Function of Prion Protein and the Family Member, Shadoo

Takashi Onodera¹*, Takuya Nishimura², Katsuaki Sugiura¹ and Akikazu Sakudo³,⁴

¹Research Center for Food Safety, Graduate School of Agricultural and Life Sciences, University of Tokyo, Tokyo, Japan.
²Pharmaceuticals and Medical Devices Agency, Tokyo, Japan.
³Laboratory of Biometabolic Chemistry, School of Health Sciences, University of the Ryukyus, Nishihara, Japan.
⁴Faculty of Veterinary Medicine, Okayama University of Science, Imabari, Ehime, Japan.

*Correspondence: takashi.onodera@riken.jp

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Abstract
Lowering cellular prion protein (PrP⁰) levels in the brain is predicted to be a powerful therapeutic strategy for the prion disease. PrP⁰ 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 PrP⁰. Using PrP⁰-knockout cell lines, the inhibition of apoptosis through stress inducible protein1 (STI1) is mediated by PrP⁰-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 PrP⁰ expression.

Neuprotein Shadoo (Sho), similarly to PrP⁰, 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 excitotoxicity-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 (PrPSc) accumulation. Sho and PrP share a stress-protective activity. The ability to adopt a toxic conformation of PrPSc seems to be specific for PrP.
PrP<sub>C</sub> protects neurons from stress-induced apoptosis

**Neurogenesis**

Recently, several reports showed that cellular prion protein (PrP<sub>C</sub>) 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 PrP<sub>C</sub> 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<sub>C</sub>. In contrast, post-mitotic neurons express high levels of PrP<sub>C</sub> 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<sub>C</sub> may be expressed exclusively in differentiated neurons (Tanji et al., 1995). Studies in vitro have shown that expression of PrP<sub>C</sub> 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<sub>C</sub> enhance neurite outgrowth and survival (Kanaani et al., 2005).

The distribution of PrP<sub>C</sub> 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<sub>C</sub> 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<sub>C</sub> induced neuritogenesis in embryonic hippocampal neurons cultured in vitro (Kanaani et al., 2005; Lopes et al., 2005). PrP<sub>C</sub> 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<sub>C</sub> 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<sub>C</sub> in the neuroepithelium and its spatial and temporal relation with neural marker nestin and MAP-2 also suggests the participation of PrP<sub>C</sub> 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 of PrP<sub>C</sub> will indicate how PrP<sub>C</sub> is up-regulated during the differentiation of stem/progenitor cells.

**Neuroprotection**

The mammalian PrP<sub>C</sub> is a highly conserved glycoprotein localized in membrane lipid rafts and anchored to cell surface by glycosphatidylinositol (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<sub>C</sub> may undergo conversion into a conformationally–altered isoform (scrapie prion protein or PrP<sub>Sc</sub>) 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<sub>Sc</sub> in prion diseases, the normal function of PrP<sub>C</sub> is poorly understood. PrP<sub>C</sub> 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<sub>C</sub> is copper-binding. The octapeptide-repeat region of
PrP<sub>C</sub> binds with Cu<sup>2+</sup> 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<sub>C</sub> displays a functional role in normal brain metabolism of copper (Brown et al., 1997). Besides binding with Cu<sup>2+</sup> at the synapse, PrP<sub>C</sub> serves as a Cu<sup>2+</sup> buffer as well (Kretzschmar et al., 2000). Overexpression of PrP<sub>C</sub> increases Cu<sup>2+</sup> uptake into cells (Brown, 1999), while PrP<sub>C</sub>-knockout mice show a lower synaptosomal Cu<sup>2+</sup> concentration than normal mice (Kretzschmar et al., 2000). On the other hand, the Cu<sup>2+</sup> rapidly and reversibly stimulates the internalization of PrP<sub>C</sub> during PrP<sub>C</sub> endocytosis (Haigh et al., 2005; Kubosaki et al., 2003; Pauly et al., 1998). Through the binding with Cu<sup>2+</sup>, PrP<sub>C</sub> 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<sup>2+</sup> metabolism appears to play an important role in not only PrP function but also the pathogenesis of prion diseases.

