Review

Dual role of cellular prion protein in normal host and Alzheimer’s disease

By Takashi ONODERA*1,†

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Abstract: Using PrPC-knockout cell lines, it has been shown that the inhibition of apoptosis through STI1 is mediated by PrPC-dependent SOD activation. Antioxidant PrPC may contribute to suppression of inflammation activation. PrPC is functionally involved in copper metabolism, signal transduction, neuroprotection, and cell maturation. Recently several reports have shown that PrPC participates in trans-membrane signaling processes associated with hematopoietic stem cell replication and neuronal differentiation. In another role, PrPC also tends to function as a neurotoxic protein. A β oligomer, which is associated with neurodegeneration in Alzheimer’s disease (AD), has also been reported to act as a ligand of PrPC. However, the physiological role of PrPC as an Aβ-binding protein is not clear. Actually, PrPC is critical in Aβ-mediated autophagy in neurons. PrPC shows a beneficial role in lipid rafts to promote autophagy. Further search for PrPC-interaction molecules using Prnp−/− mice and various types of Prnp−/− cell lines under various conditions may elucidate other important PrPC important functions.

Keywords: Alzheimer’s disease, autophagy, gene-targeting, reactive oxygen species, slow virus infection, prion

Introduction

Prion diseases or transmissible spongiform encephalopathies (TSEs) are fatal neurological disorders that include Creutzfeldt-Jakob disease (CJD) and kuru in humans, scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle, and chronic wasting disease (CWD) in cervids. For the last 30 years, we and other Japanese groups have reported Japanese scrapie and BSE cases.1)–14) Hitherto, 36 cases of BSE10),13) have been reported in Japan (Fig. 1). Global elucidation of the etiology of kuru has lead to the discovery of pre-senile dementias, CJD and its variant — with basically similar cellular lesions — which are also transmissible and may be caused by a very similar and unconventional “virus” or prion.15),16) All the transmissible cerebral amyloidoses (TCA) are formed from the LPR1: low-density lipoprotein-related protein 1; LTP: long-term potentiation; MAPK: mitogen-activated protein kinase; mGluR5: metabotropic glutamate receptor 5; NADPH: nicotinamide adenine dinucleotide phosphate; NLRP3: nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3; NMDAR: N-methyl-D-aspartate receptor; NO: nitric oxide; NOS: nitric oxide synthase; OB: olfactory bulb; PI3K: phosphatidylinositol 3-kinase; PI3KC3: phosphatidylinositol 3-kinase, catalytic subunit type 3; PKA: protein kinase A; PML: progressive multifocal leukoencephalopathy; Pint1: prion protein interacting protein 1; Prnp: prion protein gene; PRNP: human prion protein gene; PrP: prion protein; PrPSc: scrapie isoform of prion protein; RAG1: recombination activating gene 1; ROS: reactive oxygen species; RSSE: Russian spring-summer encephalitis; SOD: superoxide dismutase; STI1: stress-inducible protein 1; TCA: transmissible cerebral amyloidosis; GPI-PrP: GPI deficient PrP transgenic mouse; TSE: transmissible spongiform encephalopathy; UPR: unfolded protein response; UPS: ubiquitin-proteasome system.

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TCA amyloid precursor protein (PrP) specified on the short arm of chromosome 2 in humans and chromosome 20 in mice.

There are other slow infections of the central nervous systems in humans that are caused by conventional viruses, including measles virus, papovaviruses (JC virus and SV40-progressive multifocal leukoencephalopathies or PML), rubella virus, cytomegalovirus, herpes simplex virus, cytomegalovirus, herpes simplex virus, adenovirus types 7 and 32, Russian spring-summer encephalitis (RSSE) viruses, and the human retroviruses (human T-cell lymphotropic viruses-1: HTLV1, and human immunodeficiency viruses: HIV) (Table 1). Recently, Notkins’s group, our and other groups have demonstrated that human enteroviruses and reoviruses can be regarded as typical slow infections causing type-1 diabetes mellitus.17)–25) However, unlike these conventional viruses, the “unconventional viruses” of the subacute spongiform encephalopathies are truly slow in their replication, with long doubling times. Moreover, these alone appear to be viruses that have made it necessary to alter our conceptions of the possible range of virus structure. The process of infection appears to be a seeding by a “virus”, which is a nucleating agent inducing and the automatically accelerating the conformational transition in the amyloid subunit protein. In that process the host-specified precursor protein (cellular isoform of prion protein: PrPC) is converted to an insoluble cross-β-plated configuration (scrapie isoform of prion protein: PrPSc)26) (Table 2). Oligomers of microfilament of this subunit protein nucleate its own polymerization, crystallization, and precipitation as insoluble arrays of amyloid fibrils. Thus, proteolytic cleavage and conformational change of the precursor and oligomeric assembly of this structurally altered polypeptide produces a fibril amyloid enhancing factor27) with apparent infectious properties. In this report, the dual role of PrPC is described in diseased and normal function in the compromised host. Additionally, the etiological role of PrPC in the induction of Alzheimer’s disease (AD) is discussed.

