Function of Prion Protein and the Family Member, Shadoo

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https://doi.org/10.21775/cimb.036.067

Abstract

Lowering cellular prion protein (PrP^C) levels in the brain is predicted to be a powerful therapeutic strategy for the prion disease. PrP^C may act as an antiapoptotic agent by blocking some of the internal environmental factors that initiate apoptosis. Prion protein (PrP)-knockout methods provide powerful indications on the neuroprotective function of PrP^C. Using PrP^C-knockout cell lines, the inhibition of apoptosis through stress inducible protein1 (STI1) is mediated by PrP^C-dependent superoxide dismutase (SOD) activation. Besides, PrP-knockout exhibited wide spread alterations of oscillatory activity in the olfactory bulb as well as altered paired-pulse plasticity at the dendrodendric synapse. Both the behavioural and electro-physiological phenotypes could be rescued by neuronal PrP^C expression.

Neuprotein Shadoo (Sho), similarly to PrP^C, can prevent neuronal cell death induced by the expression of PrPΔHD mutants, an artificial PrP mutant devoid of internal hydrophobic domain. Sho can efficiently protect cells against excitotoxin-induced cell death by glutamates. Sho and PrP seem to be dependent on similar domains, in particular N-terminal (N), and their internal hydrophobic domain. ShoΔN and ShoΔHD displayed a reduced stress-protective activity but are complex glycosylated and attached to the outer leaflet of the plasma membrane via glycosylphosphatidylinositol (GPI) anchor indicating that impaired activity is not due to incorrect cellular trafficking. In Sho, overexpressed mice showed large amyloid plaques not seen in wild-type mice. However, Shadoo is not a major modulator of abnormal prion protein (PrP^{Sc}) accumulation. Sho and PrP share a stress-protective activity. The ability to adopt a toxic conformation of PrP^{Sc} seems to be specific for PrP.

PrP^C protects neurons from stress-induced apoptosis

Neurogenesis

Recently, several reports showed that cellular prion protein (PrP^C) participate in a transmembrane signalling process that is associated with haematopoietic stem cell replication and neuronal differentiation (Mouillet-Richard *et al.*, 2000; Steele *et al.*, 2006; Zhang *et al.*, 2006). Abundant expression of P,,,rP^C has been detected during mouse embryogenesis in association with the developing nervous system (Manson *et al.*, 1992; Miele *et al.*, 2003; Tremblay *et al.*, 2007). In the developing mouse brain, undifferentiated neural progenitor cells in the mitotically active ventricular zone do not express PrP^C. In contrast, post-mitotic neurons express high levels of PrP^C after their last mitosis in the neuroepithelium as they migrate towards marginal layers and differentiate (Steele *et al.*, 2006; Tremblay *et al.*, 2007). Thus, PrP^C may be expressed exclusively in differentiated neurons (Tanji *et al.*, 1995). Studies *in vitro* have shown that expression of PrP^C is positively correlated with differentiation of multipotent neuronal precursors into mature neurons (Steele *et al.*, 2006). In addition, treatment of embryonic hippocampal neurons with recombinant PrP^C enhance neurite outgrowth and survival (Kanaani *et al.*, 2005).

The distribution of PrP^C in the developing nervous system of cattle (Peralta *et al.*, 2011), as well as in mice (Tremblay *et al.*, 2007) and humans (Adle-Biassette *et al.*, 2006) suggests that PrP^C plays a functional role in neural development. While mice lacking in prion protein (PrP) display no overt neural phenotype (Beuler *et al.*, 1992), numerous subtle phenotypes have been reported (Steele *et al.*, 2007), including reduction in the number of neural precursor cells in developing mouse embryo (Steele *et al.*, 2006). Other studies have shown that PrP^C induced neuritogenesis in embryonic hippocampal neurons cultured *in vitro* (Kanaani *et al.*, 2005; Lopes *et al.*, 2005). PrP^C interacts with stress-inducible protein 1 (STI1) (Zanata *et al.*, 2002), which is a heat-shock protein (Lässle *et al.*, 1997). The interaction of PrP^C with STI1 not only activates cyclic adenosine monophosphate (cAMP)-dependent protein kinase A to transducer a survival signal but also induces phosphorylation/activation of the mitogen-activated protein kinase to promote neuritogenesis (Lopes *et al.*, 2005). The expression of mammalian PrP^C in the neuroepithelium and its spatial and temporal relation with neural marker nestin and MAP-2 also suggests the participation of PrP^C in the process of neural differentiation during early embryogenesis (Peralta *et al.*, 2011). The use of embryonic stem (ES) cells to study the potential role or PrP^C will indicate how PrP^C is up-regulated during the differentiation of stem/progenitor cells.

Neuroprotection

The mammalian PrP^C is a highly conserved glycoprotein localized in membrane lipid rafts and anchored to cell surface by glycophosphatidylinositol (GPI) (McKinley *et al.*, 1991). It is present in many cell types and is particularly abundant in neurons (Taraboulos *et al.*, 1992). Under certain conditions PrP^C may undergo conversion into a conformationally–altered isoform (scrapie prion protein or PrP^{Sc}) widely believed to be the pathogenic agent in prion disease or transmissible spongiform encephalopathies (TSE) (Caughey *et al.*, 1991; Pan *et al.*, 1993). Although much is known about the effect of PrP^{Sc} in prion diseases, the normal function of PrP^C is poorly understood. PrP^C has an alpha and beta-cleavage site during normal processing and hosts translational modifications (Mange *et al.*, 2004). The most commonly observed function of PrP^C is copper-binding. The octapeptide-repeat region of

