

**Title:** New insights into Cellular Prion Protein (PrP<sup>c</sup>) functions: the “*ying and yang*” of a relevant protein.

**Running Title:** The role of the Cellular Prion Protein: a “puzzling” protein

**Authors:** Oriol Nicolas<sup>1</sup>, Rosalina Gavín<sup>1</sup> and José A. del Río<sup>1 (\*)</sup>

**Affiliations:**

1) Molecular and Cellular Neurobiotechnology laboratory, Catalonia Bioengineering Institute (IBEC), Baldiri Reixac 15-21, and Department of Cell Biology, Faculty of Biology, University of Barcelona. Diagonal 645, E-08028 Barcelona, Spain.

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**(\*) Corresponding Author’s contact information:**

J.A. del Río, PhD  
Molecular and Cellular Neurobiotechnology laboratory  
Catalonia Bioengineering Institute (IBEC)  
Baldiri Reixac, 15-21  
E-08028 Barcelona, SPAIN  
Tel: +34-93-4020296  
Fax: +34-93-4020183  
@-mail: [jadelrio@ibec.pcb.ub.es](mailto:jadelrio@ibec.pcb.ub.es)

## **Abstract**

The conversion of cellular prion protein (PrP<sup>c</sup>), a GPI-anchored protein, into a protease-K-resistant and infective form (generally termed PrP<sup>sc</sup>) is mainly responsible for Transmissible Spongiform Encephalopathies (TSEs), characterized by neuronal degeneration and progressive loss of basic brain functions. Although PrP<sup>c</sup> is expressed by a wide range of tissues throughout the body, the complete repertoire of its functions has not been fully determined. Recent studies have confirmed its participation in basic physiological processes such as cell proliferation and the regulation of cellular homeostasis. Other studies indicate that PrP<sup>c</sup> interacts with several molecules to activate signaling cascades with a high number of cellular effects. To determine PrP<sup>c</sup> functions, transgenic mouse models have been generated in the last decade. In particular, mice lacking specific domains of the PrP<sup>c</sup> protein have revealed the contribution of these domains to neurodegenerative processes. A dual role of PrP<sup>c</sup> has been shown, since most authors report protective roles for this protein while others describe pro-apoptotic functions. In this review, we summarize new findings on PrP<sup>c</sup> functions, especially those related to neural degeneration and cell signaling.

**Section:** Cellular and Molecular Biology of Nervous System

**Keywords:** Prion, Doppel, Shadoo, cell death, cell proliferation, cell differentiation.

## **Abbreviations**

ADAM: A Disintegrin and Metalloproteinase protein  
Bcl-2: B-Cell Lymphoma 2  
BSE: Bovine Spongiform Encephalopathy  
CC: Charge Cluster domain of PrP<sup>c</sup>  
CD: Central Domain of PrP<sup>c</sup>  
CGN: Cerebellar Granule Neuron  
CJD: Creutzfeldt-Jacob Disease  
CR: Central Region of PrP<sup>c</sup>  
CWD: Chronic Wasting Disease  
Dpl: Doppel  
Eps8: Epidermal growth factor receptor Pathway Substrate 8  
GPI: Glycosyl-Phosphatidylinositol  
Grb-2: Growth factor receptor-bound protein 2  
HR: Hydrophobic Region of PrP<sup>c</sup>  
Hsp60: Heat Shock Protein 60  
MdM-2: Murine Double Minute 2  
NMDA: N-Methyl-D-Aspartic Acid  
OR: Octarepeat Region of PrP<sup>c</sup>  
PrP<sup>c</sup>: Cellular Prion Protein  
PRNP: Cellular Prion gene  
PrP<sup>Sc</sup>: Scrapie form of PrP<sup>c</sup>, used to term infectious Prion forms  
SOD: Superoxide Dismutase  
Sho: Shadoo  
STI-1: Stress-Inducible Protein 1  
TSE: Transmissible Spongiform Encephalopathy  
WT: Wild-Type

## 1. Introduction

Transmissible Spongiform Encephalopathies (TSEs), which include Creutzfeldt-Jacob disease (CJD) in humans (Bruce et al., 1997; Collinge et al., 1996; Hill et al., 1997), Bovine Spongiform Encephalopathy (BSE) in cows (Wells et al., 1987), Scrapie in sheep (McGowan, 1922), or Chronic Waste Disease (CWD) in elk and deer (Wells et al., 1987), are infective diseases promoted by the re-folding of the host-protein cellular prion (PrP<sup>c</sup>) into a pathological, protease K-resistant and  $\beta$ -sheet-enriched form (PrP<sup>sc</sup> or PrP<sup>res</sup>) (Prusiner, 1982). Despite extensive neuropathological studies into prion diseases, it is still unclear whether the toxicity of PrP<sup>sc</sup> or the loss-of-function of intrinsic PrP<sup>c</sup> is responsible for the neurodegeneration observed in these pathologies. We devote this review specifically to PrP<sup>c</sup> functions in intercellular signaling and neurodegeneration. Readers interested in immunological or other aspects of PrP<sup>c</sup> biology (e.g., transmission, detection, analyses of human diseases, etc.) are referred to recent reviews (e.g., (Aguzzi et al., 2008a; Aguzzi et al., 2007; Aguzzi et al., 2008b; Linden et al., 2008)).

In humans, PRNP is located on chromosome 20 and comprises two exons (Basler et al., 1986). Transcription of the second exon gives rise to the complete PrP<sup>c</sup> protein (Puckett et al., 1991). The structure of several mature PrP<sup>c</sup> proteins in mice, cattle, humans, and Syrian hamsters is very similar. PrP<sup>c</sup> is an extracellular protein that contains two glycosylation sites and is enriched in  $\alpha$ -helix domains (Pan et al., 1993; Riek et al., 1996), which are anchored to the detergent-resistant areas of the plasma membrane by a glycosylphosphatidylinositol (GPI)-link (Prusiner, 1998b). The protein has a long, flexible N-terminal tail (residues 23–128), three  $\alpha$ -helices, and a two-stranded antiparallel  $\beta$ -sheet flanking the first  $\alpha$ -helix. This flexible N-terminal tail is

present in most of the animal species studied, except deer and elk, and contains series of four or five repeats of eight amino acids (PHGGGWGQ). The second domain of PrP<sup>c</sup> is the central domain (CD), which includes a sequence of positively charged residues (termed Charge Cluster or CC) and a hydrophobic region (HR) (Prusiner, 1998b) (See Fig. 1). The last domain or C-terminal globular domain (Donne et al., 1997; Riek et al., 1997) contains the  $\alpha$ -helix and two parallel stranded  $\beta$ -sheets (Riek et al., 1996). PrP<sup>c</sup> presents at least three distinct topological orientations: the fully extracellular form (or (sec)PrP) (Holscher et al., 2001), and two transmembrane isoforms (called Ntm-PrP and Ctm-PrP) with opposite sequence orientations with respect to the lumen of the endoplasmic reticulum (Hegde et al., 1998).