1. PrPC is indispensable for neurological functions

1) Neuritogenesis by PrPC. The mammalian PrPC is a highly conserved glycoprotein localized in membrane lipid rafts and anchored to the cell surface by glycosylphosphatidylinositol (GPI).28),29) PrPC is located in many cell types, and is particularly abundant in neurons.30) Under certain conditions PrPC may undergo conversion into a conformationally altered isoform (PrPSc) which is widely believed to be the pathogenic agent in prion disease or TSE.31) Although much has been studied about the effects of PrPSc in prion diseases, the normal function of PrPC is poorly understood as yet. Lehmann and our group have shown that PrPC has α- and β-cleavage site during normal processing and host translational modifications.32)

Recent experimental evidence showing that PrPC interacting with β1 integrin controls focal adhesion and turnover of the actin microfilaments in neurons substantiates a role for PrPC in neurito-
Several reports show that PrPC participates in trans-membrane signaling processes associated with hematopoietic stem cell replication and neuronal differentiation. Integrins are well known in autophagy. Remarkably, during neuronal differentiation, the downregulation of Rho kinase activity is necessary for neurite sprouting. Abundant expression of PrP is detected during mouse embryogenesis in association with the developing mouse nervous system. In the developing mouse brain, undifferentiated neural progenitor cells in the mitotically active ventricular zone do not express PrP. In contrast, post-mitotic neurons express high levels of PrP after their last mitosis in the neuroepithelium as they migrate towards marginal layers and differentiate. Thus, PrP may be expressed exclusively in differentiated neurons.

Studies *in vitro* have shown that expression of PrP is positively correlated with differentiation of multipotent neuronal precursors into mature neurons. In addition, treatment of embryonic hippocampal neurons with recombinant PrP enhances neurite outgrowth and survival. The distribution of PrP in the developing nervous systems of cattle, mice, and humans suggest that PrP plays a functional role in neural development. While PrP knockout (Prnp−/−) displays no overt in neural phenotype, numerous subtle phenotypes have been

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**Table 1. Conventional virus-induced slow infections of man**

<table>
<thead>
<tr>
<th>Virus Family</th>
<th>Virus</th>
<th>Diseases</th>
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<tr>
<td><strong>RNA viruses</strong></td>
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<tr>
<td><em>Picornaviridae</em></td>
<td>Poliovirus</td>
<td>Chronic meningoencephalitis in immunodeficient patient</td>
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<td>Enterovirus</td>
<td>Virus-induced diabetes, subacute encephalitis</td>
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<td>Echovirus</td>
<td>Chronic meningoencephalitis in immunodeficient patient</td>
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<tr>
<td><em>Paramyxoviridae</em></td>
<td>Measles</td>
<td>Subacute sclerotic panencephalitis</td>
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<tr>
<td><em>Togaviridae</em></td>
<td>Rubella</td>
<td>Progressive congenital rubella</td>
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<tr>
<td><em>Flaviviridae</em></td>
<td>Tick-borne encephalitis</td>
<td>Epilepsia partialis continua (Kozhevnikov’s epilepsy), RSSE, progressive bulbar palsy in Russia</td>
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<td></td>
<td>Hepatitis C</td>
<td>Chronic hepatitis C (non-A, non-B hepatitis)</td>
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<tr>
<td><em>Retroviridae</em></td>
<td>Lentivirus HIV-1, HIV-2</td>
<td>HIV neuromyelencephalopathy</td>
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<td></td>
<td>Oncovirus HTLV-1, HTLV-2</td>
<td>Adult T-cell leukemia and mycosis fungoides, HAM/TSP neuromyelencephalopathy, Jamaican neuropathy, tropical spastic paraparesis</td>
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<tr>
<td><em>Rhabdoviridae</em></td>
<td>Rabies lyssavirus</td>
<td>Rabies encephalitis</td>
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<td><strong>DNA viruses</strong></td>
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<td><em>Adenoviridae</em></td>
<td>Adenovirus 7 and 32</td>
<td>Subacute encephalitis</td>
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<tr>
<td><em>Papoviridae</em></td>
<td>JC virus</td>
<td>Progressive multifocal leukoencephalopathy</td>
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<td>SV40</td>
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<td><em>Herpesviridae</em></td>
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<td>Herpes simplex</td>
<td>Subacute encephalitis</td>
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<td>Cytomegalovirus</td>
<td>Cytomegalovirus brain infection</td>
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<td>Epstein-Barr virus</td>
<td>Chronic infectious mononucleosis</td>
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<td></td>
<td>Varicella-Zoster virus</td>
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reported,\textsuperscript{37} including reduction in the number of neural precursor cells in developing mouse embryo.\textsuperscript{35} Other studies have shown that PrP C induced neuritogenesis in embryonic hippocampal neurons cultured \textit{in vitro}.\textsuperscript{38,42} PrPC interacts with stress-inducible protein 1 (STI1),\textsuperscript{43} which is a heat-shock protein.\textsuperscript{44} The interaction of PrPC with STI1 not only activates cyclic adenosine monophosphate (cAMP)-dependent protein kinase A (PKA) to transduce a survival signal but also induces phosphorylation/activation of the mitogen-activated protein kinase to promote neuritogenesis.\textsuperscript{42} Elucidation of the signaling pathways through which PrPC influences neurogenesis, along with an understanding of the pathways involved in other key cellular processes,\textsuperscript{42} may provide insight into how PrPC misfolding leads to devastating neurodegenerative diseases.

