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A receptor for infectious and cellular prion protein

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Abstract

Prions are an unconventional form of infectious agents composed only of protein and involved in transmissible spongiform encephalopathies in humans and animals. The infectious particle is composed by PrPsc which is an isoform of a normal cellular glycosyl-phosphatidylinositol (GPI) anchored protein, PrPc, of unknown function. The two proteins differ only in conformation, PrPc is composed of 40% α helix while PrPsc has 60% ß-sheet and 20% α helix structure. The infection mechanism is trigged by interaction of PrPsc with cellular prion protein causing conversion of the latter's conformation. Therefore, the infection spreads because new PrPsc molecules are generated exponentially from the normal PrPc. The accumulation of insoluble PrPsc is probably one of the events that lead to neuronal death. Conflicting data in the literature showed that PrPc internalization is mediated either by clathrin-coated pits or by caveolae-like membranous domains. However, both pathways seem to require a third protein (a receptor or a prion-binding protein) either to make the connection between the GPI-anchored molecule to clathrin or to convert PrPc into PrPsc. We have recently characterized a 66-kDa membrane receptor which binds PrPc *in vitro* and *in vivo* and mediates the neurotoxicity of a human prion peptide. Therefore, the receptor should have a role in the pathogenesis of prion-related diseases and in the normal cellular process. Further work is necessary to clarify the events triggered by the association of PrPc/PrPsc with the receptor.

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Introduction

A disease that affects sheep causing excitability, itching, ataxia, paralysis and death was recognized two hundred years ago and named scrapie due to the animals' behavior of rubbing against the fences of their pens. The transmissibility of the disease was accidentally demonstrated by Gordon (1) when a population of Scottish sheep was inoculated with lymphoid tissue derived from an animal with scrapie. Since then a group of neurodegenerative diseases, the transmissible spongiform encephalopathies (TSE), that affect other mammals including humans has been identified.

The human Creutzfeldt-Jackob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS) and familial fatal insomnia (FFI) are rare diseases occurring only in one of 10^6 to 10^7 individuals a year, while kuru assumed epidemic proportions in New Guinea tribes due to ritual cannibalism. The animal diseases include scrapie, bovine spongi-

form encephalopathy (BSE), transmissible mink encephalopathy and chronic wasting disease (2,3).

The physical-chemical nature of the agent causing the TSEs has been intensely studied. It was recognized that the transmissible agent has an unusual resistance to high temperature, formaldehyde treatment and UV and X-ray irradiation. These observations led Grifft (4) to hypothesize that the agent might consist of protein only, without a nucleic acid. Later on, Prusiner (5) isolated the infectious agent from brains of infected hamsters and confirmed Grifft's observations. He designated the TSE agent as "proteinaceus infectious particles" or "prions" (PrP) to distinguish it from conventional pathogens such as bacteria and viruses.

Characteristics of the infectious agent

Partial purification of the scrapie agent from infected brains identified a highly insoluble protein of 33 to 35 kDa designated PrPsc (sc from scrapie) which generates a 27-30-kDa form after protease treatment (6). PrP27-30 purification allowed the determination of its aminoterminal amino acid sequence (7) which permitted the synthesis of an isocoding mixture of nucleotides that was subsequently used to identify PrP cDNA clones (8,9). A chromosomal gene instead of an exogenous nucleic acid codes for PrP which is expressed by a variety of normal neuronal and non-neuronal tissues, independent of infection by scrapie or other TSE agents. Therefore, these results permitted the identification of a normal PrP product named PrPc, a cellular isoform of the PrPsc (8).

PrPc and PrPsc have the same amino acid sequence, although they differ significantly in conformation. The α-helix content of PrPc is about 40% with little or no ß-sheet, while PrPsc contains 50% ß-sheet and only 20% α-helix (10,11). PrPsc is highly resistant to digestion by proteinase K (12), whereas PrPc is easily degraded under the same conditions.

