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High-resolution structure of infectious prion protein: the final frontier

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Abstract

Prions are the proteinaceous infectious agents responsible for the transmission of prion diseases. The main or sole component of prions is the misfolded prion protein (PrP^{Sc}), which is able to template the conversion of the host's natively folded form of the protein (PrP^C). The detailed mechanism of prion replication and the high-resolution structure of PrP^{Sc} are unknown. The currently available information on PrP^{Sc} structure comes mostly from low-resolution biophysical techniques, which have resulted in quite divergent models. Recent advances in the production of infectious prions, using very pure recombinant protein, offer new hope for PrP^{Sc} structural studies. This review highlights the importance of, challenges for and recent progress toward elucidating the elusive structure of PrP^{Sc}, arguably the major pending milestone to reach in understanding prions.

Transmissible spongiform encephalopathies (TSEs) are infectious disorders characterized by motor and cognitive impairments, extensive brain damage and neuronal dysfunction. After typically long incubation periods, individuals affected by TSEs deteriorate rapidly and progressively once the clinical symptoms arise, with lethal consequences in all cases. TSEs were first described in sheep exhibiting such abnormal behavior as erratic involuntary movements, ataxia and excessive scratching, and the disease was called scrapie¹.

In humans, the most common TSE is Creutzfeldt-Jakob disease (CJD), which appears sporadically at a rate of one new case per million people per year². Another human TSE is Kuru, first reported in 1954 among members of the Fore tribe in Papua New Guinea², whose practice of cannibalism was thought to be a determinant for spreading the disease³. In fact, the infectious nature of TSEs quickly became evident, but early efforts to isolate the underlying agent were unsuccessful. The infectious agent was found to have unusual features, such as small size and resistance to procedures that inactivate nucleic acids⁴. More recently, an outbreak of TSE affecting cows (termed BSE) destined for human consumption raised worldwide concerns regarding potential transmission to humans⁵. This concern

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proved correct when a new variant form of CJD was identified and strongly linked to interspecies transmission from BSE^{6,7}.

The nature of the infectious entity associated with TSEs has been a matter of debate for years⁸. In 1967, John Griffith proposed that the scrapie infectious material was a self-replicating protein⁹. Decades later, experiments in animal models of TSE showed that infectivity was associated with a glycosylphosphatidylinositol (GPI)-anchored membrane protein termed prion protein (PrP)^{10,11}. It soon became clear that PrP exists in two forms: the normal protein present in healthy individuals, termed PrP^C, for cellular PrP, and the protein found in infected animals, named PrP^{Sc} after scrapie-associated PrP. There are no chemical differences between PrP^C and PrP^{Sc}, and their distinction is at the level of the structure and aggregation of the protein^{12,13}. Today the widely accepted prion hypothesis states that the infectious agent associated with TSE is a self-propagating protein in an aberrant or 'misfolded' conformation^{14,15}. Weissmann and co-workers achieved an important breakthrough for the prion hypothesis by showing that PrP knockout mice were completely resistant to scrapie¹⁶. Other supporting evidence came from experiments showing that transgenic mice expressing PrP mutations associated with fatal familial insomnia or modifications that rendered the loop at positions 166–175 more rigid develop spontaneous disease that is transmissible to wild-type animals^{17,18}. Perhaps the most important evidence came from the generation of infectious material in the test tube by *in vitro* conversion and replication of PrP^C of both mammalian and recombinant origin^{19–21}. One argument often used against the prion hypothesis is the existence of prion strains⁸, a phenomenon difficult to reconcile with an exclusively proteinaceous infectious agent. However, recent findings have shown that strain properties can be propagated *in vitro*, suggesting that all elements enciphering prion strains are encoded on the PrP^{Sc} structure²².

Despite the clear involvement of PrP^{Sc} in TSE pathogenesis, the mechanisms by which the misfolded protein causes brain damage and disease are for the most part unknown. The reasons for the disease's lethal outcome are the extensive synaptic damage, neuronal loss and widespread spongiform degeneration, but how PrP^{Sc} is implicated in these processes is unclear. The current thinking on TSE, as well as other neurodegenerative diseases associated with protein misfolding and aggregation (Box 1), is that small oligomers of the misfolded protein are mainly responsible for neurotoxicity²³. The relationship between PrP^{Sc} polymer size and infectivity has been investigated using field-flow fractionation²⁴ and sedimentation velocity²⁵, which showed that per mass of PrP monomer, the most infectious particles are small oligomers with 12–24 monomers.

