REVIEW

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The structure of the infectious prion protein
Experimental data and molecular models

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Abbreviations: 2D, two-dimensional; 3D, three-dimensional; CD, circular dichroism spectroscopy; EM, electron microscopy; EPR, electron paramagnetic resonance spectroscopy; FTIR, Fourier-transform infrared spectroscopy; GPI, glycosylphosphatidylinositol anchor; H/D exchange, hydrogen / deuterium exchange; NMR, nuclear magnetic resonance spectroscopy; PK, proteinase K; PrP, prion protein; PrP^C, cellular isoform of the prion protein; PrP^{Sc}, infectious isoform of the prion protein; PrP 27-30, N-terminally truncated form of PrP^{Sc}; recPrP, recombinant PrP; SAF, scrapie-associated fibril; SAXS, small angle X-ray scattering; Δ-GPI PrP, prion protein lacking the glycosylphosphatidylinositol anchor

The structures of the infectious prion protein, PrP^{Sc}, and that of its proteolytically truncated variant, PrP 27–30, have evaded experimental determination due to their insolubility and propensity to aggregate. Molecular modeling has been used to fill this void and to predict their structures, but various modeling approaches have produced significantly different models. The disagreement between the different modeling solutions indicates the limitations of this method. Over the years, in absence of a three-dimensional (3D) structure, a variety of experimental techniques have been used to gain insights into the structure of this biologically, medically, and agriculturally important isoform. Here, we present an overview of experimental results that were published in recent years, and which provided new insights into the molecular architecture of PrP^{Sc} and PrP 27–30. Furthermore, we evaluate all published models in light of these recent, experimental data, and come to the conclusion that none of the models can accommodate all of the experimental constraints. Moreover, this conclusion constitutes an open invitation for renewed efforts to model the structure of PrP^{Sc}.

Introduction

The cellular prion protein, PrP^C, undergoes a substantial structural rearrangement during the conversion into PrP^{Sc}, the disease-causing isoform of the mammalian prion protein. The α-helical structure of recombinant PrP (which is thought to mimic the structure of native PrP^C) has been solved repeatedly by solution NMR spectroscopy and X-ray crystallography.[1] In contrast, the molecular structure of PrP^{Sc} is poorly understood.[2] A variety of approaches have been used to study the structure of PrP^{Sc}, but its general insolubility and propensity to aggregate have limited the quality of the available results and severely curtailed the applicability of many techniques. Since the structural conversion from α-helix-rich PrP^C to β-sheet-rich PrP^{Sc} forms the basis for infection, transmission, and pathogenesis, solving the structure of PrP^{Sc} remains a key challenge in prion research.

In this review, we will discuss a number of recent studies that have contributed to our current understanding of the structure of PrP^{Sc}. In some instances the results and / or their interpretation remain controversial and, to the best of our abilities, we will try to present an unbiased view.

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Experimental Data

Spectroscopy

Fourier-transform infrared spectroscopy (FTIR), and to a lesser degree circular dichroism spectroscopy (CD), demonstrated the high β-sheet content of PrP^{Sc} and its N-terminally truncated variant, PrP 27–30 (**Table 1**).[3-5] For a long time, the FTIR data were interpreted to imply that PrP^{Sc} and PrP 27–30 retained a substantial fraction of α-helical structure from the original PrP^C-fold. However, in more recent studies Smirnovas et al. showed that the ~1660 cm^{-1} band in FTIR spectra of PrP^{Sc}, attributed to α-helices, is also present in the spectrum of amyloid fibrils formed by recombinant prion protein (recPrP),[6] which have a parallel in-register β-structure and are completely devoid of α-helices.[7] Furthermore, the ~1660 cm^{-1} FTIR band overlaps heavily with bands in the same region representing turns and coils, making the assignment of α-helical structure difficult at best. Therefore, it was concluded that the FTIR-based data do not support the presence of residual α-helices in PrP^{Sc}.[6,8] Similarly, detailed analyses of recPrP amyloid by CD provided spectra that differed with the type of amyloid preparation even though all are considered to be parallel in-register β-structures.[9] Some of these recPrP amyloids gave CD spectra that resembled those of PrP 27–30, which were interpreted to be devoid of α-helical signals.[5] Others were more ambiguous and could be interpreted to also

