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PrP interactions with nucleic acids and glycosaminoglycans in function and disease

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1. ABSTRACT

Since the first description of prion diseases, great effort has been made toward comprehending this new paradigm in biology. Despite large advances in the field, many questions remain unanswered, especially concerning the conversion of PrP^{C} into PrP^{Sc}. How this conformational transition evolves is a crucial problem that must be solved in order to attain further progress in therapeutics and prevention. Recent developments have indicated the requirement for partners of the prion protein in triggering the conversion. In the present review, we will explore the interaction of PrP with some of its most intriguing partners, such as sulfated glycans and nucleic acids. These molecules seem to play a dual role in prion biology and could be fundamental to explaining how prion diseases arise, as well as in the development of effective therapeutic approaches.

2. INTRODUCTION

2.1 Prion Diseases

According to the World Health Organization (WHO) website, between 1986 and 2002, almost 200,000 cases of bovine spongiform encephalopathy (BSE) were registered in Great Britain (WHO official website: www.who.int). The "mad cow disease" epidemic caused serious damage to the European economy at the end of the 1980s and the beginning of the 1990s and led to the death of thousands of cattle across Europe. Since the first description of variant Creutzfeldt-Jakob disease (vCJD) in humans in 1995, over 200 cases have been recorded in England, France, Ireland, Italy, and the USA.

Transmissible Spongiform Encephalopathies (TSEs) belong to a group of conformationally misfolding

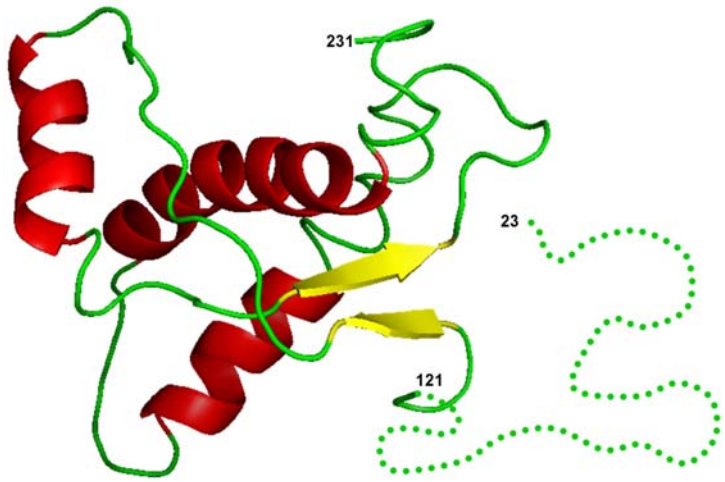

Figure 1. Mouse PrP high resolution structure. Fragment 121-231 of mouse PrP (rPrP121-231), PDB 1AG2. Alpha helixes 1, 2 and 3 are represented in red; beta strands 1 and 2 are shown in yellow; loops and turns are colored in green. The dotted line represents the unstructured amino terminal region of rPrP, containing residues 23-120. Adapted from Riek *et al.*, 1997 (17).

neurodegenerative disorders. They are rare, fatal, affect mammals (including humans), and are characterized, in general, by the loss of motor control, paralysis, and dementia (1). In addition to the hereditary and sporadic forms of prion disease, the group also possesses an infectious nature as well. All TSEs are progressive, fatal and incurable (1). The TSEs that affect humans are: Creutzfeldt-Jakob disease (CJD), the Gerstmann-Sträussler-Scheinker syndrome (GSS), Fatal Familial Insomnia (FFI) and the previously eradicated kuru.

The first description of a TSE dates back to the mid-eighteenth century, when the first cases of scrapie (the prion disease that affects sheep) compromised the expansion of the textile industry during the Industrial Revolution (2). At the beginning of the twentieth century, the first cases of Creutzfeldt-Jakob disease were reported in humans (3, 4). In the mid-twentieth century, kuru was identified as a TSE in Papua New Guinea, and resulted from contamination by the practice of ritualistic cannibalism, killing a large number of the local aborigine population (5). Subsequently, Alper and collaborators demonstrated that the infectious agent related to TSEs was highly resistant to treatments that affect nucleic acids, such as ionizing radiation and ultraviolet light (6), suggesting that a protein alone could infect and replicate in the absence of nucleic acids (7). In the 1980s, the neuroscientist Stanley Prusiner characterized the infectious agent and the concept of the prion: **pro**teinaceous **in**fectious particle (8, 9).

All TSEs, including scrapie in sheep and BSE in cow, have been described as single infectious agents that are isoforms of a constitutive protein known as the prion protein (PrP) (2). There are two main players involved in prion disease. The first is the cellular prion protein (PrP^C), an isoform that occurs naturally in cells of the host body (10). The second is a conformational variant of the first, which is involved in transmission of the disease, such as the prion scrapie (PrP^{Sc}) (11).

2.2. Cellular prion protein (PrP^C)

PrP^C (or PrP-sen, for protease-sensitive) is a constitutive and highly conserved protein among mammals (1). It is normally found in the membrane of mammalian cells in many tissues, such as kidney and skeletal muscle (2). PrP^c is found in particular abundance in the central nervous system (12), and also in lymphocytes and lymphoid organs (13). This protein is encoded by the *PRNP* gene contained in chromosome 20 in humans, and present only in one copy (12). Mature PrP contains 209 amino acid residues, two preserved glycosylation sites, and is anchored to the outer cell membrane through a glycosyl phosphatidyl inositol (GPI) anchor (1, 14).

The mouse prion protein is synthesized in the endoplasmic reticulum as a protein with 254 amino acid residues. The first 22 amino acids in the amino-terminal region localize the protein to the membrane. Post-translational processing attaches the C-terminal region of the PrP GPI anchor, fixing it to the outer portion of the cell membrane (1). There are two *N*-glycosylation sites present in asparagine residues 181 and 197 (in human PrP), to which are added a variety of sugar species (15, 16). PrP possesses a sequence of eight amino acid residues (PHGGGWGQ) in its amino-terminal region between residues 50 and 90 that are flanked by basic and hydrophobic segments (residues 23-50 and 99-120, respectively) (17, 18). This octapeptide region is highly conserved and contains four repetitions of these eight amino acids that function to bind copper (18, 19).

The tertiary structures of recombinant prion proteins ($rPrP^C$) from different species were determined by nuclear magnetic resonance (17, 20-22). The human PrP structure reveals the presence of a globular domain (residues 125 to 228) and a highly disordered amino-terminal domain (23). The globular domain contains three alpha-helices (residues 144-154; 173-194 and 200-228) and a small beta-sheet formed by two beta-strands comprising residues 128 to 131 and 161 to 164 (23) (Figure 1).

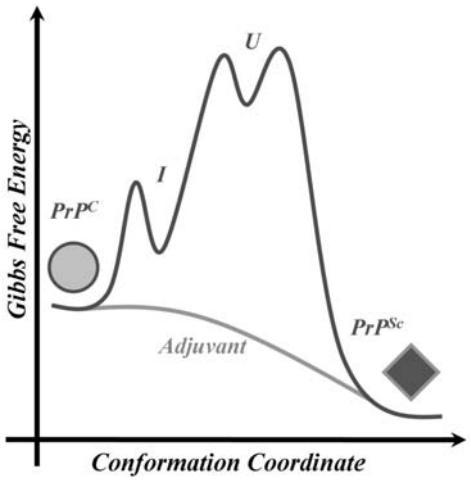

Figure 2. Conversion of PrP^{C} into the beta-sheet isoform free energy diagram. Unfolding of the cellular prion protein (PrP^{C}) and its subsequent misfolding to the scrapie isoform (PrP^{Sc}) has been implicated in transmissible spongiform encephalopathies. The two isofoms of PrP can be differentiated by their secondary structures: PrP^{C} is largely helical whereas PrP^{Sc} is enriched in beta-structure. The conformational transition is separated by a large energetic barrier that is associated with unfolding and oligomerization. I and U represent intermediate and unfolded states of the protein. For these reason, it seems likely that other molecules are crucial for prion propagation acting as an adjuvant factor by lowering the energy barrier. Adapted from Cordeiro et. al., 2001 (48).

The *Prnp* gene was identified in the 1980s. It is located on chromosome 2 in mice and 20 in humans (see (24) for review). The knockout mouse for this gene was generated in 1992 (25). Today, almost three decades after the identification of the *Prnp* gene and 16 years after construction of the first prion protein knockout animal, little is known about its physiological function. Over the years, many functions have been assigned to PrP^{C}, including immune regulation, signal transduction, copper metabolism, nucleic acid processing, synaptic transmission, protection, and induction of apoptosis (26-28).

Despite all efforts to elucidate the physiological role of PrP^{C}, a specific function has not been firmly established. Many biological partners for PrP have been identified that could be involved in its function. Based on the currently available data, it is suggested that the presence of the GPI anchor indicates the requirement for PrP to bind a transmembrane protein to transfer an extracellular signal to the intracellular space, indicating PrP may function as an accessory protein (27).

The most studied ligand of the prion protein is copper (19), which interacts with PrP through its amino-terminal domain, suggesting a function in copper metabolism (28). Some studies have suggested that PrP^{C} is able to bind extracellular matrix macromolecules, such as laminin (29, 30) and the laminin receptor (31). PrP has also been shown to interact with and modulate the activation of plasminogen (32). Other ligands for cellular PrP have been reported: the chaperones Hsp60 and GroEL; STI1 (stress-inducible protein I); nNOS (Neural nitric oxide synthase); alpha-tubulin (33-36); and nucleic acids (37, 38).

2.3. The prion scrapie (PrP^{Sc}) and the conversion reaction

PrP^{Sc} is the isoform of PrP^{C} associated with prion diseases, and is enriched in beta-sheet structures, as measured by Fourier transform infrared spectroscopy (FTIR). PrP^{Sc} presents itself as an insoluble protein that has a tendency to aggregate, and is partially resistant to digestion by proteases. For this reason it is also called PrP-res (39). The protease resistant core forms amyloid fibrils and amorphous aggregates comprising residues 90 to 231 (40). Because of the tendency to form aggregates, determination of the three-dimensional structure of PrP^{Sc} by high-resolution techniques, such as X-ray crystallography and nuclear magnetic resonance, has proven to be a challenge. However, molecular modeling studies suggest that the formation of PrP^{Sc} involves the re-folding of residues 90 to 140 into beta-sheets (41). The single disulfide bond, which links the helices on the C-terminal region, would remain intact since this bridge is necessary for the formation of PrP^{Sc} (42). A recent model included the flexible region of the prion protein (residues 89 to 175), suggesting that this domain could also fold into beta-sheets (43).

The "protein-only hypothesis" was first outlined by Griffith (7) and developed by Stanley Prusiner (1). According to this theory, the prion protein is the main agent responsible for the outbreak of TSEs. The discovery that PrP knockout mice are resistant to infection by prions (25, 44) is one of the most solid foundations that support this theory. Changes in protein secondary structure from an alpha-helical-rich conformation to a higher beta-sheet content account for the physical-chemical differences between PrP^{C} and PrP^{Sc}. The mechanisms that lead to conversion of PrP^{C} into PrP^{Sc} are not yet fully understood, but there are several proposed models. In a previous cell-free assay, incubation of PrP^{C} with large quantities of PrP^{Sc} conferred resistance to protease digestion (45, 46), suggesting that PrP^{Sc} catalyzes the conversion of PrP^{C} into newly formed, protease resistant PrP^{Sc}. Accordingly, the scrapie form induces misfolding of PrP^{C} into more PrP^{Sc}, and is therefore responsible for its own propagation (47). PrP^{Sc} could act as a molecular template by helping PrP^{C} refold incorrectly to generate more PrP^{Sc}. No post-translational modifications have been identified that distinguish the two isoforms. The mechanism by which the conversion takes place is another open question. Several groups have suggested that an additional, unknown factor may initiate or modulate the conversion of PrP^{C} to PrP^{Sc} (38, 48-52). This hypothetical co-factor molecule would function to lower the free-energy barrier between PrP^{C} and PrP^{Sc} in order to trigger conversion (1, 15) (Figure 2).

