Misfolding of the prion protein: linking biophysical and biological approaches

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Abstract – Prion diseases are a group of neurodegenerative diseases that can arise spontaneously, be inherited, or acquired by infection in mammals. The propensity of the prion protein to adopt different structures is a clue to its pathological and perhaps biological role too. While the normal monomeric PrP is well characterized, the misfolded conformations responsible for neurodegeneration remain elusive despite progress in this field. Both structural dynamics and physico-chemical approaches are thus fundamental for a better knowledge of the molecular basis of this pathology. Indeed, multiple misfolding pathways combined with extensive posttranslational modifications of PrP and probable interaction(s) with cofactors call for a combination of approaches. In this review, we outline the current physico-chemical knowledge explaining the conformational diversities of PrP in relation with postulated or putative cellular partners such as proteic or non-proteic ligands.

structure / membrane / oligomer / kinetics

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1. INTRODUCTION

Prion diseases, also known as transmissible spongiform encephalopathies (TSE) are a family of fatal neurodegenerative diseases affecting a number of mammalian species. TSE were first noted in sheep as scrapie, but also occur in cattle such as bovine spongiform encephalopathy (BSE) and in cervids such as chronic wasting disease (CWD). These diseases connected with the prion protein (PrP)

also affect humans as for instance in kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker disease and fatal familial insomnia. Prion diseases may arise spontaneously, be inherited through mutations of the prion protein gene or be acquired through exposure to prion infected tissues. While usually rare, TSE can assume epidemic proportions under still unknown conditions.

The most unusual feature of TSE, which makes these diseases unique among the pathologies caused by protein misfolding, is

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the nature of the pathogenic agent in infectivity. It is now widely accepted, although not definitely proven, that the infective agent is the PrP itself according to the so-called “protein-only hypothesis” [95]. Strong evidence indicates that the conversion of a normal soluble α -helical monomeric protein into a β -sheet-rich oligomeric structure and further fibrillar aggregation are the key events in the disease pathogenesis [9, 90]. To distinguish between these two conformers of the same protein, the terms PrP^C and PrP^{Sc} are used to name respectively the endogenous non-pathogenic form and the disease-associated isoform. The misfolded PrP^{Sc} is characterized by new biochemical properties such as protease resistance to proteinase K and forms insoluble aggregates observed as cerebral accumulations in different TSE [96]. The aberrant conformer PrP^{Sc} is believed to act as a structural template, which induces conversion of other PrP^C molecules to the pathological form [39]. Two prevalent hypotheses exist to explain the molecular mechanism of prion propagation, which has yet to be formally determined. The first one is the nucleation model which proposes that PrP^{Sc} exists in equilibrium with PrP^C but can form oligomers acting as seeds for further polymerization [51]. The second one, known as the template-assisted model, proposes the formation of PrP^C-PrP^{Sc} heterodimers which refold into a homodimer of PrP^{Sc} [29]. The “protein only hypothesis” represents a new paradigm of molecular biology since it implies that proteins rather than viruses or nucleic acids may be infectious and carry heritable information. The ability of a protein to misfold into multiple self-propagating conformations would also be at the origin of prion inheritance in yeast and fungi prions as discussed and compared to mammal prions in recent reviews [18, 21].

Evidence to sustain these hypotheses in mammal models would be to determine infectious properties of aggregates produced in vitro from recombinant PrP. Legname et al. showed that injection of the recombinant mouse PrP in the form of amyloid-like fibrils is sufficient to generate disease in mice that express the endogenous protein [60]. Wild-type animals have been infected with aggregates

produced in vitro from sonicated PrP^C-PrP^{Sc} mixtures [13]. However, one can deplore the low level of infectivity of prion aggregates produced in vitro as already suggested by May et al. [73]. Silveira et al. attempted to determine the relationship between infectivity and converting activity with the size of various misfolded PrP-containing aggregates. Their analyses suggest that non-fibrillar particles, with masses equivalent to 14-28 PrP molecules, are the most efficient initiators of TSE disease [113]. Recently, amyloid fibrils prepared in vitro from full-length mouse recombinant prion protein (recPrP) were found to be as toxic to cells as soluble oligomeric intermediates [83]. Another comprehensive study showed that soluble β -rich oligomers of PrP were toxic to neuronal cells [56, 114]. But recPrP amyloid fibrils appeared to be more biologically active than the soluble β -rich oligomers since only the former induces synaptic impairments prior to neuronal death [84].