Table 1. Comparison of spectroscopical analyses

<table>
<tr><th></th><th>PrP^{C}</th><th>recPrP 121–230</th><th>PrP 27–30</th><th>PrP 27–30</th><th>PrP 27–30</th><th>PrP^{Sc}</th><th>PrP^{Sc}</th><th>Δ-GPI PrP^{Sc}</th></tr>
<tr><td>α-helix</td><td>42%</td><td>40%</td><td>17%</td><td>21%</td><td>0%</td><td>30%</td><td>20%</td><td>0%</td></tr>
<tr><td>β-sheet</td><td>3%</td><td>7%</td><td>47%</td><td>54%</td><td>43%</td><td>43%</td><td>34%</td><td>~75%</td></tr>
<tr><td>turn</td><td>32%</td><td rowspan="2">53%</td><td>31%</td><td>9%</td><td rowspan="2">57%</td><td>11%</td><td rowspan="2">46%</td><td rowspan="2">~25%</td></tr>
<tr><td>coil</td><td>23%</td><td>5%</td><td>16%</td><td>16%</td></tr>
<tr><td>reference</td><td>4</td><td>53</td><td>3</td><td>4</td><td>5</td><td>4</td><td>5</td><td>6</td></tr>
<tr><td>Method</td><td>FTIR</td><td>NMR</td><td>FTIR</td><td>FTIR</td><td>CD</td><td>FTIR</td><td>CD</td><td>H/D exchange</td></tr>
</table>

The secondary structure assessments were taken directly from the references indicated, except for the H/D exchange data,[6] which were estimated from the figures in that reference. A large number of other studies that also employed FTIR and CD spectroscopy to determine the secondary structure content of PrP^{Sc} and PrP 27–30 were omitted in favor of brevity.

contain α-helical contributions, emphasizing the limitations of using CD spectroscopy for the analysis of highly aggregated samples.

Electron microscopy

Negative stain electron microscopy was used to first visualize PrP^{Sc} and PrP 27–30. The first observed forms of PrP^{Sc} and PrP 27–30 were labeled "scrapie-associated fibrils" (SAFs)[10] and "prion rods,"[11] respectively, reflecting the position of the authors in the debate surrounding the nature of the "scrapie agent." In both cases the morphological similarity of the PrP^{Sc} and PrP 27–30 aggregates to amyloid fibrils was noted, but interpreted differently with respect to the causal relationship between the fibrils and the underlying disease (scrapie).

The highly aggregated nature of the PrP^{Sc} and PrP 27–30 preparations limited the amount of structural information that could be gathered. The discovery of two-dimensional (2D) crystals of PrP 27–30 provided another avenue to study the structure of the infectious prion via electron crystallography.[12] By themselves, the resulting projection maps from the 2D crystals were of limited value, since they could not be interpreted in terms of molecular features of PrP 27–30. It was a fortuitous finding that a deletion mutant of the prion protein, PrP106 or "miniprion,"[13] which is capable of sustaining a bona fide prion infection,[14] formed 2D crystals that were isomorphous to the 2D crystals of PrP 27–30.[12] Difference maps between the 2D crystals of PrP 27–30 and PrP^{Sc}106 allowed to localize the N-linked oligosaccharides and the internal deletion of PrP106 (residues 141–176) on the crystal lattice, providing the first insights into the molecular architectures of both PrP 27–30 and PrP^{Sc}106.[12,15,16] FTIR spectroscopy suggested that the internal deletion of PrP106 formed part of the β-sheet structure in PrP 27–30,[14] thus allowing to pinpoint elements of the β-structure on the 2D crystal lattice. Subsequently, the data obtained from the 2D crystal studies were used to constrain molecular models for the structure of PrP^{Sc}/PrP 27–30 (see below) and a parallel β-helical conformation was predicted as a key feature of the infectious prion.[12,15]