Several biophysical approaches have been applied to characterize the thermodynamic properties of the alpha-helical PrP^{C} and beta-sheet species (53-55). The free-energy diagram depicted in Figure 2 highlights a model in

which the cellular isoform is in a metastable conformation. High-pressure FTIR and pressure perturbation calorimetry studies have demonstrated that the cellular PrP isoform is more hydrated and has a larger solvent-accessible surface area than aggregated a-rPrP obtained by thermal treatment (55-57). The role of hydration in the folding stability and amyloidogenicity of PrP has been corroborated by computer modeling and molecular dynamics (58, 59). As represented in Figure 2, binding of a cofactor or a catalytic effector (such as nucleic acid) 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-domain), and the globular C-domain is highly flexible and not well-packed. Upon binding to biological targets, disordered segments can fold and become less hydrated. This could occur with PrP^C when it binds a partner such as a glycosaminoglycan or nucleic acid. Alternatively, this could also happen by protein-protein interactions with assembling oligomers or amyloid fibrils. Below, we focus our review on the nucleic acid- and glycosaminoglycan-binding properties of the prion protein and try to clarify the importance of these ubiquitous macromolecules in prion biology, as well as in the occurrence of transmissible spongiform encephalopathies.

3. ARE COFACTORS NEEDED FOR PRION INFECTIVITY?

Since the proposal of the protein-only hypothesis, scientists have had little success generating infectious prion particles from recombinant prion protein (60, 61). Even the generation of detergent insoluble aggregates and protease resistant prion particles, which possess classical characteristics of PrP^{Sc}, failed to produce infectivity in animals (62). The lack of infectivity indicated that something was missing in the generation of infectious prion particles *in vitro*.

Through the protein-misfolding cyclic amplification (PMCA) technique first described by Saborio and collaborators in 2001, it was shown that a mixture of crude homogenates from normal and scrapie brain resulted in a 6-fold amplification of PrP-res (63, 64). However, it was necessary to use a 50-fold excess of PrP-res template to drive conversion in a cell-free system containing only purified proteins (45). These results indicate that other factors are required for efficient conversion and that brain homogenates must contain such adjuvants.

Legname and coworkers (65) published a paper that claimed to contain the definitive proof for the protein-only hypothesis. This group managed to induce the disease in transgenic animals by inoculating them with a recombinant prion protein (residues 89 to 230) in its fibrillar beta-sheet-rich form. The disease was induced in transgenic animals overexpressing the prion fragment (PrP 89-230). However, animals bearing many copies of the prion protein developed the disease later on and, in some cases, presented a sub-clinical form of scrapie. Therefore, it seems that the injected material only accelerated the process in transgenic animals expressing excess PrP.

Studies with transgenic animals also suggest that conversion of PrP^C into PrP^{Sc} could be assisted by another biological macromolecule (49). This observation could explain the species barrier in prion diseases (49). The adjuvant could be a binding cofactor or even a specific posttranslational modifier (49, 66).

It is currently well-accepted that an unknown factor is involved in prion pathology (38, 48, 49, 67). This molecule would function by lowering the free energy barrier between PrP^C and PrP^{Sc} and triggering conversion (53, 68). A great number of biological macromolecules have emerged as candidates for conversion catalysts: cellular adhesion molecules, extracellular matrix molecules, glycosaminoglycans, and nucleic acids. All of these molecules have been reported to interact with PrP and trigger structural conversion from an alpha-helical-rich structure to a beta-sheet-rich form (38, 48, 50, 69, 70). Throughout this review, we will focus mainly on two prion protein ligands: the glycosaminoglycans and nucleic acids.

4. INTERACTION WITH SULFATED POLYSACCHARIDES

Proteoglycans (PG) are glycoproteins consisting of a core protein covalently linked to one or more glycosaminoglycan chains. Glycosaminoglycans (GAGs) are linear polysaccharides comprised of a disaccharide repeat unit of a hexuronic acid linked to a hexosamine that is mainly modified by *N*-deacetylase and *N*-sulfotransferases. Sulfated polysaccharides have long been shown to interact with several proteins. They play important roles in the regulation of many physiological processes, including cell-cell and cell-matrix interactions, cell growth and proliferation, and viral infection (for review see (71)). GAGs have been implicated in many conformational diseases and have been detected in different types of amyloid deposits since the 19^{th} century (72-76). In addition, the importance of these molecules is not only restricted to spatial deposition, but also to temporal appearance (77) and the induction of conformational changes (78) in amyloidogenesis.

Numerous evidences reveal the direct binding of GAGs to PrP^C in soluble form and at the cell surface (50, 79-81). The decrease in cellular heparan sulfate content leads to a strong reduction in PrP^{Sc} formation and incorporation in cells to regulate the metabolism of prions (82, 83). These observations indicate that the interaction of HS proteoglycans and PrP^C/PrP^{Sc} is essential for the pathogenesis of prion diseases.

4.1. The paradoxical effect of sulfated glycans.

GAGs, mostly heparan sulfate (HS), have been the subject of many prion disease studies. Snow and collaborators were the first to show the presence of heparan sulfate in amyloid plaques in prion diseases like Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker syndrome, and scrapie (76, 84). Since then, efforts have

focused on the function of these carbohydrates in transmissible spongiform encephalopathy pathogenesis.

After the identification of GAGs as considerable amyloid aggregate components, some studies showed that sulfated polysaccharides can inhibit the accumulation of PrP-res in cells infected with scrapie, and can bind PrP^C (50, 85). One explanation for this finding is that exogenous sulfated compounds inhibit PrP^{Sc} production by decreasing the amount of plasma membrane PrP^C. Since no alteration in the synthesis or degradation of PrP^C was observed, the observed effect may be due to an increase in the rate of endocytosis induced by these compounds (86). Another work demonstrated that sulfated GAGs could inhibit the polymerization of synthetic prion peptides into amyloid fibrils (87). It is postulated that the interaction of PrP (PrP^C and/or PrP^{Sc}) with endogenous GAGs could be necessary for PrP^{Sc} propagation, and that exogenous GAGs act as inhibitors that block the interaction of PrP with endogenous PG (78). According to this hypothesis, endogenous GAGs at the cell membrane possess characteristics that are necessary to facilitate PrP^{Sc} formation/propagation and differ from exogenous molecules that do not cause this effect.

Wong and collaborators (78) used a cell-free conversion assay to show that sulfated glycans induce conformational changes in PrP-sen and stimulate the conversion of PrP-sen to a PrP-res isoform, which probably acts as a cofactor in the pathogenic process. Exogenous GAGs have been shown to reconstitute the formation of PrP^{Sc} in cells possessing strongly reduced membrane-associated GAGs (82). Furthermore, heparan sulfate proteoglycans (HSPG) stimulated PrP-res amplification *in vitro* (88), and heparin enhanced the aggregation of a prion protein fragment ($PrP^{185-208}$) and induced the formation of amyloid aggregates that are toxic to neuronal cells (89, 90). The ability to stimulate PrP conversion reveals the paradoxical effect of these sulfated glycans.

4.2. Function of the PrP^C:GAG interaction

PrP^C is mainly located on the cell surface where it interacts with cell surface proteoglycans and the extracellular matrix. Heparan sulfate proteoglycans (HSPG) on the cell membrane are mainly syndecans (Sdc) or glypicans (Gpc). The ability to bind GAGs may be involved not only in the pathogenesis of prions, but also in the normal function of PrP and HS.

Although PrP^C is a GPI-anchored membrane protein with no cytoplasmic domain, it appears to be involved in neurite outgrowth and neuronal survival through signal transduction pathways, including the nonreceptor Src-related family member p59 (Fyn), PI3 kinase/Akt, cAMP-dependent protein kinase A, and MAP kinase.(91). *N*-syndecans are transmembrane cell surface proteoglycans that interact with Src family kinases and mediate neurite outgrowth (92). Therefore, the effect of PrP on promoting neurite outgrowth and neuronal survival may be modulated by its interaction with HSPG.

GAGs also mediate the interaction of prion protein with other molecules, such as the 37-kDa/67-kDa laminin receptor (LRP/LR) (93, 94). Like heparan sulfate, LRP/LR is another prion ligand that is believed to be a cell membrane receptor for PrP^C and PrP^{Sc} (31, 95). Not only does heparin function as a cofactor to mediate plasminogen activity through PrP^C stimulation of tissue-type plasminogen activator (t-PA), but it may also influence cellular proteolysis (96).

Glypicans, which include PrP^C, are GPI-anchored proteins present in lipid rafts (for review see (97)). Gpc-1 recycling involves internalization via the caveolae pathway and degradation in lysosomes. This process leads to removal and either enzymatic degradation of the HS sulfate chains by heparanase or non-enzymatic degradation by (Cu(II)-/Zn(II)-dependent, nitric oxide (NO)-catalyzed deaminative cleavage. These processes generate products, such as anhydromannose oligosaccharides, that interact with oxidized proteins and the proteasome to participate in the clearance of misfolded proteins (98). Recycling Gpc-1 is also crucial for the uptake of polyamines, which are important molecules for cell growth and survival (99). Gpc-1 possesses conserved cysteine residues in the central domain that are *S*-nitrosylated in a Cu(II)-Cu(I)-dependent redox cycle during endocytosis (100). PrP^C is known to bind copper at the octapeptide repeat region of its N-terminal domain, and has been related to copper metabolism and oxidative stress (for review, see (101)). Cu(II)-loaded PrP^C transfers copper and supports NO-catalyzed Gpc-1 autodegradation *in vitro*. Ectopic expression of PrP^c in prion-null fibroblasts ($PRNP^{0/0}$) restores this process (102). Gpc-1 and PrP^C are co-internalized by Cu(II) ions. Gpc-1 expression does not influence PrP^C endocytosis, but PrP^C controls GPC-1 internalization and auto-processing (103). Sulfated polysaccharides and Cu(II) ions also stimulate PrP^C endocytosis (86, 104).

4.3. Heparin/Heparan sulfate binding sites

The presence of sulfur and carboxyl groups makes GAGs negatively charged molecules. Interactions with other proteins are mainly ionic and depend on the presence of basic amino acids throughout the protein sequence. Although the interaction might look random, GAGs have specific requirements for defined sequences. Cardin and Weintraub (105) were the first to show the presence of defined motifs in heparin-binding domains of four proteins (105). They characterized two consensus sequences, XBBXBX and XBBBXXBX, where B is a basic residue and X is any other amino acid (105). Sobel and collaborators also proposed a third consensus sequence, XBBBXXBBBXXBBX (106). These motifs are not always found as binding regions; therefore, other spatial patterns for heparin binding must exist (107, 108).

Lysine and arginine are basic amino acids that show greatest affinity for sulfated glycans, with arginine exhibiting 2.5 times greater affinity (109). Histidine residues are also important for heparin interactions, with some proteins demonstrating a strong pH and cation binding dependence (110-113).

The prion protein's conserved octapeptide repeat region at the N-terminus has a consensus sequence of PHGGGWGQ that binds Cu(II) (114). The presence of

extra octapeptide repeats is associated with prion disease (115, 116). Although this region is not a heparin-binding motif, evidence indicates that it can bind heparin. The interaction could be mediated by divalent ions bound to protonated His side chains (117, 118). Additionally, the interaction between LRP/LR and PrP is thought to be dependent on an HSPG bridge that interacts with the octarepeat region (93). A synthetic peptide of amino acids 53-93 was shown to interact with heparin/HS, as demonstrated by biosensor analysis (80).

Aside from the octapeptide region, Warner and collaborators (80) also identified two additional synthetic peptides (a.a. 23-52 and 110-128) that independently bind heparin/HS. The first region contains a Cardin-Weintraub motif with four basic residues, KKRPK. Pan and collaborators demonstrated that a synthetic peptide (a.a. 23-35) contained the only region capable of binding GAG (79). They later reported that deletion of the first 12 amino acids was sufficient to abrogate binding to GAG, and the presence of extra peptide repeats resulted in a more flexible N-terminus that bound GAG with greater affinity. A more flexible N-terminus has been suggested to expose the heparin-binding region and increase its interaction with GAGs (119-121).

Another Cardin-Weintraub motif in the N-terminal sequence of the prion protein (a.a. 112-121) was included in the third sequence identified by Warner *et al* (80). The amino acid 185-208 fragment of the prion protein has been shown to interact with heparin/HS to induce the formation of amyloid aggregates (89, 90).