Infection mechanism

The "protein only" hypothesis proposed by Prusiner (13) suggests that the prion protein is identical to PrPsc and when it is introduced into a normal cell causes the conversion in PrPc conformation. Therefore, according to this model, the infection spreads because new PrPsc molecules are generated exponentially from the normal PrPc. The accumulation of insoluble PrPsc is probably one of the events that lead to neuronal death (13).

The definite proof of PrPc participation in the infection mechanism was provided when Weissmann's group (14) generated mice devoid of the PrP gene (0/0). The animals were totally resistant to prion infection and complementation with a PrP transgene restored the phenotype. However, some controversy still exists regarding PrPsc participation in the infection process. Even in the purest samples, the ratio of PrP molecules to infectious units is about 10^5, indicating that the participation of other molecules cannot be excluded (15).

It has been observed that prions are not uniform molecules, mainly due to their differences in incubation times, distribution of lesions in brain and deposition. These features were used to define prion strains and raised the question of how a single protein could be responsible for such phenotypic variety. The conformational hypothesis postulates that each strain is associated with a different conformation of PrPsc, and each of these can convert its host's PrPc into a likeness of itself (16).

Inter-species barrier

Resistance of one specie to infection with prion particles from another has been ob-

served. Mice are normally resistant to hamster prions, and this resistance was abolished when the hamster transgene was introduced (17). The length of incubation time after inoculation with hamster prion was inversely proportional to the level of expression of the transgene. Moreover, when these animals were inoculated with mouse prions only mouse prions were produced. Conversely, animal inoculation with hamster prions led to the synthesis of only hamster prions. Therefore, PrP gene sequences might be responsible for the species barrier (18).

It has been observed that mice expressing both mouse and human PrPc were resistant to human prions while those expressing only human PrPc were susceptible (19,20). On the basis of these data, it was suggested that mouse PrPc prevented the conversion of human PrPc by binding to another mouse protein with a higher affinity than does human PrPc. This hypothetical protein was called protein X and may act as a molecular chaperone (20). However, there is no definitive proof of chaperone participation in prion formation in mammalian cells. Interestingly, BSE or "mad cow disease" is known to have caused prion disease in other species including domestic cats (21) and humans (22), presumably by ingestion of BSE-contaminated feed.

Human prion diseases

The human prion diseases occur in acquired, inherited and sporadic forms. Acquired prion diseases include kuru and iatrogenic CJD. Kuru assumed epidemic proportions in the first decades of this century in Papua, New Guinea, probably due to ritual cannibalism (23) and may have originated with the consumption of the remains of a CJD victim. Iatrogenic routes of transmission are treatment with human cadaveric pituitary-derived growth hormone or gonadotrophin, dura mater or corneal grafting and the use of inadequately sterilized neurosurgical instruments (24).

The inherited forms of prion disease are associated with coding mutation in the PrP gene. Twenty different mutations in the human PrP gene have been found that segregate with the illness. Mutations at codons 102 (Pro-Leu), 117 (Ala-Val), 198 (Phe-Ser) and 217 (Gln-Arg) are found in patients with GSS, while 178 (Asp-Asn), 200 (Glu-Lys), 208 (Arg-His) and 210 (Val-Ile) are found in patients with familial CJD (24). Fatal familial insomnia patients presented mutation at codon 178 (Asp-Asn) (25) and mutation at residue 171 has been observed by our group in one family suffering from severe psychiatric disorders (26). Moreover, a polymorphism at codon 129 appears to increase susceptibility to iatrogenic CJD (24). It has been suggested that mutations in the PrP gene produce proteins that are more susceptible to changing their own conformation and to acquiring a ß-sheet structure (3).

Sporadic forms of prion disease comprise the most cases of CJD and a small percentage of GSS (27). It is not known how the patients acquire the disease. They may acquire it by somatic mutation of the PrP gene, by spontaneous conversion of PrPc into PrPsc (28) or by horizontal transmission of prions from humans or animals (29).

Recently, the reports of 20 cases of an atypical variant CJD (vCJD) in 3 teenagers and 17 adults (30,31) in Great Britain and France raised considerable concern that bovine prions may have been passed to humans. In fact, Collinge (22) showed that vCJD has strain characteristics distinct from other types of CJD and which resemble those of the BSE transmitted to mice, domestic cats and macaques, consistent with BSE being the source of this new disease.

