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Aptamers to explore prion protein interactions with nucleic acids

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1. ABSTRACT

A misfolded isoform of the prion protein (PrP) is the essential component of the prion diseases' agent. The prion concept has progressively gained acceptance, in a large part thanks to the realization that it played a role not only in the transmissible spongiform encephalopathies, but also in the non-Mendelian propagation of self-perpetuating phenotypes of the yeast Saccharomyces cerevisiae. Uncertainties about the nature of the agent and the function of PrP have fostered searches of nucleic acid ligands of the protein. *In vitro* methods of nucleic acid evolutions have been used to identify RNAs or DNAs that bind PrP, towards the triple objective of i) setting up new diagnostic tools, ii) identifying nucleic acids with which PrP may interact, as part of its physiological or pathological function, and iii) elucidating the pathological transconformation of PrP. This review will focus on these studies, their methods, the knowledge acquired from them about the prion protein, and the possibilities that they offer in the areas of diagnosis and therapy of prion diseases.

2. INTRODUCTION

Considerable progresses have been accomplished in the past ten years in the understanding of the prion mechanisms. In spite of this, the nature of the infectious agent of transmissible spongiform encephalopathies (TSEs) is still a mystery. Besides, another largely unsolved problem in TSEs relates to the physiological function of PrP^C, the cellular prion protein (1).

For decades, there have been numerous attempts to characterize the agent of scrapie and related diseases, and to compare its properties with those of known infectious agents. Ever since the first attempts towards its molecular characterization, the unusual properties of the agent led to the hypothesis that it could lack a nucleic acid, and several models were proposed to account for an infectious agent with no genome (2-4). Indeed, many independent studies all failed to identify specific nucleic acids in the scrapie agent, including viroid-like molecules, as well as to demonstrate any infectivity associated with

nucleic acids extracted from the brains of infected animals (5-6). This is also consistent with the exceptional resistance of the agent against physical, chemical and enzymatic treatments that degrade nucleic acids (7-10). These data led Prusiner to elaborate the prion concept, according to which the infectious agent is a proteinaceous particle lacking nucleic acid and essentially composed of PrP^{Sc}, a misfolded isoform of a ~255-residue glycoprotein that is abundantly expressed by neurons and glial cells (11-12). This hypothesis remained difficult to admit since it could not easily explain the diversity of strains nor the adaptive properties of the agent. As a result, searches for an elusive nucleic acid component of the scrapie agent continued for more than two decades after the first proposal of the prion hypothesis (13-17). The discovery of several prion mechanisms in yeast has offered alternative models in which it was possible to explore many aspects of the prion hypothesis and to test its validity (18). Studies of the (PSI+) phenotype in yeast have convincingly established the validity of the prion theory: prion strains with distinct (PSI+) phenotypes are enciphered by distinct polymerization modes of the yeast protein Sup35 (19-22). Similarly, distinct prion strains in mammals seem to rely on several polymerization modes of PrP (23-25).

If the proteinaceous nature of the TSE agent now seems firmly established, the involvement of nucleic acids in triggering the initial polymerization events remains a possible scenario. In addition, mysteries still obscure the status and biological functions of the cellular protein PrP^{C}, the knowledge of which could help understand many aspects of the pathophysiology of prion diseases (1, 26). These problems have also motivated the studies of prion protein's interaction with nucleic acids. The first such study was conducted with prion peptide 106-126, which had previously been shown to polymerize into amyloid-like fibrils *in vitro*, and to trigger apoptosis of neuronal cells (27-29). Nandi showed that peptide 106-126 was able to bind DNA *in vitro*, and that this interaction induced a conformational change in the DNA, as measured by the quenched fluorescence of the DNA-bound dye TO-PRO (27). Reciprocally, DNA was shown to induce polymerization of the peptide into amyloid, leading the author to suggest that such an interaction *in vivo* could trigger the conformational conversion of PrP^{C} to PrP^{Sc} (27, 30). Based on these initial observations, many studies have then analyzed the interactions of PrP and PrP^{Sc} with nucleic acids. Instead of searching for nucleic acids in the purified infectious fraction, several authors used the newly described SELEX method (Systematic Evolution of Ligands by Exponential enrichment) to identify nucleic acid ligands of PrP from a pool of synthetic sequences.

I will here review the different studies, for their most part based on the SELEX method, that have been conducted to identify DNA or RNA sequences that bind prion proteins. This review will also expose the knowledge that was gained from these studies in understanding how PrP interacts with nucleic acids, and present the perspectives that they offer in the diagnosis and therapy of prion diseases.

3. THE SELEX METHOD

The SELEX method was first developed to isolate RNAs, termed aptamers, that bind with high affinity and specificity to small molecules or to proteins (31-32). SELEX is an *in vitro* evolution method which allows one to isolate, starting from a very large pool of random sequences, one or a few families of sequences that satisfy the selection criteria. It is most often used to isolate DNAs or RNAs that bind a given target, but can also identify nucleic acids exhibiting an enzymatic activity (33-35). The starting DNA pool contains 10^{12} to 10^{15} molecules consisting of a central core of 20-40 random nucleotides (nt), flanked by two constant sequences which contain primer-binding sites and (usually) the T7-RNA polymerase promoter. However, the vast majority of sequences in a synthetic repertoire are expected to have no biological relevance, since they lie outside of the biological domain under consideration: for instance, the human genome encodes no more than ~3 x 10^{9} distinct words of a given size, which is several orders of magnitude less than the complexity of a SELEX repertoire. On the contrary, the genomic version of SELEX allows one to focus the search onto a genome-wide repertoire that is considerably smaller and at the same time more relevant (36-38). In the selection process, RNAs or DNAs that bind the target are separated from unbound molecules through various means, e.g. filter binding, magnetic beads, affinity column or other elaborate processes (39). Each selection reduces the complexity of the population, and usually ten to fifteen iterative cycles of (transcription- selection- amplification) are performed, after which a few tens of sequences are cloned and sequenced.

Not only do these aptamers represent potential therapeutic or diagnostic tools (40, 41). The method also demonstrated its validity in answering biologically relevant questions. Indeed, in many instances the SELEX method was able to extract, from a large population of synthetic sequences, the very motif to which the protein binds *in vivo* to perform its biological function, thus demonstrating the specific interaction with a structural or sequential motif in the nucleic acid (42-44).

4. PROPERTIES OF APTAMERS AND OTHER NUCLEIC ACID LIGANDS OF PrP

4.1. General properties

Towards the purpose of isolating aptamers that could distinguish PrP^{C} from its pathological isoform PrP^{Sc}, Weiss *et al.* were the first authors to carry out the SELEX method in the prion field (45). A recombinant syrian golden hamster PrP fused to GST (GST::rPrP23-231) was immobilized on a glutathione-Sepharose column to capture RNA aptamers which specifically bind to PrP (results are presented below). The other SELEX that were carried out subsequently in other laboratories (Table 1) were directed against either full-length recombinant PrP from various species, its globular domain only, non-PK-treated Scrapie Associated Fibrils, or against synthetic peptide 90-129 (46-51). In spite of the fact that RNAs are more labile than DNAs, they are considered to adopt more complex structures, and for that reason they are often preferred to

Table 1. Published PrP-specific aptamers

First author, date (ref. n°)	nucleic acid type	n[1]	PrP species and construction
Weiss, 1997 (45)	unmodified RNA	74	syrian golden hamster, GST::PrP23-231
Proske, 2002 (51)	2'-amino-2'-deoxypyrimidine RNA	40	Human PrP 90-129 peptide
Rhie, 2003 (50)	2'-fluoro-pyrimidine RNA	49	Scrapie Associated Fibrils (hamster strain 263K, no PK-treatment)
Mercey, 2006 (48)	unmodified RNA	40	Ovine Recombinant PrP25-231
Sekiya, 2005 (47)	unmodified RNA	30	Mouse recombinant PrP23-230, and mouse SAF
Sekiya, 2006 (66)	unmodified RNA	30	Mouse recombinant PrP23-230
Nishikawa, 2007, Matsugami 2008 (63, 65)	unmodified RNA	30	Bovine recombinant PrP
Murakami, 2008 (64)	RNA	55	Bovine recombinant PrP
Takemura, 2006 (46)	ssDNA	40	Human recombinant PrP23-231
Bibby, 2008 (49)	ssDNA	45	Mouse recombinant PrP (His6-moPrP90-231)
Ogasawara 2008 (53, 107)	ssDNA		Mouse recombinant PrP23-231

[1]: length of the random sequence

DNAs, or at least more abundant in the literature (52). To overcome the problem of degradation and increase their stability against nucleases, some studies used chemically modified RNA aptamers that were transcribed in the presence of either 2'-Fluoro- or 2'-amino-2'-deoxy pyrimidine nucleotide triphosphates (50-51). Three studies chose instead to isolate DNA aptamers, which are more stable and have been shown to functionally rivalize with RNA ligands (46, 49, 53-54).

