 'virino', which is conceived of as a small, informational nucleic acid molecule susceptible to mutation which, with the host protein, PrP^{C}, combines after infection. The other view proposes that the causative agent contains only protein and derives its information from the structure of that protein. It is suggested that the progression of disease is accompanied by the conversion of normal host-encoded PrP^{C} to the abnormal form, PrP^{Sc}. According to this hypothesis, when this abnormal protein is transferred to a new host, it initiates a cascade and catalyses the modification of the new host's other PrP^{C} so that they in turn, become abnormal. Hence PrP^{Sc} is infectious.

3 Isoforms

Despite the uncertainty in the relationship between PrP^{Sc} and the causative agent(s) of the transmissible spongiform encephalopathies, one aspect appears quite clear: the main difference between normal PrP^{C} and scrapie PrP^{Sc} is conformational.

3.1 Absence of a chemical modification that might distinguish PrP^{Sc} from PrP^{C}

To date, PrP^{Sc} is the only component of the infectious prion protein identified. A post-translational process was suggested to mediate the conversion of the normal, cellular PrP isoform PrP^{C} or a precursor into abnormal PrP^{Sc} form by two findings. Throughout the course of scrapie infection, investigators found only one PrP mRNA in normal and scrapie-infected brains, and its level remained constant.[14] Furthermore, the entire PrP gene was found within a single exon, hence eliminating the possibility of alternatively spliced species of PrP during mRNA processing. PrP^{Sc} often has the same amino acid sequence as PrP^{C}, as shown by mass spectrometry and Edman degradation sequencing. Mass spectrometry showed no post-translational chemical modifications other than the GPI attached to the C-terminus and two Asn-linked oligosaccharides already known to occur on both PrP^{Sc} and PrP^{C}, indicating that conformation alone distinguishes the PrP isoforms. Indeed, a substantial conformational difference was observed using FTIR spectroscopy and circular dichroism spectroscopy. PrP^{C} consists primarily of α-helices, while PrP^{Sc} contains β-sheet.[15]

3.2 Molecular modelling of PrP suggested a four-helix bundle protein

Molecular modelling was used to predict the structure of the normal protein based solely on its amino acid sequence. Secondary structure prediction algorithms identified four potential helical regions, suggesting that PrP^{C} might be a four-helix-bundle protein (Fig. 1).[16] The four putative α-helical regions,

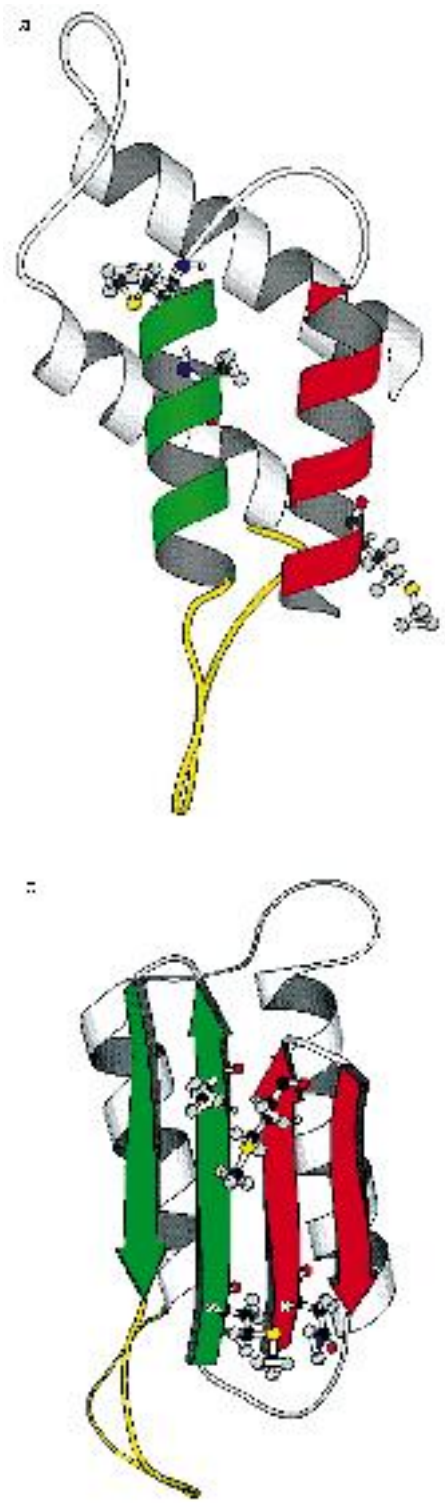

Fig. 1 Proposed models of PrP^{C} (a) and PrP^{Sc} (b).[16] Residues implicated in the species barrier[31] (Asn108, Met112, Met129 and Ala133) are shown as balls and sticks. (Reproduced with permission from ref. 16).

