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Molecular Pathogenesis of Prion Diseases

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1. Introduction

Prion diseases or transmissible spongiform encephalopathies (TSEs) are rare, fatal and incurable neurodegenerative disorders of humans and animals (Prusiner, 1998).

In humans, prion diseases occur with unique aetiology as sporadic, genetic or infectious disorders. Sporadic cases of prion diseases, which account for the majority of casualties (up to 85% of all cases), are of unknown origin; the genetic forms are less frequent (up to 15%), while the infectious cases are extremely rare with an incidence of less than 1% (Prusiner, 2001). Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker (GSS) syndrome, Fatal Familial Insomnia (FFI) are examples of human prion diseases. In animals the disease is mostly infectious and the mode of transmission is horizontal. Prion diseases include scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle, and chronic wasting disease of deer, elk, and moose (Williams, 2005).

The agents responsible for prion diseases are infectious proteins named prions. The term 'prion' was coined when Stanley B. Prusiner introduced the concept of *proteinaceous infectious particles* (Prusiner, 1982). Since the introduction of this once heretical notion, mounting evidence has strengthened its validity.

In the next sections of this chapter we present and discuss the peculiar complexity of the molecular pathogenesis of prion diseases in humans and animals.

2. Prion protein and prions

2.1 The prion protein

The prion protein (PrP) is one of the most and best-studied models for misfolding diseases. The cellular form of PrP (PrP^C) is a glysosyl-phosphatidylinositol (GPI) anchored polypeptide present on the outer leaflet of the cellular membrane of most cell types in mammals. In humans, the *PRNP* gene, located in the short arm of chromosome 20 (Liao et al., 1986), features two exons. The second exon contains the entire open reading frame (ORF), which encodes for the protein. The PrP^C is composed of 253 amino acids in humans, including 22 amino acids of endoplasmic reticulum signal sequence at the N-terminus and 23 amino acids as GPI anchoring signal at the C-terminus (Stahl et al., 1990). The N-terminal region of PrP^C encompasses five characteristic amino acid octarepeats that coordinate

copper and, to a lesser extent, other metal ions. The mature 208-residues protein possesses a single disulphide bridge between Cys179 and Cys214, and two sites for Asn-linked glycosylation within the carboxy-terminal region at position Asn181 and Asn197.

The protein is the first system where a polypeptide has been shown to exist in at least two significantly different conformations, associated with radically different functions.

The physiological function of PrP^C has not been established with certainty yet; nevertheless its evolutionarily conserved sequence suggests that it might play an important role in neuronal development and physiology. Indeed one recent finding indicates a possible involvement of this protein in neuronal differentiation and polarization (Kanaani et al., 2005). On account of additional evidence, it could also contribute to myelin formation and maintenance (Benvegnu et al., 2011a).

A strategy often employed to identify protein function is the development of transgenic mouse lines with a disabled gene. Many lines of knockout (KO) mice have been developed for PrP (Weissmann and Flechsig, 2003). In these models, typically either the entire ORF of exon 3 of *Prnp* (in mice), or the ORF as well as flanking sequences are deleted (Weissmann and Flechsig, 2003). The *Prnp* KO mice ($Prnp^{0/0}$) appear to develop and reproduce normally (Bueler et al., 1992), but their further evaluation found several abnormalities. The mice appear clinically asymptomatic yet they develop peripheral nerve demyelination, have increased susceptibility to ischemic brain injury, altered sleep and circadian rhythm, altered hippocampal neuropathology and physiology, including deficits in hippocampal-dependent spatial learning and hippocampal synaptic plasticity (Tobler et al., 1997, Nishida et al., 1999, Spudich et al., 2005, Criado et al., 2005). Mice with $Prnp^{0/0}$ are also more susceptible to oxidative stress, and PrP^C appears to play a neuroprotective role in cellular response to hypoxic-ischemic injury (Weise et al., 2006). Some $Prnp^{0/0}$ mouse lines in which the deletion extends beyond the ORF, although developing normally, acquire ataxia and Purkinje cell loss later in life (Moore et al., 1999).

Recent findings show development regional differences of the expression of PrP in mouse central nervous system (CNS), with specific white matter structures showing the earliest and highest expression of PrP^C. Indeed, all these regions are part of the thalamo-limbic neurocircuitry, hence suggesting a potential role of PrP^C in the development and functioning of this specific brain system (Benvegnu et al., 2010).