Negative stain electron microscopy was also used to measure the protofilament diameter of PrP 27–30 fibers. Several independent studies gave diameters in the 4–6 nm range,[10,17,18] and the differences may be attributed to the properties of different prion strains, stain penetration, different purification procedures, and subjective differences on how to delineate individual protofilaments. Anchorless PrP 27–30, which lacks the GPI-anchor and most of the glycosylation side chains, has an apparent molecular mass of ~18 kDa. It also has a reduced protofilament diameter of 3–4 nm,[17,19] which provides a good estimate on the relative physical sizes of the protein moiety and the N-linked oligosaccharides, respectively. The diameter of a canonical left-handed parallel β-helix lies in the order of 3 nm,[20] which is good agreement with the observed protofilament diameter of anchorless PrP 27–30.

X-ray fiber diffraction and small-angle X-ray scattering

Initial studies using X-ray fiber diffraction on purified samples of PrP 27–30 confirmed the amyloid nature of the prion rods (cross-β signal).[21] By using synchrotron based X-ray sources, purification procedures based on precipitation with phosphotungstate anions,[22,23] and improved methods to align the fibril preparations, a more detailed view on the conformational architecture of PrP 27–30 was achieved. Each molecule of PrP 27–30 was found to contain a stack of four β-strands in a cross-β configuration resulting in a repeating unit size (= molecular height) of 19.2 Å = 4 × 4.8 Å.[18] In combination with an observed lack of a prominent equatorial diffraction signal around 10 Å, these results suggest that the molecular architecture of PrP 27–30 contains a β-helix or β-solenoid structure.

At the time it was not known, how the X-ray fiber diffraction pattern of a β-solenoid structure would look like experimentally. Fortuitously, a solid-state NMR structure of the prion domain of the fungal HET-s prion (residues 218–289) showed that its structure consisted of a two-rung β-solenoid.[24,25] Subsequently, X-ray fiber diffraction on HET-s(218–289) amyloid fibrils confirmed the predicted characteristics of a β-solenoid structure,[26,27] lending further support to the assumption that the structure of PrP 27–30 also contains a β-solenoid.

Small angle X-ray scattering (SAXS) was used to characterize a suspension of PrP 27–30 fibrils purified from the brains of scrapie-sick Syrian hamsters. In the sample, the random orientation of the fibrils limited the observed data to a scattering profile that provided a measurement of the average fibril diameter.[28] The average fibril diameter was determined to be 11.0 nm ± 0.2 nm for a doublet of two protofilaments, which compared favorably with earlier measurements by negative stain

electron microscopy of Syrian hamster-based PrP 27–30 fibrils with a diameter of 5.7 nm ± 1.1 nm per protofilament, *i.e.* 11.4 nm per doublet.[18]

Limited proteolysis

Limited proteolysis has provided valuable insights on the structures of PrP^{Sc} and PrP 27–30. This technique is based on the fact that under limiting conditions, proteases nick and degrade protein stretches exhibiting accessible and flexible secondary structure, i.e., loops and α-helices, mostly sparing β-strands.[29] Limited proteolysis using proteinase K (PK) was actually one of the first techniques to provide information on the structure of PrP^{Sc}, namely, that its N-terminus is exceedingly labile, while the rest of the molecule, from residue ~90 all the way to the C-terminus is surprisingly resilient to proteolytic cleavage.[30,31] Over time, additional minor, mostly C-terminal products of PK cleavage have been described.[32-35]

Recently, Vázquez-Fernández et al. obtained a complete map of PK-susceptible sites in GPI-anchorless PrP^{Sc} using mass spectrometry.[36] PK-resistant peptides spanning from residues 116, 118, 133, 134, 141, 152, 153, 162, 169, and 179 to the C-terminus, define the putative location of loops and the β-strands that they connect. Of note, several of these sites (133/134, 152/153, and 162) are located near proline residues (P136, P157, and P164), which reinforces the notion that they mark non-β structured regions. The C-terminal half, spanning from residues 152/153 to the C-terminus, appears to be the most resistant portion of PrP^{Sc}, further corroborated by its resilience to partial unfolding by guanidine.[32,36]

In retrospect, the observation that PK fails to cleave the C-terminal portion of PrP^{Sc} while completely degrading PrP^{C}, should have been a warning of the improbability of any residual α-helical structure being present in PrP^{Sc}. Other indications that the C-terminal portion of PrP may play a more significant role in the conversion to the infectious state came from the point mutations that were found to cause familiar prion diseases. The majority of these mutations lie in the region that had been predicted to remain α-helical, which led many investigators to question how these mutations may cause disease.[1] Assuming the C-terminal part of PrP^{Sc} consists of β-structure will facilitate future studies into the molecular mechanisms by which these point mutations trigger the conversion of PrP^{C} to PrP^{Sc}.

