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Prion protein as a mediator of synaptic transmission

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Neurodegenerative disorders are characterized by synaptic and neuronal dysfunction which precedes general neuronal loss and subsequent cognitive or behavioral anomalies. Although the exact early cellular signaling mechanisms involved in neurodegenerative diseases are largely unknown, a view is emerging that compromised synaptic function may underlie the initial steps in disease progression. Much recent research has been aimed at understanding these early underlying processes leading to dysfunctional synaptic signaling, as this knowledge could identify putative sites of interventions, which could potentially slow progression and delay onset of disease. We have recently reported that synaptic function in a *Drosophila melanogaster* model can be modulated by the presence of native mouse prion protein and this modulation is negatively affected by a mutation within the protein which is associated with the Gerstmann-Sträussler-Scheinker syndrome, a human form of prion disease. Indeed, wild-type prion protein facilitates synaptic release, whereas the mutated form induced diminished phenotypes. It is believed that together with the gain-of-function of neurotoxic misfolded prion signaling, the lack of prion protein contributes to the pathology in prion diseases. Therefore, our study investigated a potential endogenous role of prion protein in synaptic signaling, the lack of which could resemble a lack-of-function phenotype in prion disease.

Keywords: neurodegeneration, neurotoxicity, prion protein, synapse, vesicle release, vesicle pools

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It is of great importance to understand the signaling pathways involved in neurodegenerative processes as the average lifespan continues to increase worldwide and with it the incidence of neurodegenerative disorders (ND) such as Parkinson and Alzheimer disease. Much research is now focused on unravelling the molecular mechanisms which lead to dysregulation of synaptic transmission by studying several synaptic proteins involved in neurotransmission. The neuronal network relies on plasticity mechanisms where reversible formation and disassembling of synaptic connections occurs in a controlled manner. It is generally accepted that in neurodegenerative conditions there is an early onset dysfunction at the synapse, opening up the possibility of intervention to manipulate neuroprotective pathways to balance between degenerative and survival signaling. It is now widely established, that the loss of presynaptic termini is a key event in the process, which initiates further axonal dysfunction and neuronal cell soma loss, resulting in cell death as a hall mark of many ND.[1-4]

Prion diseases are a form of transmissible spongiform encephalopathies (TSE), fatal ND of mammals characterized by the deposition of protease resistant misfolded prion protein. The cellular prion protein (PrP^{C}) is a cell membrane-anchored glycoprotein which plays an important role in a variety of neuronal processes including circadian rhythm, neuroprotection and neuroplasticity.[5,6] Although the physiological role of PrP^{C} remains elusive, the conversion of PrP^{C} into the neurotoxic infectious scrapie isoform of PrP (PrP^{Sc}) during prion disease and its detrimental signaling are well documented.[6-9] As a consequence of protein misfolding, several mammalian species develop neurodegenerative conditions best known as scrapie in sheep, bovine spongiform encephalopathy in cattle (BSE), chronic wasting disease (CWD) of elk and deer or Creutzfeldt-Jacob disease (CJD) and Gerstmann-Sträussler-Scheinker syndrome (GSS) in

human.[8,10,11] The unique feature of these conditions is that it can be caused by either sporadic mutations or inherited variants of the prion protein but it can also be transmitted by the scrapie isoform of PrP according to the 'protein only' hypothesis.[7,12] The early onset of the disease before manifestation of neuronal cell death may be caused by loss-of-function of the prion protein and/or by a gain-of-function of the cytotoxic PrP^{Sc}. It is therefore important to recognize the functions of PrP, especially in the synaptic context.