BOX 1

Protein misfolding in other diseases and expansion of the prion concept

TSEs are not the only diseases associated with misfolded proteins; some of the most common neurodegenerative diseases (for example, Alzheimer's and Parkinson's diseases) and many systemic disorders (for example, type 2 diabetes and secondary systemic amyloidosis) are associated with the accumulation of misfolded protein aggregates in different organs^{23,102}. There is experimental evidence for prion-like mechanisms of transmission in various protein misfolding disorders (for reviews, see

refs. 98–101). Indeed, recent studies have shown that the pathological hallmarks of various diseases, including Alzheimer's, Parkinson's and Huntington's diseases, and some forms of systemic amyloidosis, can be induced by administration of tissue homogenates carrying the respective misfolded proteins. Confirmation of these observations by human epidemiological data would indicate that the prion mechanism could be responsible for various protein misfolding disorders, a concept with broad-ranging implications for understanding disease mechanisms and for the development of strategies in disease prevention and intervention.

The widespread involvement of protein misfolding in different pathologies (Box 1) indicates a more ubiquitous phenomenon underlying protein folding regulation at the cellular level. It is likely that other diseases not yet associated with prions may have similar roots, particularly given that the ability to form the highly structured supramolecular protein arrangements called amyloids is a trait encoded within the backbone of most, if not all, proteins²³. In addition, the prion phenomenon seems to have non-pathogenic roles in certain organisms (Box 2).

BOX 2

Prions as genes

The prion paradigm of transmission of biological information by propagation of protein misfolding has been proposed as a new mechanism for non-Mendelian inheritance^{103,104}. The discovery of self-propagating proteins associated with protein-based conformational inheritance in yeast and other fungal species opened up new avenues for studying prions. Although the potential role of different yeast prions in the regulation of cellular processes is still under debate, their discovery raises the question of whether prions are more than a rarity in nature¹⁰⁵ and points to the possibility that cells may use the prion principle to propagate functional changes through auto-catalytic replication of protein-folding alterations. Indeed, a recent study has demonstrated that prions occur with a surprisingly high frequency in wild yeast and provide beneficial phenotypes under selective conditions¹⁰⁶. However, the analogies between fungal and mammalian prions are not straightforward. Fungal prions do not produce disease; moreover, they have structural features associated with highly organized, β -sheet-rich protein aggregates termed amyloids, whereas PrP^{Sc} usually has a rather amorphous supramolecular organization. In both cases, the existence of different prion 'strains' arising from the same primary sequence has led to the hypothesis that a prion can adopt multiple conformations that can themselves self-propagate through protein-protein interactions^{107,108}.

Elucidating PrP^{Sc} structure: importance and challenges

The structure of natively folded PrP^C became available in 1996 (ref. 26), but the high-resolution three-dimensional structure of the abnormal form of PrP has remained elusive, along with mechanistic details of PrP^{Sc} self-propagation. These are arguably the major remaining challenges in the prion field. Elucidating the structure of PrP^{Sc} is essential to fully understand the mechanism of prion replication, just as the discovery of DNA structure

enabled us to understand the process of genetic information transmission. Furthermore, the PrP^{Sc} structure should clarify the molecular basis of the species barrier and could allow predicting which species or strains of PrP^{Sc} can convert which PrP^C sequences. Finally, the availability of the PrP^{Sc} structure will provide a great incentive for the development of drugs to treat prion diseases.

The available evidence indicates that PrP^{Sc} is a polymer composed of PrP monomers organized in an intermolecular β -sheet structure. Prion replication probably follows a seeding-nucleation model, in which PrP^{Sc} acts as a seed to template the conversion of PrP^C, incorporating it into the growing polymer^{27–29}. The spontaneous (unseeded) formation of PrP^{Sc} would be thermodynamically unfavorable, which may explain the low frequency of sporadic disease.