PrP<sub>C</sub> 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<sub>C</sub> 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<sub>C</sub> in signal transduction may be anticipated. Discovery of several PrP<sub>C</sub>-interacting candidates has facilitated the understanding of the PrP<sub>C</sub> function (Table 2.1). PrP<sub>C</sub>-interacting molecules are most likely involved in signal transduction. In addition, a phosphorylating function of PrP<sub>C</sub>, 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<sub>C</sub> 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<sub>ε</sub> 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<sub>C</sub> 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 (Mouillet-Richard et al., 2005). In addition, PrP<sub>C</sub> binds with extracellular matrix laminin to promote genesis and maintenance of neurites (Graner et al., 2000a,b). Indeed, a recent study has discovered PrP<sub>C</sub> 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<sub>C</sub> plays an important role in neural differentiation (Steele et al., 2006). Therefore, the interaction between PrP<sub>C</sub> 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<sub>C</sub> (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<sub>C</sub> expression (Kuwahara et al., 1999). Overexpression of Bcl-2 in this cell-line reveals a functional relation of PrP<sub>C</sub> with Bcl-2 in the anti-apoptotic pathway
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Function of Prion Protein

(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<sub>C</sub>–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<sub>C</sub>-dependent SOD activation (Sakudo et al., 2005). The functional role of STI1 and PrP<sub>C</sub> 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<sub>C</sub> in these mice. Interestingly, deposition of PrP<sub>Sc</sub> 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<sub>C</sub> in the DCN of cerebellum.

A loss of PrP<sub>C</sub> function could be implicated in the pathogenesis of prion diseases and PrP<sub>C</sub>-dependent pathways might be involved in neurotoxic signalling. For example, in vivo crosslinking of PrP<sub>C</sub> by antibodies triggered neuronal apoptosis (Solforosi et al., 2004) and PrP<sub>C</sub>-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<sub>C</sub> is functionally involved in copper metabolism, signal transduction, neuroprotection and cell maturation (Fig. 2.1). Despite these published roles, mice that

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

**Synaptic plasticity**

In PrP\textsuperscript{−/−} mice, Kim \textit{et al.} (2007) have observed pathological alterations and some physiological dysfunctions in olfactory bulb (OB). Recently, Le Pichen \textit{et al.} (2009) have uncovered a significant phenotype of PrP\textsuperscript{−/−} mice in the olfactory system by utilizing a combination of genetic, behavioural 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\textsuperscript{−/−} mice exhibited impaired behaviour that was rescued in transgenic mice expressing PrP\textsuperscript{C} specifically in neurons but not in mice expressing only extra-neuronal PrP\textsuperscript{C}. PrP\textsuperscript{−/−} 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 \textit{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 \textit{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\textsuperscript{−/−} lines (Zurich I PrP knockout: Beuler \textit{et al.}, 1992; Nagasaki PrP knockout: Sakaguchi \textit{et al.}, 1996; Edinburgh PrP knockout: Manson \textit{et al.}, 1994) on alternate genetic backgrounds, indicating strong evidence of its dependence on PrP\textsuperscript{C} rather than other genetic factors. PrP knockouts also displayed altered behaviour in the habituation–dishabituation task, suggesting the phenotype

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**Figure 2.1** Gain and loss of function in prion disease.
was likely olfactory-specific. PrP<sup>−/−</sup> 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<sub>C</sub> 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<sup>−/−</sup> behavioural phenotype. Oscillatory LFPs may act to organize information flow within the olfactory system (Lledo <i>et al.</i>, 2006; Stopher <i>et al.</i>, 2007) by constraining the timing of mitral cell action potentials (Kasiwadani <i>et al.</i>, 1999). In addition, gamma oscillations are specifically implicated in behavioural performance in olfactory tasks (Beshel <i>et al.</i>, 2007; Brown <i>et al.</i>, 2005; Nusser <i>et al.</i>, 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 <i>et al.</i>, 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 <i>in vitro</i> to result from the rapid and reciprocal interactions between granule and mitral cells across the dendrodendritic synapse (Lagier <i>et al.</i>, 2007; Schoppa <i>et al.</i>, 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<sub>C</sub> expression, they cannot claim a causal link between these findings.

