**Background:**

Prions are infectious agents containing only protein (no nucleic acid). The host cell has a similar version to the prion protein. Host proteins are correctly folded and prion proteins are misfolded. Prions are thought to be the cause of the transmissible spongiform encephalopathies (TSEs). TSEs are made of PrP’s, designates the prion protein. This protein can fold in multiple, structurally distant ways. It is thought that at least one of these folds is transmissible to other prion proteins, leading to the disease that has commonality to viral infections. Bovine spongiform encephalopathy (BSE), also known as “mad cow disease”, and scrapie in sheep are thought to be caused from misfolded prions. Human prion diseases include Creutzfeldt-Jackob Disease (CJD) and its variant (vCJD), Gerstmann-Straussler-Scheinker syndrome, Fatal Familial Insomnia, and kuru (Prion Diseases, 2015). The misfolded proteins cause healthy proteins to misfold and cell death occurs as insoluble proteins accumulate in cytoplasm. All of the known human prion diseases appear to affect the brain and other neural tissue, which currently is untreatable and fatal (Prusiner, 1998).

Prions may replicate by transmitting their misfolded protein. This occurs when the prion enters into a healthy organism and causes the properly folded proteins to change into the misfolded form and this process is continuous. The incubation period of the prion disease is determined by the exponential growth rate associated with prion replication. The prion replication is a balance between breakage of aggregates and linear growth (Masel, 1999). Prions rely heavily on the presence of normally folded proteins in which to breed more of themselves. That being said, animals that do not express the normal form of the prion protein cannot develop or transmit the disease.
Prions have different forms; the normal form is the PrPC, where the “C” refers to the cellular version. The infectious form is the PrPSc, and the “Sc” stands for scrapie which is the prototypic prion disease found in sheep. Another form of a prion is the PrPres which stands for Protease-resistant PrPSc-like protein; this is an isoform of PrPc but this form is structurally changed and molded into a misfolded proteinase K-resistant form in a test tube (in vitro). The transmission method the studied prion disease is area of interest and it has been recognized that prion diseases can come about three different ways: acquired, familial or sporadic (Groschup, 2001).

**Protein X Theory:**

The “Protein X” theory centers on the transmission method the prions use, hypothesizing that an unidentified cellular protein (Protein X) enables the conversion of PrPC to PrPSc by taking a molecule of each of the two together into a complex (Telling, 1995). The theoretical transmission process consists of the following: the compact isoform PrPSc provides a template for conversion of normal cellular PrPC into a developing PrPSc through a process facilitated by the genetically proven protein X (see Figure 1) (Premzl, 2009).

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**Figure 1: Prion Protein X Theory**
The protein X theory explains that, in order for the prion replication process to be carried out, a cellular co-factor is required. The existence of a host-encoded conversion factor was first hypothesized when experiments with transgenic mice were expressing chimeric prion proteins from two different species. It was in 1982 when Stanley B. Prusiner of the University of California, San Francisco that coined the term “Protein X” in reference to the cellular co-factor. Moreover, there is no formal proof that the co-factor is even a protein at all (Soto, 2011). Leading evidence of the protein X spawned from Protein Misfolding Cyclic Amplification (PMCA) studies of PrPC to PrPSc conversion. Purified hamster PrPC was not converted when mixed with highly purified PrPSc. Furthermore, conversion was returned when the complete brain homogenate was added to the sample. The results of this experiment show that unknown factors (i.e. protein X) in the brain homogenate are vital for the conversion process (Soto, 2011).

In 2009, Premzl et al. examined that positive selection at the amino acid level in PrP might have occurred in human and related species from the superordinal group Euarchonta (including primates), as well as in bovine and related species from the superordinal clade Laurasiatheria (e.g. bats and whales). The results of the study showed evidence that positive selection at the amino acid level might have taken place in the PrP signal sequences and conformationally plastic PrP regions, as well as at the protein X binding site; providing further evidence of the existence of the protein X (Premzl, 2009).