The nucleic sequence of PrP<sup>c</sup> has been strongly conserved throughout evolution in mammals (Rivera-Milla et al., 2006), especially in those regions that encode flexible domains of the protein (Wopfner et al., 1999). However, alignments of human and mammalian PRNP sequences indicate that codons located between residues 90 and 130 influence the transmissibility of prions in mammals, including humans (Schatzl et al., 1995). In addition, some paralogue genes have been described in chicken, amphibians, reptiles and fish (Gabriel et al., 1992; Simonic et al., 2000; Strumbo et al., 2001; Suzuki et al., 2002)

## **2. Tissue and cellular expression of PrP<sup>c</sup>: from the whole organism to the neuronal synapse.**

In mammals, PrP<sup>c</sup> is expressed in several tissues, such as secondary lymphoid organs, heart, and neural tissue (see (Ford et al., 2002; Linden et al., 2008; Miele et al., 2003) for examples), with lower levels found in kidney and liver (Miele et al., 2003; Tichopad et al., 2003). In the brain, maximal *Prnp* mRNA expression is observed in the adult

neocortex and cerebellum. At the cellular level, *Prnp* is expressed in various neuronal populations of the hippocampus, thalamus and neocortex (Bailly et al., 2004; Harris et al., 1993) and in glial cells (Ford et al., 2002; Laine et al., 2001; Moser et al., 1995; Radovanovic et al., 2005). In neurons, PrP<sup>c</sup> is predominant in axons and dendrites, excluded from synapse vesicles, and detected in endosomal compartments (Mironov et al., 2003). PrP<sup>c</sup> has also been identified in elongating axons (Sales et al., 2002). However, its presence and its putative role at the synapse are controversial (Fournier et al., 1995; Laine et al., 2001; Vassallo and Herms, 2003). For example, in CJD patients, an abnormal form of PrP<sup>c</sup> accumulates at synaptic terminals (Kitamoto et al., 1992), which suggest that loss of natural function of PrP<sup>c</sup> or PrP<sup>c</sup>-mediated processes at the synaptic terminals is responsible for neuronal dysfunction and degeneration in this disease (Kitamoto et al., 1992). How PrP<sup>c</sup> participates in synaptic transmission is unknown. However, several explanations have been proposed. PrP<sup>c</sup> may help neurotransmitter release, as reported at the neuromuscular junction (Re et al., 2006). In this regard, the vesicle-associated protein Synapsin Ib has been reported to interact with PrP<sup>c</sup>; which may supports this hypothesis (Spielhaupter and Schatzl, 2001). In addition, PrP<sup>c</sup>, by Cu<sup>2+</sup> binding, may participate in the control of calcium flux and redox stage of the presynaptic terminal (Vassallo and Herms, 2003). Lastly, pioneering electrophysiological studies (using PrP<sup>c</sup> knockout slices) by Collinge established that PrP<sup>c</sup> participates in long-term potentiation (Collinge et al., 1994; Curtis et al., 2003; Maglio et al., 2006). More recently, it has been shown that the absence of PrP<sup>c</sup> alters the expression of several neurotransmitter receptors (see (Maglio et al., 2004; Rangel et al., 2007) for examples). Thus, *Prnp*<sup>0/0</sup> mice show increased neuronal excitability as well as enhanced glutamate excitotoxicity by modulation of NMDA as well as kainate receptors (Khosravani et al., 2008b). PrP<sup>c</sup> therefore has distinct functions during neurotransmitter release and receptor synthesis in neural transmission. Furthermore, among these functions in neurotransmission, an additional role in synapse formation and maintenance cannot be ruled out, since PrP<sup>c</sup> interacts with structural scaffold

proteins at the synapse (e.g., PSD95) (Yehiely et al., 1997) and recent studies point to parallel scaffolding functions in other cell types during cell-cell interactions (Morel et al., 2008). These data indicate that the structural effects of neural PrP<sup>c</sup> during synapse formation and stabilization may occur in intrinsic or extrinsic ways. Intrinsic *Prnp* expression is developmentally regulated (Miele et al., 2003) and takes place postnatally in neural tissue (Steele et al., 2006), especially during the fate restriction of multipotential cells towards the neural lineage (Mouillet-Richard et al., 1999), stages characterized by neuronal polarization and synapse formation. Conversely, extracellular PrP<sup>c</sup> may act via specific receptors in neighboring neurons to promote synapse formation and neuronal maturation (Graner et al., 2000; Kanaani et al., 2005). In this regard, PrP<sup>c</sup> is considered a dynamic cell-surface platform for the assembly of signaling molecules which may participate in neuronal processes, including neuronal differentiation (see (Linden et al., 2008) for additional review).

### **3. The arise for physiological functions of PrP<sup>c</sup>**

The repertoire of physiological functions of PrP<sup>c</sup> is beginning to be elucidated. Indeed, several studies report numerous physiological processes associated with PrP<sup>c</sup> or PrP<sup>c</sup> - mediated events. Some of these are summarized below.

#### **3.1. PrP<sup>c</sup> and Copper homeostasis: more than simple binding or a “kiss and run” phenomenon.**

By using synthetic peptides, several authors have reported that the OR region of PrP<sup>c</sup> binds Cu<sup>2+</sup>, and to a lesser extent Cu<sup>+</sup>, thereby modulating its intracellular levels (e.g., (Hornshaw et al., 1995a; Hornshaw et al., 1995b)). Indeed, synaptosomal fractions have revealed that the amount of copper in the brain of PrP<sup>c</sup> knockout mice is lower than in wild-type (WT) counterparts (Herms et al., 1999). However, other studies have

not reproduced these results, since subcellular fractions of WT and PrP<sup>c</sup> knockout mice show equal Cu<sup>2+</sup> level and Cu/Zn SOD and CitC oxidase activity (Waggoner et al., 2000). Far from these discrepancies, *in vitro* cerebellar neuron cultures revealed that PrP<sup>c</sup> modulates oxidative stress through regulating Superoxide Dismutase-1 (SOD-1) activity in a Copper-dependent manner (Brown et al., 1997b; Sorenson, 2001). Experiments using recombinant PrP<sup>c</sup> and PrP<sup>c</sup> immunoprecipitated from brain tissue has postulated SOD activity as an intrinsic property of the PrP<sup>c</sup> protein (Brown et al., 1999), although other authors were unable to find this activity *in vivo* (Hutter et al., 2003). Interestingly, at the synaptic cleft, Cu<sup>2+</sup> regulation by binding to PrP<sup>c</sup> has been associated with the regulation of redox levels to facilitate neural transmission (Brown et al., 1997a; Herms et al., 1999). However, PrP<sup>c</sup> functions appear to be more diverse than a single redox control by PrP<sup>c</sup>-Cu<sup>2+</sup> binding in the cell or in the synapse, since PrP<sup>c</sup>-Cu<sup>2+</sup> *in vitro* binding promotes clathrin-mediated endocytosis (Cheng et al., 2006; Haigh et al., 2005; Pauly and Harris, 1998). This observation suggests that PrP<sup>c</sup> or PrP<sup>c</sup>-Cu<sup>2+</sup> binding is involved in the turnover of several receptors at the plasma membrane. This process is probably mediated by the low-density lipoprotein receptor-related protein 1 (LRP1) (Taylor and Hooper, 2007) in a dynamin-dependent manner (Caetano et al., 2008). The effects of PrP<sup>c</sup>-Cu<sup>2+</sup> binding are diverse, and most require further detailed analysis. However, a common feature in these functions appears to be the maintenance of cellular homeostasis.