Mitral cells receive facilitated inhibition in PrP<sup>−/−</sup> 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<sub>C</sub> protein as well as its biochemical interactions with synaptic machinery (Criado <i>et al.</i>, 2005).

**Myelination and chronic demyelinating polyneuropathy**

A late-onset peripheral neuropathy has been identified in PrP<sub>C</sub>-deficient Nagasaki (<i>Prnp<sup>Ngsk/</sup></i><i>Ngsk</i>) and Zurich-I (<i>Prnp<sup>−/−</sup></i>) mice (Sakaguchi <i>et al.</i>, 1996; Nishida <i>et al.</i>, 1999; Büeler <i>et al.</i>, 1992). This indicates that PrP<sub>C</sub> might have a role in peripheral neuropathies. At 60 weeks of age, all <i>Prnp<sup>−/−</sup></i> mice (<i>n</i> = 52) investigated showed chronic demyelinating polyneuropathy (CDP) (Bremer <i>et al.</i>, 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 <i>Prnp</i> knockout mouse lines, <i>Prnp<sup>GFP/GFP</sup></i> (Heikenwalder <i>et al.</i>, 2008) mice and <i>Prnp<sup>Edbg/Edbg</sup></i> (Manson <i>et al.</i>, 1994) mice. <i>Prnp<sup>−/−</sup></i> and <i>Prnp<sup>Edbg/Edbg</sup></i> mice suffered from CDP despite the normal expression of Doppel (Dpl) (Moore <i>et al.</i>, 1999), indicating that Dpl regulation did not cause polyneuropathy. CDP was present in mice lacking both <i>Prnp</i> and <i>Prnd</i> (the gene for Dpl) (Genoud <i>et al.</i>, 2004), but absent from mice selectively lacking <i>Prnd</i> (Behrens <i>et al.</i>, 2002). Therefore, Dpl is not required for the maintenance of peripheral nerves. PrP<sub>C</sub> 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 myelintrophic 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 (C_C2), 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 myelintrophic 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 Prnp0/0 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 Prnp0/0 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 PrnpZH3/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 PrnpZH3/ZH3 mice on a pure genetic C57BL/6J background. Genomic, translational and phenotypic characterization of PrnpZH3/ZH3 mice failed to identify phenotypes previously described in non-co-isogenic Prnp–/– mice. However, PrnpZH3/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<sub>C</sub> binds to the G-protein-coupled receptor Adgrg6 (Gpr126) of Schwann cells, eliciting a promyelinating cAMP response <em>in vitro</em> and <em>in vivo</em> in mice and zebrafish (Küffer <em>et al.</em>, 2016). This pointed to the N-terminal fragment of PrP<sub>C</sub> as a promyelinating factor that might serve as a possible treatment in other peripheral chronic demyelinating polyneuropathies (Wulf <em>et al.</em>, 2017).

**PrP<sub>C</sub> mediates toxic signalling by PrP<sub>Sc</sub>**

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 <em>et al.</em>, 2003, 2007; White <em>et al.</em>, 2008). The same group have shown that PrP<sub>Sc</sub> replication causes sustained unfolded protein response (UPR) induction with persistent, deleterious expression of eLF2α-P in prion disease (Moreno <em>et al.</em>, 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 <em>et al.</em>, 2008; Tsaytler <em>et al.</em>, 2011). The unfolded PrP<sub>C</sub> response works as protective cellular mechanism triggered by rising levels of misfolded PrP<sub>Sc</sub> protein (Moreno <em>et al.</em>, 2012).