Beside the highly specific ligands that were selected from random libraries, PrP has been shown to bind a variety of DNAs or RNAs, which have proved very useful in uncovering many aspects of the PrP-nucleic acids interaction. These include plasmids, total mouse DNA, short DNA duplexes known to bind transcription factors or other DNA-binding proteins, HIV-1 LTR DNA and HIV-1 RNA (27, 55-59). In addition, small synthetic RNAs that were specially designed for their highly structured conformation were found to strongly bind PrP *in vitro* (60-62). The best ligands in the latter studies harboured sequences from Ap1, a previously described PrP-specific aptamer (45, 60-61).

4.2. Sequence features

Sequence comparisons and other motif searches could not reveal an obvious pattern that would be shared by all PrP-specific aptamers. However, we identified at least two interesting similarities between aptamers that were raised against sheep PrP in our laboratory and those obtained independently in other laboratories (48). First, our most frequently isolated aptamer RM-312, that was also the best ligand in our collection, contained a 21-nt motif that is also present in DP7, a 2'-aminopyrimidine-modified aptamer isolated by Proske *et al.* against prion peptide 90-129 (51). This sequence could be minimally described by the following pattern AAG (A or G) (C or U)GUCGGGG-N_{0-1}-UUGGCA-N_{0-1}-AA, where N_{0-1} is zero or one nucleotide. Secondly, aptamers from the second family in our collection shared a 14-nt motif (CUCCAAUG-N_3-UGG) with SAF-131, a 2'-Fluoro aptamer that bound scrapie-associated fibrils from hamster brain (50). Of note, the only Genbank-deposited sequences that were found to harbour the sequence motif shared by aptamers RM-312 and DP7 were those of DP7 itself and its related aptamers, which suggests that these motifs do not result from some experimental bias. On the other hand, even when allowing for one or two mismatches, this sequence pattern was rare in Genbank, which suggest that it does not represent a physiologically relevant target of PrP *in vivo*.

4.3. Structural features in PrP-specific aptamers

In spite of their sequence diversity, several PrP aptamers shared the potential to form guanine-quadruplex structures. Weiss *et al.* observed that thirty percent of their aptamers contained repeated guanosine triplets (GGG….GGG….GGG…GGG), leading the authors to suggest that they fold into a guanine quadruplex (45). This structure seemed indeed necessary for the interaction, since its disruption in aptamer Ap1 by replacing the guanosine residues with uridines completely abolished its interaction with PrP. G-quadruplexes were identified in bovPrP-specific RNA, and were also predicted in DP7, a 2'-aminopyrimidine aptamer selected by Proske *et al.* against human prion peptide 90-129 (51, 63-65). In fact, several other DNA- or RNA-aptamers raised against PrP contain at least four separate GG dinucleotides in their full-length sequence (46, 48, 66). For that reason one cannot definitely rule out the possibility that they also adopt a guanine-quadruplex structure, which is not necessarily intramolecular but can also arise from dimerization. Guanine-quadruplexes are a structural motif composed of two or more tetrads of coplanar guanosines (the G-quartets) that are stacked on top of each other (67). They are present in a significant fraction of mRNAs, and may represent a common recognition motif, since they have been found in a number of aptamers that were raised against various proteins (68-69). For instance Fragile-X Mental Retardation Protein (FMRP) was shown to bind intramolecular G quartets in its mRNAs ligands (70).

Beside G-quadruplexes, diverse structural motifs have been described in RNA- and DNA-aptamers raised against PrP (46, 48, 49, 60, 61, 66, 71). In some cases, the requirement of these structures for PrP-binding has been probed by various methods, including nuclease protection assays, truncations and nucleotide substitutions. However, the absence of any obvious relation between them precludes the identification of a consensus structural pattern.

As a last remark concerning linear or structural motifs in the aptamers, we should keep in mind that the SELEX method itself could present some biases. Firstly, the three polymerases that are commonly used in each cycle of an RNA-based SELEX (e.g. RNA polymerase,

reverse transcriptase and thermostable DNA polymerase) may filter out some sequences or structures which may be difficult to synthesize. Secondly, because it is difficult to quantify, the strength of the *in vitro* selection may be too high to identify biologically relevant sequences, whose functionality requires a balanced and reversible association (72). As a result, some functionally relevant sequences may be lost while searching for the strongest ligands.

5. BINDING PROPERTIES, AND SPECIFICITY OF PRION-NUCLEIC ACID INTERACTIONS

The specificity of the PrP-nucleic acid interaction can be considered from both the protein and nucleic acid sides. Regarding the recognition of PrP by nucleic acids, most of the aptamers that have been raised against PrP from one species also recognized PrP from other species (45, 46, 48, 49). This is not unexpected, considering the high conservation of PrP polypeptide sequence, which is close to 100% in the PrP regions involved in nucleic acid binding. In addition, most of these aptamers recognize both PrP^{C} and its pathological isoform PrP^{Sc}, except for some of them that show some specificity towards PrP^{Sc} or the beta-oligomeric isoform of recombinant PrP, as a result of the selection process (45, 50, 71).

Considering the interaction from the other side, PrP was shown to bind a large variety of nucleic acids that were either identified through SELEX, or tested individually. As discussed above, no obvious consensus sequence or structural pattern emerges from the comparison of these ligands. Indeed, these include both single-stranded and double stranded DNAs, as well as RNAs with diverse structures, some of them harbouring chemically modified nucleotides. However, this diversity should not lead to the conclusion that PrP binds any nucleic acid with no specificity, since modifications of RNA or DNA aptamers through truncation or nucleotide substitutions often reduce binding (46, 48). Moreover, whatever the target molecule, the SELEX method generally identifies several families of sequences, of which only a fraction may represent biologically relevant sequences (72). Reciprocally, genuine nucleic acid-binding proteins do not necessarily bind only one well defined sequence, but may instead functionally interact with more than one structural or sequential pattern (73, 74). Furthermore, functional interaction sometimes requires the protein to be flexible enough as to accommodate several low-affinity binding-sites, as in the case of HIV Rev protein (75).

As discussed above, the affinity of a protein-nucleic acid interaction may not necessarily correlate with functionality, since the latter often requires a reversible association. However, it is a convenient value in comparing several interactions, and in predicting the practical use of an interaction in detection assays. The K_D values that have been published for the various PrP-nucleic acid interactions (Table 2), ranging from ~10 $nM.L^{-1}$ to 10 $microM.L^{-1}$, compare well with those measured for genuine RNA- or DNA-binding proteins. These values however should be considered with caution, given the diversity of methods and binding conditions (buffer, temperature, pH, etc.) that were used to calculate them. It would indeed be worthwhile to rigorously compare the affinities of these nucleic-acid ligands by measuring them in parallel, with several types of PrPs, in the same experimental setting.

6. MAPPING THE APTAMER-BINDING SITE ON PrP

Studies with aptamers and other nucleic acid ligands have allowed a better understanding of how they interact with PrP. One important aspect of this knowledge relates to the nucleic acid binding site on PrP. Taking together the results of various studies, interaction of PrP with nucleic acids involves at least three distinct sites in the protein, two of them located at the ends of its unstructured N-terminal domain.

6.1. The N-terminal lysine cluster

Using a series of recombinant proteins in which GST was fused to successive parts of PrP, Weiss *et al.* mapped the major binding site of PrP to residues 23-52, and more precisely to residues 23-36 at the N-terminus of the mature PrP, from the results of antibody-induced supershifts in gel retardation assays (45). Aptamer Ap1 in the Weiss' study was shown to bind PrP^{C} in brain homogenate from mice, hamster and cattle, but did not bind the proteinase K resistant-PrP27-30 extracted from diseased brains, probably because proteinase K-treated PrP lacks its N-terminal part. Many independent studies also pointed to the N-terminal part of PrP as its main nucleic-acid binding domain (46, 61, 66). Our own work with a panel of PrP-derived nonapeptides identified the N-terminal lysine cluster of mature PrP ($K^{25}KRPKPGGGW^{34}$ in ovine PrP) as its major nucleic-acid binding site (48). This peptide probably binds through charge interaction with low specificity and high affinity, and likely coincides with the heparin-binding site of PrP (76). However, a rigorous demonstration that only charge interactions are at play would require studying the interaction of nucleic acids with a modified PrP in which lysines are replaced by arginines, and vice versa.

