# Prions the cellular prion protein (prpc) into

Prions are unique unconventional infectious agents lacking nucleicacid materials. They are propagated by misfolded cellular prion proteins (PrPc)which give rise to rare but devastating neurodegenerative diseases in humansand animals called Prion diseases or Transmissible Spongiform Encephalopathies(TSE). The diseased mis-folded isoformof prion proteins ( PrPSc) characterized by ?- sheet enrichment andproteinase K resistance are thought to be the causative agent of thisrare but fatal group of neurodegenerative diseases (Byungi Jang et al., 2013). Prion diseases affect a broad spectrum of mammals and have differentnames depending on the host species, including scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle, chronic wasting diseases(CWD) in cervids.

In human the disease pathologies connected with Prion proteinare kuru, Creutzfeldt- Jacob Disease (CJD), Gerstmann Straussler- Scheinker(GSS) and Fatal Familial Insomnia (FFI). Prion Diseases may arise spontaneouslywith unknown causative agent (sporadic), be inherited (genetic) or acquired byinfection (iatrogenic), which earned it the name transmissible spongiform encephalopathies(TSE) (Sigurdson CJ and Aguzzi A, 2007; Prusiner SB, 1996; Huang Z et al., 1995). The conversion of the cellular prion protein (PrPC) intomisfolded disease isoform (PrPSc) through a series of post-translationallymodification processes that aggregates in the brain of infected individualrepresent the hallmark of prion disease (Oesch B et al., 1985). Transmissible Spongiform encephalopathies arecharacterized by various neurological symptoms and common histopathologicalfeatures such as spongiform degeneration of central nervous system (CNS), astrogliosis,

neuronal apoptosis and accumulation of extra cellular proteindeposits that may have or not have properties of amyloid fibrils (Y. S Kim etal., 1990; R.

I Carp et al., 1995; Westermark P et al., 2005; Chesebro B et al.

, 2005; Aquzzi A, 2005; Aguzzi A and Heikenwalder M, 2006). Although prion diseases are thought to be caused by unconventionalinfectious pathogens, they possess two unique features that are shared withother conventional infectious agents. These features are existence of multipleprion strains and species barrier.

Theexistence of multiple prion strains gives rise to different, specific andstable disease phenotypes distinguished by clinical manifestations, incubationtime, PrPSc patterns and neuropathological features that arefaithfully maintained upon repeated passage in the same host (Prusiner SB, 1998; Collinge J, 2001; Collinge J and Clarke A. R., 2007; Cobb N. J and SurewiczWK., 2009; Laura S.

et al., 2013). Species barrier postpones the infection of aprion across other species (Hagiwara K et al., 2013). The biological effect ofspecies barrier is reflected by complete resistance or low sensitivity with alonger incubation time in some animals when inoculated with prion agents fromother species (Shi et al., 2015). Consequence of this distinct molecularconformation results in two possible scenario when prion strain from onespecies infects an animal of different species. The infectious PrPScmay have a conformation that is not compatible with the PrPC conformationof the host, resulting in non-conversion; in this case species barrier isabsolute and there is no disease transmission.

The other possibility is thatthe PrPSc conformation is compatible with the PrPC of thehost, allowing conversion and there for clinical infection. In this case theresulting strain can be identical to the infectious strain or can change into adifferent prion strain characterized by different conformation (Safar J. etal., 1998; Tanaka M. et al., 2004).

Cross species transmission of  prion agent was first demonstrated using asheep scrapie agent in laboratory rodents in the 1930s (Gao C. et al., 2016). Theonly transmissible spongiform encephalopathy proven to have crossed a speciesbarrier naturally is bovine spongiform encephalopathy, which is transmittedfrom cattle to human; the causative agent of variant CJD in humans (Agrimi U. et al., 2008). Differing opinions has been given on the likely factors responsiblefor interspecies transmission of prion species.