In another study, expression of PrP<sub>C</sub> in neuronal cells is required to mediate neurotoxic effects of PrP<sub>Sc</sub> (Chesebro <em>et al.</em>, 2005). PrP<sub>Sc</sub> might elicit a deadly signal through a PrP<sub>C</sub> 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 <em>et al.</em>, 2002). Toxicity of cytoPrP seems to be dependent on its association with cellular membranes (Wang <em>et al.</em>, 2006) and its binding to Bcl-2, an antiapoptotic protein present at the cytosolic side of ER and mitochondrial membranes (Rambold <em>et al.</em>, 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 <em>in vitro</em> and cell culture approaches, it was proposed that prion neurotoxicity is linked to PrP<sub>Sc</sub> oligomers, which translocate to the cytosol and inhibit the URS (Kristiansen <em>et al.</em>, 2007).

**Stress-inducible and toxic signalling mediated by PrP<sub>C</sub> are interconnected**

PrP<sub>C</sub> expression is indispensable for prion-induced neurotoxicity (Brandner <em>et al.</em>, 1996), implying that PrP<sub>C</sub> could be a receptor for prions to trigger detrimental signalling. Strittmatter reported that PrP<sub>C</sub> transduces the synaptic cytotoxicity of amyloid-β(Aβ) oligomers <em>in vitro</em> (Laurén <em>et al.</em>, 2009) and in Aβtransgenic mice (Gimbel <em>et al.</em>, 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 PrPC in Alzheimer’s disease (AD) pathogenesis. However, others found that the absence of PrPC 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 PrPC and the rate-limiting enzyme in the production of Aβ, the β-secretase BACE1, and two studies have also found direct links: PrPC has been reported to be a receptor for Aβ oligomers (Laurén et al., 2009) and the expression of PrPC is controlled by the amyloid intracellular domain (AICD) (Vincent et al., 2009). There are two potential roles suggested for PrPC 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, PrPC, 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 PrPC, how do the levels of PrPC compare with the brains of AD patients and age-matched control, and what is the effect of altering PrPC levels in mouse models of AD. Understanding the molecular and cellular mechanisms involved in the interactions between PrPC and APP/Aβ is crucial to the understanding of AD pathogenesis.

PrPC seems to regulate the β-secretase cleavage of amyloid precursor protein, thereby regulating the production of Aβ (Parkin et al., 2007). Besides α-secretase regulates the cleavage of PrPC, regulating an N-terminal fragment with neuroprotective activity (Cissé et al., 2005; Guillot-Sestier, et al., 2009). PrPC also binds to transmembrane proteins such as the 67-kDa laminin receptor (Rieger et al., 1997; Gauzynski 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 PrPC internalization. Remarkably, PrPC 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 PrPC 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-PrPC engagement induces an increase in intracellular Ca2+ levels. Using a best candidate approach to test potential channels involved in Ca2+ influx, Beraldo et al. (2010) found that a-bungarotoxin, a specific inhibitor for α7 nicotinic acetylcholine receptor (α7nAChR), was able to block PrPC-STI1-mediated signalling, neuroprotection, and neuritogenesis. STI1 can interact with the PrPC-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 PrPC can hijack one of the key signalling pathways related to AD. And it is possible that STI1
modulation containing a complex containing PrP\textsuperscript{C} and \alpha7nAChR may play an important role in AD.

Remarkably, PrP\textsuperscript{C} functions as a receptor or coreceptor for extracellular matrix proteins such as laminin (Vassallo \textit{et al.}, 2005) and vitronectin (Hajj \textit{et al.}, 2007) as well as ST11 (Sakudo \textit{et al.}, 2005), which has been repeatedly found by our group. These data suggest that GPI-anchored PrP\textsuperscript{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\textsuperscript{C} (Linden \textit{et al.}, 2008; Martin \textit{et al.}, 2010). PrP\textsuperscript{C} has been identified to bind A\beta oligomers (A\betaO) with high affinity and to selectively interact with high molecular mass assemblies of A\betaO in AD but not control brains (Jarosz-Griffiths \textit{et al.}, 2016). PrP\textsuperscript{C} is responsible for A\betaO-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. A\betaO-binding to PrP\textsuperscript{C} leads to activation of Fyn kinase. In addition, the A\betaO 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\textsuperscript{C}-bound A\betaO to activate Fyn (Jarosz-Griffiths \textit{et al.}, 2016). Fyn kinase phosphorylates N-methyl-D-aspartate receptor (NMDAR) and tau. Eventually NMDAR and tau (p\text{Tyr18}) induce synaptic impairment and neurodegeneration.

