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Review

Protein misfolding and disease: the case of prion disorders

C. Hetz[a,b] and C. Soto[a,*]

[a] Serono Pharmaceutical Research Institute, 14 Chemin des Aulx, 1228 Plan les Ouates (Switzerland)
[b] Instituto de Ciencias Biomédicas, Universidad de Chile, Santiago (Chile)
Current address: Serono International, 15 Chemin des Mines, 1211 Geneva, Switzerland,
e-mail: Claudio.soto@serono.com

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Abstract. Recent findings strongly support the hypothesis that diverse human disorders, including the most common neurodegenerative diseases, arise from misfolding and aggregation of an underlying protein. Despite the good evidence for the involvement of protein misfolding in disease pathogenesis, the mechanism by which protein conformational changes participate in the disease is still unclear. Among the best-studied diseases of this group are the transmissible spongiform encephalopathies or prion-related disorders, in which misfolding of the normal prion protein plays a key role in the disease. In this article we review recent data on the link between prion protein misfolding and the pathogensis of spongiform encephalopathies.

Key words. Protein conformational disorders; prion protein misfolding; transmissible spongiform encephalopathies; neuronal apoptosis; brain inflammation; prion protein function.

Introduction

Analysis of the neuropathological characteristics of several degenerative diseases has revealed common features underlying the mechanism of disease initiation and progression. In the last few years, protein misfolding has been proposed to be a central aspect of diverse diseases which are now classified as protein conformational disorders (PCDs) [1, 2]. This group includes transmissible spongiform encephalopathies (TSEs), Alzheimer disease, diabetes type 2, Hungtington disease, serpin-deficiency disorders, Parkinson disease, amyloid polyneuropathy, haemodialysis-related amyloidosis and several others (table 1). Despite the very different clinical manifestations of diverse PCDs, there are many similarities at the molecular level. In the cases of PCDs in which the protein involved is known, the pathological conformation is rich in β-sheet structure and shows a capacity to oligomerize or aggregate due to protein stabilization by intermolecular β-sheet interactions [2]. This leads to accumulation of different forms of protein aggregates in diverse tissues of affected individuals (table 1). Among the PCDs that have been extensively studied in terms of the involvement of protein misfolding in the pathogenesis of the disease is TSE, or prion disease.

TSEs are rare fatal neurodegenerative diseases of humans and other animals [3, 4]. Primary symptoms include progressive dementia and ataxia [5]. The hallmark pathological features of TSEs are spongiform degeneration of the brain, accompanied by extensive astrogliosis, and accumulation of the abnormal, protease-resistant prion protein (PrP) isoform in the central nervous system, which sometimes forms amyloid-like plaques [3, 6]. TSEs in humans can be divided in three groups: familial, sporadic and infectious. Human familial TSEs are all associated with different mutations in the PrP gene, and include some forms of Creutzfeldt-Jakob disease (CJD), Gertmann-Straussler-Sheinker (GSS) syndrome and fa-

* Corresponding author.

Table 1. Common features of some neurodegenerative diseases that have been classified in the group of protein conformational disorders.

Disease	Protein involved	Type of aggregates	Brain inflammation	Neuronal apoptosis	Proposed function normal protein
TSEs	Prion protein	oligomers/plaques	extensive	extensive	signal transduction, antioxidant, copper binding
Alzheimer	amyloid-β	amyloid plaques	extensive	sporadic	neurite outgrowth, synaptic vesicle transport
Parkinson	α-synuclein	Lewy bodies	sporadic	extensive	regulation of membrane stability/or turnover
Huntington	Huntington	nuclear inclusions	N.D.	extensive	transcriptional regulation

N.D., not well determined in human samples.

tal familial insomnia (FFI) [7]. Sporadic CJD has not been associated with any known mutation and occurs worldwide with an incidence of 0.5–1.5 new cases per 1 million people each year [8]. Infectious TSE diseases include kuru, which was propagated by ritualistic cannibalism, and iatrogenic CJD, which is spread by tissue transplantation, contamination of surgical tools or inoculation with materials derived from CJD-infected tissues [4]. New variant CJD (vCJD) is a novel infectious disease which was first described in 1996 [9]. In animals, the most common disease is scrapie in sheep and goats, and bovine spongiform encephalopathy (BSE) or 'mad cow disease' in cattle [3]. There is strong evidence linking the appearance of vCJD in humans to exposure to the BSE agent, most possibly through dietary contamination with BSE tissue [10, 11].

Despite the fact that TSEs are relatively rare diseases, they have gained significant attention from the scientific community and society in general [12], affecting medical, agricultural, economic and political issues in Europe. Although an estimated 750,000 BSE-infected cattle were eaten by humans between about 1980 and 1996, it is impossible to make any well-founded predictions about the future number of vCJD cases, because insufficient information is currently available regarding the incubation time and the actual level of exposure to contaminated material [13].

In the present article, we review the putative mechanism by which protein misfolding and aggregation is associated with the tissue damage and organ dysfunction using TSEs as a model. The major aim is to put together findings related to the cellular mechanism by which misfolding of the PrP is associated with neurodegeneration in TSEs. Although we describe briefly some aspects of the structure of the PrP, the mechanism of prion replication and the nature of the infectious agent, more in-depth reviews about these aspects can be found elsewhere [3, 4, 12, 14–17].

Disease propagation by replication of PrP misfolding

As mentioned above, a hallmark feature of TSE is accumulation in the brain of affected individuals of PrP^{SC}, a misfolded form of normal prion protein. Human PrP is the product of a single gene which leads to synthesis of a protein of 253 amino acids containing five octapeptide repeats near the amino-terminal, two glycosylated sites and one disulfide bridge (fig. 1A). In addition, a glycosylphosphatidylinositol anchor (GPI) attaches the protein to the outer surface of the cell membrane [18]. The *PrP* gene is constitutively expressed in the brain and other tissues of healthy people and animals. No sequence or post-translational differences have been detected between the normal host cell surface PrP, termed PrP^{C}, and the pathological PrP^{SC} isoform [4]. The conversion of PrP^{C} into PrP^{SC} involves a conformational change whereby the α-helical content diminishes and the amount of β sheet increases [19] (fig. 1B). This structural transition is accompanied by profound changes in the biochemical properties of the protein: PrP^{C} is soluble in nondenaturating detergents, whereas PrP^{SC} is insoluble and forms aggregates in infected brain parenchyma. PrP^{C} is readily digested by proteases, while PrP^{SC} is partially resistant. However, even though PrP^{SC} has a marked tendency to aggregate in vitro, forming amyloid prion rods, accumulation of amyloid plaques in the brain is observed only in a small percentage of TSE cases [4]. The tridimensional structure of several fragments as well as the full-length recombinant PrP has been obtained from different species [20–24]. Nuclear magnetic resonance (NMR) studies of the human recombinant protein show a structured globular domain extending from residues 125 to 228 and a N-terminal flexible and disordered region. The globular domain contains three α helices comprising residues 144–154, 173–194, and 200–228, and an antiparallel β sheet consisting of two short strands comprising residues 128–131 and 161–164 [22]. Recently, the crystalline structure of a dimeric form of recombinant human PrP^{C} was obtained by X-ray diffraction [25]. The dimer results from the three-dimensional (3D) swapping of the third α