How prions damage cells and the precise nature of the neurotoxic agent in TSE are not fully understood. The synaptic loss is a prominent feature of prion diseases, which is observed at the early pre-clinical stages [25, 40, 52] while neuronal death is typically observed at late clinical stages [16]. Other authors underlined the difficulties to establish the identification of the early molecular events associated with neuronal degeneration by a valuable biological model system [50, 84].

Because neurotoxicity and infectivity are so intimately related to a protein that may exist in at least two different conformational stable states and multiple meta stable oligomeric states, large interest has been paid over the last 15 years to establish the structure of PrP both in the native and in its various pathological forms and to understand the folding/misfolding pathway. Biophysical approaches (i.e. structural and protein physical-chemistry) could help for a better understanding of prion pathology and neurotoxicity at the molecular level since prion diseases originate from a protein folding problem and depend on its internal dynamics.

2. NATURAL AND RECOMBINANT PRION PROTEINS

PrP is encoded by a single exon as a polypeptide chain of 250 to 260 amino acids depending on the species [86]. The post-translational modification includes the cleavage of the N-terminal signal sequence of 22 residues and the C-terminal signal (23 residues) of the glycosylphosphatidylinositol (GPI) anchor. PrP also contains two sites of N-glycosylation [118]. When PrP is purified from natural sources, it was in some instance observed as a mixture of nonglycosylated, monoglycosylated, and biglycosylated species. Furthermore, the number of glycosylated variants (glycoforms) was estimated up to 50 different bi-, tri-, and tetra-antennary N-linked oligosaccharides [103]. PrP inserts into the cellular plasma membrane through the GPI anchor attached to the C-terminus [116].

The structural characterization of extractive PrP^C failed until now essentially due to its rarity and its poor purification yield. For these reasons, most of the biophysical investigations were performed on the recombinant PrP (recPrP) produced in *Escherichia coli*. This strategy leads to the purification of several milligrams of recPrP, which is neither glycosylated nor GPI anchored. Hornman et al. demonstrated the physico-chemical equivalence of recPrP and the extractive PrP through NMR, circular dichroism (CD) spectroscopies and thermal unfolding analysis [46].

3. STRUCTURAL ANALYSIS OF THE PRP

The first 3D structure of the PrP C-terminal domain was determined by nuclear magnetic resonance (NMR) in Wüthrich's laboratory; this comprehended the 121-231 globular domain of murine PrP [101]. Since then, several NMR structures from different mammalian species were resolved [62, 64]. All showed the same global fold (Fig. 1a), consisting in two short anti-parallel β -sheets (S1 and S2), a small α -helix (H1) and two anti parallel helices (H2 and H3) linked by the single disulfide bond.

More recently, high resolution crystallographic structures were established for sheep PrP variants [32, 43]. They showed the same

global fold as other mammalian species determined by NMR. However, these high resolution structures led to establish the molecular bases of the difference in the previously described energetic unfolding [32, 98]. Furthermore, the use of an antibody (Fab-VRQ 14) in the crystallization trials helps to identify the segment of ovine PrP (OvPrP) with an unchanged secondary structure in the PrP^C-to-PrP^{Sc} conversion. Indeed the Fab of VRQ 14 antibody recognized the C-terminal end of helix H2 and the N-terminal part of the H2-H3 loop (Fig. 1b). ELISA titrations with normal or infected brain homogenates from the mouse, hamster, and macaque indicate that the VRQ 14 Fab epitope is also found in these species and is conserved in both normal and pathological isoforms of their prion proteins.

