Mini review

PRION PROTEIN AND ITS ROLE IN SIGNAL TRANSDUCTION

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Abstract: Prion diseases are a class of fatal neurodegenerative disorders that can be sporadic, genetic or iatrogenic. They are characterized by the unique nature of their etiologic agent: prions (PrP^{Sc}). A prion is an infectious protein with the ability to convert the host-encoded cellular prion protein (PrP^{C}) into new prion molecules by acting as a template. Since Stanley B. Prusiner proposed the "protein-only" hypothesis for the first time, considerable effort has been put into defining the role played by PrP^{C} in neurons. However, its physiological function remains unclear. This review summarizes the major findings that support the involvement of PrP^{C} in signal transduction.

Key words: Prion, PrP^{C}, PrP^{Sc}, Src, MAP kinases, PKA, PKC, AKT, Calcium, Signaling

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THE "PROTEIN-ONLY" HYPOTHESIS

Prion diseases, or transmissible spongiform encephalopathies (TSEs), are a group of incurable neurological disorders characterized by spongiform vacuolization and progressive degeneration of the central nervous system (CNS) [1]. Examples include Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS) and fatal familial insomnia (FFI) in humans, and bovine spongiform encephalopathy (BSE), chronic wasting disease (CWD) and scrapie in animals. Prion diseases can manifest in three forms – sporadic, inherited or iatrogenic – and are caused by the accumulation of prions in the CNS. Prions, alternatively named PrP^{Sc}, are pathogenic agents described as proteinaceous infectious particles with the unique capacity to self-replicate. According to the "protein-only" hypothesis, PrP^{Sc} is a pathological conformer of the host-encoded cellular prion protein (PrP^{C}), which is converted into new prion particles during the progress of the disease [2]. The main event in the conversion of PrP^{C} into PrP^{Sc} is a post-translational modification in which some α-helix and random-coil elements of PrP^{C} are refolded into β-sheet-enriched secondary structures [3, 4]. Structural changes dramatically alter the biochemical properties of PrP^{C}, which becomes insoluble, prone to aggregation and amyloid formation, and partially resistant to the effects of proteases [3, 5].

THE CELLULAR PRION PROTEIN

Enzymatic digestion of brain extracts from affected animals and subsequent size-exclusion chromatographic analysis led to the isolation of a protein with a molecular weight of 27-30 kDa, designated PrP^{27-30}, which represents the resistant core of PrP^{Sc} [6]. Edman sequencing of the PrP^{27-30} N-terminal domain allowed Oesch *et al.* [7] to synthesize oligonucleotide probes and screen cDNA libraries derived from healthy and scrapie-infected hamster brains. A single chromosomal gene with a similar restriction pattern in both normal and affected animals was detected by Southern blot. The same gene was later found in humans, mice and other organisms [8]. The gene, named *Prnp*, is highly conserved across species and encodes for a protein of about 250 amino acids. The open reading frame of PrP^{C} is contained into a single exon in all the known *Prnp* genes [9, 10].

PrP^{C} is a ubiquitous protein, prevalently expressed in neurons [11]. Structurally, it consists of an N-terminal flexible tail containing binding sites for copper ions, known as octarepeats [12], and a C-terminal domain, prevalently folded in α-helices, as determined by nuclear magnetic resonance [13]. A signal peptide of 22 amino acids located at the N-terminus targets PrP^{C} to the secretory pathway, where it undergoes a glycosylation process through the addition of two N-linked carbohydrate chains. Additionally, a second signal sequence of 23 amino acids mediates the attachment of a glycophosphatidylinositol (GPI) anchor to the C-terminal end of the protein (Fig. 1) [14].

In its mature form, PrP^C is a membrane protein linked to the outer layer of the cell surface through the GPI moiety. It is particularly abundant at the level of the detergent-insoluble membrane microdomains known as lipid rafts, which are enriched in cholesterol and sphingolipids [15, 16]. PrP^C is naturally recycled between the cell surface and the endocytic compartment [17]. In some cells, PrP^C undergoes endocytosis through the caveolae, which are invaginations of the plasma membrane containing the protein caveolin-1 [18]. In cells lacking caveolin-1, such as neurons, PrP^C is translocated out of rafts upon copper binding and internalized through clathrin-coated pits [19]. Endocytosis of PrP^C is also relevant in prion disease pathology, since the endosomal compartment has been identified as a putative conversion site for prions [20, 21].
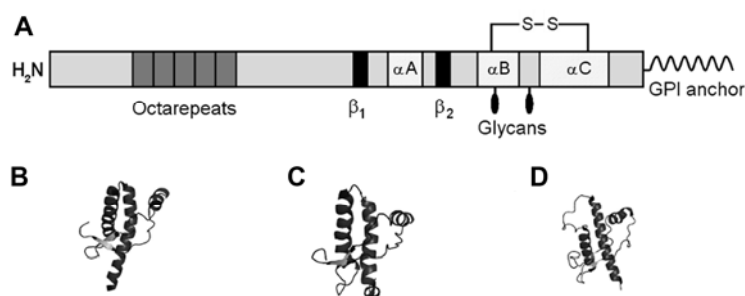

Fig. 1. Structural features of cellular prion protein (PrP^C). A – The secondary structure of mature PrP^C consists of a flexible N-terminal domain containing five octarepeats and a globular C-terminal domain composed of 3 α-helices (αA, αB, αC) and two β-strands (β1 and β2). In addition, it contains two N-glycosylation sites, a disulfide bridge, and a GPI moiety connecting PrP^C to the cell membrane. The tertiary structure of the globular domain is evolutionarily conserved, as seen by comparing the 3-dimensional structures of representative species. B – Human prion protein (pdb ID: 1QM0). C – Mouse prion protein (pdb ID: 1AG2). D – Chicken prion protein (pdb ID: 1U3M).

THE PHYSIOLOGICAL FUNCTION OF PRION PROTEIN – INSIGHTS FROM KNOCKOUT MODELS

In the early 1990s, several groups attempted to define the physiological function of PrP^C by generating different PrP^C-deficient mouse models. The first knockout mice for PrP^C, Zurich I and Edinburgh, were created by disrupting the *Prnp* gene with the *neo* cassette sequence by means of homologous recombination [22, 23]. Those mice failed to show any gross pathological phenotype in terms of development and behavior. Only subtle abnormalities in circadian rhythms, memory and learning were described [24-26]. By contrast, another set of knockout models, Ngsk and Rcm0 mice, developed progressive ataxia due to extensive cerebellar atrophy and Purkinje cell loss [27, 28]. Further analyses demonstrated how the knockout procedure led to the formation of a chimeric transcript between *Prnp* and a paralog gene, named *Prnd*, located 16 kb

downstream from the *Prnp* locus. The ectopic overexpression of *Prnd* in the brain, which encodes a protein called Doppel, induced the ataxic phenotype in Ngsk and Rcm0 mice by triggering apoptosis in cerebellar neurons [28, 29]. To exclude any compensatory mechanism occurring during the development of PrP^C-null mice, Mallucci *et al.* [30] created a conditional knockout model to explore the effects of PrP^C depletion on neuronal survival and function in the adult brain. No evidence for histological changes or neurodegeneration was found up to 15 months post knockout. The only differences were detected in the excitability of hippocampal CA1 neurons. Similarly, disruption of *Prnp* expression in cattle and goats resulted in no apparent developmental, physiological or anatomical abnormalities [31, 32].

Data from *in vivo* models substantially failed to pinpoint any important role for PrP^C in neuronal physiology, except for its role in prion propagation. Indeed, PrP^C-null animals are resistant to prion infection [31, 33]. Only recently, the knockdown of the *Prnp* hortologs in zebrafish embryos showed a strong loss-of-function phenotype characterized by a loss of cell adhesion and arrested gastrulation [34]. Due to the discrepancies between the different experimental models, many groups around the world have spent the past decade trying to solve this fascinating biological enigma. Several functions have been proposed for PrP^C. A role in copper uptake and homeostasis has been hypothesized, considering that the octarepeats in the unstructured N-terminal domain bind copper *in vivo* [35]. Copper binding also seems to confer a protective role against oxidative stress to PrP^C [36]. The slight abnormalities in circadian rhythms found in Zurich I null mice also suggested an involvement of PrP^C in the control of molecular clocks and sleep-wakefulness patterns [24, 37]. A role in hippocampal-dependent spatial learning and memory formation has been proposed on the basis of the small impairments detected in Zurich I and Edinburgh mice [25, 26]. In the same mouse models, a participation in myelin maintenance in the peripheral nervous system and in the correct processing of sensory information by the olfactory system has been recently proven [38, 39]. In addition to its contribution to nervous system functions, evidence of PrP^C modulating the immune system response has also been published [40, 41].