### **3.2. PrP<sup>c</sup> ligands and intracellular signaling**

Numerous studies have determined several PrP<sup>c</sup> ligands but the function of most of these interactions is still unclear. Moreover, the results of these studies are not always reproducible. Most of the interactions have been determined by co-immunoprecipitation, two-yeast assays or other biochemical techniques. Among the



myriad of interactions described, Hsp60 (Edenhofer et al., 1996), ST11 (Zanata et al., 2002), Bcl-2 (Kurschner and Morgan, 1995), and Grb2 (Spielhauer and Schatzl, 2001) have been proposed as PrP<sup>c</sup> ligands. However, only a small proportion of these related pathways are functional in a physiological context (see (Lee et al., 2003) or (Westergard et al., 2007) and (Linden et al., 2008) for reviews). Two *in vitro* studies demonstrate that PrP<sup>c</sup> binds to the laminin receptor 67K (Gauczynski et al., 2001) and the adhesion molecule N-CAM (Santuccione et al., 2005; Schmitt-Ulms et al., 2001), both transducing survival signals or promoting neurite outgrowth. In addition, PrP<sup>c</sup> mediates neuritogenesis in a laminin-mediated manner (Graner et al., 2000), thereby suggesting that the PrP<sup>c</sup>-laminin interaction is relevant for neuronal development and further plasticity-related mechanisms. Indeed, *Prnp* is overexpressed by cortical neurons during axon regeneration and sprouting (JADR et al., unpublished data). Conversely, some of the interactions described for PrP<sup>c</sup> are also associated with neurodegeneration in prion diseases. As an example, the interaction of this protein with the precursor of the laminin receptor (Rieger et al., 1997), or its interaction with glycosaminoglycans, such as chondroitin sulphate A/B, heparin and hyaluronic acid (Pan et al., 2002), have been postulated to facilitate the PrP<sup>c</sup> to PrP<sup>sc</sup> conversion *in vitro* (Wong et al., 2001). Whether these interactions play similar roles *in vivo* is unknown.

Furthermore, several *in vitro* studies have addressed PrP<sup>c</sup>-mediated signaling (see (Westergard et al., 2007) for review). In this sense, we would like to remark two sets of studies which opens the notion of PrP<sup>c</sup> as a signaling molecule. In the first, using PrP<sup>c</sup> aggregation strategies, Mouillet-Richard and coworkers reported that PrP<sup>c</sup>-mediated signaling by P59Fyn kinase modulates cell survival in 1C11 cell line (Mouillet-Richard et al., 2000; Santuccione et al., 2005). This data is crucial since it linked the survival properties of PrP<sup>c</sup> to a specific signaling pathway. In this regard, the yeast two-hybrid system pointed to Grb2 and other proteins as interactive partners in signal

transduction, although this interaction has not been fully demonstrated in functional terms (Spielhaupter and Schatzl, 2001). The second set of studies was conducted by V.R. Martin's group, who described the interaction of the Stress-inducible protein 1 (STI1) with PrP<sup>c</sup> in neuronal hippocampal cultures (Lopes et al., 2005). These results are also important since ST1 is a co-chaperone located at the cell surface that binds PrP<sup>c</sup>, thereby inducing several responses in distinct types of cell, such as neuroprotection or neuritogenesis. Most effects are mediated through cAMP-dependent protein kinase A (PKA) and stress-related kinase ERK1/2 signaling (Lopes et al., 2005). For the intracellular transduction of PrP<sup>c</sup>-STI1-mediated signaling, endocytosis of PrP<sup>c</sup> is required (Americo et al., 2007; Caetano et al., 2008). For this reason, a strong correlation between PrP<sup>c</sup> trafficking alterations and prion pathologies have been suggested in other studies (Borchelt et al., 1992; Kiachopoulos et al., 2004; Pimpinelli et al., 2005; Shyng et al., 1995). Lastly, a recent study has reported PrP<sup>c</sup>-independent functions of STI1 in cell proliferation (Arruda-Carvalho et al., 2007). However, this observation appears to be only specific to retinal cells, although the interaction of STI1/PrP<sup>c</sup> takes place in most neurons as well as malignant glial cells.

### **3.3 PrP<sup>c</sup>, cell cycle regulation and differentiation: jumping the G1 phase**

The presence of PrP<sup>c</sup> is crucial for the proliferative stage of several subsets of cells along the body axis. However, the expression of this protein in proliferating cells differs and may explain the particular behavior of these cells in relation to PrP<sup>c</sup> expression. For example, PrP<sup>c</sup> expression *in vitro* leads to lymphoid cell proliferation (Cashman et al., 1990; Li et al., 2001) or promotes human hematopoietic cell renewal (Zhang et al., 2006). In addition, PrP<sup>c</sup> is directly involved in tumor cell progression in several tissues. A specific isoform of PrP<sup>c</sup> is highly expressed during G1 phase in a human glioblastoma cell line (Kikuchi et al., 2002). Indeed, in gastric cancer, PrP<sup>c</sup> mRNA and protein levels are overexpressed in diverse tumor cell lines (Liang et al., 2007).

However, contradictory results using other neoplastic and viral-transformed cell lines show that PrP<sup>c</sup> expression in G1 correlates with growth arrest or terminal differentiation (Gougoumas et al., 2001; Kniazeva et al., 1997). These observations open the question as to whether additional mechanisms acting together with PrP<sup>c</sup> regulate cell proliferation and G1 transition. For example, a transcriptomic study by Satoh determined that PrP<sup>c</sup> knockout fibroblasts showed a decreased expression of cell cycle-involved proteins such as cyclin D1, Eps8, and CD44 compared to control cells (Satoh et al., 2000).

Neural tissue is an interesting case as PrP<sup>c</sup> is differentially regulated in proliferating cells. In mice, PrP<sup>c</sup> expression starts during development around embryonic day E8,5-9 in postmitotic neurons (Miele et al., 2003) and around day E13,5-16,5 in specific non-neuronal populations (Manson et al., 1992). In the adult, PrP<sup>c</sup> is not expressed by proliferating cells in the subventricular zone of the lateral ventricle (Steele et al., 2006). However, unpublished studies by our group indicate that adult oligodendrocyte precursors express PrP<sup>c</sup> (Fontana et al., unpublished data). As reported by Steele and coworkers, neural stem cell proliferation in the subventricular zone is positively regulated by increasing PrP<sup>c</sup> expression levels in Tg20-overexpressing mice. In contrast, proliferating cells in the hippocampus show lower proliferative rates in PrP<sup>c</sup> knockout mice, and no relevant effect of PrP<sup>c</sup> expression is observed in the proliferation of hippocampal progenitors, since enhanced expression of this protein does not correlate positively with increased proliferation with respect to WT mice (Steele et al., 2006). Surprisingly, previous *in vitro* studies showed that PrP<sup>c</sup>-deficient neural cell lines derived from embryonic hippocampus display higher proliferative rates than WT cells in culture (Kim et al., 2005). As indicated above, recent results from our group demonstrate PrP<sup>c</sup> expression in remnant oligodendrocyte progenitor cells located in the adult neural parenchyma that normally proliferate (Fontana et al., unpublished data). This particular population showed a higher proliferative rate in PrP<sup>c</sup>