In order to distinguish between these 2, not exclusive, possibilities it is crucial to investigate the endogenous functions of PrP^{C} itself. PrP^{C} is ubiquitously expressed in the body, reaching the highest levels in the nervous system.[13-15] Morphological studies suggest that PrP^{C} is preferentially located along axons and in presynaptic terminals[15,16] but postsynaptic localization and signaling has also been reported.[17,18] Evidence demonstrates that neuroprotective roles of PrP^{C} are essential[19,20] as loss-of-function in $Prnp^{-/-}$ animals or mutations in PrP^{C} lead to neuronal dysfunction.[21-23] Interestingly, $Prnp^{-/-}$ animals exhibit phenotypes with impaired long-term potentiation[24-26], abnormal circadian rhythm[27] and glutamatergic synaptic signaling.[28,29] In addition, compromised dopaminergic transmission[30] but also more severe characteristics such as Purkinje cell degeneration and demyelination of peripheral nerves leading to ataxia have been reported in $Prnp^{-/-}$ animals.[21,31] Comparisons of wild-type with $Prnp^{-/-}$ mice have revealed that PrP^{C} expression at synapses contributes to hippocampal synaptic function[32] and exerts neuroprotection by modulating neuronal excitability.[33-35] In particular, PrP^{C} has been shown to inhibit *N*-methyl-D-aspartate receptors (NMDAR) containing the NR2D subunit[33,36], the activation of which has direct links to the general neurotoxic signaling mediated *via* the NMDAR-nitric oxide pathway.[37] However, the above studies have investigated prion protein functions in neuronal networks, which include pre- and postsynaptic compartments making it difficult to unambiguously define specific roles of PrP.

In order to study the functions of the prion protein in more detail and to isolate pre- and postsynaptic mechanisms, model systems other than mouse have been recently utilised. In particular, non-mammalian neurodegeneration models have been employed[38] with expression of wild-type or mutant prion proteins in *Drosophila melanogaster* or *C. elegans* allowing investigations of prion protein function in host organisms that do not have a direct prion protein ortholog.[39-45]

We recently showed that presynaptic expression of a wild-type mouse prion protein at a glutamatergic synapse, the *Drosophila* neuromuscular junction (NMJ), leads to an enhanced release of synaptic vesicles as a result of larger functional vesicle pools sizes.[46] This positive modulation of transmitter release was accompanied by increased presynaptic vesicle sizes leading to an overall augmentation of transmission. We did not observe any effects on the NMJ morphology, including numbers of release sites following prion protein expression, consistent with previous data.[39] We hypothesized in this study that expression of wild-type prion protein has a gain-of-function effect at presynaptic signaling, which corroborates previous findings in neurons of the mammalian central nervous system.[28]

In our study we asked the question whether the observed functional effects of wild-type mouse PrP could be diminished by expressing a mutated form of this prion protein, in which proline 101 was substituted with leucine (P101L). This conserved P102L mutation has been linked to the human prion disorder GSS syndrome. In our hands, expression of this mutated prion protein resulted in a lack of some functional phenotypes seen following expression of wild-type prion protein. Importantly, neither protein form showed any proteinase K resistance suggesting that the functional observations are not due to a cytotoxic gain-of-function of misfolded prion (PrP^{Sc}) but rather due to direct effects of the non-misfolded proteins. This is in contrast to studies using the same P101L mutant expressed in *Drosophila*, in which aged flies showed characteristics of protein misfolding and clear phenotypes of neurodegeneration reminiscent of the GSS syndrome.[40] Other data also indicate that expression of hamster and mouse (although to a lesser degree) PrP in *Drosophila* causes neurodegeneration in aged flies[41] suggesting an age- and species- dependent difference in prion protein signaling. However, our study provided important evidence that prion protein signaling, in the absence of misfolding and aggregation, has fundamental effects on synaptic transmission. It further suggests that in prion disease, due to the conversion of unfolded native PrP^{C} into PrP^{Sc}, neurons face both, a continuously diminishing prion protein function as well as an increasingly additional cytotoxic PrP^{Sc} function. So how can prion protein contribute to synaptic function? There are several lines of evidence suggesting that endogenous prion protein can modulate transmitter release *via* multiple pathways. Studies in mouse NMJs and hippocampal CA1 neurons showed that PrP^{C} potentiates synaptic release[28,47] consistent with PrP^{C} expression at presynaptic terminals.[48] PrP^{C} has been reported to interact with synapsin[49] (**Fig. 1**) and its internalisation is mediated *via* clathrin-coated pits[50] in a dynamin-dependent process.[51] So it is conceivable to suggest that PrP^{C} may play a role in endocytosis, vesicle replenishment and release, which is likely to impact on vesicle pool availabilities. This interaction offers a new functional explanation of how PrP^{C} can modulate transmitter release and how a consequent conversion of PrP^{C} into PrP^{Sc} could lead to synaptic dysfunction. An essential part of synaptic transmission is synaptic Ca^{2+} homeostasis in which Ca^{2+} influx through Ca^{2+} channels determines the release of neurotransmitter. Reports showed that a mutation in PrP^{C} leads to impaired membrane delivery of the $\alpha_2\delta{-}1$ subunit of voltage-gated Ca^{2+} channels (VGCC) in cerebellar granule neurons.[23] This caused reduced Ca^{2+} currents and a defective glutamate release suggesting that PrP^{C} function is directly required for synaptic Ca^{2+} signaling and vesicular release.

In conclusion, given the fact that PrP interacts with proteins involved in synaptic release[49] and additionally with various metabotropic and ionotropic neurotransmitter receptors[20,52-55] and

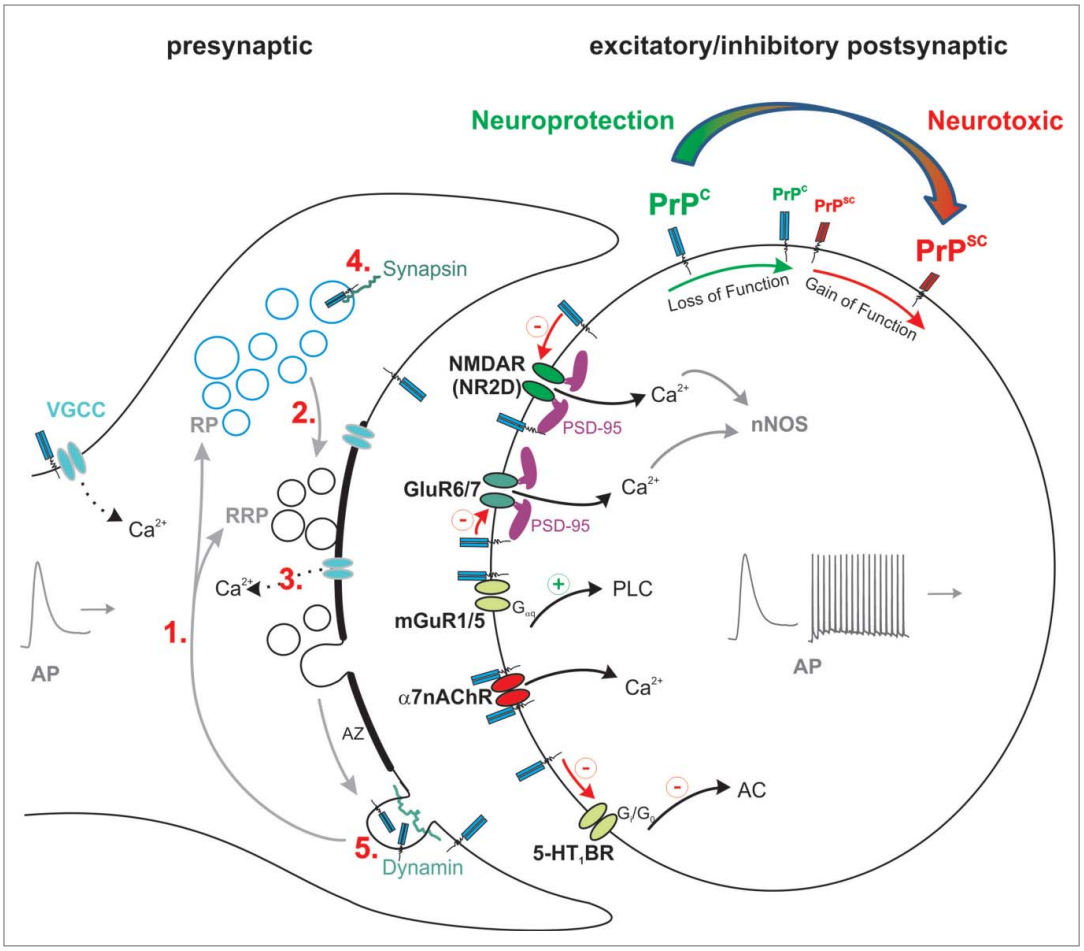

Figure 1. Prion protein signaling at the synapse. **General:** PrP^C possesses a normal, physiological activity, which is neuroprotective and is lost upon conversion to PrP^{Sc} leading to a loss-of-function phenotype. Secondly in prion disease, the toxic gain-of-function mechanism: PrP^{Sc} possesses a novel neurotoxic activity that is independent of the normal function of PrP^C. **Presynaptic signaling:** Prion protein is widely expressed at presynaptic sites. PrP^C is involved in vesicle pool maintenance. It potentially contributes to vesicle recycling/cycling leading to distinct availabilities of vesicles and function pool sizes (**1**) and vesicle recruitment (**2**). It is further involved in trafficking of a VGCC subunit ($\alpha_2\delta-1$) to the membrane, thereby facilitating Ca^{2+}-dependent neurotransmitter release (**3**). Its interaction with synapsin, a vesicular protein involved in transmitter release, also implicates PrP^C in vesicle fusion mechanisms (**4**). PrP^C internalization is dependent upon activity of dynamin I, a key mechano-enzyme involved with the fission of endocytotic vesicles from the plasma membrane (**5**). **Postsynaptic signaling (excitatory or inhibitory):** PrP^C has been found to interact with several receptors and postsynaptic molecules. It is associated with postsynaptic densities (PSD-95) and has been shown to directly interact with NMDARs (NR2D) and glutamate receptors (GluR6/7) thereby attenuating nNOS/NO-dependent excitotoxicity by inhibiting the receptors. The interaction with the α7nAChR complex promotes receptor signaling. PrP^C modulates G-protein coupled receptor signaling (activation of metabotropic glutamate receptor [mGluR1/5] signaling *via* direct PrP-mGluR interaction) and leads to inhibition of the serotonin 1B receptor ($5\text{-}HT_1BR$). All of the above prion protein-mediated functions will ultimately affect synaptic signaling, action potential propagation and physiology with its dysfunction potentially contributing to neurodegenerative phenotypes. Abbreviations: $5\text{-}HT_1BR$ – Serotonin 1B receptor, AC – adenylyl cyclase, AP – action potential, AZ – active zone, α7nAChR – α-7 nicotinic acetylcholine receptor, mGluR – metabotropic glutamate receptor, NMDAR – N-methyl-D-aspartate receptor, nNOS – neuronal nitric oxide synthase, NO – nitric oxide, PLC – phospholipase C, RRP – ready releasable pool, RP – reserve pool, VGCC – voltage gated Ca^{2+} channels.