Several lines of evidence suggest that PrP^C can bridge stimuli between the extracellular environment and the cellular milieu. This particular aspect of prion biology, with its highlights and implications in pathogenesis, will be discussed in more detail later in this article.

PRION PROTEIN AND THE RECEPTOR-LIGAND PARADIGM – LOOKING FOR INTERACTORS

The preferential localization of PrP^C in lipid rafts suggested its possible involvement in signal transduction, as with other raft-associated GPI-anchored proteins [42]. Indeed, rafts are classically considered "hot spots" for signal transduction due to the local high concentration of signaling molecules [43]. The

first event in signal transduction involves interaction between a ligand and a specific receptor. Usually, receptors are transmembrane proteins with a cytosolic domain that can transfer the signals from the outside to the inside of the cell [44]. In terms of PrP^C as a receptor, the principal anomaly is the lack of an intracellular domain activating the cascade of molecular events in response to external stimuli. Therefore, many attempts have been made to identify the physiological ligands of PrP^C that could serve as co-receptors and overcome the absence of transmembrane and cytoplasmic portions. A considerable number of interacting proteins have been associated with PrP^C (for a comprehensive list see Aguzzi *et al.* [45]). However, the biological significance of many of them still needs to be validated to exclude technical artefacts, especially for those proteins that are only present in the cytosol or in the nucleus. The principal interactors that have shown relevance in PrP^C-mediated signal transduction are presented in this paper.

Schmitt-Ulms *et al.* [46] used mild *in situ* cross-linking to preserve the microenvironment of PrP^C on the membrane of murine neuroblastoma N2a cells. High molecular weight protein complexes containing PrP^C were isolated and three splice variants of the neural cell adhesion molecule (NCAM) were discovered in the complexes through liquid chromatography. Following the yeast two-hybrid assay approach, the laminin receptor precursor (LRP) and laminin receptor (LR) were discovered as cell-surface binding partners of PrP^C [47]. Additionally, the interaction between PrP^C and the extracellular matrix glycoprotein laminin was characterized using *in vitro* binding assays between radiolabeled laminin and prion protein [48]. Later, by taking advantage of the complementary hydropathy theory, antibodies raised against a peptide mimicking the docking site of PrP^C in a ligand [49] were used to look for interactors in Western blots from two-dimensional gels. The co-chaperone stress-inducible protein 1 (STI1) was identified in the screening as a cell-surface antigen, and its binding with PrP^C was confirmed using pull-down assays and co-immunoprecipitation *in vitro* [50]. Since paraformaldehyde cross-linking and yeast two-hybrid assay are methods that can yield a high number of false positives, Rutishauser *et al.* [51] proposed a more physiological approach to characterize the interactome of PrP^C. They carried out immunopurifications under native conditions of protein complexes from transgenic mice overexpressing a Myc-tagged version of PrP^C. Several interactors were identified by quantitative mass spectrometry analysis. A group of them was identified as participating in axomyelinic maintenance, and seven of these co-eluted equimolarly with PrP^C, suggesting they could be part of a multiprotein complex [51].

Recently, Lauren *et al.* [52] identified PrP^C as a functional receptor for Aβ-oligomers, the molecular species mediating toxicity in Alzheimer's disease (AD), via unbiased genome-wide expression cloning in COS-7 cells. This discovery attracted significant attention because it represented a potential link between TSEs and AD. However, while independent groups have confirmed the

interaction between the two proteins, the role in AD pathophysiology remains controversial [53-55]. Table 1 summarizes the most important PrP^C-interacting proteins.

As membrane proteins, all the aforementioned binding partners share the same sub-cellular environment with PrP^C. Moreover, their interactions have been functionally validated as triggers of signal cascades that regulate important cell functions, such as neuroprotection, cell death and neurite outgrowth. All of the pathways controlled by PrP^C will be discussed in this paper.

Table 1. Physiological ligands of PrP^C

Interactor	Cellular function	Method of screening
neural cell adhesion molecule (NCAM)	cell adhesion, neuritogenesis, synaptic plasticity, memory and learning	formaldehyde *in situ* cross-linking, liquid chromatography
laminin receptor (LR), laminin receptor precursor (LRP)	cell adhesion	yeast two-hybrid assay
laminin	cell adhesion, differentiation, migration, neuritogenesis	radioactive binding assay
stress inducible protein 1 (STI1)	heat shock protein	complementary hydropathy, co-immunoprecipitation assay
M6-a, neurofascin, 2',3'-cyclic nucleotide 3'-phophodiesterase, P0 glycoprotein, myelin-associated glycoprotein	formation, maintenance and degeneration of myelin sheaths	native immunoprecipitation assay of Myc-PrP^C, isotope-coded affinity tagging (ICAT) mass spectrometry
Aβ-oligomers	the principal mediator of AD dysfunctions	expression cloning in COS cells using biotin-tagged oligomers

PRION PROTEIN AND THE SRC FAMILY

The first evidence of a direct involvement of PrP^C in signal transduction was provided by Mouillet-Richard *et al.* [56], who used antibodies (Abs) against prion protein to mimic a putative physiological ligand of PrP^C. They were able to describe the activation of Fyn upon Ab-mediated cross-linking of PrP^C on the membrane of 1C11 cells, a murine line that can be differentiated in either serotonergic or noradrenergic neurons. Fyn is a member of the Src protein family, a group of nine non-receptor tyrosine kinases that play a pivotal role in the regulation of different aspects of cell physiology [57]. Fyn was also shown to mediate NCAM-induced neurite outgrowth [58]. Given that PrP^C interacts with NCAM on the neuronal cell surface, Santuccione *et al.* [59] investigated whether PrP^C plays a role in NCAM-mediated signaling. Using primary hippocampal neurons as a model, the authors demonstrated that PrP^C directly interacts with NCAM and recruits its transmembrane isoforms in lipid rafts to activate Fyn through the receptor protein tyrosine phosphatase α (RPTPα) and to promote neurite outgrowth (Fig. 2). Fyn-dependent axon elongation is enhanced not only

by NCAM-PrP^C interactions in *cis*, but also by *trans* interactions with recombinant PrP added to the culture medium, as shown in primary rat hippocampal neurons by Kanaani *et al.* [60]. These findings represented the first indicator of an involvement of PrP^C as a signaling molecule in nervous system development.
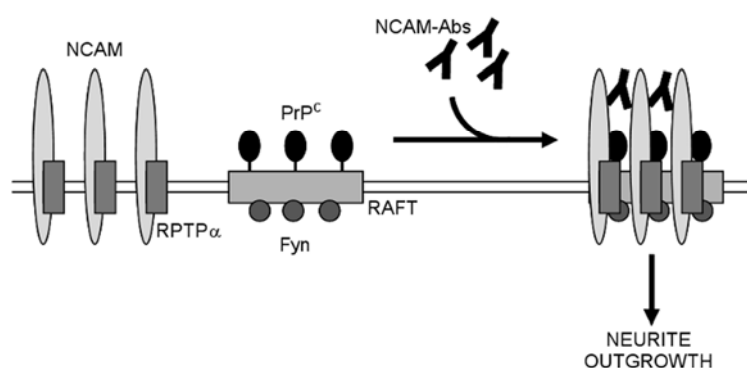

Fig. 2. The role of cellular prion protein in NCAM-mediated neurite outgrowth. According to the proposed model [59], in resting conditions (on the left) NCAM and the Fyn activator RPTPα interact outside the rafts that are enriched in PrP^C and Fyn. With specific stimuli, such as Ab-mediated cross-linking, NCAM is activated by palmitoylation, which promotes its recruitment into lipid rafts (on the right). Inside the rafts, PrP^C stabilizes NCAM clusters, triggering NCAM-mediated Fyn activation via RPTPα. In its active form, Fyn enhances neurite outgrowth.