2) Neuroprotection by PrP C. In this chapter we describe how our group has spent much effort to understand the normal function of PrP C using \textit{Prnp}\textsuperscript{−/−} mice. Interestingly we observed that PrP C worked in an \textit{in vitro} system favorably provide neuroprotection for mice under the oxidative stress or virus infection by suppressing the apoptosis. In this chapter and Chapter 4, we show the same neuroprotective role of PrP C in an \textit{in vivo} mouse system. The most commonly observed function on PrP C is copper-binding. Previous and our studies by our laboratories and others have demonstrated that the octapeptide-repeat region of PrP C binds with Cu\textsuperscript{2+} within the physiological concentration range.\textsuperscript{45–47} Furthermore, PrP C displays a functional role in normal brain metabolism of copper.\textsuperscript{48} Besides binding with Cu\textsuperscript{2+} at the synapse, PrP C serves as Cu\textsuperscript{2+} buffer as well.\textsuperscript{49} Overexpression of PrP C increases Cu\textsuperscript{2+} uptake into cells\textsuperscript{50} while PrP C-knockout (\textit{Prnp}\textsuperscript{−/−}) mice show a lower synaptosomal Cu\textsuperscript{2+} concentration than normal mice.\textsuperscript{49} However, Cu\textsuperscript{2+} rapidly and reversibly stimulates the internalization of PrP C during PrP C endocytosis.\textsuperscript{51,52} Through binding with Cu\textsuperscript{2+}, PrP C displays superoxide dismutase (SOD) activity \textit{in vitro}.\textsuperscript{50,53–57} Intriguingly, treatment with copper chelator cuprizone induces TSE-like spongiform degeneration.\textsuperscript{58} Therefore, Cu\textsuperscript{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 internal or environmental factors that initiate apoptosis.\textsuperscript{59,60} Mature PrP C trends to localize in lipid rafts of cells.\textsuperscript{30} As lipid rafts are membrane structures specialized in signaling, a potential role of PrP C in signal transduction may be anticipated. Aβ on the raft is the most important interacting protein among them (see the next chapter: section 3). Such a signaling platform or signalosome is based on PrP C. PrP C localizes to cholesterol- and sphingolipid-rich, detergent-resistant lipid rafts due to the saturated acryl chains in its GPI anchor and to an N-terminal targeting

Table 2. Transmissible spongiform encephalopathies with mutations responsible for inherited genetic forms of the disease

<table>
<thead>
<tr>
<th>In humans</th>
<th>In Animals</th>
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<tbody>
<tr>
<td>Kuru</td>
<td>Scrapie (sheep, goats, mouflon)</td>
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<tr>
<td>Creutzfeldt-Jakob disease</td>
<td>Transmissible mink encephalopathy</td>
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<tr>
<td>Sporadic</td>
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<tr>
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<td>Bovine spongiform encephalopathy</td>
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<tr>
<td>Familial (178asn, 200lys, 210ile, octapeptides inserts 2, 4-9)</td>
<td>Exotic ungulate spongiform encephalopathy (nyala, gemsbok, Arabian oryx, greater kudu, eland)</td>
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signal interacting with heparin sulfate proteoglycan, glypican-1. PrP C has been proposed as a key scaffolding protein for dynamic assembly of cell surface signaling modules, and PrPC, along with the microdomain-forming flotillin, or caveolin proteins, may lead to the local assembly of membrane protein complexes at sites involved in cellular communication, such as cell-cell contacts, focal adhesions, the T-cell cap, and synapses.

In addition, a phosphorylating function of PrP C, mediated by caveolin-1 to indirectly increase Fyn (a member of the Src family of tyrosine kinase) phosphorylation, governs the downstream production of NADPH-oxidase-dependent reactive oxygen species and activation of the extracellular regulated kinase 1/2 has been demonstrated.30,61 PrP C interacts with normal phosphoprotein synapsin Ib and cytoplasmic adaptor protein Grb2 without being deciphered with prion interactor Pint1.62 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.63

Upon engagements with PrP C binding peptides and certain antibodies, PrP C transduces neuroprotective signals that affect the sensitivity to induce cell death.64 Linden et al. have suggested that the engagement of PrP C transduces neuroprotective signals through a cAMP/PKA-dependent pathway. According to their theory, PrP C may function as a trophic receptor, where the activation of which leads to a neuroprotective state.60 In addition, PrP C binds with extracellular matrix laminin to promote genesis and maintenance of neurites. We have shown that PI3K is activated by PrP C through the intercellular transportation of copper molecules.65 In fact, a study has discovered PrP C to induce self-renewal of long-term populating hematopoietic stem cells.34 Furthermore, another study has revealed that PrP C 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.39 Therefore, the interaction between PrP C and various signal transduction molecules speaks well for important functions (such as differentiation and cell survival) in the living system (Fig. 2).

A stress-protective activity has also been assigned to PrP C based on results with primary neuronal cultures. Neuronal cells derived from Prnp −/− mice are more sensitive to oxidative stress and serum deprivation than wild-type cells. Moreover, after ischemic brain injury, Prnp −/− mice reveal enlarged infarct volumes.60 In our studies Prnp −/− cell line (HpL3-4), immortalized from hippocampal neuronal precursor cells in Rikn Prnp −/− mice (Fig. 3), is sensitive to serum deprivation-induced apoptosis, although it is activated and/or survives
with PrPC expression. The same effect was observed in another Prnp-deficient cell line (NpL2), immortalized from hippocampal neuronal cells in Zrch I Prnp!/~ mice. Overexpression of B-cell lymphoma 2 protein (Bcl-2) in this cell line reveals a functional relation of PrPC with Bcl-2 in the anti-apoptotic pathway. Martin et al. have shown that prevention of cell death in cultured retinal explants from neonatal rats and mice induced by anisomycin (a protein synthesis inhibitor) unfurls the effect associated with PrPC-STI1 interactions. Li and our group show that the production of another type of heat-shock protein (Hsp 70) is enhanced when PrP levels elevate during hyperglycemia.