6.2. The second lysine cluster and the 90-130 region of PrP

Our data with the panel of PrP-derived nonapeptides (48) clearly identified a second nucleic-acid binding site, consisting of the second lysine cluster ($W^{101}NKPSKPKT^{110}$ in sheep PrP). Interestingly, both lysine-clusters contain alternating proline and basic residues, a feature that is reminiscent of the binding motifs found in proteins that are known to interact with nucleotides and nucleic acids (77). Our data suggested that binding to this second lysine cluster does not rely solely on charge interaction but may also involve stacking interactions with tryptophan residue 101, since the overlapping peptide $K^{103}PSKPKTNM^{112}$, lacking residue W^{101}, did not bind in spite of its three basic lysine residues. Therefore, this lysine cluster may form the core of a larger binding site, and this view is supported by the identification through nuclear magnetic resonance (NMR) of PrP residues involved in DNA-binding (78). From chemical shift measurements of DNA-bound prion protein (Syrian

Table 2. Binding properties of the aptamers and other PrP-binding nucleic acids

First author, date (ref. n°)	K_D [1] ($nM.L^{-1}$)	K_D determination method, or binding-assay	PrP species recognized [2,3]	PrP binding site(s)	Nucleic acid
Weiss, 1997 (45)	ND	Gel-shift of labelled aptamer	Mouse, hamster, cow (PrP^3 in brain homogenates)	[23-36]	RNA-aptamer
Proske, 2002 (51)	100	Filter-binding assay	Syrian hamster, mouse, human, and chimeric mouse-hamster-mouse PrP (recombinant PrPs)	[90 -129] = Selex target	RNA-aptamer
Rhie, 2003 (50)	16	homologous competition binding assay	recBov-PrP in beta-oligomeric form or alpha-helical conformation, PK-untreated SAF, PK-treated SAF	N-terminus and conformation-specific site in [110-230]	RNA-aptamer
Sayer, 2004 (71)	6.8	Equilibrium binding	recBov-PrP		RNA-aptamer
Mercey, 2006 (48)	15	Surface plasmon resonance, filter-binding assay	recOv-PRP (ARR, VRQ, AHQ, ARQ), recMo-PrP, recBov-PrP	[25-34] and [101-110]	RNA-aptamer
Sekiya, 2005 (47)			recMo-PrP(23-230), and mouse SAF	[23-108] of MoPrP	RNA-aptamer
Sekiya, 2006 (66)	5.6	Filter-binding assay	recMo-PrP(23-230), recBov-PrP, Mouse PrP^3 in brain homogenate	[23-108] (23-88)	RNA-aptamer
Takemura, 2006 (46)	16	End-point titration method in microplate, gel-shift, dot-blot	recHum-PrP(23-231), PrP^3 immunoprecipitated from brain homogenates of sheep, calves, pigs, deer, PK-untreated PrP from ScN2A cells	[23-89]	DNA-aptamer
Nishikawa, 2007, Matsugami 2008 (63, 65)	ND		recBov-PrP	ND	RNA-aptamer
Bibby, 2008 (49)	18	saturation binding using PrP-coated Ni-NTA beads	recMo-PrP (His6-moPrP90-231), recOv-PrP and recHum-PrP(90-231)	[90-230] region	DNA-aptamer
Ogasawara 2008 (53, 107)	100	Surface plasmon resonance	recMo-PrP(23-231)	ND	DNA-aptamer
Murakami, 2008 (64)	31	Filter-binding assay	recBov-PrP	[125-231]	RNA-aptamer
Nandi, 1997 (27)	250	Fluorescent dye displacement	recHum-PrP 106-126		Plasmid DNA
Gabus, 2001 (58)	ND	Gel shift	recHum-PrP (23-231 and 23-144)	N-terminus	HIV-1 LTR DNA (1000 bp), $tRNALys^3$
Gabus, 2001 (59)	ND	Gel shift	recHum-PrP (23-231), recOv-PrP (25-234)	N-terminus	HIV-1 5'-leader RNA (415 nt)
Cordeiro 2001 (56)	25	Fluorescence polarization	recMo-PrP (23-231)		Short double-stranded DNAs (Lexcons, recA1/2, E2DBS)
Lima 2006 (78)	90	Fluorescence anisotropy	recHa-PrP (23-231)		Short double-stranded DNAs (E2DBS)
Adler 2003 (60, 61)	3.8	Gel shift, filter-binding assay	recHum-PrP, PrP^3 from brain homogenates of mouse, rat and hamster	N-terminus	Small, highly structured RNAs
Bera 2007 (57)	1100	Fluorescence polarization	recHum-PrP(23-231)	N-terminus	Short double-stranded DNAs (NC-DNA and Lef-DNA)

[1]: when several aptamers were described, K_D value is that of the best interaction. [2] **:** when several types of PrP were assayed in binding experiments, PrP-type with the lowest K_D value is the first indicated. [3]: recOv-, revMo-, recBov-, recHum-PrP : recombinant ovine, bovine, murine and human PrP, respectively.

hamster PrP^{90-231}), Lima *et al.* identified several residues in a ~20 amino-acid region (N^{97} to A^{116} in hamster PrP) encompassing this lysine cluster, that are involved in the interaction of PrP with nucleic acid (Figure 1). Data from two other studies also argue in favour of an interaction of nucleic acids with peptide 90-130 of PrP. Firstly, the earliest demonstration of a PrP-DNA interaction was conducted with prion peptide 106-126 (KTNMKHMAGAAAAGAVVGGLG), which partially overlaps the second lysine cluster (27). Secondly, the 90-129 region of PrP was chosen as a target to isolate PrP-specific RNAs, based on the rationale that it belongs to an unstructured epitope known to be involved in the PrP^C to PrP^{Sc} conversion: DP7, together with other aptamers from the same study, was obtained from a SELEX against peptide 90-129 from human PrP (51). It is likely that this region acts in cooperation with the first lysine cluster, at the other end of PrP's unstructured domain, to bind one nucleic-acid molecule, but a simultaneous interaction with two or more molecules cannot be ruled out.

Interestingly, this second nucleic-acid binding site harbours several residues whose substitution is associated with susceptibility to familial and transmissible prion diseases. In several instances, these substitutions involve only subtle differences between hydrophobic residues. In humans, substitutions P102L, P105L, A117V and G131V are all associated with Gerstmann-Sträussler-

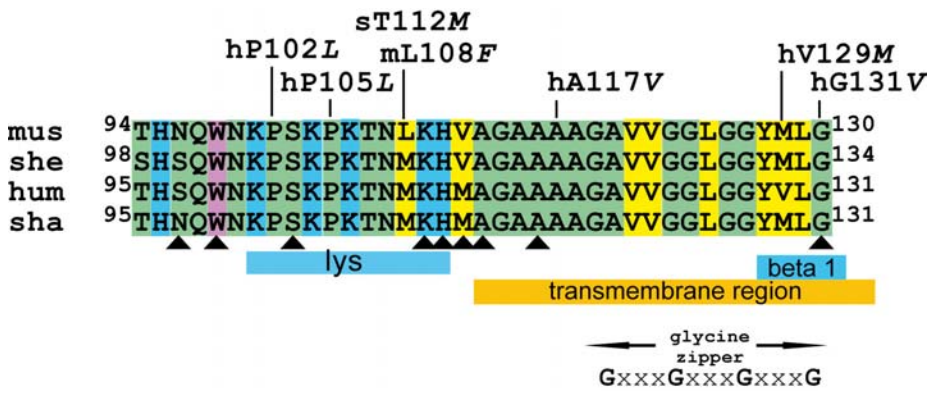

Figure 1. The second lysine cluster in the prion protein. The lysine cluster and the neighboring peptide region (residues 94 to 130) of the murine (top), sheep, human Val129 and Syrian hamster (bottom) prion proteins were aligned. Amino-acids are color-coded as follows : small, uncharged, polar and proline residues are green (G, A, N, S, T, Q, P), hydrophobic residues are yellow (Y, F, V, I, L, M), basic residues are blue (R, K, H), red : acidic (E, D), purple : rare (W, C), (123). Triangles underneath indicate residues in syrian hamster PrP that were predicted to interact with DNA, on the basis of chemical shifts measurements between the NMR spectra of free and DNA-bound ShaPrP90-231 (78). Polymorphic residues associated with familial diseases or susceptibility to prion diseases are indicated above the alignment. Since numbering varies between species, these are denoted as follows: the first letter (lowercase), indicating the species, is followed by the common amino-acid at the given position, followed by the position number and the susceptibility-linked residue. Thus, in humans P102L, P105L, A117V and G131V are all associated with the familial Gerstmann-Straüssler-Scheinker disease, whereas M129 confers susceptibility to vCJD. In sheep (sT112M), residue T112 is not common, but it is associated with resistance to scrapie in sheep of the ARQ genotype (81). In mouse, substitution L108F (together with T189V) is associated with a prolonged incubation period (80).