Certain groups support thehypothesis that Interspecies transmission is possible primarily because thereis compatibility between the conformation of PrPSc of the infectiousagent and the PrPC of the new host (Bruce M. et al., 1994; Bruce ME. Et al., 2002; Hill AF and Collinge J.

, 2004). Even though the primary structureof the cellular prion protein is conserved between species, some amino acidicresidues are different resulting in PrPC with distinct molecularconformations. Notably, ovine and murine prion protein have the same aminoacids at position 154 and 169, while bovine PrP differs only at codon 154, having H instead of Y.  Position 154 and159 are quite variable among mammalian prion proteins. Human and bovinesequences are 154H-169S, sheep and goat 154Y-169S, elk and deer 145Y-169N(Agrimi U. et al., 2008).  Othersbelieved apart from the

homology between PrP sequences, certain unknown factorseither in the host microenvironment or the prion protein itself may directly orindirectly be responsibility for susceptibility to prion strains duringcross-species transmission (Shi et al.

, 2015). Nicolas et al., (2017) fromtheir research findings reported that PrPC is not the sole factorinvolved in the region –specific conversion of PrP. In their supplementationexperiments using homogenates prepared with brain areas from PrP knock-outmice, they suggested the presence of region-specific modulators of PrPconversion, other than PrPC. Timothy D. Kurt and colleagues (2017)reported that five key residue positions markedly impacted prion conversion, four of which were in steric zipper segments where side chains from amino acidstightly interdigitate in a dry interface.

Their findings suggested that prionconversion between PrP sequences does not require an exact match in the sidechain between PrPC and PrPSc, nonetheless side chaincomplementarity in the amyloid-prone segments is essential. Their resultssupported the hypothesis that certain interspersed asparagine and glutamineresidues may facilitate the anchoring of PrPC to PrPSc throughstrengthening a replicative interface, driving prion conversion betweendissimilar sequences and lowering the barrier for aggregation. Also, newfindings supports the hypothesis that sialylation of prion proteins may havecontrolling effect on the rate of prion amplification and cross speciestransmission (Katorcha E. et al., 2014). Previous studies have demonstrated that infectious prion strainscan be discriminated from one another based on their differing biochemicalproperties. These studies utilizes

biochemical properties such as global and/or local secondary structure (Caughey, B et al.

, 1998; Baron, G. S eta l., 2011), electrophoretic mobility of protease resistance core of PrPSc, the relative proteinase K resistance of PrPSc (Kascsak RJ et al., 1985;  Kuczius T et al.

, 1999; Jacobs JGet al., 2007), glycosylation profile, exposure of surface-exposed epitopes (Safar J et al., 1998)  and conformationalstability as defined by resistance to denaturation by temperature and/ orchemical agents such as Guanidine Hydrochloride ( Bett, C et al., 2013; Ayers, J. 1 et al.

, 2011; Peretz , D et al., 2002; Legname G  et al., 2006; Colby , D. W et al., 2009; Bett, C et al., 2012).  A conformationalstability assay (CSA), combining guanidine hydrochloride denaturation withlimited proteolysis using Proteinase K, measures the progressive loss of PrPScproteinase K resistance after exposure to increasing concentration of GuanidineHydrochloride.

It showed that different prion strains may exhibit distinctdenaturation profiles (Peretz, D et al., 2001). A conformation-dependentimmunoassay (CDI) demonstrated the existence of multiple strain –specified PrPScconformers by quantifying the immunoreactivity of native and denatured PrPScof eight hamster prion isolates and also showed that prion-infected brainscontain both protease-sensitive (sPrPSc)  and protease-resistant PrPSc (r PrPSc)( Safar J et al.

, 1998, 2005). Conformational Stability and Solubility Assay(CSSA) based on differential solubility of PrPc and PrPSc measures PrPSc solubility as afunction of increased exposure to Guanidine Hydrochloride in the absence ofproteinase K, giving provision to study both protease sensitive and protease-resistantPrPSc ( Pirisinu, L et al., 2010). Legname and fellow researchers, (2006) reported that a reduced resistanceto GdnHCl denaturation, indicative of a reduced conformational stability, correlates with a shorter incubation time in mouse adapted prion strains asless stable mice prions replicates more rapidly.