We have demonstrated that the inhibition of apoptosis through STI1 is mediated by PrPC-dependent SOD activation. This neuroprotective role of PrPC has been linked to cell signaling events. The interaction of PrPC with the STI1 generates neuroprotective signals that rescue cells from apoptosis (Fig. 4). The functional role of STI1 and PrPC have been confirmed in both murine and bovine systems.

![Fig. 3. Phenotypes of Prnp!/~ mice established in several laboratories in the world. Different kinds of targeting cassette for Prnp are shown. The structures of constructs used to produce six lines of Prnp!/~ mice are explained. The structure of wild-type (WT) Prnp exon3 and PrP coding region (open box) is shown at the top. The selection markers are indicated by black boxes. The presence and absence of 3 splicing acceptors are correlated with ectopic expression of Doppel (Dpl) and development of late-onset ataxia induced by loss of Purkinje cells. Chronic demyelinating polyneuropathy (CDP) was observed in type-1 and type-2 Prnp!/~ mice. The selection markers were: PGK, mouse phosphoglycerate kinase promoter; NEO, neomycin phosphotransferase; HPRT, mouse hypoxanthine phosphoribosyltransferase; TK, human herpes simplex virus type 1 thymidine kinase promoter; MT, mouse metallothionein promoter; loxP, a 34 bp recombination site from phage P1. The restriction enzyme sites were: E, EcoRI; X, XbaI, S.A., splicing acceptor. Zurich type-2 knockout mouse produces while type-1 mouse does not produce doppel proteins. NT: not tested.](image-url)

![Fig. 4. Capacity of truncated Prnp!/~ genes to rescue apoptosis when reintroduced to Prnp!/~ cells. Introducing empty vector (EM) to Prnp!/~ cells shows strong apoptosis. However, introducing Δ#1 (octarepeat region (OR); aa 53-94 truncated) induces more potent apoptosis, showing the dominant negative effect of Δ#1 gene. Similarly, introducing Δ#2 (STI1 binding area; N-terminal half of hydrophobic region; aa 95-132 truncated) failed to rescue Prnp!/~ cells from apoptosis.](image-url)
The late onset of severe ataxia and loss of cerebellar Purkinje cells in several Prnp−/− mouse lines (70–72) from several laboratories suggest the lack of cerebellar protection by PrPC in these mice. Interestingly, deposition of PrPSc has been located in the deep cerebellar nuclei (DCN) of scrapie-infected sheep. (73) Future studies with a microarray analysis (74) by our group applied in eye-blink-conditioning of mice may provide insight into understanding the normal function of PrPC in the DCN of the cerebellum.

A loss of PrPC function could be implicated in the pathogenesis of prion diseases and PrPC-dependent pathways might be involved in neurotoxic signaling. For example, in vivo crosslinking of PrPC by antibodies triggered neuronal apoptosis (75) and PrPC-dependent receptors were postulated to explain the neurotoxic effect of a PrP mutant lacking the hydrophobic domain (see next sections 2 and 3). (76) Still available data indicated that PrPC is a critical element of the network that controls the sensitivity to programmed cell death in both the nervous system and several other cell types. The outcome of the engagement of PrPC on either cell death or survival is likely dictated by its available ligands. These ligands in fact determine the array of intervening signaling pathways.

Taken together, PrPC is functionally involved in copper metabolism, signal transduction, neuroprotection, and cell maturation. Despite these putative roles, mice null for PrPC display no consistent phenotype apart from complete resistance to TSE infection. (41,77) Further searches for PrPC-interaction molecules using Prnp−/− mice and various types of Prnp−/− cell lines under various conditions may elucidate the PrPC functions.

3) Enhanced synaptic plasticity by PrPC. In Rikn Prnp−/− mice, Kim et al. in our group have observed pathological alterations and some physiological dysfunctions in the olfactory bulb (OB). (78) Recently, Le Pichon et al. have proposed that electrophysiological alterations at the dendrodendritic synapse in the OB could underlie the behavior phenotypes. (79) In detail, the cookie finding phenotype was manifested in three Prnp−/− lines (Zrch I Prnp−/− mice, 41) Ngsk Prnp−/− mice, 72) Npu Prnp−/− mice, 80) on alternate genetic backgrounds, indicating strong evidence of its dependence on PrPC rather than other genetic factors. Prnp−/− mice also displayed altered behavior in the habituation-dishabituation task, suggesting the phenotype is most likely to be olfactory-specific. (78,79) Prnp−/− mice exhibited wide spread alterations of oscillatory activity in the OB as well as altered paired-pulse plasticity at the dendrodendritic synapse. Importantly, both the behavioral and electrophysiological phenotypes could be rescued by neuronal PrPC expression. (79) These data suggest a critical role for PrPC in the normal processing of sensory information by the olfactory system.

Kim et al. and Le Pichon et al. employed a so-called “cookie finding task”, a test of broad olfactory acuity, to analyze a battery of mice including PrP knockout from multiple genetic backgrounds and transgenic mice in which Prnp expression was driven by cell type-specific promoters. (59,79) Prnp−/− mice exhibited impaired behavior that was rescued in transgenic mice expressing PrPC specifically in neurons but not in mice expressing only extra-neuronal PrPC. Prnp−/− mice displayed altered behavior in an additional olfactory test (habituation-dishabituation) which was also rescued by transgenic neuronal PrP expression suggesting that the phenotype was olfactory-specific. Additionally, the odor-evoked electrophysiological properties of the OB of Prnp−/− mice have been studied. (79) In these mice, alterations in the patterns of oscillatory activity in the OB were detected. The plasticity of dendrodendritic synaptic transmission was altered between the granule cells and mitral cells.