H1–H4, were found to reside within residues 90–231 of the protease-resistant core of PrP 27–30.

3.3 Synthetic peptides corresponding to three of the four putative α-helical regions of PrP can fold into β-sheets

The four putative helices were synthesised as peptides of 13–17 amino acids and their secondary structures found by FTIR spectroscopy. When the peptides were dried from a helix-promoting solvent such as 1,1,1,3,3,3-hexafluoropropan-2-ol, they had spectra indicative of α-helices with amide I bands showing maxima at 1650–1660 cm^{-1}. However, on addition of aqueous buffers, only the spectrum of H2 remained unchanged while the other three α-helical regions immediately adopted β-sheet conformations.[17] Furthermore, the three peptides that folded into β-sheet conformation were individually observed to precipitate slowly from aqueous solution as amyloid fibrils.

3.4 β-Sheet structures aggregate into amyloid fibrils enhanced by mutations

Some synthetic PrP peptides were found to have a high β-sheet content and be capable of amyloid formation. Mutations in these regions enhanced their ability to aggregate. PrP molecules that arise from mutated genes probably do not adopt the abnormal, scrapie conformation as soon as they are synthesised, or people with mutant genes would become ill in their early childhood or before birth. It was therefore suspected that mutations in the PrP gene made the proteins produced from these mutations more susceptible to flipping from an α-helical to a β-sheet shape.

3.5 Peptides in their β-sheet form can induce α-helix to β-sheet conformational transitions

Further evidence supporting the proposition that PrP^{Sc} can induce the switching of an α-helical PrP molecule to a β-sheet form came from studying the conformational transition with synthetic peptides.[18] It was found that the highly conserved hydrophobic peptide H1 (residues 109–122) was capable of inducing a β-sheet structure in other helical PrP peptides. These include peptide H2 (residues 129–141) and the longer, more hydrophilic peptides containing the H1 sequence such as 104H1 (residues 104–122), both of which have a coil or α-helical structure in solution.

3.6 *In vitro* conversion of PrP^{C} into PrP^{Sc}

By identifying conditions which allowed the reversible denaturation of PrP^{Sc}, a cell-free system was set up to test if *de novo* conversion of PrP^{C} to PrP^{Sc} could be observed *in vitro*.[19] Treatment of PrP^{Sc} preparations with a high concentration (6 M) of the denaturant GuHCl irreversibly destroyed its ability to induce the conformational conversion and its resistance to proteinase K. On the other hand, the converting activity and protease-resistance of PrP^{Sc} could be restored by diluting the denaturant to concentrations less than 3 M. This incomplete denaturation suggested that the converting activity and recovery of the characteristic protease-resistance of PrP^{Sc} requires the maintenance of some native PrP^{Sc} structure and that irreversible denaturation can only be achieved by exceeding the threshold denaturant concentration at which the native structure was completely destroyed.

^{35}S-Labelled PrP^{C} from an uninfected source was mixed with PrP^{Sc} in order to evaluate whether the conversion of PrP^{C} to a proteinase K-resistant form could occur during PrP^{Sc} renaturation. After two days incubation, proteinase K digestion was shown to eliminate the original full-length ^{35}S-PrP^{C} and produce ^{35}S-labelled proteinase K-resistant PrP. This observation established that PrP^{C} can be converted selectively to proteinase K-resistant forms similar to PrP^{Sc} in a cell-free system.

3.7 Presence of a β-sheet in an NMR structure of mouse PrP

While the model predictions of the structure PrP^{C} suggested that it was comprised entirely of α-helices,[16] another group of investigators found a β-sheet in an NMR structure of an autonomously folding mouse prion protein domain comprising residues 121–231, PrP(121–231) (Fig. 2).[20] Attempts to express PrP(108–231) in the periplasm of *E. coli* resulted in proteolytic cleavage after residues 112, 118 and 120. PrP(121–231) was found to contain three α-helices and a two-stranded antiparallel β-sheet. It is possible that the short β-sheet in PrP(121–231) serves as a nucleation site for the conformational transition from α-helix to β-sheet seen in the PrP^{C} to PrP^{Sc} conversion. No folds similar to PrP(121–231) could be identified in the Brookhaven Protein Data Bank. The orientation of the three helices in PrP(121–231) (Fig. 2) was distinctly different from the proposed four helix bundle model of PrP(109–217) [Fig. 1(a)]. Invariant residues in PrP sequences are found within the hydrophobic core and the two glycosylation sites are located on the protein surface. All six residues for which mutation is believed to be associated with inherited prion disease are located in, or near, regular structural elements. The residues that vary with familial prion diseases are all solvent accessible.

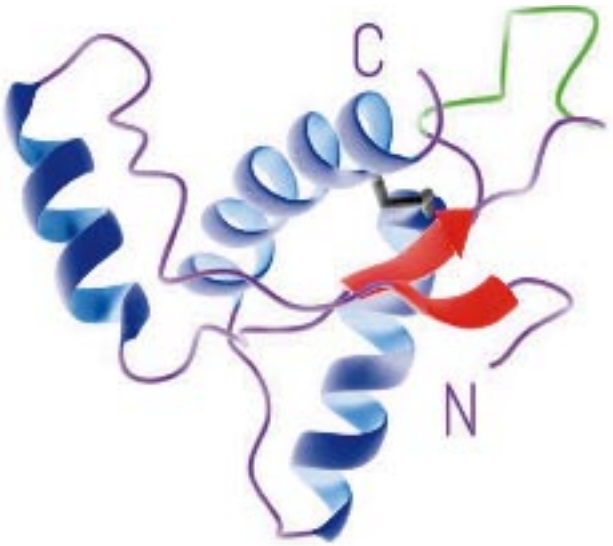

Fig. 2 NMR structure of PrP(121–231). (Reproduced with permission from ref. 20.)

4 Diversity

A difficulty exists in the 'protein-only' hypothesis for the infectious agent of prion diseases: the existence of multiple prion strains with markedly different, and apparently inheritable, characteristics. These various strains can be distinguished on the basis of species response, incubation period, clinical disease, neuropathological manifestations and PrP^{Sc} distribution in brain tissue.[21] About twenty phenotypically different strains of scrapie and BSE have been isolated in mice. This discovery of 'strain variation' therefore poses an interesting challenge to the 'protein-only' hypothesis as to date only pathogens containing nucleic acids are known to occur in multiple strains.

4.1 Each strain could be associated with a different PrP conformer

A possible explanation for prion diversity is that prions can adopt multiple conformations. A prion might be able to convert normal PrP into the infectious form highly efficiently when folded in a particular conformation, but when folded another way, its efficiency of conversion might be decreased. Similarly, one 'conformer' could be attracted to the neurons in one specific

part of the brain, whereas another conformer resides in a different site, thus exhibiting different symptoms. PrP^{C} and PrP^{Sc} have been shown to adopt at least two different structures but the exact number of conformations a PrP can adopt is unknown.

4.2 New variant CJD—evidence for BSE transmission to humans

New variant Creutzfeldt–Jakob disease is a form of human prion disease recently reported in the United Kingdom, affecting, unusually, young people and having a highly consistent and unique pathological pattern.[22] These patients were homozygotes (for methionine) at residue 129 of PrP. This important finding could be an indication of the arrival of a new risk factor for CJD in the United Kingdom, possibly through dietary exposure to bovine offals.

In order to differentiate new variant CJD from the other three forms (iatrogenic, sporadic and acquired, see Table 1), molecular analyses involving mainly the use of Western blots, proteinase K denaturation and transgenic technology were performed.[22] Sporadic and iatrogenic CJD were demonstrated to be associated with three distinct patterns of human PrP^{Sc} on Western blots after proteolytic cleavage by their differing band sizes. Types 1 and 2 were related to different phenotypes of sporadic CJD. A third type was seen in some CJD cases, in which the route of exposure to prions was through intramuscular injection of human hormones. On the other hand, iatrogenic CJD resulting from CNS exposure was found to typically resemble classical sporadic CJD. Despite showing similarity to the PrP^{Sc} band sizes of Type 3 CJD, the new variant CJD could be distinguished from the other three types of CJD by a characteristic pattern of band intensities. This unique molecular marker has clearly separated new variant CJD from sporadic CJD and confirmed the proposal that new variant CJD exists as a distinct and new subtype of prion disease. A glycoform pattern similar to that of new variant CJD was observed in BSE itself, experimental mice, naturally transmitted BSE in domestic cats and experimental BSE in macaques. These results strongly support the hypothesis that new variant CJD has resulted from the transmission of BSE to humans. The cause of sporadic CJD remains unclear but may involve spontaneous conversion of PrP^{C} to PrP^{Sc} as a rare random event.