The functional interaction of prion protein with NCAM was also investigated in the light of TSEs, in order to assess whether the NCAM-mediated Fyn pathway is involved in prion propagation. *In vivo* experiments on knockout animals proved that neither *NCAM*$^{-/-}$ mice nor *Fyn*$^{-/-}$ mice exhibit differences in terms of incubation time and PrP^{Sc} deposition compared to wild-type mice [46, 61]. These data suggest that NCAM interactions are not necessary for prion conversion. Interestingly, increased levels of phosphorylated Fyn were detected in chronically prion-infected cell lines and in two separate animal models of prion disease [62-64]. Tentatively, the aberrant activation of Fyn upon prion infection could be explained by postulating a higher affinity of PrP^{Sc} for NCAM, leading to an increment in its recruitment into lipid rafts. In the alternative, PrP^{Sc} might trigger Fyn through still unknown interactions.

It was recently shown that the deleterious effects on synaptic function due to PrP^C and Aβ-oligomer interactions also depend on Fyn activation, which leads to phosphorylation of the NR2B subunit of NMDA receptors and to their subsequent loss from the post-synaptic membrane [65].

PRION PROTEIN AND THE MAP KINASES

In 2003, after discovering functional connections between PrP^C and Fyn, the same group identified the extracellular signal-related kinase 1/2 (ERK1/2) complex as the downstream target of PrP^C-Ab-mediated Fyn activation [66]. ERK1/2 takes part in the mitogen-activated protein kinase (MAPK) cascades, a group of signaling pathways that are strongly conserved in eukaryotes. They govern a broad range of cellular processes, from cell proliferation and differentiation to apoptosis and inflammation [67]. Different interacting partners have been shown to activate the ERK pathway upon PrP^C binding on the cell surface. Mouse hippocampal neurons treated with either full-length STI1 or its PrP^C-binding fragment STI1$_{230-245}$ show increased levels of the active form of ERK1/2 [68]. The treatment affects both neuritogenesis and neuronal survival. However, neuroprotection resulted independently from ERK cascade activation, which only regulates axonal elongation. Surprisingly, PrP^C-dependent neurite outgrowth in rat hippocampal neurons is not blocked by ERK inhibitors [60], suggesting that PrP^C could modulate the same biological effect through different pathways in different species and cytotypes, according to the specific subset of interactors expressed by the cell. For example, Schneider *et al.* [66] highlighted how the presence or the absence of a functional PrP^C-caveolin-Fyn ternary complex in different cell types might result in alternative PrP^C-dependent pathways converging on the MEK-ERK1/2 module.

As for Fyn kinase, many attempts have been made to connect MAPK signaling to prion pathology. The discovery that ERK1/2 activation is controlled by NADPH oxidase-dependent reactive oxygen species (ROS) production offered an interesting link between the two fields [66]. Pietri *et al.* [69] further explored the hypothesis of a possible alteration of the redox equilibrium after prion infection. They found that the treatment of 1C11 and GT1 cells with the neurotoxic and amyloidogenic PrP$_{106-126}$ peptide leads to overproduction of ROS and sustained activation of MAPK cascades, which are associated with apoptotic signaling. Studies by other groups supported that hypothesis. Indeed, neuronal cells exposed to PrP^{Sc} resulted in aberrant ERK pathway activation [64, 70], which also occurs in the brains of prion-infected hamsters [71-73]. These findings reveal a possible scenario in which prion conversion promotes the hyper-activation of specific MAPK pathways leading to neuronal death through ROS production (Fig. 3). In addition, considering the protective activity of PrP^C against oxidative stress, prion replication might also subtract an important ROS buffer from the cell, as proved by the augmented susceptibility of PrP^C-deficient cells to oxidants [74].

Notably, pharmacological inhibitors of ERK phosphorylation were shown to clear PrP^{Sc} from prion-infected cells [75], suggesting a direct role for the ERK pathway in prion formation. By contrast, Uppington *et al.* [76] have shown that inhibiting the ERK pathway in prion-infected cells results in increased cell death

and suggested that the increased activity of the ERK pathway has a protective role against prion-induced neurotoxicity.
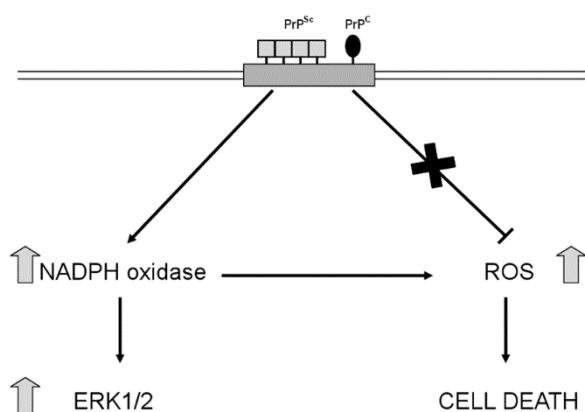

Fig. 3. A model of prion-induced cell death through ROS production. Prion replication stimulates ERK1/2 complex activation by NADPH oxidase. The activity of NADPH oxidase promotes the accumulation of ROS in prion-infected cells. The withdrawal of PrP^C due to prion conversion enhances the cytotoxic effects of ROS by increasing the susceptibility of the cell to oxidative stress. The combination of the two effects eventually leads to cell death.

PRION PROTEIN AND PROTEIN KINASES A AND C

The cAMP-dependent protein kinase A (PKA) cascade is one of most studied and well known signaling pathways [77]. It is a powerful cell regulator that controls a myriad of different cell functions, such as cytoskeletal dynamics, cell migration and adhesion [78]. In prion biology, PKA seems to mediate principally neuroprotective signals, as demonstrated for the first time by Chiarini *et al.* [79] in their work on retinal neurons. Indeed, they showed how treatment with a PrP^C-binding peptide could rescue anisomycin-induced apoptosis in organotypical retinal explants from wild-type mice but not from *Prnp* knockout animals. The exposure to the peptide leads to an increase in the intracellular concentration of cAMP and the competitive inhibitors of PKA consistently block the neuroprotective effects. Neuroprotection was also obtained in hippocampal neurons exposed to either recombinant STI1 or the STI1$_{230-245}$ peptide [68]. ST1-PrP^C interactions are responsible for the activation of both ERK and PKA pathways. The latter mediates neuronal survival, which is abrogated by PKA inhibitors, while ERK inhibitors have no effect. On the contrary, the PKA and ERK pathways have been recently shown to act synergistically in mediating memory processing through PrP^C-laminin interactions on the neuronal surface [80]. While the participation of PKA signaling in PrP^C biology is consolidated, there is much less evidence in the literature about a possible involvement of the phospholipid-dependent serine/threonine protein kinase C (PKC) [81, 82]. Using

splenocytes from wild-type and PrP^C-deficient mice, Mazzoni *et al.* [83] discovered that *Prnp*$^{0/0}$ splenocytes display a defective PKCα/β phosphorylation that affects splenocyte mitosis. PKC also seems to be involved in mediating axon elongation in primary rat hippocampal neurons exposed to recombinant PrP, as PKC inhibitors block its effects on neurite outgrowth [60]. Moreover, a putative interaction between PrP^C and PKC was shown by immunoprecipitation in prion protein-enriched fractions from cerebellar granule cells [84]. Recently, a possible role for PKC in prion pathology was described in a murine model of BSE [85]. Decreased levels of phospholipase Cβ1 and PKCδ were detected in BSE-infected bovine-PrP^C transgenic mice 270 days post-inoculation, at a time when pathological signs start manifesting.

PRION PROTEIN AND THE PI3K/AKT PATHWAY

The phosphatidylinositol 3-kinase/AKT (PI3K/AKT) pathway is mainly involved in the regulation of fundamental cellular processes and in cancer progression [86, 87]. The first evidence of a biological effect mediated by PrP^C through the PI3K/AKT cascade was provided by Chen *et al.* [88], who showed that the PI3K inhibitor wortmannin is able to block axon elongation induced by the fusion protein PrP-Fc in mouse cerebellar granules.

In subsequent years, the main role ascribed to the PI3K/AKT pathway was conveying PrP^C-dependent neuroprotective signals in response to different injuries. Indeed, in a model of ischemic brain injury, *Prnp*$^{0/0}$ mice showed increased infarct volume and reduced phospho-AKT levels after ischemia compared to the controls [89]. Moreover, caspase-3 activation was more pronounced in the *Prnp*$^{0/0}$ mouse cortex and basal ganglia than in wild-type animals [89]. The same group later showed that PrP^C overexpression could reduce ischemic areas, but not change post-ischemic AKT phosphorylation [90]. Reduced AKT phosphorylation in response to oxidative stress was also detected in two PrP^C-deficient cell lines, hippocampal HpL and neuroblastoma N2a cells, compared to the PrP^C-bearing counterpart [91, 92]. Interestingly, the effects of PrP^C on PI3/AKT activation seem to rely on copper binding through the octapeptide repeats [91].