knockout than in WT or PrP<sup>c</sup>-overexpressing mice. Taken together, these observations suggest that PrP<sup>c</sup> has a cell-specific function in cell proliferation. Most probably, PrP<sup>c</sup> acts with other factor/s to modulate cell proliferation and differentiation. In this scenario, several questions remain unanswered. Does increased expression of PrP<sup>c</sup> in overexpressing mice affect proliferating cells in the subventricular zone that do not express PrP<sup>c</sup> in these mice? And more relevantly, if increased PrP<sup>c</sup> expression in neuronal cultures potentiates neuronal differentiation, polarization and synapse formation, does it increase cell proliferation in the subventricular zone? (Kanaani et al., 2005). Answers to these questions have not been given, but we can hypothesize that PrP<sup>c</sup> acts as an “inducer” of the intrinsic proliferative properties of these cells in “neuronal niches”, probably by forming part of or acting on a “neurotrophic” complex, and that PrP<sup>c</sup> expression inhibits cell proliferation in other neural cell types that do not show this “neurotrophic” complex. In this regard, a recent study reports a dual role of PrP<sup>c</sup> in cell proliferation and cell polarization (e.g., in the formation of desmosomal junctions) in epithelial cells (Morel et al., 2008), thereby expanding the functions of this protein in cell proliferation and differentiation.

### **3.4.- Emerging roles of PrP<sup>c</sup> modulating A $\beta$ deposition and Alzheimer’s disease progression**

During several years, several studies showed by different ways a putative interaction between prionopathies and Alzheimer’s disease (AD) (e.g., (Checler and Vincent, 2002)). Thus coexistence of PrP<sup>sc</sup> and A $\beta$ (1-42) in the same amyloid plaque has been described in patients with Creutzfeldt-Jakob or Gerstmann-Straussler-Scheinker diseases (e.g., (Ferrer et al., 2001; Preusser et al., 2006; Tsuchiya et al., 2004)). Thus several studies aimed to determine a putative interaction between A $\beta$  deposition and PrP<sup>c</sup> functions. For example an study of Debatin and coworkers reported the co-

occurrence of pathological changes typical for sporadic CJD and Alzheimer's disease in combination with the inverse association between accumulation of A $\beta$  and PrP<sup>Sc</sup> in a subgroup of sporadic CJD patients as indicative of pathways involved in the generation or clearance of A $\beta$  and PrP<sup>Sc</sup> in a subgroup of sporadic CJD patients (Debatin et al., 2008). This study was in contrast results obtained in mouse models where the inoculation with PrP<sup>Sc</sup> increased A $\beta$  deposition in AD models (Baier et al., 2008) or studies reporting increased A $\beta$  accumulation in PrP<sup>C</sup> mutant models (Schwarze-Eicker et al., 2005). In a first study, Parkin and coworkers determined that PrP<sup>C</sup> modulated BACE1 activity being a negative regulator of A $\beta$  production (Parkin et al., 2007). Last month, a study of Landis et al., determined the interaction of PrP<sup>C</sup> with  $\beta$ -amyloid oligomers (Lauren et al., 2009). In this study, the authors claimed that PrP<sup>C</sup> is a mediator of amyloid- $\beta$ -oligomer-induced synaptic dysfunction and A $\beta$ -deposition, and PrP<sup>C</sup>-specific pharmaceuticals may have therapeutic potential for Alzheimer's disease (Lauren et al., 2009). This point might reinforce the data of Debatin et al., concerning the mechanism of clearance of A $\beta$  deposits. Thus, the conversion between PrP<sup>C</sup> and PrP<sup>Sc</sup>, may interfere with this clearance by a putative reduction of PrP<sup>C</sup> in brain parenchyma since this process is independent of PrP<sup>Sc</sup> as indicated by Lauren et al. (Lauren et al., 2009). Indeed, the study opens the notion that additional factors than PrP<sup>C</sup> might also affect AD progression and to whether PrP<sup>C</sup>-mediated function affects the phosphorylation of Tau an additional hallmark of the AD not analyzed in the study of Landis and coworkers. On the other hand, a function of PrP<sup>C</sup> inducing A $\beta$  oligomerization may explain the studies of Schwarze-Eicker et al., and Baier et al., (Fig. 2). In this respect, it is well known that PrP<sup>C</sup> bind amyloid fibrils (see (Li et al., 2007b) for comments).

#### **4. *Prnp* knockout mice: the arise for its functions in mice models**

To ascertain the putative roles of PrP<sup>c</sup>, diverse groups have generated several mouse models that lack PrP<sup>c</sup> expression and carry several copies of the *Prnp* gene. More recently, mice that express a number of modified PrP<sup>c</sup> proteins have also been produced. All these studies have opened new avenues of research as various phenotypes observed in some of these mice were unexpected. In addition, recent studies have revised previous descriptions for some of these rodents, especially those first produced (see below).

#### **4.1. *Prnp*-deficient mice**

The generation of two lines of mice by disrupting *Prnp* expression was carried out in the early 1990s: Zürich I (outbred) (Bueler et al., 1992) and Edinburgh I (inbred) (Manson et al., 1994) lines (see (Weissmann and Aguzzi, 1999) for review). *Prnp*-knockout mice are resistant to prion infection (Bueler et al., 1993) but surprisingly do not show major phenotypical defects. However, it was finally demonstrated that Edinburgh *Prnp*-knockout mice show alterations in circadian rhythms (Tobler et al., 1996) and cognitive deficits (Criado et al., 2005). In addition, both lines showed deficits in synaptic transmission (Collinge et al., 1994; Herms et al., 1995) and increased sensitivity to oxidative stress (Brown et al., 2002). Recent studies indicate that these mice also show increased susceptibility to glutamate excitotoxicity (Khosravani et al., 2008a; Rangel et al., 2007; Walz et al., 1999; Walz et al., 2002) as a result of changes in the expression of glutamate receptor subunits (Khosravani et al., 2008a; Lledo et al., 1996; Rangel et al., 2007). More recently, a conditional knockout of PrP<sup>c</sup> has been generated (Mallucci et al., 2002). These mice do not show the behavioral alterations of infected mice unless PrP aggregates are formed (Mallucci et al., 2003; Mallucci et al., 2007).