ion channels[23] (**Fig. 1**), our data provides further evidence for a direct functional role of presynaptic prion protein signaling.[46] This study highlights the ability of prion protein to modulate vesicles and release properties leading to enhanced synaptic strength and transmission thereby corroborating and extending information gained from mouse models.[28-30,32] The use of the *Drosophila* NMJ system allows detailed investigations of presynaptic PrP functions to support studies in other model systems. Thus, our data point toward a physiological role of prion protein in synaptic function and will thereby help understanding the fundamental signaling pathways of prion proteins and their involvement in prion pathogeneses.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Conforti L, Adalbert R, Coleman MP. Neuronal death: where does the end begin? Trends Neurosci 2007; 30:159-66; PMID:17339056; http://dx.doi.org/10.1016/j.tins.2007.02.004
2. Milnerwood AJ, Raymond LA. Early synaptic pathophysiology in neurodegeneration: insights from Huntington's disease. Trends Neurosci 2010; 33:513-23; PMID:20850189; http://dx.doi.org/10.1016/j.tins.2010.08.002
3. Picconi B, Ghiglieri V, Bagetta V, Barone I, Sgobio C, Calabresi P. Striatal synaptic changes in experimental parkinsonism: role of NMDA receptor trafficking in PSD. Parkinsonism Relat Disord 2008; 14(Suppl 2): S145-9; PMID:18583173; http://dx.doi.org/10.1016/j.parkreldis.2008.04.019
4. Marcello E, Epis R, Saraceno C, Di Luca M. Synaptic dysfunction in Alzheimer's disease. Adv Exp Med Biol 2012; 970:573-601; PMID:22351073; http://dx.doi.org/10.1007/978-3-7091-0932-8_25
5. Linden R, Martins VR, Prado MA, Cammarota M, Izquierdo I, Brentani RR. Physiology of the prion protein. Physiol Rev 2008; 88:673-728; PMID:18391177; http://dx.doi.org/10.1152/physrev.00007.2007
6. Caughey B, Baron GS, Chesebro B, Jeffrey M. Getting a grip on prions: oligomers, amyloids, and pathological membrane interactions. Ann Rev Biochem 2009; 78:177-204; PMID:19231987; http://dx.doi.org/10.1146/annurev.biochem.78.082907.145410
7. Prusiner SB. Prions. Proc Natl Acad Sci U S A 1998; 95:13363-83; PMID:9811807; http://dx.doi.org/10.1073/pnas.95.23.13363
8. Collinge J, Clarke AR. A general model of prion strains and their pathogenicity. Science 2007; 318:930-6; PMID:17991853; http://dx.doi.org/10.1126/science.1138718
9. Shorter J, Lindquist S. Prions as adaptive conduits of memory and inheritance. Nat Rev Genet 2005; 6:435-50; PMID:15931169; http://dx.doi.org/10.1038/nrg1616
10. Lloyd SE, Mead S, Collinge J. Genetics of prion diseases. Curr Opin Genet Dev 2013; 23:345-51; PMID:23518043; http://dx.doi.org/10.1016/j.gde.2013.02.012
11. Aguzzi A. Prion diseases of humans and farm animals: epidemiology, genetics, and pathogenesis. J Neurochem 2006; 97:1726-39; PMID:16805779; http://dx.doi.org/10.1111/j.1471-4159.2006.03909.x
12. Aguzzi A, Calella AM. Prions: protein aggregation and infectious diseases. Physiol Rev 2009; 89:1105-52; PMID:19789378; http://dx.doi.org/10.1152/physrev.00006.2009