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 behavioral phenotype in Prnp−/− mice. Oscillatory LFPs may act to organize information flow within the olfactory system (81,82) by constraining the timing of mitral cell action potentials. In addition, gamma oscillations are specifically implicated in behavioral performance in olfactory tasks. (83,84) Therefore, alterations in oscillatory timing during odor exposure may perturb OB output to higher centers by disrupting how information is packaged within a breathing cycle.

Altering the dendrodendritic synapse may have multiple functional consequences. This synapse may mediate lateral inhibition between ensembles of mitral cells, and be critical for olfactory discrimination. (85,86) Additionally, because granule cells receive convergent information onto their proximal dendritic arbor from multiple higher brain areas, (87) disruption of the dendrodendritic synapse may alter the transmission of centrifugal modulation of OB mitral cells.

High-frequency oscillations in the OB are shown to result from the rapid and reciprocal interactions between granule and mitral cells across the dendro-
dendritic synapse in vitro.88) Therefore, Le Pichon’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 PrPC expression, they cannot claim a causal link between these findings. Mitral cells receive facilitated inhibition in Prnp−/− mice. Future work should determine the precise synaptic localization of the PrPC protein as well as its biochemical interactions with synaptic machinery.

At least two definite molecular interactions of PrPC with hippocampal cell surface proteins, STI1 and laminin, can mediate the effect of PrPC on memory consolidation.89) Moreover, it is likely that PrPC modulates memory retention through both these interactions. Further support of the hypothesis that PrPC plays important roles in memory and cognition is found in humans.89) The presence of Val at codon 129 of PRNP (human prion protein gene) in at least one allele is associated with worsened cognitive performance in elderly subjects, with early cognitive decline.89) Conversely, healthy young adults expressing Met at codon 129 in at least one allele exhibit better long-term memory than those with Val in this codon, although short-term memory was unaffected. Thus, polymorphism at codon 129, a site that is highly important for protein structure, seems to be strongly related to cognitive performance.89)

Another report shows the potential impact of the temporary disruption of the cerebellar circuit during the time window critical for sensorimotor development in Zrch I Prnp−/− mice.90) In that investigation, the authors considered the effect of PrP gene knock-out on cerebellar neural circuits, which show intense PrP expression during development and selective affinity for PrP. Zrch I Prnp−/− mice showed low performance in the accelerating rotorod and runway test and the functioning of 40% of granule cells was abnormal. This phenomenon may have a reflection in some of the late motor, cognitive, and emotional abnormalities in Zrch I Prnp−/− mice.90) Their results suggest that PrP plays an important role in granule cell development to eventually regulate cerebellar network and motor control.

4) Myelination in peripheral nerves by PrPC.

A late-onset peripheral neuropathy has been identified in PrPC-deficient Ngsk Prnp−/− and Zrch I Prnp−/− mice(41,72,91) (Fig. 4). This indicates that PrPC might have a role in peripheral neuropathies. At 60 weeks of age, all investigated Zrch I Prnp−/− mice (n = 52) showed chronic demyelinating polyneuropathy (CDP).92) CDP was 100% penetrating and conspicuous in all investigated peripheral nerves (sciatic and trigeminal nerves, dorsal and ventral spinal roots) (Fig. 4). Besides, CDP was associated with another 2 independently targeted Prnp knock-out mouse lines, GFP Prnp−/− mice(93) and Npu Prnp−/− mice.80)

Zrch I Prnp−/− and Npu Prnp−/− mice suffered from CDP despite normal expression of Doppel (Dpl),70) indicating that Dpl regulation did not cause polyneuropathy. CDP was present in mice lacking both Prnp and Prnd (the gene for Dpl),94) but absent from mice selectively lacking Prnd.95) Therefore, Dpl is not required for the maintenance of peripheral nerves. PrPC might interact with myelin component directly or through other axonal proteins. Certain reported PrPC interacting proteins have roles in homeostasis,96) and represent possible candidates for mediation of its myeliotrophic effects. The octapeptide repeat region was not required for myelin maintenance, whereas mice PrP lacking central domain (aa 94-134) developed CDP.97) It is worthy to note that the hydrophobic core, but not the charge cluster (CC3), of this central PrPC domain was essential for peripheral myelin maintenance.

Our and many other recent reports have shown that PrPC undergoes regulated proteolysis in late secretory compartments.32,81,100) Bremer et al.92) have observed an association between the presence of CDP and lack of C1 fragment in the sciatic nerves. All PrPC mutants in which CDP was rescued produced abundant C1.92) CDP was prevented by PrPC variants that undergo proteolytic amino-terminal cleavage,32) but not by variants that undergo nonpermissive cleavage, including secreted PrPC lacking its glycolipid membrane anchor. Cleavage of PrPC appeared therefore to be linked to its myeliotrophic function. This conjecture might also explain the requirement for membrane anchoring of PrPC uncovered in GPI-deficient PrP transgenic (GPI-PrP) mice,101) as anchorless PrPC did not undergo regulated proteolysis. These results indicate that neuronal expression and regulated proteolysis of PrPC are essential for myelin maintenance.92)
and central myelinopathies, PrPC deficiency has been reported to affect synaptic function, however, the amplitudes of foot muscle compound action potentials following distal stimulation are not significantly altered in 53-week-old Zrch I Prnp−/− mice; arguing against an important synaptic defect in the neuromuscular synaptic junction.