PrP^C binds with Cu^{2+} within the physiological concentration range (Hornshaw *et al.*, 1995; Kramer *et al.*, 2001; Miura *et al.*, 1999; Prusiner, 1997; Zeng *et al.*, 2003). Furthermore, PrP^C displays a functional role in normal brain metabolism of copper (Brown *et al.*, 1997). Besides binding with Cu^{2+} at the synapse, PrP^C serves as a Cu^{2+} buffer as well (Kretzschmar *et al.*, 2000). Overexpression of PrP^C increases Cu^{2+} uptake into cells (Brown, 1999), while PrP^C-knockout mice show a lower synaptosomal Cu^{2+} concentration than normal mice (Kretzschmar *et al.*, 2000). On the other hand, the Cu^{2+} rapidly and reversibly stimulates the internalization of PrP^C during PrP^C endocytosis (Haigh *et al.*, 2005; Kubosaki *et al.*, 2003; Pauly *et al.*, 1998). Through the binding with Cu^{2+}, PrP^C displays superoxide dismutase (SOD) activity *in vitro* (Brown *et al.*, 1999; Vassallo *et al.*, 2003). Interestingly, treatment with copper chelator cuprizone induces TSE-like spongiform degeneration (Pattison *et al.*, 1973). Therefore, Cu^{2+} metabolism appears to play an important role in not only PrP function but also the pathogenesis of prion diseases.

PrP^C may act as an antiapoptotic agent by blocking some of the factors that initiate apoptosis (Bounhar *et al.*, 2001; Roucou *et al.*, 2005). Mature PrP^C tend to localize in lipid raft of cells (Taraboulos *et al.*, 1992). As lipid rafts are membrane structures that specialize in signalling, a potential role of PrP^C in signal transduction may be anticipated. Discovery of several PrP^C-interacting candidates has facilitated the understanding of the PrP^C function (Table 2.1). PrP^C-interacting molecules are most likely involved in signal transduction. In addition, a phosphorylating function of PrP^C, mediated by caveolin-1 to indirectly increase Fyn (a member of Src family of tyrosine kinase) phosphorylation, governs the downstream production of nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase-dependent reactive oxygen species and activation of the extracellular regulated kinase 1/2 has been demonstrated (Mouillet-Richard *et al.*, 2000; Schneider *et al.*, 2003). PrP^C interacts with normal phosphoprotein synapsin Ib and cytoplasmic adaptor protein Grb2 without being deciphered with prion interactor Pint1 (Spielhaupter and Schätzl, 2001). Bovine PrP strongly interacts with the catalytic α/α¢ subunit of protein kinase CK2 to increase the phosphotransferase activity of CK2, thus leading to the phosphorylation of calmodulin (Maggio *et al.*, 2000).

Recently, PrP^C has been demonstrated to modulate serotonergic receptor-signalling in the inducible serotonergic 1C115-HT cell line, viz. modulation of 5-hydroxytryptamine (5-HT) receptor coupling to activate G-protein functions, as well as acting as a protagonist to promote homeostasis of serotonergic neurons (Moulliet-Richard *et al.*, 2005). In addition, PrP^C binds with extracellular matrix laminin to promote genesis and maintenance of neurites (Graner *et al.*, 2000a,b). Indeed, a recent study has discovered PrP^C to induce self-renewal of long term populating haematopoietic stem cells (Zhang *et al.*, 2006). Furthermore, another study has revealed that PrP is expressed on the multipotent neural precursors and mature neurons without being detected in glia, suggesting that PrP^C plays an important role in neural differentiation (Steele *et al.*, 2006). Therefore, the interaction between PrP^C and various signal transduction molecules speaks well for its importance (such as differentiation and cell survival) within the living system.

PrP-knockout methods provide useful hints on the neuroprotective function of PrP^C (Sakudo *et al.*, 2006). A PrP gene (*Prnp*)-deficient cell line (HpL3-4), perpetuated from hippocampal neuronal precursors, is sensitive to serum deprivation-induced apoptosis but is activated/survived with PrP^C expression (Kuwahara *et al.*, 1999). Overexpression of Bcl-2 in this cell-line reveals a functional relation of PrP^C with Bcl-2 in the anti-apoptotic pathway

Table 2.1 Proteins interacting with PrP

Proteins	Methods	References
Stress-inducible protein 1	Complementary hydropathy	Martins *et al.* (1997)
Tubulin	Cross-linking by bis(sulfosuccinimidyl)-suberate	Nieznanski *et al.* (2005)
Neural adhesion molecule (N-CAM)	Cross-linking by formaldehyde	Schmitt-Ulms *et al*. (2001)
Dystroglycan	Detergent-dependent immunoprecipitation	Keshet *et al.* (2000)
Neuronal isoform of nitric oxide synthase (nNOS)	Detergent-dependent immunoprecipitation	Keshet *et al.* (2000)
Grp94	Immunoprecipitation	Capellari *et al.* (1999)
Protein disulphide isomerase	Immunoprecipitation	Capellari *et al.* (1999)
Calnexin	Immunoprecipitation	Capellari *et al.* (1999)
Calreticulin	Immunoprecipitation	Capellari *et al.* (1999)
ZAP-70	Immunoprecipitation	Mattei *et al.* (2004)
NF-E2 related factor 2 (Nrf2)	Interaction with PrP23-231-alkaline phosphatase probe	Yehiely *et al.* (1997)
Amyloid precursor protein-like protein 1 (Aplp1)	Interaction with PrP23-231-alkaline phosphatase probe	Yehiely *et al.* (1997)
F-box protein-6	Interaction with PrP23-231-alkaline phosphatase probe	Yehiely *et al.* (1997)
Neural F-box protein 42 kDa (NFB42)	Interaction with PrP23-231-alkaline phosphatase probe	Yehiely *et al.* (1997)
Postsynaptic density 95 kDa (PSD-95)/SAP-90 associated protein	Interaction with PrP23-231-alkaline phosphatase probe	Yehiely *et al.* (1997)
Protein tyrosine phosphatase, non-receptor type-21	Interaction with PrP23-231-alkaline phosphatase probe	Yehiely *et al.* (1997)
Predicted protein KIAA0443	Interaction with PrP23-231-alkaline phosphatase probe	Yehiely *et al.* (1997)
Glial fibrillary acidic protein (GFAP)	Interaction with radioisotope-labelled PrP27-30	Oesch *et al.* (1990)
Hsp60 of *Brucella abortus*	Pull-down assay	Watarai *et al.* (2003)
Bcl-2	Yeast two-hybrid system	Kurschner and Morgan (1995)
Heat shock protein 60 kDa	Yeast two-hybrid system	Edenhofer *et al.* (1996)
37 kDa laminin receptor protein (LRP)	Yeast two-hybrid system	Rieger *et al.* (1997)
Pint1	Yeast two-hybrid system + immunoprecitation	Spielhaupter and Schätzl (2001)
Synapsin Ib	Yeast two-hybrid system + immunoprecitation	Spielhaupter and Schätzl (2001)
Neuronal phosphoprotein Grb2	Yeast two-hybrid system + immunoprecitation	Spielhaupter and Schätzl (2001)

Table 2.1 Continued

Proteins	Methods	References
Neurotrophin receptor interacting MAGE homolog	Yeast two-hybrid system + *in vitro* binding assay + immunoprecipitation	Bragason *et al.* (2005)
Potassium channel tetramerization domain containing 1 (KCTD1) protein	Yeast two-hybrid system	Huang *et al.* (2012)
Rab7a	Coimmunoprecipitation + immunofluorescence	Zafar *et al.* (2011)
Rab9	Coimmunoprecipitation + immunofluorescence	Zafar *et al.* (2011)
HS-1 associated protein X-1 (HAX-1)	Yeast two-hybrid system	Jing *et al.* (2011)
Histone H1	Far Western immunoblotting	Strom *et al.* (2011)
Histone H3	Far Western immunoblotting	Strom *et al.* (2011)
Lamin B1	Far Western immunoblotting	Strom *et al.* (2011)
14-3-3beta protein	Immunoprecipitation + pull-down assays	Liu *et al.* (2010)
Casein kinase II	Immunoprecipitation + pull-down assays	Chen *et al.* (2008)
Tetraspanin-7	Yeast two-hybrid system + immunoprecipitation	Guo *et al.* (2008)
2P domain K+ channel TREK-1 protein	Bacterial two-hybrid + immunoprecipitation	Azzalin *et al.* (2006)
ADAM23	Immunoprecipitation + pull-down assay	Costa *et al.* (2009)

(Kurschner *et al.*, 1995; Kuwahara *et al.*, 1999). Prevention of cell death in cultured retinal explants from neonatal rats and mice induced by anisomysin (a protein synthesis inhibitor) unfurls and the effect is associated with PrP^C–STI1 interactions (Zanata *et al.*, 2002). The production of another type of heat-shock protein (Hsp 70) is enhanced when PrP levels elevate during hyperglycaemia (Shyu *et al.*, 2005). According to findings in another study, the inhibition of apoptosis through STI1 is mediated by PrP^C-dependent SOD activation (Sakudo *et al.*, 2005). The functional role of STI1 and PrP^C has been confirmed in both murine and bovine systems (Hashimoto *et al.*, 2000). The late onset of severe ataxia and loss of cerebellar Purkinje cells in several knockout mouse lines (Moore *et al.*, 1999; Rossi *et al.*, 2001; Sakaguchi *et al.*, 1996) suggest a lack of protection of cerebellum by PrP^C in these mice. Interestingly, deposition of PrP^{Sc} has been located in the deep cerebellar nuclei (DCN) of scrapie-infected sheep (Ersdal *et al.*, 2003). Future studies with a microarray analysis (Park *et al.*, 2006) applied in eye-blink conditioning of mice may provide insight into understanding the normal function of PrP^C in the DCN of cerebellum.

A loss of PrP^C function could be implicated in the pathogenesis of prion diseases and PrP^C-dependent pathways might be involved in neurotoxic signalling. For example, *in vivo* crosslinking of PrP^C by antibodies triggered neuronal apoptosis (Solforosi *et al.*, 2004) and PrP^C-dependent receptors were postulated to explain the neurotoxic effect of a PrP mutant lacking the hydrophobic domain (see next sections) (Winklhofer *et al.*, 2008).

Taken together, PrP^C is functionally involved in copper metabolism, signal transduction, neuroprotection and cell maturation (Fig. 2.1). Despite these published roles, mice that

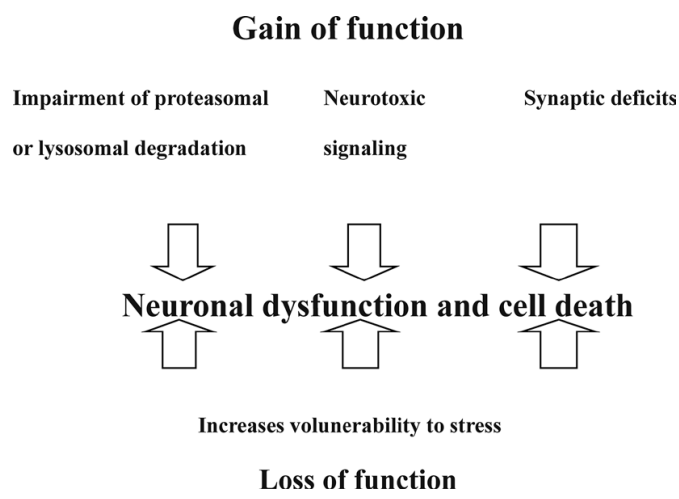

Figure 2.1 Gain and loss of function in prion disease.

are lacking PrP^C display no consistent phenotype apart from complete resistance to TSE infection (Büeler *et al.*, 1992, 1993). Further search for PrP^C-interaction molecules using *Prnp*$^{-/-}$ mice and various types of *Prnp*$^{-/-}$ cell lines under various conditions may elucidate the PrP^C functions.

Synaptic plasticity

In $PrP^{-/-}$ mice, Kim *et al.* (2007) have observed pathological alterations and some physiological dysfunctions in olfactory bulb (OB). Recently, Le Pichen *et al.* (2009) have uncovered a significant phenotype of $PrP^{-/-}$ mice in the olfactory system by utilizing a combination of genetic, behavioural and physiological and physiological techniques in a systems approach. They employed a so-called 'cookie finding task', a test of broad olfactory acuity, to analyse a battery of mice including PrP knockout on multiple genetic backgrounds and transgenic mice in which *Prnp* expression was driven by cell type-specific promoters. $PrP^{-/-}$ mice exhibited impaired behaviour that was rescued in transgenic mice expressing PrP^C specifically in neurons but not in mice expressing only extra-neuronal PrP^C. $PrP^{-/-}$ mice displayed altered behaviour in an additional olfactory test (habituation–dishabituation) which was also rescued by transgenic neuronal PrP expression suggesting that the phenotype was olfactory specific.

Besides, the odour-evoked electrophysiological properties of the OB of PrP knockouts were studied (Le Pichon *et al.*, 2009). In these mice, alterations in the patterns of oscillatory activity in the OB were detected. The plasticity of dendrodendritic synaptic transmission was altered between granule cells and mitral cell. Le Pichon *et al.* propose that electrophysiological alterations at the dendrodendritic synapse in the OB could underlie the behaviour phenotypes.

In detail, the cookie finding phenotype was manifest in three $PrP^{-/-}$ lines (Zurich I PrP knockout: Beuler *et al.*, 1992; Nagasaki PrP knockout: Sakaguchi *et al.*, 1996; Edinburgh PrP knockout: Manson *et al.*, 1994) on alternate genetic backgrounds, indicating strong evidence of its dependence on PrP^C rather than other genetic factors. PrP knockouts also displayed altered behaviour in the habituation–dishabituation task, suggesting the phenotype

was likely olfactory-specific. $PrP^{-/-}$ mice exhibited wide spread alterations of oscillatory activity in the OB as well as altered paired-pulse plasticity at the dendrodendritic synapse. Both the behavioural and electrophysiological phenotypes were rescued by neuronal PrP^{C} expression.

Disruption was observed in local field potential (LFP) oscillation and in the plasticity of the dendrodendritic synapse, either, or both, of which could contribute to the $PrP^{-/-}$ behavioural phenotype. Oscillatory LFPs may act to organize information flow within the olfactory system (Lledo *et al.*, 2006; Stopher *et al.*, 2007) by constraining the timing of mitral cell action potentials (Kasiwadani *et al.*, 1999). In addition, gamma oscillations are specifically implicated in behavioural performance in olfactory tasks (Beshel *et al.*, 2007; Brown *et al.*, 2005; Nusser *et al.*, 2001). Therefore, alterations in oscillatory timing during odour exposure may perturb OB output to higher centres by disrupting how information is packaged within a breathing cycle.

Altering the dendrodendritic synapse may have multiple functional consequence. This synapse may mediate lateral inhibition between ensembles of mitral cells, and be critical for olfactory discrimination (Urban, 2002; Yokoi *et al.*, 1995). Additionally, because granule cells receive convergent information onto their proximal dendritic arbour from multiple higher brain areas (Shepherd, 2003), disruption of the dendrodendritic synapse may alter the transmission of centrifugal modulation of OB mitral cells.

High frequency oscillations in the OB (gamma and high-gamma) are shown *in vitro* to result from the rapid and reciprocal interactions between granule and mitral cells across the dendrodendritic synapse (Lagier *et al.*, 2007; Schoppa *et al.*, 2006). Therefore, Le Picheon's observation could imply that increased facilitation of mitral cell inhibitory postsynaptic potential (IPSP) following repetitive spiking, decreases the dynamic range and increases the duration of gamma oscillations across the boundaries of breath. Although both oscillatory and synaptic effects could be reversed by neuronal PrP^{C} expression, they cannot claim a causal link between these findings.

Mitral cells receive facilitated inhibition in $PrP^{-/-}$ mice. This facilitation could result from either pre- and/or post-synaptic changes to the dendrodendritic synapse. Future work should determine the precise synaptic localization of the PrP^{C} protein as well as its biochemical interactions with synaptic machinery (Criado *et al.*, 2005).

Myelination and chronic demyelinating polyneuropathy

A late-onset peripheral neuropathy has been identified in PrP^{C}-deficient Nagasaki ($Prnp^{Ngsk/Ngsk}$) and Zurich-I ($Prnp^{-/-}$) mice (Sakaguchi *et al.*, 1996; Nishida *et al.*, 1999; Büeler *et al.*, 1992). This indicates that PrP^{C} might have a role in peripheral neuropathies. At 60 weeks of age, all $Prnp^{-/-}$ mice ($n = 52$) investigated showed chronic demyelinating polyneuropathy (CDP) (Bremer *et al.*, 2010). CDP was 100% penetrant and conspicuous in all investigated peripheral nerves (sciatic and trigeminal nerves, dorsal and ventral spinal roots). Besides, CDP was associated with another two independently targeted *Prnp* knockout mouse lines, $Prnp^{GFP/GFP}$ (Heikenwalder *et al.*, 2008) mice and $Prnp^{Edbg/Edbg}$ (Manson *et al.*, 1994) mice.

$Prnp^{-/-}$ and $Prnp^{Edbg/Edbg}$ mice suffered from CDP despite the normal expression of Doppel (Dpl) (Moore *et al.*, 1999), indicating that Dpl regulation did not cause polyneuropathy. CDP was present in mice lacking both *Prnp* and *Prnd* (the gene for Dpl) (Genoud *et al.*, 2004), but absent from mice selectively lacking *Prnd* (Behrens *et al.*, 2002). Therefore, Dpl is not required for the maintenance of peripheral nerves. PrP^{C} might interact with the

myelin component directly or through other axonal proteins. Some of the reported PrP^C interacting proteins have roles in homeostasis (Rutinshauser *et al.*, 2009), and represent possible candidates for mediation of its myelinotrophic effects. The octapeptide repeat region was not required for myelin maintenance, whereas mice PrP lacking central domain (aa 94–134) developed CDP (Baumann *et al.*, 2007). The hydrophobic core, but not the charge cluster (CC_2), of this central PrP^C domain was essential for peripheral myelin maintenance.