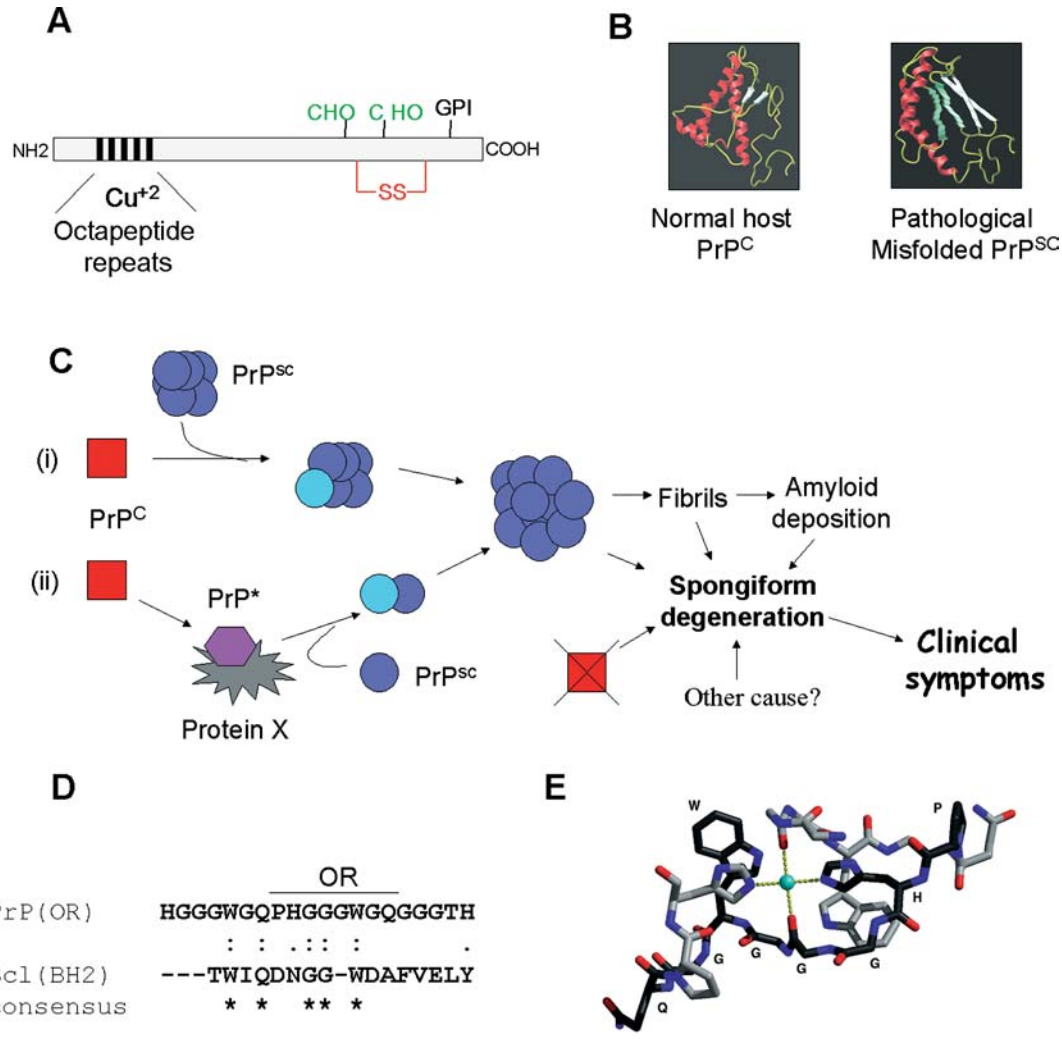

Figure 1. Mechanism of neurodegeneration in prion diseases. (*A*) Schematic representation of the primary structure of PrP. (*B*) 3D structures of PrP^{C} and PrP^{SC}. The image of PrP^{C} corresponds to the experimentally determined structure of the recombinant protein by NMR [17], while PrP^{SC} represents a computational model based on secondary structure calculations. (*C*) A model for TSE progression. The hallmark event in the disease is misfolding of the normal PrP^{C} to form the β-sheet-rich PrP^{SC}. Two alternative mechanism have been proposed to explain this process [12, 15, 31], (i) the nucleation/polymerization model in which conformational changes are induced by protein oligomerization, and (ii) the template-assisted conversion model, in which protein misfolding is independent of aggregation, which is a nonnecessary end point of conformational changes. Regardless of the conversion mechanism, it is clear that the misfolded protein has a high tendency to aggregate, forming amyloid prion rods which might be the precursor of cerebral amyloid plaques seen in some TSE cases. The final cause of clinical symptoms associated with TSEs is the extensive spongiform degeneration of the brain. It is unclear whether neurodegeneration is due to lack of PrP^{C} function, to gain of toxic activity of some soluble or aggregated forms of PrP^{SC}, or to another cause unrelated to PrP. Red squares represent PrP^{C}, blue circles PrP^{SC} (light blue is newly formed PrP^{SC} and dark blue is old PrP^{SC}) and the pink hexagon represents the putative conformational intermediate PrP*. The gray star corresponds to the proposed chaperone protein X. (*D*) Sequence comparison of prion octapeptide repeats (OR) and BH2 domain of Bcl-2. (*E*) Model of copper binding sites on the octapeptide repeats of PrP.

helix and rearrangement of the disulfide bond. A new antiparallel β sheet is formed at the dimer interface comprising strands from each of the monomers. The crystal structure suggests a potential mechanism of PrP oligomerization involving 3D domain swapping.

It has been proposed that PrP^{C} is required for development of infection since PrP-knockout mice are resistant to prion infection and are also unable to generate PrP^{SC} [26]. In addition, in vivo studies using experimental scrapie models in mice or hamsters demonstrate that from the time of infection to the period in which clinical symptoms are detectable there is an enormous increase on the amount of PrP^{SC} in the brain. These findings suggest that the disease is propagated in vivo by the conversion of PrP^{C} into PrP^{SC}, catalyzed by infectious PrP^{SC}. This hypothesis is supported by experiments showing that PrP^{SC} is capable of directing the conversion of PrP^{C} into a protease-resistant PrPSc-like form in vitro [27–30]. These results demonstrate that PrP^{SC} contains the information necessary to replicate itself by transferring its misfolded

conformation to the normal host PrP^{C} protein. The exact mechanism of the conversion is not known. Two alternative models have been proposed to explain this process [12, 15, 31] (fig. 1C). First, the 'nucleation/polymerization' model [31, 32] proposes that PrP^{SC} is formed upon oligomerization with other monomers or oligomers. In this model the infectious agent is a multimeric, highly ordered aggregate of PrP^{SC}, and the slow step is formation of a nucleus that acts as a seed for further stabilization of PrP^{SC}. Second, the 'template-assisted conversion' model postulates that PrP^{SC} is thermodynamically more stable than PrP^{C}, but kinetically inaccessible [15]. PrP^{C} exists in equilibrium with a transient conformational intermediate (named PrP*), which after interaction with a cellular chaperone (protein X) is able to make a heterodimer with PrP^{SC}. This heterodimer is spontaneously converted into a PrP^{SC} homodimer consisting of the old and newly formed PrP^{SC} molecules. The homodimer can dissociate to form two templates or can further polymerize to form higher-order aggregates.

Neuronal apoptosis in prion diseases: Is prion conversion the cause?

Neuronal loss is a salient feature of prion diseases [33]. However, its cause and mechanism, particularly its relationship to the prion replication process, is still unclear. PrP conversion may promote the disease either by a gain of a toxic activity of PrP^{SC} or by the lack of the biological function of the natively folded PrP^{C} due to disease-associated conformational changes.

A number of studies suggest that neuronal dysfunction occurs through a process of programmed cell death or apoptosis [33–38]. In situ end-labeling studies and morphological characterization of the brain areas affected by prion infections have shown that neuronal cell death due to murine scrapie occurs by apoptosis, mainly in terminally ill animals, and in those brain areas that show vacuolation [35, 36, 39]. Neuronal loss and apoptosis have been described also in experimental models for CJD in mice [40].

Although it seems unlikely based on the evidence [4], we cannot completely rule out the possibility that PrP conversion might be an epiphenomenon, not directly involved in TSE neurodegeneration. Arguing against this view is the fact that all individuals who develop TSE have PrP^{SC} in their brains [4]. In addition, all mutations associated with familial forms of prion diseases are located in the PrP gene [7]. Moreover, transgenic animal models have been generated in which overexpression of mutant PrP was shown to induce spontaneous neurologic disease with spongiform degeneration [41]. Furthermore these abnormalities could be transmitted to normal mice by inoculation of the disease-affected brain homogenate. Finally, good correlation has been found between PrP^{SC} accumulation and neuronal apoptosis in an experimental scrapie model. However, different authors have shown that in postmortem samples of humans affected with FFI [42] and CJD [33, 37, 38], apoptotic cell death of neurons does not correlate well with PrP deposition (reviewed in [43]).