It has been suggested that PrPC has various roles in immunity, and lymphocytes are important in mouse models of hereditary demyelinating neuropathies. As the CDP in our mutant mice was not modulated by Rag1 removal, lymphocytes were not involved in its pathogenesis. The combined results of restricting expression of PrPC of neurons and of selectively depleting PrPSc from neurons indicate that the expression of PrPC by the neuron is essential for the long-term integrity of peripheral myelin sheaths. Not only was the trophic function of PrPC exerted in trans, but also correlated with the proteolytic processing of in diverse transgenic mouse models. These findings identify PrPSc as a critical messenger of transcellular axomyelinic communication, and indicate that regulated proteolysis of axonal PrPC might expose domains that interact with Schwann cell receptors. Recently, Aguzzi’s group has reported that PrPC promotes myelin homeostasis through flexible tail (23-50)-mediated Gpr126 agonism, and Gpr126 is crucial for peripheral nerve development. Gpr126−/− mice exhibit drastic hypomyelination. Clarifying the molecular basis of these phenomena might lead to better understanding of peripheral neuropathies, particularly those with late-onset, and might help to uncover new therapeutic targets.

2. PrPSc mediates toxic signaling by PrPSc

Mice with prion disease show PrPSc accumulation and develop extensive neurodegeneration, in contrast to mouse models of AD or Parkinson’s disease, where neuronal loss is rare. Therefore, prion-infected mice allow access to mechanisms linking protein misfolding to neuronal death. Studies of both neuronal and nonneuronal cells substantiate the coupling of PrPSc to signaling effectors involved in cell survival, redox equilibrium and homeostasis (e.g., ERK1/2, NADPH oxidase, cyclic AMP-responsive element binding protein (CREB) transcription factor and metalloproteinases) (Fig 2). According to these studies, PrPC functions as a dynamic cell surface platform for assembly of signaling molecules. However, the sequence of cellular and molecular events that leads to neuronal cell demise in TSEs remains obscure. At present, we envision that neuronal cell death results from several parallel, interacting or sequential pathways involving protein processing and proteasome dysfunction, oxidative stress, apoptosis, and autophagy.

The fact that PrPSc levels alone cannot serve as a marker for tissue infectivity suggests that it may be useful to adapt current protocols of prion detection in tissues, since they are so far largely based on the detection of bona fide PrPSc. Malucci’s group has previously shown rescue of neuronal loss and reversal of early cognitive and morphological changes in prion-infected mice by depleting PrPSc in neurons, preventing prion replication and abrogating neurotoxicity. Recently, the same group has shown that PrPSc replication causes sustained unfolded protein response (UPR) induction with persistent, deleterious expression of eLF2-P in prion disease. The resulting chronic blockade of protein synthesis leads to synaptic failure, spongiosis, and neuronal loss. Promoting eLF2-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 diseases. The unfolded PrPC response works as a protective cellular mechanism triggered by rising levels of misfolded PrPSc protein.

In another study, expression of PrPC in neuronal cells is required to mediate neurotoxic effects of PrPSc, which might elicit a deadly signal through a PrPC-dependent signaling pathway. Spontaneous neurodegeneration in transgenic mice expressing a PrPSc mutant without the N-terminal ER-targeting sequence indicated the toxic potential of PrPSc when located in the cytosolic compartment (cytoPrP). Toxicity of cytoPrP seems to be dependent on its association with cellular membranes and its binding to Bcl-2, an antiapoptotic protein present at the cytosolic side of ER and mitochondrial membranes. Might the toxic potential of misfolded PrPC in the cytosol be relevant to the pathogenesis of prion diseases? The most recent finding has revealed an impairment of the ubiquitin-proteasome system (UPS) in prion-infected mice. In conjunction with in vitro and cell culture approaches, it has been proposed that prion neurotoxicity is linked to PrPSc oligomers, which translocate to the cytosol and inhibit the UPS. Another study shows that a reduction in autophagy combined with endosomal/lysosomal dysfunction has indeed been proposed for the development of prion diseases.
3. Role of PrPC in AD

PrPC is anchored to the cell surface by GPI. Since the 1980s, PrP-related studies have mainly focused on the mechanisms by which PrPC is converted into PrPSc that is responsible for transmissible spongiform encephalopathies (TSE) and causes fatal neurodegeneration and aggregation. Concomitantly, the physiology of PrPC has also been studied, including the role in the cellular trafficking of copper ions, hippocampal morphology, cognition, oxidative stress and apoptosis \(^\text{131,132}\) (Fig. 5). Depending on cell context or physiological conditions, PrPC is beneficial or harmful to the cell. Many studies have indicated that PrPC can rescue neurons from various stressful situations. \(^\text{133,134}\) Evidence from Prnp-knockout mice studies has provided information regarding subtle phenotypes (e.g., mild neuropathological, cognitive and behavioral deficits). \(^\text{135}\) and enhanced brain injury after ischemia. \(^\text{136,137}\) In addition, the N-terminal region of PrPC displays a neuroprotective function. \(^\text{138}\) Conversely, PrPC also tends to function as a neurotoxic protein. \(^\text{139}\)

