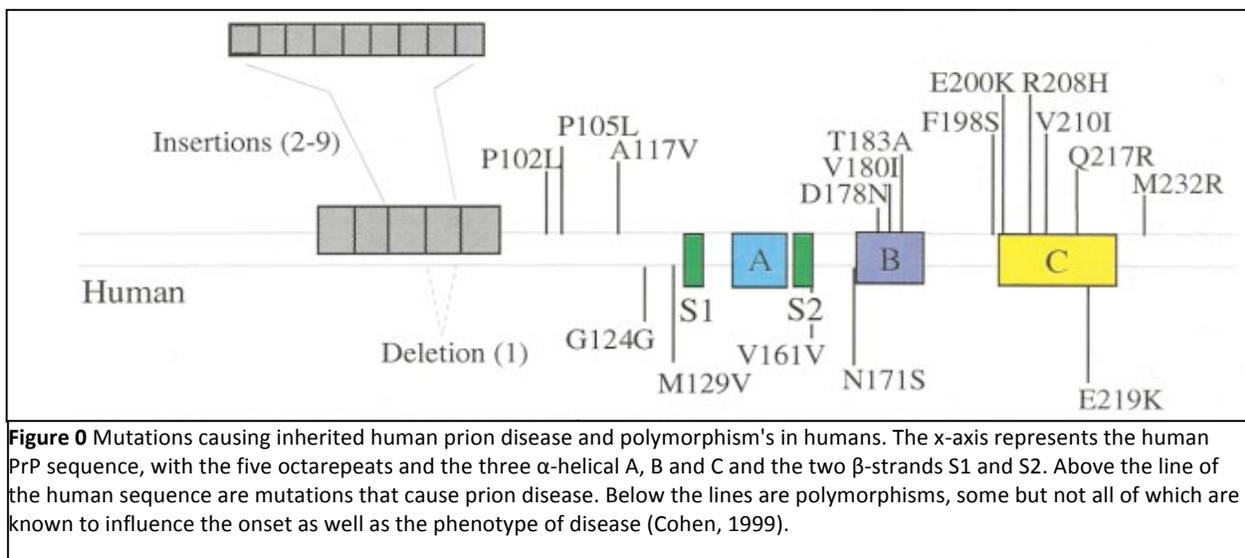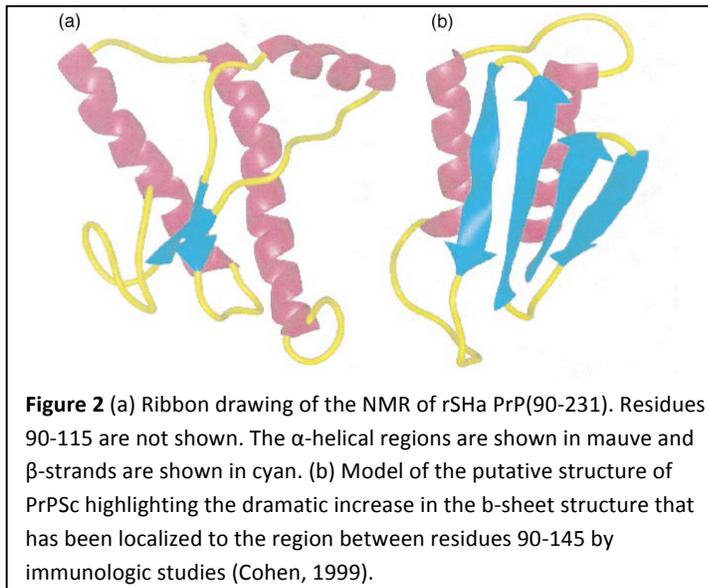Justin Vincent
BIOC 118Q
Prof. Doug Brutlag

Human Prion Diseases: Classic Definitions, Problems, and New Directions in Research

Human prion diseases are a class of neurodegenerative diseases unique in the sense that they possess sporadic, familial, and infectious etiologies. The diseases, Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler syndrome (GSS), fatal familial insomnia (FFI), and kuru, are all associated with a misfolded isoform of the prion protein (PrP), the protein product of the *prnp* gene located on chromosome 20 (see **Fig. 1**). There have been strong correlations between particular point mutations and prion pathologies, while some polymorphisms, particularly M129V, are related to latency and incubation times (Hu W, 2007).