Scheinker disease, whereas M129, as opposed to V129, confers susceptibility to vCJD, the variant form of Creutzfeldt-Jakob disease (79). In mice, L108F is one of the two polymorphisms that control scrapie incubation time (the other being T189V) (80). And strikingly, a recent study described a new polymorphism in ovine PrP, M112T, which is structurally equivalent to residue 108 in murine PrP (see figure 1). In sheep, susceptibility to scrapie is governed by three major polymorphisms at residues 136, 154 and 171, with $A^{136}R^{154}Q^{171}$ being one of the most susceptible alleles. However, sheep carrying one or two copies of the rare $T^{112}A^{136}R^{154}Q^{171}$ allele, instead of the common $M^{112}A^{136}R^{154}Q^{171}$, showed a dramatically prolonged scrapie-incubation time (81).

6.3. The structured domain of PrP

Binding experiments with peptides may be too stringent and therefore identify only the strongest, charge-dependent interactions. Indeed, beside these two interaction sites in its disordered N-terminal domain, the structured domain of PrP also contributes to nucleic acid binding, and studies based on small angle X-ray scattering and nuclear magnetic resonance spectroscopy have shown that that the globular and unstructured domains of PrP are both involved in its interaction with nucleic acids (78). Chemical shifts between the NMR spectra of free and DNA-bound hamster PrP^{90-231} point to several residues in the structured domain, from arginine 136 to alanine 224, that interact with E2DBS, a model 18 bp DNA consisting of the E2 DNA-binding domain of human papillomavirus. This is also supported by an independent study by Rhie *et al.*, using a SAF-specific aptamer (50). These authors suggested the presence of a conformation-specific "aptatope" downstream of residue 110 in order to explain the behaviour of the interaction observed with one of their aptamers: SAF-93, which was raised against PK-untreated SAFs, bound equally well to both PK-treated and PK-untreated SAFs, with a K_D close to 25 nM. This suggested that aptamer SAF-93 bound to a site downstream from residue 90, since the proteinase treatment usually removes the N-terminal part by cleaving in the vicinity of residue 90 (82). Indeed, SAF-93 was shown to bind both recombinant bovine PrP^{90-230} and a $PrP^{110-230}$ peptide derived from it; however this interaction was conformation-specific since it only occurred when the recombinant protein was refolded in a beta-oligomeric isoform (50, 71).

Each of these three sites in PrP may cooperate in binding nucleic acids through either of the two main modes through which proteins are known to interact with nucleic acids: i) binding to the groove of a nucleic-acid helix, through charge interactions with the backbone, and ii) sequence-specific recognition of unstacked bases into binding pockets of the protein (83, 84). These two modes may be used simultaneously or sequentially: for instance, charge interactions may first bring the two partners in close proximity, thus favouring in a second step the establishment of more specific interactions (85). As a last remark, whereas it is convenient to study in vitro the interaction of one protein with one nucleic acid, one should not forget that in vivo, this simple bimolecular interaction may represent only part of a larger and more complex physiological interaction. Indeed, most interactions involve more than one protein and are cooperatively established within large ribonucleoprotein complexes, as in the case of ribosomes.

7. BIOLOGICAL RELEVANCE OF THE PrP-NUCLEIC ACID INTERACTION

7.1. Aptamers as clues to functional ligands of PrP *in vivo*

Information gained from SELEX can be used to identify functional motifs to which a protein binds *in vivo* (42, 44). Therefore, sequence analysis of the published PrP-specific aptamers could reveal some sequence patterns to which PrP could bind *in vivo*, assuming that such interactions do exist. As discussed above, no obvious sequence motif emerges from the comparison of the various PrP-aptamers, whatever the set considered (DNA aptamers, RNA aptamers, or both). Although one can find in Genbank some of the sequence patterns that happen to be shared by independently obtained aptamers, these patterns are rare, at least when they are searched with high stringency. For instance, the pattern "AAG (A or G) (C or U)GUCGGGG-N_{0-1}-UUGGCA-N_{0-1}-AA", which minimally describes the shared sequence between RNA-aptamers RM-312 and DP-7 was very rare in Genbank-deposited sequences (48, 51). Combined to the fact that, when this pattern is found in a given gene, it is not conserved between mammalian species that are susceptible to prion diseases, this might indicate that it does not play a major role in the pathophysiology of prion diseases. However, this sequence motif is probably too stringent and too long, compared with the short motifs that are usually required for sequence-specific interactions of proteins with DNA or RNA. Indeed, the binding site for most RNA binding proteins rarely exceeds ten nucleotides (73, 86). Therefore, the biologically relevant sequence, if any, may be a shorter sequence embedded in that pattern, possibly combined to structural constraints. Alternatively, and not exclusively, PrP might also interact with specific patterns in DNA, or with nucleic acids bearing some defined structural feature such as guanine-quadruplexes. For instance, like FMRP, PrP might physiologically interact with a specific subset of RNAs bearing guanine-quadruplexes (70).

7.2. Alternative methods to isolate functional nucleic acid ligands of PrP?

To date, SELEX has been used essentially to identify high affinity aptamers from libraries of synthetic sequences, and probably there is room for other, more functional, ligands. Three distinct approaches could potentially fill this gap. Firstly, as discussed above, physiologically relevant sequences may be lost while searching for high affinity ligands, and it may instead be necessary to characterize sets of ligands with low, medium and high affinities, in order to decipher the sequence patterns that make up functional ligands. This can be achieved through lowering both the stringency of the selection method and the number of SELEX cycles, and characterizing larger sets of ligands by high throughput sequencing (87-88). Secondly, the genomic version of the SELEX method could identify new targets, from a genome-wide repertoire of RNAs or DNAs. This to our knowledge has not been done, and our own attempts to isolate potential ligands among total RNAs prepared from mouse brain were unsuccessful (unpublished). Thirdly, as an alternative to SELEX, cross-linking and immunoprecipitation (CLIP) of RNAs has the potential to isolate RNA-PrP complexes that are formed *in vivo* (89). This method was used recently, combined with high-throughput sequencing, to establish a genome-wide map of the RNAs that interact *in vivo* with Nova, a neuron-specific splicing factor (90). Applying this novel method to PrP may reveal functional interactions of PrP with RNAs *in vivo*.

7.3. Uncovering possible functions of PrP

Many reports have shown that interaction of PrP with nucleic acids can modify the structure of the latter. Binding of PrP to model double-stranded DNAs induced bending and destabilization of the DNA helix, in a manner analogous to the distortion of DNA by transcription factors (56-57, 91). Distortion facilitates melting and unwinding of the two strands, which probably accounts for the DNA strand-transfer properties of PrP *in vitro* (58). During this strand-transfer, which is required to complete the synthesis of HIV-1 minus strand cDNA, a small cDNA is unwound from the 5' end of its HIV-1 RNA template, then transferred and annealed to its 3'end. Recombinant PrP was shown to functionally replace HIV-1 protein NCp7 in this strand transfer, as well as in other steps of the reverse transcription *in vitro*, thus showing its RNA-chaperone properties (59, 92). All these activities required the presence of the unstructured N-terminal domain of PrP, since they were not observed with N-terminally truncated PrPs (57-59). What is the biological relevance of these observations? It would be somewhat surprising that none of these specific activities has any counterpart *in vivo*. But how could PrP, a glycosylated, membrane-anchored protein, physiologically interact with nucleic acids? As a surface-anchored extracellular protein, PrP^C is not likely to encounter nucleic acids, except for nucleotide neurotransmitters (93). However, PrP^C has been observed to adopt other subcellular topologies. Cytosolic PrP was observed in some neurons of the hippocampus, neocortex, and thalamus, and was found to accumulate in cytosolic inclusions in the endocrine beta-cells of pancreatic islets, in relation with glucose dysregulation (94-95). A nuclear form of PrP was also shown to interact with lectin CBP70 in the nucleus of a human promyelocytic leukemia cell line (96). In addition, transmembrane isoforms of PrP^C have been observed, with a transmembrane domain mapped to residues 110-135 (97-98). The presence of an extended glycine-zipper in this domain (**G**^{119}AVV**G**GLG**G**YML**G**^{131}) also suggests that PrP has the potential to form transmembrane oligomers (99). Therefore, both intracellular and transmembrane forms of PrP^C can potentially interact with nucleic acids, perhaps in some specialized cells or subcellular compartments where PrP could participate in various RNA-mediated regulations through its RNA-chaperone activity. On the other hand, an interaction of the pathological PrP^{Sc} with nucleic acids could also be relevant to the pathophysiology of prion diseases, and indeed a pathological, PK-resistant PrP has been detected in the nuclei of prion-infected neuroblastoma cells, and shown to interact with chromatin *in vivo* (100). In this study using N2A cells chronically infected with the 22L prion strain, PrP^{Sc} was found to selectively accumulate in highly transcribed euchromatin areas, and was deduced

to be associated with DNA, since it was released after DNase I treatment. This is also consistent with the fact that PrP^{Sc} from diseased brains could be captured by an anti-DNA antibody, as well as by a DNA-binding protein (101). These observations led the authors to propose that PrP^{Sc} could thereby interfere with transcription and alter gene expression, and one may wonder whether this could reflect a related, yet unknown, activity of PrP^{C} under physiological conditions.