The neuroprotective activity of the PI3K/AKT pathway has been demonstrated to be impaired upon exposure to the neurotoxic PrP$_{106-126}$ peptide in neuronal cells [93]. Prion replication seems to interfere with neuronal protein synthesis of survival factors, which is positively stimulated by PI3K [94]. All of the experimental data outline a key role for the PI3K/AKT pathway in the TSE phenotype. Indeed, the disruption of PI3K signaling might explain the cell death that occurs in prion diseases. It could represent a potential target for therapeutic treatments of TSEs.

PRION PROTEIN AND CALCIUM SIGNALING

Intracellular calcium signaling is a universal and versatile regulator of many cellular processes. Several extracellular agonists are able to trigger free intracellular calcium concentration increases by opening selective channels located on the cell membrane and on the membranes of cell organelles [95]. Especially for neurons, local calcium fluctuations are important for synaptogenesis, synaptic transmission, plasticity and cell survival [96].

The first clue of a possible role of prion protein in the regulation of calcium homeostasis was provided by electrophysiological studies on *Prnp* knockout mice. Indeed, disruption of calcium-activated K^+ currents was recorded in hippocampal CA1 pyramidal cells and cerebellar Purkinje cells from PrP^C-deficient mice [97-99]. Herms *et al.* [100] proposed that the lack of PrP^C does not affect the K^+ channels directly, but modulates their activity by controlling intracellular calcium homeostasis via calcium influx through L-type voltage-sensitive calcium channels. This hypothesis was corroborated by several experimental observations. Synaptosomes treated with recombinant PrP show a dose-dependent increase in the intracellular concentration of calcium that is fully blocked by gadolidium chloride, a potent inhibitor of voltage-sensitive calcium channels [101]. Recombinant PrP was also demonstrated to modulate voltage-sensitive calcium channels in primary cerebellar granule cells [102]. A few years later, the initial hypothesis was revisited by Powell *et al.* [103], who explained the differences in calcium homeostasis seen in PrP^C-deficient mice as a consequence of calcium buffering in the endoplasmic reticulum (ER). Lazzari *et al.* [104] described a dramatic increase of cytosolic calcium in PrP^C-deficient granule neurons due to lower expression of PMCA and SERCA, the two major calcium-extruding pumps respectively located on the cell membrane and the ER. Both hypotheses, direct control of calcium homeostasis through modulation of the activity of calcium channels on the cell membrane or indirect control through regulation of the expression of calcium-related proteins in the cytosol, seem realistic. It is not excluded that both mechanisms may concur to the final regulation exerted by PrP^C.

Another piece of evidence of the tight connection between prion biology and calcium signaling is that prion diseases are characterized by derangements in calcium homeostasis. Not surprisingly, dysmetabolism of calcium is a common feature of various neurodegenerative diseases [105]. Kristensson *et al.* [106] were the first to show that prion infection consistently reduces or fully blocks calcium increases upon bradykinin treatment in mouse neuroblastoma N2a and hamster brain HaB cells. A similar impairment was found in calcium-activated K^+ currents in prion-infected mice and hamsters [107, 108]. Notably, prions were shown to impair neuronal excitability and viability by blocking L-type voltage-sensitive calcium channels [109-111]. In addition, alterations in ER calcium homeostasis were registered in infectious and familial models of TSEs [112]. Although not all of the cellular events of PrP^C-mediated calcium signaling

are well understood, perturbations of calcium homeostasis are likely to concur to the neuronal loss observed in TSEs.

PRION PROTEIN SIGNALING: IS THERE A COMMON PATTERN?

Understanding the physiological function of PrP^C is not just a matter of basic science. The identification of the cellular activities in which PrP^C takes part will also help in defining the molecular mechanisms of neurodegeneration elicited by its pathological counterpart. However, the lesson that we can learn from the study of all of the pathways controlled by PrP^C is not immediately obvious. Indeed, the analysis of the whole body of experimental evidence does not lead to a conclusion of an unambiguous role for PrP^C in cell signaling. A considerable number of different and apparently unrelated cellular processes seem to be modulated by PrP^C through different pathways (Fig. 4). Nevertheless, if we focus our attention on the commonalities shared by the different cascades, it can be reasonably stated that PrP^C activity is somehow beneficial for the organism and its absence makes the cells more vulnerable to different types of stress. In particular, PrP^C seems to promote differentiation and cell survival in neuronal and non-neuronal cells via multiple pathways. In this light, it can be speculated that the loss-of-function of PrP^C (due to PrP^C-PrP^{Sc} conversion) might also contribute to the TSE phenotype rather than the sole neurotoxic activity of PrP^{Sc}.
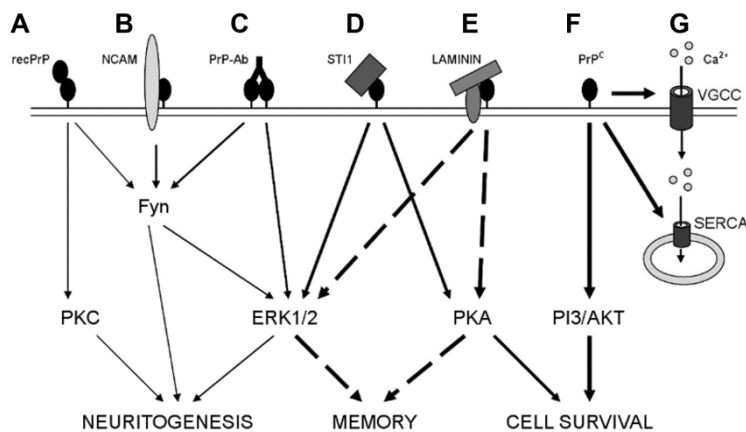

Fig. 4. The role of cellular prion protein in signal transduction. The diagram summarizes the pathways modulated by prion protein in different experimental conditions. In particular, the triggering of signal transduction is shown. This can be due to soluble recombinant PrP (A); Ab-mediated PrP^C dimerization (C); or interactions with physiological ligands such as NCAM (B), STI1 (D) or laminin (E). Moreover, effects on signaling have been detected through comparisons between wild-type and PrP^C-deficient animal models (F). In general, the main biological functions mediated by PrP^C pertain to differentiation (neuritogenesis), cell survival and memory. A role in calcium homeostasis (F) has been also proposed for PrP^C, through action on voltage-gated calcium channels (VGCCs) on the cell membrane or on the SERCA calcium pump on the endoplasmic reticulum.

This hypothesis is supported by the evidence that no strict correlation exists between cell death and PrP^{Sc} deposition within the CNS of TSE patients [113]. The lack of a strong pathological phenotype in PrP^{C}-deficient animal models contrasts with the proposed scenario and still needs to be explained. One possible interpretation could be the existence of compensatory mechanisms supplying PrP^{C} function in steady-state conditions. Alternatively, it may be hypothesized that PrP^{C} is an ancillary protein with no autonomous function, but its presence in the organism is still important because it might work as a fine tuner of specific cellular responses to particular stimuli. In both cases, the investigation of dynamic responses under defined stress conditions may be the key to detecting a clear *Prnp* knockout phenotype.