The amino acid sequence and saccharide domains of the polysaccharide chain are important for the protein:GAG interaction. The uronic acid residues of Heparin/HS can be 2-*O*-sulfated or unsubstituted. Glucosamine can be 6-*O*-sulfated, *N*-acetylated, unsubstituted, or *N*-sulfated. An *N*-unsubstituted GlcN and a 3-*O*-sulfate group (an unusual modification encountered in GlcN) are involved in the binding of HS to herpes simplex gD (122). The *N*-sulfated GlcN, IdoA, 6-*O*-sulfate, and 3-*O*-sulfate groups are required for antithrombin binding (123). Both the size and the degree of sulfate content in heparan mimetics are important for their ability to inhibit PrP endocytosis (124). Not only is sulfate density important for the interaction, but it also affects the properties of the glycan backbone. Chondroitin sulfate and kappa-carrageenan possess similar sulfate densities, but they do not have the same effect (85). Using competition assays, Warner and collaborators showed that removal of the 2-*O*-sulfate group reduced the capacity to compete for interaction with PrP^{C}-heparin. Removal of the 6-*O*-sulfate group had only a minor effect. Hence, the 2-*O*-sulfates of heparin play an important role in substrate recognition (80).

5. PRP INTERACTION WITH NUCLEIC ACIDS

Nucleic acid molecules, both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), form a peculiar group of PrP ligands. Due to the "protein-only" hypothesis, these molecules were excluded from prion biology for decades (1, 9), but they are now being considered for several new roles: instead of carrying genetic information, DNA and RNA molecules can participate in prion diseases as adjuvants in the conversion of PrP^{C} into PrP^{Sc}, and they may generate toxic species (38, 48, 50, 69, 125, 126). On the other hand, they have been shown to prevent PrP aggregation and accumulation in cultured cells (127, 128). The participation of nucleic acids as prion protein cofactors was first considered by Weissmann in 1991 when he raised the idea that another molecule, such as a nucleic acid, could play a crucial role in the propagation of prion proteins (10). Nucleic acid binding of recombinant PrP to DNA was described by Nandi & Leclerc (129). In 2001, we provided the first experimental evidence for the catalytic participation of a nucleic acid molecule in prion protein conversion (48).

Some research groups have also proposed the use of modified NA molecules as prototypes for prion diagnosis (130, 131). Another interesting feature of the interplay between prions and NAs is that the former might participate in nucleic acid processing as an NA chaperone (38, 132-134). DNA and RNA molecules present similarities and differences in their effects on prion structure, conversion, aggregation, and propagation (69). In this section, we focus on PrP interactions with DNA and RNA separately. We will also examine new possibilities and questions raised by the experimental observations of these molecules in prion biology.

5.1. Outline: a decade of interesting findings

5.1.1. DNA

The interaction between PrP and DNA molecules has captured the attention of scientists for over a decade, and was first described by P. K. Nandi in 1997. Through fluorescence measurements, he observed that the human PrP-derived peptide $PrP^{106-126}$ could bind a small DNA sequence derived from the papilloma virus with micromolar affinity. This finding was similar to that observed for the retroviral protein p10 (129).

Two years later, Nandi observed the polymerization of a full-length recombinant murine prion protein (rPrP) in nucleic acid solution (135). In 2001, our group showed that the same prion protein can bind DNA sequences with high affinity *in vitro* (48). This interaction can prevent the aggregation of a highly hydrophobic prion peptide derived from the Syrian hamster PrP sequence ($ShaPrP^{109-149}$) in a concentration-dependent manner. It can also induce a change in the rPrP conformation, converting it from an alpha-helical-rich structure to an alternative, beta-sheet-rich conformation (48). We proposed that the PrP-nucleic acid complex could act as a catalyst. When the full-length prion protein was added to the rPrP-DNA complex, aggregation did not occur (Figure 3); however, when a large amount of prion β-sheet-rich aggregate was added to a solution containing the DNA complex, aggregation increased drastically (Figure 3). These results led us to propose that the rPrP-DNA complex catalyzes aggregate formation (48). However, in addition to the PrP-DNA complex, a considerable amount of protein in the scrapie conformation was required. Without excess PrP^{Sc},

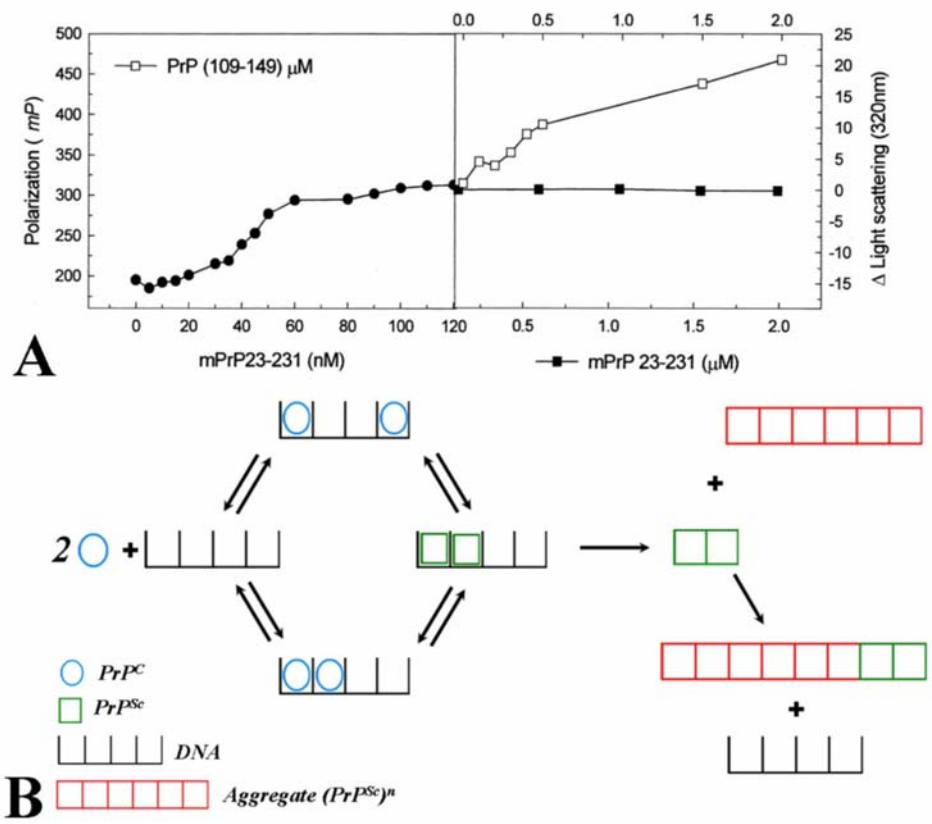

Figure 3. DNA can act as catalyst in PrP^{Sc} formation. (A) ShaPrP 109-149 aggregation is increased by rPrP:DNA complex. *Left,* addition of increasing concentrations of rPrP 23–231 to a solution at pH 5.0 containing 25 nM E2DBS labeled with rhodamine at the 3' end. *Right,* light scattering at 320 nm when ShaPrP 109–149 (*open squares*) or rPrP 23–231 (*filled squares*) was added to the rPrP:E2DBS complex. The LS values plotted are the difference between the conditions in the presence and in the absence of the complex. (B) rPrP:DNA complex facilitates PrP^{C}/PrP^{Sc} conversion together with the perturbation of an excess of PrP^{Sc} aggregates. Adapted from Cordeiro *et al.*, 2001 (48).

spontaneous aggregation of the α-helical PrP^{c} form would occur if PrP bound nucleic acids.

Following this observation, the interaction of rPrP with synthetic double-stranded DNAs was confirmed to lead to partial unfolding of the protein into an amyloid-like structure. This was characterized by binding to amyloid-specific dyes of Congo red and thioflavin T (136). These amyloid-like structures were also found to be spherical and resistant to proteinase-K (PK) digestion, similar to those in infected brains (137).

Subsequently, we showed that double-stranded DNA sequences also interact with the rrPrP construct, which lacked most of the unfolded N-terminal domain (PrPΔ32-121). This construct contains a deletion of amino acids 32 to 121 (138). The structural data obtained from Small Angle X-ray Scattering (SAXS) and Nuclear Magnetic Resonance (NMR) measurements showed that rPrP interacts with DNA through the globular domain (Figure 4). NMR measurements further identified chemical shift changes in amino acids both in the C and N domains of the protein, suggesting a restructuring of the protein upon DNA binding (138).

Mangé *et al.* reported translocation and accumulation of misfolded scrapie-like prions in the nuclei of infected cells, and that this transport was independent of the proteasome machinery. They also reported that the misfolded PrP has the capacity to interact with chromatin (139). Recently, the interaction of exogenous DNA and recombinant PrP was shown to result in DNA internalization and expression in mammalian cells in the presence of Ca^{2+} (140). Membrane-attached PrP^{C} binds DNA during DNA internalization, but not during DNA expression (140). Further investigation of DNA interactions with PrP led to the identification of anti-DNA antibodies that were specific to the PrP^{Sc} of diseased human brain tissues. This raised the possibility of using antibodies as markers of prion disease (131). These data indicate nucleic acids might be physiological ligands of PrP (38).

The use of modified, more stable oligonucleotides represents another strategy used to identify PrP isoform-specific ligands for use as aptamers in diagnostic and therapeutic procedures. Kocisko and coworkers designed degenerate phosphorothioate oligonucleotides that reduce PrP^{Sc} formation *in vivo* (141). Recently, King and collaborators described DNA

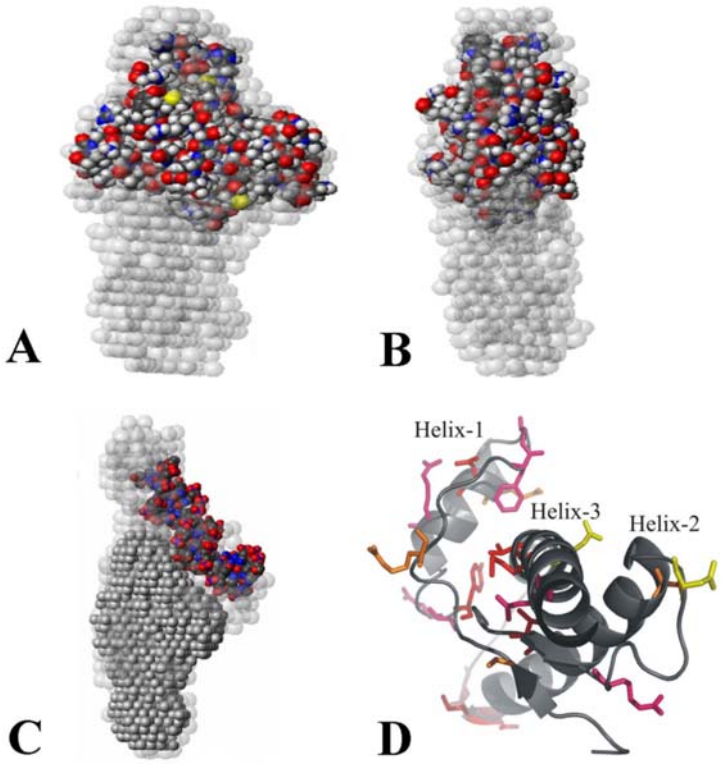

Figure 4. Models for the mouse prion protein complexed with DNA. (A and B) Three-dimensional model of rPrP reconstituted from SAXS measurements. Superposition of the rPrP Del32-121 model obtained from SAXS experiments and the NMR structure of the murine prion protein globular domain, residues 121-231 (PDB entry 1AG2). **(C)** rPrP Del32-121-DNA complex envelopes superimposed onto rPrP Del32-121 envelope and crystal structure of a 16 base pair DNA (PDB entry 2BOP). The DNA used in this superposition is smaller than the used in the measurements and complete superposition was not realized. (D) Adapted from Lima *et al.*, 2006 (138).

thioaptamers that bind with high affinity to different mammalian prion proteins and short phosphorothioate DNA molecules to decrease the PrP^{C} and PrP^{Sc} content of infected neuroblastoma cells (128, 142).

5.1.2. RNA

In 1997, Weiss and colleagues isolated RNA aptamers that specifically recognize recombinant prion protein (143). An interaction was observed for the full-length Syrian golden hamster protein, but not a construct lacking residues 23 to 90, suggesting that the amino-terminal region is required (143). Adler and colleagues (144) isolated highly structured RNA molecules that bind recombinant human PrP with high affinity in the presence of bovine serum *in vitro*. They also observed that this interaction formed oligomers that remained complexed with the RNA, protecting it from ribonuclease A degradation (144). A few years later, Vasan and coworkers observed that bovine PrP, in the absence of bovine serum, interacted with their RNA aptamers to produce soluble oligomers. These oligomers were sensitive to PK digestion, but were potentially toxic, according to *in vitro* testing (125).