Furthermore, the transcriptome during development for the CNS of mice lacking a functional *Prnp* gene has recently been compared with that of wild-type animals (Benvegnu et al., 2011b). To assess the influence of PrP^C on gene expression profile in the mouse brain, a microarray analysis was undertaken using RNA isolated from the hippocampus at two different developmental stages: newborn (4.5-day-old) and adult (3-month-old) mice, both from wild-type and *Prnp* KO animals. Based on the comparison of these datasets, *commonly* co-regulated genes and *uniquely* de-regulated genes during postnatal development were identified. The absence of PrP^C affected several biological pathways, the most representative ones being cell signaling, cell-cell communication and transduction processes, calcium homeostasis, nervous system development, and synaptic transmission and cell adhesion. However, there was only a moderate alteration of the gene expression profile in our animal models. PrP^C deficiency did not lead to a dramatic alteration of gene expression profile, and produced moderately altered gene expression levels from young to adult animals. Hence,

these results further support silencing endogenous PrP^C as therapeutic approach to prion diseases (Benvegnu et al., 2011b).

Concerning PrP^C cellular function, experiments have recently shown that PrP^C regulates the cleavage of neuregulin-1 proteins (NRG1). Neuregulins provide key axonal signals, which regulate processes, including glial cells proliferation, survival and myelination. Interestingly, *Prnp*$^{0/0}$ mice have recently been reported to have a late-onset demyelinating disease in the peripheral nervous system (PNS), but not in the CNS (Bremer et al., 2010). The comparison of wild-type and *Prnp*$^{0/0}$ mice showed that the NRG1 processing is developmentally regulated in the PNS and influenced by PrP^C in old but not in young animals. In addition, it has been found that neuregulin-3 processing — another neuregulin family member — is altered in the PNS of *Prnp*$^{0/0}$ mice. These differences in neuregulin proteins processing are not paralleled in the CNS, thus suggesting a different cellular function for PrP^C between the CNS and the PNS (Benvegnu et al., 2011a).

2.2 Prions and the biology of the conversion mechanism

Prion diseases are caused by changes in the conformation of the endogenous PrP^C, which turns into an alternatively folded, disease-causing form called the prion, or PrP^{Sc}. The normal PrP^C contains three α-helixes and two short β-sheet structures in its globular domain, whereas PrP^{Sc} contains fewer α–helical and mostly β-sheet structures (Prusiner, 1998). PrP^C and PrP^{Sc} possess the same primary polypeptide sequence, but different secondary and tertiary structures. PrP^{Sc} is produced by the conversion of existing PrP^C into PrP^{Sc}.

The process leading to PrP^{Sc} production from PrP^C is not completely understood. It is believed that this occurs when PrP^C comes into contact with PrP^{Sc} and is thus induced to take on the shape of PrP^{Sc} (Prusiner, 1998). The fact that mice devoid of PrP^C are resistant to infection, as they are unable to replicate prions, provides strong evidence that PrP^C is necessary for prion disease (Bueler et al., 1993). Although it is clear that PrP^C is necessary for prion disease, it is still debated whether other proteins or molecules are involved in the conformational change *in vivo* (Telling et al., 1995, Deleault et al., 2003). The conversion of monomeric PrP^C into insoluble, protease-resistant PrP^{Sc} is a process that seems to occur in structures denominated caveolae-like domains (CLDs) (Gorodinsky and Harris, 1995), and the resulting PrP^{Sc} subsequently traffics to other membranous compartments such as endosomes and lysosomes (Marijanovic et al., 2009). The membranes of CLDs seem to be composed of cholesterol-rich rafts and presumably provide the cellular environment for the formation of PrP^{Sc}. Two conversion and replication models have been proposed: (i) a nucleation-polymerization reaction, and (ii) a template-assisted conversion process. In the first model, the rate-limiting step is the formation of a critical amount of PrP^{Sc} to form a *seed* for the polymerization of PrP^{Sc}. In the template-assisted model, PrP^C must first undergo conversion toward a transition state that presumably corresponds to a partially destabilized structure (Aguzzi and Calella, 2009). The structural transition could be mediated by an auxiliary molecule, which facilitates the conversion to a nascent prion (Telling et al., 1995, Deleault et al., 2003). In disease-affected brain homogenates, limited proteolysis completely hydrolyzes PrP^C and produces a protease-resistant PrP^{Sc} molecule of about 140 amino acids, designated PrP27–30. In the presence of detergent, PrP27–30 polymerizes into amyloid (McKinley et al., 1991). Prion amyloids, or rods, formed by limited proteolysis and detergent

extraction, are indistinguishable from the filaments that aggregate to form PrP amyloid plaques in the CNS, exhibiting similar ultrastructural morphology and tinctorial characteristics after staining with Congo red dye (Prusiner et al., 1983). So far, little is known about the structure of prions. Several models have been proposed, attempting to satisfy all available biophysical, biochemical and immunochemical data on infectious prions (Govaerts et al., 2004). The discovery that recombinant PrP, expressed in *Escherichia coli*, is infectious to mice when polymerized into amyloid fibrils has opened new avenues for research in the prion field (Legname et al., 2004). Characterization of these synthetic prions revealed novel distinctiveness associated with neuropathological changes in mouse models of prion disease (Legname et al., 2005). The conformational changes acquired by the synthetic prions confer increasing stability to PrP^{Sc}, as measured by the amount of chaotropic agents necessary to completely unfold PrP^{Sc}. Moreover, a linear correlation is established when the measure of stability of any particular isolate is expressed as a function of mouse survival times to the disease (Legname et al., 2006).