CONCLUDING REMARKS

If we consider the set of biological processes controlled by PrP^{C}, a significant question remains. What is the biological function of prion protein? Based on the available data, this question could have more than one correct answer. The modular structure, the variety of binding partners and the strategic localization in lipid rafts suggest that PrP^{C} could serve as a dynamic platform on the cell surface with the capability to assemble multicomponent complexes through its different domains and trigger different signaling pathways, according to the physiological state of the cell and the specific cytotype (Fig. 5). The idea that
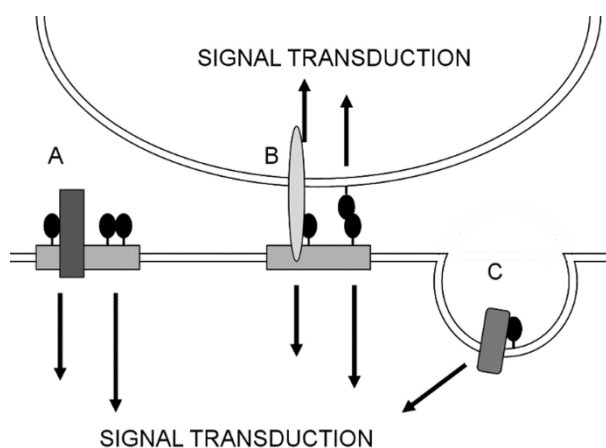
Fig. 5. Cellular prion protein works as a scaffold protein on the cell membrane. Prion protein is able to interact with different binding partners at the cell surface and it takes part in different signal cascades. It can be engaged in homophilic and heterophilic interactions either in *cis* (A) or in *trans* (B). According to the stimuli, it can also be recruited outside of lipids rafts in different membrane sub-domains, such as the endocytic compartment (C).

a single pathway could mediate all the functions associated with PrP^C is counterintuitive. Furthermore, other membrane proteins have shown a broad spectrum of functions contributing to cellular and tissue homeostasis, such as the molecules of the major histocompatibility complex (MHC), which have various functions beyond their classical role in presenting antigens to T cells [114].

Thus, once we accept the idea of PrP^C as a scaffold protein, a reductionist approach may be argued not to represent the optimum strategy to investigate its pleiotropic nature. In this light, the "omic" tools that are now available could greatly help in providing an unbiased global view of the network of processes co-regulated by PrP^C under defined physiological conditions. This systemic approach would aid not only in shedding light on one of the most intriguing puzzles of biology, but also in finding more effective therapies to treat TSEs.

REFERENCES

1. Gajdusek, D.C. Unconventional viruses and the origin and disappearance of kuru. **Science** 197 (1977) 943-960.
2. Prusiner, S.B. Molecular structure, biology, and genetics of prions. **Adv. Virus. Res.** 35 (1988) 83-136.
3. Pan, K.M., Baldwin, M., Nguyen, J., Gasset, M., Serban, A., Groth, D., Mehlhorn, I., Huang, Z., Fletterick, R.J. and Cohen, F.E. Conversion of alpha-helices into beta-sheets features in the formation of the scrapie prion proteins. **Proc. Natl. Acad. Sci. USA** 90 (1993) 10962-10966.
4. Benetti, F. and Legname, G. De novo mammalian prion synthesis. **Prion** 3 (2009) 213-219.
5. Cohen, F.E., Pan, K.M., Huang, Z., Baldwin, M., Fletterick, R.J. and Prusiner, S.B. Structural clues to prion replication. **Science** 264 (1994) 530-531.
6. Prusiner, S.B., Groth, D.F., Bolton, D.C., Kent, S.B. and Hood, L.E. Purification and structural studies of a major scrapie prion protein. **Cell** 38 (1984) 127-134.
7. Oesch, B., Westaway, D., Wälchli, M., McKinley, M.P., Kent, S.B., Aebersold, R., Barry, R.A., Tempst, P., Teplow, D.B., Hood, L.E., Prusiner, S.B. and Weissmann, C. A cellular gene encodes scrapie PrP 27-30 protein. **Cell** 40 (1985) 735-746.
8. Gabriel, J.M., Oesch, B., Kretzschmar, H., Scott, M. and Prusiner, S.B. Molecular cloning of a candidate chicken prion protein. **Proc. Natl. Acad. Sci. USA** 89 (1992) 9097-9101.
9. Schatzl, H.M., Da Costa, M., Taylor, L., Cohen, F.E. and Prusiner, S.B. Prion protein gene variation among primates. **J. Mol. Biol.** 245 (1995) 362-374.
10. Basler, K., Oesch, B., Scott, M., Westaway, D., Wälchli, M., Groth, D.F., McKinley, M.P., Prusiner, S.B. and Weissmann, C. Scrapie and cellular PrP isoforms are encoded by the same chromosomal gene. **Cell** 46 (1986) 417-428.

11. Kretzschmar, H.A., Prusiner, S.B., Stowring, L.E. and DeArmond, S.J. Scrapie prion proteins are synthesized in neurons. **Am. J. Pathol.** 122 (1986) 1-5.

12. Viles, J.H., Cohen, F.E., Prusiner, S.B., Goodin, D.B., Wright, P.E. and Dyson, H.J. Copper binding to the prion protein: structural implications of four identical cooperative binding sites. **Proc. Natl. Acad. Sci. USA** 96 (1999) 2042-2047.

13. Zahn, R., Liu, A., Luhrs, T., Riek, R., von Schroetter, C., Garcia, F.L., Billeter, M., Calzolai, L., Wider, G. and Wüthrich, K. NMR solution structure of the human prion protein. **Proc. Natl. Acad. Sci. USA** 97 (2000) 145-150.

14. Stahl, N., Baldwin, M.A., Burlingame, A.L. and Prusiner, S.B. Identification of glycoinositol phospholipid linked and truncated forms of the scrapie prion protein. **Biochemistry** 29 (1990) 8879-8884.

15. Vey, M., Pilkuhn, S., Wille, H., Nixon, R., DeArmond, S.J., Smart, E.J., Anderson, R.G.W., Taraboulos, A. and Prusiner, S.B. Subcellular colocalization of the cellular and scrapie prion proteins in caveolae-like membranous domains. **Proc. Natl. Acad. Sci. USA** 93 (1996) 14945-14949.

16. Naslavsky, N., Stein, R., Yanai, A., Friedlander, G. and Taraboulos, A. Characterization of detergent-insoluble complexes containing the cellular prion protein and its scrapie isoform. **J. Biol. Chem.** 272 (1997) 6324-6331.

17. Shyng, S.L., Huber, M.T. and Harris, D.A. A prion protein cycles between the cell surface and an endocytic compartment in cultured neuroblastoma cells. **J. Biol. Chem.** 268 (1993) 15922-15928.

18. Anderson, R.G. The caveolae membrane system. **Annu. Rev. Biochem.** 67 (1998) 199-225.

19. Taylor, D.R. and Hooper, N.M. The prion protein and lipid rafts. **Mol. Membr. Biol.** 23 (2006) 89-99.

20. Borchelt, D.R., Taraboulos, A. and Prusiner, S.B. Evidence for synthesis of scrapie prion proteins in the endocytic pathway. **J. Biol. Chem.** 267 (1992) 16188-16199.

21. Didonna, A., Vaccari, L., Bek, A. and Legname, G. Infrared microspectroscopy: a multiple-screening platform for investigating single-cell biochemical perturbations upon prion infection. **ACS Chem. Neurosci.** 2 (2011) 160-174.

22. Bueler, H., Fischer, M., Lang, Y., Bluethmann, H., Lipp, H.P., DeArmond, S.J., Prusiner, S.B., Aguet, M. and Weissmann, C. Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. **Nature** 356 (1992) 577-582.

23. Manson, J.C., Clarke, A.R., Hooper, M.L., Aitchison, L., McConnell, I. and Hope, J. 129/Ola mice carrying a null mutation in PrP that abolishes mRNA production are developmentally normal. **Mol. Neurobiol.** 8 (1994) 121-127.

24. Tobler, I., Gaus, S.E., Deboer, T., Achermann, P., Fischer, M., Rülicke, T., Moser, M., Oesch, B., McBride, P.A. and Manson, J.C. Altered circadian

activity rhythms and sleep in mice devoid of prion protein. **Nature** <u>380</u> (1996) 639-642.

25. Coitinho, A.S., Roesler, R., Martins, V.R., Brentani, R.R. and Izquierdo, I. Cellular prion protein ablation impairs behavior as a function of age. **Neuroreport** <u>14</u> (2003) 1375-1379.

26. Criado, J.R., Sánchez-Alavez, M., Conti, B., Giacchino, J.L., Wills, D.N., Henriksen, S.J., Race, R., Manson, J.C., Chesebro, B. and Oldstone, M.B. Mice devoid of prion protein have cognitive deficits that are rescued by reconstitution of PrP in neurons. **Neurobiol. Dis.** <u>19</u> (2005) 255-265.