PrP^C undergoes regulated proteolysis in late secretory compartments (McMahon *et al.*, 2001; Sunyach *et al.*, 2007; Walmsley *et al.*, 2009; Watt *et al.*, 2005). Bremer *et al.* (2010) observed an association between the presence of CDP and lack of C1 fragment in sciatic nerves. All PrP mutants in which CDP was rescued produced abundant C1. Cleavage of PrP^C appeared, therefore, to be linked to its myelinotrophic function. This conjuncture might also explain the requirement for membrane anchoring of PrP^C uncovered in mice (Chesebro *et al.*, 2005), as anchorless PrP^C did not undergo regulated proteolysis.

Prion diseases mainly affect the central nervous system (CNS), myelin degeneration in optic nerves, corpus callosum or spinal cords was not detected in 60-week-old $Prnp^{-/-}$ mice (Bremer *et al.*, 2010). Nevertheless, subliminal myelin pathologies might extend to central myelin in $Prnp^{0/0}$ mice (Nazor *et al.*, 2007), and transgenic mice expressing toxic PrP^C show both peripheral and central myelinopathy (Baumann *et al.*, 2007; Radovanovic *et al.*, 2005). PrP^C deficiency affected synaptic function (Collinge *et al.*, 1994; Mallucci *et al.*, 2002). However, the amplitudes of foot muscle compound action potentials following distal stimulation were not significantly altered in 53-week-old $Prnp^{0/0}$ mice thus arguing against an important synaptic defect in neuromuscular synaptic junction.

PrP^C show various roles in immunity (Isaacs *et al.*, 2006), and lymphocytes are important in mouse models of hereditary demyelinating neuropathies. As the CDP in our mutant mice was not modulated by removal of *Rag1*, lymphocytes are not involved in its pathogenesis. The combined results of restricting expression of PrP^C of neurons and of selectively depleting PrP^C from neurons indicate that the expression of PrP^C by the neuron is essential for the long-term integrity of peripheral myelin sheaths (Bremer *et al.*, 2010). Not only was the trophic function of PrP^C exerted *in trans*, but also correlated with the proteolytic processing of in diverse transgenic mouse models. These findings identify PrP^C as a critical messenger of transcellular axomyelinic communication and indicate that regulated proteolysis of axonal PrP^C might exposed domains that interact with Schwann cell receptors. Clarifying the molecular basis of these phenomena might lead to a better understanding of peripheral neuropathies – particularly those of late onset – and might help to uncover new therapeutic targets.

Recent reports show that PrP^C-deficient mice of five different PrP^C-knockout strains, including the $Prnp^{ZH3/ZH3}$ mice (co-isogenic to BL/6 mice), develop a late-onset peripheral neuropathy, indicating that peripheral myelin maintenance is a bona fide physiological function of PrP^C (Bremer *et al.*, 2010; Nishida *et al.*, 1999; Wulf *et al.*, 2017). Nuvolone *et al.* (2016) used TALEN-mediated genomic editing in fertilized mouse oocytes to create $Prnp^{ZH3/ZH3}$ mice on a pure genetic C57BL/6J background. Genomic, translational and phenotypic characterization of $Prnp^{ZH3/ZH3}$ mice failed to identify phenotypes previously described in non-co-isogenic $Prnp^{-/-}$ mice. However, $Prnp^{ZH3/ZH3}$ mice developed a CDP, confirming the crucial involvement of PrP^C in peripheral myelin maintenance.

Neuronal PrP^C expression and amino-proximal cleavage are necessary for the promyelinating signal (Bremer *et al.*, 2010). It has been discovered that very N-terminal polycationic

cluster of PrP^C binds to the G-protein-coupled receptor Adgrg6 (Gpr126) of Schwann cells, eliciting a promyelinating cAMP response *in vitro* and *in vivo* in mice and zebrafish (Küffer *et al.*, 2016). This pointed to the N-terminal fragment of PrP^C as a promyelinating factor that might serve as a possible treatment in other peripheral chronic demyelinating polyneuropathies (Wulf *et al.*, 2017).

PrP^C mediates toxic signalling by PrP^{Sc}

Mice with prion disease show misfolded PrP accumulation and developed extensive neurodegeneration, in contrast to mouse models of Alzheimer's disease (AD) or Parkinson's disease (PD), in which neuronal loss is rare. Therefore, prion-infected mice allow access to mechanism linking protein misfolding to neuronal death. Mallicci's group have previously shown the rescue of neuronal loss and the reversal of early cognitive and morphological changes in prion-infected mice by depleting PrP in neurons, preventing prion replication and abrogating neurotoxicity (Mallucci *et al.*, 2003, 2007; White *et al.*, 2008). The same group have shown that PrP^{Sc} replication causes sustained unfolded protein response (UPR) induction with persistent, deleterious expression of eLF2α-P in prion disease (Moreno *et al.*, 2012). The resulting chronic blockade of protein synthesis leads to synaptic failure, spongiosis and neuronal loss. Promoting eLF2α-P dephosphorylation rescues vital translation rates and is thereby neuroprotective, whereas preventing this further reduces translation and enhances neurotoxicity. The data support the development of generic proteostatic approaches to therapy in prion (Balch *et al.*, 2008; Tsaytler *et al.*, 2011). The unfolded PrP^C response works as protective cellular mechanism triggered by rising levels of misfolded PrP^{Sc} protein (Moreno *et al.*, 2012).