Normal function of PrPc

The function of PrPc is still unknown, mice lacking the gene that encodes the cellular prion protein (PrP 0/0) developed quite

normally (32). However, PrP 0/0 mice produced by another group presented an impaired γ-aminobutyric acid type A ($GABA_A$) receptor-mediated fast inhibition, a decrease in long-term potentiation in the hippocampus (33) and alteration on circadian rhythms (34). A third group of PrP (0/0) animals presented an extensive loss of Purkinje cells from cerebellum and progressive signs of ataxia that lead to premature death (35). PrPc appears to be involved in mitogen-induced activation in lymphocytes (36) and regulation of intracellular calcium concentration in neuronal cells (37).

Recent work suggested that copper ions bind to the N-terminal sequences in PrPc molecules (38,39). Copper analysis showed that the metal levels were 20 times lower in tissues from PrP 0/0 than in wild type animals, indicating that this association is physiological (39). Binding to copper is important for the catalytic activity of many enzymes involved in oxidative stress, including superoxide dismutase (40). Since cerebellar cells lacking PrPc are more sensitive to oxidative stress than wild type cells (41), it has been speculated that PrPc might act as a "shuttle" for copper ions destined to bind to enzymes that prevent oxidative stress (41). Copper ion may be important in protein conformation and alterations in its levels may contribute to the pathogenicity of the infective strains or to the senile dementias (38). Intensive work is still necessary to clarify the real function of PrPc.

A PrPc/PrPsc receptor protein

Mature PrPc is anchored to the outer surface of the plasma membrane by a glycosyl-phosphatidylinositol (GPI) moiety (42) and undergoes endocytosis (43) and recycling (44). Conflicting data have shown that its internalization is mediated by clathrin-coated pits (45) or by caveolae-like membranous domains (46). However, both pathways seem to require a third protein (a receptor or a prion-binding protein) to make the connection between the GPI-anchored molecule to clathrin (45) or to convert PrPc into PrPsc (46,47).

Our group has recently identified a specific receptor for cellular prion protein (48). Using the complementary hydropathy theory, which predicts that peptides encoded by complementary DNA strands can bind one another (49-51), we designed a hypothetical peptide complementary to the prion peptide previously described to be neurotoxic in primary neuronal culture (52) and responsible for PrPc internalization (53).

Serum raised against the prion complementary peptide recognized a 66-kDa membrane protein which binds recombinant and cellular PrPc *in vitro* and *in vivo*. Conversely, blocking the receptor with serum against the hypothetic peptide inhibited cell death produced by the prion neurotoxic peptide (48).

Further work is required to establish the physiological consequences of PrPc docking to the receptor. It is possible that the role of the receptor is to allow internalization of PrPc (53), as well as prion, aiding the latter in promoting the structural modifications of the former which lead to accumulation and disease (54). It is, however, also tempting to speculate that, since PrPc tends to accumulate in postsynaptic vesicles (55) both PrPc and its receptor participate in heterophilic inter-neuronal cell adhesion leading to neuronal networking (Figure 1).

Peripheral blood cell participation in prion infection

The most effective experimental transmission of prions is direct delivery to the host's brain. However, most cases of transmission were due to intramuscular injection (contaminated hormones) and oral uptake (kuru and BSE). The lymphoreticular system has been considered to be a reservoir for prion replication. PrPsc has been demonstrated in tonsil biopsies from infected indi-

viduals (56) and in intestinal Peyer's patches almost immediately after oral administration of prions (57).

Using a panel of immunodeficient mice, Aguzzi's group (58) has recently shown that animals lacking T lymphocytes develop disease when peripherally inoculated with the scrapie agent, while those without B lymphocytes do not fall ill under the same conditions. Prion agent replication in B lymphocytes is unknown, but these cells should be important at least for transportation of the agent to the peripheral nervous system (58). It is possible that after contact with the peripheral nerve endings prions can further spread trans-synaptically and along fibre tracts (59).