Solving the high-resolution structure of PrP^{Sc} faces many, so far insurmountable, obstacles:

1. PrP^{Sc} consists of a large collection of interconvertible polymers of different sizes in dynamic equilibrium in solution^{24,25}. Any particular prion strain is known to exist as a diverse population of PrP species with different buoyancy-associated densities²⁵, which may correspond to different degrees of polymerization of an as-yet-unknown basic PrP^{Sc} molecular unit.
2. PrP^{Sc} aggregates typically have a high molecular weight³⁰. Although most infective PrP species seem to be rather small oligomers, these are still in the 400- to 600-kDa (ref. 24) range, which creates a substantial hurdle for classical biophysical analysis.
3. PrP^{Sc} aggregates are mainly hydrophobic. Like other amyloid-like aggregates, prions are water-insoluble particles³¹, and under the conditions required for structural studies, PrP^{Sc} forms non-crystalline aggregates that cannot be efficiently solubilized by any detergent tested³¹. Some degree of solubilization has been achieved with combinations of chemicals and heat treatments, but the resulting samples show a pronounced decrease in the infectivity titer, indicating that these procedures can change prion structure and its ability to self-propagate^{32,33}. More recent reports have described the isolation of partially detergent-soluble infectious PrP^{Sc} oligomers that are markedly more protease sensitive than classical prions and may prove a suitable substrate for biophysical characterization³⁴.
4. PrP^{Sc} particles probably contain a mixture of PrP molecules with different degrees of glycosylation (di-, mono- and non-glycosylated)³⁵.
5. Despite various protocols to purify PrP^{Sc}, its sticky nature results in the capture of many contaminants inside the prion particle, including other proteins, lipids and nucleic acids³⁶.
6. Current techniques to produce infectious prions *in vitro* have relatively low yield, which makes it difficult to generate sufficient material for biophysical studies.

Production of synthetic prions for structural studies

The difficulty in obtaining PrP^{Sc} for structural studies by using brain-derived material from diseased animals has led to attempts to produce synthetic PrP^{Sc} with the biochemical, biological and infectious properties of bona fide prions. Such efforts included either chemically or physically altering the conformation of recombinant PrP produced in bacteria (recPrP)^{37–41}. Whereas *in vitro* generation of amyloid-like aggregates by using recPrP is relatively straightforward, those aggregates typically lack infectivity⁴².

The first landmark in synthetic prion generation was achieved by Prusiner and colleagues in 2004, who reported that *in vitro*-assembled recPrP amyloid fibrils could produce prion-like symptoms when injected into transgenic mice overexpressing a truncated form of PrP, after long incubation times⁴³. However, the same fibrils lacked infectivity when injected into wild-type mice, raising justified concerns, given that transgenic animals overexpressing PrP have a well-known propensity to spontaneously develop prion-like diseases^{44–46}. Follow-up studies showed that disease can be transmitted to wild-type mice after multiple passaging in transgenic mice⁴⁷. Moreover, aggregates prepared under various different conditions resulted in distinct strain properties upon serial passages in wild-type mice^{47,48}. Using a similar approach, Baskakov and colleagues reported that recPrP amyloid fibrils produced infectious prions after two successive passages in wild-type mice⁴⁹. Although the animals did not show any symptoms upon direct injection of fibrils, a PrP^{Sc}-like protease-resistant signal was detected by immunoblot analysis in several brains, after long incubation times.

Although these results are encouraging, the very long incubation periods and the need for various *in vivo* passages before the agent showed the typical properties of prions point to differences between *in vitro*-produced recPrP aggregates and *in vivo*-generated PrP^{Sc}. This is further supported by studies using X-ray fiber diffraction, hydrogen exchange and atomic force microscopy, showing that recPrP fibrils and PrP^{Sc} appear to have substantially different cross- β -spine architectures^{50–52}.

At least three scenarios can explain the differences between *in vitro*-generated recPrP aggregates and *in vivo*-generated PrP^{Sc} (ref. 50): (i) recPrP aggregates may correspond to an ‘immature’ conformation that undergoes specific structural rearrangements *in vivo* toward a more infectious form that is equivalent to PrP^{Sc}; (ii) *in vitro* aggregation of recPrP results in a highly heterogeneous mixture of structures, of which only a minority has the folding and properties of infectious PrP^{Sc}; and (iii) some of the recPrP aggregates may inhibit replication of bona fide PrP^{Sc}, resulting in the reduction of infectivity, increase of the incubation period and the inability to infect wild-type animals in the first passage. Regardless of which scenario is correct, at this point, it is clear that recPrP aggregates are not a suitable model to study the structure of infectious PrP^{Sc}.