#### **4.2. Other PrP<sup>c</sup> mutant mice: the puzzle of functions increases.**

Following the Zürich I and Edinburgh I strains, other mouse models were generated. The Nagasaki I line was developed in 1996. These mice show selective death of Purkinje Cells (PCs) and subsequent progressive cerebellar-associated ataxia (Katamine et al., 1998; Sakaguchi et al., 1996). The Rcm0 line (Moore et al., 1999; Silverman et al., 2000) displays a large deletion in the *Prnp* gene and unexpectedly overexpresses a homolog PrP<sup>c</sup> protein, called Doppel (Dpl, from the German *double*). Rcm0 mice show severe PC death and ataxia. Finally, a Zürich II line, generated in 2001, was found to be very similar to Rcm0 regarding the *Prnp* deletion and the phenotypic ataxic syndrome (Rossi et al., 2001). These studies led to the discovery of Dpl and it has been proposed that the functions of this protein are opposed or complementary to those of PrP<sup>c</sup>. In fact, Dpl-mediated toxicity of PCs is reversed by the introduction of WT PrP<sup>c</sup> (Yamaguchi et al., 2004). However, the role of Dpl is not clear since it shows low expression in adult brain and the studies performed to date have focused on its roles during development and in other organs (Behrens and Aguzzi, 2002) and also its functions in prion pathogenesis (Behrens et al., 2001; Mo et al., 2001)

Dpl protein shares with PrP<sup>c</sup> the C-terminal portion of the molecule but lacks the OR and CD, although it conserves the GPI anchor (Moore et al., 1999). On the basis of these observations, several studies were directed to analyzing the phenotype of mice expressing truncated forms of PrP<sup>c</sup> (lacking some portions of the N-terminal domains). As expected, in comparison with Dpl mice, in N-terminal truncated mice, the neocortex as well as other brain regions were preserved, but the cerebellum was strongly affected and cerebellar neurons showed degeneration (Li et al., 2007b; Shmerling et al., 1998). Whether these effects in Dpl mice are specifically associated with the cerebellum is unknown, but differential expression of PrP<sup>c</sup> or Dpl together with the presence of putative ligands/receptors might be involved. In this regard, histoblots have revealed

that either Fc-labelled Dpl or PrP<sup>c</sup> bind specifically to cerebellar granule neurons (CGNs) (Legname et al., 2002), which may explain why the cerebellum suffers degeneration in Dpl-mediated degeneration while other brain structures remain healthy.

*Prnp* knockout mice overexpressing the C-terminal portion of PrP<sup>c</sup> (lacking OR and CD) of the N-terminal domain ( $\Delta$ F35 mice) display CGN death, white matter pathology and a progressive and early lethal ataxic phenotype termed Shmerling syndrome (Radovanovic et al., 2005; Shmerling et al., 1998). PCs present no signs of degeneration because of enhancer-mediated splicing that prevents  $\Delta$ F35 expression in these neurons (Shmerling et al., 1998). However, targeted-PC expression of the  $\Delta$ F35 construct by a specific promoter induces PC death and ataxia (Anderson et al., 2004; Flechsig et al., 2003). Similarly to Dpl-mediated degeneration, Shmerling syndrome is rescued by reintroducing the full-length *Prnp* (Shmerling et al., 1998). However, it has been proposed that myelin pathology induced by N-terminally truncated PrP<sup>c</sup> acts through independent mechanisms, in contrast to CGN depletion (Radovanovic et al., 2005). Recent studies have shown that overexpression of PrP<sup>c</sup> devoid of CD or a fragment of CD (residues 105-125) in PrP<sup>c</sup> knockout mice promotes an exacerbated ataxic syndrome, causing lethality at approximately postnatal day 20 (Baumann et al., 2007; Li et al., 2007b). More in-depth studies using these knockout mice would be useful to elucidate the physiological functions of the domains of PrP<sup>c</sup>, and also to improve our understanding of the putative signaling pathways involved in prion-related pathologies.

#### **4.3. PrP<sup>c</sup> expression and prion pathology**

The generation of mice devoid of PrP<sup>c</sup> implied a reinforcement of the "Protein only hypothesis", proposed by Stanley Prusiner in 1982 (Prusiner, 1982), since knockout



mice showed prion infection resistance and could not replicate prion infection (Brandner et al., 1996; Bueler et al., 1993). In 2003, it was reported that PrP<sup>c</sup> depletion in mice stops scrapie-mediated neuronal degeneration (Mallucci et al., 2003), and also recovers behavioral and cognitive symptoms (Mallucci et al., 2007). In addition, PrP<sup>sc</sup> does not infect neurospheres derived from PrP<sup>c</sup> knockout mice *in vitro* (Giri et al., 2006), and *Prnp* null mice are re-sensitized to prion infection even when several amino-truncated PrP<sup>c</sup> constructs are expressed (Aguzzi and Heikenwalder, 2006; Aguzzi and Polymenidou, 2004; Fischer et al., 1996; Flechsig et al., 2000). Regarding the presence of PrP<sup>c</sup> for prion transmission, GPI-anchoring has been proposed as a key factor in scrapie infection; in fact, mice lacking GPI fail to reproduce clinical scrapie, although amyloid deposition is present (Chesebro et al., 2005). These studies demonstrate that the presence of PrP<sup>c</sup> is indispensable for prion pathologies and that the interaction of this protein with the plasma membrane contributes to neurodegeneration. Despite of this helpful data, more simple models altering certain PrP<sup>c</sup> properties (like the expression levels) could improve our understanding of PrP<sup>sc</sup> toxicity and transmissibility.

In this sense, it has been reported that modulation or restriction of PrP<sup>c</sup> expression in some prion models has aroused as an important tool focused on the understanding of prion infection/invasion mechanisms. For example, generation of *Prnp* knockout mice with restricted PrP<sup>c</sup> expression to B or T cells have been useful to elucidate important questions in prion neuroinvasion (Brandner et al., 1999; Raeber et al., 2001). In addition, these models could be complemented using neuroendocrine cell lines which have been proposed as models for the studying of physiological mechanisms of PrP<sup>sc</sup> counteracting (Aguib et al., 2008). In consequence, the generation of neuronal lines has supposed new models for the *in vitro* study of the PrP<sup>c</sup>-PrP<sup>sc</sup> interaction. For example, a neural line derived from Zürich I mice which do not express Dpl have shown neuronal features (Kim et al., 2005). Thus, it would be feasible to induce

differential levels of PrP<sup>c</sup> expression or to alter other important factors (like PrP<sup>c</sup> cytoplasm/membrane localization, trafficking or glycosilation) in order to study how PrP<sup>sc</sup> invasion/toxicity is modulated. In this regard, we would point out certain studies that revealed interesting data about PrP<sup>c</sup> expression and properties; in fact, further studies based on these findings could be helpful to understand the progression of prion diseases. For example, *in vitro* experiments showed that the inhibition of PrP<sup>c</sup> glycosilation promoted the formation of PrP<sup>sc</sup>-like molecules (Ma and Lindquist, 1999), and other PrP<sup>sc</sup>-like molecules accumulates in aggresomes (Cohen and Taraboulos, 2003). In addition, the study of the cytosolic form of PrP<sup>c</sup> have provided new insights regarding which signaling pathways could be presumably involved in prion disorders; for example, in aged transgenic mice overexpressing PrP<sup>c</sup>, mitochondrial localization of PrP<sup>c</sup> promotes neuronal apoptosis (Hachiya et al., 2005) and cytosolic PrP<sup>c</sup> failed to rescue Bax-induced cell death in yeast (Li and Harris, 2005), supporting the toxic properties of cytosolic PrP<sup>c</sup> in neurons (Rambold et al., 2006). However, it is necessary to take into account which of these related pathways are directly implicated in the “real” human or animal prion disease (Chiesa et al., 2005). In the other hand, prion pathology triggers primarily dysfunction and activation of glial cells rather than neuronal death, in contrast with other degenerative disorders (Prusiner, 1991; Prusiner, 1998a). In consequence, several studies have focused in the study of the role of glial cells in prion diseases. As mentioned, although neuronal apoptosis is important in prion-associated neural degeneration (Gray et al., 1999), microglial/astroglial recruitment is crucial for the progression of the disease (Baker et al., 2002; Depino et al., 2003; Giese et al., 1998; Giese and Kretzschmar, 2001; Marella and Chabry, 2004; Schultz et al., 2004). In consequence, transgenic models such as PrP<sup>c</sup> knockout mice that ectopically overexpress Dpl have elucidated important issues in the mechanisms of glial activation. This study strongly suggest that ectopic Dpl expression in the absence of PrP<sup>c</sup> is actively involved in the glial-cell activation in the brain the glial cell activation was notable well before the onset of the Purkinje cell degeneration in these mice. (Atarashi

et al., 2001). However, more refined *in vitro* studies modeling neuronal degeneration and glial proliferation would be useful to strongly link morphological and histological features observed in *postmortem* brains with specific signaling pathways related in neuronal degeneration and survival.