One of the strongest arguments for the existence of prions is the link between inherited prion diseases and mutations in the *PRNP* gene. Currently, almost 60 pathogenic mutations and several polymorphisms have been identified in the *PRNP* gene (Kovacs et al., 2002). They include missense point mutations, mostly located in the globular part, insertion or deletion mutations involving the N-terminal domain, and non-sense mutations resulting in the premature termination of PrP synthesis. Twelve polymorphisms are silent, while four of them alter the amino acid sequence. The most important one that markedly influences the disease is the M/V polymorphism at codon 129 (Collinge, 2001). The M/V polymorphism at position 129 is common; the homozygous M/M and V/V and the heterozygous M/V subjects account for 43%, 8% and 49%, respectively, in the Caucasian population (Zimmermann et al., 1999). This polymorphism is a key determinant of genetic susceptibility to acquired and sporadic prion diseases, the large majority of which occur in homozygous individuals (Collinge et al., 1991, Palmer et al., 1991, Windl et al., 1996). The *PRNP* heterozygotes appear to be protected from sporadic CJD (sCJD) compared to the *PRNP* homozygotes (Kobayashi et al., 2009, Baker et al., 1991, Hsiao et al., 1992). The M/V polymorphism at position 129 affects the disease phenotype when it is located on the mutant allele: D178N-129V causes familial CJD (fCJD), while D178N-129M is responsible for familial FFI. The M/V polymorphism located on the normal allele affects the age onset and duration of the disease. Patients carrying either M or V 129 codon have been observed in all inherited prion diseases. The altered conformation observed in human PrP mutants might lead to a different affinity for extracellular matrix components and cellular membranes and, consequently, to an aberrant localization of PrP in different cellular compartments, favoring formation of altered pathogenic topologies (Hegde et al., 1999). Independent evidence derived from cell culture studies, expressing some of the disease-linked mutants, showed that these mutations may affect folding and maturation of PrP^{C} in the secretory pathway of neuronal cells (Ashok and Hegde, 2009).

How mutations and polymorphisms can structurally modulate the diseases is not clear. In fact, until recently there was no evidence of a pathological point mutation causing substantial structural differences in PrP folding.

To shed new light on the role of pathological point mutations on PrP structure, a high-resolution 3D structure of the truncated recombinant human PrP containing the pathological

Q212P mutation has recently been determined and examined (Ilc et al., 2010). This mutation is responsible for a GSS syndrome characterized by mild amyloid PrP deposition in patients (Piccardo et al., 1998, Young et al., 1998). The high-resolution NMR structure of Q212P mutant revealed unique conformational features compared to the known structures of either human or other mammalian PrP^C (Christen et al., 2009, Christen et al., 2008, Gossert et al., 2005, Lopez Garcia et al., 2000, Riek et al., 1996).

The most remarkable differences involved the C-terminal end of the protein and the β_2–α_2 loop region. The Q212P mutant is the first known example of PrP structure where the α_3 helix between E200 and Y226 is broken into two helices. This breakage brings about dramatic changes in the hydrophobic interactions between the α_3 helix and the β_2–α_2 loop region. In the wild-type protein, long-range interactions between Y225 and M166 define the position of the β_2–α_2 loop and thus the tertiary structure of the protein. In this protein type, the solvent-exposed surface of the β_2–α_2 loop and the α_3 helix region is smaller, and Y169 is buried inside the hydrophobic cluster (Ilc et al., 2010).

When these structural findings are compared with the already resolved NMR structures of human PrP, carrying respectively the CJD-related E200K (Zhang et al., 2000) and the artificial R220K mutation, the α_3 helix appears well ordered up to the point mutation (Calzolai et al., 2000). After this mutation, the α_3 helix shows increased flexibility and significantly less order. At the same time, the R220K mutation does not alter the hydrophobic interactions between the aromatic residues of the β_2–α_2 loop and the α_3 helix.