Reciprocally, interaction of PrP with nucleic acids also results in modifications of the secondary and tertiary structures of the protein: binding induces a conformational shift of PrP to a beta-rich structure and promotes oligomerization or aggregation of the protein. However, the extent of polymerization depends on the nucleic acid and conditions of interaction (see 8.3 below). While long molecules of RNAs and DNAs generally induce the formation of large nucleoprotein aggregates, interaction with short RNAs or DNAs results in a soluble or oligomerized beta-sheet PrP (56, 58, 102). Although transition of PrP to a beta-rich structure is generally viewed as pathological, the possibility exists that, to a certain extent, it may also represent a physiological mechanism. Firstly, the conversion of PrP to a soluble beta-sheet rich conformation could represent a functional switch whereby a charge-mediated, unspecific interaction, triggers the structural transition which could be a prerequisite for a sequence-specific binding. Indeed, sequence-specific interactions of proteins with RNA often involve beta-sheet regions, where unstacked bases of the RNA are pulled into binding pockets of the protein (83-84). But is this conformational transition always the same, or instead could it depend on the structure and sequence of the nucleic acid, similar to the sequence-specific allosteric regulation of glucocorticoid receptor (103)? And, if several conformational transitions co-exist, are there pathological ones? Determining the structure of PrP in complex with various nucleic acids, although technically challenging, would probably help resolve these issues. Furthermore, it would be interesting to compare these structural modifications with those induced in PrP by copper and other metal ions. Indeed, uncovering the physiological functions of PrP^{C} will probably require considering its complex relations with its multiple ligands, in order to build a meaningful biological network (1, 26).

Secondly, oligomerization following this conformational switch could endow PrP with a new function, and there are now a number of examples where biological function requires oligomerization of the protein (75, 104, 105). Given the key role of polymerization in the prion mechanism and the existence of several distinct pathological "conformers" of PrP^{Sc}, it is of utmost importance to determine whether distinct nucleic acids can promote distinct modes of polymerization, and whether there could exist some physiological pathways of oligomerization, along with pathological ones. Elucidating these complex interactions between PrP and nucleic acids would certainly improve our understanding of both the biological function of PrP^{C} and the pathology of prion diseases.

8. APTAMERS AS TOOLS

8.1. Detection of PrP^{Sc}

Most PrP-aptamers were raised against various forms of recombinant PrP, and as a result recognize both PrP^{C} and its pathological isoform PrP^{Sc} (45). A parallel can be made with the difficulty in obtaining monoclonal antibodies that specifically recognize PrP^{Sc} (106). Since most aptamers bound primarily to the N-terminal unstructured domain of PrP, they did not bind to PrP27-30, the proteinase K-resistant core of PrP^{Sc}. Indeed, proteinase K-treament removes the main part of this N-terminal domain, by cleaving one or more sites that are present upstream and within the second lysine cluster (82). However, at least one of the aptamers that were raised against Scrapie-Associated Fibrils exhibited some specificity towards the pathological isoform: as discussed above, aptamer SAF-93 specifically recognized a beta-oligomeric isoform of recombinant bovine PrP^{90-230}, as well as the $PrP^{110-230}$ peptide derived from it (50). Thus, SAF-93 and possibly other aptamers could have a potential application in the detection of PrP^{Sc}, and one potential advantage of nucleic acid aptamers over antibodies resides in their possible amplification, by PCR, RT-PCR, or even direct amplification of suitably designed RNAs by an RNA-dependent RNA polymerase such as Q-beta replicase (61). Following another approach; Ogasawara *et al.* designed an aptamer-based system to detect prion protein in solution (107). In this system, PrP induced a conformational change in a PrP-specific DNA aptamer, allowing it to anneal to a biotinylated capture-DNA that was immobilized in the wells of a streptavidin-coated 96-well microplate. Promising as they are, these tools have not yet reached a wide practical application, since all the commercial tests officially in use to date still rely on immunological methods using one or two PrP-specific antibodies. A PCR amplification has been used to detect and quantify very low levels of PrP^{Sc} in biological samples, but the nucleic acid used in this assay was not an aptamer but a biotinylated reporter DNA that was indirectly bridged through streptavidin to the biotinylated secondary PrP-specific antibody of the sandwich-ELISA (108-109).

8.2. Removal and concentration of PrP^{Sc}

Beside detection of PrP, aptamers and other nucleic acid ligands of PrP can also be used to concentrate PrP from fluids. A set of small, highly structured RNAs were designed for that purpose (61). One such small RNA ligand, immobilized on a column cartridge, efficiently bound PrP and PrP^{Sc}. This device was able to concentrate PrP from biological fluids, thus showing its potential in improving the current diagnostic tests by lowering the detection threshold (61). A similar principle was recently tested using magnetic nanoparticles functionalized with a PrP-specific DNA aptamer (46, 110). In spite of their experimentally demonstrated potential, to our knowledge these aptamer-based tools have not yet been commercially developed.

8.3. Preventing PrP misfolding: therapy perspectives

Nucleic acids have generally been shown to trigger the polymerization of PrP into amyloid (55).

However, depending on their sequence, length and concentration, nucleic acids can have distinct effects on PrP. Thus, addition of a complex mixture of cellular RNAs (extracted from neuroblastoma cells) to recombinant mouse PrP23-231 induced the formation of aggregates in which PrP was partially resistant to proteolysis. Furthermore, these aggregates were toxic when added to neuroblastoma cells (102). Aggregation is probably favored by long nucleic acids, which can bind several molecules of PrP, even with low-affinity interactions. In addition, PrP itself through its chaperone properties could accelerate this aggregation process, by promoting the annealing of complementary sequences in a complex population of nucleic acids (59). This is consistent with the observation that total RNA prepared from mammals stimulates the *in vitro* amplification of PrPres (111). Intriguingly, ribosomal RNA was shown to bear a protein folding activity that is inhibited by two anti-prion compounds (112-113). Whether this could have a bearing on the RNA-induced polymerization of PrP is open to speculation. On the other hand, nucleic acids may act by accelerating the aggregation process, which would otherwise occur on longer timescales and is notably favoured by high concentrations of protein, acidic pH and the presence of salt (114-115).

On the contrary, short RNAs or DNAs can efficiently bind PrP and even induce a conformational shift to a beta-rich structure, but they often prevent aggregation. Thus, small RNAs of known sequences, including aptamer SAF-93, induced the formation of small oligomers that were not toxic to cultured neuroblastoma cells, contrary to the total RNA-induced PrP aggregates of the same study (102, 116). In another study, binding of the short 18-bp E2 DNA binding sequence to mouse recombinant PrP23-231 induced its conversion to a soluble beta-sheet structure, and at the same time prevented its aggregation, suggesting that tight binding to specific ligands, as opposed to non-specific ones, may prevent aggregation by favoring the formation of a stable nucleic acid-PrP complex (56, 117). By forming soluble complexes of 1:1 stoechiometry with a DNA ligand, PrP was found to be stabilized, notably against aggregation, in a broad pH range (118). How exactly is this stabilization achieved is not precisely known. However, several mechanisms can be proposed. Firstly, interaction, particularly with high-affinity ligands, could capture PrP in one single folded state where the intrinsically disordered N-terminal domain of PrP also acquires structure. This in turn may render the protein less prone to aggregation. Secondly, interaction may bury hydrophobic patches at the protein-nucleic acid interface, preventing them from participating in aggregation pathways. A third possibility is that interaction with small, high-affinity nucleic acid ligands, as opposed to long molecules, could prevent a "domain swapping" process, which is one of the known mechanisms that lead to protein aggregation (119-120). By taking advantage of this property, aptamers can be used to prevent PrP aggregation *in vivo*. Indeed, SAF-specific aptamer SAF-93 was shown to inhibit the accumulation of PrP^{res} in a cell-free conversion assay, whereas DP7, another PrP-aptamer, was found to reduce the generation of PrP^{Sc} aggregates in a persistently infected neuroblastoma cell line, even after a short duration (16 hrs) of treatment (50-51). Put in parallel with the promising applications of newly developed therapeutic aptamers, these data could pave the way towards some possible therapies of prion diseases (41).

9. SUMMARY AND PERSPECTIVES

Through its ability to isolate high affinity ligands from a very large pool of random sequences, the SELEX method can provide informations about the target protein and its interactions with nucleic acids. Indeed, the protocols that were applied in different laboratories have allowed the isolation of RNA- or DNA-ligands that bind PrP with high affinity. Comparison of their sequences has revealed i) conserved sequence patterns that were present in a few independently obtained aptamers, and ii) a relatively frequent structural feature consisting of a guanine quadruplex. These aptamers and other nucleic acid ligands of PrP have been used to investigate the interactions of PrP with nucleic acids, allowing to draw a complex picture that deserves further investigation. These studies have shown that at least three regions in PrP are involved in its interaction with nucleic acids.