PrPSC neurotoxicity: The gain-of-function hypothesis

The mechanism by which PrP^{SC} may induce neuronal cell dysfunction remains controversial and unclear [4]. Several reports have claimed that the PrP^{SC} may acquire neurotoxic activity upon aggregation [44], similar to other peptides responsible for neurodegeneration processes such as β-amyloid peptide, implicated in Alzheimer disease [45, 46]. However, it is unknown whether soluble or deposited PrP^{SC} induces neurodegeneration observed in TSE diseases. In other amyloid-related disorders, the appearance of amyloid deposits is a common feature that has been associated with the disease [47, 48]. However, recent evidence suggests that soluble misfolded proteins might induce neuronal dysfunction and hence be more directly related to the severity of clinical symptoms [49–51].

Different strategies have been developed to understand the relationship between PrP misfolding and neuronal dysfunction. Hereditary prion diseases, which include 10% of the cases of CJD and all cases of GSS syndrome and FFI, are inherited in an autosomal dominant fashion and are linked to point and insertional mutations in the PrP gene on chromosome 20 [7]. These mutations are presumed to favor spontaneous conversion of PrP^{C} to the PrP^{SC} state [15]. Recently, a transgenic mouse model of familial prion diseases has been developed by expressing the PrP homologue of a nine-octapeptide insertional mutation described in human patients [52]. This insertional mutation is associated with a prion disease characterized by dementia and ataxia, and by the presence of PrP-containing amyloid plaques in the cerebellum and basal ganglia. Interestingly, these transgenic mice present accumulation of protease-resistant PrP^{SC} and apoptotic cell death of cerebellar granule cells in addition to progressive ataxia [53]. On the other hand, transgenic mice expressing PrP fragments die spontaneously by ataxia, showing accumulation of protease-resistant PrP within neuronal dendrites and cell bodies, apparently causing apoptosis [54, 55]. In some inherited cases of prion disease, it has been reported that the predominant form of PrP detectable in the brain is not PrP^{SC}, but rather a transmembrane form of the prion protein, named ^{CTM}PrP [56]. This phenomenon is associated with a subset of patients with GSS syndrome that carry the mutation A117V. Interest-

ingly, this mutation leads to degeneration in transgenic mice in the absence of PrP^{SC} generation and infectivity when inoculated in the brain of rodents.

Attempts to understand the molecular bases of neuronal dysfunction in prion diseases have led to the search for in vitro models to analyse the putative neurotoxic activity of PrP^{SC}. Some authors have shown that a hypothalamic neuronal cell line persistently infected with scrapie prions has the capability to replicate endogenous PrP^{C} into PrP^{SC}, and exhibits typical morphological and biochemical features of apoptosis [57]. However, most of the neuronal lines chronically infected with prions, show no signs of cellular death. The same has been observed in cells transfected with mutant forms of PrP associated with inherited prion diseases [58, 59]. Although those cells produce a form of PrP resistant to low concentrations of proteases, they do not exhibit cellular apoptosis. The PrP fragment spanning the sequence 106–126, corresponding to a putative transmembrane region of PrP^{C}, has been extensively used to induce cell death in neuronal cultures [60–62]. PrP106–126 showed a high intrinsic ability to polymerize in vitro and form amyloid fibrils, reminiscent of the scrapie fibrils purified from infected brain [63]. In addition, PrP106–126 is partially resistant to proteinase K and contains a β sheet-enriched structure [63, 64]. The PrP106–126 peptide has been shown to be cytotoxic in vitro via the programmed cell death pathway [65]. Recent data suggest that PrP106–126 also induces apoptotic-mediated cell death in vivo, using as a model retinal neurons treated with intravitreous injections of PrP fragments [66]. Interestingly, in this study neither apoptosis nor retinal electrical activity modifications were observed using nonaggregated peptides. Both in vitro and in vivo, the toxicity of PrP^{SC} and PrP106–126 appears to depend upon neuronal expression of PrP^{C} and on microglial activation [26, 67].

Some studies in neuroblastoma cell lines have established that apoptotic cell death mediated by this PrP peptide depends on at least two different signaling pathways involving the participation of apoptosis-related cysteine proteases such as caspase-3, and activation of calpain due to intracellular calcium release and mitochondrial dysfunction [68]. In addition, other authors have recently proposed that PrP106–126 toxicity can be modulated by p38 MAP kinase inhibitors [69]. It seems that mitochondrial dysfunction due to modification of intracellular calcium levels is a central feature of PrP^{SC} toxicity. Calcium homeostasis is disrupted in the hypothalamic gonadotropin-releasing hormone neuronal cell line treated with PrP106–126 through impairment of L-type voltage-sensitive calcium channels [70, 71]. Intracellular calcium increase is known to be a common mediator of apoptosis in response to many apoptotic stimuli or under different pathological conditions [72–74]. Other authors have proposed an alternative model, in which the neurotoxic properties of PrP106–126 are related to membrane depolarization, probably due to ionic channel formation [74, 75]. Interestingly, Yan and co-workers have shown that PrP peptide fibrils interact with receptor for advanced glycation end product (RAGE) receptors, inducing cellular stress and nuclear factor kappa B (NF-κB) activation [76]. RAGE has been extensively studied for its contribution to amyloid-β toxicity in different cellular systems, and seems to be a general receptor for amyloidogenic peptides.

The major drawback with the studies using PrP106–126 as a model is that although this sequence is present in several peptides isolated from cerebral amyloid plaques of patients suffering from GSS syndrome, the relevance of this peptide to TSE diseases remains to be determined. Only limited data are available on the direct effects of PrP^{SC} on neuronal cells. Potential effects include changes in plasma membrane properties and caspase activation due to mitochondrial dysfunction. In addition, activated microglia cells release proinflammatory cytokines and reactive oxygen species in response to PrP^{SC}. Extensive analysis must be performed using full-length PrP^{SC} to analyse the molecular pathways related to neuronal dysfunction and PrP misfolding.

PrP^{C} biology and prion diseases: Loss of beneficial activity

In addition to direct or indirect toxic effects of PrP^{SC}, a loss-of-function of PrP^{C} may contribute to neuronal cell death. Potential mechanisms include disturbances of antioxidative defense mechanism or loss of a putative anti-apoptotic activity of PrP^{C}.

Although involvement of the infectious form of PrP in a number of human and animal neurodegenerative diseases has been extensively studied, the normal function of PrP^{C} is still poorly understood. The protein is mainly expressed on neurons and is evolutionarily conserved [4]. To gain some insights about the putative function of PrP^{C}, several groups have generated knockout mice in which normal PrP expression was eliminated. The first two null mice generated were viable and had no obvious neurological problems [77–79], whereas two generated subsequently developed cerebellar degeneration and ataxia [79, 80]. These findings led to the discovery of a gene located downstream of PrP, termed Prnd, which encodes a protein named Doppel (Dpl) [81]. Dpl has partial sequence and structural homology to PrP, and its overexpression in the brain appears to be responsible for the cerebellar degeneration observed in some of the mice [79, 81, 82]. The molecular mechanism by which Dpl overexpression in the brain leads to cerebellar degeneration remains unclear. Interestingly, in the PrP-null mice overexpressing Dpl, the neurological deficit was eliminated by expression of a normal PrP gene, suggesting that the two

proteins compete for a common target protein important in cerebellar structure and function [79]. Dpl knockout mice have now been generated, and lack of Dpl expression does not appear to influence the pathogenesis of TSE disease [83]. Dpl, as well as PrP, is a glycosyl-phosphatidylinositol (GPI)-anchored cell surface glycoprotein [84] with the ability to bind copper [85]. Structural studies of Dpl show that the protein has a high helical content (40%), is thermodynamically stable, soluble and sensitive to proteinase K digestion. Therefore, Dpl possesses biochemical properties similar to those of recombinant PrP [86].