Decreasing PrPScstability increases the fragmentation of PrPSc molecules, thereforeallowing the exposure of more PrPSc surface , which is able to bindto PrPc , resulting in an increased rate of PrPSc formationand hence a shortening of incubation times. In contrast, a different result wasreported for hamsters as short incubation period strains were characterized bymore stable PrPSc (Ayers, J. I et al., 2011).

Also Conformationalstability has been correlated with ability of different prion strains to invadethe central nervous system (CNS). Conformationally unstable prions has beenfound to be highly neuro-invasive, efficiently generate PrPSc moleculeswithin a short incubation period and also form diffuse, toxic non-fibrillar PrPaggregates in the CNS hence rapidly progressing to terminal disease while moreconformationally stable prions are weakly neuro-invasive and form dense, congophilic, fribillar plaques with mice progressing to terminal disease moreslowly (Bett C et al., 2012; Silveira  JRet al., 2005). Scrapie, a transmissible neurodegenerative disease

in sheep andgoats is the oldest known Animal prion diseases (Shi et al., 2012).

It has beenfound to overcome species barriers to experimentally infect other rodentsthrough interspecies transmission. Basedon incubation time, clinical manifestations, neuropathological features, distribution of PrPSc in the central nervous system, electrophoreticmobility and glycosylation pattern, over twenty scrapie strains seriallypassaged and adapted to laboratory animals have been described till date(Morales R. et al., 2007). Some are ME7, 22L, 139A, 139H, 263K, RML, 22C, 87A, 87V, 79A, 79V, 301C, 301V (L. J.

M. van Keulen et al., 2015).

Each scrapie strainexhibits tropism targeting specific brain regions. 22L targets the cerebellum, 139A, the cerebral cortex and ME7, hippocampus. ME7scrapie strain was originally isolated from a scrapie infected sheep in the Suffolk flock of MoredunResearch Institute in the UK(Zlotnik I and Rennie JC., 1963).

The primary feature of ME7 strain is markedneuronal loss in the CA1 Field of the hippocampus. ME7 infected animals havebeen found to show marked vacuolation of the hippocampal, septal and thalamicneutrophil. Hippocampal CA1 pyramidal neuron loss was discovered to be relativelysignificant in ME7 (C.

Cunningham et al., 2005). Mouse adapted ME7predominantly target the polymorph layer of the dentate gyrus and stratumlucidum, the CA3 field and

the pyramidal layer with granular labeling and smallaggregates. It displayed a pattern of predominantly widespread granulardeposition within the neuropil and the presence of small aggregates (Beck etal., 2012).

In ME7 model of prion disease, proteinase K resistant PrPSc is readily detected at late stage of thedisease and is widespread throughout the hippocampus. Also, at the advancedstage of the disease, there is a clear reduction in the volume of hippocampalformation, hippocampal cell loss indicated by thinning of the stratum pyramidalof CA1 and a decrease in the intensity and disorganization of staining ofseveral synaptic markers, including the synaptic vesicle proteinSynaptophysin. (Ayodeji A.

Asuni etal., 2014). Also peculiar to ME7 infection is the observation thatbehavioral changes and hippocampal synaptic loss occurs several weeks prior toneuronal cell death (C. Cunningham et al., 2003). Mouse adapted ME7 scrapie strain serially passaged in C57BL/6induces neurodegeneration and gliogenesis in the hippocampus brain region ofthe infected animals. The re-transmissibility of mouse adapted strain totransgenic mice expressing ovine prion protein remains unknown.

Hence in this studythe re-transmissibility and the distinct disease phenotype generated due to interspeciestransmission of mouse adapted ME7 scrapie strain to Ovine prion protein transgenicmice was investigated.