Gabus and colleagues demonstrated that PrP possesses the characteristics of RNA-binding chaperones and, similar to the nucleocapsid protein of HIV-1, forms structures similar to nucleocapsid retroviral proteins. Based on their finding, the group suggested that PrP could participate in the metabolism of nucleic acids (133).

In 2003, several groups investigated the interaction of PrP with RNA *in vivo* and *in vitro* to demonstrate that the presence of RNA molecules could stimulate the conversion of PrP^{C} to PrP-res in hamster brain homogenates. They also showed that treatment of these homogenates with RNase could inhibit the conversion (52). In the same year, Rhie and colleagues characterized RNA aptamers that preferentially bind to the infectious form of PrP and inhibit the conversion of the cellular isoform. The aptamer is thought to bind the scrapie form to prevent an interaction between PrP^{C} and PrP^{Sc} (130). Following this study, other groups presented a series of PrP-binding RNA aptamers (145, 146).

The *in vivo* evidence that RNA molecules induce PrP conversion to a PrP^{Sc}-like form was provided by Deleault and coworkers (88, 126). Through a modified PMCA *in vitro* reaction, they demonstrated that synthetic RNAs could generate PrP-res formation (88). This is consistent with our *in vitro* DNA-binding results suggesting that nucleic acid molecules could be involved in prion

conversion (48). In their study, inoculation of commercially available synthetic RNAs, along with purified PrP and co-purified lipids caused neurodegeneration in healthy wild type hamsters (126). Geoghegan and colleagues co-localized hamster PrP extracellular aggregates with RNA molecules, suggesting that RNA molecules incorporate into forming prion aggregates (147).

Recently, our group characterized the biophysical and structural features of the interaction between recombinant mouse PrP and RNA molecules. We investigated PrP:RNA complex formation through structural changes in PrP during RNA binding. The reagents used include: recombinant full-length rPrP, two constructs lacking different portions of the amino-terminal domain (rPrPΔ23-121 and rPrPΔ51-90), total RNA extracts from prokaryotic and eukaryotic cells, and two small RNA sequences synthesized *in vitro*. Our results revealed that the full-length rPrP aggregates upon RNA binding, and immediately loses most of its alpha-helical content. This phenomenon was observed for all RNA samples tested. RNA extracted from neuroblastoma cells (N2aRNA) elicited the most drastic effects. Complex formation with N2aRNA protected both the protein and RNA from degradation with proteinase K and ribonuclease A, respectively (69). NMR measurements with the synthetic sequence, $SAF93^{43-59}$, presented a surprising result: after 3 days of incubation, the soluble portion of PrP recovered most of its original fold, but with distinct changes in NMR. The N-terminal deletion constructs revealed that RNA interacts with rPrP at the N-terminal, octapeptide region of the protein between residues 50 and 90. In this study, we also performed viability assays to verify the toxicity of PrP:RNA aggregates. Incubation of cultured neuroblastoma cells with the aggregates revealed that the toxicity of PrP:RNA aggregates was restricted to total RNA extract from N2a cells. Synthetic sequences were not able to induce toxicity, suggesting that an unidentified component present in the total RNA extract of N2a cells is responsible for PrP aggregation and PrP:N2aRNA complex toxicity (69).

5.1.3. New insights on RNA structure and function

For many decades, RNA molecules were classically described as accessories of the cellular machinery involved in DNA translation and transcription (148). The human genome project showed, however, that only a small portion of our DNA is actually transcribed into protein and the majority does not encode functional sequences. In the past decade, evidence that this portion of our genome is actually largely transcribed has emerged (148, 149). These versatile transcripts are being revisited, and new functions for them are being proposed. Non-coding RNAs (ncRNA), for example, exercise a variety of important functions in eukaryotic cells, such as post-translational regulation, cell morphogenesis, and participation in embryo and neural development (for review see (148)). RNA is a flexible molecule, and its structural properties allow it to exercise different roles through a broad range of mechanisms, including cell to cell communication (150).

As discussed in this review, the interaction of PrP with RNA molecules has been widely reported, and the secondary structure of RNA has been suggested to be important for this interaction (69, 130, 144-146). The particular RNA structure necessary for prion binding remains elusive, but the flexibility of such molecules certainly account for these interactions.

5.2. DNA *vs.* RNA: Similarities and differences

All experimental evidence for PrP:NA interactions suggests the partnership of these molecules *in vivo*. Both DNA and RNA produce misfolded isoforms of PrP. Interestingly, the misfolded PrP isoforms generated by DNA and RNA are not identical. DNA usually produces aggregates with amyloid properties, such as high beta-sheet content and fiber formation (135, 136). Under some conditions, soluble species are produced (48, 138). Depending on the RNA origin, PrP:RNA complexes can form large aggregates or small oligomers (69, 125), but they adopt an amorphous morphology (69). DNA and RNA also bind PrP at different sites: DNA interacts mostly with the C-terminal region, but causes significant changes in the N-terminal domain (138). Experiments performed with RNA suggest that the RNA binding site is located in the PrP unfolded N-terminal domain; PrP constructs lacking residues 50-90 do not interact with RNA (69, 143). The toxicity of PrP:NA complexes was observed only for PrP:RNA aggregates (69, 126).

These findings suggest that a nucleic acid molecule could be involved in prion conversion. Emerging evidence for the extracellular functions of RNA molecules (150) also contribute to the idea that a nucleic acid molecule might be implicated in the generation of PrP^{Sc}.

Some groups reported that both DNA and RNA molecules can prevent prion misfolding and propagation in infected cells (48, 128, 131, 141, 142), and that these molecules could preferentially recognize PrP^{C} or PrP^{Sc} (125, 130). The experimental evidence raises the possibility for further development of new molecules for TSE diagnosis and therapy.

Binding of NA molecules to PrP can alter the structure of the protein, as well as the structure of the nucleic acid. NA binding can induce modifications in the amino-terminal region of PrP (48, 69, 138). The interaction with PrP bends DNA molecules and facilitates strand transfer (151). This property of PrP is consistent with the activity of proteins involved in protein synthesis (151). These observations, along with the finding that PrP exhibits characteristics of NA-binding proteins (132, 133), suggest a possible role as an NA chaperone (151, 152). To confirm this assumption, however, more experimental clues are needed.

5.3. NA chaperone and PrP^{Sc} generation

After decades of intense study, spongiform encephalopathies are clinically well-described, but they remain incurable. The spontaneous generation of PrP^{Sc} in sporadic prion diseases, along with the capacity of transmission is still an obscure subject. Despite the

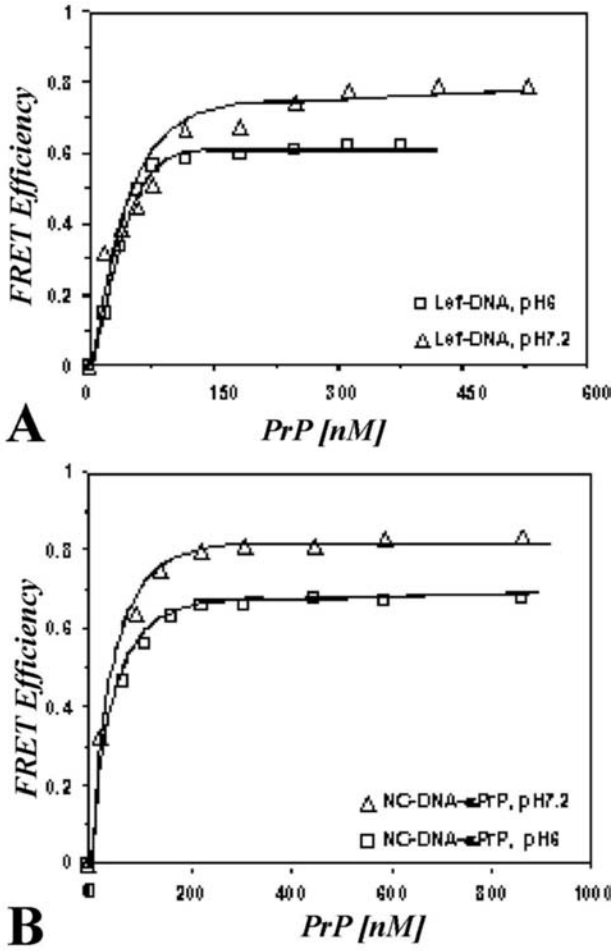

Figure 5. Dependence of FRET efficiency (TE = $(I_{DO}-I_D)/I_{DO}$) on alpha-PrP concentration at pH 6 and 7.2**.** (A) FAM−Lef-DNA−TAMRA. (B) FAM−NC-DNA−TAMRA. At both pHs, the FRET efficiency is greater for the NC-DNA albeit to a smaller extent, and the efficiency is more at pH 7.2 than that at pH 6. Extracted from Bera *et al.*, 2007(155).

significant efforts of the scientific community, the physiological role of PrP^C remains unknown (153). PrP interaction with nucleic acid molecules presents an interesting avenue for understanding prion physiology and the occurrence of TSEs.

Recent studies suggest that the function of PrP^C is related to NA metabolism. PrP has been reported to share similarities with viral NA-binding proteins, such as the Ncp7 of HIV1 (133). Using a methodology to predict DNA interaction sites in proteins, Tjong and coworkers identified 23 residues as possible DNA-binding sites in the murine PrP globular domain (154). Identification of this binding region is consistent with the findings of our group using chemical shift variation analysis of DNA binding to rPrP via examination of rPrP HSQC spectra (138) (Figure 4). Anchored PrP^C enables DNA translocation to the intracellular space (140), and recombinant prion proteins can bend small double-stranded DNA molecules and promote DNA expression (140, 155). The FRET experiments (Figure 5) performed by Nandi's group provide clear-cut evidence that the prion protein modifies the structure of nucleic acids. The observation that the prion protein has a disordered N-terminal domain is consistent with other nucleic acid chaperones (156) and suggests a nucleic acid chaperone function for PrP.

A considerable amount of evidence suggests that nucleic acids can be involved in the conversion of PrPC to PrPSc. Both DNA and RNA can convert recombinant or purified PrP into misfolded isoforms that possess PrPSc characteristics. These studies reported a series of PrP:NA aggregates, some of which induced cell death and neurodegeneration (38, 48, 50, 69, 125, 126, 135-137). However, the size, composition, and secondary structure of nucleic acid molecules necessary for binding and converting PrPC to PrPSc are still open for debate. The different effects observed for DNA and RNA molecules indicate that these molecules exercise distinct roles in prion biology. For example: both DNA and RNA can generate aggregated forms of PrP but, until recently, toxicity was only observed in association with PrP:RNA aggregates (69, 126).

If PrP^C is indeed involved in nucleic acid metabolism, how do nucleic acids convert PrP^C into PrP^{Sc}? Under what conditions does this interaction become dangerous? The answers to these questions could explain the sporadic incidence of prion disease cases.

6. PERSPECTIVES IN THERAPY AND DIAGNOSIS

A series of therapeutic strategies have been tested to find an effective treatment against TSEs. However, there is still no efficient approach to preventing disease development and death (for a review on TSE drug therapies, see (157). A broad variety of compounds have been tested in an attempt to reverse or prevent the formation of PrP^{Sc}, such as Congo Red, amphotericin B, porphyrins, polyamines, and sulfated polyanions (2). These drugs seem to function directly or indirectly in the conversion of PrP^C to PrP^{Sc}, and thus prevent the spread of the infectious form. Unfortunately, the compounds tested were not effective in inhibiting the spread of disease when given after the first appearance of symptoms (2). In 2001, Korth and colleagues reported that some phenotiazine and acridine-derived compounds were able to inhibit PrP^{Sc} formation when administered to infected neuroblastoma cells (ScN2a) (158). Unfortunately, when administered over a long period or at high doses, these drugs caused side effects in humans, such as liver damage (159), and they were not suitable for prion disease treatment. Further work described a screening assay for PrP^{Sc} inhibitors and characterized a group of polyphenols that inhibit the cell-free conversion reaction (160). In 2004, our group reported that the naphthalene derivative compound 4,4'-dianilino-1,1'-binaphthyl-5,5'-sulfonate (bis-ANS) can inhibit Syrian hamster PrP peptide ShaPrP(109-149) aggregation (161). This compound is also able to convert rPrP23-231 from its normal alpha-helical form to an alternative, beta-sheet-rich structure. This dual effect was similar to that observed in response to small double-stranded DNA sequences (48). Moreover, binding of bis-ANS to full-length rPrP was reduced by the addition of nanomolar concentrations of oligonucleotides, demonstrating that they compete for the same binding site.