Some reports describe abnormalities of PrP knockout mice at the neuronal level, such as altered long-term potentiation and defects in GABA-ergic receptor-mediated synaptic inhibition [78]. Other studies found no electrophysiological differences in the hippocampal region of PrP null mice compared with wild-type mice [87]. However, if loss of function has a causal effect in prion disease, it is likely that mice lacking PrP^{C} expression would show abnormalities. In potential agreement with this hypothesis, Brown and co-workers have shown that lack of PrP expression results in neuronal sensitivity to oxidative stress; however, this did not lead to neurodegeneration [88]. The biochemical changes of these mice include increased levels of NF-κB and Mn dismutase, decreased levels of p53, altered melatonin levels, and increased expression of apoptosis-related genes Bax and Bcl-2 and phospho-ERKs (extracellular signal-related kinases) [88]. The authors suggested that compensatory responses within different signaling pathways in PrP knock-out mice might attenuate the expected phenotype.

Searching for PrP-interacting proteins using different approaches has also been used in an attempt to understand the function of PrP^{C}. Candidate ligands are Bcl-2 [89], Hsp60 [90] and the laminin receptor [91, 92]. Recently, new interacting proteins have been described, such as the neuronal signaling-associated proteins phosphoprotein synapsin Ib and the adapter protein Grb2, as well as the still-uncharacterized prion interactor protein Pint1 [93]. In addition, PrP^{C} has been suggested to play a role in signal transduction since PrP is located in caveolae-like domains [94], and cross-linking of PrP^{C} leads to activation of tyrosine kinase Fyn, which is involved in intracellular signaling [95]. Others reports have shown that PrP^{C} can bind to DNA in vitro and has nucleic acid chaperone activity [96, 97].

Interestingly, PrP-null neuronal cell lines are more susceptible to serum deprivation-induced apoptosis. Bcl-2 overexpression can attenuate this sensitivity, suggesting that PrP^{C} has neuroprotective activity [98]. In addition, PrP fusion proteins interact with Bcl-2, as shown by yeast two-hybrid experiments and by coimmunoprecipitations [89]. A link between PrP and the Bcl-2 family is further suggested by the similarity between the highly conserved octapeptide repeats of PrP and the Bcl-2 homology domain 2 (BH2) of Bcl-2 family proteins (fig. 1D). This domain is crucial for the antiapoptotic function of Bcl-2 and its interaction with the proapoptotic protein Bax (for reviews see [99–101]), suggesting that PrP might be a member of the antiapoptotic family. Indeed, studies of the possible role of PrP^{C} in regulating neuronal apoptosis have shown that PrP^{C} can protect human neurons against Bax-induced apoptosis [102]. This neuroprotective activity was not observed with mutant proteins bearing deletions of the octapeptide repeats or with some C-terminal PrP mutations associated with inherited prion diseases [102]. These results are consistent with the observation on transgenic mice expressing mutant PrP devoid of the octapeptide repeats, which show alterations in the incubation time and histopathology during infections with scrapie [103]. In addition, recent reports suggest that the signal transduction events associated with PrP^{C} lead to neuroprotection [104]. Taken together, these results suggest that PrP^{C} loss of function may be involved in TSEs, probably due to alterations in signaling pathways related to neuronal survival.

On the other hand, recent data indicate that both recombinant and native PrP^{C} are able to bind copper [105] and acquire antioxidant activity as a result (review in 106]). This enzymatic property is mainly dependent on the copper binding to the octapeptide repeats [107] (fig. 1E). Mouse PrP^{C}, expressed as recombinant protein or immunoprecipitated from brain tissues, has superoxide dismutase activity [108–110]. Interestingly, interaction between PrP^{C} and PrP106–126 or PrP^{SC} inhibits this activity, suggesting that PrP misfolding with the concomitant loss of function may contribute to neuronal dysfunction [106, 111]. Finally, research using PrP knockout mice also provides evidence for a link between PrP^{C} copper binding and resistance to oxidative stress [88].

Role of brain inflammation in prion diseases

The protein misfolding and aggregation hypothesis for PCDs proposes that accumulation of protein aggregates with aberrant conformation ultimately leads to neuronal dysfunction. In the case of prion diseases, one interpretation of this hypothesis is that PrP^{SC} is directly neurotoxic. An alternative or complementary interpretation is that PrP^{SC} may be neurotoxic by an indirect pathway, such a chronic brain inflammation, leading to the pathological destruction of neurites and cell bodies. Indeed, a prominent feature of TSE neuropathology is the presence of reactive astrocytes and activated microglia [33, 112]. Supporting the inflammatory hypothesis, previous in vitro reports have shown that neurotoxicity of the peptide PrP106–126, as well as of partially purified prion preparations, is dependent on the presence and activation of

microglial cells [67, 113, 114]. Microglial activity has described as the major component of the host reaction during interspecies transmission of a CJD agent [115]. Proinflammatory cytokines such as interleukin-1β (IL-1β), tumor necrosis factor α (TNF-α) and IL-6 are elevated during the course of experimentally induced mouse scrapie [39, 116, 117]. In addition, other inflammation-related compounds, such as prostaglandins and lipocortins [117], and inducible nitric oxide synthase are produced in elevated amounts [118, 119]. It has been suggested that cytokine production may be associated with microglia rather than astrocytes during the development of scrapie [39, 117]. However, the precise nature of the cells producing cytokines as well as the specific contribution of either type of glial cell to the neurodegenerative process of TSEs in vivo is still uncertain. In other pathological conditions, such as Alzheimer disease, the most abundant source of proinflammatory cytokines appears to be activated microglia, even though astrocytes, endothelial cells and even neurons can also produce inflammatory cytokines (for a review see [120]). A careful analysis has been made during the development of