The non-pathologic, cellular PrP (PrP^c) is ubiquitous throughout the body's tissues, though found in the highest concentrations in the brain.   The *prnp* gene consists of a single reading frame, with three exons, coding for a 253 amino acid polypeptide (Prusiner, 1997). The protein is modified through the removal of 22 amino acids from the N-terminus and 23 from the C-terminus with the addition of a glycophosphatidyl inositol (GPI) anchor that secures it in the

Figure 0 Mutations causing inherited human prion disease and polymorphism's in humans. The x-axis represents the human PrP sequence, with the five octarepeats and the three α-helical A, B and C and the two β-strands S1 and S2. Above the line of the human sequence are mutations that cause prion disease. Below the lines are polymorphisms, some but not all of which are known to influence the onset as well as the phenotype of disease (Cohen, 1999).

Figure 2 (a) Ribbon drawing of the NMR of rSHa PrP(90-231). Residues 90-115 are not shown. The α-helical regions are shown in mauve and β-strands are shown in cyan. (b) Model of the putative structure of PrPSc highlighting the dramatic increase in the b-sheet structure that has been localized to the region between residues 90-145 by immunologic studies (Cohen, 1999).

cellular membrane and glycosylation at two asparagine residues (Flechsig, 2004). The N-terminus contains a 5 octarepeat region that has been shown to bind metal ions, in particular Cu^{2+}, suggesting a role in mediating copper toxicity (Mastrangelo, 2001). Insertions in the octapeptide region are a positive marker for the familial component of CJD. Tertiary structure consists of a "flexible" N-terminal region of about 100 amino acids and a C-terminal region consisting of 3 α-helices and one small parallel β-sheet (Cohen, 1999).

Prion disease seems to be the result of conformational change in PrP^c from the largely helical cellular form to a β-sheet rich form prone to the formation of amyloid plaque in neural tissue; though controversy remains as to whether this is the causative agent or a byproduct of some other unknown mechanism (Caramelli, 2006). The mechanism by which PrP^c is converted to PrP^{Sc}, the pathologic form, remains elusive; however, knockout and transgenic studies in mice have provided much insight. Different strains of $Prnp^{0/0}$ mice have revealed that the normal physiological PrP is a necessary precursor to PrP^{Sc}. $Prnp^{0/0}$ mice inoculated with prion infected homogenate do not develop prion disease. Most knockout lines developed and lived normally presenting with only clinically inconsequential demyelination later in life. One line did develop substantial Purkinje cell loss and ataxia, but further research showed the problem due to ectopic up regulation of the downstream *Dpl* gene as a result of extensive deletion in the areas flanking

Prnp (Mastrangelo, 2001). Expression of PrP^c in transgenic mice expressing Dpl alleviated the problem (Flechsig, 2004).

A general mechanism has been proposed by Dobson for the formation of amyloid plaques in conformational diseases (2001). Since protein folding *in vivo* normally involves a nascent polypeptide chain achieving its most stable, lowest energy conformation the question is raised, how does the protein achieve its pathogenic form in the first place? There must be some thermodynamic or kinetic disparity between the different isoforms that is some way tipped to favor the pathogenic form.
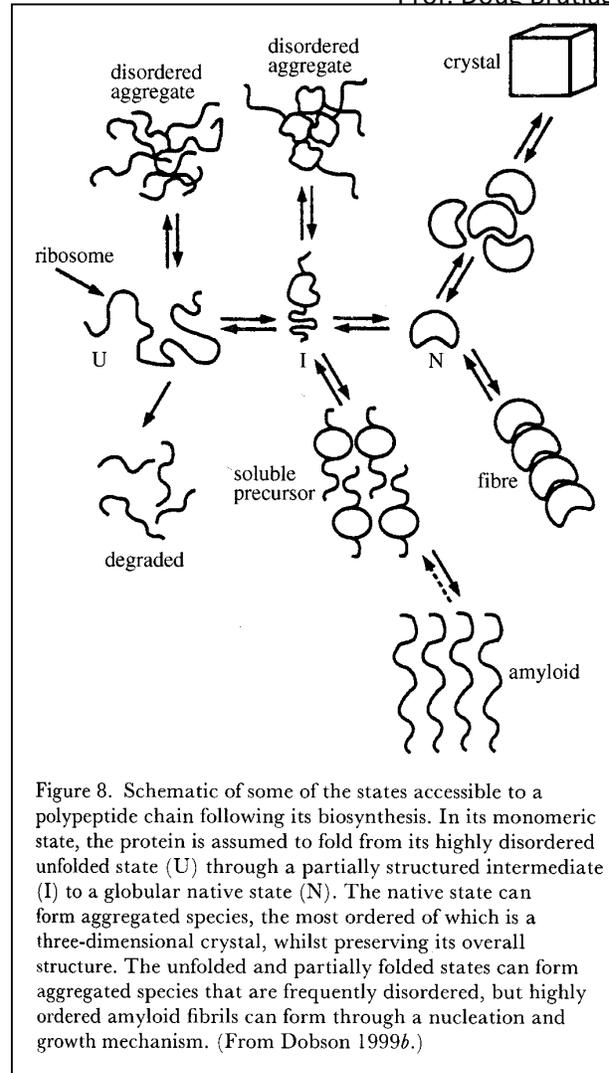