Other groups described the capacity of some NA molecules to reduce PrP^{Sc} formation and to decrease PrP^{Sc} content in infected cells (128, 130, 141, 142). This interesting property of NA molecules as potential anti-prion compounds opens the possibility of new drugs based on PrP:NA interactions (128, 130, 141, 142).

Aside from the use of nucleic acids, glycosaminoglycans have long been considered as a candidate for prion therapy. Sulfated glycans were first used for TSE treatment as antiviral drugs when the pathogen was thought to be a virus. Sulfated glycans were able to prolong the incubation time of the disease and prevent symptoms in mice when administered prophylactically (162-165). Intraventricular infusion of pentosan polysulfate, an analog of heparin, was reported to increase survival of vCJD patients (70, 166). Due to the diverse biological functions of heparan sulfate and its involvement in prion diseases, many heparan sulfate mimetics (HMs) are being developed and tested as anti-prion drugs. HMs are dextran-based molecules whose hydroxyl groups are substituted with carboxymethyl ether, sulfate esters, or hydrophobic groups. These molecules inhibit PrP^{Sc} accumulation in cell culture without affecting the level of PrP^C, and they prolong the survival of scrapie-infected hamsters with no cytopathic or cytostatic effects (167-169). HMs could interact with the prion to lower its binding to cellular HS, and increase the length and mediate the degree of sulfation. The introduction of hydrophobicity could positively modulate this effect (124).

Another developing area in prion diseases is the improvement of new methodologies for TSE diagnosis; PrP:NA interactions could elucidate new possibilities in this field. Some anti-DNA antibodies are able to detect PrP^{Sc} *in vivo* (131), and RNA aptamers can bind selectively to PrP^C and PrP^{Sc} (145, 146). The establishment of an effective TSE diagnosis may allow for detection of PrP^{Sc} in pre symptomatic states of prion diseases, and facilitate clearance of PrP aggregates before they become highly toxic. We expect great developments in this field in the years to come.

7. CONCLUSIONS AND PERSPECTIVES

There is increasing evidence that prions have other additional accomplices that chaperone their activity in converting the normal, cellular form of protein into the disease-causing isoform. The most likely candidates for this partnership are nucleic acids and glycosaminoglycans. The catalytic effect of RNA or DNA on the $PrP^C \rightarrow PrP^{Sc}$ conversion would depend on the sequence and structure of the nucleic acids and their ability to provide a protective effect. The potential therapeutic use of modified nucleic acids has recently been demonstrated by different groups. In this review, we examined recent research seeking to understand how nucleic acids and GAGs bind to prions, and the resultant implications for cellular toxicity and prion conversion. Figure 6 summarizes possible mechanisms by which a nucleic acid or GAG could affect conversion of the prion protein.

There are, however, many questions that remain to be explored. The nucleic-acid binding properties of the prion protein (both RNA and DNA) might have broader implications for its native function than for disease. The great abundance of RNA in the cytosol that acts in a variety of cellular processes may hint at the physiological target of prion protein. In a recent article, Beaudoin and coworkers (170) described large ribonucleoprotein particles induced by cytoplasmic PrP, which share striking similarities with the chromatoid body. The chromatoid body is an RNA granule that is predicted to function in post-transcriptional gene regulation. Their findings indicate that PrP functions in the assembly and RNA processing center.

The formation of a complex between non-infectious PrP and RNA may be just part of the story. The connection between PrP^C, NA, and PrP^{Sc} could be a side effect of the prion protein's native cellular function. The implications of these interactions are causing a paradigm

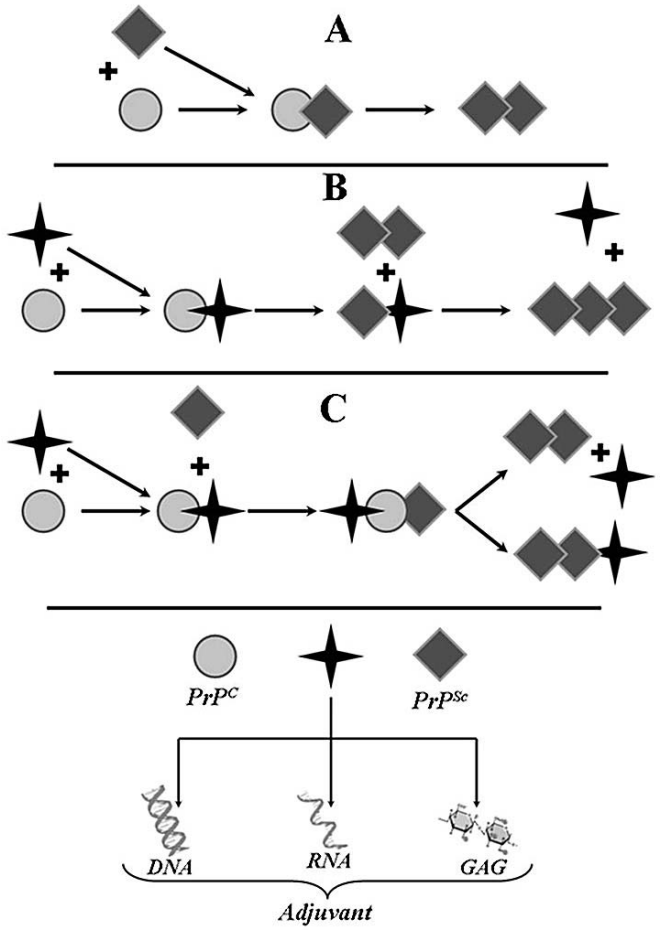

Figure 6. Models for PrP^{C} conversion into PrP^{Sc}. (A) PrP^{Sc} is the only responsible for structural conversion of PrP^{C} into new PrP^{Sc}. (B) A macromolecule (DNA, RNA or GAG) is responsible for PrP^{C} conversion into PrP^{Sc}. **(C)** An adjuvant molecule acts as a catalyst, facilitating PrP^{C} conversion into PrP^{Sc}. Adapted from Aguzzi and Polymenidou, 2004 and Silva *et al.*, 2008 (2, 38).

shift in the area of prion research and we can anticipate new findings in the years ahead

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9. REFERENCES

1. S.B. Prusiner: Prions. *Proc Natl Acad Sci U S A* 95, 13363-13383 (1998)

2. A. Aguzzi and M. Polymenidou: Mammalian prion biology: one century of evolving concepts. *Cell* 116, 313-327 (2004)

3. H.G. Creutzfeldt: Über eine eigenartige herdförmige erkrankung des zentralnervensystems. *Z Ges Neurol Psychiatr* 57, 1-19 (1920)

4. A. Jakob: Über eigenartige erkrankungen des zentralnervensystems mit bemerkenswertem anatomischem befunde (spastische pseudosklerose-encephalomyelopathie mit disseminierten degenerationsherden). *Z Ges Neurol Psychiatr* 64, 147-228 (1921)

5. D.C. GAJDUSEK and V. ZIGAS: Degenerative disease of the central nervous system in New Guinea; the endemic occurrence of kuru in the native population. *N Engl J Med* 257, 974-978 (1957)

6. T. Alper, W.A. Cramp, D.A. Haig and M.C. Clarke: Does the agent of scrapie replicate without nucleic acid? *Nature* 214, 764-766 (1967)

7. J.S. Griffith: Self-replication and scrapie. *Nature* 215, 1043-1044 (1967)

8. S.B. Prusiner, D.F. Groth, S.P. Cochran, F.R. Masiarz, M.P. McKinley and H.M. Martinez: Molecular properties, partial purification, and assay by incubation period measurements of the hamster scrapie agent. *Biochemistry* 19, 4883-4891 (1980)

9. S.B. Prusiner: Novel proteinaceous infectious particles cause scrapie. *Science* 216, 136-144 (1982)

10. S.B. Prusiner, D.F. Groth, D.C. Bolton, S.B. Kent and L.E. Hood: Purification and structural studies of a major scrapie prion protein. *Cell* 38, 127-134 (1984)

11. B. Caughey and B. Chesebro: Transmissible spongiform encephalopathies and prion protein interconversions. *Adv Virus Res* 56, 277-311 (2001)

12. B. Oesch, D. Westaway, M. Walchli, M.P. McKinley, S.B. Kent, R. Aebersold, R.A. Barry, P. Tempst, D.B. Teplow, L.E. Hood and .: A cellular gene encodes scrapie PrP 27-30 protein. *Cell* 40, 735-746 (1985)

13. N.R. Cashman, R. Loertscher, J. Nalbantoglu, I. Shaw, R.J. Kascsak, D.C. Bolton and P.E. Bendheim: Cellular isoform of the scrapie agent protein participates in lymphocyte activation. *Cell* 61, 185-192 (1990)

14. N. Stahl, D.R. Borchelt, K. Hsiao and S.B. Prusiner: Scrapie prion protein contains a phosphatidylinositol glycolipid. *Cell* 51, 229-240 (1987)

15. P.M. Rudd, T. Endo, C. Colominas, D. Groth, S.F. Wheeler, D.J. Harvey, M.R. Wormald, H. Serban, S.B. Prusiner, A. Kobata and R.A. Dwek: Glycosylation differences between the normal and pathogenic prion protein isoforms. *Proc Natl Acad Sci U S A* 96, 13044-13049 (1999)

16. P.M. Rudd, M.R. Wormald, D.R. Wing, S.B. Prusiner and R.A. Dwek: Prion glycoprotein: structure, dynamics, and roles for the sugars. *Biochemistry* 40, 3759-3766 (2001)

17. R. Riek, S. Hornemann, G. Wider, R. Glockshuber and K. Wuthrich: NMR characterization of the full-length recombinant murine prion protein, mPrP(23-231). *FEBS Lett* 413, 282-288 (1997)

18. J.H. Viles, F.E. Cohen, S.B. Prusiner, D.B. Goodin, P.E. Wright and H.J. Dyson: Copper binding to the prion protein: structural implications of four identical cooperative binding sites. *Proc Natl Acad Sci U S A* 96, 2042-2047 (1999)

19. D.R. Brown, K. Qin, J.W. Herms, A. Madlung, J. Manson, R. Strome, P.E. Fraser, T. Kruck, A. von Bohlen, W. Schulz-Schaeffer, A. Giese, D. Westaway and H. Kretzschmar: The cellular prion protein binds copper *in vivo*. *Nature* 390, 684-687 (1997)

20. D.G. Donne, J.H. Viles, D. Groth, I. Mehlhorn, T.L. James, F.E. Cohen, S.B. Prusiner, P.E. Wright and H.J. Dyson: Structure of the recombinant full-length hamster prion protein PrP(29-231): the N terminus is highly flexible. *Proc Natl Acad Sci U S A* 94, 13452-13457 (1997)

21. D.A. Lysek, C. Schorn, L.G. Nivon, V. Esteve-Moya, B. Christen, L. Calzolai, C. von Schroetter, F. Fiorito, T. Herrmann, P. Guntert and K. Wuthrich: Prion protein NMR structures of cats, dogs, pigs, and sheep. *Proc Natl Acad Sci U S A* 102, 640-645 (2005)

22. L. Calzolai, D.A. Lysek, D.R. Perez, P. Guntert and K. Wuthrich: Prion protein NMR structures of chickens, turtles, and frogs. *Proc Natl Acad Sci U S A* 102, 651-655 (2005)

23. R. Zahn, A. Liu, T. Luhrs, R. Riek, C. von Schroetter, G.F. Lopez, M. Billeter, L. Calzolai, G. Wider and K. Wuthrich: NMR solution structure of the human prion protein. *Proc Natl Acad Sci U S A* 97, 145-150 (2000)

24. J.C. Watts and D. Westaway: The prion protein family: diversity, rivalry, and dysfunction. *Biochim Biophys Acta* 1772, 654-672 (2007)

25. H. Bueler, M. Fischer, Y. Lang, H. Bluethmann, H.P. Lipp, S.J. DeArmond, S.B. Prusiner, M. Aguet and C. Weissmann: Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. *Nature* 356, 577-582 (1992)

26. A. Aguzzi and M. Heikenwalder: Pathogenesis of prion diseases: current status and future outlook. *Nat Rev Microbiol* 4, 765-775 (2006)