Prion strain variation therefore involves post-translational modifications of PrP, which persist or can be converted between isolated strains (in the case when PrP genotypes are mismatched). This is consistent with the model of prion propagation whereby strain variation results in post-translational modification of PrP (such as a conformational change) with no nucleic acid involved.

4.3 Barriers to prion infection between species

The phenomenon known as the 'species barrier' has been observed in prion research since the mid-1980s. It is the observation that it is difficult for prions made by one species to cause disease in animals of another species. For instance, difficulties were observed in the transmission of scrapie from one animal species to another as well as in transmission of human diseases, such as Kuru and CJD to various animal species, including non-human primates. This could be partly due to the reduced efficiency of interactions between endogenous PrP^{C} and exogenous PrP^{Sc} which differ in their amino acid sequence. One notable example of the 'species barrier' is the BSE epidemic or 'Mad Cow Disease' in Great Britain in early 1986, an indication of the first transmission of a prion to a new species showing lower infectivity and longer incubation time than subsequent passages in that species. Furthermore, the transmission of BSE to the marmoset and macaques has since raised the possibility of BSE transmission to humans. Worryingly, bovine PrP has recently been noted to be more homologous with human PrP than is sheep PrP in the region between codons 96 and 167. However, it is still unknown whether the differences in the amino acid sequence between bovine and sheep PrP in this central domain could account for the apparent different susceptibility of humans to bovine and sheep prions.

5 Replication

The structural findings that demonstrated the conformational difference between PrP^{C} and PrP^{Sc} have also led to suggestions of two plausible models for prion replication which could account for the pathogenesis of infectious, inherited, and sporadic forms of prion disease. Any such conformational model requires that one amino acid sequence can code for at least two conformations, depending on its state of complex and surrounding environment. The proposed models raised an important issue in the PrP^{C} to PrP^{Sc} conversion as to whether the presence of PrP^{Sc} polymers/multimers is required or whether the formation of PrP^{Sc} aggregate is solely a consequence of the overproduction of PrP^{Sc}.

5.1 Model 1: a catalytic or template-assisted model (Fig. 3)

This conformational model[23] proposes that random fluctuations in the structure of PrP^{C} result in the reversible generation of a partially unfolded monomer, an intermediate designated PrP*, involved in the formation of PrP^{Sc}. The concentration of PrP* would normally be low and PrP^{Sc} formed in insignificant amounts.

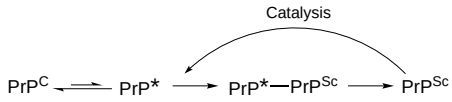

Fig. 3 Catalytic model for prion replication

Exogenous prions containing PrP^{Sc} act as templates to induce the conversion of PrP* into PrP^{Sc} in the infectious form of prion disease. However, the insolubility of PrP^{Sc} renders this process irreversible and so the formation of PrP* followed by PrP^{Sc} promotes increasing concentration of PrP^{Sc}. Mutations in PrP could cause destabilisation of PrP^{C}, inducing its conversion into the intermediate PrP* state resulting in formation of PrP^{Sc}.

In the case of sporadic prion diseases, the production of PrP^{Sc} could result from sufficient accumulation of PrP* under rare situations. Indeed, the development of spongiform degeneration is observed in transgenic mice overexpressing the wild-type PrP gene. Somatic mutations could also play a part in this form of disease by destabilising PrP^{C} and promoting its conversion into PrP^{Sc} through PrP*. For instance, both Met and Val are commonly found at amino acid 129 in human PrP. Homozygosity (Met-Met) at codon 129 was found to predispose to sporadic CJD in these studies. Further experiments showed that those patients carrying the Asp178 to Asn mutation would have insomnia if the codon 129 specifies Met in the mutant allele; if the codon 129 specifies Val, they would have dementia.