The structural characterization of the N-terminal as well as by NMR or CD spectroscopy reveals that this region is unfolded in solution [29]. However, four repetitions of eight amino acids called the octapeptide domain present a high affinity for Cu²⁺ ions. The interaction of this octapeptide and Cu²⁺ leads to a partial structuration of this region [1].

4. LINKAGE BETWEEN PRP CONFORMATIONAL DYNAMICS AND PRION PATHOLOGY

The conformational diversity of PrP was proposed to be at the origin of all prion pathology [126]. In practice, PrP can adopt several structures through multiple folding pathways leading to the formation of some conformers associated to the apparition of pathological symptoms [4]. Several studies converge in supporting the diversity of pathological conformers: the first one and, probably, the most evident with respect to physico-chemical considerations, is the species barrier [93], and the second one is the anatomopathological diversity linked to the strain phenomena [23]. The species barrier resulting from PrP sequence variation implicates that the infectious conformer can harbor diverse conformations modulated by its primary structure [93]. This type of modulation was also reported in a given species and called "the polymorphism barriers" [53, 74]. The best example of this

Figure 1. Structural characterization of the recombinant PrP. (a) The first characterization of PrP in 1996 by Riek et al. [101] reveals two short β -strands (S1 and S2), a small α -helix and two anti-parallel α -helices linked by a disulfide bond. (b) Recently, crystallographic approaches give high-resolution 3D structures of different mammalian prion proteins. Here a crystallographic structure obtained by the co-crystallization of VRQ 14 Fab with ARQ sheep variant. (A color version of this figure is available online at www.vetres.org.)

modulation was found in sheep species for which just a single mutation (Q171R) can protect against the sheep scrapie agent [10].

Several approaches were actually adopted to investigate the PrP conformational diversities. The use of a low chaotropic concentration (~ 2 M urea or guanidium chloride) is one of the strategies which can lead to the formation of amyloid fibrils [4, 76, 122]. Recently the Baskakov's group showed that fragmentation of amyloid fibrils under different physico-chemical constraints such as temperature or shaking rate leads to the formation of fibrillar fragments whose size is correlated to the conformational stability of prion fibrils [68, 120].

Comparing the PrP 3D structures leads to extract information about its folding dynamics and its structural diversity. The unfolding studies of recombinant PrP induced by temperature [99], pressure [69] or by urea or guanidine hydrochloride [8, 76, 122] revealed that the monomeric and α -helical conformer can be refolded into several oligomeric conformers presenting a high content of β -sheet.

According to calorimetric studies it appears that the PrP unfolding leads to the formation of an unidentified transient intermediate below pH 4.5 and over pH 6.0 giving rise to beta oligomers and/or amyloid fibrils (Fig. 2) [98]. Clearly, the protonation states of some PrP residues can particularly affect the folding pathway of the protein.

5. REGIONS INVOLVED IN THE STRUCTURAL CHANGE

As cited previously, the prion pathology belongs to diseases due to protein misfolding families. The misfolding term means that the protein can exist at least in two structures. Taking into account the fundamental biophysical principles of a macromolecule's behavior, the passage of one conformer to another can only occur through at least a partial unfolding process. Another important point, which remains obscure, is the region of PrP that is involved in the structural conversion. This lack in information is mostly due to the absence of the PrP^{Sc} high resolution 3D structure.

Figure 2. The diversity of PrP folding pathway explored by differential scanning calorimetry. The calorimetric thermograms of the unfolding process at different pH reveal different unfolding pathways. At a pH below 4.5 and over pH 6.0, unfolding occurs with the existence of an intermediate, characterized as an oligomeric state of PrP [98].

Proteolysis fingerprinting performed on extractive PrP^{Sc} reveals that the N-terminal region is completely accessible to proteases [89]. Furthermore, transgenic mice whose N-terminal PrP domain was deleted, were able to replicate the infectious agent [36, 102]. All these observations indicate that the N-terminal part of PrP plays a minor role in the conversion process. However, in transgenic mice the deletion of this region affects the anatomopathological distribution of the lesions as well as the incubation period.