Hydrogen/deuterium (H/D) exchange

The secondary structure of proteins can also be probed through the exchange of hydrogen for deuterium ions (H/D exchange) at backbone amides. Unstructured regions are characterized by a rapid exchange compared with the relatively slow exchange of the amides involved in systematically hydrogen-bonded structures such as β-sheets, with α-helices exhibiting intermediate rates. Smirnovas et al. probed GPI-anchorless PrP^{Sc} via H/D exchange and found that the entire region from residue ~80–90 to the C-terminus exhibits exceedingly low exchange rates typical of β-strands.[6] Within this region, only the stretch spanning from 224 to 231 was found to be somewhat less protected. These results reinforce the idea that PrP^{Sc} consists of β-strands connected by short turns and/or loops, with no substantial α-helices remaining. The location of loops/turns could not be determined, given the resolution limit of the technique, which relied on mass-spectrometric analysis of pepsin fragments.

Surface reactivity

Functional groups on the surface of a protein can be derivatized by a variety of chemistries. Reacting a target protein with specific reagents followed by mass spectrometry analysis can identify surface-exposed residues. One such approach relied on reacting purified PrP 27–30 with a bi-functional cross-linker, bis(sulfosuccinimidyl) suberate.[37] The most reactive sites were G82, G86, and G90, which represent the N-terminus of PrP 27–30 after cleavage with PK. Intermolecular crosslinks that trapped dimers of PrP 27–30 were found to involve mostly G90, thereby localizing the N-terminus of PK-treated PrP 27–30 at a surface accessible contact site between two molecules. In another study, clarified brain extracts from prion-infected mouse or hamster brains were reacted with activated esters of N-hydroxysuccinimide.[38] Here the surface exposure of reactive residues was determined through the use of monoclonal antibodies and the masking of their epitopes.

Nitration of surface exposed tyrosines residues can be used to probe the local structure of PrP 27–30, in particular when compared with results obtained with PrP^{C}. Mass spectrometry revealed that tyrosines Y225 and Y226 become less exposed in Syrian hamster PrP 27–30, while being completely accessible to small reagents in PrP^{C}.[39] This supports the notion that a major rearrangement of the C-terminus takes places during conversion of PrP^{C} to PrP^{Sc}. Also, tyrosines Y162 and/or Y163 become exposed in PrP^{Sc}. Interestingly, these residues are part of an "YYR" epitope that is exposed in misfolded forms of PrP (including PrP^{Sc}), while being inaccessible in PrP^{C}.[40] Monoclonal antibodies that are targeted against the "YYR" epitope also recognize acid-misfolded forms of recombinant PrP, which show no infectivity.[40] The latter is a commonly observed problem with antibodies that are claimed to be specific for PrP^{Sc}, but also recognize other misfolded forms of PrP that are not infectious.[41]

As alluded to in the preceding paragraphs, monoclonal antibodies are a useful tool to probe the structure of small, surface-accessible segments of the prion protein either as linear or discontinuous epitopes. A rigorous analysis is needed to ascertain if a particular antibody recognizes PrP^{C}, PrP^{Sc}, or other conformers of PrP, which is more difficult than commonly appreciated.[41] In addition, motif-grafted antibodies that use specific sequences of the prion protein in the HCDR3 subdomain have demonstrated a remarkably high affinity for PrP^{Sc} and PrP 27–30.[42] This approach allows to probe which parts of the prion protein are responsible for intermolecular contacts between PrP^{C} and PrP^{Sc}, providing further insights into their structure.