It is likely that further information could be gathered by thorough comparisons of these aptamers, as well as from modified SELEX protocols combined with high throughput methods. Firstly, these aptamers should ideally be compared to one another in a single study to rigorously evaluate their relative binding affinities and to map more precisely their binding sites on PrP. Secondly, characterization of larger sets of low and medium affinity ligands, as opposed to high affinity aptamers, may help identify sequence patterns and possible functional ligands. Thirdly, the identification of putative functional binding sequences could also benefit from the genomic version of SELEX. And finally the recently described CLIP method (89) has the potential to isolate functional RNA-PrP interactions *in vivo*, should they exist.

A number of observations have shown indeed that PrP^{C} interacts with nucleic acids *in vivo* (121). However, what is the function of PrP in these interactions essentially remains to be established, as well as how PrP^{Sc} could act through either a gain- or loss-of-function. A better knowledge of the functional ligands of PrP would certainly help in elucidating this issue, and could provide important clues to the pathophysiology of prion diseases. In that respect, the 90-130 region of PrP is intriguingly involved in many physiological and pathological aspects of PrP: i) it contains the transmembrane region and its glycine zipper, which also partially overlaps the neurotoxic and amyloid-forming peptide 106-126, ii) several residues in that region, including the lysine cluster, are involved in interactions with nucleic acids, and iii) it harbors determining polymorphisms that are associated with Gerstmann-Sträussler-Scheinker disease in humans and susceptibility to transmitted prion diseases in animals (1, 79). On the other hand, elucidating the structure of PrP in complex with various nucleic acid ligands would certainly improve our understanding of this interaction, in relation with the biological and pathological functions of PrP.

On the practical side, prion-specific aptamers could eventually lead to valuable applications in the diagnosis and treatment of prion diseases. They can be used to capture and enrich PrP from biological fluids, thus allowing a more powerful detection. In addition, they could also be combined with classical immunological methods to design new detection assays. *In vivo*, the ability of some aptamers to prevent PrP aggregation could be put to use in new therapeutic approaches, which could be combined with an RNA-interference treatment to reduce PrP expression (122).

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11. REFERENCES

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

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

3. Gibbons R. A, G. D. Hunter: Nature of the scrapie agent. *Nature* 215, 1041-1043 (1967)

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

5. Marsh R. F, J. S. Semancik, K. C. Medappa, R. P. Hanson, R. R. Rueckert: Scrapie and transmissible mink encephalopathy: search for infectious nucleic acid. *J Virol* 13, 993-996 (1974)

6. Hunter G. D, S. C. Collis, G. C. Millson, R. H. Kimberlin: Search for scrapie-specific RNA and attempts to detect an infectious DNA or RNA. *J Gen Virol*, 32, 157-162 (1976)

7. Alper T, D. A. Haig, M. C. Clarke: The scrapie agent: evidence against its dependence for replication on intrinsic nucleic acid. *J Gen Virol* 41, 503-516 (1978)

8. Ward R. L, D. D. Porter, J. G. Stevens: Nature of the scrapie agent: evidence against a viroid. *J Virol* 14, 1099-1103 (1974)

9. Diener T. O, M. P. McKinley, S. B. Prusiner: Viroids and prions. *Proc Natl Acad Sci U S A* 79, 5220-5224 (1982)

10. Bellinger-Kawahara C, T. O. Diener, M. P. McKinley, D. F. Groth, D. R. Smith, S. B. Prusiner: Purified scrapie prions resist inactivation by procedures that hydrolyze, modify, or shear nucleic acids. *Virology* 160, 271-274 (1987)

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

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

13. Borras T, C. J. Gibbs, Jr.: Molecular hybridization studies with scrapie brain nucleic acids. I. Search for specific DNA sequences. *Arch Virol* 88, 67-78 (1986)

14. Sasaki K, K. Tanaka, M. Ito, H. Isomura, M. Horiuchi, N. Ishiguro, M. Shinagawa: Biological activity of subfractions from scrapie-associated fibrils. *Virus Res* 23, 241-251 (1992)

15. Aiken J. M, R. F. Marsh: The search for scrapie agent nucleic acid. *Microbiol Rev* 54, 242-246 (1990)

16. Oesch B, D. F. Groth, S. B. Prusiner, C. Weissmann: Search for a scrapie-specific nucleic acid: a progress report. *Ciba Found Symp* 135, 209-223 (1988)

17. Safar J. G, K. Kellings, A. Serban, D. Groth, J. E. Cleaver, S. B. Prusiner, D. Riesner: Search for a prion-specific nucleic acid. *J Virol* 79, 10796-10806 (2005)

18. Tuite M. F, B. S. Cox: Propagation of yeast prions. *Nat Rev Mol Cell Biol* 4, 878-890 (2003)

19. Tanaka M, P. Chien, N. Naber, R. Cooke, J. S. Weissman: Conformational variations in an infectious protein determine prion strain differences. *Nature* 428, 323-328 (2004)

20. Toyama B. H, M. J. Kelly, J. D. Gross, J. S. Weissman: The structural basis of yeast prion strain variants. *Nature* 449, 233-237 (2007)

21. Shewmaker F, R. B. Wickner, R. Tycko: Amyloid of the prion domain of Sup35p has an in-register parallel beta-sheet structure. *Proc Natl Acad Sci U S A* 103, 19754-19759 (2006)

22. King C. Y, R. Diaz-Avalos: Protein-only transmission of three yeast prion strains. *Nature* 428, 319-323 (2004)

23. Cobb N. J, F. D. Sonnichsen, H. McHaourab, W. K. Surewicz: Molecular architecture of human prion protein amyloid: a parallel, in-register beta-structure. *Proc Natl Acad Sci U S A* 104, 18946-18951 (2007)

24. Cobb N. J, W. K. Surewicz: Prion strains under the magnifying glass. *Nat Struct Mol Biol* 14, 882-884 (2007)

25. Legname G, H. O. Nguyen, D. Peretz, F. E. Cohen, S. J. DeArmond, S. B. Prusiner: Continuum of prion protein structures enciphers a multitude of prion isolate-specified phenotypes. *Proc Natl Acad Sci U S A* 103, 19105-19110 (2006)

26. Marc D, R. Mercey, F. Lantier: Scavenger, transducer, RNA chaperone? What ligands of the prion protein teach us about its function. *Cell Mol Life Sci* 64, 815-829 (2007)

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

28. Forloni G, N. Angeretti, R. Chiesa, E. Monzani, M. Salmona, O. Bugiani, F. Tagliavini: Neurotoxicity of a prion protein fragment. *Nature* 362, 543-546 (1993)

29. Selvaggini C, L. De Gioia, L. Cantu, E. Ghibaudi, L. Diomede, F. Passerini, G. Forloni, O. Bugiani, F. Tagliavini, M. Salmona: Molecular characteristics of a protease-resistant, amyloidogenic and neurotoxic peptide homologous to residues 106-126 of the prion protein. *Biochem Biophys Res Commun* 194, 1380-1386 (1993)

30. Nandi P. K: Polymerization of human prion peptide HuPrP 106-126 to amyloid in nucleic acid solution. *Arch Virol* 143, 1251-1263 (1998)

31. Ellington A. D, J.W. Szostak: In vitro selection of RNA molecules that bind specific ligands. *Nature* 346, 818-822 (1990)

32. Tuerk C, L. Gold: Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science* 249, 505-510 (1990)

33. Bartel D. P: Re-creating an RNA replicase. In: The RNA World. Eds: Gesteland R. F, Cech T. R, Atkins J. F, Cold Spring Harbor Laboratory Press, NY (1999)

34. Lawrence M. S, D. P. Bartel: New ligase-derived RNA polymerase ribozymes. *Rna* 11, 1173-1180 (2005)

35. Piganeau N, V. Thuillier, M. Famulok: In vitro selection of allosteric ribozymes: theory and experimental validation. *J Mol Biol* 312, 1177-1190 (2001)

36. Salehi-Ashtiani K, A. Luptak, A. Litovchick, J. W. Szostak: A genomewide search for ribozymes reveals an HDV-like sequence in the human CPEB3 gene. *Science* 313, 1788-1792 (2006)

37. Shtatland T, S. C. Gill, B. E. Javornik, H. E. Johansson, B. S. Singer, O. C. Uhlenbeck, D. A. Zichi, L. Gold: Interactions of Escherichia coli RNA with bacteriophage MS2 coat protein: genomic SELEX. *Nucleic Acids Res* 28, E93 (2000)

38. Lorenz C, F. von Pelchrzim, R. Schroeder: Genomic systematic evolution of ligands by exponential enrichment (Genomic SELEX) for the identification of protein-binding RNAs independent of their expression levels. *Nat Protoc* 1, 2204-2212 (2006)

39. Yang Y, D. Yang, H. J. Schluesener, Z. Zhang: Advances in SELEX and application of aptamers in the central nervous system. *Biomol Eng* 24, 583-592 (2007)

40. Alvarez-Salas L. M: Nucleic acids as therapeutic agents. *Curr Top Med Chem* 8, 1379-1404 (2008)

41. Kaur G, I. Roy: Therapeutic applications of aptamers. *Expert Opin Investig Drugs* 17, 43-60 (2008)

42. Sterba K. M, S. G. Mackintosh, J. S. Blevins, B. K. Hurlburt, M. S. Smeltzer: Characterization of Staphylococcus aureus SarA binding sites. *J Bacteriol* 185, 4410-4417 (2003)

43. Liang X, T. J. Nazarenus, J. M. Stone: Identification of a consensus DNA-binding site for the Arabidopsis thaliana SBP domain transcription factor, AtSPL14, and binding kinetics by surface plasmon resonance. *Biochemistry* 47, 3645-3653 (2008)

44. Symensma T. L, L. Giver, M. Zapp, G. B. Takle, A. D. Ellington: RNA aptamers selected to bind human immunodeficiency virus type 1 Rev in vitro are Rev responsive in vivo. *J Virol* 70, 179-187 (1996)

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

46. Takemura K, P. Wang, I. Vorberg, W. Surewicz, S. A. Priola, A. Kanthasamy, R. Pottathil, S. G. Chen, S. Sreevatsan: DNA aptamers that bind to PrP(C) and not PrP(Sc) show sequence and structure specificity. *Exp Biol Med (Maywood)* 231, 204-214 (2006)

47. Sekiya S, F. Nishikawa, K. Noda, P. K. Kumar, T. Yokoyama, S. Nishikawa: In vitro selection of RNA aptamers against cellular and abnormal isoform of mouse prion protein. *Nucleic Acids Symp Ser (Oxf)* 361-362 (2005)

48. Mercey R, I. Lantier, M. C. Maurel, J. Grosclaude, F. Lantier, D. Marc: Fast, reversible interaction of prion protein with RNA aptamers containing specific sequence patterns. *Arch Virol*, 151:2197-214 (2006)

49. Bibby D. F, A. C. Gill, L. Kirby, C. F. Farquhar, M. E. Bruce, J. A. Garson: Application of a novel in vitro selection technique to isolate and characterise high affinity DNA aptamers binding mammalian prion proteins. *J Virol Methods* 151, 107-115 (2008)

50. Rhie A, L. Kirby, N. Sayer, R. Wellesley, P. Disterer, I. Sylvester, A. Gill, J. Hope, W. James, 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)

51. Proske D, S. Gilch, F. Wopfner, H. M. Schatzl, E. L. Winnacker, M. Famulok: Prion-protein-specific aptamer reduces PrPSc formation. *Chembiochem* 3, 717-725 (2002)

52. Stoltenburg R, C. Reinemann, B. Strehlitz: SELEX--a (r)evolutionary method to generate high-affinity nucleic acid ligands. *Biomol Eng* 24, 381-403 (2007)

53. Ogasawara D, H. Hasegawa, K. Kaneko, K. Sode, K. Ikebukuro: Screening of DNA aptamer against mouse prion protein by competitive selection. *Prion* 1, 248-254 (2007)

54. Ellington A. D, J. W. Szostak: Selection in vitro of single-stranded DNA molecules that fold into specific ligand-binding structures. *Nature* 355, 850-852 (1992)

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

56. Cordeiro Y, 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)

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

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

59. Gabus C, E. Derrington, P. Leblanc, J. Chnaiderman, D. Dormont, W. Swietnicki, M. Morillas, W. K. Surewicz, D. Marc, P. Nandi, 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)

60. Adler V, B. Zeiler, V. Kryukov, R. Kascsak, R. Rubenstein, 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)

61. Zeiler B, V. Adler, V. Kryukov, A. Grossman: Concentration and removal of prion proteins from biological solutions. *Biotechnol Appl Biochem* 37, 173-182 (2003)

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

63. Matsugami A, T. Mashima, F. Nishikawa, K. Murakami, S. Nishikawa, K. Noda, T. Yokoyama, M. Katahira: Structural analysis of r(GGA)4 found in RNA aptamer for bovine prion protein. *Nucleic Acids Symp Ser (Oxf)* 179-180 (2008)

64. Murakami K, F. Nishikawa, K. Noda, T. Yokoyama, S. Nishikawa: Anti-bovine prion protein RNA aptamer containing tandem GGA repeat interacts both with recombinant bovine prion protein and its beta isoform with high affinity. *Prion* 2, 73-80 (2008)

65. Nishikawa F, K. Murakami, K. Noda, T. Yokoyama, S. Nishikawa: Detection of structural changes of RNA aptamer containing GGA repeats under the ionic condition using the microchip electrophoresis. *Nucleic Acids Symp Ser (Oxf)*, 397-398 (2007)

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

67. Keniry M. A: Quadruplex structures in nucleic acids. *Biopolymers*, 56:123-46 (2000)

68. Huppert J. L, A. Bugaut, S. Kumari, S. Balasubramanian: G-quadruplexes: the beginning and end of UTRs. *Nucleic Acids Res* 36, 6260-6268 (2008)

69. Gatto B, M. Palumbo, C. Sissi: Nucleic Acid aptamers based on the g-quadruplex structure: therapeutic and diagnostic potential. *Curr Med Chem* 16, 1248-1265 (2009)

70. Darnell J. C, K. B. Jensen, P. Jin, V. Brown, S. T. Warren, R. B. Darnell: Fragile X mental retardation protein targets G quartet mRNAs important for neuronal function. *Cell* 107, 489-499 (2001)

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

72. Shultzaberger R. K, T. D. Schneider: Using sequence logos and information analysis of Lrp DNA binding sites to investigate discrepanciesbetween natural selection and SELEX. *Nucleic Acids Res* 27, 882-887 (1999)

73. Hogan D. J, D. P. Riordan, A. P. Gerber, D. Herschlag, P. O. Brown: Diverse RNA-binding proteins interact with functionally related sets of RNAs, suggesting an extensive regulatory system. *PLoS Biol* 6, e255 (2008)

74. Wu H, A. Henras, G. Chanfreau, J. Feigon: Structural basis for recognition of the AGNN tetraloop RNA fold by the double-stranded RNA-binding domain of Rnt1p RNase III. *Proc Natl Acad Sci U S A* 101, 8307-8312 (2004)

75. Daugherty M. D, I. D'Orso, A. D. Frankel: A solution to limited genomic capacity: using adaptable binding surfaces to assemble the functional HIV Rev oligomer on RNA. *Mol Cell* 31, 824-834 (2008)

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

77. Rajendran K. S, P. D. Nagy: Characterization of the RNA-binding domains in the replicase proteins of tomato bushy stunt virus. *J Virol* 77, 9244-9258 (2003)

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

79. Kovacs G. G, G. Trabattoni, J. A. Hainfellner, J. W. Ironside, R. S. Knight, H. Budka: Mutations of the prion protein gene phenotypic spectrum. *J Neurol* 249, 1567-1582 (2002)

80. Westaway D, P. A. Goodman, C. A. Mirenda, M. P. McKinley, G. A. Carlson, S. B. Prusiner: Distinct prion proteins in short and long scrapie incubation period mice. *Cell* 51, 651-662 (1987)

81. Laegreid W. W, M. L. Clawson, M. P. Heaton, B. T. Green, K. I. O'Rourke, D. P. Knowles: Scrapie resistance in ARQ sheep. *J Virol* 82, 10318-10320 (2008)

82. Hayashi H. K, T. Yokoyama, M. Takata, Y. Iwamaru, M. Imamura, Y. K. Ushiki, M. Shinagawa: The N-terminal cleavage site of PrPSc from BSE differs from that of PrPSc from scrapie. *Biochem Biophys Res Commun* 328, 1024-1027 (2005)

83. Draper D. E: Themes in RNA-protein recognition. *J Mol Biol* 293, 255-270 (1999)

84. Shulman-Peleg A, M. Shatsky, R. Nussinov, H. J. Wolfson: Prediction of interacting single-stranded RNA bases by protein-binding patterns. *J Mol Biol* 379, 299-316 (2008)

85. Bokinsky G, L. G. Nivon, S. Liu, G. Chai, M. Hong, K. M. Weeks, X. Zhuang: Two distinct binding modes of a protein cofactor with its target RNA. *J Mol Biol* 361, 771-784 (2006)

86. Fairbrother W. G, R. F. Yeh, P. A. Sharp, C. B. Burge: Predictive identification of exonic splicing enhancers in human genes. *Science* 297, 1007-1013 (2002)

87. Liu J, G. D. Stormo: Combining SELEX with quantitative assays to rapidly obtain accurate models of protein-DNA interactions. *Nucleic Acids Res* 33, e141 (2005)