PrPC expression is indispensable for prion-induced neurotoxicity to develop \(^\text{140}\) implying PrPC could be a receptor for prions to trigger detrimental signaling. Strittmatter has reported that PrPC transduces the synaptic cytotoxicity of amyloid-\(\beta\) (A\(\beta\)) oligomers \(^\text{in vitro}\) \(^\text{141}\) and in A\(\beta\)-transgenic mice. \(^\text{142}\) Moreover, different anti-PrP antibodies or their antigen-binding fragments that disrupt the PrP-A\(\beta\) interaction are able to block the A\(\beta\)-mediated disruption of synaptic plasticity. These findings are important because they suggest the involvement of PrPC in AD pathogenesis. However, others have found that the absence of PrPC does not prevent deficits in hippocampal-dependent behavioral tests upon intracerebral A\(\beta\) injection. \(^\text{143}\) It has been suggested that variations in copper availability may contribute to these discrepancies. \(^\text{144}\)

PrPC seems to regulate the \(\beta\)-secretase cleavage of amyloid precursor protein, thereby regulating the production of A\(\beta\). \(^\text{145}\) In addition, \(\alpha\)-secretase regulates the cleavage of PrPC, regulating an N-terminal fragment with neuroprotective activity. \(^\text{146,147}\) PrPC also binds to transmembrane proteins such as the 67-kDa laminin receptor \(^\text{148}\) neural cell adhesion molecules, \(^\text{149,150}\) G protein-coupled serotonergic receptors, \(^\text{151}\) and low density lipoprotein receptor-related protein 1 (LPR1), \(^\text{152,153}\) which are able to promote intracellular signaling-mediated neuronal adhesion and differentiation as well as PrPC internalization. Remarkably, PrPC functions as a receptor or co-receptor for extracellular matrix proteins such as laminin \(^\text{154}\) and vitronectin, \(^\text{155}\) as well as ST11 \(^\text{156}\) which has been repeatedly found by our group. These data suggest that GPI-anchored PrPC is a potential scaffold receptor in a multiprotein, cell surface, and signaling complex that may serve as the basis for the multiple neuronal functions ascribed to PrPC. \(^\text{157,158}\)

PrPC has been identified to bind A\(\beta\) oligomers (A\(\beta\)O), but not monomers or fibrils, with high affinity and to selectively interact with high molecular mass assemblies of A\(\beta\)O in AD but not control brains. \(^\text{159}\) PrPC is responsible for A\(\beta\)O-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\(\beta\)O-binding to PrPC leads to activation of Fyn kinase. In addition, the A\(\beta\)O activation of Fyn leads to tau phosphorylation. Both metabotropic glutamate receptor 5 (mGluR5) and LPR1 have been identified as co-receptors required for the PrPC-binding A\(\beta\)O to activate Fyn. \(^\text{160}\) Fyn kinase phosphorylates N-methyl-D-aspartate receptor (NMDAR) and tau. Eventually NMDAR and tau (pTyr18) induce synaptic impairment and neurodegeneration (Fig. 6).

Recently, A\(\beta\)\(12\), which is associated with neurodegeneration in AD, has also been reported to act as a ligand of PrPC. \(^\text{161}\) However, the physiological role of PrPC as an A\(\beta\)\(12\)-binding protein is not clear. Actually, Jung and our group have demonstrated that PrPC is critical in A\(\beta\)\(12\)-mediated autophagy in neurons. \(^\text{162}\) The interaction of PrPC 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\(\beta\)\(12\), showing a beneficial role of PrPC as a positive regulator of the BECN1-PI3KC3 complex in lipid rafts.

4. PrPC and viral or bacterial infection

Microglia is one of the major cell types that produce NO, which in fact plays a role in active cellular protection. Keshet et al. and our group have reported that NOS activity is significantly reduced in adult Zurich I Prnp\(^{-/-}\) mice (age \(>100\) days), whereas NOS activity in young Zrch I Prnp\(^{-/-}\) mice (\(\leq30\)-day-old) is similar to that of wild-type Prnp\(^{+/+}\) mice. \(^\text{163,164}\) To further elucidates the role of PrPC, we have infected wild type prion protein gene (Prnp\(^{+/+}\)) and Zrch I or Rikn Prnp\(^{-/-}\) mice with encephalomyocarditis virus (EMCV)-B via an intra-
Fig. 5. Summarized schematic representation of phenotypes of Prnp<sup>−/−</sup> mice. Under the stressful conditions, Prnp<sup>−/−</sup> mice showed various phenotypes. Detail of these phenotypes is summarized in reference 119. In vivo studies have demonstrated that Prnp<sup>−/−</sup> mice are more prone to seizure, depression, and induction of epilepsy as well as experience extensive cerebral damage following ischemic challenge or viral infection. In experimental autoimmune encephalomyelitis, Prnp<sup>−/−</sup> mice reportedly have a more aggressive disease onset and clinical improvement during chronic phase than wild-type mice. Prnp<sup>−/−</sup> mice demonstrated significantly greater increase in blood glucose concentration after intraperitoneal injection of glucose than wild-type mice. In mice given oral dextran sulfate, PrPC indicated a potential protective role against inflammatory bowel disease.