Deletion mutants

The early observation that digestion with PK cleaves off the N-terminus of PrP^{Sc}, thereby generating PrP 27–30, without affecting infectivity, indicated that not all parts of PrP are necessary for the infectious state.[30,43] Genetically engineered variants of the prion protein that lack part of the N-terminus and mimic PrP 27–30—PrP(Δ32–80) and PrP(Δ32–92)—can be converted to the infectious state and induce a bona fide prion

disease.[44] Additional deletion mutants, in which other parts of the molecule have been deleted, were screened for the presence of PK-resistant PrP in prion infected N2a cells.[13,45] Successful candidates were tested in transgenic mice and PrP106 (Δ23–88, Δ141–176) was able to propagate RML prions within the same genetic background.[14] The deletion of the GPI-anchor signal sequence produced GPI-anchorless PrP, which also supported prion propagation while producing copious amounts of PrP amyloid.[19,46] Nevertheless, a variety of other deletion mutants failed to support prion propagation either in infected cell cultures or transgenic mouse models: e.g., PrP61 (Δ23–88, Δ141–221).[45,47] A caveat of the cell culture experiments lies in the selection criterion: PK-resistance, since it is an imperfect surrogate marker that is neither necessary nor sufficient for prion infectivity.[22,48-50]

Another limitation of the deletion mutant approach lies in the sensitivity of the prion protein to changes in its primary structure, which can impair the conversion to the infectious state or delay the appearance of disease symptoms beyond the lifespan of laboratory rodents.[51] Many studies address the question of delayed transmission based on differences in the primary structure between different species, also known as "species barrier," but this complex topic falls outside of the scope of this review.

Molecular Models

In the following paragraphs, we are going to list all serious attempts to model the structures of PrP^{Sc} and PrP 27–30. These models were based to varying degrees on the experimental data described above or alternatively on concepts and ideas that were deemed of particular interest. At the end of each paragraph we will give reasons why we believe these models to be inaccurate representations of the true (and as of yet unknown) structure of PrP^{Sc} / PrP 27–30. Ultimately, we come to the conclusion that none of the published models fit all currently available, experimental data, which serves as an open invitation to devise new molecular models for the structure of PrP^{Sc}.

(1) The first attempt to model the molecular structure of PrP 27–30 was done on a slightly truncated version: PrP(108–218)[52] and predates the first NMR structure of recombinant PrP.[53] Therefore, this model was based on an earlier model[54] for the structure of PrP^{C} and on the limited amount of experimental data that was available on the structure of PrP^{Sc}/PrP 27–30. This first model predicted PrP 27–30 to consist of a four-stranded β-sheet plus two C-terminal α-helices.[52] A key criticism for this model, which will be repeated for many of the models that follow (see below), rests with the prediction that PrP^{Sc} would retain substantial amounts of α-helical structure. This prediction was based on the interpretation of FTIR measurements, which are no longer supported by recent experimental observations.[6,8,36]

(2) The next entry into the modeling realm is rather unconventional in that it predicts PrP^{Sc} and PrP 27–30 to adopt an antiparallel, intertwined "β-helix."[55] In this model, strands of antiparallel β-sheets project from an antiparallel, intertwined core, which itself spans the height of eight β-strands. This unconventional model has no counterpart among the known protein structures in the PDB database and it does not conform to the definitions that are used to describe parallel β-helical structures.[20,56] Therefore, it is difficult to compare this unusual modeling result with known protein folds or other models for PrP^{Sc} and PrP 27–30. This model predicts the molecular height of PrP^{Sc} and PrP 27–30 to be approximately 38.4 Å = the height of eight β-strands, which is in clear contradiction with the X-ray fiber diffraction results.[18]

(3) The next modeling effort was precipitated by the comparison of the 2D crystals of PrP 27–30 and PrP^{Sc}106.[12] The tight packing of the PrP 27–30 molecules in the crystal lattice and the inferred locations of the N-linked oligosaccharides and the β-sheet structure led to the idea that PrP 27–30 contains a parallel β-helix at its core. The limited resolution of the electron crystallography results did not allow a distinction between putative trimeric or hexameric assemblies and subsequently the molecular models included both possibilities: left- and right-handed parallel β-helices. Among several weaknesses, these models have to be disqualified for their adherence to the C-terminal α-helices, which are no longer supported by the experimental observations.[6,8,36]