A more successful approach to generate PrP^{Sc} *in vitro* has been to mimic prion replication in the test tube templated by brain-isolated PrP^{Sc}. Initial attempts led to the cell-free conversion assay, developed by Caughey and colleagues⁵³, in which radioactively labeled PrP^C was incubated with a molar excess of PrP^{Sc}, usually in the presence of a chaotropic denaturing reagent. This resulted in small amounts of newly converted misfolded PrP that

was resistant to proteolytic degradation, but its infectivity could not be tested, owing to the low efficiency of the system and the inability to distinguish newly formed PrP^{Sc} from the original PrP^{Sc} inoculum.

More recently, an efficient *in vitro* prion-replication system was developed, termed the protein misfolding cyclic amplification (PMCA) assay⁵⁴. In this system, prions are replicated by mixing minute amounts of brain homogenates containing PrP^{Sc} with healthy brain homogenates harboring PrP^C. The replication of PrP^{Sc} can be amplified exponentially, as PrP^{Sc} polymers are fragmented by sonication, multiplying the number of seeds for conversion⁵⁴. The newly converted PrP^{Sc} has physicochemical properties identical to those of brain-derived PrP^{Sc} and, more importantly, is highly infectious in wild-type animals¹⁹. PMCA allows faithful replication of prion strain properties²², including complex characteristics such as species barrier, strain adaptation and strain memory^{55,56}.

PMCA has become a powerful tool to culture prions *in vitro*, providing information on the nature of the infectious agent and the mechanism of prion replication, and serving as a highly sensitive prion-detection system. However, the use of brain homogenates limits its usefulness to provide structural information on the conversion process. Using highly purified PrP^C from healthy brains as a substrate for PMCA, Supattapone and colleagues generated infectious prions with only the addition of synthetic polyanions²⁰. This was the first time prions were generated from pure components, but the need for polyanionic molecules and the presence of co-purifying lipids raised questions about the involvement of non-PrP components during conversion. Although initial attempts to use recPrP as a substrate for PMCA were unsuccessful⁵⁷, Wang and co-workers have reported the formation of prions from recPrP that were highly infectious to wild-type mice²¹. Notably, PrP^{Sc} formation required not only recPrP but also synthetic lipids and mouse-isolated total RNA. The same authors reported that endogenous RNA can be replaced by synthetically produced RNA polynucleotide⁵⁸. In other studies, recPrP prions were generated by PMCA, using only a combination of buffers and detergents⁵⁹, but these showed a low-infectivity titer, reflected in highly variable attack rates (proportion of animals showing clinical symptoms) and long incubation times.

Altogether, these findings clearly indicate that non-protein components participate in prion replication, at least *in vitro*. The questions then are what specific functions do these non-PrP molecules have and which molecules fulfill these functions *in vivo*^{15,60,61}. Cofactor molecules can influence PrP misfolding through at least two different mechanisms (Fig. 1). In the first model, the cofactor may act as a catalytic molecule that binds both the normal and misfolded PrP forms and brings them together, lowering the activation energy for the conversion process (Fig. 1a). Upon binding, the cofactor may also induce conformational changes in PrP^C and/or PrP^{Sc} that facilitate the interaction and conversion process. In the second model, the infectious PrP^{Sc} conformation would be stabilized by the cofactor (Fig. 1b). In biological terms, the main difference is whether the cofactor is a molecule provided by the host or a component of the infectious particle. In the latter case, the infectious agent would not be 'protein-only' but rather would consist of a complex between PrP^{Sc} and the cofactor. This difference is not only important for clarifying the nature of the infectious agent, it is also crucial for the elucidation of the PrP^{Sc} structure.