27. A. Aguzzi, C. Sigurdson and M. Heikenwaelder: Molecular mechanisms of prion pathogenesis. *Annu Rev Pathol* 3, 11-40 (2008)

28. V.R. Martins, R. Linden, M.A. Prado, R. Walz, A.C. Sakamoto, I. Izquierdo and R.R. Brentani: Cellular prion protein: on the road for functions. *FEBS Lett* 512, 25-28 (2002)

29. E. Graner, A.F. Mercadante, S.M. Zanata, O.V. Forlenza, A.L. Cabral, S.S. Veiga, M.A. Juliano, R. Roesler, R. Walz, A. Minetti, I. Izquierdo, V.R. Martins and R.R. Brentani: Cellular prion protein binds laminin and mediates neuritogenesis. *Brain Res Mol Brain Res* 76, 85-92 (2000)

30. E. Graner, A.F. Mercadante, S.M. Zanata, V.R. Martins, D.G. Jay and R.R. Brentani: Laminin-induced PC-12 cell differentiation is inhibited following laser inactivation of cellular prion protein. *FEBS Lett* 482, 257-260 (2000)

31. S. Gauczynski, J.M. Peyrin, S. Haik, C. Leucht, C. Hundt, R. Rieger, S. Krasemann, J.P. Deslys, D. Dormont, C.I. Lasmezas and S. Weiss: The 37-kDa/67-kDa laminin receptor acts as the cell-surface receptor for the cellular prion protein. *EMBO J* 20, 5863-5875 (2001)

32. V. Ellis, M. Daniels, R. Misra and D.R. Brown: Plasminogen activation is stimulated by prion protein and regulated in a copper-dependent manner. *Biochemistry* 41, 6891-6896 (2002)

33. A. Barret, L. Forestier, J.P. Deslys, R. Julien and P.F. Gallet: Glycosylation-related gene expression in prion diseases: PrPSc accumulation in scrapie infected GT1 cells

depends on beta-1,4-linked GalNAc-4-SO4 hyposulfation. *J Biol Chem* 280, 10516-10523 (2005)

34. S.M. Zanata, M.H. Lopes, A.F. Mercadante, G.N. Hajj, L.B. Chiarini, R. Nomizo, A.R. Freitas, A.L. Cabral, K.S. Lee, M.A. Juliano, E. de Oliveira, S.G. Jachieri, A. Burlingame, L. Huang, R. Linden, R.R. Brentani and V.R. Martins: Stress-inducible protein 1 is a cell surface ligand for cellular prion that triggers neuroprotection. *EMBO J* 21, 3307-3316 (2002)

35. G.I. Keshet, O. Bar-Peled, D. Yaffe, U. Nudel and R. Gabizon: The cellular prion protein colocalizes with the dystroglycan complex in the brain. *J Neurochem* 75, 1889-1897 (2000)

36. K. Nieznanski, H. Nieznanska, K.J. Skowronek, K.M. Osiecka and D. Stepkowski: Direct interaction between prion protein and tubulin. *Biochem Biophys Res Commun* 334, 403-411 (2005)

37. A. Aguzzi, F. Baumann and J. Bremer: The prion's elusive reason for being. *Annu Rev Neurosci* 31, 439-477 (2008)

38. J.L. Silva, L.M. Lima, D. Foguel and Y. Cordeiro: Intriguing nucleic-acid-binding features of mammalian prion protein. *Trends Biochem Sci* 33, 132-140 (2008)

39. K.M. Pan, M. Baldwin, J. Nguyen, M. Gasset, A. Serban, D. Groth, I. Mehlhorn, Z. Huang, R.J. Fletterick, F.E. Cohen and S.B. Prusiner: Conversion of alpha-helices into beta-sheets features in the formation of the scrapie prion proteins. *Proc Natl Acad Sci U S A* 90, 10962-10966 (1993)

40. S.B. Prusiner, M.P. McKinley, K.A. Bowman, D.C. Bolton, P.E. Bendheim, D.F. Groth and G.G. Glenner: Scrapie prions aggregate to form amyloid-like birefringent rods. *Cell* 35, 349-358 (1983)

41. Z. Huang, S.B. Prusiner and F.E. Cohen: Scrapie prions: a three-dimensional model of an infectious fragment. *Fold Des* 1, 13-19 (1996)

42. L.M. Herrmann and B. Caughey: The importance of the disulfide bond in prion protein conversion. *Neuroreport* 9, 2457-2461 (1998)

43. C. Govaerts, H. Wille, S.B. Prusiner and F.E. Cohen: Evidence for assembly of prions with left-handed beta-helices into trimers. *Proc Natl Acad Sci U S A* 101, 8342-8347 (2004)

44. H. Bueler, A. Aguzzi, A. Sailer, R.A. Greiner, P. Autenried, M. Aguet and C. Weissmann: Mice devoid of PrP are resistant to scrapie. *Cell* 73, 1339-1347 (1993)

45. D.A. Kocisko, J.H. Come, S.A. Priola, B. Chesebro, G.J. Raymond, P.T. Lansbury and B. Caughey: Cell-free formation of protease-resistant prion protein. *Nature* 370, 471-474 (1994)

46. D.A. Kocisko, S.A. Priola, G.J. Raymond, B. Chesebro, P.T. Lansbury, Jr. and B. Caughey: Species specificity in the cell-free conversion of prion protein to protease-resistant forms: a model for the scrapie species barrier. *Proc Natl Acad Sci U S A* 92, 3923-3927 (1995)

47. C. Weissmann: The state of the prion. *Nat Rev Microbiol* 2, 861-871 (2004)

48. Y. Cordeiro, F. Machado, L. Juliano, M.A. Juliano, R.R. Brentani, D. Foguel and J.L. Silva: DNA converts cellular prion protein into the beta-sheet conformation and inhibits prion peptide aggregation. *J Biol Chem* 276, 49400-49409 (2001)

49. G.C. Telling, M. Scott, J. Mastrianni, R. Gabizon, M. Torchia, F.E. Cohen, S.J. DeArmond and S.B. Prusiner: Prion propagation in mice expressing human and chimeric PrP transgenes implicates the interaction of cellular PrP with another protein. *Cell* 83, 79-90 (1995)

50. B. Caughey, K. Brown, G.J. Raymond, G.E. Katzenstein and W. Thresher: Binding of the protease-sensitive form of PrP (prion protein) to sulfated glycosaminoglycan and congo red [corrected]. *J Virol* 68, 2135-2141 (1994)

51. B. Caughey and G.S. Baron: Prions and their partners in crime. *Nature* 443, 803-810 (2006)

52. N.R. Deleault, R.W. Lucassen and S. Supattapone: RNA molecules stimulate prion protein conversion. *Nature* 425, 717-720 (2003)

53. I.V. Baskakov, G. Legname, S.B. Prusiner and F.E. Cohen: Folding of prion protein to its native alpha-helical conformation is under kinetic control. *Journal of Biological Chemistry* 276, 19687-19690 (2001)

54. A.C. Apetri and W.K. Surewicz: Atypical effect of salts on the thermodynamic stability of human prion protein. *J Biol Chem* 278, 22187-22192 (2003)

55. Y. Cordeiro, J. Kraineva, R. Ravindra, L.M. Lima, M.P. Gomes, D. Foguel, R. Winter and J.L. Silva: Hydration and packing effects on prion folding and beta-sheet conversion. High pressure spectroscopy and pressure perturbation calorimetry studies. *J Biol Chem* 279, 32354-32359 (2004)

56. Y. Cordeiro, J. Kraineva, M.P. Gomes, M.H. Lopes, V.R. Martins, L.M. Lima, D. Foguel, R. Winter and J.L. Silva: The amino-terminal PrP domain is crucial to modulate prion misfolding and aggregation. *Biophys J* 89, 2667-2676 (2005)

57. Y. Cordeiro, J. Kraineva, R. Winter and J.L. Silva: Volume and energy folding landscape of prion protein revealed by pressure. *Braz J Med Biol Res* 38, 1195-1201 (2005)

58. A. De Simone, G.G. Dodson, C.S. Verma, A. Zagari and F. Fraternali: Prion and water: tight and dynamical

hydration sites have a key role in structural stability. *Proc Natl Acad Sci U S A* 102, 7535-7540 (2005)

59. A. De Simone, G.G. Dodson, F. Fraternali and A. Zagari: Water molecules as structural determinants among prions of low sequence identity. *FEBS Lett* 580, 2488-2494 (2006)

60. A.F. Hill, M. Antoniou and J. Collinge: Protease-resistant prion protein produced *in vitro* lacks detectable infectivity. *J Gen Virol* 80 (Pt 1), 11-14 (1999)

61. S.B. Prusiner, D. Groth, A. Serban, N. Stahl and R. Gabizon: Attempts to restore scrapie prion infectivity after exposure to protein denaturants. *Proc Natl Acad Sci U S A* 90, 2793-2797 (1993)

62. G.M. Shaked, G. Fridlander, Z. Meiner, A. Taraboulos and R. Gabizon: Protease-resistant and detergent-insoluble prion protein is not necessarily associated with prion infectivity. *J Biol Chem* 274, 17981-17986 (1999)

63. G.P. Saborio, B. Permanne and C. Soto: Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. *Nature* 411, 810-813 (2001)

64. R. Lucassen, K. Nishina and S. Supattapone: *In vitro* amplification of protease-resistant prion protein requires free sulfhydryl groups. *Biochemistry* 42, 4127-4135 (2003)

65. G. Legname, I.V. Baskakov, H.O. Nguyen, D. Riesner, F.E. Cohen, S.J. DeArmond and S.B. Prusiner: Synthetic mammalian prions. *Science* 305, 673-676 (2004)

66. K. Kaneko, L. Zulianello, M. Scott, C.M. Cooper, A.C. Wallace, T.L. James, F.E. Cohen and S.B. Prusiner: Evidence for protein X binding to a discontinuous epitope on the cellular prion protein during scrapie prion propagation. *Proc Natl Acad Sci U S A* 94, 10069-10074 (1997)

67. B. Caughey and D.A. Kocisko: Prion diseases - A nucleic-acid accomplice? *Nature* 425, 673-674 (2003)

68. Y. Cordeiro and J.L. Silva: The hypothesis of the catalytic action of nucleic acid on the conversion of prion protein. *Protein Pept Lett* 12, 251-255 (2005)

69. M.P. Gomes, T.A. Millen, P.S. Ferreira, N.L. Silva, T.C. Vieira, M.S. Almeida, J.L. Silva and Y. Cordeiro: Prion protein complexed to N2a cellular RNAs through its N-terminal domain forms aggregates and is toxic to murine neuroblastoma cells. *J Biol Chem* 283, 19616-19625 (2008)

70. L. Horonchik, S. Tzaban, O. Ben Zaken, Y. Yedidia, A. Rouvinski, D. Papy-Garcia, D. Barritault, I. Vlodavsky and A. Taraboulos: Heparan sulfate is a cellular receptor for purified infectious prions. *J Biol Chem* 280, 17062-17067 (2005)

71. I. Capila and R.J. Linhardt: Heparin-protein interactions. *Angew Chem Int Ed Engl* 41, 391-412 (2002)

72. R. Virchow: Zur cellulosefrage. *Virchows Arch Pathol Anat Physiol* 6, 416-426 (1854)

73. A.D. Snow and R. Kisilevsky: Temporal Relationship Between Glycosaminoglycan Accumulation and Amyloid Deposition During Experimental Amyloidosis - A Histochemical-Study. *Laboratory Investigation* 53, 37-44 (1985)

74. I.D. Young, J.P. Willmer and R. Kisilevsky: The Ultrastructural-Localization of Sulfated Proteoglycans Is Identical in the Amyloids of Alzheimers-Disease and Aa, Al, Senile Cardiac and Medullary Carcinoma-Associated Amyloidosis. *Acta Neuropathologica* 78, 202-209 (1989)

75. I.D. Young, L. Ailles, S. Narindrasorasak, R. Tan and R. Kisilevsky: Localization of the Basement-Membrane Heparan-Sulfate Proteoglycan in Islet Amyloid Deposits in Type-Ii Diabetes-Mellitus. *Archives of Pathology & Laboratory Medicine* 116, 951-954 (1992)

76. A.D. Snow, R. Kisilevsky, J. Willmer, S.B. Prusiner and S.J. DeArmond: Sulfated Glycosaminoglycans in Amyloid Plaques of Prion Diseases. *Acta Neuropathologica* 77, 337-342 (1989)