Figure 8. Schematic of some of the states accessible to a polypeptide chain following its biosynthesis. In its monomeric state, the protein is assumed to fold from its highly disordered unfolded state (U) through a partially structured intermediate (I) to a globular native state (N). The native state can form aggregated species, the most ordered of which is a three-dimensional crystal, whilst preserving its overall structure. The unfolded and partially folded states can form aggregated species that are frequently disordered, but highly ordered amyloid fibrils can form through a nucleation and growth mechanism. (From Dobson 1999*b*.)

Exactly how and why this happens remains largely unclear. Single point mutations rarely disturb a protein's free energy by greater than 2-3 kcal (Cohen 1999). This could explain why the mutated PrP^c is so much more likely to undergo the change to PrP^{Sc}. If the mutant PrP^c is at a higher free energy level than wild type PrP^c and the mutant PrP^{Sc} is sufficiently more stable than the "c" condition the phenomenon could be explained. Unfortunately, data do not support this conclusion (Cohen, 1999). From a kinetic point of view, if the energy of activation, E_A, were large enough then the energy difference between PrP^c and PrP^{Sc} could be negligible. A mutation could increase the energy of the substrate, decreasing E_A, sufficiently to greatly increase the

likelihood of making the change. If this is the case, then the nucleation/growth mechanism put forth by Dobson is feasible. This, along with the idea of PrP^{Sc} acting as a template to assist in the conversion from "c" to "Sc," seems to be the case.

While the body of knowledge surrounding PrP is growing, the actual cellular function remains unclear. In the CNS, PrP has been shown to promote neuronal survival. In cell culture PrP has been shown to protect against Bax mediated cell death, a pathway that releases apoptogenic factors from the mitochondria (Hu W, 2007). A protective role in ischemic stroke has also been implied. Recent research has started getting away from analysis strictly relevant to the CNS, showing constitutive expression of PrP^{c} in the membranes of dendritic cells and monocytes in levels close to that of the MHC II complex molecules involved in antigen presentation (Hu W, 2007). Studies using PrP^{c} deficient mice have yielded conflicting results in *in vivo* and *in vitro* studies, showing increased and decreased rates of phagocytosis respectively (Hu W, 2007). Furthermore, PrP has been shown to affect T cell activation and proliferation through a number of pathways.

Of the prion diseases found in Humans CJD is the most common, occurring in one person per million per year. With sporadic, familial, and acquired forms CJD remains a difficult disease to diagnose and treat. Cases of sCJD, accounting for about 10-15% of all cases, usually present in the sixth decade of life and usually result in death within two years, with 70% of patients succumbing within 6 months (Collinge, 2001). There are a broad range of symptoms including ataxia, dementia, myoclonus, fatigue, insomnia, and cortical blindness. Since the outbreak of variant CJD (vCJD) due to the ingestion of prion infected beef in England a number of new health risks have come to the surface. Because of the differences in incubation times afforded by

the M129V polymorphism, as first identified in kuru, there remains the possibility of another outbreak in future years. This means there is an at risk population that is essentially acting as carriers of the disease in the mean time. This population could unwittingly be propagating the disease through donating blood, as blood transfusion has been identified as the source of disease in some patients (Collinge, 2001). Dura matter grafts and cadaver derived human growth hormone have also been identified as transmission vehicles. PrP^{Sc} has been shown to associate with LDL and apoB in blood (Safar, 2006), giving the possibility of screening, though more development is required. Treating prion disease remains a most difficult task because by the time symptoms present the pathology is already quite advanced. There have been many compounds identified that will interact and interfere with PrP^{Sc} propagation. Unfortunately, they are not good models for treatment because of toxicity and inability to cross the blood brain barrier (Caramelli, 2006).