Although far from conclusive, the available evidence leans toward a scaffolding role for the cofactor (model 1). Negatively charged molecules (particularly nucleic acids, lipid particles and heparin sulfate proteoglycans) have long been proposed as PrP partners during conversion^{62–64}, and aggregation of PrP in the presence of DNA or RNA is well known^{62,65,66}. In addition, infectious prions form nuclease- and protease-resistant protein–nucleotide complexes *in vitro*. The scaffolding role to catalyze prion replication is also consistent with the observation that short-length nucleotides are highly inefficient in PMCA assays that are run with pure components⁶⁷. PrP^{Sc}-templated conversion of pure PrP^C by PMCA in the presence of light-cleavable nucleotides generated infectious PrP that showed no differences in titer and strain properties when the nucleotides were hydrolyzed after conversion⁶⁸, suggesting that polyanions act during conversion and do not need to be part of the infectious agent. Finally, though many molecules can be found associated with PrP^{Sc} particles, no specific molecules are present in high quantity in the infectious material.

It is therefore likely that polyanionic molecules act as two-dimensional catalytic scaffolds that efficiently gather PrP^C and PrP^{Sc}, increasing the likelihood of conversion⁶³. Still, the lack of high-resolution structural data makes it impossible to rule out the stabilizing role of a cofactor as an integral part of the infectious agent. In addition, cofactors could be involved in prion conversion through alternative pathways, as described elsewhere^{15,60}.

Probing the prion structure with low-resolution techniques

The unique properties of prion aggregates pose challenges for X-ray crystallization and NMR. Similar obstacles also exist for most amyloid systems, yet remarkable breakthroughs have been achieved with short peptides that form amyloid fibrils and are amenable to crystallization⁶⁹. Those structures revealed unique peptide arrangements called steric zippers, that is, pairs of β -sheets that are stabilized by tight interdigitation^{69,70}. However, extrapolating these observations to full-length proteins is not trivial. The structure of the prion-forming domain of fungal prion Het-S has been solved⁷¹, revealing cross- β in-register amyloid-like structures. The relevance of these findings to mammalian prions is unclear, as infectious PrP^{Sc} aggregates are typically not amyloid fibrils⁴².

As an alternative, several groups have used low-resolution biophysical and biochemical techniques to gather structural information on PrP^{Sc}. Although these approaches do not provide information about tertiary contacts and the overall arrangement of PrP^{Sc}, they can provide useful information for building structural models.

Initial efforts relied on classical spectroscopic techniques such as CD spectroscopy and FTIR spectroscopy, and revealed the predominantly β -sheet composition of PrP^{Sc} isolated from diseased brains, in contrast to the mainly α -helical nature of normally folded PrP^{13,72,73}. Indeed, characteristic IR spectral bands between 1,615 and 1,636 cm^{–1} associated with β -sheet structures are typically observed in prion samples. These findings were corroborated by FTIR for PrP^{Sc} from many different strains and species^{74–76}. These studies have also uncovered prion strain–associated differences in the secondary structure of PrP^{Sc}^{75,76}. However, the presence of complex glycans attached to PrP^{Sc}, and its C-terminal GPI anchor, added considerable interference to the data. Recently, GPI-less transgenic mice

able to replicate and produce infectious anchorless PrP^{Sc} (ref. 77) were developed, and the PrP^{Sc} obtained was also mostly non-glycosylated. FTIR analyses of this material showed no differences between wild-type and anchorless PrP^{Sc} of a particular strain^{52,78}, indicating that glycans and GPI do not affect the overall prion structure.

Limited proteolysis has also provided structural information on PrP^{Sc}. As early as the time of the first identification of PrP in the infectious material, it was clear that PrP^{Sc} was substantially resistant to proteolysis¹¹. Proteinase K treatment removes a fragment of about 12 kDa from the N terminus of PrP^{Sc} (ref. 79), resulting in a truncated form that retains infectious properties⁸⁰ and is often referred to as PrP27-30 because of the apparent size of the monomer in western blots. These observations suggest that the N-terminal region of PrP (up to around amino acid 90) is not essential for self-propagation. Experiments using transgenic mice expressing different PrP truncations confirmed that the minimal region required for sustaining PrP^{Sc} *in vivo* propagation starts from residue ~90 all the way up to the C-terminal part of PrP⁸¹. Interestingly, distinct prion strains show different resistance to proteolytic degradation, and the cleavage site can also vary between distinct strains⁸². The latter has been used to argue that the folding and packing of PrP associated with distinct strains is different.

Antibody mapping studies have examined a panel of monoclonal antibodies with known epitopes in recognizing PrP^{Sc} untreated or treated with denaturing agents, to investigate the accessibility of those sequences within the polymer. These studies showed that the region spanning residues ~90 to ~120 is not accessible to antibodies unless PrP^{Sc} is completely denatured⁸³, whereas segments located C-terminal to this region, such as the sequences 152–163 and 225–231, are accessible⁸⁴.