The expression of a specific prion ligand on the surface of B lymphocytes which should be absent in T lymphocytes may explain the differences between these cells regarding prion transportation. This seems to be the case for the receptor we have characterized (Martins VR, Nomizo R, Zanata SM, Reis LFL and Brentani RR, unpublished data). The results regarding prion transport by B lymphocytes have raised an important problem that should be extensively discussed, i.e., the screening of prions in blood to be used for transfusions.

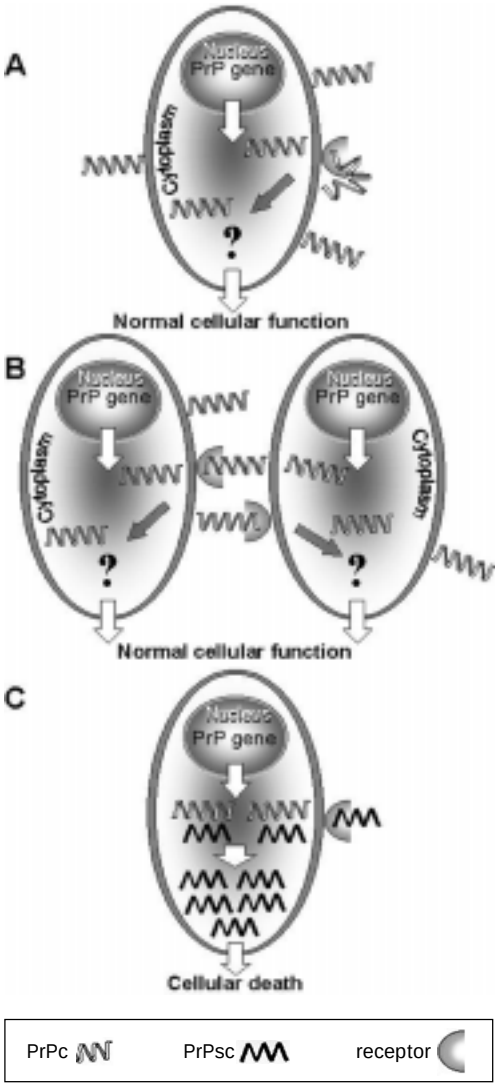

Figure 1 - Proposed mechanism of action for PrPc/PrPsc receptor in the normal (A and B) and infectious processes (C). In the normal cell, PrPc and receptor from the same cell (A) or from different cells (B) can interact and promote signal transduction, triggering their physiological function. The infectious agent should use the same receptor (C) which allows its internalization, facilitating the interaction with PrPc and the conformational changes, leading to PrPsc accumulation and cell death.

What is next?

Cloning the PrPc/PrPsc receptor is important both to design drugs that should be used to prevent the infectious agent from entering the cell or being transported to the central nervous system and to characterize the normal cellular function of PrPc. We believe that the cellular function of PrPc involves its association with other cellular proteins besides the receptor. Therefore, the characterization of prion binding to proteins with known function and the signals triggered by these interactions may help solve the mystery of PrPc cellular function.

References

1. Gordon WS (1946). Advances in veterinary research. *Veterinary Record*, 58: 518-525.
2. Prusiner SB (1991). Molecular biology of prion diseases. *Science*, 252: 1515-1522.
3. Prusiner SB (1993). Genetic and infectious prion diseases. *Archives of Neurology*, 50: 1129-1153.
4. Grifft JS (1967). Self replication and scrapie. *Nature*, 215: 1043-1044.
5. Prusiner SB (1982). Novel proteinaceous infectious particles cause scrapie. *Science*, 216: 136-144.
6. Bolton DC, McKinley MP & Prusiner SB (1982). Identification of a protein that purifies with the scrapie prion. *Science,* 218: 1309-1311.
7. Prusiner SB, Groth DF, Bolton DC, Kent