5.2 Evidence for structural differences between the PrP^{Sc} structure involved in prion infectivity and amyloid formation

Organic solvents that perturb protein conformation were employed in the study of the structure of PrP amyloid.[24] 1,1,1,3,3,3-Hexafluoropropan-2-ol (HFIP), a solvent known to promote α-helix formation, was observed to modify the structure of PrP amyloids from their original rod shape into flattened ribbons with a more regular substructure. Increasing the concentration of HFIP resulted in a steady reduction of β-sheet content, proteinase K resistance of PrP27–30 and prion infectivity. Congo red dye green gold birefringence under polarised light is characteristic of amyloid. HFIP was found to have reversibly decreased the binding of Congo red to the rods while inactivating prion infectivity in an irreversible manner. A

structurally related solvent, 1,1,1-trifluoropropan-2-ol, however, did not inactivate prion infectivity, but was shown to exhibit similar effects as HFIP by altering the morphology of the rods and abolishing Congo red binding. These results obtained from the use of electron microscopy and differential Congo red binding have distinguished the specific β-sheet-rich structures required for prion infectivity from those needed for amyloid formation hence indicating that amyloid formation is not essential for prion infectivity, in conflict with the nucleation-dependent polymerisation model described below.

5.3 Model 2: a nucleation-dependent polymerisation model (Fig. 4)

The slow onset of neurodegeneration is a characteristic of both the human prion diseases and Alzheimer's disease.[11] The brain pathology of these diseases shows similarity by the appearance of aggregated peptides, often in the form of amyloid plaques. Amyloid exists as ordered non-crystalline polymers and can be defined as a one-dimensional crystal in which the intermolecular packing in the plane perpendicular to the direction of fibril growth is non-uniform. Amyloid is capable of forming different types of insoluble, amorphous aggregates which can form rapidly when the protein concentration exceeds the solubility. There is a kinetic barrier to amyloid formation caused by a lag in forming an amyloid nucleus which can subsequently propagate. This rate-determining step was proposed to be mechanistically relevant to that accelerated by the infective agent of scrapie during amyloid formation. This led to the hypothesis of a possible replication mechanism for the conversion of PrP^{C} into PrP^{Sc} which assumes that PrP^{Sc} is an aggregate in which an alternative conformer of PrP is stabilised by intermolecular interactions.[11] In this model, the initial slow reversible formation of a nucleating PrP^{Sc} multimer leads to a seeding process, during which PrP monomers are added to the growing polymer in an abnormal PrP conformation.[25] The growing polymers can break apart, generating new seeds. In the sporadic form of disease, the reversible nucleation process is normally slow; on the other hand, PrP^{Sc} acts as a ready-made nucleation seed in the infectious form of disease and inherited mutations might contribute to the increase in affinity of the abnormal conformation of PrP for the polymer seed. Both replication and infection involve the nucleation of polymerisation according to this mechanism. Nucleation-dependent polymerisation is common among many well-characterised processes including protein crystallisation, flagellum assembly, microtubule assembly, sickle-cell haemoglobin fibril formation and actin polymerisation. The process is similar to crystallisation from a metastable supersaturated solution.

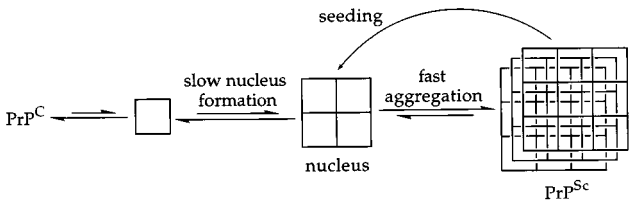

Fig. 4 Nucleation-dependent polymerisation model for prion replication[11]

Three distinctive features are characteristic of a nucleation-dependent polymerisation mechanism. First, there is a time lag before the aggregates become detectable. Secondly, there is a critical concentration. After completion of polymerisation, the solution contains mainly monomers and high polymers at equilibrium. The monomer concentration at this point is referred to as the critical concentration, below which polymerisation will not occur. Thirdly, a supersaturated solution can be seeded by a preformed nucleus, resulting in immediate polymerisation. In contrast, the growth of a linear polymer neither requires nucleation nor can be seeded. Linear polymerisation is characterised by the accumulation of intermediates in a sequential manner: no time lag is observed, and supersaturated solutions are unstable and rapidly aggregate.