The ultrastructural features of prions have been studied by transmission electron microscopy (TEM)^{30,85} and more recently by atomic force microscopy⁸⁶. Brain-isolated PrP^{Sc} molecules usually appear as amorphous aggregates of heterogeneous sizes. Upon exhaustive purification procedures, including prolonged protease treatment, the aggregates acquire more defined structures called prion rods. Rods are typically in the range of 10- to 100-nm long and 5-nm wide and are usually shorter than classical amyloid fibrils⁴⁷. TEM analyses do not show appreciable differences between distinct strains. However, sedimentation velocity experiments have shown that size-distribution patterns differ between distinct strains, and the size of the polymers tends to correlate with infectivity properties²⁵. This agrees with the observation that strains containing higher proportions of oligomeric species self-propagate quickly and more efficiently in animal models²⁴. Yet, prion strain isolates composed of larger aggregates were recently shown to propagate better *in vivo*⁸⁷. The explanation was that larger aggregates may be more prone to fragmentation and may therefore spread prion seeds at higher rates. On the other hand, extensive sonication of PrP^{Sc} aggregates right before intracerebral inoculation did not alter any of the strain properties of a particular isolate (263K), including the incubation period⁸⁸. A plausible compromise interpretation is that prion strain differences lie within unique secondary and/or tertiary structural elements that give rise to strain-specific quaternary arrangements upon *in vivo* spreading. Therefore, the size distribution of a particular strain will be faithfully recovered upon injection even with low amounts of highly disrupted material. It is also important to

consider that extensive manipulation of prions, including simple brain extraction in mild detergents, may well yield changes in the size distribution of aggregates. Finally, the highly dynamic interconversion of aggregates makes it difficult to evaluate the biological properties of isolated aggregates.

Although PrP^{Sc} isolated from the brain of diseased animals does not form crystals amenable to X-ray crystallography, low-resolution diffraction patterns can be obtained by X-ray fiber diffraction⁸⁹. This technique relies on the quasi-symmetrical scattering of fiber-like macromolecular aggregates upon X-ray bombardment. The data obtained are useful to study the packing of the core regions and the overall organization of the aggregates. This technique has been widely used to study the fiber-like organization of amyloids, and has revealed a motif called the cross- β -sheet, in which parallel β -sheets are stacked perpendicularly to the fiber axis. A similar motif was identified in PrP^{Sc}. The fiber diffraction data of fibrils formed with protease-treated PrP^{Sc} (PrP27-30) showed a sharp, albeit weak, meridional 4.72-Å cross- β -reflection, typical of amyloid aggregates, but the equatorial 10-Å reflection typical of amyloids was absent in PrP^{Sc}, replaced by a weak and broad 8-Å signal⁵⁰. These data suggest that PrP^{Sc} has a structure with cross- β -packing similar to that in amyloid fibrils but with considerable differences.

Electron crystallography has also provided some clues about PrP^{Sc} structural organization. This technique proves useful when very small crystals (usually with 2D spatial arrangements) are available, as electrons can interact more strongly with the protein crystal lattice than X-rays in thin samples, producing better beam diffraction. Using such an approach, combined with computational threading, Govaerts *et al.* produced one of the first structural models for PrP^{Sc}, the β -helix structure⁹⁰ (discussed in the next section).

Alternative methods to obtain residue-level structural constraints can also yield information on the PrP^{Sc} structure. EPR relies on the use of paramagnetic probes attached to certain amino acids in the protein that can report about site-specific structures as well as intra- and intermolecular distances⁹¹. EPR studies based on labeled recPrP subjected to *in vitro* misfolding showed evidence for a parallel, in-register β -sheet arrangement, similar to that of classical amyloids⁹², but the lack of infectivity of these samples raises questions about the extrapolation of these findings to the PrP^{Sc} structure. Hydrogen-deuterium exchange coupled to either MS (HX-MS) or NMR (HX-NMR) also provides residue-specific structural constraints by means of the degree of accessibility to water hydrogens within specific regions. Recently, the use of HX-MS was successful in showing that brain-isolated prions have a highly water-inaccessible core composed mainly of β -sheets and small loops spanning from residue ~90 to the C-terminal⁵². Moreover, several different strains had subtle differential exchanges in the region of residues 90 to 140, suggesting that specific conformational differences may be involved in prion strains⁵².