In earlier 2000, the molecular dynamics tried to determine the PrP region presenting a dual structure. The first target was helix H1, which contains about 10 amino acids. Morrissey and Shakhnovich [78] first predicted that H1 might be involved in the seeding of prion aggregates, noting that H1 is an unusually hydrophilic α -helix with few contacts with the remainder of the molecule, whereas H2 and H3 are hydrophobic and linked by a disulfide bond. The particular interest for the H1 helix was mostly due to the nucleation theory [88], which predicts that PrP^C to PrP^{Sc} conversion can be initiated by the propagation of a β -structure starting from the two short PrP^C β -strands (S1 and S2). According to this the-

ory, H1 constitutes the junction between these two β -strands (Fig. 3) and thus its conformation and stability may play an important role in the conversion kinetics. Other experimental data support the evidence that the H1 helix is a possible site of association of PrP^{Sc} with PrP^C during formation of the PrP^{Sc}-PrP^C complex. For instance, an antibody directed against H1 cured prion infections in two separate cell lines chronically infected with PrP^{Sc} [34, 45, 92]. It is noteworthy that antibodies obtained against other regions of PrP have also been reported to inhibit the replication of the infectivity [35, 85], suggesting that PrP^{Sc} replication may involve different regions of the prion protein.

More recently and in parallel to the particular interest focused on H1, the H2 and H3 helices solicited a regain of interest. Dima and Thirumalai first showed by molecular dynamics that some segments of H2 and H3 presented significant instability [28]. Then they proposed that some structural changes in these two helices might be at the origin of transition of PrP^C to PrP^{Sc} although a disulfide bond linked these two helices covalently. Furthermore, H2 and H3 helices may play an important role in the conversion process as proposed from hydrogen/deuterium exchange

Figure 3. The structural basis of PrP^C-to-PrP^{Sc} conversion. The two β -strands S1 and S2 are proposed to play the role of a “seed” for elongation during H1 unfolding. This hypothesis has been exploited to design inhibitor molecules (called “beta breakers”) for PrP^C-to-PrP^{Sc} conversion. (A color version of this figure is available online at www.vetres.org.)

experiments and from the hydration properties of the unfolded state of PrP investigated by molecular dynamics [26, 63].

6. STRUCTURAL DYNAMICS OF PrP CONVERSION

Several biochemical and physico-chemical approaches well described the structural difference between PrP^C and PrP^{Sc}. However, an important question remains concerning the early structural events during PrP^C-to-PrP^{Sc} conversion.

Recently we tried to address this question by two approaches: the first one was to establish a kinetic scheme describing the recombinant PrP oligomerization at acidic pH, and the second one was to introduce some physical constraints in defined PrP regions so as to study the impact on the oligomerization pattern (Fig. 4a) [33]. The kinetic investigation showed that PrP can generate different types of oligomers according to a parallel oligomerization pathway (Fig. 4b). Only one of these oligomers was identified to be amyloidogenic. By locking sub-domains of PrP with disulfide bonds, we showed that the S1-H1-S2 domain must physically separate from the H2-H3 domain prior to oligomerization. This opening mechanism illustrated in Figure 4c implies a conformational change in the S2-H2 hinge-loop corresponding to residues 168-173. Altogether kinetic and structural data reported by Eghiaian et al. are consistent with observations related to the PrP^C/PrP^{Sc} conversion in vivo [33]. The conformation of the S2-H2 loop seems to play an important role in PrP^C/PrP^{Sc} conversion. (i) Protein X was putatively proposed to target the S2-H2 loop and to promote

the opening of the molecule like a lever [54]; (ii) in sheep, mutation Q171R located in the S2-H2 loop confers a resistance phenotype to sheep scrapie [49]; (iii) the conformation of this loop was proposed as the basis of TSE susceptibility differences in domestic animals [41]. Since the separation of H1-S2 from the rest of the protein is a prerequisite unfolding event for oligomerization, all mutations affecting the H1-S2 expansion from the H2-H3 bundle may be involved in the PrP^C/PrP^{Sc} conversion as for the human Q217R mutation.