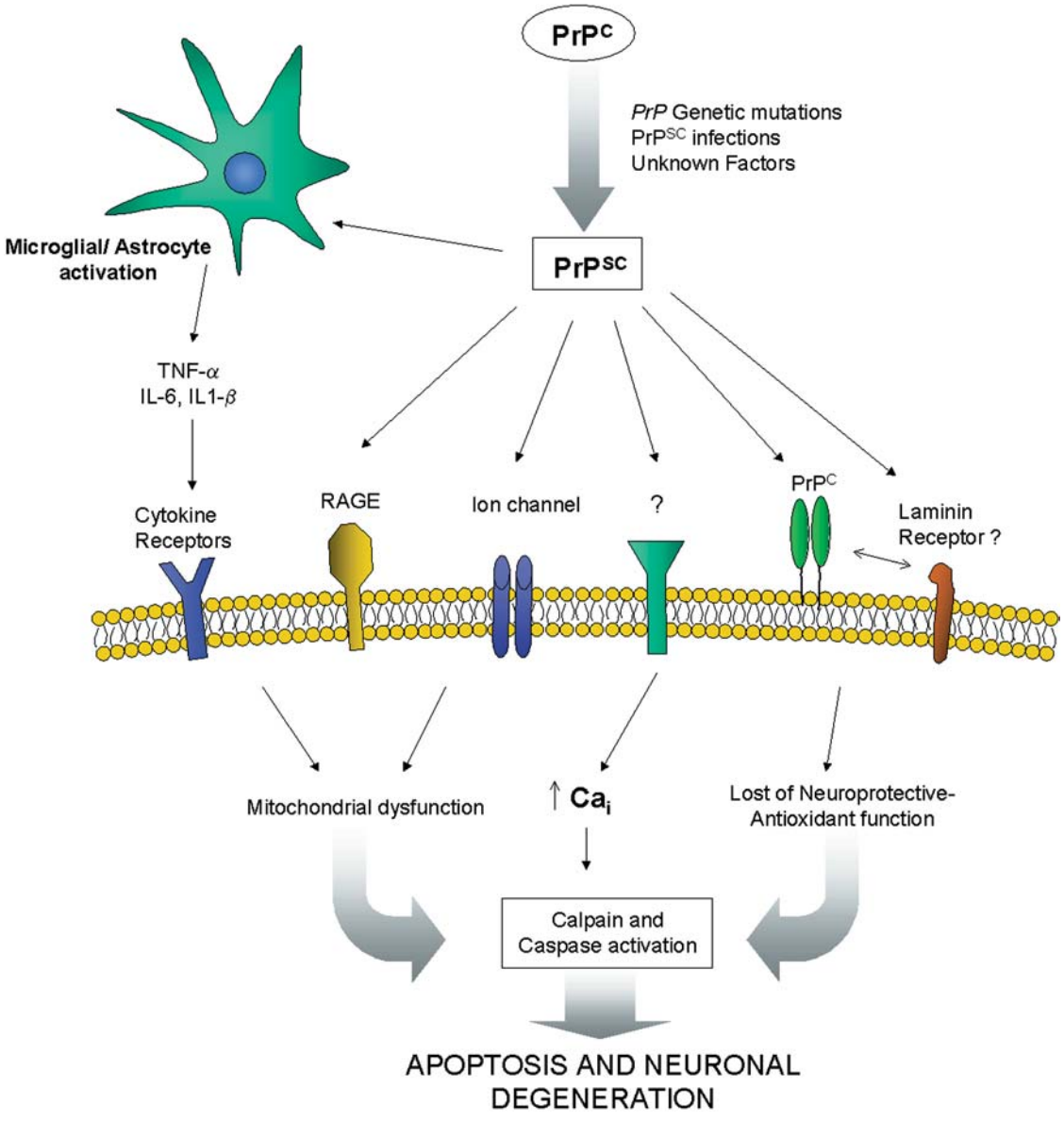

Figure 2. Cellular pathways of prion protein neurotoxicity in TSEs. Conformational changes of PrP^{C} induced by prion infections, mutations or unknown factors lead to the production and accumulation of the misfolded PrP^{SC} protein. The pathological protein may interact with different neuronal cell surface receptors and with microglia and astrocytes, triggering signal transduction cascades which result in caspase activation due to mitochondrial dysfunction. An increase in intracellular calcium (Ca_i^{2+}) might activate calpain proteases, which in turn can contribute to induction of neuronal apoptosis. In addition, the conformational transition could lead to losts of beneficial activity of the natively folded protein, as an antiapoptotic or/and an antioxidant activity.

murine scrapie, showing that neuroglial activation and cytokine production precede PrP^{SC} deposition and occur before spongiform degeneration of the brain [39]. In addition, different transgenic models for familial prion diseases develop extensive astrogliosis, reflected in microglial and astrocyte activation [53, 121].

In vitro studies have shown that murine microglial cells treated with PrP106–126 in fibrilar form produce inflammatory cytokines such as IL-1β and IL-6, but not TNF-α [67, 122]. Others have shown that PrP106–126 stimulated the induction of iNOS (inducible nitric oxide synthase) with the concomitant production of nitric oxide in human microglia in a TNF-α-dependent manner [123]. In addition, Bate and co-workers showed that microglia secrete more IL-6 when they are incubated with PrP106–126-treated neurons than when they are incubated with the PrP peptides alone, suggesting that microglia recognize some peptide-induced changes in neurons [67]. However, experimental manipulation of inflammatory processes during the development of prion diseases must be performed to understand the precise contribution of this phenomenon to the neuropathology of prion diseases.

Concluding Remarks

Protein misfolding depends on conformational changes which are stabilized by protein oligomerization. The starting point in PCDs is normally a natural folded protein which is converted into a pathological form that can promote the diseases either by gain of toxic activity or by the loss of biological activity of the normal protein. In this review we have discussed the most relevant aspects related to the involvement of protein misfolding in neurodegeneration, using prion diseases as an example of PCDs. Interestingly, despite the lack of sequence or structural homology among the known proteins implicated in PCDs, a striking feature of these proteins is their ability to adopt at least two different stable conformations, which in most cases are rich in β-sheet structures [2]. This conformational transition is normally associated with aberrant protein aggregation and accumulation of amyloid-like deposits in different organs. However, it is still a matter of controversy whether the deposits of aggregated proteins are the culprits of disease progression or an inseparable epiphenomen on [2, 12, 124].

Many alternative possibilities have been proposed to explain the involvement of prion protein misfolding in neurodegeneration observed in prion diseases. Fig. 2 shows a summary of the different molecular pathways by which PrP might be implicated in the development of TSEs. This schema could be applied to other pathological conditions that form part of PCDs. The model proposes three alternative pathways: gain of negative activity by generation of toxic misfolded proteins; loss of beneficial activity of the native normal proteins; or an indirect mechanism involving initiation of inflammatory reaction induced by the presence of the pathological misfolded protein. Assuming that protein misfolding is the triggering event in the development of PCDs, therapeutic approaches that prevent or reverse this process might have beneficial consequences for disease treatment.

1 Carrell R. W. and Lomas D. A. (1997) Conformational disease. Lancet **350:** 134–138
2 Soto C. (2001) Protein misfolding and disease; protein refolding and therapy. FEBS Lett. **498:** 204–207
3 Collinge J. (2001) Prion diseases of humans and animals: their causes and molecular basis. Annu. Rev. Neurosci. **24:** 519–550
4 Prusiner S. B. (1998) Prions. Proc. Natl. Acad. Sci. USA **95:** 13363–13383
5 Ironside J. W. and Bell J. E. (1997) Pathology of prion diseases In: Prion Diseases pp. 57–88, Collinge J. and Palmer M. S. (eds), Oxford University Press, Oxford
6 Budka H., Aguzzi A., Brown P., Brucher J. M., Bugiani O., Gullotta F. et al. (1995) Neuropathological diagnostic criteria for Creutzfeldt-Jakob disease (CJD) and other human spongiform encephalopathies (prion diseases). Brain Pathol. **5:** 459–466
7 Prusiner S. B. and Scott M. R. (1997) Genetics of prions. Annu. Rev. Gent. **286:** 593–606
8 Johnson R. T. and Gibbs C. J. Jr (1998) Creutzfeldt-Jakob disease and related transmissible spongiform encephalopathies. N. Engl. J. Med. **339:** 1994–2004
9 Will R. G., Ironside J. W., Zeidler M., Cousens S. N., Estibeiro K., Alperovitch A. P. et al. (1996) A new variant of Creutzfeldt-Jakob disease in the UK. Lancet **347:** 925
10 Collinge J. (1999) Variant Creutzfeldt-Jakob disease. Lancet **354:** 317–323
11 Bruce M. E. (2000) 'New variant' Creutzfeldt-Jakob disease and bovine spongiform encephalopathy. Nat. Med. **6:** 258–259
12 Soto C. and Saborio G. P. (2001) Prions: disease propagation and disease therapy by conformational transmission. Trends Mol. Med. **7:** 109–114
13 Balter M. (2001) Infectious diseases. Uncertainties plague projections of vCJD toll. Science **294:** 770–771
14 Prusiner S. B., Scott M., DeArmond S. J. and Cohen F. E. (1998) Prion protein biology. Cell **93:** 337–348
15 Cohen F. E. and Prusiner S. B. (1998) Pathologic conformations of prion proteins. Ann. Rev. Biochem. **67:** 793–819
16 Caughey B. (2001) Interactions between prion protein isoforms: the kiss of death? Trends Biochem. Sci. **26:** 235–242
17 Wuthrich K. and Riek R. (2001) Three-dimensional structures of prion proteins. Adv. Protein Chem. **57:** 55–82
18 McKinley M. P., Taraboulos A., Kenaga L., Serban D., Stieber A., DeArmond S. J. et al. (1991) Ultrastructural localization of scrapie prion proteins in cytoplasmic vesicles of infected cultured cells. Lab. Invest. **65:** 622–630
19 Pan K. M., Baldwin M., Njuyen J., Gassett M., Serban A., Groth D. et al. (1993) Conversion of alpha-helices into β-sheets features in the formation of scrapie prion poteins. Proc. Natl. Acad. Sci. USA **90:** 10962–10966
20 Riek R., Hornemann S., Wider G., Billeter M., Glockshuber R. and Wüthrich K. (1996) NMR structure of the mouse prion protein domain PrP(121-231). Nature **382:** 180–182
21 Lopez Garcia F., Zahn R., Riek R. and Wüthrich K. (2000) NMR structure of the bovine prion protein. Proc. Natl. Acad. Sci. USA **97:** 8334–8339