SB & Hood LE (1984). Purification and structural studies of a major scrapie prion protein. *Cell,* 38: 127-134.
8. Oesch B, Westaway D, Walchl M, McKinley MP, Kent SB, Aebersold R, Barry RA, Tempst P, Teplow DB, Hood LE, Prusiner SB & Weissmann C (1985). A cellular gene encodes scrapie PrP 27-30 protein. *Cell*, 40: 735-746.
9. Chesebro B, Race R, Wehrly K, Nishio J, Bloom M, Lechner D, Bergstrom S, Robbins K, Mayer L, Keith JM, Garon C & Haase A (1985). Identification of scrapie prion protein-specific mRNA in scrapie infected and uninfected brain. *Nature*, 315: 331-333.
10. Pan KM, Baldwin M, Nguyen J, Gasset M, Serban A, Groth D, Mehlhorn I, Huang Z, Fletterick RJ, Cohen FE & Prusiner S (1993). Conversion of alpha-helices into beta-sheet features in the formation of the scrapie prion proteins. *Proceedings of the National Academy of Sciences, USA*, 90: 10962-10966.
11. Riek R, Hornemann S, Wider G, Billeter M, Glockshuber R & Wuthrich K (1996). NMR structure of the mouse prion protein domain PrP (121-231). *Nature*, 382: 180-182.
12. McKinley MP, Bolton DC & Prusiner SB (1983). A protease-resistant protein is a structural component of the scrapie prion. *Cell,* 35: 57-62.
13. Prusiner SB (1989). Scrapie prions. *Annual Review of Microbiology*, 43: 345-374.
14. Bueler H, Aguzzi A, Sailer A, Greiner RA, Autenried P, Aguet M & Weissmann C (1993). Mice devoid of PrP are resistant to scrapie. *Cell*, 73: 1339-1347.
15. Horwich AL & Weissmann JS (1997). Deadly conformations - Protein misfolding in prion disease. *Cell*, 89: 499-510.
16. Aguzzi A & Weissmann C (1997). Prions research: the next frontiers. *Nature*, 389: 795-798.
17. Scott M, Foster P, Mirenda C, Serban D, Coufal F, Walchli M, Tarchia M, Groth D, Carlson G, DeArmond SJ, Westaway D & Prusiner SB (1989). Transgenic mice expressing hamster prion protein produce species-specific scrapie infectivity and amyloid plaques. *Cell*, 59: 847-857.
18. Prusiner SB, Scott M, Foster D, Pan KM, Groth D, Mirenda C, Torchia M, Yang SL, Serban D, Carlson GA, Hoppe PC, Westaway D & DeArmond SJ (1990). Transgenetic studies implicate interactions between homologous PrP isoforms in scrapie prion replication. *Cell,* 63: 673-686.
19. Telling GC, Scott M, Hsiao KK, Foster D, Yang SL, Torchia M, Sidle KC, Collinge J, DeArmond SJ & Prusiner SB (1994). Transmission of Creutzfeldt-Jakob disease from humans to transgenic mice expressing chimeric human-mouse prion protein. *Proceedings of the National Academy of Sciences, USA,* 91: 9936-9940.
20. Telling GC, Scott M, Mastrianni J, Gabizon R, Torchia M, Cohen FE, DeArmond SJ & Prusiner SB (1995). Prion propagation in mice expressing human and chimeric PrP transgenes implicates the interaction of cellular PrP with another protein. *Cell,* 83: 79-90.
21. Bruce M, Chree A, McConnell I, Foster J, Pearson G & Fraser H (1994). Transmission of bovine spongiform encephalopathy and scrapie to mice: strain variation and the species barrier. *Philosophical Transactions of the Royal Society of London, Series B. Biological Sciences*, B343: 405-411.
22. Collinge J, Sidle KCL, Meads J, Ironside J & Hill AF (1996). Molecular analysis of prion strain variation and the aetiology of "new variant" CJD. *Nature*, 383: 685-690.
23. Hadlow WJ (1959). Scrapie and Kuru. *Lancet*, 2: 289-290.
24. Prusiner SB (1996). Molecular biology and pathogenesis of prion diseases. *Trends in Biochemical Sciences*, 21: 482-487.