13. Benvegnu S, Poggiolini I, Legname G. Neurodevelopmental expression and localization of the cellular prion protein in the central nervous system of the mouse. J Comp Neurol 2010; 518:1879-91; PMID:20394048; http://dx.doi.org/10.1002/cne.22357
14. Fournier JG, Escaig-Haye F, Billette de Villemeur T, Robain O, Lasmezas CI, Deslys JP, Dormont D, Brown P. Distribution and submicroscopic immunogold localization of cellular prion protein (PrPc) in extracerebral tissues. Cell Tissue Res 1998; 292:77-84; PMID:9506914; http://dx.doi.org/10.1007/s004410051036
15. Moya KL, Sales N, Hassig R, Creminon C, Grassi J, Di Giamberardino L. Immunolocalization of the cellular prion protein in normal brain. Microsc Res Tech 2000; 50:58-65; PMID:10871549; http://dx.doi.org/10.1002/1097-0029(20000701)50:1%3c58::AID-JEMT9%3e3.0.CO;2-5
16. Barmada S, Piccardo P, Yamaguchi K, Ghetti B, Harris DA. GFP-tagged prion protein is correctly localized and functionally active in the brains of transgenic mice. Neurobiol Dis 2004; 16:527-37; PMID:15262264; http://dx.doi.org/10.1016/j.nbd.2004.05.005
17. Um JW, Nygaard HB, Heiss JK, Kostylev MA, Stagi M, Vortmeyer A, Wisniewski T, Gunther EC, Strittmatter SM. Alzheimer amyloid-beta oligomer bound to postsynaptic prion protein activates Fyn to impair neurons. Nat Neurosci 2012; 15:1227-35; PMID:22820466; http://dx.doi.org/10.1038/nn.3178
18. Um JW, Kaufman AC, Kostylev M, Heiss JK, Stagi M, Takahashi H, Kerrisk ME, Vortmeyer A, Wisniewski T, Koleske AJ, et al. Metabotropic glutamate receptor 5 is a coreceptor for Alzheimer abeta oligomer bound to cellular prion protein. Neuron 2013; 79:887-902; PMID:24012003; http://dx.doi.org/10.1016/j.neuron.2013.06.036
19. Wu G, Nakajima K, Takeyama N, Yukawa M, Taniuchi Y, Sakudo A, Onodera T. Species-specific anti-apoptotic activity of cellular prion protein in a mouse PrP-deficient neuronal cell line transfected with mouse, hamster, and bovine Prnp. Neurosci Lett 2008; 446:11-5; PMID:18809465; http://dx.doi.org/10.1016/j.neulet.2008.09.020
20. Carulla P, Bribian A, Rangel A, Gavin R, Ferrer I, Caelles C, Del Río JA, Llorens F. Neuroprotective role of PrPC against kainate-induced epileptic seizures and cell death depends on the modulation of JNK3 activation by GluR6/7-PSD-95 binding. Mol Biol Cell 2011; 22:3041-54; PMID:21757544; http://dx.doi.org/10.1091/mbc.E11-04-0321
21. Hetz C, Maundrell K, Soto C. Is loss of function of the prion protein the cause of prion disorders? Trends Mol Med 2003; 9:237-43; PMID:12829011; http://dx.doi.org/10.1016/S1471-4914(03)00069-8
22. Machado CF, Beraldo FH, Santos TG, Bourgeon D, Landemberger MC, Roffe M, Martins VR. Disease-associated mutations in the prion protein impair laminin-induced process outgrowth and survival. J Biol Chem 2012; 287:43777-88; PMID:23132868; http://dx.doi.org/10.1074/jbc.M112.428235