22 Zahn R., Liu A., Luhrs T., Riek R., von Schroetter C., Lopez Garcia F. et al. (2000) NMR solution structure of the human prion protein. Proc. Natl. Acad. Sci. USA **97:** 145–150

23 James T. L., Liu H., Ulyanov N. B., Farr-Jones S., Zhang H., Donne D. G. et al. (1997) Solution structure of a 142-residue recombinant prion protein corresponding to the infectious fragment of the scrapie isoform. Proc. Natl. Acad. Sci. USA **94:** 10086–10091

24 Donne D. G., Viles J. H., Groth D., Mehlhorn I., James T. L., Cohen F. E. et al. (1997) Structure of the recombinant full-length hamster prion protein PrP(29-231): the N terminus is highly flexible. Proc. Natl. Acad. Sci. USA **94:** 13452–13457

25 Knaus K. J., Morillas M., Swietnicki W., Malone M., Surewicz W. K. and Yee V. C. (2001) Crystal structure of the human prion protein reveals a mechanism for oligomerization. Nat. Struct. Biol. **8:** 770–774

26 Bueler H., Aguzzi A., Sailer A., Greiner R. A., Autenried P., Aguet M. et al. (1993) Mice devoid of PrP are resistant to Scrapie. Cell **73:** 1339–1347

27 Kocisko D. A., Come J. H., Priola S. A., Chesebro B., Raymond G. J., Lansbury P. T. et al. (1994) Cell-free formation of protease-resistant prion protein. Nature **370:** 471–474

28 Caughey B. (2000) Formation of protease-resistant prion protein in cell-free systems. Curr. Issues Mol. Biol. **2:** 95–101

29 Saborio G. P., Soto C., Kascsak R. J., Levy E., Kascsak R., Harris D. A. et al. (1999) Cell-lysate conversion of prion protein into its protease-resistant isoform suggests the participation of a cellular chaperone. Biochem. Biophys. Res. Commun. **258:** 470–475

30 Saborio G. P., Permanne B. and Soto C. (2001) Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. Nature **411:** 810–813

31 Harper J. D. and Lansbury P. T. Jr (1997) Models of amyloid seeding in Alzheimer's disease and scrapie: mechanistic truths and physiological consequences of the time-dependent solubility of amyloid proteins. Annu. Rev. Biochem. **66:** 385–407

32 Brown P., Goldfarb L. G. and Gajdusek D. C. (1991) The new biology of spongiform encephalopathy: infectious amyloidoses with a genetic twist. Lancet **337:** 1019–1022

33 Gray F., Chretien F., Adle-Biassette H., Dorandeu A., Ereau T., Delisle M. B. et al. (1999) Neuronal apoptosis in Creutzfeldt-Jakob disease. J. Neuropathol. Exp. Neurol. **58:** 321–328

34 Peretz D., Williamson R. A., Matsunaga Y., Serban H., Pinilla C., Bastidas R. B. et al. (1997) A conformational transition at the N-terminus of the prion protein features in the formation of the scrapie isoform. J. Mol. Biol. **273:** 614–622

35 Lucassen P. J., Williams A., Chung W. C. and Fraser H. (1995) Detection of apoptosis in murine scrapie. Neurosci. Lett. **198:** 185–188

36 Giese A., Groschup M. H., Hess B. and Kretzschmar H. A. (1995) Neuronal cell death in scrapie-infected mice is due to apoptosis. Brain Pathol. **5:** 213–221

37 Ferrer I. (1999) Nuclear DNA fragmentation in Creutzfeldt-Jakob disease: does a mere positive in situ nuclear end-labeling indicate apoptosis? Acta Neuropathol. **97:** 5–12

38 Gray F., Adle-Biassette H., Chretien F., Ereau T., Delisle M. B. and Vital C. (1999) [Neuronal apoptosis in human prion diseases]. Bull. Acad. Natl. Med. **183:** 305–320

39 Williams A., Lucassen P. J., Ritchie D. and Bruce M. (1997) PrP deposition, microglial activation, and neuronal apoptosis in murine scrapie. Exp. Neurol. **144:** 433–438

40 Jesionek-Kupnicka D., Buczynski J., Kordek R. and Liberski P. P. (1999) Neuronal loss and apoptosis in experimental Creutzfeldt-Jakob disease in mice. Folia Neuropathol. **37:** 283–286

41 Prusiner S. B. (1996) Transgenetics of prion diseases. Curr. Topics Microbiol. Immunol. **206:** 275–304

42 Dorandeu A., Wingertsmann L., Chretien F., Delisle M. B., Vital C., Parchi P. et al. (1998) Neuronal apoptosis in fatal familial insomnia. Brain Pathol. **8:** 531–537

43 Chretien F., Dorandeu A., Adle-Biassette H., Ereau T., Wingertsmann L., Brion F. et al. (1999) [A process of programmed cell death as a mechanisms of neuronal death in prion diseases]. Clin. Exp. Pathol. **47:** 181–191

44 Safar J. (1996) The folding intermediate concept of prion protein formation and conformational links to infectivity. Curr. Topics Microbiol. Immunol. **207:** 69–76

45 Soto C., Branes M. C., Alvarez J. and Inestrosa N. C. (1994) Structural determinants of the Alzheimer's amyloid beta-peptide. J. Neurochem. **63:** 1191–1198

46 Yankner B. A. (1996) Mechanisms of neuronal degeneration in Alzheimer's disease. Neuron **16:** 921–932

47 Selkoe D. J. (2000) Toward a comprehensive theory for Alzheimer's disease. Hypothesis: Alzheimer's disease is caused by the cerebral accumulation and cytotoxicity of amyloid beta-protein. Ann. N.Y. Acad. Sci. **924:** 17–25

48 Soto C. (1999) Plaque busters: strategies to inhibit amyloid formation in Alzheimer's disease. Mol. Med. Today **5:** 343–350

49 Lue L. F., Kuo Y. M., Roher A. E., Brachova L., Shen Y., Sue L. et al. (1999) Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. Am. J. Pathol. **155:** 853–862

50 Lambert M. P., Barlow A. K., Chromy B. A., Edwards C., Freed R., Liosatos M. et al. (1998) Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins. Proc. Natl. Acad. Sci. USA **95:** 6448–6453

51 Hartley D. M., Walsh D. M., Ye C. P., Diehl T., Vasquez S., Vassilev P. M. et al. (1999) Protofibrillar intermediates of amyloid beta-protein induce acute electrophysiological changes and progressive neurotoxicity in cortical neurons. J. Neurosci **19:** 8876–8884

52 Chiesa R., Piccardo P., Ghetti B. and Harris D. A. (1998) Neurological illness in transgenic mice expressing a prion protein with an insertional mutation. Neuron **21:** 1339–1351

53 Chiesa R., Drisaldi B., Quaglio E., Migheli A., Piccardo P., Ghetti B. et al. (2000) Accumulation of protease-resistant prion protein (PrP) and apoptosis of cerebellar granule cells in transgenic mice expressing a PrP insertional mutation. Proc. Natl. Acad. Sci. USA **97:** 5574–5579

54 Supattapone S., Bouzamondo E., Ball H. L., Wille H., Nguyen H. O., Cohen F. E. et al. (2001) A protease-resistant 61-residue prion peptide causes neurodegeneration in transgenic mice. Mol. Cell Biol. **21:** 2608–2616

55 Supattapone S., Bosque P., Muramoto T., Wille H., Aagaard C., Peretz D. et al. (1999) Prion protein of 106 residues creates an artifical transmission barrier for prion replication in transgenic mice. Cell **96:** 869–878