## **5. Dissecting PrP<sup>c</sup> domains and cell death: the N-terminal domain**

The N-terminal domain of PrP<sup>c</sup> is a flexible "tail" compared to the C-terminal portion (Donne et al., 1997; Riek et al., 1996), N-terminal fragments are present in brains affected by scrapie and correlate with the presence of protease-resistant PrP<sup>c</sup> forms (Pan et al., 2005). In addition, aberrant N-terminal truncated forms of PrP<sup>c</sup> tethered to the cell membrane alter the response to oxidative stress and become protease-resistant (Zeng et al., 2003). Moreover, processing of the putative N-terminal transmembrane fragment could be determinant in neurodegenerative diseases, since mice expressing a PrP variant (mutation that alters topological orientation) promote neuronal degeneration (Hegde et al., 1998; Stewart et al., 2001). The overexpression of an N-terminal truncated PrP<sup>c</sup> lacking the OR region in an immortalized PrP<sup>c</sup>-deficient cell line promotes cell death through the caspase-3 pathway. This effect correlates with SOD inhibition, thereby suggesting a relationship between oxidative stress and the cell death triggered by the PrP<sup>c</sup>-lacking the OR (Sakudo et al., 2003). Accordingly, the insertion of three octarepeats alters the conformation of PrP<sup>c</sup>, thereby promoting greater cell susceptibility to oxidative insults *in vitro* (Yin et al., 2006). Indeed, PrP<sup>c</sup> lacking the OR fails to undergo oxidative-mediated  $\beta$ -cleavage, thereby promoting an *in vitro* increase in cell susceptibility to oxidative radicals (Watt et al., 2005). In PrP<sup>c</sup> knockout mice, the OR domain contributes to pathologies induced by N-terminal truncation. In fact, OR-inserted PrP<sup>c</sup> does not completely overcome the  $\Delta F35$  ataxic syndrome (Li et al., 2007c). On the basis of these observations, these authors

concluded that OR domain integrity is crucial to the prevention of  $\Delta F35$ -evoked gain-of-function.

## **6. Dissecting PrP<sup>c</sup> domains and cell death: the Central Domain**

Like the OR, the Central Domain (CD) of PrP<sup>c</sup> plays a decisive role in cell homeostasis. This domain is formed by a positively-charged cluster (from 95 to 110 residues) and a hydrophobic transmembrane region (from 112 to 133) located in the N-terminal domain of the protein (Fig. 1). Two specific sequences are crucial to membrane topology. Residues 110 to 135 confer hydrophobic properties to PrP<sup>c</sup>, and mutations in this part alter endoplasmic reticulum regulation and promote cell degeneration (Hegde et al., 1998; Stewart et al., 2001) while the Stop Transfer Effector sequence (residues 103 to 111) directs PrP<sup>c</sup> chains to the transmembrane (Yost et al., 1990). Furthermore, the transmembrane part of PrP<sup>c</sup> holds specific residues that regulate chain orientation when the protein is anchored to the cell membrane (Ott et al., 2007). All the above observations point to the relevance of the CD in PrP<sup>c</sup> transmembrane signaling and in PrP<sup>c</sup> to PrP<sup>Sc</sup> conversion.

The central sequence of the CD (residues 105-125 in mice and 106-126 in humans), which is the most conserved part of PrP<sup>c</sup>, is essential for the biological functions of the protein. It has been extensively reported that peptides mimicking this part of PrP<sup>c</sup> promote neurotoxicity in neuronal cell cultures (Forloni et al., 1993) because of their self-aggregative properties. However, these studies are subject to debate since the requirement of PrP<sup>c</sup> expression for synthetic peptide toxicity is controversial (see for example (Brown, 2000; Fioriti et al., 2005; Gavin et al., 2005; Kunz et al., 1999; Singh et al., 2002)).

N-terminally truncated constructions, like  $\Delta F35$  or Dpl protein, lack residues 105-125 (Luhrs et al., 2003; Mo et al., 2001; Shmerling et al., 1998).  $\Delta F35$  and Dpl overexpression promotes neural cell death in several mouse models, thereby implicating the 105-125 region in survival signaling and indicating that its absence promotes the activation of several death-related pathways. Moreover, mice lacking the 105-125 (PrP $_{\Delta CR}$ ) region develop an exacerbated ataxic syndrome (Li et al., 2007b), similar to that observed in  $\Delta F35$ , PrP $_{\Delta CD}$  and Dpl models. These three "PrP $^c$ -like" proteins appear to trigger the same death pathways; the histological characteristics of the degenerating cerebellum in these mice are similar (regarding CGN/PC death, white matter vacuolization), as are their biochemical properties (membrane attachment and vesicular trafficking). A copy of *Prnp* rescues these neurodegenerative processes, thereby pointing to a common ligand-mediated toxicity mechanism rather than a misfolding protein (Baumann et al., 2007; Li et al., 2007b; Shmerling et al., 1998). However, to some extent, the toxic activity of PrP $^{Sc}$  and the protective activity of PrP $^c$  are interconnected by the HR region (Rambold et al., 2008). Using *in vitro* methods, these authors described that the HR plays a key role in the formation of PrP $^c$  dimers, which correlate with its neuroprotective role, and, more relevantly, that in scrapie-infected cells the number of PrP $^c$  dimers decrease in parallel with the loss of resistance to stress (Rambold et al., 2008). Although performed *in vitro*, this study is one of the first descriptions of a direct relationship between the protective functions of PrP $^c$  and scrapie-mediated neurotoxicity and it provides new insight into the functions of the HR domain.

## **7. Ligands for the CD and neural cell death**

N-terminal truncated PrP $^c$  and Dpl mice display a large degree of neurodegeneration. Biochemical studies have shown that Dpl and  $\Delta F35$  are glycosylated and membrane-

located like native PrP<sup>c</sup> (Behrens and Aguzzi, 2002), thereby suggesting a "non-prionic" mechanism of degeneration. Interestingly, the C-terminal fragment of PrP (residues 121-231) is converted to a  $\beta$ -enriched structure in a specific *in vitro* system (Hornemann and Glockshuber, 1998); however, no "scrapie-like" PrP<sup>c</sup> forms have been found in any *Prnp* knockout or  $\Delta F35$  mice. These observations suggest that the pathology of *Dpl*/ $\Delta F35$  models is caused by the absence of natural PrP<sup>c</sup> functions.