27. Sakaguchi, S., Katamine, S., Nishida, N., Moriuchi, R., Shigematsu, K., Sugimoto, T., Nakatani, A., Kataoka, Y., Houtani, T., Shirabe, S., Okada, H., Hasegawa, S., Miyamoto, T. and Noda, T. Loss of cerebellar Purkinje cells in aged mice homozygous for a disrupted PrP gene. **Nature** <u>380</u> (1996) 528-531.

28. Moore, R.C., Lee, I.Y., Silverman, G.L., Harrison, P.M., Strome, R., Heinrich, C., Karunaratne, A., Pasternak, S.H., Chishti, M.A., Liang, Y., Mastrangelo, P., Wang, K., Smit, A.F., Katamine, S., Carlson, G.A., Cohen, F.E., Prusiner, S.B., Melton, D.W., Tremblay, P., Hood, L.E. and Westaway, D. Ataxia in prion protein (PrP)-deficient mice is associated with upregulation of the novel PrP-like protein doppel. **J. Mol. Biol.** <u>292</u> (1999) 797-817.

29. Didonna, A., Sussman, J., Benetti, F. and Legname, G. The role of Bax and caspase-3 in doppel-induced apoptosis of cerebellar granule cells. **Prion** <u>6</u> (2012) 309-316.

30. Mallucci, G.R., Ratte, S., Asante, E.A., Linehan, J., Gowland, I., Jefferys, J.G. and Collinge, J. Post-natal knockout of prion protein alters hippocampal CA1 properties, but does not result in neurodegeneration. **EMBO J.** <u>21</u> (2002) 202-210.

31. Richt, J.A., Kasinathan, P., Hamir, A.N., Castilla, J., Sathiyaseelan, T., Vargas, F., Sathiyaseelan, J., Wu, H., Matsushita, H., Koster, J., Kato, S., Ishida, I., Soto, C., Robl, J.M. and Kuroiwa, Y. Production of cattle lacking prion protein. **Nat. Biotechnol.** <u>25</u> (2007) 132-138.

32. Yu, G., Chen, J., Xu, Y., Zhu, C., Yu, H., Liu, S., Sha, H., Chen, J., Xu, X., Wu, Y., Zhang, A., Ma, J. and Cheng, G. Generation of goats lacking prion protein. **Mol. Reprod. Dev.** <u>76</u> (2009) 3.

33. Bueler, H., Aguzzi, A., Sailer, A., Greiner, R.A., Autenried, P., Aguet, M. and Weissmann, C. Mice devoid of PrP are resistant to scrapie. **Cell** <u>73</u> (1993) 1339-1347.

34. Malaga-Trillo, E., Solis, G.P., Schrock, Y., Geiss, C., Luncz, L., Thomanetz, V. and Stuermer, C.A. Regulation of embryonic cell adhesion by the prion protein. **PLOS Biol.** <u>7</u> (2009) e55.

35. Brown, D.R., Qin, K., Herms, J.W., Madlung, A., Manson, J., Strome, R., Fraser, P.E., Kruck, T., von Bohlen, A., Schulz-Schaeffer, W., Giese, A., Westaway, D. and Kretzschmar, H. The cellular prion protein binds copper in vivo. **Nature** <u>390</u> (1997) 684-687.

36. Brown, D.R., Clive, C. and Haswell, S.J. Antioxidant activity related to copper binding of native prion protein. **J. Neurochem.** <u>76</u> (2001) 69-76.
37. Tobler, I., Deboer, T. and Fischer, M. Sleep and sleep regulation in normal and prion protein-deficient mice. **J. Neurosci.** <u>17</u> (1997) 1869-1879.
38. Le Pichon, C.E., Valley, M.T., Polymenidou, M., Chesler, A.T., Sagdullaev, B.T., Aguzzi, A. and Firestein, S. Olfactory behavior and physiology are disrupted in prion protein knockout mice. **Nat. Neurosci.** <u>12</u> (2009) 60-69.
39. Bremer, J., Baumann, F., Tiberi, C., Wessig, C., Fischer, H., Schwarz, P., Steele, A.D., Toyka, K.V., Nave, K.A., Weis, J. and Aguzzi, A. Axonal prion protein is required for peripheral myelin maintenance. **Nat. Neurosci.** <u>13</u> (2010) 310-318.
40. Zhang, C.C., Steele, A.D., Lindquist, S. and Lodish, H.F. Prion protein is expressed on long-term repopulating hematopoietic stem cells and is important for their self-renewal. **Proc. Natl. Acad. Sci. USA** <u>103</u> (2006) 2184-2189.
41. Isaacs, J.D., Jackson, G.S. and Altmann, D.M. The role of the cellular prion protein in the immune system. **Clin. Exp. Immunol.** <u>146</u> (2006) 1-8.
42. Shmerling, D., Hegyi, I., Fischer, M., Blättler, T., Brandner, S., Götz, J., Rülicke, T., Flechsig, E., Cozzio, A., von Mering, C., Hangartner, C., Aguzzi, A. and Weissmann, C. Expression of amino-terminally truncated PrP in the mouse leading to ataxia and specific cerebellar lesions. **Cell** <u>93</u> (1998) 203-214.
43. Simons, K. and Toomre, D. Lipid rafts and signal transduction. **Nat. Rev. Mol. Cell Biol.** <u>1</u> (2000) 31-39.
44. Pawson, T. Protein modules and signalling networks. **Nature** <u>373</u> (1995) 573-580.
45. Aguzzi, A., Baumann, F. and Bremer, J. The prion's elusive reason for being. **Annu. Rev. Neurosci.** <u>31</u> (2008) 439-477.
46. Schmitt-Ulms, G., Legname, G., Baldwin, M.A., Ball, H.L., Bradon, N., Bosque, P.J., Crossin, K.L., Edelman, G.M., DeArmond, S.J., Cohen, F.E. and Prusiner, S.B. Binding of neural cell adhesion molecules (N-CAMs) to the cellular prion protein. **J. Mol. Biol.** <u>314</u> (2001) 1209-1225.
47. Gauczynski, S., Peyrin, J.M., Haïk, S., Leucht, C., Hundt, C., Rieger, R., Krasemann, S., Deslys, J.P., Dormont, D., Lasmézas, C.I. and Weiss, S. The 37-kDa/67-kDa laminin receptor acts as the cell-surface receptor for the cellular prion protein. **EMBO J.** <u>20</u> (2001) 5863-5875.
48. Graner, E., Mercadante, A.F., Zanata, S.M., Forlenza, O.V., Cabral, A.L., Veiga, S.S., Juliano, M.A., Roesler, R., Walz, R., Minetti, A., Izquierdo, I., Martins, V.R. and Brentani, R.R. Cellular prion protein binds laminin and mediates neuritogenesis. **Brain Res. Mol. Brain Res.** <u>76</u> (2000) 85-92.
49. Martins, V.R., Graner, E., Garcia-Abreu, J., de Souza, S.J., Mercadante, A.F., Veiga, S.S., Zanata, S.M., Neto, V.M. and Brentani, R.R. Complementary hydropathy identifies a cellular prion protein receptor. **Nat. Med.** <u>3</u> (1997) 1376-1382.

50. Zanata, S.M., Lopes, M.H., Mercadante, A.F., Hajj, G.N.M., Chiarini, L.B., Nomizo, R., Freitas, A.R.O., Cabral, A.L.B., Lee, K.S., Juliano, M.A., de Oliveira, E., Jachieri, S.G., Burlingame, A., Huang, L., Linden, R., Brentani, R.R. and Martins, V.R. Stress-inducible protein 1 is a cell surface ligand for cellular prion that triggers neuroprotection. **EMBO J.** 21 (2002) 3307-3316.

51. Rutishauser, D., Mertz, K.D., Moos, R., Brunner, E., Rulicke, T., Calella, A.M. and Aguzzi, A. The comprehensive native interactome of a fully functional tagged prion protein. **PLOS One** 4 (2009) e4446.

52. Lauren, J., Gimbel, D.A., Nygaard, H.B., Gilbert, J.W. and Strittmatter, S.M. Cellular prion protein mediates impairment of synaptic plasticity by amyloid-beta oligomers. **Nature** 457 (2009) 1128-1132.

53. Kessels, H.W., Nguyen, L.N., Nabavi, S. and Malinow, R. The prion protein as a receptor for amyloid-beta. **Nature** 466 (2010) E3-4; discussion E4-5.