77. P.A. McBride, M.I. Wilson, P. Eikelenboom, A. Tunstall and M.E. Bruce: Heparan sulfate proteoglycan is associated with amyloid plaques and neuroanatomically targeted PrP pathology throughout the incubation period of scrapie-infected mice. *Exp Neurol* 149, 447-454 (1998)

78. C. Wong, L.W. Xiong, M. Horiuchi, L. Raymond, K. Wehrly, B. Chesebro and B. Caughey: Sulfated glycans and elevated temperature stimulate PrP(Sc)-dependent cell-free formation of protease-resistant prion protein. *EMBO J* 20, 377-386 (2001)

79. T. Pan, B.S. Wong, T. Liu, R. Li, R.B. Petersen and M.S. Sy: Cell-surface prion protein interacts with glycosaminoglycans. *Biochem J* 368, 81-90 (2002)

80. R.G. Warner, C. Hundt, S. Weiss and J.E. Turnbull: Identification of the heparan sulfate binding sites in the cellular prion protein. *J Biol Chem* 277, 18421-18430 (2002)

81. R. Gabizon, Z. Meiner, M. Halimi and S.A. Ben Sasson: Heparin-like molecules bind differentially to prion-proteins and change their intracellular metabolic fate. *J Cell Physiol* 157, 319-325 (1993)

82. O. Ben Zaken, S. Tzaban, Y. Tal, L. Horonchik, J.D. Esko, I. Vlodavsky and A. Taraboulos: Cellular heparan sulfate participates in the metabolism of prions. *J Biol Chem* 278, 40041-40049 (2003)

83. N. Hijazi, Z. Kariv-Inbal, M. Gasset and R. Gabizon: PrPSc incorporation to cells requires endogenous glycosaminoglycan expression. *J Biol Chem* 280, 17057-17061 (2005)

84. A.D. Snow, T.N. Wight, D. Nochlin, Y. Koike, K. Kimata, S.J. DeArmond and S.B. Prusiner: Immunolocalization of heparan sulfate proteoglycans to the prion protein amyloid plaques of Gerstmann-Straussler syndrome, Creutzfeldt-Jakob disease and scrapie. *Lab Invest* 63, 601-611 (1990)

85. B. Caughey, D. Ernst and R.E. Race: Congo red inhibition of scrapie agent replication. *J Virol* 67, 6270-6272 (1993)

86. S.L. Shyng, S. Lehmann, K.L. Moulder and D.A. Harris: Sulfated glycans stimulate endocytosis of the cellular isoform of the prion protein, PrPC, in cultured cells. *J Biol Chem* 270, 30221-30229 (1995)

87. M. Perez, F. Wandosell, C. Colaco and J. Avila: Sulphated glycosaminoglycans prevent the neurotoxicity of a human prion protein fragment. *Biochem J* 335 (Pt 2), 369-374 (1998)

88. N.R. Deleault, J.C. Geoghegan, K. Nishina, R. Kascsak, R.A. Williamson and S. Supattapone: Protease-resistant prion protein amplification reconstituted with partially purified substrates and synthetic polyanions. *J Biol Chem* 280, 26873-26879 (2005)

89. M. Cortijo-Arellano, J. Ponce, N. Durany and J. Cladera: Amyloidogenic properties of the prion protein fragment PrP(185-208): comparison with Alzheimer's peptide Abeta(1-28), influence of heparin and cell toxicity. *Biochem Biophys Res Commun* 368, 238-242 (2008)

90. B. Klajnert, M. Cortijo-Arellano, M. Bryszewska and J. Cladera: Influence of heparin and dendrimers on the aggregation of two amyloid peptides related to Alzheimer's and prion diseases. *Biochem Biophys Res Commun* 339, 577-582 (2006)

91. S. Chen, A. Mange, L. Dong, S. Lehmann and M. Schachner: Prion protein as trans-interacting partner for neurons is involved in neurite outgrowth and neuronal survival. *Mol Cell Neurosci* 22, 227-233 (2003)

92. T. Kinnunen, M. Kaksonen, J. Saarinen, N. Kalkkinen, H.B. Peng and H. Rauvala: Cortactin-Src kinase signaling pathway is involved in N-syndecan-dependent neurite outgrowth. *J Biol Chem* 273, 10702-10708 (1998)

93. C. Hundt, J.M. Peyrin, S. Haik, S. Gauczynski, C. Leucht, R. Rieger, M.L. Riley, J.P. Deslys, D. Dormont, C.I. Lasmezas and S. Weiss: Identification of interaction domains of the prion protein with its 37-kDa/67-kDa laminin receptor. *EMBO J* 20, 5876-5886 (2001)

94. S. Gauczynski, D. Nikles, S. El Gogo, D. Papy-Garcia, C. Rey, S. Alban, D. Barritault, C.I. Lasmezas and S. Weiss: The 37-kDa/67-kDa laminin receptor acts as a receptor for infectious prions and is inhibited by polysulfated glycanes. *J Infect Dis* 194, 702-709 (2006)

95. C. Leucht, S. Simoneau, C. Rey, K. Vana, R. Rieger, C.I. Lasmezas and S. Weiss: The 37 kDa/67 kDa laminin receptor is required for PrP(Sc) propagation in scrapie-infected neuronal cells. *EMBO Rep* 4, 290-295 (2003)

96. M. Praus, G. Kettelgerdes, M. Baier, H.G. Holzhutter, P.R. Jungblut, M. Maissen, G. Epple, W.D. Schleuning, E. Kottgen, A. Aguzzi and R. Gessner: Stimulation of plasminogen activation by recombinant cellular prion protein is conserved in the NH2-terminal fragment PrP23-110. *Thromb Haemost* 89, 812-819 (2003)

97. L.A. Fransson, M. Belting, F. Cheng, M. Jonsson, K. Mani and S. Sandgren: Novel aspects of glypican glycobiology. *Cell Mol Life Sci* 61, 1016-1024 (2004)

98. K. Mani, F. Cheng and L.A. Fransson: Heparan sulfate degradation products can associate with oxidized proteins and proteasomes. *J Biol Chem* 282, 21934-21944 (2007)

99. M. Belting, S. Persson and L.A. Fransson: Proteoglycan involvement in polyamine uptake. *Biochem J* 338 (Pt 2), 317-323 (1999)

100. K. Ding, K. Mani, F. Cheng, M. Belting and L.A. Fransson: Copper-dependent autocleavage of glypican-1 heparan sulfate by nitric oxide derived from intrinsic nitrosothiols. *J Biol Chem* 277, 33353-33360 (2002)

101. G.L. Millhauser: Copper and the prion protein: methods, structures, function, and disease. *Annu Rev Phys Chem* 58, 299-320 (2007)

102. K. Mani, F. Cheng, B. Havsmark, M. Jonsson, M. Belting and L.A. Fransson: Prion, amyloid beta-derived Cu(II) ions, or free Zn(II) ions support S-nitroso-dependent autocleavage of glypican-1 heparan sulfate. *J Biol Chem* 278, 38956-38965 (2003)

103. F. Cheng, J. Lindqvist, C.L. Haigh, D.R. Brown and K. Mani: Copper-dependent co-internalization of the prion protein and glypican-1. *J Neurochem* 98, 1445-1457 (2006)

104. P.C. Pauly and D.A. Harris: Copper stimulates endocytosis of the prion protein. *J Biol Chem* 273, 33107-33110 (1998)

105. A.D. Cardin and H.J. Weintraub: Molecular modeling of protein-glycosaminoglycan interactions. *Arteriosclerosis* 9, 21-32 (1989)

106. M. Sobel, D.F. Soler, J.C. Kermode and R.B. Harris: Localization and characterization of a heparin binding domain peptide of human von Willebrand factor. *J Biol Chem* 267, 8857-8862 (1992)

107. H. Margalit, N. Fischer and S.A. Ben Sasson: Comparative analysis of structurally defined heparin binding sequences reveals a distinct spatial distribution of basic residues. *J Biol Chem* 268, 19228-19231 (1993)

108. J.R. Fromm, R.E. Hileman, J.M. Weiler and R.J. Linhardt: Interaction of fibroblast growth factor-1 and related peptides with heparan sulfate and its oligosaccharides. *Arch Biochem Biophys* 346, 252-262 (1997)

109. J.R. Fromm, R.E. Hileman, E.E. Caldwell, J.M. Weiler and R.J. Linhardt: Differences in the interaction of heparin with arginine and lysine and the importance of these basic amino acids in the binding of heparin to acidic fibroblast growth factor. *Arch Biochem Biophys* 323, 279-287 (1995)

110. A. Abedini, S.M. Tracz, J.H. Cho and D.P. Raleigh: Characterization of the heparin binding site in the N-terminus of human pro-islet amyloid polypeptide: implications for amyloid formation. *Biochemistry* 45, 9228-9237 (2006)

111. J. Hallgren, S. Backstrom, S. Estrada, M. Thuveson and G. Pejler: Histidines are critical for heparin-dependent activation of mast cell tryptase. *J Immunol* 173, 1868-1875 (2004)

112. A. Sebollela, T.C. Cagliari, G.S. Limaverde, A. Chapeaurouge, M.H. Sorgine, T. Coelho-Sampaio, C.H. Ramos and S.T. Ferreira: Heparin-binding sites in granulocyte-macrophage colony-stimulating factor. Localization and regulation by histidine ionization. *J Biol Chem* 280, 31949-31956 (2005)

113. R.A. Pixley, Y. Lin, I. Isordia-Salas and R.W. Colman: Fine mapping of the sequences in domain 5 of high molecular weight kininogen (HK) interacting with heparin and zinc. *J Thromb Haemost* 1, 1791-1798 (2003)

114. F. Wopfner, G. Weidenhofer, R. Schneider, A. von Brunn, S. Gilch, T.F. Schwarz, T. Werner and H.M. Schatzl: Analysis of 27 mammalian and 9 avian PrPs reveals high conservation of flexible regions of the prion protein. *J Mol Biol* 289, 1163-1178 (1999)

115. L.G. Goldfarb, P. Brown, W.R. McCombie, D. Goldgaber, G.D. Swergold, P.R. Wills, L. Cervenakova, H. Baron, C.J. Gibbs, Jr. and D.C. GAJDUSEK: Transmissible familial Creutzfeldt-Jakob disease associated with five, seven, and eight extra octapeptide coding repeats in the PRNP gene. *Proc Natl Acad Sci U S A* 88, 10926-10930 (1991)

116. S. Yu, S. Yin, C. Li, P. Wong, B. Chang, F. Xiao, S.C. Kang, H. Yan, G. Xiao, P. Tien and M.S. Sy: Aggregation of prion protein with insertion mutations is proportional to the number of inserts. *Biochem J* 403, 343-351 (2007)

117. R. Gonzalez-Iglesias, M.A. Pajares, C. Ocal, J.C. Espinosa, B. Oesch and M. Gasset: Prion protein interaction with glycosaminoglycan occurs with the formation of oligomeric complexes stabilized by Cu(II) bridges. *J Mol Biol* 319, 527-540 (2002)

118. D.B. Brimacombe, A.D. Bennett, F.S. Wusteman, A.C. Gill, J.C. Dann and C.J. Bostock: Characterization and polyanion-binding properties of purified recombinant prion protein. *Biochem J* 342 Pt 3, 605-613 (1999)

119. S. Yin, S. Yu, C. Li, P. Wong, B. Chang, F. Xiao, S.C. Kang, H. Yan, G. Xiao, J. Grassi, P. Tien and M.S. Sy: Prion proteins with insertion mutations have altered N-terminal conformation and increased ligand binding activity and are more susceptible to oxidative attack. *J Biol Chem* 281, 10698-10705 (2006)

120. S. Yin, N. Pham, S. Yu, C. Li, P. Wong, B. Chang, S.C. Kang, E. Biasini, P. Tien, D.A. Harris and M.S. Sy: Human prion proteins with pathogenic mutations share common conformational changes resulting in enhanced binding to glycosaminoglycans. *Proc Natl Acad Sci U S A* 104, 7546-7551 (2007)

121. S. Yu, S. Yin, N. Pham, P. Wong, S.C. Kang, R.B. Petersen, C. Li and M.S. Sy: Ligand binding promotes prion protein aggregation--role of the octapeptide repeats. *FEBS J* 275, 5564-5575 (2008)