Special interest in prion biology is therefore focused on the epitope formed by the β_2–α_2 loop and the α_3 helix, as this surface has been implicated in interactions with a hypothetical facilitator of prion conversion involved in the development of TSEs (Kaneko et al., 1997, Telling et al., 1995). Therefore, the plasticity of the loop may modulate the susceptibility to prion disease of a given species. While in PrP^C from most mammalian species this loop is flexible, it is well defined in PrP^C of elk (Gossert et al., 2005), bank vole (*Clethrionomys glareolus*) (Christen et al., 2008), tammar wallaby (Christen et al., 2009) and, as found out very recently, horse (Perez et al., 2010) and rabbit (Wen et al., 2010). Interestingly, elk and bank vole are highly susceptible to TSEs, whereas there have been no cases of prion diseases either in marsupials, horses or rabbits. The structure-function relationship suggested by these works may provide the molecular basis for understanding the generation of PrP^{Sc} in inherited prion diseases. In fact, the characterization of high-resolution structures of PrP pathological mutants and their comparison with the wild-type overall folding, highlights important regions in these proteins that could be involved in early events of PrP misfolding. This may also provide a molecular explanation for prion formation in the sporadic forms of prion disease.

3. Molecular pathogenesis of prion diseases

3.1 PrP^{Sc} conformers in human and animal prion disorders

Human and animal TSEs exist as different prion strains characterized by distinct biological properties. A prion strain is defined using several criteria, such as incubation time and lesion profile after transmission, as well as by physico-chemical characteristics of pathological PrP^{Sc} conformers (Bruce et al., 1994, Aguzzi et al., 2008).

Several studies demonstrated that prion strains can be distinguished based on different biochemical properties of PrP^{Sc}, encompassing conformation, glycoform profile, degrees of protease-resistance under different denaturing conditions, thus allowing a molecular strain typing classification of PrP^{Sc} (Wadsworth and Collinge, 2011). Treatment of PrP^{Sc} with proteinase K (PK) generates a large PK-resistant C-terminal core fragment termed PrP27-30 which is considered the pathogenic and infectious core of PrP^{Sc}. Full-length PrP^{Sc} and PrP27-30 are associated to the naturally occurring infectious agent causing prion diseases and are thought to be the primary cause of the histological changes in brains of subjects with prion diseases.

In human and animal prion disorders, the remarkable heterogeneity of disease phenotypes is influenced by the combination of either PrP^{Sc} type or relevant *PRNP* polymorphisms (Gambetti et al., 2011).

3.2 The biochemical phenotype of PrP^{Sc} in human and animal TSEs

In human prion disorders, several different types of PrP^{Sc} have been recognized. PrP^{Sc} types are distinguished based on the electrophoretic migration and the glycosylation profile of PrP27-30. This is composed of a major triplet of bands which represent the differently glycosylated isoforms of PrP^{Sc}.

Additional minor C-terminally truncated fragments (CTFs) resistant to proteases have been reported and they contribute to define the *biochemical strains* of PrP^{Sc}. The combination of PrP27-30 and CTFs is representative of specific patterns and correlates to distinct disease phenotypes (Zou et al., 2003, Zanusso et al., 2004).

In sCJD, three distinct PrP^{Sc} types have been described: type 1, type 2A and type U (Fig. 1A). Type 1 and type 2 PrP^{Sc} are distinguished based on the different electrophoretic mobility of the unglycosylated form of approximately 21kDa in type 1, and 19kDa in type 2, respectively (Parchi et al., 1999, Gambetti et al., 2003). In contrast, type U PrP^{Sc} shares apparent gel mobility with type 1, though it lacks the diglycosylated isoforms (Zanusso et al., 2007).

In variant CJD (vCJD), an unglycosylated band migrating at 19kDa and a highly glycosylated-dominant profile characterize PrP^{Sc}; wheresas type 2A PrP^{Sc} in sCJD is distinguished by the highly glycosylated-dominant profile. Accordingly, the current nomenclature defines type 2A-PrP^{Sc} associated to sCJD, and type 2B-PrP^{Sc} associated to vCJD, thus identifying BSE agent in humans (Collinge et al. 1996). In familial forms of CJD (E200K-129V) and FFI forms, a type 2B-like pattern is observed (Fig. 1A). The exception to the conventional definition of PrP^{Sc} typing is observed in GSS mutations. In GSS, PrP27-30 is absent and pathological PrP is composed of intermediate fragments (IFs) of ~11kDa and ~8kDa spanning residues ~90-150 and ~60-150 (Tagliavini et al., 1991, Tagliavini et al., 1994, Piccardo et al., 1998). According to their sequence, these IFs lack PrP post-translational modifications, including GPI-anchor.

However, in GSS P102L mutation, a hybrid phenotype of PrP^{Sc} is observed. In P102L, PrP^{Sc} is characterized by the presence of the 8kDa intermediate fragment (PrP8), as in other GSS, but also of PrP27-30 (Parchi et al., 1998) (Fig. 1A).