In another study, expression of PrP^C in neuronal cells is required to mediate neurotoxic effects of PrP^{Sc} (Chesebro *et al.*, 2005). PrP^{Sc} might elicit a deadly signal through a PrP^C dependent signalling pathway. Spontaneous neurodegeneration in transgenic mice expressing a PrP mutant without the N-terminal endoplasmic reticulum (ER)-targeting sequence indicated a toxic potential of PrP when located in cytosolic compartment (cytoPrP) (Ma *et al.*, 2002). Toxicity of cytoPrP seems to be dependent on its association with cellular membranes (Wang *et al.*, 2006) and its binding to Bcl-2, an antiapoptotic protein present at the cytosolic side of ER and mitochondrial membranes (Rambold *et al.*, 2006). Might the toxic potential of misfolded PrP in the cytosol be relevant to the pathogenesis of prion diseases? Most recent information revealed an impairment of the ubiquitin-proteasome system (UPS) in prion-infected mice. In conjunction with *in vitro* and cell culture approaches, it was proposed that prion neurotoxicity is linked to PrP^{Sc} oligomers, which translocate to the cytosol and inhibit the URS (Kristiansen *et al.*, 2007).

Stress-inducible and toxic signalling mediated by PrP^C are interconnected

PrP^C expression is indispensable for prion-induced neurotoxicity (Brandner *et al.*, 1996), implying that PrP^C could be a receptor for prions to trigger detrimental signalling. Strittmatter reported that PrP^C transduces the synaptic cytotoxicity of amyloid-β(Aβ) oligomers *in vitro* (Laurén *et al.*, 2009) and in Aβtransgenic mice (Gimbel *et al.*, 2010). Moreover, different anti-PrP antibodies or their antigen-binding fragment that disrupt the PrP–Aβ

interaction were able to block the Aβ-mediated disruption of synaptic plasticity. These findings were important because they suggest the involvement of PrP^C in Alzheimer's disease (AD) pathogenesis. However, others found that the absence of PrP^C did not prevent deficits in hippocampal-dependent behavioural tests on intracerebral Aβ injection (Balducci *et al.*, 2010). Variations in copper availability could contribute to these discrepancies (Stys *et al.*, 2012).

Parkin *et al.* (2007) reported an interaction between PrP^C and the rate-limiting enzyme in the production of Aβ, the β-secretase BACE1, and two studies have also found direct links: PrP^C has been reported to be a receptor for Aβ oligomers (Laurén *et al.*, 2009) and the expression of PrP^C is controlled by the amyloid intracellular domain (AICD) (Vincent *et al.*, 2009). There are two potential roles suggested for PrP^C in AD: one, a role in the physiological regulation of amyloid precursor protein (APP) via interaction with BACE1; and two, a role in the pathological progression of AD by mediating Aβ toxicity by binding Aβ42-oligomers. The feedback loop between, PrP^C, BACE1, APP and AICD are described, and provides a model linking these recent observations (Kellett *et al.*, 2009). However, several questions remain to be answered, including, what effect does Aβ42-oligomer binding have on the functions of PrP^C, how do the levels of PrP^C compare with the brains of AD patients and age-matched control, and what is the effect of altering PrP^C levels in mouse models of AD. Understanding the molecular and cellular mechanisms involved in the interactions between PrP^C and APP/Aβ is crucial to the understanding of AD pathogenesis.

PrP^C seems to regulate theβ-secretase cleavage of amyloid precursor protein, thereby regulating the production of Aβ (Parkin *et al.*, 2007). Besides α-secretase regulates the cleavage of PrP^C, regulating an N-terminal fragment with neuroprotective activity (Cissé *et al.*, 2005; Guillot-Sestier, *et al.*, 2009). PrP^C also binds to transmembrane proteins such as the 67-kDa laminin receptor (Rieger *et al.*, 1997; Gauczynski *et al.*, 2001; Hundt *et al.*, 2001), neural cell adhesion molecules (Schmitt-Ulms *et al.*, 2001; Santuccione *et al.*, 2005), G protein-coupled serotonergic receptors (Mouillet-Richard *et al.*, 2005), and low density lipoprotein receptor-related protein 1 (Taylor *et al.*, 2007; Parkyn *et al.*, 2008), which are able to promote intracellular signalling-mediated neuronal adhesion and differentiation as well as PrP^C internalization. Remarkably, PrP^C functions as receptor or co-receptor for extracellular matrix proteins such as laminin (Graner *et al.*, 2000a, 2000b) and vitronectin (Hajj *et al.*, 2007), as well as STI1 (Zanata *et al.*, 2002). These data suggest that glycosylphosphatidylinositol-anchored PrP^C is a possible scaffold receptor in a multiprotein, cell surface, signalling complex (Linden *et al.*, 2008, 2009; Martins *et al.*, 2010).

In hippocampal neurons STI1-PrP^C engagement induces an increase in intracellular Ca^{2+} levels. Using a best candidate approach to test potential channels involved in Ca^{2+} influx, Beraldo *et al.* (2010) found that α-bungarotoxin, a specific inhibitor for α7 nicotinic acetylcholine receptor (α7nAChR), was able to block PrP^C-STI1-mediated signalling, neuroprotection, and neuritogenesis. STI1 can interact with the PrP^C·α7nAChR complex to promote signalling and provide a potential target for modulation of the effect of prion protein in neurodegenerative diseases. The drugs that prevent bindings of Aβ1-42 toα7nAChR seem to be beneficial in a model of AD (Wang *et al.*, 2009). It seems that STI1 binding to PrP^C can hijack one of the key signalling pathways related to AD. And it is possible that STI1

modulation containing a complex containing PrP^C and α7nAChR may play an important role in AD.

Remarkably, PrP^C functions as a receptor or coreceptor for extracellular matrix proteins such as laminin (Vassallo *et al.*, 2005) and vitronectin (Hajj *et al.*, 2007) as well as STI1(Sakudo *et al.*, 2005), which has been repeatedly found by our group. These data suggest that GPI-anchored PrP^C is a potential scaffold receptor protein, cell surface, and signalling complex. These processes may serve as the basis for the multiple neuronal functions ascribed to PrP^C (Linden *et al.*, 2008; Martin *et al.*, 2010). PrP^C has been identified to bind Aβ oligomers (AβO) with high affinity and to selectively interact with high molecular mass assembles of AβO in AD but not control brains (Jarosz-Griffiths *et al.*, 2016). PrP^C is responsible for AβO-mediated inhibition of long-term potentiation (LTP) in hippocampal slices and is also required for the manifestation of memory impairment in an AD mouse model. AβO-binding to PrP^C leads to activation of Fyn kinase. In addition, the AβO activation of Fyn leads to phosphorylation of tau. Both metabotropic glutamate receptor 5 (mGluR5) and LPR1 have been identified as co-receptors required for the PrP^C-bound AβO to activate Fyn (Jarosz-Griffiiths *et al.*, 2016). Fyn kinase phosphorylates *N*-methyl-D-aspartate receptor (NMDAR) and tau. Eventually NMDAR and tau (pTyr18) induce synaptic impairment and neurodegeneration.