7. CONVERSION OF PRIONS IS LIKELY TO OCCUR AT THE MEMBRANE SURFACE

Under normal physiological conditions, cellular PrP is incorporated in the outer layer of the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor. Like other GPI-anchored proteins, PrP^C is localized in membrane microdomains enriched in cholesterol and sphingomyelin so-called rafts. Depletion of cholesterol from cellular membranes prevents PrP^{Sc} formation [5, 12, 123]. In contrast, depletion of sphingolipid increases the PrP^{Sc} formation in prion-infected neuroblastoma cells [82]. PrP^C is easily cleaved and released from the cell surface after digestion with phosphatidylinositolphospholipase C (PI-PLC), whereas PrP^{Sc} is resistant to release [106, 117]. The effect of GPI anchor on the conversion of PrP expressed in fibroblasts has been tested [3]. Both GPI-anchored and GPI-deficient PrP^C are associated into raft domains. But the GPI-anchored PrP could not be converted into PrP^{Sc} without the action of PI-PLC or addition of a membrane-fusion agent, while the GPI-deficient PrP

Figure 4. An artificial disulfide bond was used to explore the structural basis of the recombinant PrP conversion (a). The covalent linkage of the S1-H1-S2 domain to the H2-H3 domain through an artificial S-S bond (Y160C, M209C) leads to the complete inhibition of PrP oligomerization as shown by Size Exclusion Chromatograms (b). A diagram showing that the expanding of the S1-H1-S2 domain from the rest of the protein is required prior to oligomerization (c). (A color version of this figure is available online at www.vetres.org.)

undergoes conversion without PI-PLC or fusogenic agent. These findings suggest that lipid-PrP interaction could form anchorless intermediates playing a role in the conversion of PrP^C into PrP^{Sc} provided the interacting PrP^C is still membrane-anchored via the GPI moiety.

Although the majority of PrP^{Sc} generated in infected animals contains a GPI anchor, soluble forms of PrP released from the cell surface are GPI deficient and observed in murine neuroblastoma or in mice cell culture [9, 14, 15] or directly in mice brains [91]. Besides in scrapie-infected cells the absence of the GPI anchor reduces conversion [102], which

suggests that conversion involves membrane-bound GPI-anchored PrP. The role of the anchor-deficient form of PrP in regards with susceptibility to TSE infection in vivo is still under debate. In transgenic mice, combined expression of GPI-anchored and GPI-deficient PrP increases clinical scrapie whereas expression of solely GPI-deficient PrP produces infectious particles without clinical signs [19]. But it cannot be excluded that soluble intermediates of PrP could play a role in the conversion process [19, 59, 60].

Only a few in vitro biophysical studies have been dedicated to lipid-prion protein interaction, generally employing a recombinant

full-length or N-truncated polypeptide of PrP^C. Although these two recombinant forms are neither glycosylated nor GPI anchored, they undergo a structural transition from an α -helical state to a β -sheeted structure that mimick on a conformational point of view the PrP^C to PrP^{Sc} conversion. The interaction of α -helical recombinant PrP with model lipid membranes has been characterized [75, 107] as well as those of the β -sheeted form [24, 55].

The native and β -sheeted forms have different binding affinities to lipid membranes. Both native and converted forms bind to negatively charged lipid membranes in a pH-dependent manner [24, 55, 75, 107]. No binding occurs when prion protein interacts with pure zwitterionic phosphatidylcholine (PC) lipids. But both native and β -sheeted conformers bind to model raft membranes constituted of PC, cholesterol and sphingomyelin [55, 107]. The native conformer interacts with rafts only at pH 7, while the β -sheeted form binds strongly at both neutral and acidic pH [24].