122. D. Shukla, J. Liu, P. Blaiklock, N.W. Shworak, X. Bai, J.D. Esko, G.H. Cohen, R.J. Eisenberg, R.D. Rosenberg and P.G. Spear: A novel role for 3-O-sulfated heparan sulfate in herpes simplex virus 1 entry. *Cell* 99, 13-22 (1999)

123. B. Casu and U. Lindahl: Structure and biological interactions of heparin and heparan sulfate. *Adv Carbohydr Chem Biochem* 57, 159-206 (2001)

124. M.O. Ouidja, E. Petit, M.E. Kerros, Y. Ikeda, C. Morin, G. Carpentier, D. Barritault, J. Brugere-Picoux, J.P. Deslys, K. Adjou and D. Papy-Garcia: Structure-activity studies of heparan mimetic polyanions for anti-prion therapies. *Biochem Biophys Res Commun* 363, 95-100 (2007)

125. S. Vasan, P.Y. Mong and A. Grossman: Interaction of prion protein with small highly structured RNAs: detection and characterization of PrP-oligomers. *Neurochem Res* 31, 629-637 (2006)

126. N.R. Deleault, B.T. Harris, J.R. Rees and S. Supattapone: Formation of native prions from minimal components *in vitro*. *Proc Natl Acad Sci U S A* 104, 9741-9746 (2007)

127. N. Daude, M. Marella and J. Chabry: Specific inhibition of pathological prion protein accumulation by small interfering RNAs. *J Cell Sci* 116, 2775-2779 (2003)

128. M.V. Karpuj, K. Giles, S. Gelibter-Niv, M.R. Scott, V.R. Lingappa, F.C. Szoka, D. Peretz, W. Denetclaw and S.B. Prusiner: Phosphorothioate oligonucleotides reduce PrP levels and prion infectivity in cultured cells. *Mol Med* 13, 190-198 (2007)

129. P.K. Nandi: Interaction of prion peptide HuPrP106-126 with nucleic acid. *Arch Virol* 142, 2537-2545 (1997)

130. A. Rhie, L. Kirby, N. Sayer, R. Wellesley, P. Disterer, I. Sylvester, A. Gill, J. Hope, W. James and A. Tahiri-Alaoui: Characterization of 2'-fluoro-RNA aptamers that bind preferentially to disease-associated conformations of prion protein and inhibit conversion. *J Biol Chem* 278, 39697-39705 (2003)

131. W.Q. Zou, J. Zheng, D.M. Gray, P. Gambetti and S.G. Chen: Antibody to DNA detects scrapie but not normal prion protein. *Proc Natl Acad Sci U S A* 101, 1380-1385 (2004)

132. C. Gabus, S. Auxilien, C. Pechoux, D. Dormont, W. Swietnicki, M. Morillas, W. Surewicz, P. Nandi and J.L. Darlix: The prion protein has DNA strand transfer properties similar to retroviral nucleocapsid protein. *J Mol Biol* 307, 1011-1021 (2001)

133. C. Gabus, E. Derrington, P. Leblanc, J. Chnaiderman, D. Dormont, W. Swietnicki, M. Morillas, W.K. Surewicz, D. Marc, P. Nandi and J.L. Darlix: The prion protein has RNA binding and chaperoning properties characteristic of nucleocapsid protein NCP7 of HIV-1. *J Biol Chem* 276, 19301-19309 (2001)

134. J.L. Silva, L.M. Lima, D. Foguel and Y. Cordeiro: Response to Radulescu and Brenig: Infectious nucleic acids in prion disease: halfway there. *Trends Biochem Sci* 34, 5-6 (2009)

135. P.K. Nandi and E. Leclerc: Polymerization of murine recombinant prion protein in nucleic acid solution. *Arch Virol* 144, 1751-1763 (1999)

136. P.K. Nandi, E. Leclerc, J.C. Nicole and M. Takahashi: DNA-induced partial unfolding of prion protein leads to its polymerisation to amyloid. *J Mol Biol* 322, 153-161 (2002)

137. P.K. Nandi and J.C. Nicole: Nucleic acid and prion protein interaction produces spherical amyloids which can function *in vivo* as coats of spongiform encephalopathy agent. *J Mol Biol* 344, 827-837 (2004)

138. L.M. Lima, Y. Cordeiro, L.W. Tinoco, A.F. Marques, C.L. Oliveira, S. Sampath, R. Kodali, G. Choi, D. Foguel, I. Torriani, B. Caughey and J.L. Silva: Structural insights into the interaction between prion protein and nucleic acid. *Biochemistry* 45, 9180-9187 (2006)

139. A. Mange, C. Crozet, S. Lehmann and F. Beranger: Scrapie-like prion protein is translocated to the nuclei of infected cells independently of proteasome inhibition and interacts with chromatin. *J Cell Sci* 117, 2411-2416 (2004)

140. S. Yin, X. Fan, S. Yu, C. Li and M.S. Sy: Binding of recombinant but not endogenous prion protein to DNA causes DNA internalization and expression in mammalian cells. *J Biol Chem* 283, 25446-25454 (2008)

141. D.A. Kocisko, A. Vaillant, K.S. Lee, K.M. Arnold, N. Bertholet, R.E. Race, E.A. Olsen, J.M. Juteau and B. Caughey: Potent antiscrapie activities of degenerate phosphorothioate oligonucleotides. *Antimicrob Agents Chemother* 50, 1034-1044 (2006)

142. D.J. King, J.G. Safar, G. Legname and S.B. Prusiner: Thioaptamer interactions with prion proteins: sequence-specific and non-specific binding sites. *J Mol Biol* 369, 1001-1014 (2007)

143. S. Weiss, D. Proske, M. Neumann, M.H. Groschup, H.A. Kretzschmar, M. Famulok and E.L. Winnacker: RNA aptamers specifically interact with the prion protein PrP. *J Virol* 71, 8790-8797 (1997)

144. V. Adler, B. Zeiler, V. Kryukov, R. Kascsak, R. Rubenstein and A. Grossman: Small, highly structured RNAs participate in the conversion of human recombinant PrP(Sen) to PrP(Res) *in vitro*. *J Mol Biol* 332, 47-57 (2003)

145. S. Sekiya, K. Noda, F. Nishikawa, T. Yokoyama, P.K. Kumar and S. Nishikawa: Characterization and application of a novel RNA aptamer against the mouse prion protein. *J Biochem* 139, 383-390 (2006)

146. N.M. Sayer, M. Cubin, A. Rhie, M. Bullock, A. Tahiri-Alaoui and W. James: Structural determinants of conformationally selective, prion-binding aptamers. *J Biol Chem* 279, 13102-13109 (2004)

147. J.C. Geoghegan, P.A. Valdes, N.R. Orem, N.R. Deleault, R.A. Williamson, B.T. Harris and S. Supattapone: Selective incorporation of polyanionic molecules into hamster prions. *J Biol Chem* 282, 36341-36353 (2007)

148. F.F. Costa: Non-coding RNAs: new players in eukaryotic biology. *Gene* 357, 83-94 (2005)

149. D. Kampa, J. Cheng, P. Kapranov, M. Yamanaka, S. Brubaker, S. Cawley, J. Drenkow, A. Piccolboni, S. Bekiranov, G. Helt, H. Tammana and T.R. Gingeras: Novel RNAs identified from an in-depth analysis of the transcriptome of human chromosomes 21 and 22. *Genome Res* 14, 331-342 (2004)

150. M.E. Dinger, T.R. Mercer and J.S. Mattick: RNAs as extracellular signaling molecules. *J Mol Endocrinol* 40, 151-159 (2008)

151. A. Bera and P.K. Nandi: Biological polyamines inhibit nucleic-acid-induced polymerisation of prion protein. *Arch Virol* 152, 655-668 (2007)

152. M.P. Gomes, Y. Cordeiro and J.L. Silva: The peculiar interaction between mammalian prion protein and RNA. *Prion* 2, 64-66 (2008)

153. R. Linden, V.R. Martins, M.A. Prado, M. Cammarota, I. Izquierdo and R.R. Brentani: Physiology of the prion protein. *Physiol Rev* 88, 673-728 (2008)

154. H. Tjong and H.X. Zhou: DISPLAR: an accurate method for predicting DNA-binding sites on protein surfaces. *Nucleic Acids Res* 35, 1465-1477 (2007)

155. A. Bera, A.C. Roche and P.K. Nandi: Bending and unwinding of nucleic acid by prion protein. *Biochemistry* 46, 1320-1328 (2007)

156. P. Tompa and P. Csermely: The role of structural disorder in the function of RNA and protein chaperones. *FASEB J* 18, 1169-1175 (2004)

157. N.R. Cashman and B. Caughey: Prion diseases--close to effective therapy? *Nat Rev Drug Discov* 3, 874-884 (2004)

158. C. Korth, B.C. May, F.E. Cohen and S.B. Prusiner: Acridine and phenothiazine derivatives as pharmacotherapeutics for prion disease. *Proc Natl Acad Sci U S A* 98, 9836-9841 (2001)

159. S.J. Collins, V. Lewis, M. Brazier, A.F. Hill, A. Fletcher and C.L. Masters: Quinacrine does not prolong survival in a murine Creutzfeldt-Jakob disease model. *Ann Neurol* 52, 503-506 (2002)

160. D.A. Kocisko, G.S. Baron, R. Rubenstein, J. Chen, S. Kuizon and B. Caughey: New inhibitors of scrapie-associated prion protein formation in a library of 2000 drugs and natural products. *J Virol* 77, 10288-10294 (2003)

161. Y. Cordeiro, L.M. Lima, M.P. Gomes, D. Foguel and J.L. Silva: Modulation of prion protein oligomerization, aggregation, and beta-sheet conversion by 4,4'-dianilino-1,1'-binaphthyl-5,5'-sulfonate (bis-ANS). *J Biol Chem* 279, 5346-5352 (2004)

162. R.H. Kimberlin and C.A. Walker: Suppression of scrapie infection in mice by heteropolyanion 23, dextran sulfate, and some other polyanions. *Antimicrob Agents Chemother* 30, 409-413 (1986)

163. C.F. Farquhar and A.G. Dickinson: Prolongation of scrapie incubation period by an injection of dextran sulphate 500 within the month before or after infection. *J Gen Virol* 67 (Pt 3), 463-473 (1986)

164. H. Diringer and B. Ehlers: Chemoprophylaxis of scrapie in mice. *J Gen Virol* 72 (Pt 2), 457-460 (1991)

165. A. Ladogana, P. Casaccia, L. Ingrosso, M. Cibati, M. Salvatore, Y.G. Xi, C. Masullo and M. Pocchiari: Sulphate polyanions prolong the incubation period of scrapie-infected hamsters. *J Gen Virol* 73 (Pt 3), 661-665 (1992)

166. N.V. Todd, J. Morrow, K. Doh-ura, S. Dealler, S. O'Hare, P. Farling, M. Duddy and N.G. Rainov: Cerebroventricular infusion of pentosan polysulphate in human variant Creutzfeldt-Jakob disease. *J Infect* 50, 394-396 (2005)

167. K.T. Adjou, S. Simoneau, N. Sales, F. Lamoury, D. Dormont, D. Papy-Garcia, D. Barritault, J.P. Deslys and C.I. Lasmezas: A novel generation of heparan sulfate mimetics for the treatment of prion diseases. *J Gen Virol* 84, 2595-2603 (2003)

168. O. Schonberger, L. Horonchik, R. Gabizon, D. Papy-Garcia, D. Barritault and A. Taraboulos: Novel heparan mimetics potently inhibit the scrapie prion protein and its endocytosis. *Biochem Biophys Res Commun* 312, 473-479 (2003)

169. C. Larramendy-Gozalo, A. Barret, E. Daudigeos, E. Mathieu, L. Antonangeli, C. Riffet, E. Petit, D. Papy-Garcia, D. Barritault, P. Brown and J.P. Deslys: Comparison of CR36, a new heparan mimetic, and pentosan polysulfate in the treatment of prion diseases. *J Gen Virol* 88, 1062-1067 (2007)

170. S. Beaudoin, B. Vanderperre, C. Grenier, I. Tremblay, F. Leduc and X. Roucou: A large ribonucleoprotein particle induced by cytoplasmic PrP shares striking similarities with the chromatoid body, an RNA granule predicted to function in posttranscriptional gene regulation. *Biochim Biophys Acta* 1793, 335-345 (2009)

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