In light of the new research showing correlations between PrP and various cellular processes it seems that a wealth of new information lay just beyond the horizon. Given this information, along with recent developments in molecular-genetic techniques, a broad based genome-wide association study focusing on all known prion diseases is in order. One such study has been published on vCJD which has revealed a handful of SNPs worth investigation (Mead, 2009); although a small sample size due to the rarity of the disease could have precluded the researchers from making more significant connections. Because vCJD affects primarily European and Japanese populations a more broad analysis of all prion diseases, which affect all populations worldwide, is definitely needed.

Justin Vincent
BIOC 118Q
Prof. Doug Brutlag

Even with the promises of new research the prion hypothesis remains highly controversial. There remains compelling evidence that a virion, or slow virus, may be the infectious particle while the amyloid deposits of PrP^{Sc} are a secondary result of infection (Manuelidis, 2007). Manuelidis maintains that while the prion concept was sensationalized by the exotic character of the kuru epidemic and the more recent outbreaks of vCJD in Europe, a large body of experimental evidence pointing towards a viral pathogen has been largely overlooked by the scientific community as a whole. She holds that with new and rapid advances being made in cell and molecular biological techniques the nature of such a virus can be more readily accessed than ever before.

Whether the infectious agent be a virus or the prion particle the fact remains that the diseases associated with them are devastating and completely untreatable. With the epidemiologic threat of another large outbreak of vCJD in future years it is imperative that researchers remain diligent in seeking answers. Genome-wide association studies have the potential to unlock some of the mysteries behind PrP function. Characterization of the roles of cellular PrP will prove an invaluable tool in making strides towards understanding these diseases. Research into the possibility of a viral cause also needs to be taken seriously as the presence of a virus has the potential to explain many of the discrepancies in current prion etiology.

Justin Vincent
BIOC 118Q
Prof. Doug Brutlag

Works Cited

Caramelli, M. e. (2006). Prion Diseases: Current Understanding of Epidemiology and Pathogenesis, and Theraputic Advances. *CNS Drugs , 20* (1), 15-28.

Cohen, F. E. (1999). Protein Misfolding and Prion Diseases. *J. Mol. Biol , 293*, 313-320.

Collinge, J. (2001). Prion Diseases of Humans and Animals: Their Causes and Molecular Basis. *Annu. Rev. Neurosci , 24*, 519-50.

Dobson, C. (2001). The Structural Basis of Protein Folding and its Link to Human Disease. *Phil. Trans. R. Soc. Lond. , B*, 133-145.

Flechsig, E. a. (2004). The Role of PrP in Health and Disease. *Current Molecular Medicine , 4*, 337-353.

Hu W, R. R. (2007). Prion Proteins: a biological role beyond prion diseases. *Acta Neurol Scand , 116*, 75-82.

Manuelidis, L. (2007). A 25nm Virion is Likely the Cause of Transmissible Spongioform Encephalopathies. *Journal of Cellular Biochemistry , 100*, 897-915.

Mastrangelo, P. a. (2001, October 5). *Biology of the Prion Gene Complex*. Retrieved March 9, 2009, from NRC Research Press Web-site: http://bcb.nrc.ca

Mead, S. e. (2009). Genetic risk factors for variant Creutzfeldt–Jakob disease:a genome-wide association study. *Lancet Neurol , 8*, 57-66.

Prusiner, S. a. (1997). Genetics of Prions. *Annu. Rev. Genet. , 31*, 139-75.

Safar, J. a. (2006). Human Prions and Plasma Lipoproteins. *PNAS , 103* (30), 11312-17.

Justin Vincent
BIOC 118Q
Prof. Doug Brutlag