23. Senatore A, Colleoni S, Verderio C, Restelli E, Morini R, Condliffe SB, Bertani I, Mantovani S, Canovi M, Micotti E, et al. Mutant PrP suppresses glutamatergic neurotransmission in cerebellar granule neurons by impairing membrane delivery of VGCC alpha(2)delta-1 Subunit. Neuron 2012; 74:300-13; PMID:22542184; http://dx.doi.org/10.1016/j.neuron.2012.02.027
24. Collinge J, Whittington MA, Sidle KC, Smith CJ, Palmer MS, Clarke AR, Jefferys JG. Prion protein is necessary for normal synaptic function. Nature 1994; 370:295-7; PMID:8035877; http://dx.doi.org/10.1038/370295a0
25. Maglio LE, Martins VR, Izquierdo I, Ramirez OA. Role of cellular prion protein on LTP expression in aged mice. Brain Res 2006; 1097:11-8; PMID:16730679; http://dx.doi.org/10.1016/j.brainres.2006.04.056
26. Rangel A, Madronal N, Gruart A, Gavin R, Llorens F, Sumoy L, Torres JM, Delgado-García JM, Del Río JA. Regulation of GABA(A) and glutamate receptor expression, synaptic facilitation and long-term potentiation in the hippocampus of prion mutant mice. PloS One 2009; 4:e7592; PMID:19855845; http://dx.doi.org/10.1371/journal.pone.0007592
27. Tobler I, Gaus SE, Deboer T, Achermann P, Fischer M, Rulicke T, Moser M, Oesch B, McBride PA, Manson JC. Altered circadian activity rhythms and sleep in mice devoid of prion protein. Nature 1996; 380:639-42; PMID:8602267; http://dx.doi.org/10.1038/380639a0
28. Carleton A, Tremblay P, Vincent JD, Lledo PM. Dose-dependent, prion protein (PrP)-mediated facilitation of excitatory synaptic transmission in the mouse hippocampus. Pflugers Arch 2001; 442:223-9; PMID:11417218; http://dx.doi.org/10.1007/s004240100523
29. Prestori F, Rossi P, Bearzatto B, Laine J, Necchi D, Diwakar S, Schiffmann SN, Axelrad H, D'Angelo E. Altered neuron excitability and synaptic plasticity in the cerebellar granular layer of juvenile prion protein knock-out mice with impaired motor control. J Neurosci 2008; 28:7091-103; PMID:18614678; http://dx.doi.org/10.1523/JNEUROSCI.0409-08.2008
30. Rial D, Pamplona FA, Moreira EL, Moreira KM, Hipolide D, Rodrigues DI, Dombrowski PA, Da Cunha C, Agostinho P, Takahashi RN, et al. Cellular prion protein is present in dopaminergic neurons and modulates the dopaminergic system. Eur J Neurosci 2014; 40:2479-86; PMID:24766164; http://dx.doi.org/10.1111/ejn.12600
31. Martins VR, Beraldo FH, Hajj GN, Lopes MH, Lee KS, Prado MA, Linden R. Prion protein: orchestrating neurotrophic activities. Curr Issues Mol Biol 2010; 12:63-86; PMID:19767651
32. Maglio LE, Perez MF, Martins VR, Brentani RR, Ramirez OA. Hippocampal synaptic plasticity in mice devoid of cellular prion protein. Brain Res Mol Brain Res 2004; 131:58-64; PMID:15530652; http://dx.doi.org/10.1016/j.molbrainres.2004.08.004
33. Khosravani H, Zhang Y, Tsutsui S, Hameed S, Altier C, Hamid J, Chen L, Villemaire M, Ali Z, Jirik FR, et al. Prion protein attenuates excitotoxicity by inhibiting NMDA receptors. J Cell Biol 2008; 181:551-65; PMID:18443219; http://dx.doi.org/10.1083/jcb.200711002