In 2007, Hachiya et al. conducted research to examine the possible role of protein X in regulating a physiological endoproteolytic cleavage of the cellular prion protein. The binding of PrPC to protein X is accompanied by a conformational change (i.e. unfolding) in PrPC, similar to a study on a molecular chaperone-leading the change from one form to another. The intermediate has been identified as PrP. The PrPSc/PrP/protein X complex is modulated by an unknown
process, followed by PrP conversion into PrPSc and protein X release (Hachiya, 2007). Amino acid residues overlap at the physiological cleavage site where another putative molecular chaperone is assumed to target and operate in conjunction with PrP proteases. The amino acid residues are noted to be important because they assist in the target site of protein X for the pathological conversion into PrPSc. It is hypothesized that these molecules show a chaperone-like (i.e. unfolding) activity against the same hydrophobic residues in PrPC could be similar (Hachiya, 2007). Furthermore, if it is assumed that protein X exists only for the pathological condition for prion replication, but this is unlikely. A current hypothesis examines physiological endoproteolytic cleavage pf PrPC and its pathological conversion to PrPSc (Hachiya, 2007). The identification of this putative molecular chaperone working in conjunction with proteases that focuses on PrPC is vital because it may be also directed toward identifying protein X in prion replication (Hachiya, 2007).

**Prion Diseases related to Protein X:**

Transmissible spongiform encephalopathies (TSEs) or prion diseases are degenerative disorders that affect the central nervous system and can lead to motor dysfunction, dementia and death. The prion diseases as mentioned earlier include scrapie of sheep, bovine spongiform encephalopathy (BSE) in cattle, and the human diseases including Crutzfeldt-Jakob disease (CJD), Gerstmann-Straussler-Scheinker syndrome, and fatal familial insomnia (Weissmann, 2002). PrPSc mainly accumulates in the brain and is thought to be the principal or only constituent of the prion. There have been differences in the primary structure of the PrPC and PrPSc have found. This could mean that there are differences in their conformations. The tertiary structure of the PrPC is well known but the same cannot be stated for PrPSc. However, the beta-
sheet content of PrPSc is higher, while the beta-sheet content of PrPc is relatively low and provides for information for further research (Weissmann, 2002).

Weissmann et al., theorized that protein X may have a role when essential cellular components are required for prion uptake and/or replication. This can be seen in the ectopic overexpression of PrP in T or B lymphocytes of PrPolo mice does not render these cells vulnerable to infection in vivo. In addition, PrP expression is not the only feature needed for vulnerability of N2a neuroblastoma cells to prions in vitro. Furthermore, it thought that prion protein does not have a nucleic acid due to its unusual resistance to UV irradiation (Weissmann, 2002).

In 2007, Lee et al. attempted to disapprove the protein X theory. The goal was to evaluate the effect of the Q218K variant of full length recombinant prion protein (Q218K rPrP), one of the dominant-negative mutants, on cell-free polymerization of wild-type rPrP into amyloid fibrils. Results showed that both Q218K and wild-type (WT) rPrPs were incorporated into fibrils when incubated as a mixture; although the yield of polymerization was substantially decreased in the presence of Q218K rPrP. In conclusion of the study Lee et al., demonstrated that the Q218K variant exhibits the dominant-negative effect in cell-free conversion in the absence of protein X, and that this effect is controlled by physical interactions between WT rPrP and Q218K through the polymerization process (Lee, 2007).

**Conclusion:**

Prions are infectious agents containing only protein (no nucleic acid). Prion proteins interact with host proteins, causing host proteins to misfold and become non-functional and insoluble. The prion protein exists in two isoforms, the cellular form PrPC and the disease form
PrPC is rich in alpha-helices and PrPSc is rich in beta-structures (Hachiya, 2007). Many studies showed that single-point mutations of the prion protein displayed dominant negative effects on prion replication. These dominant-negative effects may be controlled by protein X. The protein X theory assumes that there is an unknown cellular cofactor mediating the prion replication process (Lee, 2007). Current literature explains different perspectives on the protein X theory, on the functionality of it or its existence entirely. What is unknown is the mechanism from which PrPC is transformed into PrPSc. Once the mechanism is understood, it can be interrupted or even stopped completely; and then human diseases such as Creutzfeldt - Jakob disease (CJD), its variant (vCJD), Gerstmann-Straussler-Scheinker syndrome, Fatal Familial Insomnia, and kuru can be better controlled or eliminated from human future completely.

References:


