Review

Intriguing nucleic-acid-binding features of mammalian prion protein

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In transmissible spongiform encephalopathies, the infectious material consists chiefly of a protein, the scrapie prion protein PrP^{Sc}, that carries no genetic coding material; however, prions are likely to have accomplices that chaperone their activity and promote the conversion of the cellular prion protein PrP^{C} into the disease-causing isoform (PrP^{Sc}). Recent studies from several laboratories indicate that PrP^{C} recognizes many nucleic acids (NAs) with high affinities, and we correlate these findings with a possible pathophysiological role for this interaction. Thus, of the chaperones, NA is the most likely candidate for prions. The participation of NAs in prion propagation opens new avenues for developing new diagnostic tools and therapeutics to target prion diseases, as well as for understanding the function of PrP^{C}, probably as a NA chaperone.

Prions: a trespasser in the world of infectious agents

The concept that proteins are the causative agents for transmissible spongiform encephalopathies (TSEs) challenged the traditional paradigm that disease transmission results solely from an agent that carries genetic information. Mammalian prions are infectious agents that cause neurodegenerative diseases affecting humans and animals; these diseases can be infectious, sporadic or inherited [1]. As they present an infectious character, these TSEs (or prion diseases) are unique among neurodegenerative conditions that are also linked to structural changes in normal cellular proteins, such as Alzheimer's Disease (AD) and Parkinson's Disease (PD) [2,3]. To date, this infectivity is attributed to conversion of the cellular prion protein (PrP^{C}) into an abnormal structural isoform, the scrapie PrP (PrP^{Sc}). PrP^{C} is a glycoprotein expressed in different mammalian cells, and its turnover includes transit from the outer cell membrane, where PrP^{C} is attached through a glycosylphosphatidyl inositol bridge, to the cytosol. In its normal, α-helical-rich form, PrP^{C} is sensitive to proteinase-K treatment and has a defined structure, with a globular C-terminal domain and an unfolded N-terminal domain [4]. The PrP^{C} isoform binds copper ions at its N terminus, and it also binds other ligands that might be relevant for deciphering the still unknown function of this protein [5].

PrP^{Sc} was named from the first well-characterized prion disease in animals, scrapie, which affects sheep. This isoform is highly resistant to proteinase-K digestion, occurs as aggregates in infected animals, and has a greater proportion of β-sheet secondary structure than PrP^{C} [6,7]. To unravel the mechanisms through which prion diseases occur, it is vital to address how PrP^{Sc} is formed and how a protein can possess an infectious character in the absence of any genetic material.

The 'protein-only hypothesis' was originally based on the mechanistic model proposed by J. S. Griffith involving seeding, catalyzing conversion and oligomerization [8]. The 'protein-only hypothesis' gained great support when the group of the Nobel laureate Stanley Prusiner identified a protein that could propagate the disease when injected into healthy animals [9]. This hypothesis postulates that prion protein is the main agent that causes TSEs [8,9]. Prusiner named the protein 'prion' owing to the description of this agent as a proteinaceous infectious particle. His discovery was a hallmark not only for the understanding of these unusual diseases but also because it changed the molecular biology paradigm whereby a protein could never be an infectious agent without an associated nucleic acid, which was the only known replicative unit at that time. The construction of PrP-knockout mice helped to establish this hypothesis – PrP^{C} knockout mice are resistant to prion infection [10]. This result decisively established that endogenous PrP is necessary for prion propagation and infection and helped us to understand the role of PrP^{C} in the development of the disease. A definitive proof of the 'protein-only' hypothesis is still lacking today, however, because one cannot exclude the possibility that other factors from the host, apart from PrP^{C}, could participate in prion propagation.

Although no additional molecule implicated in the pathogenesis has been found in PrP^{Sc} samples, the presence of a cofactor is still not completely ruled out. In parallel with numerous discussions about the presence or absence of any non-proteinaceous material in PrP^{Sc} samples, several host macromolecules have been suggested as candidates for conversion catalysts [11]. Cellular adhesion molecules, nucleic acids (NAs), basal membrane molecules and sulfated glycans, among other biological macromolecules, have been reported to interact with PrP^{C} and to induce or modulate its conversion into a β-sheet-rich structure that shares many features with the infectious PrP [12–14].

Here, we focus on evidence gained over the past eight years regarding the NA-binding properties of PrP

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0968-0004/$ – see front matter doi:10.1016/j.tibs.2007.11.003 Available online 19 February 2008

Table 1. Interaction of prion protein with nucleic acids: timeline of key findings

Year	Scientific achievement	Refs
1967	Griffith postulates that a protein might be responsible for the propagation of scrapie	[8]
1974	Ward *et al.* report strong evidence against the possibility of scrapie being a viroid	[70]
1982	Prusiner shows that a protein is the main agent responsible for scrapie propagation	[9]
1999	Nandi and Leclerc observe oligomerization of PrP^{C} into proteinase-K-resistant structures induced by DNA	[18]
2001	Cordeiro *et al.* demonstrate high specificity and affinity for DNA sequences and the catalytic action of the PrP–DNA complex on the PrP^{C}–PrP^{Sc} conversion	[13]
2003	Rhie *et al.* characterize RNA aptamers capable of inhibiting PrP conversion	[47]
2003	Deleault *et al.* show that RNA molecules stimulate PrP conversion *ex vivo*	[42]
2004	*Ex vivo* measurements identify PrP^{Sc} bound to chromatin in the nuclei of scrapie-infected cells	[62]
2004	Cordeiro *et al.* show that binding of NA to PrP^{C} competes with binding of aniline naphthalene sulfonate compounds	[49]
2005	Safar *et al.* demonstrate new experiments reinforcing the absence of DNA-based infectivity in prion purifications	[27]
2005	Castilla *et al.* generate infectious scrapie prions *in vitro*	[71]
2006	Lima *et al.* present the low resolution structure of a PrP:DNA complex and show that the high-affinity PrP interaction with DNA is mediated chiefly by the globular domain and by part of the disordered domain	[44]
2006	Mercey *et al.* demonstrate the reversible nature of PrP interaction with RNA aptamers	[72]
2006	Kocisko *et al.* develop modified oligonucleotides with potent anti-scrapie activity	[55]
2007	In a high-throughput screening, King *et al.* map a sequence and structural recognition consensus in DNA aptamers for PrP binding	[40]
2007	Deleault *et al.* show that *in vitro* propagation of infectious PrP^{Sc} can be accomplished with a minimal set of components, including native PrP^{C}, co-purified lipid molecules, and synthetic NA	[51]
2007	Immunization experiments by Kaiser-Schulz *et al.* indicate that both prophylactic and therapeutic immunization can be achieved using the complex of dimeric PrP and CpG oligonucleotides	[68]

(Table 1), and try to clarify the importance of these ubiquitous macromolecules in prion biology and in the occurrence of the transmissible spongiform encephalopathies.

Can prion protein alone cause disease?

A remaining challenge in the biology of TSEs is to understand how PrP^{C}, an α-helical-rich protein, undergoes profound conformational changes to acquire higher β-sheet content to become PrP^{Sc}. In the sporadic and most common form of the disease, no mutations have been identified in the gene that encodes PrP, and both isoforms – PrP^{C} and PrP^{Sc} – share the same amino acid sequence [1,15]. Moreover, no covalent modifications have been identified that can distinguish between the two isoforms. The conversion process is poorly understood and the main consensus in the field postulates that PrP^{Sc} 'multiplies' by catalyzing the conversion of PrP^{C} into a likeness of itself, thus becoming responsible for its own propagation [16]. However, the mechanism by which this event takes place is still an open question, and it has been suggested by several groups that an additional, as yet unknown, factor might initiate or modulate the PrP^{C}-to-PrP^{Sc} conversion [13,17–19]. This hypothetical cofactor molecule would act by lowering the free-energy barrier between PrP^{C} and PrP^{Sc}, thus triggering conversion [20,21] (Figure 1).

Biophysical studies have provided a detailed characterization of the thermodynamic properties of the α-helical PrP^{C} and *in-vitro*-generated β-sheet-rich species presenting PrP^{Sc}-like characteristics [22–24]. High-pressure fourier transform infrared spectroscopy and pressure perturbation calorimetry studies have demonstrated that PrP^{C} is more hydrated and has a larger solvent-accessible surface area than aggregated β-sheet-rich recombinant PrP (β-rPrP) obtained by thermal treatment [23]. The role of hydration in the folding stability and aggregation of PrP is supported by molecular dynamics [25]. As represented in Figure 1, binding of a cofactor or a catalytic effector would lead to a decrease in solvent-accessible surface area and a decrease in the level of hydration. The finding that PrP^{C} is highly hydrated is consistent with the observation that the protein has a long disordered segment (the N-terminal domain) and the globular C-terminal domain is structured but relatively flexible and not well packed when compared with classical globular proteins [23,25]. Some protein domains, as well as some full-length proteins, are intrinsically unstructured [26], but functional, such as the transcriptional activator cyclic-AMP-response-element binding protein (CREB)-binding protein (CBP) [26] and α-synuclein, the protein related to the pathogenesis of PD [2].

On binding to biological targets, disordered segments can fold, becoming less hydrated. This could happen for PrP^{C} either during binding to another molecule, such as a nucleic acid, or during assembly into oligomers or amyloid fibrils. Either the protein–water interactions would be

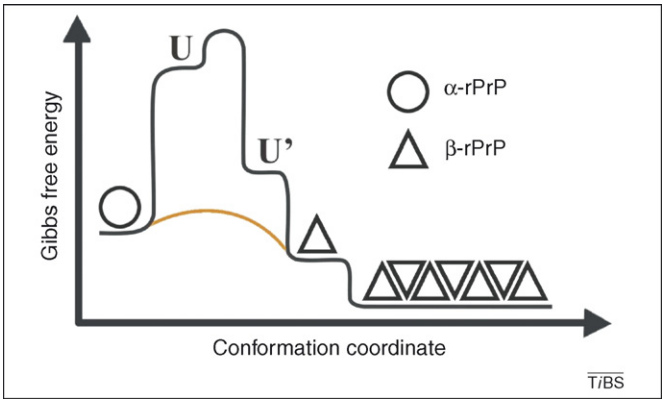

Figure 1. Free-energy profile for effector-catalyzed conversion of α-helical PrP into the β-sheet isoform. This free-energy diagram highlights a model in which the cellular isoform (represented here by α-helical recombinant PrP, α-rPrP, depicted by a circle) is in a metastable conformation, in a slightly higher chemical potential than β-rPrP (this form accounts for β-sheet-rich recombinant PrP species, depicted by a triangle). This diagram considers that α-rPrP is slightly less stable than β-rPrP, suggesting that the folding of the α-helical conformation is under kinetic control [20]. U and U′ represent unfolded states of the protein. The catalytic effector (CE, depicted in red) is a hypothetical cofactor molecule that would lower the free-energy barrier between α-rPrP and β-rPrP, triggering conversion (Figure modified from Refs [21,23].).

replaced by protein–nucleic-acid or by protein–protein interactions.

Nucleic acid binding properties of PrP^C

Since the proposal of the protein-only hypothesis [8,9], the participation of NA molecules in prion propagation has been excluded based on findings that PrP^{Sc} infectivity is decreased by protein-modification protocols, but not by nucleic-acid-modification treatments. In a broad study, Prusiner's group showed that an infectious unit could not contain any NA larger than 100 nt, which ruled out the possibility of a viral particle being involved [27]. Several groups have demonstrated, however, that PrP can bind NAs *in vitro* and *ex vivo* with varying affinities, and have shown that the interaction of PrP with DNA or RNA can lead to conformational changes both to the protein and to the NA molecule [13,28–30].

RNA aptamers that bind Syrian hamster recombinant PrP have been described [31], and PrP displays NA binding and activity comparable to that observed for retroviral nucleocapsid proteins [32,33]. Indeed, Gabus *et al.* [33] reported that human PrP possesses DNA strand transfer properties similar to those of HIV-1 retroviral nucleocapsid protein. Several groups have described the formation of large nucleoprotein complexes following PrP–NA binding (RNA or DNA) [28,29]. These findings led to the suggestion that PrP might be involved in NA metabolism *in vivo*, another possible function for PrP^C that awaits experimental verification.

It is interesting to speculate whether PrP^C possesses NA chaperoning properties: NA-binding by PrP^C can lead to structural rearrangements in both binding partners, with folding/refolding of the NA and folding/oligomerization of the protein [13,18,30,32] (Box 1). NA chaperones correspond to a growing family of ubiquitous proteins with key biological functions in DNA maintenance, RNA transport and translation, and more recently, in short interfering RNA (siRNA)-mediated silencing [34,35]. Moreover, these proteins also play essential functions in virus replication and diversity [36]. RNA-chaperoning activity includes specific and non-specific RNA binding [37]; therefore, it is an open possibility that the prion protein might lay on the latter class – because to date there is known no single specific NA sequence recognized by PrP (Box 1).

As recently emphasized by Caughey and Baron, many ligands for PrP^C have been ascribed [11]. It is noteworthy, however, that nanomolar affinities are observed for some complexes with DNA or RNA, suggesting that these molecules represent interesting candidates for *in vivo* PrP binding [13,38–40]. Binding of PrP to small double-stranded NA sequences increases the β-sheet content of the protein and, depending on the PrP:NA molar ratio, protein aggregates can form [13,29]. The aggregates formed upon the interaction of PrP with nucleic acids are mainly amorphous although, under some conditions, NA binding can promote partial unfolding of PrP, triggering formation of an amyloid-like structure that resembles PrP^{Sc} in its resistance to proteinase-K digestion [13,29,41,42].

To date, several high-resolution structures of PrP have been reported [4,43]. Until recently, however, no structural data had been obtained for PrP complexed to NA. A year ago, we reported the low-resolution structure of PrP in complex with an 18 bp double-stranded DNA (dsDNA), derived from small-angle X-ray scattering (SAXS) and NMR measurements [44] (Figure 2). The full-length mouse recombinant PrP binds to this dsDNA sequence with an affinity in the nanomolar range at physiological ionic strength. SAXS studies indicate that the C-terminal globular domain of PrP is important in the formation of the complex, and NMR HSQC spectra reveal changes that are clustered in two major regions: one in the disordered N-terminal portion of PrP and the other in the C-terminal globular domain. Recently, an *in silico* approach [45] yielded a prediction of the PrP DNA-binding site consistent with the NMR chemical shift perturbation data [44] and supported the idea that the PrP globular domain recognizes DNA mainly through the helix 1 (Figure 2). Clearly, higher-resolution structural studies on PrP complexed to NAs are required as the structural description of a PrP–NA complex is an important pre-requisite for understanding

Box 1. From prion–nucleic-acid interaction to a putative NA-chaperone function

Several nucleic-acid-binding proteins have been studied for decades, and classified according to their structural recognition binding motif [73]. Some proteins are able to recognize a plethora of sequences, such as the tumor suppressor p53 and polymerases, and others bind specifically a responsive element, such as the nuclear receptors and regulatory proteins from viruses and other microorganisms. Protein–NA dissociation constants for these proteins range from picomolar (in very low stringency conditions) to nanomolar levels, and suggest a hierarchical preference of binding. However, even for those DNA-binding proteins said to be specific, with well-characterized sequence and structural motifs from both elements involved in complexation, there are non-specific, scrambled DNA sequences that can be bound by these proteins with relatively high affinity (nanomolar levels). The stable union of PrP^C and polynucleic acids (single-stranded and dsRNA/DNA) has been demonstrated by independent research groups using a variety of cellular, biochemical and biophysical approaches. The concomitant presence of PrP^C, PrP^{Sc}, RNA and DNA both in the cytosol and in the cell nucleus raises the possibility of formation of different assemblies, which might lead into conformational changes in the protein and in the nucleic acid (bending, unwinding and folding). In the presence of PrP^{Sc}, the likely effect is cell death caused by the toxic aggregates. In the absence of PrP^{Sc}, a yet-to-be-identified nucleic acid chaperone function is the most probable. As reviewed by Tompa and Csermely [46], there is a remarkable high frequency of disordered domains in nucleic acid chaperones. Ordering of the chaperone with a concomitant unfolding of the substrate (entropy transfer) has been demonstrated for several RNA chaperones [46]. Binding of NA by the prion protein occurs with ordering of the N-terminal domain [44] as well as structural modification of the NA [13,30]. This malleability of the prion protein has been alluded to elegantly in a recent book by Jonah Lehrer [74] in the context of the long-term memory, its fallibility, and in the light of Marcel Proust's literary masterpiece *In Search of Lost Time*. Lehrer conveys the plastic properties of the prion protein and of a related protein, CPEB (cytoplasmic polyadenylation element binding protein), which has a repetitive domain similar to that found in yeast prions [75]. Curiously CPEB is a NA-binding protein that acts as a translational regulator, one that activates dormant mRNAs by elongating their poly (A) tails. The question of which nucleic acids (ss/ds, RNA or DNA) can serve as the natural partners of PrP^C, and indeed what might be the cellular function of the resulting complex, are targets for future research.

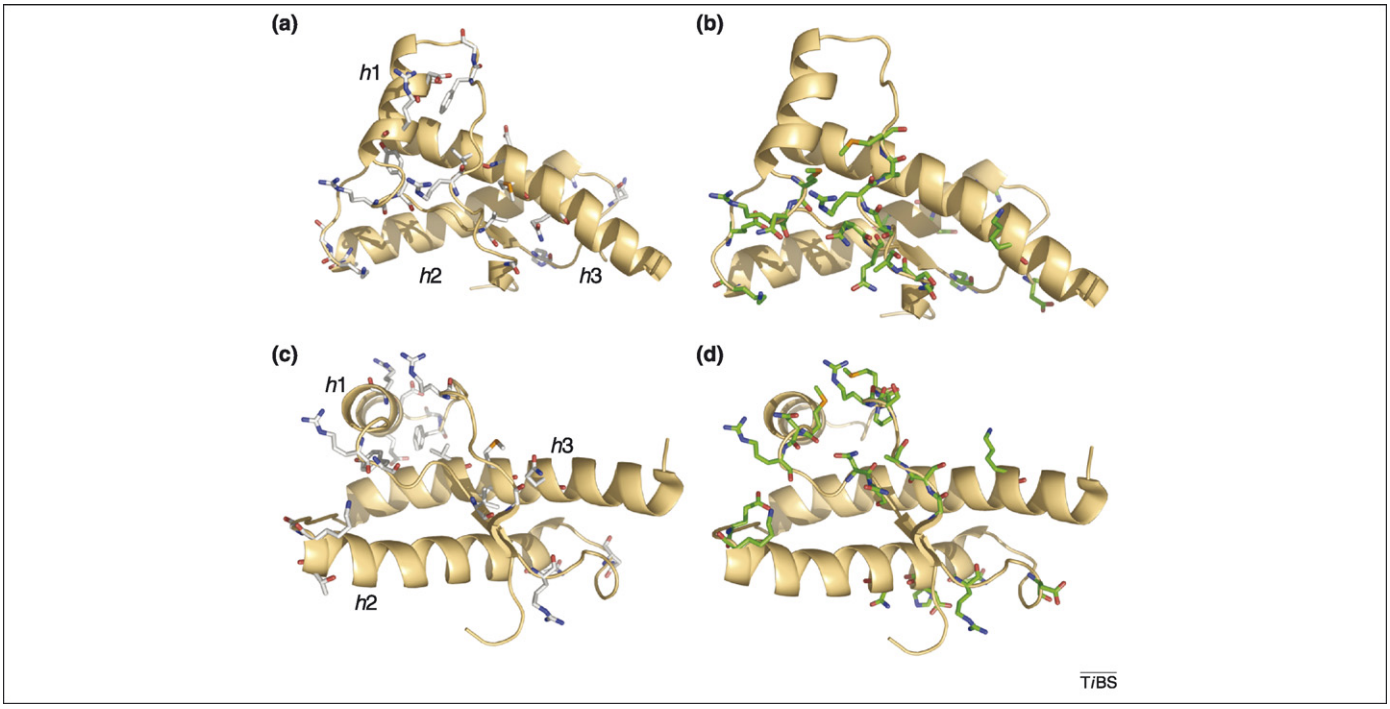

Figure 2. Mapping DNA-binding regions of prion protein using direct NMR measurement and predictive analysis. Insights into the most probable NA-binding sites in the PrP have been recently achieved using NMR chemical shift perturbation [44] and computational predictive analysis [45]. The figure represents two topological views (upper and lower panels, rotated 90°) of mapped DNA-binding regions of prion protein. Depicted amino acids correspond to those assigned by the methods mentioned above, which accounts for the DNA interaction. **(a,c)** PrP NMR chemical shift changes induced by DNA binding [44]. **(b,d)** Computer-aided predictive analysis of DNA binding sites in PrP [45]. Images were generated using PyMol (http://pymol.sourceforge.net/). *h1*, *h2* and *h3* are the three helices in PrP^{C}.

how oligonucleotides bind PrP and for the design of anti-prion compounds based on nucleic acids.

Catalysis of the conversion of PrP by nucleic acids

One of the more intriguing characteristics of the interaction between PrP and NAs is the finding that some NA sequences can act as catalysts for the formation of a scrapie-like PrP conformation. NA-induced polymerization of recombinant PrP and NA condensation was first observed upon rPrP–NA interaction by Nandi and Leclerc [18]. In 2001, we demonstrated that the high-affinity binding of some small dsDNAs to recombinant murine PrP (rPrP23–231) converted it from the α-helical conformation (cellular isoform) into the soluble, β-sheet isoform [13]. Whereas in high concentrations, the DNA sequences inhibited aggregation of prion peptides, the soluble complex PrP–DNA could catalyze the conversion and induce aggregation. These results led us to first propose the hypothesis of NA-catalyzed PrP conversion [13]. Accordingly, host NAs might catalyze the conversion between PrP^{C} and PrP^{Sc} by acting as a scaffold and thereby making the protein–protein interactions more likely [21]. As pointed out in the diagram in Figure 1, the free energy barrier between PrP^{C} and PrP^{Sc} would be lowered, probably by rearrangement of the protein without necessarily passing through the unfolded state. Both aggregation reaction and NA binding occur with a decrease in hydration [23,24,44], which might explain the bypass of the unfolded state. As discussed above, these mutual coupled rearrangements are typical of NA chaperones [46] (Box 1).

Our results have been confirmed and supported by other groups, who have also reported the specific interaction of PrP^{c} with NAs (Table 1). PrP^{C} interacts with mammalian RNA preparations *in vitro* and subsequently acquires resistance to protease digestion [42]. Highly structured RNAs can also convert human PrP^{C} into PrP^{Sc}-like forms [28], and some RNA aptamers bind with high specificity to PrP^{Sc} [38,47]. The N terminus of PrP^{C} is important for this interaction, because mutants lacking different portions of the N terminus bind some RNA aptamers with lower affinity, or not at all [31,48].

NAs might serve as a catalyst for prion propagation (Figure 3). This model is consistent with *in vitro* biophysical studies [13,29,41,44,49], as well as with the fact that polyanions, especially NAs, increase the level of protease-resistant PrP (PrPres, a fraction of the disease-associated prion protein PrP^{Sc} that is partially resistant to proteolysis) amplification *in vitro* [42,50,51]. In the latter studies, Deleault *et al.* [51] provided compelling evidence for the PrP-host derived nucleic acid hypothesis for prion replication using the protein misfolding cyclic amplification (PMCA) technique. PMCA involves the amplification of PrPres from a small PrP^{Sc} fraction mixed with PrP^{C} until no more original PrP^{Sc} units are found in the infectious material [52]. Using this approach with a preparation containing only native PrP^{C} and co-purified lipid molecules, the authors showed that successful PMCA propagation of PrP^{Sc} molecules in a purified system required accessory polyanion molecules (synthetic RNA). Moreover, *de novo* formation of PrP^{Sc} molecules from these defined molecules in the absence of pre-existing prions was observed and inoculation of purified, *in vitro*-generated PrP^{Sc} molecules into hamsters caused disease [51]. This seminal work shows