25. Tateishi J, Brown P, Kitamoto T, Hoque ZM, Roos R, Wollman R, Cervenakova L & Gajdusek DC (1995). First experimental transmission of fatal familial insomnia. *Nature*, 376: 434-435.
26. Samaia HB, Mari JJ, Vallada HP, Moura RP, Simpson ATG & Brentani RR (1997). A prion-linked psychiatric disorder. *Nature*, 390: 241.
27. Hsiao K, Baker HF, Crow TJ, Poulter M, Owen F, Terwilliger JD, Westaway D, Ott J & Prusiner SB (1989). Linkage of a prion protein missense variant to Gerstmann-Straussler syndrome. *Nature,* 338: 342-355.
28. Hsiao K, Meiner Z, Kahana E, Cass C, Kahana I, Avrahami D, Scarlato G, Abramsky O, Prusiner SB & Gabizon R (1991). Mutation of the prion protein in Libyan Jews with Creutzfeldt-Jakob disease. *New England Journal of Medicine,* 324: 1091-1097.
29. Gajdusek DC (1977). Unconventional viruses and the origin and disappearance of kuru. *Science*, 197: 943-960.
30. Bateman D, Hilton D, Love S, Zeidler M, Beck J & Collinge J (1995). Sporadic Creutzfeldt-Jakob disease in an 18-year-old in the UK. *Lancet*, 346: 1155-1156.
31. Cousens SN, Vynnycky E, Zeidler M, Will RG & Smith PG (1997). Predicting the CJD epidemic in humans. *Nature*, 385: 197-198.
32. Bueler H, Fischer M, Lang Y, Bluethmann H, Lipp HP, DeArmond SJ, Prusiner SB, Aguet M & Weissmann C (1992). Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. *Nature*, 356: 577-582.
33. Collinge J, Whittington MA, Sidle KC, Smith CJ, Palmer MS, Clarke AR & Jefferys JG (1994). Prion protein is necessary for normal synaptic function. *Nature*, 370: 295-297.
34. Tobler I, Gaus SE, Deboer T, Achermann P, Fischer M, Rulicke T, Moser M, Oesch B, McBride PA & Manson JC (1996). Altered circadian activity rhythms and sleep in mice devoid of prion protein. *Nature*, 380: 639-642.
35. Sakaguchi S, Katamine S, Nishida N, Moriuchi R, Shigematsu K, Sugimoto T, Nakatani A, Kataoka Y, Houtani T, Shirabe S, Okada H, Hasagawa S, Miyamoto T & Noda T (1996). Loss of cerebellar Purkinje cells in aged mice homozygous for a disrupted PrP gene. *Nature*, 380: 528-531.
36. Cashman NR, Loertsche R, Nalbantoglu J, Shaw I, Kascsak RJ, Bolton DC & Bendheim PE (1990). Cellular isoform of the scrapie agent protein participates in lymphocyte activation. *Cell*, 61: 185-192.
37. Whatley SA, Powell JF, Politopoulou G, Campbell IC, Brammer MJ & Percy NS (1995). Regulation of intracellular free calcium levels by the cellular prion protein. *NeuroReport*, 6: 2333-2337.
38. Miura T, Hori-i A & Takeuchi H (1996). Metal-dependent alpha-helix formation promoted by the glycine-rich octapeptide region of prion protein. *FEBS Letters*, 396: 248-252.
39. Brown DR, Qin K, Herms JW, Madlung A, Manson J, Strome R, Fraser PE, Kruck T, von Bohlen A, Schulz-Schaeffer W, Giese A, Westaway D & Kretzschmar H (1997). The cellular prion protein binds copper *in vivo. Nature*, 390: 684-687.
40. Karlin KD & Tyeklar Z (1993). *Bioinorganic Chemistry of Copper*. Chapman and Hall, London.
41. Brown DR, Schulz-Schaeffer WJ, Schmidt B & Kretzschmar HA (1997). Prion protein-deficient cells show altered response to oxidative stress due to decreased SOD-1 activity. *Experimental Neurology*, 146: 104-112.
42. Stahl N, Borchelt DR, Hsiao K & Prusiner SB (1987). Scrapie prion protein contains a phosphatidylinositol glycolipid. *Cell*, 51: 229-240.