Recently, Aβ42, which is associated with neurodegeneration in AD, has also been reported to act as a ligand of PrP^C (Nah *et al.*, 2013). Jung and our group have demonstrated that PrP^C is critical in Aβ42-mediated autophagy in neurons (Nah *et al.*, 2013). The interaction of PrP^C with Beclin (BECN)1 facilitates the localization of BECN1 into lipid rafts and thus allows the activation of phosphatidylinositol 3-kinase (catalytic subunit type-3 or PI3KC3) complex in response to Aβ42, showing a beneficial role of PrP^C as a positive regulator of the BECN1–PI3KC3 complex in lipid rafts (Fig. 2.2).

Several studies have reported that β-sheet-rich amyloid protein (including α-synuclein)

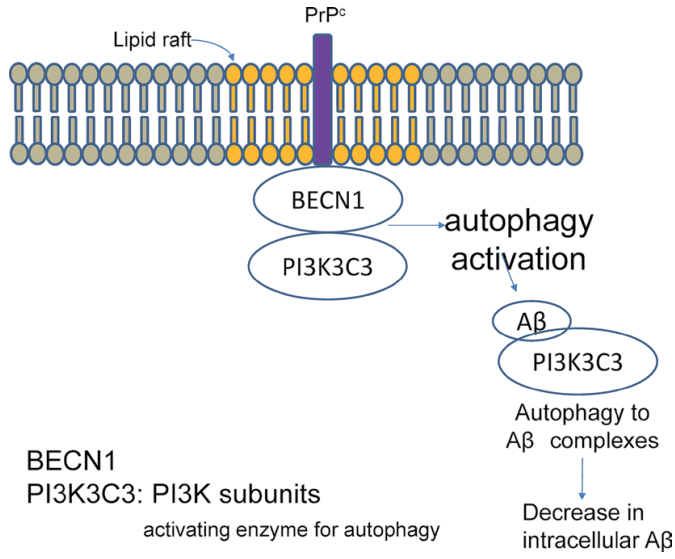

Figure 2.2 BECN1 (beclin 1) is supporting for intracellular decrease of Aβ. In elderly mice, the amount of cellular BECN1 is decreased. PI3K3C3 is a subunit of PI3K, and activating enzyme for autophagy to Aβ complexes, working with BECN1.

can interact with plasma membrane (Monsellier *et al.*, 2016). Although this interaction might be involved in amyloid internalization leading to cytotoxicity, 'docking' receptor-mediated interaction activities at plasma membrane might support most of the physiological activities of the oligomeric proteinaceous species (Linden *et al.*, 2017). PrP^C can bind with numerous membrane-associated molecules including adhesion molecules, growth factor receptors, and neurotransmitter receptors, among others. Abnormal α-synuclein aggregates appear, in addition to PD, in various α-synucleinopathies such as dementia with Lewy bodies and multiple system atrophy (Masuda-Suzukake *et al.*, 2014). In these disorders, aggregates are deposited in the brain in a filamentous form displaying a β-sheet structure (Serpell *et al.*, 2000) which is abnormally phosphorylated at Serine129 (α-synuclein) and is also ubiquitinated (Urrea *et al.*, 2017).

Shadoo, a highly conserved glycoprotein with similarities to PrP^C

In the search for homologous/paralogues of PrP^C, a new gene was identified termed *Sprn*, encoding for a protein denoted Shadoo (Sho) (Premzl *et al.*, 2003). Sho is highly conserved from fish to mammals. The sequence homology between Sho and PrP is restricted to the internal hydrophobic domain. However, certain features, such as a N-terminal repeat region and a C-terminal glycosylphosphatidylinositol (GPI) anchor, are conserved, suggesting that Sho and PrP may be functionally related. Experimental evidence for the post-translational modifications and cell surface localization of Sho was first presented for zebrafish Sho (Miesbauer *et al.*, 2006) and afterwards, also, for mouse Sho (Watts *et al.*, 2007). Similarly to PrP^C, Sho can prevent neuronal cell death induced by the expression of PrPΔHD (hydrophobic domain) mutants, an artificial PrP mutant devoid of internal hydrophobic domain (Watts *et al.*, 2007). The stress-protective activity of Sho is not restricted to counteracting the toxic effects of PrPΔHD. Sakthivelu *et al.* (2011) employed glutamate as a physiologically relevant stressor to show Sho can efficiently protect cells against excitotoxin-induced cell death. Deletion mutants revealed that the stress-protective activity of Sho and PrP seems to be dependent on similar domains, in particular, the N-terminal and their internal hydrophobic domain. ShoΔN (N-terminal) and ShoΔHD displayed a reduced stress-protective activity but are complex glycosylated and attached to the outer leaflet of the plasma membrane via GPI anchor, indicating that the impaired activity is not due to incorrect cellular trafficking.

The N-terminal domain of PrP is intrinsically disordered, and these disordered domains are involved in protein–protein interactions (Tompa *et al.*, 2009). Thus, it will be an attractive idea to assume that the N-terminal domains of PrP^C and Sho mediate interaction with an, as yet, unknown co-receptor required for intracellular signal transmission. The HD is the only domain with significant sequence homologies between Sho and PrP^C. The hydrophobic domain (HD) prompted dimerization of both Sho and PrP^C and was part of dimer interface. It is worth mentioning that dimerization is a common feature of many cell surface receptors. Therefore, it can be speculated that dimer formation is involved in signal transmission of PrP^C and Sho-dependent pathways.