The lipid membrane-prion interaction results either in a conformational change of the protein and/or in a destabilization of the lipid membrane, depending on pH, membrane composition and initial conformation of the PrP species. Both native and β -sheeted forms of the N-truncated PrP gain in β -structure upon binding to negatively charged phosphatidylglycerol (PG) vesicles leading to total release of the lipid vesicles [55, 107]. These interactions with negatively charged vesicles result in PrP aggregation whatever the initial conformation of the PrP in any pH studied. With raft model membranes, the native helical conformer of N-truncated PrP is more folded in helical structure and does not lead to PrP aggregation while β -conformer upon binding is unfolded and leads to fibrillization of PrP. However, full length PrP bearing a synthetic GPI anchor in the α -helical state remains structurally rather unchanged when interacting with raft vesicles [30], suggesting that raft domains could protect the cellular helical conformer from β -conversion (Fig. 5). However, endocytosis studies have shown that in vivo PrP^C leaves rafts and transits outside rafts via clathrin coated pits before being re-

cycled back to the plasma membrane [121]. During transit outside rafts, PrP could bind to negatively charged lipids, leading to a precursor in β -rich structure prone to aggregation. The higher affinity of the β -conformer towards raft microdomains enables to speculate the accumulation of these β -rich intermediates in the vicinity of PrP^C embedded in the lipid membrane. Thus, both PrP^C and PrP^{Sc} should be localized in raft domains to ensure the prion propagation in vivo and to alter the normal trafficking of PrP^C in neuronal cells. In infected cells, PrP^C would be internalized with bound pathological PrP, leading to further β -conversion. The localization of the β -conversion could be either at the membrane or in endosomes/lysosomes. As hypothesized in Figure 5, the transconformed PrP poisons several other cell compartments, affecting cellular machinery (especially protein synthesis and degradation), and, due to its protease-resistance, accumulates in aggregates.

8. PrP CONVERSION MEDIATED BY OTHER MOLECULES

In order to unravel the normal role of PrP or to find cofactors (e.g. protein X according to Telling et al.) involved in transconformation from PrP^C to PrP^{Sc}, several molecules interacting with PrP were identified [125]. These molecules are listed in Table I and can be subdivided into two groups:

(1) Non-proteic ligands. PrP can bind to nucleic acids. Co-precipitations of PrP and DNA were performed. PrP can also interact with membrane lipids and can be converted into β -sheeted structure, or can keep its native conformation when inserted in rafts. Peptides derived from PrP induce liposome fusion (118-135) [17] or can form ionic channels (106-126) [57]. These peptides induce apoptosis of cells by interacting with biological membranes. It was shown that heparan sulfate can bind PrP [38, 111]. These ligands are components of the extracellular matrix and were shown to be of great importance for neuron growth, a function postulated for the prion protein. Interestingly, heparan sulfate mimetics are also promising drugs for prion disease curing. PrP was shown to bind metals selectively and

Figure 5. Model for the cellular toxicity of oligomeric PrP species: (A) The normal PrP^C isoform is embedded in rafts via the GPI anchor. β-rich intermediates, either soluble oligomers or PrP^{Sc} itself, have high affinities towards raft microdomains leading to their accumulation in the vicinity of PrP^C. The oligomeric species could interact with the normal protein leading to structural conversion and/or change in signal properties. (B) In non-infected cells, PrP^C trafficking is normal, as described in the literature and text. In infected cells, PrP^C is internalized with bound PrP^{Sc}. Transconversion could occur either at the membrane or in endosomes/lysosomes. (C) In infected cells, due to its resistance to proteolytic digestion, PrP^{Sc} accumulates more and more and "poisons" several cell compartments [20, 65, 66, 114]. Abbreviations and symbols used are the following: Agr (PrP^{Sc} aggregates), Cy (cytoskeleton), End (endosome), N (nucleus), Lys (lysosome), Ps (proteasome), Mt (mitochondrion), ER (endoplasmic reticulum), G (Golgi apparatus). Arrows indicate the cellular compartments/functions affected by infection. (A color version of this figure is available online at www.vetres.org.)

especially copper. A function in cell redox regulation was proposed.