Structural models for PrP^{Sc}

The information obtained from low-resolution biophysical techniques has been used to develop structural models for PrP^{Sc}, with several proposed in the last decade. Here we describe and discuss some of these models.

The β -helix

This is one of the most popular models that was proposed based on EM data on 2D crystals⁹⁰. The authors found that a left-handed β -helix would best fit the experimental data. A model was then constructed by threading the PrP sequence through a known β -helix motif (Fig. 2a). In this model, a trimeric arrangement constitutes the basic symmetrical unit for PrP^{Sc}, with the N-terminal residues of PrP27-30 (~90–175) forming left-handed β -helices that are horizontally stacked and include a long unstructured loop encompassing residues 145–163 (Fig. 2a). Larger aggregates are formed by vertically stacking PrP trimers along the β -helical axis. In addition to a major refolding within the N-terminal region of PrP27-30, the model is characterized by only minor structural rearrangements in the C-terminal part of the protein, which retains most of its native secondary structure, except for the first small α -helix that switches to a loop (Fig. 2a). Interestingly, the β -helical motif has been observed in other proteins that exhibit biochemical features reminiscent of PrP^{Sc}, such as partial resistance to protease degradation and aggregation propensity^{93,94}. Remarkably, the fungal prion HET-s was shown to form a β -solenoid arrangement of β -sheets that is structurally similar to β -helices⁷¹.

The β -spiral

This model was proposed by Daggett and colleagues based on molecular dynamics simulations of PrP conformational fluctuations under amyloidogenic conditions (low pH), using the natively folded structure as the starting point⁹⁵ (Fig. 2b). The model consists of a spiraling core of extended sheets, comprising three short β -strands (spanning residues 116–119, 129–132 and 160–164) and an isolated strand (residues 135–140) (Fig. 2b). The advantage of this model is that the structural scaffold was not chosen arbitrarily; instead, the model is the result of a putative conversion pathway from the monomer to the misfolded oligomer. As with the β -helical model, formation of β -strands involves the natively unfolded N-terminal region of PrP27-30, whereas most of the C-terminal remains intact, preserving the three α -helices characteristic of PrP^C. Fibrils are symmetrically arranged in a way that resembles spiral-like amyloid organization (Fig. 2b). This model satisfies many of the observations obtained by low-resolution techniques, except perhaps the proteolysis and the HX-MS data.

The extended in-register β -sheet

In this radically different model, proposed by Surewicz and colleagues, PrP^{Sc} is represented as a stack of parallel β -sheets that form an in-register arrangement, allowing for indefinite growth of the fibrils (Fig. 2c). This model is based on structural constraints obtained by HX-MS studies from recPrP fibrils⁹² and with brain-derived PrP^{Sc} (ref. 52). In the latter study, the authors used PrP27-30 isolated from prion-infected transgenic mice expressing mostly non-glycosylated PrP lacking the GPI anchor⁷⁷ to avoid interference from these post-translational modifications in the HX-MS studies. In this model, PrP^{Sc} consists of β -strands and relatively short turns and/or loops, with no α -helices present (Fig. 2c). Therefore, PrP conversion would involve refolding of the entire protein, and PrP^{Sc} would not preserve any of the structural motifs of PrP^C. The overall structure of the aggregates would resemble that of typical amyloid assemblies.

It is difficult to determine which of these three models is a closer representation of the PrP^{Sc} structure, as they are all based on data from low-resolution biophysical experiments. Nevertheless, the fact that these models are so substantially different reflects how little we know about the structural details of PrP^{Sc}.

A point of contention is the structural fate of the C-terminal domain, which is globular in PrP^C, with well-defined and stable α -helices. In both the β -helical and the β -spiral models, the C-terminal domain retains most of its structure upon misfolding, whereas in the extended in-register β -sheet model, the entire protein refolds into a mainly β -sheet conformation. The latter model fits the proteolysis data better, as it is difficult to understand the high resistance to proteolytic degradation of the C-terminal part of PrP^{Sc} if its structure is not substantially different from PrP^C, in which this region is easily cleaved by proteases. Indeed, in both the β -helix and spiral models, the α -helical domains face the outside of the polymer, hence they should be at least partially accessible to proteases.