43. Borchelt DR, Scott M, Taraboulos A, Stahl N & Prusiner SB (1990). Scrapie and cellular prion proteins differ in their kinetics of synthesis and topology in cultured cells. *Journal of Cell Biology*, 110: 743-752.
44. Shyng SL, Huber MT & Harris DA (1993). A prion protein cycles between the cell surface and an endocytic compartment in cultured neuroblastoma cells. *Journal of Biological Chemistry,* 268: 15922-15928.
45. Shyng S-L, Heuser JE & Harris DA (1994). A glycolipid-anchored prion protein is endocytosed via clathrin-coated pits. *Journal of Cell Biology,* 125: 1239-1250.
46. Vey M, Pilkuhn S, Wille H, Nixon R, DeArmond SJ, Smart EJ, Anderson RG, Taraboulos A & Prusiner SB (1996). Subcellular colocalization of the cellular and scrapie prion proteins in caveolae-like membranous domains. *Proceedings of the National Academy of Sciences, USA,* 93: 14945-14949.
47. Yehiely F, Bamborough P, Da Costa M, Perry BJ, Thinakaran G, Cohen FE, Carlson GA & Prusiner SB (1997). Identification of candidate proteins binding to prion protein. *Neurobiology of Disease,* 3: 339-355.
48. Martins VR, Graner E, Garcia-Abreu J, Souza SJ, Mercadante AF, Veiga SS, Zanata SM, Neto VM & Brentani RR (1997). Complementary hydropathy identifies a cellular prion protein receptor. *Nature Medicine*, 3: 1376-1382.
49. Brentani RR (1988). Biological implications of complementary hydropathy of amino acids. *Journal of Theoretical Biology*, 135: 495-499.
50. Brentani RR (1990). Complementary hydropathy and the evolution of interacting polypeptides. *Journal of Molecular Evolution*, 31: 239-243.
51. Baranyi L, Campbell W, Ohshima K, Fujimoto S, Boros M & Okada H (1995). The antisense homology box: A new motif within proteins that encodes biological active peptides. *Nature Medicine*, 1: 894-901.
52. Forloni G, Angeretti N, Chiesa R, Monzani E, Salmona M, Bugiani O & Tagliavini F (1993). Neurotoxicity of a prion protein fragment. *Nature*, 362: 543-546.
53. Shyng S-L, Moulder KJ, Laesko A & Harris DA (1995). The N-terminal domain of a glycolipid-anchored prion protein is essential for its endocytosis via clathrin-coated pits. *Journal of Biological Chemistry*, 270: 14793-14800.
54. Gasset M, Baldwin MA, Lloyd DH, Gabriel JM, Holtzman DM, Cohen F, Fletterick R & Prusiner SB (1992). Predicted alpha-helical regions of the prion protein when synthesized as peptides form amyloid. *Proceedings of the National Academy of Sciences, USA*, 89: 10940-10944.
55. Askanas V, Bilak M, Engel WK, Leclec A & Tome F (1993). Prion protein is strongly immunolocalized at the postsynaptic domain of the human neuromuscular junctions. *Neuroscience Letters*, 159: 111-114.
56. Hill AF, Zeidler M, Ironside J & Collinge J (1997). Diagnosis of new variant Creutzfeldt-Jakob disease by tonsil biopsy. *Lancet,* 349: 99-100.
57. Kimberlin RH & Walker CA (1989). Pathogenesis of scrapie in mice after intragastric infection. *Virus Research,* 12: 213-220.
58. Klein MA, Frigg R, Flechsig E, Raeber AJ, Kalinke U, Bluethmann H, Bootz F, Suter M, Zinkernagel RM & Aguzzi A (1997). A crucial role for B cells in neuroinvasive scrapie. *Nature,* 390: 687-690.
59. Brandner S, Raeber A, Sailer A, Blattler T, Fischer M, Weissmann C & Aguzzi A (1996). A normal host prion protein (PrPC) is required for scrapie spread within the central nervous system. *Proceedings of the National Academy of Sciences, USA*, 93: 13148-13151.