56 Jamieson E., Jeffrey M., Ironside J. W. and Fraser J. R. (2001) Apoptosis and dendritic dysfunction precede prion protein accumulation in 87V scrapie. Neuroreport **12:** 2147–2153

57 Schatzl H. M., Laszlo L., Holtzman D. M., Tatzelt J., DeArmond S. J., Weiner R. I. et al. (1997) A hypothalamic neuronal cell line persistently infected with scrapie prions exhibits apoptosis. J. Virol. **71:** 8821–8831

58 Lehmann S. and Harris D. A. (1995) A mutant prion protein displays an aberrant membrane association when expressed in cultured cells. J. Biol. Chem. **270:** 24589–24597

59 Lehmann S. and Harris D. A. (1996) Two mutant prion proteins expressed in cultured cells acquire biochemical properties reminiscent of the scrapie isoform. Proc. Natl. Acad. Sci. USA **93:** 5610–5614

60 Tagliavini F., Prelli F., Verga L., Giaccone G., Sarma R., Gorevic P. et al. (1993) Synthetic peptides homologous to prion protein residues 106-147 form amyloid-like fibrils in vitro. Proc. Natl. Acad. Sci. USA **90:** 9678–9682

61 Forloni G., Angeretti N., Chiesa R., Monzani E., Salmona M., Bugiani O. et al. (1993) Neurotoxicity of a prion protein fragment. Nature **362:** 543–546

62 Brown D. R., Herms J. and Kretzschmar H. A. (1994) Mouse cortical cells lacking cellular PrP survive in culture with a neurotoxic PrP fragment. Neuroreport **5:** 2057–2060
63 Selvaggini C., De Gioia L., Cantu L., Ghibaudi E., Diomede L., Passerini et al. (1993) Molecular characteristics of a protease-resistant, amyloidogenic and neurotoxic peptide homologous to residues 106-126 of the prion protein. Biochem. Biophys. Res. Commun. **194:** 1380–1386
64 De Gioia L., Selvaggini C., Ghibaudi E., Diomede L., Bugiani O., Forloni et al. (1994) Conformational polymorphism of the amyloidogenic and neurotoxic peptide homologous to residues 106-126 of the prion protein. J. Biol. Chem. **269:** 7859–7862
65 Forloni G., Chiesa R., Smiroldo S., Verga L., Salmona M., Tagliavini F. et al. (1993) Apoptosis mediated neurotoxicity induced by chronic application of beta amyloid fragment 25-35. Neuroreport **4:** 523–526
66 Ettaiche M., Pichot R., Vincent J. P. and Chabry J. (2000) In vivo citotoxicity of the prion protein fragment 106–126. J. Biol. Chem. **275:** 36487–36490
67 Bate C., Reid S. and Williams A. (2001) Killing of prion-damaged neurones by microglia. Neuroreport **12:** 2589–2594
68 O'Donovan C. N., Tobin D. and Cotter T. G. (2001) Prion protein fragment PrP-(106-126) induces apoptosis via mitochondrial disruption in human neuronal SH-SY5Y cells. J. Biol. Chem. **276:** 43516–43523
69 Thellung S., Villa V., Corsaro A., Arena S., Millo E., Damonte G. et al. (2002) p38 MAP kinase mediates the cell death induced by PrP106-126 in the SH-SY5Y neuroblastoma cells. Neurobiol. Dis. **9:** 69–81
70 Thellung S., Florio T., Villa V., Corsaro A., Arena S., Amico C. et al. (2000) 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:** 299–309
71 Thellung S., Florio T., Corsaro A., Arena S., Merlino M., Salmona M. et al. (2000) Intracellular mechanisms mediating the neuronal death and astrogliosis induced by the prion protein fragment 106-126. Int. J. Dev. Neurosci. **18:** 481–492
72 Nicotera P. and Orrenius S. (1998) The role of calcium in apoptosis. Cell Calcium **23:** 173–180
73 McConkey D. J. and Orrenius S. (1997) The role of calcium in the regulation of apoptosis. Biochem. Biophys. Res. Commun. **239:** 357–366
74 Kawahara M., Kuroda Y., Arispe N. and Rojas E. (2000) Alzheimer's beta-amyloid human islet amylin and prion protein fragment evoke intracellular free calcium elevations by a common mechanism in a hypothalamic GnRH neuronal cell line. J. Biol. Chem. **275:** 14077–14083
75 Lin M. X., Mirzabekov T. and Kagan B. L. (1997) Channel formation by a neurotoxic prion protein fragment. J. Biol. Chem. **272:** 44–47
76 Yan S. D., Zhu H., Zhu A., Golabek A., Du H., Roher A. E. et al. (2000) Recepto-dependent cell stress and amyloid accumulation in systemic amyloidosis. Nat. Med. **6:** 643–651
77 Bueler H., Fischer M., Lang Y., Bluethmann H., Lipp H. P., DeArmond S. J. et al. (1992) Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. Nature **356:** 577–582
78 Collinge J., Whittington M. A., Sidle K. C., Smith C. J., Palmer M. S., Clarke A. R. et al. (1994) Prion protein is necessary for normal synaptic function. Nature **370:** 295–297
79 Behrens A. and Aguzzi A. (2002) Small is not beautiful: antagonizing functions for the prion protein PrP(C) and its homologue Dpl. Trends Neurosci. **25:** 150–154
80 Sakaguchi S., Katamine S., Nishida N., Moriuchi R., Shigematsu K., Sugimoto T. et al. (1996) Loss of cerebellar Purkinje cells in aged mice homozygous for a disrupted PrP gene. Nature **380:** 528–531
81 Moore R. C., Lee I. Y., Silverman G. L., Harrison P. M., Strome R., Heinrich C. et al. (1999) Ataxia in prion protein (PrP)-deficient mice is associated with upregulation of the novel PrP-like protein doppel. J. Mol. Biol. **292:** 797–817
82 Moore R. C., Mastrangelo P., Bouzamondo E., Heinrich C., Legname G., Prusiner S. B. et al. (2001) Doppel-induced cerebellar degeneration in transgenic mice. Proc. Natl. Acad. Sci. USA **98:** 15288–15293
83 Tuzi N. L., Gall E., Melton D. and Manson J. C. (2002) Expression of doppel in the CNS of mice does not modulate transmissible spongiform encephalopathy disease. J. Gen. Virol. **83:** 705–711
84 Silverman G. L., Qin K., Moore R. C., Yang Y., Mastrangelo P., Tremblay P. et al. (2000) Doppel is an N-glycosylated, glycosylphosphatidylinositol-anchored protein. Expression in testis and ectopic production in the brains of Prnp(0/0) mice predisposed to Purkinje cell loss. J. Biol. Chem. **275:** 26834–26841
85 Westaway D. and Carlson G. A. (2002) Mammalian prion proteins: enigma, variation and vaccination. Trends Biochem. Sci. **27:** 301–307
86 Lu K., Wang W., Xie Z., Wong B. S., Li R., Petersen R. B. et al. (2000) Expression and structural characterization of the recombinant human doppel protein. Biochem. **39:** 13575–13583
87 Lledo P. M., Tremblay P., DeArmond S. J., Prusiner S. B. and Nicoll R. A. (1996) Mice deficient for prion protein exhibit normal neuronal excitability and synaptic transmission in the hippocampus. Proc. Natl. Acad. Sci. USA **93:** 2403– 2407
88 Brown D. R., Nicholas R. S. and Canevari L. (2002) Lack of prion protein expression results in a neuronal phenotype sensitive to stress. J. Neurosci. Res. **67:** 211–224
89 Kurschner C. and Morgan J. I. (1996) Analysis of interaction sites in homo- and heteromeric complexes containing Bcl-2 family members and the cellular prion protein. Brain Res. Mol. Brain Res. **37:** 249–258
90 Edenhofer F., Rieger R., Famulok M., Wendler W., Weiss S. and Winnacker E. L. (1996) Prion protein PrP^c interacts with molecular chaperones of the Hsp60 family. J. Virol. **70:** 4724–4728
91 Gauczynski S., Peyrin J. M., Haik S., Leucht C., Hundt C., Rieger R. et al. (2001) The 37-kDa/67-kDa laminin receptor acts as the cell-surface receptor for the cellular prion protein. EMBO J. **20:** 5863–5875
92 Hundt C., Peyrin J. M., Haik S., Gauczynski S., Leucht C., Rieger R. et al. (2001) Identification of interaction domains of the prion protein with its 37-kDa/67-kDa laminin receptor. EMBO J. **20:** 5876–5886
93 Spielhaupter C. and Schatzl H. M. (2001) PrPC directly interacts with proteins involved in signaling pathways. J. Biol. Chem. **276:** 44604–44612
94 Vey M., Pilkuhn S., Wille H., Nixon R., DeArmond S. J., Smart E. J. et al. (1996) Subcellular colocalization of the cellular and scrapie prion proteins in caveolae-like membranous domains. Proc. Natl. Acad. Sci. USA **93:** 14945– 14949
95 Mouillet-Richard S., Ermonval M., Chebassier C., Laplanche J. L., Lehmann S., Launay J. M. et al. (2000) Signal transduction through prion protein. Science **289:** 1925–1928
96 Gabus C., Derrington E., Leblanc P., Chnaiderman J., Dormont D. et al. (2001) The prion protein has RNA binding and chaperoning properties characteristic of nuclear capsid protein NCP7 of HIV-1. J. Biol. Chem. **276:** 19301–19309
97 Gabus C., Auxilien S., Pechoux C., Dormont D., Swietnicki W., Morillas M. et al. (2001) The prion protein has DNA strand transfer properties similar to retroviral nucleocapsid protein. J. Mol. Biol. **307:** 1011–1021
98 Kuwahara C., Takeuchi A. M., Nishimura T., Haraguchi K., Kubosaki A., Matsumoto Y. et al. (1999) Prions prevent neuronal cell-line death. Nature **400:** 225–226