34. Rangel A, Burgaya F, Gavin R, Soriano E, Aguzzi A, Del Rio JA. Enhanced susceptibility of Prnp-deficient mice to kainate-induced seizures, neuronal apoptosis, and death: Role of AMPA/kainate receptors. J Neurosci Res 2007; 85:2741-55; PMID:17304577; http://dx.doi.org/10.1002/jnr.21215
35. Spudich A, Frigg R, Kilic E, Kilic U, Oesch B, Raeber A, Bassetti CL, Hermann DM. Aggravation of ischemic brain injury by prion protein deficiency: role of ERK-1/-2 and STAT-1. Neurobiol Dis 2005; 20:442-9; PMID:15893468; http://dx.doi.org/10.1016/j.nbd.2005.04.002
36. You H, Tsutsui S, Hameed S, Kannanayakal TJ, Chen L, Xia P, Engbers JD, Lipton SA, Stys PK, Zamponi GW. Abeta neurotoxicity depends on interactions between copper ions, prion protein, and N-methyl-D-aspartate receptors. Proc Natl Acad Sci U S A 2012; 109:1737-42; PMID:22307640; http://dx.doi.org/10.1073/pnas.1110789109
37. Steinert JR, Chernova T, Forsythe ID. Nitric oxide signaling in brain function, dysfunction, and dementia. Neuroscientist 2010; 16:435-52; PMID:20817920; http://dx.doi.org/10.1177/1073858410366481
38. Gama Sosa MA, De Gasperi R, Elder GA. Modeling human neurodegenerative diseases in transgenic systems. Hum Genet 2012; 131:535-63; PMID:22167414; http://dx.doi.org/10.1007/s00439-011-1119-1
39. Park Y, Kim W, Kim AY, Choi HJ, Choi JK, Park N, Koh EK, Seo J, Koh YH. Normal prion protein in Drosophila enhances the toxicity of pathogenic polyglutamine proteins and alters susceptibility to oxidative and autophagy signaling modulators. Biochem Biophys Res Commun 2011; 404:638-45; PMID:21146501; http://dx.doi.org/10.1016/j.bbrc.2010.12.030

40. Gavin BA, Dolph MJ, Deleault NR, Geoghegan JC, Khurana V, Feany MB, Dolph PJ, Supattapone S. Accelerated accumulation of misfolded prion protein and spongiform degeneration in a Drosophila model of Gerstmann-Straussler-Scheinker syndrome. J Neurosci 2006; 26:12408-14; PMID:17135402; http://dx.doi.org/10.1523/JNEUROSCI.3372-06.2006
41. Fernandez-Funez P, Zhang Y, Casas-Tinto S, Xiao X, Zou WQ, Rincon-Limas DE. Sequence-dependent prion protein misfolding and neurotoxicity. J Biol Chem 2010; 285:36897-908; PMID:20817727; http://dx.doi.org/10.1074/jbc.M110.174391
42. Rincon-Limas DE, Casas-Tinto S, Fernandez-Funez P. Exploring prion protein biology in flies: genetics and beyond. Prion 2010; 4:1-8; PMID:20083902; http://dx.doi.org/10.4161/pri.4.1.10504
43. Thackray AM, Muhammad F, Zhang C, Di Y, Jahn TR, Landgraf M, Crowther DC, Evers JF, Bujdoso R. Ovine PrP transgenic Drosophila show reduced locomotor activity and decreased survival. Biochem J 2012; 444:487-95; PMID:22435640; http://dx.doi.org/10.1042/BJ20112141
44. Sanchez-Garcia J, Arbelaez D, Jensen K, Rincon-Limas DE, Fernandez-Funez P. Polar substitutions in helix 3 of the prion protein produce transmembrane isoforms that disturb vesicle trafficking. Hum Mol Genet 2013; 22:4253-66; PMID:23771030; http://dx.doi.org/10.1093/hmg/ddt276