Sho is stress-protective, however does not mediate PrP^{Sc}-induced toxicity

Expression of murine Sho gene (*Sprn*) transgene significantly increased brain Sho protein levels in generated mice (Wang *et al.*, 2011). Following infection with mouse-adapted scrapie strain 22L, all transgenic mice tested exhibited characteristics of scrapie disease. Importantly, there was no correlation between the expression level or incubation time of Sho with disease phenotypes. Although the function of Sho are, as yet, little characterized, the gain of function experiments seems to be essential for CNS development in mice. Wang *et al.* (2011) generated mice overexpressing Sho to determine the role of Sho in the pathogenesis of transmissible spongiform encephalopathy (TSE). Wang reported that Sho overexpression has no correlation with the incubation period of scrapie disease or with disease progression. There is no possible relationship between levels of Sho expression and scrapie pathology.

To evaluate the survival time, 22L strain of scrapie was injected intracerebrally into the brains of wild-type and *Sprn* over-expressed mice with mouse PrP-promoter (TgMo*Sprn*). All 16 prion-infected wild-type mice showed abnormal behaviour such as tremors and ataxia by 85 days. All mice had died by 149 days. The disease incubation period in infected wild-type mice was not significantly different from those of infected TgMo*Sprn* mice; three lines totalled to 40 mice.

In Sho over-expressed transgenic mice, Wang *et al.* (2011) detected large amyloid plaques not seen in wild-type mice. Recent work has shown that reduction in levels of Sho was not a direct or simple consequence of PrP^{Sc} accumulation. Instead, Sho protein levels are specific for the inoculated TSE agent and were not an intrinsic and invariant host process (Miyazawa and Manuelidis, 2010). Overexpression of Sho does not affect PrP, indicating that Sho has an alternate function. Other studies have shown that Sho exhibit no clear protective role in infected mice (Jeffrey *et al.*, 1997; Lloyd *et al.*, 2009; Miyazawa and Manuelidis, 2010) with no reduction in the time from incubation to neurological disease (Gossner *et al.*, 2009). In PrP knockout-mouse brain there was no significant change in expression of Sho (Watts *et al.*, 2007), further demonstrating that Sho protein and PrP protein are independent. The unaltered survival time of scrapie infected TgMo*Sprn* mice is not in accordance with a neuroprotective effect of Sho, but it is not completely ruled out as there might be possible interference with a Sho-overexpressing phenotype. Anyway, Sho is not a major modulator of PrP^{Sc} accumulation and scrapie pathogenesis.

Sho mutants devoid of the internal hydrophobic domain do not acquire a toxic potential

Studies in transgenic mice revealed the unexpected finding that PrP can acquire a neurotoxic potential by deleting the internal hydrophobic domain (Shmerling *et al.*, 1998; Baumann *et al.*, 2007; Li *et al.*, 2007). The neurotoxic potential of PrPΔHD is independent of the propagation of infectious prions, a phenomenon also seen for other neurotoxic PrP mutants (Winklhofer *et al.*, 2008). Although the underlying mechanism of PrPΔHD -induced toxicity are still elusive, co-expression of wild type PrP^{C} completely prevents toxic effects of PrPΔHD. Based on this intriguing observation, it has been hypothesized that stress-protective signalling of PrP^{C} and the neurotoxic signalling of PrPΔHD are transmitted through a common co-receptor, which remains to be identified (Rambold *et al.*, 2008;

Shmerling *et al.*, 1998; Baumann *et al.*, 2007; Li *et al.*, 2007). Co-transfection experiment with PrP-deficient cerebellar granule neurons indicated that Sho has a PrP^C-like activity to alleviate toxic effects of PrPΔHD expression (Watts *et al.*, 2007). Sakthivelu *et al.* (2011) have been able to recapitulate the toxic activity of PrPΔHD expression in their cell culture model and demonstrate the protective activity of PrP and Sho against PrPΔHD-induced toxicity. In addition, Sakthivelu *et al.* (2011) showed that ShoΔHD lost its ability to protect against stress-induced cell death. However, ShoΔHD did not acquire a toxic activity, at least not under the experimental conditions tested. In summary, Sho and PrP share a stress-protective activity. However, the ability to adopt a toxic conformation seems to be specific for PrP.

Ablation of PrP in higher organism

Any phenotypic effects of PrP^C loss are readily studied in higher organisms. Cattle lacking PrP have been generated and apparently free of clinical physiological, pathological, immunological, and reproductive abnormalities, at least up to 20 months of age (Richt *et al.*, 2007). PrP knockout goats have also been produced and appear to be developmentally normal (Yu *et al.*, 2009).

Systemic lipopolysaccharide (LPS) challenge induced characteristic signs of sickness behaviour that was prolonged by about two hours in PrP-deficient ($Prnp^{Ter/Ter}$) goats after the initial dose of LPS (Salvesen *et al.*, 2017). This is a noble clinical loss-of-function phenotype, pointing to a more inflammatory response in the absence of PrP^C. Transcriptome data revealed that in the absence of PrP^C, LPS induced an increased expression of numbers of genes downstream of type I interferons. It will be interesting to examine the peripheral nervous system in elderly knockout cows and goats to see if the role of PrP^C in the maintenance of peripheral nerve myelination is conserved in higher organisms (Watts *et al.*, 2018).

In humans, large-scale exome sequencing efforts have uncovered individuals carrying early stop codon mutations within one copy of their *Prnp* gene (Minikel *et al.*, 2016). The location of these mutations predicts that only one functional copy of PrP^C would be produced, and thus, these individuals would be expected to express approximately half of the normal level of PrP^C in their brains. The limited phenotypic data available for these individuals, who are between the ages of 52 and 79, suggest the absence of any overt neurological diseases. More in-depth analysis of people who are partially or fully deficient for PrP^C expression will be required to determine whether PrP^C is also dispensable in humans.

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