99 Wisniewski T., Aucouturier P., Soto C. and Frangione B. (1998) The prionoses and other conformational disorders. Amyloid **5:** 212–224
100 Yin X. M., Oltvai Z. N. and Korsmeyer S. J. (1994) BH1 and BH2 domains of Bcl-2 are required for inhibition of apoptosis and heterodimerization with Bax. Nature **369:** 321–323
101 Yin X. M., Oltvai Z. N., Veis-Novack D. J., Linette G. P. and Korsmeyer S. J. (1994) Bcl-2 gene family and the regulation of programmed cell death. Cold Spring Harb. Symp. Quant. Biol. **59:** 387–393
102 Bounhar Y., Zhang Y., Goodyer C. G. and LeBlanc A. (2001) Prion protein protects human neurons against Bax-mediated apoptosis. J. Biol. Chem. **276:** 39145–39149
103 Flechsig E., Shmerling D., Hegyi I., Raeber A. J., Fischer M., Cozzio A. et al. (2000) Prion protein devoid of the octapeptide repeat region restores susceptibility to scrapie in PrP knockout mice. Neuron **27:** 399–408
104 Martins V. R., Linden R., Prado M. A., Walz R., Sakamoto A. C., Izquierdo I. and Brentani R. R. (2002) Cellular prion protein: on the road for functions. FEBS Lett. **512:** 25–28
105 Kramer M. L., Kratzin H. D., Schmidt B., Romer A., Windl O., Liemann S. et al. (2001) Prion protein binds copper within the physiological concentration range. J. Biol. Chem. **276:** 16711–16719
106 Wong B. S., Pan T., Liu T., Li R., Petersen R. B., Jones I. M. et al. (2000) Prion disease: a loss of antioxidant function? Biochem. Biophys. Res. Commun. **275:** 249–252
107 Ruiz F. H., Silva E. and Inestrosa N. C. (2000) The N-terminal tandem repeat region of human prion protein reduces copper: role of tryptophan residues. Biochem. Biophys. Res. Commun. **269:** 491–495
108 Brown D. R., Wong B. S., Hafiz F., Clive C., Haswell S. J. and Jones I. M. (1999) Normal prion protein has an activity like that of superoxide dismutase. Biochem. J. **344 Pt 1:** 1–5
109 Wong B. S., Clive C., Haswell S. J., Williamson R. A., Burton D. R., Gambetti P. et al. (2000) Copper has differential effect on prion protein with polymorphism of position 129. Biochem. Biophys. Res. Commun. **269:** 726–731
110 Wong B. S., Pan T., Liu T., Li R., Gambetti P. and Sy M. S. (2000) Differential contribution of superoxide dismutase activity by prion protein in vivo. Biochem. Biophys. Res. Commun. **273:** 136–139
111 Brown D. R. (2000) PrPSc-like prion protein peptide inhibits the function of cellular prion protein. Biochem. J. **352 Pt 2:** 511–518
112 Budka H. and Hainfellner H. A. (1996) The human prion diseases: neuropathology, pathobiology and molecular genetics – Vienna, Austria, 16 October 1995. Neuropathol. Appl. Neurobiol. **22:** 171–173
113 Brown D. R., Schmidt B. and Kretzschmar H. A. (1996) Role of microglia and host prion protein in neurotoxicity of a prion protein fragment. Nature **380:** 345–347
114 Giese A., Brown D. R., Groschup M. H., Feldmann C., Haist I. and Kretzschmar H. A. (1998) Role of microglia in neuronal cell death in prion disease. Brain Pathol. **8:** 449–457
115 Manuelidis L., Fritch W. and Xi Y. G. (1997) Evolution of a strain of CJD that induces BSE-like plaques. Science **277:** 94–98
116 Williams A., Van Dam A. M., Ritchie D., Eikelenboom P. and Fraser H. (1997) Immunocytochemical appearance of cytokines, prostaglandin E2 and lipocortin-1 in the CNS during the incubation period of murine scrapie correlates with progressive PrP accumulations. Brain Res. **754:** 171–180
117 Williams A. E., Van Dam A. M., Man A. H. W., Berkenbosch F., Eikelenboom P. and Fraser H. (1994) Cytokines, prostaglandins and lipocortin-1 are present in the brains of scrapie-infected mice. Brain Res. **654:** 200–206
118 Kim J. I., Ju W. K., Choi J. H., Choi E., Carp R. I., Wisniewski H. M. et al. (1999) Expression of cytokine genes and increased nuclear factor-kappa B activity in the brains of scrapie-infected mice. Brain Res. Mol. Brain Res. **73:** 17–27
119 Lee D. W., Sohn H. O., Lim H. B., Lee Y. G., Kim Y. S., Carp R. I. et al. (1999) Alteration of free radical metabolism in the brain of mice infected with scrapie agent. Free Radic. Res. **30:** 499–507
120 Akiyama H., Barger S., Barnum S., Bradt B., Bauer J., Cole G. M. et al. (2000) Inflammation and Alzheimer's disease. Neurobiol. Aging **21:** 383–421
121 Hsiao K. K., Scott M., Foster D., Groth D. F., DeArmond S. J. and Prusiner S. B. (1990) Spontaneous neurodegeneration in transgenic mice with mutant prion protein. Science **250:** 1587–1590
122 Peyrin J. M., Lasmezas C. I., Haik S., Tagliavini F., Salmona M., Williams A. et al. (1999) Microglial cells respond to amyloidogenic PrP peptide by the production of inflammatory cytokines. Neuroreport **10:** 723–729
123 Fabrizi C., Silei V., Menegazzi M., Salmona M., Bugiani O., Tagliavini F. et al. (2001) The stimulation of inducible nitric-oxide synthase by the prion protein fragment 106 – 126 in human microglia is tumor necrosis factor-alpha- dependent and involves p38 mitogen-activated protein kinase. J. Biol. Chem. **276:** 25692–25696
124 Carrell R. W. and Gooptu B. (1998) Conformational changes and disease – serpins, prions and Alzheimer's. Curr. Opin. Struct. Biol. **8:** 799–809