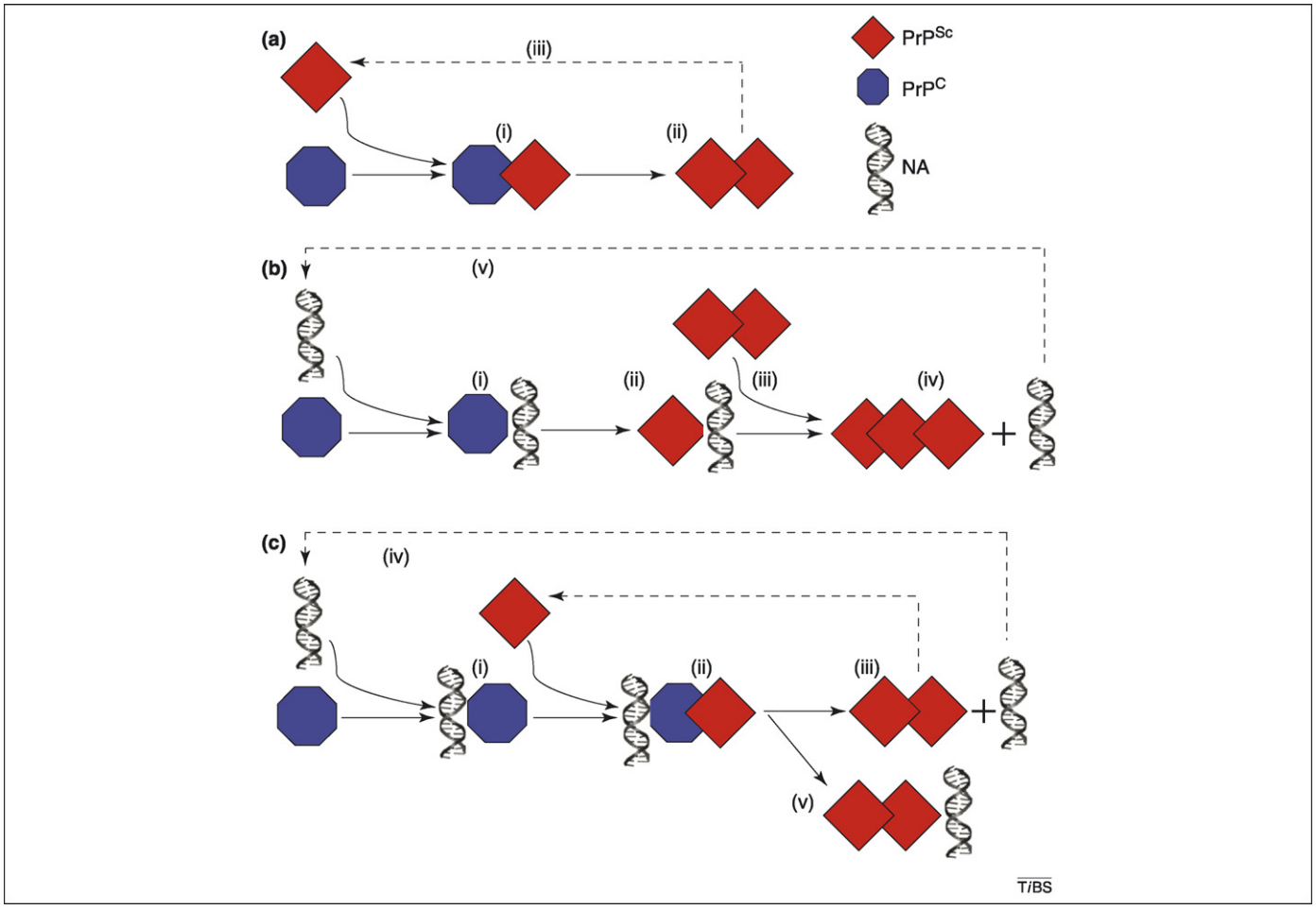

Figure 3. Models for nucleic acid-mediated prion propagation. The models depicted in this figure propose how a NA (gray) can act as a catalyst and/or molecular chaperone for the PrP^{C} (blue) to PrP^{Sc} (red) conversion. **(a)** The template-assisted refolding model for PrP^{C} conversion to PrP^{Sc}. PrP^{Sc} (exogenous PrP^{Sc} or generated from spontaneous conversion of PrP^{C} into PrP^{Sc}) interacts directly with PrP^{C} (i) and helps the latter to assume the misfolded, PrP^{Sc}-like conformation (ii). Formed PrP^{Sc} is further able to catalyze conversion of more PrP^{C} into the infectious species (iii). **(b)** Binding of NA by PrP^{C} (i) induces the acquisition of a PrP^{Sc}-like, β-sheet-rich conformation (ii). Following the addition of PrP^{Sc} oligomers (iii), an aggregate is formed and the nucleic acid is released from the complex (iv). It is then able to catalyze another conversion event (v). **(c)** Formation of the PrP^{C}–NA complex (i) would act as a scaffold for binding of PrP^{Sc} to the protein part of the complex (ii), leading to conversion of the innocuous, NA-bound form into a scrapie-like conformation, giving rise into the net conversion of more PrP^{Sc} (iii). The NA could be released from the complex, after PrP^{Sc} formation (iii) being able to further convert PrP^{C} into PrP^{Sc} (iv), or could be irreversibly incorporated into the infectious aggregated particle (v). In the *in vitro* studies, the molar ratio between PrP and NA dictates the extent of aggregation, so that an apparently irreversible process in the direction of PrP aggregation predominates in a high PrP to NA ratio. It must be stressed that there are no experimental data to date that compare affinities of PrP^{C} and PrP^{Sc} towards the same nucleic acid. Therefore, the models represented in this figure are tentative and the main idea is to provide the reader with a general view of the possibilities of PrP conversion catalyzed by NA.

that infectious prions can be formed from a minimal set of components including native PrP^{C} molecules, co-purified lipid molecules, and a synthetic NA, stressing the possible role of nucleic acids in PrP^{Sc} generation. Their results corroborate the hypothesis that endogenous polyanions (such as NAs) can affect the rate of prion propagation by acting as scaffolds or surfaces that facilitate the interaction between PrP^{C} and PrP^{Sc} molecules, as previously proposed [13].

The models proposed (Figure 3b,c) show how a NA can act as a catalyst and/or molecular chaperone for the PrP^{C}-to-PrP^{Sc} conversion in a process that does not rely on the encoded genetic information within the NA [13,21]. The precise mechanism behind this catalyzed conversion is still not completely understood, but the highly charged environment around the NA (as in the case of glycosaminoglycans [GAGs]) could contribute to the conversion. In both models, the formation of large aggregates of PrP^{Sc} would need the presence of exogenous PrP^{Sc} (probably the non-fibrillar oligomeric species, with masses equivalent to 14–28 PrP molecules, recently described by Silveira *et al.*, [53]), which would rescue the already converted PrP^{Sc} from the NA. The freed NA would then be able to catalyze another conversion, amplifying the process.

Interestingly, a hydrophobic naphthalene-derived compound (bis-ANS, 4,4′-bis[1-anilinonaphthalene 8-sulfonate]) can inhibit the aggregation of small prion domains (residues 109–141 and 109–149 from Syrian hamster PrP) or result in limited oligomerization of the full-length protein [49]. To some extent, these effects are analogous to those caused by NAs [13]. In fact, bis-ANS and dsDNA oligonucleotides compete for binding to PrP^{C} [49]. It is commonly believed that small oligomers perform a crucial function for the assembly and stabilization of larger aggregates in general [2,3] and oligomers of the amyloid peptide Aβ are also stabilized by bis-ANS and other sulfonated hydrophobic molecules [54].

Prions: the missing link between the protein and nucleic acid worlds?

It is quite extraordinary that the PrP^{Sc}, which, as an infectious agent that carries no genomic nucleic acid, stands as the only known exception to the molecular biology dogma, is also derived from an isoform (PrP^{C}) that binds NAs with relatively high affinity. Clearly, there is no correlation between the nucleic acid segments (DNA or RNA) that bind PrP^{C} and those of a genomic NA (which would encode the PrP protein and coat the protein, as in a viral particle). The described NA-dependent effects occur via sequences with no known cellular functions and which are also much smaller than those that could carry enough genetic information for subsequent PrP transcription and translation [13,40,47,55]. The full human PrP gene sequence contains 35 522 bp [56]. Taking out the intron, the two remaining exons contain around 2500 nt; and the single-coding exon (~2 kb) is approximately at least one order of magnitude larger than the sequences that have been described to bind or to affect conversion.

So is there any *in vivo* functional implication for the NA-binding properties of PrP^{C}? Although PrP^{C} is indispensable for replication of the infectious prion agent and for the initiation of prion disease [1,10,57], the physiological function of PrP^{C} remains unclear. Many different functions have been attributed to PrP^{C}, including signal transduction, copper binding, synaptic transmission, regulation of the immune system, pro-apoptotic or anti-apoptotic effects [5,11,13,58], and participation in NA metabolism [29,32,33]. It is still unclear, however, how these activities are related to the ability of PrP^{C} to interact with other proteins, glycosaminoglycans and nucleic acids.

The interaction of PrP^{C} with small, non-coding transcripts (e.g. microRNAs) as part of the functional or pathogenic cycle of the protein cannot be ruled out. To date, there are around 500 known human microRNA sequences (each ~21 nt), which bind to multiple mRNA targets [59], modulating their coding. There are no systematic studies on the interaction between PrP and noncoding RNAs, however. In most experiments with DNA and RNA, the highest affinities for PrP^{C} were obtained for small NA sequences [13,28,40,47]. With the capacity to bind both small RNAs and DNAs, PrP might exert its function by participating in the gene regulation array at the post-transcriptional level, probably acting as a NA chaperone [30] (Box 1). Of interest, PrP has been shown to interfere with the synthesis of HIV-1 proteins [60], implying an interaction with retroviral RNA. Moreover, very recent studies provide strong support for an essential function of PrP in the control of activated endogenous retroviruses [61].

It is intriguing that PrP^{Sc} can migrate to the nucleus and interact with chromatin in a mouse neuroblastoma cell line (N2a) infected with PrP^{Sc} [62]. Although it has been shown that proteasome inhibition can promote PrP cytosolic accumulation and cellular toxicity [63], PrP^{Sc} accumulates in the nucleus of prion-infected cells via a microtubule-dependent transport pathway, even when proteasome function is not impaired [62].

The cellular turnover of PrP takes ~10 h [64], and during this period of time PrP transits from the cell membrane to the cytoplasm and perhaps to the nucleus [62,65]. In Figure 4, the potential sites of interaction of PrP with NAs are indicated. From the evidence accumulated in our own studies and those of others, binding of PrP with a NA *per se* would not lead to conversion [13,42,51]. The net formation of PrP^{Sc} would also require the presence of seeding amounts of PrP^{Sc} in addition to NA, as outlined in Figure 3c. These triple encounters (PrP^{C}–NA–PrP^{Sc}) might occur either at the outer membrane (Figure 4d) or inside the cell (Figure 4h). If they occur in the cytoplasm, it is much more reasonable to suppose that an RNA molecule would be the culprit for conversion rather than DNA.

The use of modified oligonucleotides for early diagnosis and therapy of prion diseases

A particularly interesting aspect of the ready association of NAs with PrP concerns the possibility of using NAs as diagnostic tools. Some NA sequences bind differentially to PrP^{C} and PrP^{Sc}, and are apparently able to recognize PrP^{Sc} in diseased animals [38,39,47,48,55]. This observation means that it might be possible to develop reagents that allow the early diagnosis of TSE in animals and also in the pre-clinical stage in humans. Recently, Zou *et al*. [66] reported that an anti-DNA antibody captured PrP^{Sc}, demonstrating that PrP^{Sc} forms a complex with NA.

Two recent studies reinforce the view that modified NAs could be useful as therapeutic compounds against prion diseases. Kocisko *et al.* [55] showed that degenerate phosphorothioate oligonucleotides (PS-ONs) at nanomolar concentrations act as PrP^{C}–to-PrP^{Sc} conversion inhibitors and strongly reduced the level of PrPres associated with membranes in an *ex vivo* assay. PS-ONs also prolonged the lives of scrapie-infected mice if administered prophylactically and were capable of neutralizing scrapie titers in infected brain inocula. The maximum benefit was obtained with oligonucleotides of 20 to 30 bases, consistent with previous results showing that the PrP–oligonucleotide interaction is sterically defined.

More recently, Prusiner's group found that the binding of ssDNA thioaptamers to PrP occurs on at least two different sites on the protein [40]. Selection against recombinant Syrian hamster $PrP_{90–231}$ (recSHaPrP) identified a 12-bp consensus sequence within a series of 20 thioaptamers, all of which comprise 40 bp, and one thioaptamer bound to recSHaPrP with extremely high affinity (0.58 nM) [40]. The same group reported that the same short phosphorothioate DNA (PS-DNA) oligonucleotides diminish the levels of both PrP^{C} and PrP^{Sc} in prion-infected neuroblastoma (ScN2a) cells [67]. The reduction of PrP levels was independent of the nucleotide sequence and the effective PS-DNA concentration required to achieve half-maximal reduction in PrP^{Sc} was significantly lower than for PrP^{C}-level reduction. This finding indicates that diminished levels of PrP^{Sc} after exposure to PS-DNA are unlikely to be caused by decreased PrP^{C} levels. Moreover, bioassays in transgenic mice demonstrated a substantial loss of prion infectivity after injection of lysates of ScN2a cells previously exposed to PS-DNAs in these rodents [67]. It remains to be established whether such modified oligonucleotides will be useful in the treatment of prion disease in humans or livestock. The potential for therapeutic use of these molecules against TSEs underscores the