(4) In the next modeling approach the fold of PrP^{Sc} was modeled on the human TATA box-binding protein containing a five-stranded β-sheet, while retaining the C-terminal α-helices.[57] This modeling effort was inspired by the X-ray crystallography-based observation that recombinant PrP can form dimers in which the third, C-terminal α-helix is swapped between the monomers.[58] From this model it is not readily apparent how PrP^{Sc} amyloid fibrils would form and what their molecular height would be. Nevertheless, the retention of the C-terminal α-helices clashes with recent experimental data (see above).

(5) Molecular dynamics was used to devise the next model for the structure of PrP^{Sc}.[59] Here the structure of recombinant PrP was used as the starting point to simulate the conversion of PrP at an acidic pH. The resulting "spiral model" retained all three α-helices of the original structure, but extended the number of β-strands to four. In a model for the structure of a PrP^{Sc} amyloid fibril, which was built from the monomers of the spiral model, the β-strands are oriented at angles that are not perpendicular to the fibril axis. This particular feature renders the fibril model incompatible with the X-ray fiber diffraction results.[18] In addition, the spiral model has the highest proportion of α-helical structure from all published models and one of the lowest β-sheets contents, which conflicts with the re-interpreted FTIR results and the limited proteolysis data.[6,8,36]

(6) The Govaerts et al. model[15] reinvestigated the β-helical models that were proposed earlier.[12] Higher resolution electron micrographs of the PrP 27–30 and PrP^{Sc}106 2D crystals resulted in improved projection and difference maps, which showed that the crystal lattice is composed of trimeric unit cells (p3 symmetry). A more stringent analysis of the properties of the β-helical folds (left-handed vs. right-handed) and the improved electron crystallography results restricted the models to a left-handed parallel β-helical fold for the N-terminal part

of the prion protein.[15] The C-terminal portion of the molecule (starting at the position of the disulfide bond (Cys 179–Cys 214)) was left in its α-helical state, which is also the main weakness of this model now.

(**7**) For the next model of PrP^{Sc} molecular dynamics was used to test and refine the left-handed parallel β-helical fold.[60] This study rejected the earlier assumption that a hexameric assembly of β-helices could account for the observed densities in the 2D crystals.[12] Furthermore, it independently arrived at many conclusions that Govaerts et al. had reached.[15] The main differences between the "Stork" and the '"Govaerts" models lay in the threading of the PrP primary structure onto the β-helical template, but the "Stork" model also retained the C-terminal α-helices, which is no longer considered to be accurate (see above).

(**8**) Another attempt to improve the β-helical model of PrP^{Sc} focused on the stability of the proposed β-helical fold,[61] which was perceived to be insufficient to explain the extraordinary stability of the infectious prion. In order to increase the stability of the β-helical architecture a 3-fold domain swap between the individual protein molecules in the trimeric unit cell was proposed. Molecular dynamics was used to show that the domain-swapped trimer model had an intrinsic stability that far exceeded that of the non-entangled trimer. The most obvious weakness of this model is again the reliance on an α-helical structure for the C-terminal part of the molecule (see above).

(**9**) The next attempt to improve the β-helical model of PrP^{Sc} also targeted the lack of stability in the proposed fold.[62] Here, the intended solution reduced the number of β-helix rungs per PrP^{Sc} molecule from four to two, which helped to overcome problems with the packing density that arose from a stretch of PrP sequence (residues 106–126) that is rich in small amino acids (glycine and alanine). Again, molecular dynamics simulations were used to ascertain the increased stability of the new model compared with the old one. The reduction in the number of β-helix rungs also brings a reduction in the height for each PrP^{Sc} molecule from 19.2 Å (four rungs) to 9.6 Å (two rungs), which runs contrary to the experimentally observed molecular height of 19.2 Å.[18] Furthermore, this model also maintained the C-terminal α-helices.