Fig. 6. PrP<sup>C</sup>-based lipid raft signaling platform. The co-receptors LRP1 and mGluR5 clusters with PrP<sup>C</sup> upon Aβ/O binding leads to activation of Fyn kinase. Fyn kinase phosphorylates NMDAR and tau. Subsequently tau becomes pTyr18 (phospho-Tyr-18). NMDAR becomes pTyr1482NR2 (phospho-Tyr-1482 NMDAR). NMDAR and tau (pTyr18) then induce synaptic impairment and neurodegeneration.
cranial route. With regard to the inflammatory response in EMCV-B-infected mouse brains, mild to severe inflammatory changes have been observed only in 15-week-old but not in 6-week-old Prnp<sup>+/−</sup> mice. There is histopathological evidence of suppressed microglial response in Prnp<sup>−/−</sup> mice, whereas these are more enlarged apoptotic brain lesions after EMCV infection in Prnp<sup>−/−</sup> mice.  

Decreases in the activities of certain enzymes (superoxide dismutase and catalase) have been observed in the brains of Zrch I Prnp<sup>−/−</sup> mice, whereas these are more enlarged apoptotic brain lesions after EMCV infection. Prnp<sup>−/−</sup> mice have impaired ability to clear Streptococcus pyogenes at the inoculation site in the thigh muscle (an event that is largely neutrophil-dependent), these mice were protected from bacteremia and sepsis. This protection was accompanied by a standard decrease in most serum cytokine concentrations, the exceptions being IL-9 and interferon-α. It is tempting to speculate that the inability of Zrch I Prnp<sup>−/−</sup> mice to mount a full-blown cytokine response may confer some protection from full-scale bacterial sepsis. Their findings are noteworthy in the context of the neutrophil response to zymosan-induced peritonitis in Zrch I Prnp<sup>−/−</sup> mice, which may be caused by deficient IL-17 production leading to inadequate neutrophil migration.

Recently, it has been reported that mitochondria control the activation of NLRP3 (nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3) inflammasome, and that the inflammasome activation is negatively regulated by autophagy and positively regulated by reactive oxygen species (ROS). PrP<sup>C</sup> has SOD activity and protects neurons from oxidative stress. Antioxidant PrP<sup>C</sup> may contribute to suppressing inflamma-


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Takashi Onodera was born in 1947. He graduated from the University of Tokyo, School of Agricultural and Life Sciences in 1969 with a doctorate degree in veterinary medicine (DVM). After his graduation from the veterinary school, he majored in zoonosis and received his Ph.D. degree in the Graduate Student Training Program in 1974 from the Institute of Medical Science, and Graduate School of Agricultural and Life Sciences of the University of Tokyo. He worked as an Assistant Professor at the Department of Animal Pathology, Institute of Medical Science, in the University of Tokyo between 1974–1977. He was given a Visiting Fellowship at the National Institute of Dental Research (NIDR), National Institutes of Health (NIH) in the United States (U.S.A.) in 1974. In 1977 he was a Visiting Associate in the Laboratory of Oral Medicine (LOM) in NIDR, NIH, and in 1981 he was promoted to serve as an Expert in LOM, NIDR, NIH. During his days at NIH, U.S.A., he worked for Dr. Abner Louis Notkins, Chief of LOM, to investigate the virus-induced diabetes mellitus and autoimmunity. Upon his accomplishment, he received the NIH Director’s Award (titled: Innovative studies on the role of viruses in autoimmunity) in 1983. He was appointed as the Chief of Laboratory of Immunology, National Institute of Animal Health in Ministry of Agriculture, Forestry and Fisheries (MAFF) in Japan in 1986, and served as the Chief of Laboratory of Immune Cytology, National Institute of Animal Health in 1988. He assumed a chair, and became the Professor and Chairman of the Department of Molecular Immunology, School of Agricultural and Life Sciences, University of Tokyo in Tokyo, Japan (1991–2010). During his career in MAFF between 1984–1991, he received the Bifidus Award from the Japanese Society for Intestinal Microbiology for his study of prion diseases. He has a distinguished research career in unfurling the virological and immunopathological mechanisms controlling slow virus infection as well as contributing to the development of prion infectiology. At the U.S. NIH, his pioneering works covered a broad range of immunopathology of virus infections, such as in the fields of dentistry, virology and vaccinology. While in Japan, his scientific contribution has focused on the field of slow virus associated with normal function of prion protein in prion diseases and Alzheimer’s diseases, and epidemiological analysis of Japanese BSE. Currently he is working on chemicals to treat and prevent for prions and Alzheimer’s disease. Besides his career at the University of Tokyo, he served as a Government Advisor for MAFF, Ministry of Health, Labor and Welfare, and Cabinet Office. He assumed the Chair in the Advisory Committee on Food, Agriculture and Rural Policy’s Subcommittee on Prion Disease, MAFF in 2000, and became a Member of the Expert Committee on Prions, Food Safety Commission in the Cabinet Office in 2003. During his career as a Chairperson in MAFF, the Prion Disease Subcommittee spotted the first Japanese BSE case, which was also the first domestic BSE case outside Europe. For these achievements and scientific contributions, he was awarded the Japan Agricultural Science Award, and the Yomiuri Agriculture Award (titled: Animal model for prion diseases) in 2009. He is now a Project Professor in Research Center for Food Safety, University of Tokyo (2010–present), and is also working as a Visiting Scientist in the Brain Research Institute of Riken (1994–present). He has served as a Member in Institut de France (French National Academy of Science and Art, titled: Veterinary Science) in Paris since 2012, and is still actively duty-bound to said position. He further serves as a Board Member of the Japanese Society of Bio-defense and of the Human Genome Research Center, Institute of Medical Science of the University of Tokyo. He has also served as a President for the following organizations: the Japanese Society for Neurovirology, and the Japanese Society for Veterinary Immunology.

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