Although prion diseases are defined based on the presence of a disease-associated protease-resistant PrP that has been proven to retain infectivity, Gambetti *et al.* shifted this dogmatic definition. They reported on a series of individuals with dementia and spongiform

encephalopathy but with a PrP resistant to minimal amount of PK, thus designating this novel form of prion disease as *variably protease-sensitive prionopathy* (or VPSPr) (Gambetti et al., 2008). Interestingly, in these cases the biochemical pattern of PrP is variable, as it contains both C-terminal fragments and intermediate fragments with molecular masses ranging from 20kDa to 7kDa (Zou et al., 2010) (Fig. 1A).

Over the last few years, the PrP^{Sc} biochemical types described in animal prion disorders have consistently increased, following the systematic testing of over 30-month-old cattle, carried out by several European countries in 2001. The diagnostic test relied on the detection of protease-resistant PrP by Western blot in the animals' brain tissue. This preventive measure allowed to detect all BSE-affected cattle, but also to recognize two additional BSE-associated PrP^{Sc} types, distinguished from classical BSE (C-BSE) for the different electrophoretic migration and pattern of PrP glycosylation. According to the higher and lower electrophoretic migration of the unglycosylated fragment of PrP^{Sc} compared to C-BSE, these "atypical" BSE forms were named H-type and L-type BSE, respectively (Biacabe et al., 2004, Casalone et al., 2004) (Fig. 1B).

The wide scale screening testing for prions on small ruminants resulted in similar findings, since additional PrP^{Sc} types were also found in scrapie. In particular, beside the classical scrapie strain, other atypical forms — originally named Nor 98 — have been reported; these are characterized by small intermediate truncated fragments of PrP associated to PrP27-30 (Benestad et al., 2003, Benestad et al., 2008) (Fig. 1C).

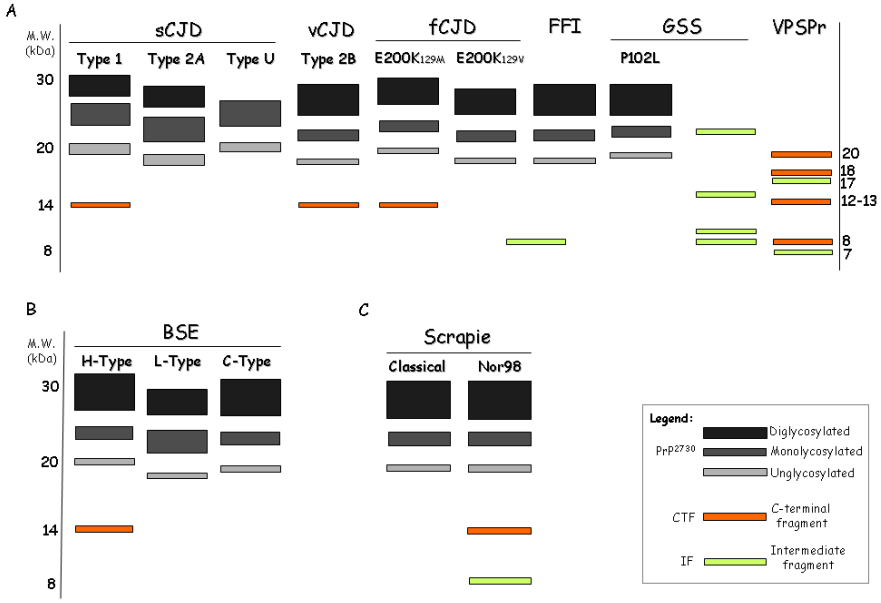

Fig. 1. PrP^{Sc} fragment patterns in human and animal prion disorders. Gel pattern of PrP^{Sc} in human forms (A), in BSE affected cattle (B), and in scrapie in sheep (C). Strains of TSE are defined in part by biochemical properties of PrP^{Sc} (glycosylation and size), pattern of PrP^{Sc} deposition in brain, and average age of disease onset.