54. Balducci, C., Beeg, M., Stravalaci, M., Bastone, A., Sclip, A., Biasini, E., Tapella, L., Colombo, L., Manzoni, C., Borsello, T., Chiesa, R., Gobbi, M., Salmona, M. and Forloni, G. Synthetic amyloid-beta oligomers impair long-term memory independently of cellular prion protein. **Proc. Natl. Acad. Sci. USA** 107 (2010) 2295-2300.

55. Calella, A.M., Farinelli, M., Nuvolone, M., Mirante, O., Moos, R., Falsig, J., Mansuy, I.M. and Aguzzi, A. Prion protein and Abeta-related synaptic toxicity impairment. **EMBO Mol. Med.** 2 (2010) 306-314.

56. Mouillet-Richard, S., Ermonval, M., Chebassier, C., Laplanche, J.L., Lehmann, S., Launay, J.M. and Kellermann, O. Signal transduction through prion protein. **Science** 289 (2000) 1925-1928.

57. Yeatman, T.J. A renaissance for SRC. **Nat. Rev. Cancer** 4 (2004) 470-480.

58. Beggs, H.E., Soriano, P. and Maness, P.F. NCAM-dependent neurite outgrowth is inhibited in neurons from Fyn-minus mice. **J. Cell Biol.** 127 (1994) 825-833.

59. Santuccione, A., Sytnyk, V., Leshchyns'ka, I. and Schachner, M. Prion protein recruits its neuronal receptor NCAM to lipid rafts to activate p59fyn and to enhance neurite outgrowth. **J. Cell Biol.** 169 (2005) 341-354.

60. Kanaani, J., Prusiner, S.B., Diacovo, J., Baekkeskov, S. and Legname, G. Recombinant prion protein induces rapid polarization and development of synapses in embryonic rat hippocampal neurons in vitro. **J. Neurochem.** 95 (2005) 1373-1386.

61. Schwarz, A., Burwinkel, M., Riemer, C., Schultz, J. and Baier, M. Unchanged scrapie pathology in brain tissue of tyrosine kinase Fyn-deficient mice. **Neurodegener. Dis.** 1 (2004) 266-268.

62. Nixon, R.R. Prion-associated increases in Src-family kinases. **J. Biol. Chem.** 280 (2005) 2455-2462.

63. Gyllberg, H., Lofgren, K., Lindegren, H. and Bedecs, K. Increased Src kinase level results in increased protein tyrosine phosphorylation in scrapie-infected neuronal cell lines. **FEBS Lett.** 580 (2006) 2603-2608.

64. Didonna, A. and Legname, G. Aberrant ERK 1/2 complex activation and localization in scrapie-infected GT1-1 cells. **Mol. Neurodegener.** <u>5</u> (2010) 29.

65. Um, J.W., Nygaard, H.B., Heiss, J.K., Kostylev, M.A., Stagi, M., Vortmeyer, A., Wisniewski, T., Gunther, E.C. and Strittmatter, S.M. Alzheimer amyloid-beta oligomer bound to postsynaptic prion protein activates Fyn to impair neurons. **Nat. Neurosci.** <u>15</u> (2012) 1227-1235.

66. Schneider, B., Mutel, V., Pietri, M., Ermonval, M., Mouillet-Richard, S. and Kellermann, O. NADPH oxidase and extracellular regulated kinases 1/2 are targets of prion protein signaling in neuronal and nonneuronal cells. **Proc. Natl. Acad. Sci. USA** <u>100</u> (2003) 13326-13331.

67. Suganuma, T. and Workman, J.L. MAP kinases and histone modification. **J. Mol. Cell. Biol.** <u>34</u> (2010) 1543-1551.

68. Lopes, M.H., Hajj, G.N., Muras, A.G., Mancini, G.L., Castro, R.M., Ribeiro, K.C., Brentani, R.R., Linden, R. and Martins, V.R. Interaction of cellular prion and stress-inducible protein 1 promotes neuritogenesis and neuroprotection by distinct signaling pathways. **J. Neurosci.** <u>25</u> (2005) 11330-11339.

69. Pietri, M., Caprini, A., Mouillet-Richard, S., Pradines, E., Ermonval, M., Grassi, J., Kellermann, O. and Schneider, B. Overstimulation of PrPC signaling pathways by prion peptide 106-126 causes oxidative injury of bioaminergic neuronal cells. **J. Biol. Chem.** <u>281</u> (2006) 28470-28479.

70. Marella, M., Gaggioli, C., Batoz, M., Deckert, M., Tartare-Deckert, S. and Chabry, J. Pathological prion protein exposure switches on neuronal mitogen-activated protein kinase pathway resulting in microglia recruitment. **J. Biol. Chem.** <u>280</u> (2005) 1529-1534.

71. Lee, H.P., Jun, Y.C., Choi, J.K., Kim, J.I., Carp, R.I. and Kim, Y.S. Activation of mitogen-activated protein kinases in hamster brains infected with 263K scrapie agent. **J. Neurochem.** <u>95</u> (2005) 584-593.

72. Carimalo, J., Cronier, S., Petit, G., Peyrin, J.M., Boukhtouche, F., Arbez, N., Lemaigre-Dubreuil, Y., Brugg, B. and Miquel, M.C. Activation of the JNK-c-Jun pathway during the early phase of neuronal apoptosis induced by PrP106-126 and prion infection. **Eur. J. Neurosci.** <u>21</u> (2005) 2311-2319.

73. Thellung, S., Villa, V., Corsaro, A., Pellistri, F., Venezia, V., Russo, C., Aceto, A., Robello, M. and Florio, T. ERK1/2 and p38 MAP kinases control prion protein fragment 90-231-induced astrocyte proliferation and microglia activation. **Glia** <u>55</u> (2007) 1469-1485.

74. Brown, D.R., Schulz-Schaeffer, W.J., Schmidt, B. and Kretzschmar, H.A. Prion protein-deficient cells show altered response to oxidative stress due to decreased SOD-1 activity. **Exp. Neurol.** <u>146</u> (1997) 104-112.

75. Nordstrom, E.K., Luhr, K.M., Ibanez, C. and Kristensson, K. Inhibitors of the mitogen-activated protein kinase kinase 1/2 signaling pathway clear prion-infected cells from PrPSc. **J. Neurosci.** <u>25</u> (2005) 8451-8456.

76. Uppington, K.M. and Brown, D.R. Resistance of cell lines to prion toxicity aided by phospho-ERK expression. **J. Neurochem.** <u>105</u> (2008) 842-852.

77. Pidoux, G. and Tasken, K. Specificity and spatial dynamics of protein kinase A signaling organized by A-kinase-anchoring proteins. **J. Mol. Endocrinol.** 44 (2010) 271-284.

78. Howe, A.K. Cross-talk between calcium and protein kinase A in the regulation of cell migration. **Curr. Opin. Cell Biol.** 23 (2011) 554-561.

79. Chiarini, L.B., Freitas, A.R., Zanata, S.M., Brentani, R.R., Martins, V.R. and Linden, R. Cellular prion protein transduces neuroprotective signals. **EMBO J.** 21 (2002) 3317-3326.

80. Coitinho, A.S., Freitas, A.R., Lopes, M.H., Hajj, G.N., Roesler, R., Walz, R., Rossato, J.I., Cammarota, M., Izquierdo, I., Martins, V.R. and Brentani, R.R. The interaction between prion protein and laminin modulates memory consolidation. **Eur. J. Neurosci.** 24 (2006) 3255-3264.

81. Kang, J.H., Toita, R., Kim, C.W. and Katayama, Y. Protein kinase C (PKC) isozyme-specific substrates and their design. **Biotechnol. Adv.** 30 (2012) 1662-1672.

82. Zhang, B. and Xia, C. 12-O-tetradecanoylphorbol-1, 3-acetate induces the negative regulation of protein kinase B by protein kinase Calpha during gastric cancer cell apoptosis. **Cell. Mol. Biol. Lett.** 15 (2010) 377-394.

83. Mazzoni, I.E., Ledebur, H.C.,Jr., Paramithiotis, E. and Cashman, N. Lymphoid signal transduction mechanisms linked to cellular prion protein. **Biochem. Cell. Biol.** 83 (2005) 644-653.

84. Botto, L., Masserini, M., Cassetti, A. and Palestini, P. Immunoseparation of prion protein-enriched domains from other detergent-resistant membrane fractions, isolated from neuronal cells. **FEBS Lett.** 557 (2004) 143-147.