Studies using  $\Delta F35$  mice, Shmerling et al., as Moore et al. using *Dpl*-overexpressing mice, argued the similarities between the two "PrP<sup>c</sup>-like" proteins, and proposed that the overexpression of these forms might arrest a putative ligand ( $L_{PrP}$ ) required for cell survival (Moore et al., 2001; Shmerling et al., 1998). It was also proposed that a "PrP<sup>c</sup>-like" protein, termed " $\pi$ ", binds to this ligand in a "low-affinity manner", partially replacing physiological PrP<sup>c</sup> functions in *Prnp* knockout mice. This hypothesis would explain why mice devoid of PrP<sup>c</sup> do not show neural degeneration (Shmerling et al., 1998). *Dpl*/ $\Delta F35$  hypothetically antagonizes this effect by acting as a dominant inhibitor and preventing PrP<sup>c</sup>-mediated signaling. In addition, *Dpl*/ $\Delta F35$  trigger death pathways (Shmerling et al., 1998). This model has recently been completed with the generation of PrP<sup>c</sup> knockout mice overexpressing a PrP<sup>c</sup> isoform that lacks the CD (residues 94-134), the PrP <sub>$\Delta$ CD</sub> line. These mice present an exacerbated lethal ataxic syndrome, which is counteracted by the introduction of the complete PrP<sup>c</sup> or PrP<sup>c</sup> protein devoid of the OR region (Baumann et al., 2007). PrP <sub>$\Delta$ CD</sub> mice constitute a stabilized dominant negative, and the OR region stabilizes PrP<sup>c</sup>-PrP<sup>c</sup> receptor interaction, but does not contribute directly to signaling (Baumann et al., 2007) (see Fig. 3A). A similar model is the *Prnp* knockout mouse model that overexpresses a N-terminal truncated PrP lacking the most evolutionary conserved residues (105-125), which is known as the PrP <sub>$\Delta$ CR</sub> (Li et al., 2007b). Like other mutants, these mice develop severe ataxia and similar histological characteristics as  $\Delta F35$  and  $\Delta_{CD}$  mice, and this phenotype is rescued by

expressing supraphysiological levels of PrP<sup>c</sup>. All of these observations indicate that the 105-125 region of PrP<sup>c</sup> underlies relevant neuroprotective properties, and that its absence triggers neurotoxic pathways that promote CGN death, cerebellar atrophy and white-matter degeneration (Li et al., 2007b). The model proposed by Li et al. differs from the previous models in several ways. However, all the models share PrP<sup>c</sup> binding to its putative receptor by two binding sites; one in the N-terminal and a second located in the C-terminal domain. Thus, in this model, PrP<sub>ΔCR</sub> has a higher affinity than WT PrP<sup>c</sup> or ΔF35/Dpl for the possible PrP<sup>c</sup> receptor (because of the amount of PrP<sup>c</sup> required to rescue the pathological phenotype). Furthermore, the toxicity of PrP<sub>ΔCR</sub> is dependent on its capacity to activate in a "pathological" form PrP<sup>c</sup> receptor when only C-terminal binding site is occupied (Li et al., 2007b) (see Fig. 3B). Despite this models have emerged as a reasonable explanation for the neuronal pathology induced by truncated forms of PrP<sup>c</sup>, today there is no evidence to strongly fix these hypotheses. Further experiments trying to report proteins that could perform "π" or L<sub>PrP</sub> functions would be interesting to follow investigations in this way. If not, other hypotheses (like ΔF35 could play a toxic role per se, independently of PrP<sup>c</sup> modulation) would arise as new models to focalize other kind of studies.

## **8.- Is Shadoo the "π" protein?**

A new gene named SPRN (shadow of prion protein) was described in 2003 (Premzl et al., 2003). SPRN comprises two exons, with the open reading frame (ORF) contained within exon 2, and codes for a protein of 130-150 amino acids named Shadoo (Sho, Japanese shadow) present in mammals as well as other vertebrates (Premzl et al., 2004). In a recent study, Watts and coworkers, described that Shadoo shared protective properties with PrP<sup>c</sup> (Watts et al., 2007), and stated that Sho functions counteract neurotoxic effects of either Doppel or ΔF35 (Watts et al., 2007). In this

study, Sho expression is decreased after PrP<sup>Sc</sup> formation, which may also decrease the neuroprotection and enhance the development of the disease in a loss of function hypothesis of the PrP<sup>C</sup> roles (Fig. 4). Whether, Shadoo is the “ $\pi$ ” protein warrant further study but especially studies in Sho-deficient mice are needed. More relevantly if we take into account that a null allele of SPRN has been associated with CJD (Beck et al., 2008).

## 9. Bcl-2/BAX and PrP<sup>C</sup>-truncated mouse models

Little is known about the intracellular cascades that lead to cell death in N-terminal truncated PrP<sup>C</sup> mouse models. However, a protein similar to  $\Delta$ F35 is generated in the healthy human brain when PrP<sup>C</sup> is endoproteolyzed into 110/111 residues through ADAMs in a Protein kinase C (PKC)-dependent way, thereby generating a C-terminal fragment termed C1 (Cisse et al., 2005; Chen et al., 1995; Harris et al., 1993; Vincent et al., 2000; Vincent et al., 2001). A shorter fragment, within the OR region (residues 90/91), the C2, is generated in pathological conditions and poor data have yet been provided about this cleavage (Chen et al., 1995). A recent study by Sunyach and co-workers established that stable C1 expression, but not C2, sensitizes HEK293 to apoptotic stimuli like staurosporine and that this effect is fully dependent on p53 activity (Sunyach et al., 2007). Similarly,  $\Delta$ F35 mice show increased p53 phosphorylation during the pre-degenerate stage (76 days), as well increased activity of stress-related kinases such as ERK1/2 or p38 (Nicolas et al., 2007). Accordingly, p38 and Mdm-2 activate p53 and promote death in neuronal cultures in hypoxic conditions (Zhu et al., 2002). Furthermore, lentiviral p53-mediated caspase-3 activation is delayed in BAX-deficient CGNs (Cregan et al., 1999). All these data implicate p53 and other stress-related proteins in cell death mediated by truncated forms of PrP<sup>C</sup>.



Bcl-2 overexpression and BAX suppression have been extensively used to prevent cell death in several pathologic situations; for example, Bcl-2 overexpression *in vivo* largely delays clinical onset in a Familial Amyotrophic Lateral Sclerosis model (Kostic et al., 1997), and BAX suppression prevents neuronal depletion in a prion disease model (Chiesa et al., 2005). Consequently, four studies have recently reported that cell death in N-terminal truncated overexpressing mice and in the Nagasaki line is regulated in a Bcl-2/BAX manner (Heitz et al., 2008; Heitz et al., 2007; Li et al., 2007a; Nicolas et al., 2007) (see Table 1). Surprisingly, contradictory results have been obtained in the latter strain, regarding the contribution of BAX depletion to ataxic syndrome rescue (Dong et al., 2007; Heitz et al., 2007). Taken together, these findings indicate that Bcl-2/BAX regulation is not the only switch in neuronal death mediated by N-terminal truncation and that other pathways are involved in CGN and PC degeneration. In this regard, Miller and co-workers demonstrated in CGN apoptosis (*in vitro* conditions), although Bcl-2/BAX regulation is present, additional caspase-independent signaling pathways are required to promote cell death (Miller et al., 1997). Consequently,  $\Delta_{CR}$  mice are not recovered by BAX suppression, thereby suggesting that the predominant cell death pathway in this model is not mitochondrial-dependent (Li et al., 2007b). Thus, it would be of interest to evaluate the implication of BAX-independent regulation factors in neural death (Cheung et al., 2005) in these apoptotic (or necrotic) processes.