(2) Proteic ligands involved in various cellular functions. Computational data¹ indicate a limited number of interactions for PrP while the literature shows more PrP interactors. Two chaperones were proposed to intervene in the

α/β conversion of PrP, the cytoplasmic Hsp73 and curiously, the bacterial GroEL, while the important Hsp70 chaperone had no role in the PrP conversion [112]. Peptides corresponding to internal sequences of PrP (109-122 and 109-141) were shown to interact in vitro with apolipoprotein E [6]. Receptor proteins playing a role in signal transduction were found to interact directly such as laminin receptor [128] or indirectly like kinase Fyn [79], with internal

¹ String, EMBL: <http://string.embl.de> [consulted 22/05/2008].

Table I. Non-exhaustive summary of PrP or PrP-derived peptide interactions with various ligands and proposed roles for these interactions.

Ligand	Identification method	Possible role	References
Non-proteinic ligands			
Heparan sulfate	Synthetic peptides, CD Cultured cells	Component of amyloid structures Competition for copper binding	[38, 111]
Gangliosides	Neurons and lymphocyte labeling	PrP ^C -GM3 complex involved in T cell activation and other membrane functions	[72]
Lectin CBP70	Immunofluorescence, flow cytometry, Western blots	Helps conversion of PrP ^C into PrP ^{Sc}	[104]
Nucleic acids	Co-aggregation ADN-PrP. Binding of ARN aptamers	Could give a role for anucleic acid in infection. Useful for a diagnostic using ARN ?	[80, 81, 128]
Membrane lipids	Tryptophane fluorescence	Insertion in membrane, conformation change into structure and leakage of vesicle content. In rafts, conformation into structure. A role in transconformation of PrP	[107]
Membrane lipids	PrP (118-135) destabilizes membranes	Induces apoptosis of rat cortical neurons	[94]
POPG > DPPC > rafts	BIAcore	The strength of the association of PrP with lipid membranes depends on the protein conformation and pH	[24]
Copper	Circular dichroism and tryptophane fluorescence	Copper binds on octarepeat. A role in transport of cellular Cu, a SOD fonction	[47, 119]
Proteins			
Apolipoprotein E	Thioflavine fluorescence	Interaction with PrP (109-122) and (109-141). A role in amyloidogenicity ?	[6]
Pli45, Pli110	Ligand blot		[87]
Pli3, Pli4, Pli5, Pli6, Pli7, Pli8	PrP-Alkaline phosphatase screening		[129]
37 kDa/67 kDa laminin receptor	Double hybrid in yeast. Cell binding	PrP receptor	[48, 100]
Laminin	Binding on PC12 cells, in presence of NGF	Neuronal plasticity	[42]
66 kDa protein	Complementary hydropathy, binding competition	Receptor to PrP before internalization and signal transduction	[71, 70]
Bcl2	Double hybrid in yeast	Bcl2 protects cells from apoptosis. Binding with PrP inhibits the protective role	[58, 97]
Plasminogen	Binding, co-precipitations	Specific binding of PrP ^{Sc}	[37, 67]

Table II. Continued.