On the other hand, the extended in-register β -sheet model is in conflict with CD and FTIR studies indicating a substantial amount of α -helical structure in PrP^{Sc}. Indeed, different groups have consistently reported that PrP^{Sc} is 15–35% α -helical^{13,19,72–76}. The majority of these experiments were conducted with FTIR, in which peaks at $\sim 1,556$ – $1,661$ cm⁻¹ were attributed to α -helices. However, this assignment is not always straightforward because other structures, including turns, loops and unordered segments, can also give rise to amide I bands in this frequency range⁹⁶. Indeed, bands around $1,656$ – $1,658$ cm⁻¹ have been observed in FTIR spectra of proteins that had no α -helices, according to X-ray crystallography or NMR spectroscopy data^{96,97}. Moreover, a report using FTIR to analyze both wild-type and GPI anchorless PrP^{Sc} has cast doubt on the presence of α -helices⁷⁸. Finally, the extended in-register β -sheet model has many similarities to the high-resolution structures of short peptides aggregated into amyloid fibrils and of yeast prions, suggesting that this is a plausible model for misfolded aggregates that have the ability to self-propagate. Nevertheless, these similarities represent a double-edged sword because, as discussed above, the infectious folding of PrP^{Sc} seems to be different from that of classical amyloid fibrils.

Conclusions and perspectives

The ability of proteins to self-propagate specific conformations and associated biological functions continues to fascinate researchers. The once heretical hypothesis that a protein can act as an infectious agent to propagate disease is now widely accepted, and the prion principle is being extended to other degenerative diseases associated with the accumulation of misfolded proteins^{98–101}. However, several key questions about prion biology, including the mechanisms of toxicity, the molecular basis of *in vivo* prion propagation and the detailed PrP^{Sc} structure, remain unanswered. Moreover, it is possible that new findings about the role of as-yet-unidentified cofactor molecules may undermine this already controversial hypothesis by demonstrating the participation of an essential non-protein component in PrP self-replication and infectivity.

From the key unanswered questions in the prion field, determination of the high-resolution structure of PrP^{Sc} will undoubtedly be a major step in understanding the mechanism by

which proteins can propagate biological information. The structure of PrP^{Sc} should also reveal the mysterious relationship between prion strains and PrP^{Sc} conformation and enable the rational design of much-needed treatments for these devastating diseases.

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Figure 1. Potential roles of non-PrP cofactor molecules during conversion of PrP^C into PrP^{Sc}. **(a)** Template-based conversion of PrP^C (blue triangles) into PrP^{Sc} (red triangles) requires surpassing a large energetic barrier that may preclude efficient misfolding during experimental timescales. In the presence of certain cofactor molecules (red line), the conversion will be greatly enhanced by reduction in the free energy of activation ($\Delta G^\ddagger$), as in typical surface-catalyzed chemical reactions. **(b)** The formation of an infection-competent misfolded PrP conformation depends on permanent binding of a cofactor molecule (blue hexagon) to PrP^{Sc}, leading to the stabilization of this structure. The resulting complex is able to propagate and produce disease upon *in vivo* transmission, whereas in the absence of this molecule, PrP^{Sc}-only aggregates (blue trapezoids) are unable to propagate *in vivo*.

Figure 2.

Alternative models proposed for the structure of PrP^{Sc}. **(a)** In the β -helical model, a major refolding of the N-terminal region of PrP²⁷⁻³⁰ into a β -helix motif from residues 90 to 177 (light green) is proposed. The C-terminal region (residues 178–230, dark green) maintains the α -helical secondary structure organization, as in PrP^C. **(b)** The β -spiral model developed by molecular dynamics simulation consists of a spiraling core of extended sheets comprising short β -strands, spanning residues 116–119, 129–132, 135–140 and 160–164. In this model, the three α -helices in PrP^C maintain this conformational motif. **(c)** The parallel in-register extended β -sheet model of PrP^{Sc} proposes a thorough refolding of PrP^C into a structure composed mainly of β -sheets. To facilitate comparison, the same color assignment for structural motifs has been used in all panels. The figure for the spiral model was kindly provided by W. Chen and V. Daggett.