85. Rodriguez, A., Martin, M., Albasanz, J.L., Barrachina, M., Espinosa, J.C., Torres, J.M. and Ferrer, I. Group I mGluR signaling in BSE-infected bovine-PrP transgenic mice. **Neurosci. Lett.** 410 (2006) 115-120.

86. Bartholomeusz, C. and Gonzalez-Angulo, A.M. Targeting the PI3K signaling pathway in cancer therapy. **Expert Opin. Ther. Targets** 16 (2012) 121-130.

87. Huang, J.G., Xia, C., Zheng, X.P., Yi, T.T., Wang, X.Y., Song, G. and Zhang, B. 17beta-Estradiol promotes cell proliferation in rat osteoarthritis model chondrocytes via PI3K/Akt pathway. **Cell. Mol. Biol. Lett.** 16 (2011) 564-575.

88. Chen, S., Mange, A., Dong, L., Lehmann, S. and Schachner, M. Prion protein as trans-interacting partner for neurons is involved in neurite outgrowth and neuronal survival. **Mol. Cell. Neurosci.** 22 (2003) 227-233.

89. Weise, J., Sandau, R., Schwarting, S., Crome, O., Wrede, A., Schulz-Schaeffer, W., Zerr, I. and Bahr, M. Deletion of cellular prion protein results in reduced Akt activation, enhanced postischemic caspase-3 activation, and exacerbation of ischemic brain injury. **Stroke** 37 (2006) 1296-1300.

90. Weise, J., Doeppner, T.R., Muller, T., Wrede, A., Schulz-Schaeffer, W., Zerr, I., Witte, O.W. and Bahr, M. Overexpression of cellular prion protein alters postischemic Erk1/2 phosphorylation but not Akt phosphorylation and

protects against focal cerebral ischemia. **Restor. Neurol. Neurosci.** 26 (2008) 57-64.

91. Vassallo, N., Herms, J., Behrens, C., Krebs, B., Saeki, K., Onodera, T., Windl, O. and Kretzschmar, H.A. Activation of phosphatidylinositol 3-kinase by cellular prion protein and its role in cell survival. **Biochem. Biophys. Res. Commun.** 332 (2005) 75-82.

92. Schmalzbauer, R., Eigenbrod, S., Winoto-Morbach, S., Xiang, W., Schutze, S., Bertsch, U. and Kretzschmar, H.A. Evidence for an association of prion protein and sphingolipid-mediated signaling. **J. Neurochem.** 106 (2008) 1459-1470.

93. Seo, J.S., Seol, J.W., Moon, M.H., Jeong, J.K., Lee, Y.J. and Park, S.Y. Hypoxia protects neuronal cells from human prion protein fragment-induced apoptosis. **J. Neurochem.** 112 (2010) 715-722.

94. Roffe, M., Beraldo, F.H., Bester, R., Nunziante, M., Bach, C., Mancini, G., Gilch, S., Vorberg, I., Castilho, B.A., Martins, V.R. and Hajj, G.N. Prion protein interaction with stress-inducible protein 1 enhances neuronal protein synthesis via mTOR. **Proc. Natl. Acad. Sci. USA** 107 (2010) 13147-13152.

95. Munaron, L. and Scianna, M. Multilevel complexity of calcium signaling: Modeling angiogenesis. **World J. Biol. Chem.** 3 (2012) 121-126.

96. Peggion, C., Bertoli, A. and Sorgato, M.C. Possible role for Ca2+ in the pathophysiology of the prion protein? **Biofactors** 37 (2011) 241-249.

97. Colling, S.B., Collinge, J. and Jefferys, J.G. Hippocampal slices from prion protein null mice: disrupted Ca(2+)-activated K+ currents. **Neurosci. Lett.** 209 (1996) 49-52.

98. Herms, J.W., Korte, S., Gall, S., Schneider, I., Dunker, S. and Kretzschmar, H.A. Altered intracellular calcium homeostasis in cerebellar granule cells of prion protein-deficient mice. **J. Neurochem.** 75 (2000) 1487-1492.

99. Herms, J.W., Tings, T., Dunker, S. and Kretzschmar, H.A. Prion protein affects Ca2+-activated K+ currents in cerebellar purkinje cells. **Neurobiol. Dis.** 8 (2001) 324-330.

100. Fuhrmann, M., Bittner, T., Mitteregger, G., Haider, N., Moosmang, S., Kretzschmar, H. and Herms, J. Loss of the cellular prion protein affects the Ca2+ homeostasis in hippocampal CA1 neurons. **J. Neurochem.** 98 (2006) 1876-1885.

101. Whatley, S.A., Powell, J.F., Politopoulou, G., Campbell, I.C., Brammer, M.J. and Percy, N.S. Regulation of intracellular free calcium levels by the cellular prion protein. **Neuroreport** 6 (1995) 2333-2337.

102. Korte, S., Vassallo, N., Kramer, M.L., Kretzschmar, H.A. and Herms, J. Modulation of L-type voltage-gated calcium channels by recombinant prion protein. **J. Neurochem.** 87 (2003) 1037-1042.

103. Powell, A.D., Toescu, E.C., Collinge, J. and Jefferys, J.G. Alterations in Ca2+-buffering in prion-null mice: association with reduced afterhyperpolarizations in CA1 hippocampal neurons. **J. Neurosci.** 28 (2008) 3877-3886.

104. Lazzari, C., Peggion, C., Stella, R., Massimino, M.L., Lim, D., Bertoli, A. and Sorgato, M.C. Cellular prion protein is implicated in the regulation of local Ca2+ movements in cerebellar granule neurons. **J. Neurochem.** 116 (2011) 881-890.

105. Mattson, M.P. Calcium and neurodegeneration. **Aging Cell** 6 (2007) 337-350.

106. Kristensson, K., Feuerstein, B., Taraboulos, A., Hyun, W.C., Prusiner, S.B. and DeArmond, S.J. Scrapie prions alter receptor-mediated calcium responses in cultured cells. **Neurology** 43 (1993) 2335-2341.

107. Barrow, P.A., Holmgren, C.D., Tapper, A.J. and Jefferys, J.G. Intrinsic physiological and morphological properties of principal cells of the hippocampus and neocortex in hamsters infected with scrapie. **Neurobiol. Dis.** 6 (1999) 406-423.

108. Johnston, A.R., Fraser, J.R., Jeffrey, M. and MacLeod, N. Alterations in potassium currents may trigger neurodegeneration in murine scrapie. **Exp. Neurol.** 151 (1998) 326-333.

109. Florio, T., Thellung, S., Amico, C., Robello, M., Salmona, M., Bugiani, O., Tagliavini, F., Forloni, G. and Schettini, G. Prion protein fragment 106-126 induces apoptotic cell death and impairment of L-type voltage-sensitive calcium channel activity in the GH3 cell line. **J. Neurosci. Res.** 54 (1998) 341-352.

110. Thellung, S., Florio, T., Villa, V., Corsaro, A., Arena, S., Amico, C., Robello, M., Salmona, M., Forloni, G., Bugiani, O., Tagliavini, F. and Schettini, G. Apoptotic cell death and impairment of L-type voltage-sensitive calcium channel activity in rat cerebellar granule cells treated with the prion protein fragment 106-126. **Neurobiol. Dis.** 7 (2000) 299-309.

111. Sandberg, M.K., Wallen, P., Wikstrom, M.A. and Kristensson, K. Scrapie-infected GT1-1 cells show impaired function of voltage-gated N-type calcium channels (Ca(v) 2.2) which is ameliorated by quinacrine treatment. **Neurobiol. Dis.** 15 (2004) 143-151.

112. Torres, M., Castillo, K., Armisen, R., Stutzin, A., Soto, C. and Hetz, C. Prion protein misfolding affects calcium homeostasis and sensitizes cells to endoplasmic reticulum stress. **PLOS One** 5 (2010) e15658.

113. Gray, F., Adle-Biassette, H., Chretien, F., Ereau, T., Delisle, M.B. and Vital, C. Neuronal apoptosis in human prion diseases. **Bull. Acad. Natl. Med.** 183 (1999) 305-320; discussion 320-301.

114. Ojcius, D.M., Delarbre, C., Kourilsky, P. and Gachelin, G. MHC and MHC-related proteins as pleiotropic signal molecules. **FASEB J.** 16 (2002) 202-206.