Ligand	Identification method	Possible role	References
Proteins (continued)			
N-CAM	Cross-linking in situ, mass spectrometry, KO mice		[109]
and tubulin	Cell cultures		[11]
Synapsin Ib	Double hybrid in yeast and co-immunoprecipitation	Presynaptic vesicle control. A role in signal transduction ?	[115]
Grb2	Double hybrid in yeast and co-immunoprécipitation	Synaptic vesicle control. A role in signal transduction ?	[115]
Pint1	Double hybrid in yeast and co-immunoprécipitation		[115]
Fyn	Cross-linking with antibodies	Signal transduction via a PrP receptor	[79]
P	PrP KO mice, PrP with N-ter deletions	Hypothetical, role similar to PrP	[110]
Calcineurin B	BIAcore	Ser/Thr phosphatase	[77]
"kringle" domain	Phage display cDNA expression library (ScN2a)		[105]
STI1	In vivo, antibodies	Signal transduction activation; neuritogenesis and neuroprotection	[61]
vitronectin	co localisation in vivo	Axonal growth	[44]
caveolin-1	in vitro interaction	Aggregation PrP-cav1 induces signaling cascade	[127]
Rdj2		chaperonin	[7]
TREK-1 2P domain	Double hybrid in bacteria, transfected cells	Interaction modulates electrophysiological-dependent cellular response	[2]
Chaperonins			
Hsp70	Thermal shock on NT-2, simultaneous increase of Hsp70 and PrP ARNm. Interaction with HSE1 et HSE2	Folding of translocated proteins. No effect on PrP conversion	[112]
Hsp60	Stabilization of folding	Postulated "Protein X"	[31]
Hsp73	Heat shock in N2a and ScN2a cells	A link between expression of Hsp73 and PrP ^{Sc} accumulation	[124]
Hsp104	Circular dichroism	Interaction of PrP with Hsp104 changes the structure of Hsp104 and inhibits its ATPase activity	[108]
GroEL	Double hybrid and peptide mapping. In vitro, cell-free conversion	Hsp104 and GroEL affects in vitro, the conversion of the hamster PrP ^C into PrP ^{Sc}	[27, 31]

cell machinery. α - and β -tubulin and N-CAM, cytoskeleton or close to cytoskeleton proteins were also found to interact with PrP.

Proteins implicated in neuronal functions were described to interact with PrP; synapsin Ib and Grb2, both proteins involved in presynaptic vesicle control, co-precipitate with PrP in a microsome fraction, calcineurin B, a ser/thr phosphatase located in synaptosomes interact with PrP. These proteins may also play a role in signal transduction.

Some intracellular proteins like Bcl-2 having an anti-apoptotic function and several other proteins without defined functions such as Pli, [129] and Pint1 [115] interact with PrP. Plasminogen, found in intercellular medium and in blood, binds PrP^{Sc} but not PrP^C [67].

The recent studies of PrP interactions seem to point towards interactions of PrP with extracellular proteins such as vitronectin [44] or glycosaminoglycans (heparin) and with proteins located in rafts like caveolin-1 [127] to transduce signals via the Fyn pathway. These signals could induce neuroprotection or neurogenesis [61] or even short term and long-term memory [22].

It seems difficult to conclude definitely on the specificity of most of the interactions detected and their role in PrP transconformation due to the conformation of the protein used for assays (with or without non-structured N-termini, glycosyl moiety or GPI-anchor) and the kind of assay performed (co-precipitation, yeast double hybrid etc.).

9. CONCLUSION

Like other amyloidopathies, prion disease is characterized by conformational changes leading to the apparition of new physico-chemical properties. Although, the structural characterization of PrP^C through its recombinant form has been well documented, PrP^{Sc} suffers from the absence of structure and biochemical characterization. Thus poor information is available concerning the molecular bases of PrP^C-to-PrP^{Sc} conversion. One of the examples of this lack is the relation between PrP folding dynamics, structural diversities and strain phenomenon. The link between these biophysical and biological aspects remains to be exper-

imentally established although multiple PrP folding pathways offer a rationale to correlate structural dynamics and physical hypothesis about the origin of prion strains. So far, the strain phenomenon is only attributed to biochemical and anatomopathology diversity.

Last, biological and cellular approaches show the complexity of establishing a biologically significant PrP interaction network. Currently, the relation between conformational change and cell death remains obscure. For example how can a cell in a cell culture model replicate the infectious agent massively without an effective toxicity.

Finally, it must be noted that a single approach cannot give a definitive answer on the mechanisms of prion infection and toxicity and it can be emphasized that multiple coordinated approaches should be helpful in unravelling the prion complexity.

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