## **10. Conclusions: the function of PrP<sup>C</sup> remains a mystery in the 21<sup>th</sup> century.**

The functions attributed to PrP<sup>C</sup> have increased steadily in recent years. However, a new finding opens up an unexpected avenue and increases the complexity of this protein. New experimental approaches have revealed novel functions of PrP<sup>C</sup> in processes such as cell proliferation and differentiation, cell homeostasis, and cell death

and survival. In addition, the discovery of Doppel, and more recently Shadoo, opens up new fields of research, since the neural functions of PrP<sup>c</sup> in the healthy or damaged brain remain elusive.

Although sometimes controversial, emerging data converge to support the generally accepted notion that PrP<sup>c</sup> plays a crucial role in regulating the basal homeostatic equilibrium of neurons. Indeed, the imbalance of PrP<sup>c</sup> expression (absence or overexpression) or changes in PrP<sup>c</sup>-mediated interactions with other partners may predispose neurons to programmed cell death. This point is relevant since the participation of PrP<sup>c</sup> in other neurodegenerative diseases, such as AD, has recently been described. Thus, in the light of new findings in deficient mice (e.g., higher susceptibility to several types of stress effectors), the modification of PrP<sup>c</sup> expression levels as a putative therapeutic intervention should be considered with care. Concerning cell homeostasis, from the first studies describing the contribution of the OR region to Cu<sup>2+</sup> binding, new domains of the protein have been reported to be involved in the maintenance of low cell stress. Here we have reviewed recent studies demonstrating that various mouse models of PrP<sup>c</sup> acquire neurotoxic potential in the absence of PrP<sup>sc</sup>. However, the most devastating effects are observed in mutant mice lacking the CR (including some residues of the HR) of the PrP<sup>c</sup> sequence. In addition, the HR region is involved in endoplasmic reticulum regulation and its absence promotes cell degeneration. The question remains as to why this region induces these effects. Recent results indicate that the HR region promotes the dimerization of PrP<sup>c</sup>, thereby increasing the stress-protective activity of the protein. The data available indicate that the unfolded pathogenic form of PrP<sup>c</sup> affects cell survival by blocking the formation of these dimers, thereby decreasing the natural neuroprotective functions of PrP<sup>c</sup>, and by direct neurotoxic effects. In parallel, these effects may also be exacerbated by the conversion of the intrinsic PrP<sup>c</sup> to PrP<sup>sc</sup>. These results should be corroborated *in vivo* especially in scrapie-inoculated mice lacking the HR domain in

asymptomatic stages, but more relevantly, in conditional expression mouse models lacking distinct regions of the CD. However, we cannot rule out that other unknown factors or ligands could also play a parallel role in modulating the neurodegenerative phenotype in these mice since mice lacking particular sequences of the CD of PrP<sup>c</sup> show distinct viability (ranging from weeks to months). In addition, both apoptotic and non-apoptotic processes are present in these mice.

In the search for cues, could Sho be considered the “ $\pi$ ” protein? Studies in Sho-deficient mice are required to answer this question. Also, does PrP<sup>c</sup> or Sho modulate neurotrophic signaling by interacting with neurotrophic receptors or by modulating their intracellular signaling cascades? These are additional unsolved questions. These new studies demonstrate that the biological tools currently available, such as single mutant mice or synthetic peptides, are not powerful enough to fully ascertain PrP<sup>c</sup>, Dpl or Sho functions. Prion research is evolving daily and researchers need to develop new tools, such as conditional expression models of the truncated form of PrP<sup>c</sup>, together with extensive biochemical and morphological analysis to address these challenging questions. Within a few years it may be possible for us to determine the cues responsible for the current “*ying and yang*” (positive and negative) functions of PrP<sup>c</sup>. This will be an important step forward; however, we are still unable to determine the molecular cues responsible for the loss of basic brain functions in CJD patients and in prion-infected mice. Future studies are likely to reveal that the “*ying and yang*” phenomena of PrP<sup>c</sup> are also interconnected in CJD patients.

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## Figure Legends

### Figure 1

Scheme of PrP<sup>c</sup>, Dpl and several N-terminal truncated forms of PrP<sup>c</sup> ( $\Delta$ F35, PrP <sub>$\Delta$ CD</sub> and PrP <sub>$\Delta$ CR</sub>). Overexpression of Dpl and truncated PrP<sup>c</sup> forms in absence of intrinsic PrP<sup>c</sup> promote neuronal degeneration and ataxia (see Section 3).

### Figure 2

Scheme illustrating the recently reported data about, PrP<sup>c</sup> and A $\beta$  oligomer removal. 1) Normal trafficking of the PrP<sup>c</sup> from lipid raft into the cell is mediated by Cu<sup>2+</sup> and a co-receptor (Taylor and Hooper, 2007). Interaction with A $\beta$  oligomers (2) may modify the interaction of PrP<sup>c</sup> with its co-receptor leading to a putative endocytic process (3) (Lauren et al., 2009). However, whether this process takes place and A $\beta$  oligomers are degraded or is able to modify signal transduction as indicated warrant further study. In addition, a link between these interactions and tau phosphorylation is missing. Lastly, a putative interaction of PrP<sup>c</sup> with A $\beta$  oligomers to enhance fibril formation and A $\beta$  deposition could take place (4).

### Figure 3

Scheme illustrating a putative scenario for PrP<sup>c</sup> and Sho in neuroprotection. In normal mice both proteins transduce neuroprotective signals (1). In *prnp* <sup>-/-</sup> mice, although PrP<sup>c</sup> is missing Sho is able to induce a decreased neuroprotective signal, and some modified phenotype is present in these mice (2). In PrP<sup>Sc</sup> infected mice levels of Sho are reduced (Watts et al., 2007). Therefore the neurodegenerative process observed in infected animals may appear in the sum of factors among which we find the loss of neuroprotective function of PrP<sup>c</sup> and Sho joined with the neurodegenerative effect induced by the PrP<sup>Sc</sup> (3).

## Figure 4

Proposed models of PrP<sup>c</sup>/receptor interaction. A) Scheme proposed by Baumann and coworkers which suggests that PrP<sub>ΔCD</sub> interaction with a putative PrP<sup>c</sup> receptor could trigger neuronal degeneration. This interaction could play as a stabilized dominant negative (adapted from Baumann et al., 2007). B) Model suggested by Li and coworkers for PrP<sub>ΔCR</sub> form. PrP<sub>ΔCR</sub> activates a putative PrP<sup>c</sup> receptor in a “pathological” manner, transducing an exacerbated death signal. PrP<sub>ΔCR</sub>, as proposed, has more interaction affinity for PrP<sup>c</sup> receptor than intrinsic PrP<sup>c</sup> (adapted from Li et al., 2007).