3.3 Correlation between biochemical phenotypes of PrP^{Sc}, disease-phenotypes and prion strain biological properties

Several studies indicate that distinct PrP^{Sc} patterns represent the molecular signature of prion and have relevant biological implications including neuropathological phenotype and transmissibility. For instance, the occurrence of spongiform changes or amyloid deposits are strictly dependent on PrP^{Sc} species in brain tissue (Table 1).

	sCJD, fCJD, iCJD	vCJD	FFI	GSS		VPSPr
				Classic	P102L	
Clinical Phenotype	Subacute dementing illness with visual, cerebellar and/or extrapyramidal signs, myoclonus	Psychiatric features, painful distal sensations, cerebellar signs	Sleep disruption, dysautonomia, motor abnormalities	Slow progressive dementia and ataxia, pyramidal and extrapyramidal signs	As sCJD or classic GSS	Cognitive decline, mood or behavioural changes
Disease Duration	Weeks, months, less than two years	Months or years	15 months (6-42)	5-6 years	36 months (3-72)	20 months (10-60)
Pathological Phenotype	SD, astrogliosis, neuronal loss, amyloid plaques	SD, astrogliosis, neuronal loss, florid amyloid plaques	SD, astrogliosis, neuronal loss, mainly thalamic	Widespread amyloid deposits, neuronal loss, astrogliosis, NFTs	SD, astrogliosis or as classic GSS	SD, minimal astrogliosis
Pattern of PrP Deposition	Synaptic/punctate, and/or amyloid plaques	Synaptic/ punctate, florid plaques	Fine punctuate staining	Multi-centric amyloid plaques	As sCJD or in classic GSS	Intense staining, plaque- and dot-like
PrP^{Sc} Biochemical Phenotype	Type 1 Type 2A CTF12-14	Type 2B CTF12-14	Type 2B-like CTF12-14	PrP8 and 11kDa IFs	PrP27-30 and PrP8	CTFs IFs
Transmissible	Yes	Yes	Yes	No	Yes/No	Pending

Legend: iCJD: iatrogenic CJD; SD: spongiform degeneration; NFTs: neurofibrillary tangles; CTFs: C-terminal fragments; IFs: intermediate fragments; PsPr: protease-sensitive PrP;

Table 1. Disease characteristics of human prion disorders

However, this assumption is not fulfilled in GSS, since subjects carrying mutations, which segregate with GSS, except P102L, show a different disease phenotype, lacking PrP27-30 (Ghetti et al., 2003). As expected, spongiform changes are not observed and the intermediate fragments promote a PrP amyloidogenesis process widespread to all brain tissue.

Experimental studies *in vitro* showed the high propensity of these peptides to form amyloid aggregates (Salmona et al., 2003). Further, GSS does not propagate as a spongiform encephalopathy. In other words, unlike other prion diseases, GSS shares the disease characteristics of several other non-transmissible neurodegenerative disorders (Fig. 2).

A first link between PrP pattern and pathological phenotype involves PrP27-30 and the detection of spongiform degeneration. In general all prion disorders associated with PrP27-30, either in humans or in animals, are characterized by spongiform degeneration and astrogliosis. Further, the presence of PrP27-30 is related to transmissibility in susceptible recipients (Fig. 2).

In contrast, in P102L mutation spongiform changes and diffuse multicentre amyloid plaques are observed, sharing disease characteristics of both CJD and GSS. These findings correlate to the presence of both PrP27-30 and PrP8 (Wadsworth et al., 2006), and only P102L cases with PrP27-30 transmitted the disease, whereas others did not. In particular, a spongiform encephalopathy was observed only in transgenic mice challenged with P102L human cases showing spongiform degeneration and PrP27-30 (Piccardo et al., 2007).

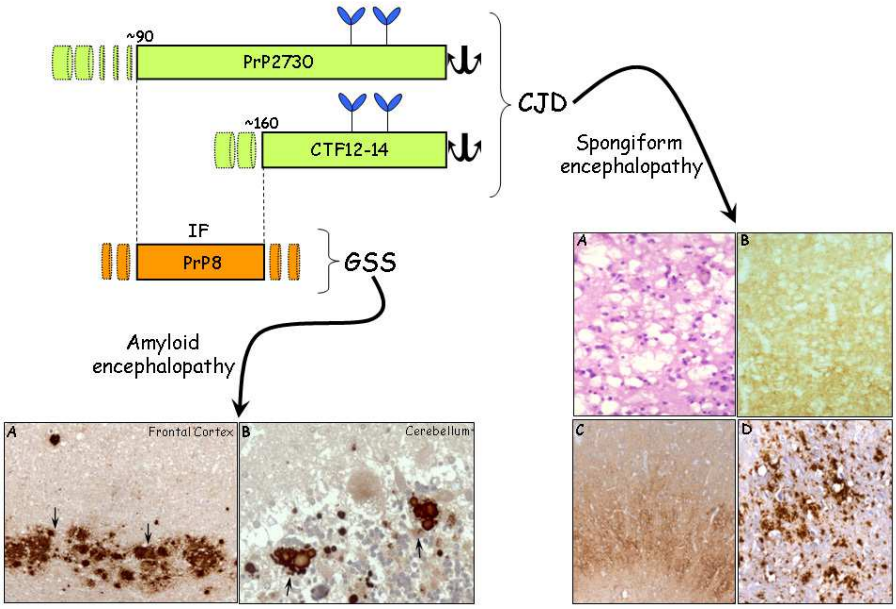

Fig. 2. Correlative analysis between PrP^{Sc} fragments and pathological phenotype.