88. Roulet E, S. Busso, A. A. Camargo, A. J. Simpson, N. Mermod, P. Bucher: High-throughput SELEX SAGE method for quantitative modeling of transcription-factor binding sites. *Nat Biotechnol* 20, 831-835 (2002)

89. Ule J, K. Jensen, A. Mele, R. B. Darnell: CLIP: a method for identifying protein-RNA interaction sites in living cells. *Methods* 37, 376-386 (2005)

90. Licatalosi D. D, A. Mele, J. J. Fak, J. Ule, M. Kayikci, S. W. Chi, T. A. Clark, A. C. Schweitzer, J. E. Blume, X. Wang, J. C. Darnell, R. B. Darnell: HITS-CLIP yields genome-wide insights into brain alternative RNA processing. *Nature* 456, 464-469 (2008)

91. Guazzaroni M. E, T. Krell, P. Gutierrez del Arroyo, M. Velez, M. Jimenez, G. Rivas, J. L. Ramos: The transcriptional repressor TtgV recognizes a complex operator as a tetramer and induces convex DNA bending. *J Mol Biol* 369, 927-939 (2007)

92. Derrington E, C. Gabus, P. Leblanc, J. Chnaidermann, L. Grave, D. Dormont, W. Swietnicki, M. Morillas, D. Marc, P. Nandi, J. L. Darlix: PrPC has nucleic acid chaperoning properties similar to the nucleocapsid protein of HIV-1. *C R Acad Sci III* 325, 17-23 (2002)

93. Gourine A. V, E. Llaudet, N. Dale, K. M. Spyer: ATP is a mediator of chemosensory transduction in the central nervous system. *Nature* 436, 108-111 (2005)

94. Mironov A. Jr., D. Latawiec, H. Wille, E. Bouzamondo-Bernstein, G. Legname, R.A. Williamson, D. Burton, S. J. DeArmond, S. B. Prusiner, P. J. Peters: Cytosolic prion protein in neurons. *J Neurosci* 23, 7183-7193 (2003)

95. Strom A, G. S. Wang, R. Reimer, D. T. Finegood, F. W. Scott: Pronounced cytosolic aggregation of cellular prion protein in pancreatic beta-cells in response to hyperglycemia. *Lab Invest* 87, 139-149 (2007)

96. Rybner C, S. Finel-Szermanski, M. Felin, T. Sahraoui, C. Rousseau, J. G. Fournier, A. P. Seve, J. Botti: The cellular prion protein: a new partner of the lectin CBP70 in the nucleus of NB4 human promyelocytic leukemia cells. *J Cell Biochem* 84, 408-419 (2002)

97. Stewart R. S, D. A. Harris: A transmembrane form of the prion protein is localized in the Golgi apparatus of neurons. *J Biol Chem* 280, 15855-15864 (2005)

98. Hegde R. S, J. A. Mastrianni, M. R. Scott, K. A. DeFea, P. Tremblay, M. Torchia, S. J. DeArmond, S. B. Prusiner, V. R. Lingappa: A transmembrane form of the prion protein in neurodegenerative disease. *Science* 279, 827-834 (1998)

99. Kim S, T. J. Jeon, A. Oberai, D. Yang, J. J. Schmidt, J. U. Bowie: Transmembrane glycine zippers: Physiological and pathological roles in membrane proteins. *Proc Natl Acad Sci U S A* 102, 14278-14283 (2005)

100. Mange A, C. Crozet, S. Lehmann, 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)

101. 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*, 101:1380-5 (2004)

102. Gomes M. P, T. A. Millen, P. S. Ferreira, N. L. e Silva, T. C. Vieira, M. S. Almeida, J. L. Silva, 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)

103. Meijsing S. H, M. A. Pufall, A. Y. So, D. L. Bates, L. Chen, K. R. Yamamoto: DNA binding site sequence directs glucocorticoid receptor structure and activity. *Science* 324, 407-410 (2009)

104. Korennykh A. V, P. F. Egea, A. A. Korostelev, J. Finer-Moore, C. Zhang, K. M. Shokat, R. M. Stroud, P. Walter: The unfolded protein response signals through high-order assembly of Ire1. *Nature* 457, 687-693 (2009)

105. Lee J. R, S. S. Lee, H. H. Jang, Y. M. Lee, J. H. Park, S. C. Park, J. C. Moon, S. K. Park, S. Y Kim, S. Y. Lee, H. B.
Chae, Y. J. Jung, W. Y. Kim, M. R. Shin, G. W. Cheong, M. G. Kim, K. R. Kang, K. O. Lee, D. J. Yun: Heat-shock dependent oligomeric status alters the function of a plant-specific thioredoxin-like protein, AtTDX. *Proc Natl Acad Sci U S A* 106, 5978-5983 (2009)

106. Pan T, B. Chang, P. Wong, C. Li, R. Li, S.C. Kang, J.D. Robinson, A. R. Thompsett, P. Tein, S. Yin, G. Barnard, I. McConnell, D. R. Brown, T. Wisniewski, M. S. Sy: An aggregation-specific enzyme-linked immunosorbent assay: detection of conformational differences between recombinant PrP protein dimers and PrP(Sc) aggregates. *J Virol* 79, 12355-12364 (2005)

107. Ogasawara D, N. S. Hachiya, K. Kaneko, K. Sode, K. Ikebukuro: Detection system based on the conformational change in an aptamer and its application to simple bound/free separation. *Biosens Bioelectron* 24, 1372-1376 (2008)

108. Gofflot S, M. Deprez, B. el Moualij, A. Osman, J.F. Thonnart, O. Hougrand, E. Heinen, W. Zorzi: Immunoquantitative PCR for prion protein detection in sporadic Creutzfeldt-Jakob disease. *Clin Chem* 51, 1605-1611 (2005)

109. Barletta J. M, D. C. Edelman, W. E. Highsmith, N. T. Constantine: Detection of ultra-low levels of pathologic prion protein in scrapie infected hamster brain homogenates using real-time immuno-PCR. *J Virol Methods* 127, 154-164 (2005)

110. Kouassi G. K, P. Wang, S. Sreevatan, J. Irudayaraj: Aptamer-mediated magnetic and gold-coated magnetic nanoparticles as detection assay for prion protein assessment. *Biotechnol Prog* 23, 1239-1244 (2007)

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

112. Samanta D, D. Mukhopadhyay, S. Chowdhury, J. Ghosh, S. Pal, A. Basu, A. Bhattacharya, A. Das, D. Das, C. DasGupta: Protein folding by domain V of Escherichia coli 23S rRNA: specificity of RNA-protein interactions. *J Bacteriol* 190, 3344-3352 (2008)

113. Tribouillard-Tanvier D, S. Dos Reis, F. Gug, C. Voisset, V. Beringue, R. Sabate, E. Kikovska, N. Talarek, S. Bach, C. Huang, N. Desban, S. J. Saupe, S. Supattapone, J. Y. Thuret, S. Chedin, D. Vilette, H. Galons, S. Sanyal, M. Blondel: Protein folding activity of ribosomal RNA is a selective target of two unrelated antiprion drugs. *PLoS ONE* , 3, e2174 (2008)

114. Swietnicki W, M. Morillas, S. G. Chen, P. Gambetti, W. K. Surewicz: Aggregation and fibrillization of the recombinant human prion protein huPrP90-231. *Biochemistry* 39, 424-431 (2000)

115. Hornemann S, R. Glockshuber: A scrapie-like unfolding intermediate of the prion protein domain PrP(121-231) induced by acidic pH. *Proc Natl Acad Sci U S A* 95, 6010-6014 (1998)

116. Yin S, X. Fan, S. Yu, C. Li, 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, 5446-5454 (2008)

117. Cordeiro Y, 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)

118. Marques A. F, Y. Cordeiro, J. L. Silva, L. M. Lima: Enhanced prion protein stability coupled to DNA recognition and milieu acidification. *Biophys Chem* 141, 135-139 (2009)

119. Bennett M. J, M. R. Sawaya, D. Eisenberg: Deposition diseases and 3D domain swapping. *Structure* 14, 811-824 (2006)

120. Whisstock J. C, S. P. Bottomley: Structural biology: Serpins' mystery solved. *Nature* 455, 1189-1190 (2008)

121. Beaudoin S, K. Goggin, C. Bissonnette, C. Grenier, X. Roucou: Aggresomes do not represent a general cellular response to protein misfolding in mammalian cells. *BMC Cell Biol* 9, 59 (2008)

122. White M. D, M. Farmer, I. Mirabile, S. Brandner, J. Collinge, G. R. Mallucci: Single treatment with RNAi against prion protein rescues early neuronal dysfunction and prolongs survival in mice with prion disease. *Proc Natl Acad Sci U S A* 105, 10238-10243 (2008)

123. Dyson H. J, P. E. Wright: Intrinsically unstructured proteins and their functions. *Nat Rev Mol Cell Biol* 6, 197-208 (2005)

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