45. Murali A, Maue RA, Dolph PJ. Reversible symptoms and clearance of mutant prion protein in an inducible model of a genetic prion disease in Drosophila melanogaster. Neurobiol Dis 2014; 67:71-8; PMID:24686303; http://dx.doi.org/10.1016/j.nbd.2014.03.013
46. Robinson SW, Nugent ML, Dinsdale D, Steinert JR. Prion protein facilitates synaptic vesicle release by enhancing release probability. Hum Mol Genet 2014; 23:4581-96; PMID:24722203; http://dx.doi.org/10.1093/hmg/ddu171
47. Re L, Rossini F, Re F, Bordicchia M, Mercanti A, Fernandez OS, Barocci S. Prion protein potentiates acetylcholine release at the neuromuscular junction. Pharmacol Res 2006; 53:62-8; PMID:16256362; http://dx.doi.org/10.1016/j.phrs.2005.09.002
48. Fournier JG, Escaig-Haye F, Grigoriev V. Ultrastructural localization of prion proteins: physiological and pathological implications. Microsc Res Tech 2000; 50:76-88; PMID:10871551; http://dx.doi.org/10.1002/1097-0029(20000701)50:1%3c76::AID-JEMT11%3e3.0.CO;2-
49. Spielhaupter C, Schatzl HM. PrPC directly interacts with proteins involved in signaling pathways. J Biol Chem 2001; 276:44604-12; PMID:11571277; http://dx.doi.org/10.1074/jbc.M103289200
50. Shyng SL, Heuser JE, Harris DA. A glycolipid-anchored prion protein is endocytosed via clathrin-coated pits. J Cell Biol 1994; 125:1239-50; PMID:7911471; http://dx.doi.org/10.1083/jcb.125.6.1239
51. Magalhaes AC, Silva JA, Lee KS, Martins VR, Prado VF, Ferguson SS, Gomez MV, Brentani RR, Prado MA. Endocytic intermediates involved with the intracellular trafficking of a fluorescent cellular prion protein. J Biol Chem 2002; 277:33311-8; PMID:12070160; http://dx.doi.org/10.1074/jbc.M203661200
52. Hernandez-Rapp J, Martin-Lanneree S, Hirsch TZ, Pradines E, Alleaume-Butaux A, Schneider B, Baudry A, Launay JM, Mouillet-Richard S. A PrP(C)-caveolin-Lyn complex negatively controls neuronal GSK3beta and serotonin 1B receptor. Scient Rep 2014; 4:4881; PMID:24810941; http://dx.doi.org/10.1038/srep04881
53. Beraldo FH, Arantes CP, Santos TG, Machado CF, Roffe M, Hajj GN, Lee KS, Magalhães AC, Caetano FA, Mancini GL, et al. Metabotropic glutamate receptors transduce signals for neurite outgrowth after binding of the prion protein to laminin gamma1 chain. FASEB J 2011; 25:265-79; PMID:20876210; http://dx.doi.org/10.1096/fj.10-161653
54. Black SA, Stys PK, Zamponi GW, Tsutsui S. Cellular prion protein and NMDA receptor modulation: protecting against excitotoxicity. Front Cell Dev Biol 2014; 2:45; PMID:25364752; http://dx.doi.org/10.3389/fcell.2014.00045
55. Beraldo FH, Arantes CP, Santos TG, Queiroz NG, Young K, Rylett RJ, Markus RP, Prado MA, Martins VR. Role of alpha7 nicotinic acetylcholine receptor in calcium signaling induced by prion protein interaction with stress-inducible protein 1. J Biol Chem 2010; 285:36542-50; PMID:20837487; http://dx.doi.org/10.1074/jbc.M110.157263