In sCJD, the biochemical phenotype of PrP^{Sc} comprises both PrP27-30 and CTFs, resulting in a spongiform encephalopathy and different patterns of PrP deposition.

Conversely, in GSS, where PrP deposits consist of an intermediate fragment (PrP8), which lacks post-translational modifications including GPI anchor, the pathological phenotype is characterized by PrP amyloid multicentric plaques (arrows).

VPSPr-affected subjects have a weakly PK-resistant PrP and neuropathologically they show the distinct feature of a spongiform encephalopathy. As mentioned above, both PrP C-terminal fragments — indicating that most of them are GPI-anchored — consist mainly of those fragments forming the PrP pattern. Since IFs do not generate spongiform changes, these findings indicate that GPI-anchored PrP molecules might be associated with spongiform degeneration. As known from transgenic GPI anchorless mice, GPI anchorless PrP is able to replicate inducing an amyloidotic disease but not a spongiform encephalopathy (Chesebro et al., 2005).

3.4 The biological properties of prion strains are enciphered in the biochemical pattern of PrP^{Sc}: A lesson from two-dimensional analysis

In sCJD, a sextet of subtypes was recognized, characterized by disease phenotypic heterogeneity, which results from the combination of two PrP^{Sc} types and the polymorphism M/V at codon 129 (Parchi et al., 1999).

A decade ago we performed a 2D analysis — which separates proteins by molecular weight and isoelectric point — aimed at introducing a technique to better define the biochemical phenotype of PrP^{Sc}, beyond the conventional patterns obtained by SDS-PAGE (Zanusso et al., 2002). In particular, we argued whether additional PrP conformers might be observed within a given PrP27-30 band. In all sCJD cases associated with type 1 PrP^{Sc}, regardless of the polymorphism at codon 129, the 2D pattern of PrP27-30 and C-terminal fragments (CTFs) is identical (Fig. 3A). Conversely, we showed that type 2 PrP^{Sc} separated as two distinct patterns, one in MM2 cortical (MM2C) and the other in MV2 and VV2, which correlated with distinct pathological phenotypes. In particular, MM2 is characterized by a severe SD in the cerebral cortex with a relative spare of the cerebellum and a coarse pattern of PrP deposition, while in MV2 and VV2 the distribution of lesions is more diffuse, mostly concentrated in the cerebellum, with abundant amyloid plaques (Zanusso et al., 2004) (Fig. 3B and 3C).

PrP27-30 core fragment is depicted in black. The different spots composing PrP27-30 represent the N-terminally truncated fragments. The 16-17-kDa and the 12-14 kDa truncated fragments are seen in sCJD with type 1, and correlate to a synaptic PrP staining seen in the frontal cortex and cerebellum. MM2C and MV2/VV2 subtypes show distinct PrP 27-30 patterns and CTFs. In MM2C, CTFs are composed of 12-14 kDa species, while in MV2/VV2 these consist of 16-17kDa fragments. These biochemical patterns correlate to distinct pathological phenotypes.

These results were subsequently confirmed by transmission studies. In transgenic mice targeting and expressing different forms of *PRNP* (MM, MV, VV), MM1 and MV1 isolates showed similar biological properties, while the strain associated with an MV2 patient could

not be distinguished from the VV2 strain, and MM2 isolate transmitted poorly (Bishop et al., 2010). In the bank vole, MM1 and MV1 CJD also behaved as similar agents, and differed from MM2 CJD. MV2 and VV2 did not transmit (Nonno et al., 2006).