5.4 Evidence for nucleation-dependent *in vitro* amyloid formation

PrP and the β-amyloid protein of Alzheimer's disease share a similar sequence that may be responsible for the initiation of protein aggregation and amyloid formation *in vivo* in both cases.[26] Part of the PrP sequence resembles the amyloidogenic C-terminal portion of the β-amyloid protein of Alzheimer's disease including amino acids 96–111 and is also highly conserved across species. Two synthetic peptides corresponding to PrP residues 96–111 (PrP96–111M and PrP96–111V) were synthesised. These peptides were observed to be sparingly soluble and formed rigid, unbranched amyloid fibrils, as shown by electron microscopy (EM), FTIR, Congo Red staining, and X-ray diffraction.[10] The existence of a kinetic barrier to amyloid formation was demonstrated by both peptides in thermodynamic solubility measurements, aggregation kinetics and seeding experiments. This suggested that the rate-determining step for aggregation is the formation of an ordered nucleus. Furthermore, seeding was shown to occur only with the amyloid fibrils of PrP96–111M and PrP96–111V but not with a related peptide with a shuffled sequence. This effect was consistent with the proposal outlined above that the aggregation of PrP involves a nucleation–polymerisation event analogous to the seeding of a crystallisation experiment.

5.5 Nucleation-dependent polymerisation mechanism accounts for predisposition of PrP homozygotes to CJD

A nonpathogenic polymorphism is known to occur at amino acid 107 (codon 129) within PrP. This polymorphism involves the conservative substitution of valine for methionine and both homozygous genotypes are predisposed to sporadic and infectious CJD. For instance, 21 in 22 individuals with sporadic CJD were homozygous for methionine or valine at position 107, while the population in general is approximately 50% homozygous.[27] The chemical basis for this genetic effect has been investigated using peptide models of PrP. Peptides derived from the PrP 118–133 sequence (containing methionine or valine at position 129) were observed to form amyloid *via* a nucleation-dependent mechanism,[10] suggesting that homogeneous peptide amyloid (Met129 or Val129) is more stable than heterogeneous amyloid (Met129 and Val129).[10]

Further studies were carried out with the objective of modelling possible mechanistic differences in prion formation between position 129 homozygotes and heterozygotes.[28] This was achieved by studying amyloid fibril formation from supersaturated peptide solutions and comparing homogeneous solutions with heterogeneous mixtures (1 : 1 Met129 : Val129) at the same total peptide concentration. These experiments demonstrated that heterogeneous supersaturated solutions showed longer nucleation times than the homogeneous solutions, indicative of a less stable heterogeneous nucleus. In addition, this phenomenon suggested the formation of non-productive heterogeneous oligomers (hence the slower nucleation rate) and that Met129 inhibits growth of Val129 fibrils (and *vice versa*). Seeding was observed when Met129 fibrils were added to supersaturated homogeneous solutions of Met129 or Val129. This was followed by a measurement of a comparable growth rate[10] which indicated that seeding, unlike nucleus formation, is insensitive to the polymorphism. Seeding of the heterogeneous solution with Met129 fibrils also proved successful, but with a slower observed growth rate, thus providing further evidence for the proposal of mutual growth inhibition. In an attempt to compare their thermodynamic stability, the solubilisation rates of fibrils formed from homo- and heterogeneous solutions were measured.[28] The results showed that the fibrils from the heterogeneous solution dissolved at approximately twice the rate of the homogeneous fibrils and reached a higher solubility than the homogeneous solutions. This sug-

gested that the two peptides formed separate homogeneous fibrils in the heterogeneous solution. By influencing nucleation time and critical concentration, a conservative sequence polymorphism (Met *vs*. Val) can therefore exert a dramatic effect on amyloid formation. In summary, because the slower nucleation and higher solubility of the peptide mixtures are analogous to the higher PrP critical concentration for nucleus formation in a heterozygote, the PrP concentration *in vivo* would therefore be more likely to be below the critical concentration. In this way, heterozygotes would be protected against amyloid formation.

5.6 Nucleation-dependent polymerisation could account for virus-like features of prion

The existence of prion strains has been difficult to explain by the protein-only hypothesis as strain variation has hitherto been explained by sequence variation in DNA or RNA. According to the nucleation-dependent polymerisation model, strain variation could results from different growth faces in prion fibrils.[11] The strain dependence of scrapie incubation time could be a result of imperfect complementarity between the interacting surface of the foreign seed and host PrP, leading to slower initial growth and delayed disease onset. After growth using host PrP, the face of the seed assumes the characteristics of the host, hence accounting for the observation that scrapie infectivity takes on the strain of the host. In this way, scrapie strains act as alternative conformations or packing arrangements of PrP^{Sc} polymers, analogous to the alternative crystal forms observed for many proteins.