Recently, A\beta42, which is associated with neurodegeneration in AD, has also been reported to act as a ligand of PrP\textsuperscript{C} (Nah \textit{et al.}, 2013). Jung and our group have demonstrated that PrP\textsuperscript{C} is critical in A\beta42-mediated autophagy in neurons (Nah \textit{et al.}, 2013). The interaction of PrP\textsuperscript{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 A\beta42, showing a beneficial role of PrP\textsuperscript{C} as a positive regulator of the BECN1–PI3KC3 complex in lipid rafts (Fig. 2.2).

Several studies have reported that \beta-sheet-rich amyloid protein (including \alpha-synuclein)

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{Figure2_2.png}
\caption{BECN1 (beclin 1) is supporting for intracellular decrease of A\beta. In elderly mice, the amount of cellular BECN1 is decreased. PI3K3C3 is a subunit of PI3K, and activating enzyme for autophagy to A\beta complexes, working with BECN1.}
\end{figure}
can interact with 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\textsuperscript{C} can bind with numerous membrane-associated molecules including adhesion molecules, growth factor receptors, and neurotransmitter receptors, among others. Abnormal \(\alpha\)-synuclein aggregates appear, in addition to PD, in various \(\alpha\)-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 \(\beta\)-sheet structure (Serpell et al., 2000) which is abnormally phosphorylated at Serine129 (\(\alpha\)-synuclein) and is also ubiquitinated (Urrea et al., 2017).

**Shadoo, a highly conserved glycoprotein with similarities to PrP\textsuperscript{C}**

In the search for homologous/paralogues of PrP\textsuperscript{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\textsuperscript{C}, Sho can prevent neuronal cell death induced by the expression of PrP\textsuperscript{ΔHD} (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 PrP\textsuperscript{ΔHD}. 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. Sho\textsuperscript{ΔN} (N-terminal) and Sho\textsuperscript{ΔHD} 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\textsuperscript{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\textsuperscript{C}. The hydrophobic domain (HD) prompted dimerization of both Sho and PrP\textsuperscript{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\textsuperscript{C} and Sho-dependent pathways.
Sho is stress-protective, however does not mediate PrPSc-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 (TgMoSprn). 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 TgMoSprn 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 PrPSc 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 TgMoSprn 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 PrPSc 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 PrPC completely prevents toxic effects of PrPΔHD. Based on this intriguing observation, it has been hypothesized that stress-protective signalling of PrPC 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\textsuperscript{C}-like activity to alleviate toxic effects of PrP\DeltaHD expression (Watts et al., 2007). Sakthivelu et al. (2011) have been able to recapitulate the toxic activity of PrP\DeltaHD expression in their cell culture model and demonstrate the protective activity of PrP and Sho against PrP\DeltaHD-induced toxicity. In addition, Sakthivelu et al. (2011) showed that Sho\DeltaHD lost its ability to protect against stress-induced cell death. However, Sho\DeltaHD 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\textsuperscript{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 (\textit{Prnp}\textsuperscript{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\textsuperscript{C}. Transcriptome data revealed that in the absence of PrP\textsuperscript{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\textsuperscript{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 \textit{Prnp} gene (Minikel et al., 2016). The location of these mutations predicts that only one functional copy of PrP\textsuperscript{C} would be produced, and thus, these individuals would be expected to express approximately half of the normal level of PrP\textsuperscript{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\textsuperscript{C} expression will be required to determine whether PrP\textsuperscript{C} is also dispensable in humans.

**References**


Function of Prion Protein


Onodera et al.