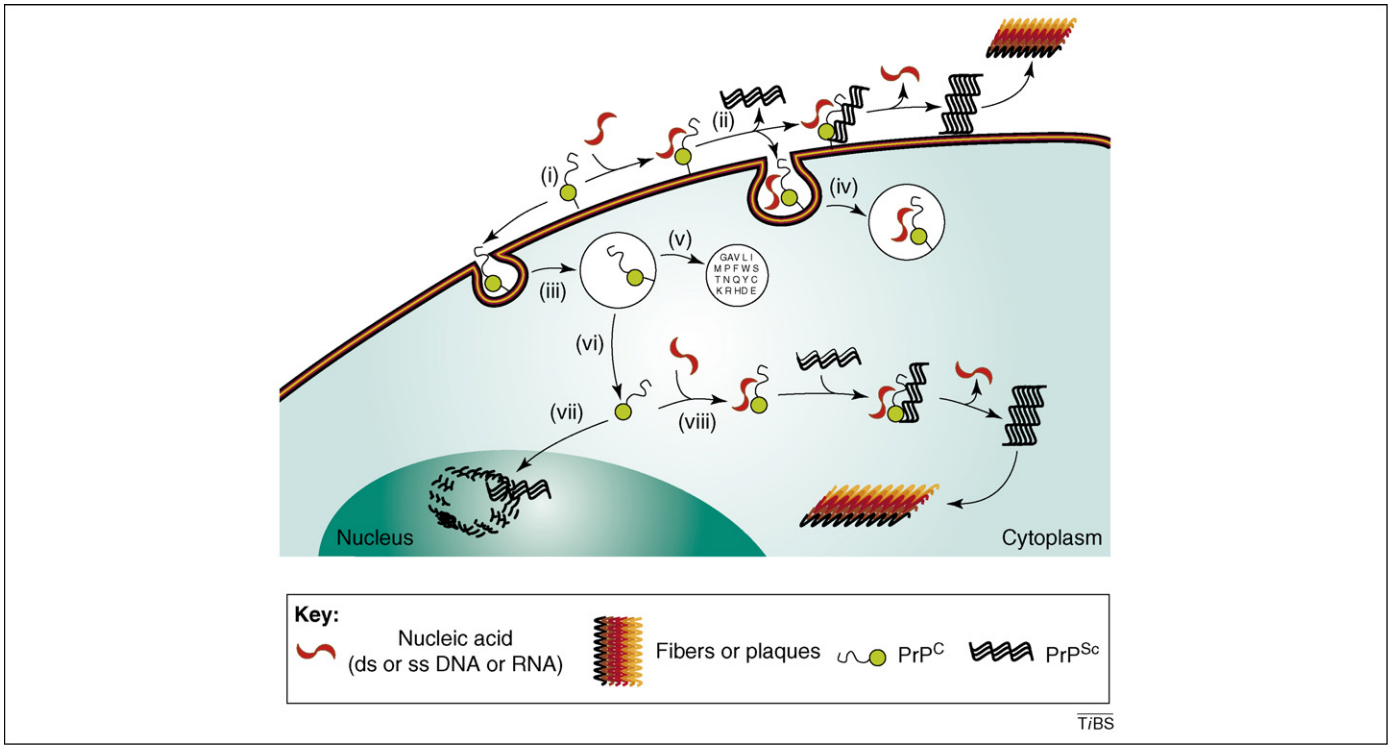

Figure 4. Hypothetical steps involved in nucleic-acid-binding by PrP in the cell. In the past decade much evidence has been accumulated suggesting that PrP can bind a NA either outside or inside the cell. There are several questions that need to be clarified, as outlined here: (1) Would there be a function associated with the NA binding properties of PrP^{C} and PrP^{Sc}? (2) How would the PrP^{C} exit the endocytic vesicles into the cytoplasm? (3) Both PrP^{C} and PrP^{Sc} have been found in the nucleus under some conditions [62,65], but is this physiologically relevant? (4) What is the main site where PrP binding to NA takes place? **(i)** Starting from the cytoplasmatic-membrane-anchored form, **(ii)** PrP^{C} might follow an extracellular pathway for NA-induced conversion and plaque formation), **(iii)** or both could be internalized in the apo or **(iv)** in the NA-complexed form to an unknown destination. **(v)** Inside the endoplasmic vesicle, the apo PrP^{C} would follow the usual proteolytic pathway, **(vi)** or, through mechanisms not elucidated, be translocated to the cytoplasm), **(vii)** and from there to the nucleus, **(viii)** or follow an intracellular pathway for NA-induced conversion and plaque formation. ss, single-stranded.

importance of understanding the interaction of PrP protein with different NAs at the molecular level.

Concluding remarks: a nucleic acid molecule is a likely cofactor for PrP conversion

The field of prion research has experienced great progress in recent years. However, it remains unclear how PrP^{C} and any cofactors, such as nucleic acids, lead to misfolding and conversion to infectious prions. A large number of studies using biochemical and biophysical techniques have led to a consensus that PrP binds to NAs with affinities in the nanomolar range. Continued investigation of the effects of modified nucleic acids on prion conversion offers promise for the development of successful TSE therapies in the near future. The prion–NA hypothesis offers an explanation for both the genetic and structural features of prion diseases [13,21]. A given NA sequence would act as a catalyst for the conversion of PrP^{C} into PrP^{Sc}, consistent with the model depicted in Figure 1 [20].

Our proposal that host NAs may exert their effects by reducing the protein's mobility and by making protein–protein interactions more likely has been reinforced recently by immunization experiments using dimeric PrP (tandem PrP) and CpG–oligonucleotide [68]. Kaiser-Schulz *et al*. propose that both prophylactic and therapeutic immunization can be achieved effectively using a complex of tandem PrP with the CpG oligonucleotide [68]. The recent finding that PrP^{C} protects against DNA damage induced by reactive oxygen species is also intriguing [69] and cannot rule out the possibility of a direct protective effect of PrP^{C} on DNA, which would require binding of the prion protein.

Altogether, the new biological and structural information on the PrP bound to RNA or DNA provides clues to how NAs can stimulate the conversion of PrP from a cellular protein into an infectious agent, and opens new avenues for the design of compounds that target prion diseases. Many pieces of the prion puzzle are still not yet in place, however. The NA-binding properties of the PrP (both RNA and DNA) may have much broader implications than those discussed here. A clear consensus is that PrP^{C} binds small DNAs and RNAs; and it is exactly the studies on small sequences of RNA that have undergone a big explosion in the past couple of years, with the conception that there are few cellular processes that do not happen under the surveillance of RNAs. The recruitment of non-infectious PrP by RNA could be just part of the story. The triple encounter between PrP^{C}, NA and PrP^{Sc} would be a side effect of the cellular function of the prion protein. We might be facing a paradigm shift in the area that will keep biochemists, neuroscientists and biologists busy for many years ahead.

Acknowledgements

We thank Martha M. Sorenson for carefully reading the manuscript. We also thank Diucenio A. R. do Carmo and Marco Aurélio C. Silva for help with the illustrations. The work in our laboratories was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Millenium Institute for Structural Biology in Biomedicine and Biotechnology (CNPq Millenium Program), Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro

(FAPERJ), Financiadora de Estudos e Projetos (FINEP) of Brazil, and by a grant from L'Oréal to Y. C.

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