(**10**) An independent analysis of recombinant PrP amyloid via site-directed spin labeling and EPR spectroscopy revealed a parallel, in-register β-sheet structure.[7] Here, each molecule of PrP contributes only 4.8 Å to the length of the amyloid fibril. The authors themselves refrain from claiming that this structure would be a good representation for the structure of PrP^{Sc}. Nevertheless, other researchers have adopted this structure as their favorite model of PrP^{Sc},[63] irrespective of the fact that it fails to account for the repeating unit size of 19.2 Å (= 4 β-strands high) in X-ray fiber diffraction experiments on PrP^{Sc} and PrP 27–30.[18]

(**11**) The most recent, complete models for the structure of PrP^{Sc} extend the idea of a parallel β-helix to the C-terminal portion of the PrP molecule.[64] Recombinant forms of PrP that comprise only the C-terminal α-helices (H2 and H3) had shown that this part of the molecule is prone to fibrillization and conversion to β-structure.[65] Here, a whole family of molecular models is discussed with separate β-helices being proposed for the N- and C-terminal domains, thereby eliminating any α-helical structure from the models. Individual models vary with respect to the position of the parallel β-helices (N-terminal and C-terminal), the number of β-helix rungs (three vs. four), and the threading of the prion protein sequence onto the β-helix templates. Little effort was made to assemble the many different models into in silico amyloid fibrils, which makes it difficult to judge if any of them would fit with the X-ray fiber diffraction data.[18] Furthermore, all these models share as a basic feature the presence of a long unstructured loop connecting the two (N- and C-terminal) β-helical domains, which was introduced to render the models compatible with EM data of recPrP amyloid fibers exhibiting a ladder-like appearance.[66] However, this ladder-like appearance is exclusive to that particular sample preparation, and has not been observed in PrP^{Sc} or PrP 27–30 fiber samples. Also, none of the models appear to be able to satisfy the constraints provided by limited proteolysis with PK,[36] but then this particular analysis has only been done on one prion strain. Other strains may give cleavage patterns that differ with respect to the individual cleavage sites, and thus be more permissive.

In summary, the molecular models for the structures of PrP^{Sc} and PrP 27–30 span a wide range of architectures and generally do not agree on a single feature or type of β-structure to explain the properties of the infectious prion. Nevertheless, these models provided valuable tools to interpret and discuss experimental data that were obtained with various techniques, preparations, and constructs of PrP. The disagreements between the different models also made for lively discussions in the literature and at conferences, to say the least. In any case, recent advances in experimental approaches and the interpretation of previously collected data have effectively ruled out all published models, and thereby issued an invitation for renewed efforts to model the structure of PrP^{Sc}.

Future Approaches

As mentioned above, molecular modeling will have a role to play in future approaches to understand the structure of PrP^{Sc}, but new experimental methods will be needed to provide data to restrain and test those models.

Experimental techniques that are poised to provide new insights into the structure of PrP^{Sc} include electron tomography and helical reconstructions of electron micrographs of individual PrP^{Sc} and PrP 27–30 amyloid fibrils. Neither of these techniques is going to provide high-resolution structural information, nevertheless recent technical advances will permit to extract unprecedented levels of detail from the images of individual fibrils, allowing to visualize molecular features that define the structure of PrP^{Sc}.

Another promising approach is based on the development of techniques that allow for the efficient production of PrP^{Sc} from recombinant sources in vitro.[67-69] H/D exchange in combination with mass spectrometry[70] or solid-state NMR spectroscopy[71]

should be able to provide structural information on a local level complementary to the data available by electron microscopy.

Prions were found to escape the selection pressure of anti-prion compounds in vivo through the selection of drug-resistant, structural variants.[72,73] Based on this observation, each prion strain appears to be a mixture of slightly different and structurally related conformers, which has been subsumed under the term "quasi species."[74] Within a quasi species ensemble the most efficient replicators will dominate based on their adaptation to the local environment. Unfortunately, this finding spells trouble for the structural analysis of prions, since most techniques rely on bulk measurements that average over large number of molecules. Except for techniques, such as EM, that can analyze exceedingly small samples, it will be a challenge to deal with this particular problem.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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