6 Conclusions

Intensive research on prion biology and diseases both currently and over the past three decades has involved a wide spectrum of disciplines including virology, neurology, neuropathology, molecular biology, cell biology and protein chemistry. These studies have taken on new urgency since the accumulation of evidence that a new prion strain has crossed the 'species barrier' to humans from BSE infected cattle.

6.1 Nature

While a large accumulation of evidence supports the involvement of prion or PrP in the pathogenesis of these transmissible spongiform encephalopathies, it is still not completely certain that there is no nucleic acid within the transmissible agent of prion-related diseases, though it appears unlikely.

Of particular significance is the demonstration that PrP^{C} could be converted into PrP^{Sc} under defined conditions *in vitro*, a useful piece of evidence in proposition of the 'protein-only' hypothesis. Nevertheless, the cell-free production of amyloid fibrils from either precursor proteins or peptides observed in most amyloid diseases has failed to transmit disease by the amyloid fibrils themselves.[29] On the other hand, the existence of a nucleic acid component might be convincingly ruled out if the generation of a new form of prion-related disease infectivity could be produced in a cell-free system.

6.2 Misfolding

The prediction of protein folds from their amino acid sequences is an impressively long-standing challenge in molecular biology and biophysics, also commonly known as the Protein Folding Problem. Protein aggregation is a widespread phenomenon that can occur during protein folding *in vivo* as well as *in vitro*. The likelihood of intermediates within a folding pathway generating incorrectly folded species is dependent on several factors, including protein concentration, pH, temperature, ionic strength and redox environment. Kinetic competition which may result in formation of aggregates also exists between the correct and misfolded forms. Prion-related diseases can be characterised as 'protein folding diseases' involving a misfolded, abnormal form of the infectious agent, that is covalently indistinguishable but conformationally distinct from the normal form. The pathogenic mechanisms of some of these diseases are also known to involve aggregation.

In prion diseases, considerable evidence has accumulated in support of the importance of PrP(90–145), and in particular PrP(109–122), in the α to β conversion during PrP^{Sc} formation. These regions have been implicated as the key regions in mediating the interaction of PrP^{C} and PrP^{Sc} during the conformational transition as well as producing effects that are indicative of the prion disease species barrier. Furthermore, elucidation of the PrP(121–231) NMR-derived tertiary structure provides additional structural details of the conversion process. While the exact molecular mechanism of PrP^{Sc} replication is still unclear, an existing challenge remains to relate the misfolding of the prion protein to the resulting cellular pathology such that the three different (inherited, infectious and sporadic) forms of prion diseases can be accounted for.

6.3 Analogues

Some striking similarities with prion diseases exist in neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease. In all these diseases, the more widespread ills mostly occur sporadically but also sometimes in a familial manner. All these disorders only appear during the middle to later stages of life and are marked by similar pathology: degeneration of neurons, aggregation of protein deposits as plaques, the growth of larger glial cells (which serve to support and nourish nerve cells) in response to neuronal damage in the brain and an absence of white blood cells in the brain. Therefore, ongoing research on prion diseases might provide a crucial relevance to other more common neurodegenerative disorders.

6.4 Therapies

A thorough understanding of the molecular processes underlying the conformational transition from PrP^{C} to PrP^{Sc} is a precondition to devising rational approaches or therapeutics to the prevention and treatment of prion disease. These approaches could involve interfering with the PrP^{C} to PrP^{Sc} structural change, stabilisation of the PrP^{C} form or reduction of the PrP concentration. In a recent study, reagents which stabilise proteins in their native state have been found to interfere with the conversion process of PrP by reducing the rate and extent of PrP^{Sc} formation.[30] The reagents appeared to have no effect on the existing population of PrP^{Sc} molecules in scrapie-infected mouse cells, but instead interfered with the formation of PrP^{Sc} from newly synthesised PrP^{C}. This observation therefore led to the suggestion that the action of the reagents was to stabilise the α-helical conformation of PrP^{C}, hence inhibiting the conformational conversion into the β-sheet form of PrP^{Sc}. The further observations that long-term glycerol treatment appeared to slow down or even prevent PrP^{Sc} formation could possibly reveal potential strategies for treatment of prion-related diseases. Identification of more potent stabilising reagents and development of novel ways of introducing them into the central nervous system are likely to result in effective therapies and treatments for prion disease in mammals.

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