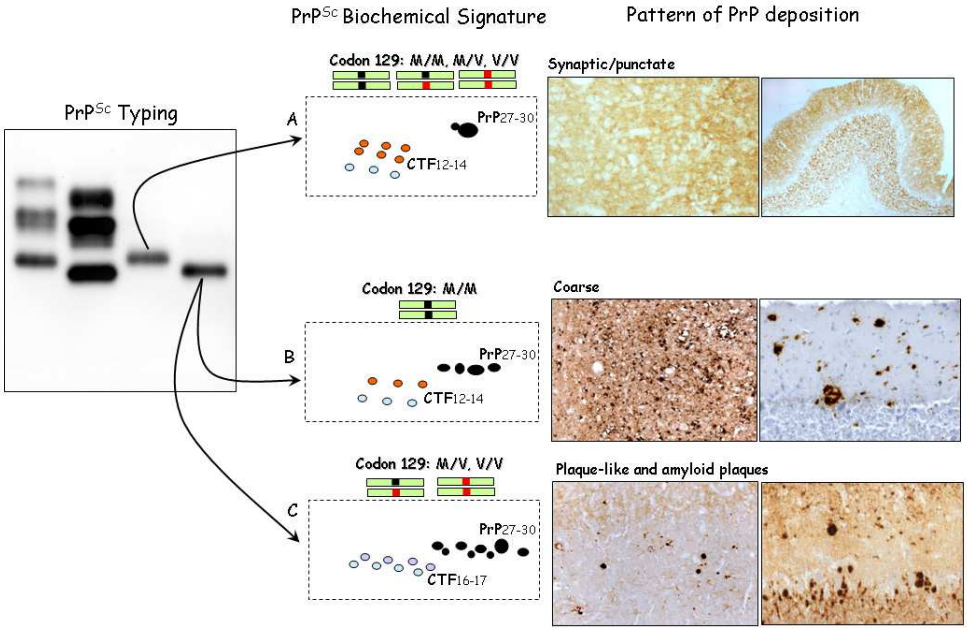

Fig. 3. Schematic diagram of PK-resistant C-terminal PrP core fragments in sCJD subtypes.

3.4.1 The biochemical link between animal and human prion forms is enciphered in PrP^{Sc}

We firstly proposed a parallel between sCJD in humans and BSE in cattle, by showing that MV2 sCJD subtype shared molecular similarities with cattle affected with bovine amyloidotic spongiform encephalopathy (BASE) including the pathological phenotype (Casalone et al., 2004, Brown et al., 2006). As in humans, two presumably sporadic forms of BSE are observed in cattle in addition to typical/classical BSE. Moreover, the apparent molecular weight of the unglycosylated band of bovine amyloidotic spongiform encephalopathy is identical to that of type 2A, while H-type BSE corresponds to type 1 PrP^{Sc} (Fig. 4). Of course, the link between C-BSE and vCJD had been largely demonstrated.

Wemheuer et al. proposed a correlation between classical and atypical/Nor98 scrapie in sheep and sCJD, showing that the two scrapie types share a number of striking similarities with human PrP^{Sc} types in sCJD (Wemheuer et al., 2009) (Fig. 4).

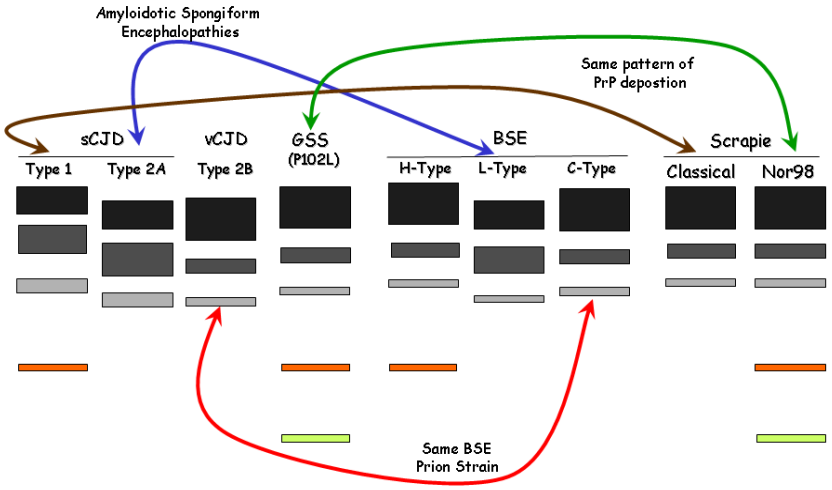

Fig. 4. Correlative analysis of PrP^{Sc} biochemical phenotype in different BSE, CJD and scrapie isolates. Arrows link human and animal forms.

4. Conclusion

The existence of different PrP^{Sc} types might be a common denominator of prion diseases in humans and animals and it might suggest the existence of different conformers within a given prion disorders. Further, although the biochemical approach is important for large-scale studies, it should be clear that for strain recognition and comparative analysis purposes, PrP^{Sc} biochemical similarity is only apparent evidence, and it does not reflect the biological properties of a given strain. These are revealed only after transmission.

In addition, the demonstration that synthetic prion strains can be made in the laboratory has ushered in a large set of experimental investigations that may help deciphering the structural determinants linked to different PrP^{Sc} types.

5. Acknowledgment

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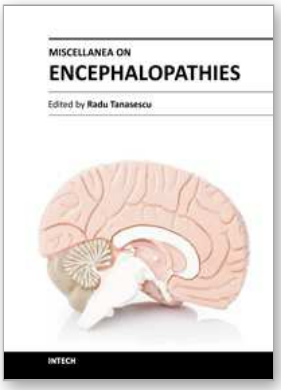

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