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THE PRION

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Transmissible spongiform encephalopathies (TSE) have been documented in livestock for centuries but the nature of the putative causative agent as a contagious, mutant form of a host-encoded protein is a very recent discovery whose nuances remain unclear. In its normal conformation, the Prion is believed to be a short-lived uptake protein ubiquitous in nervous tissues. In contrast, the mutant Prion usually has an identical primary structure, but has a radically different tertiary and quaternary structure that confers on it unusual stability and resistance to the normal post-translational reactions. Most importantly, the mutant protein binds to the normal Prion protein and alters its conformation to the mutant form. Transmission of TSE from host to host has been observed to occur primarily through ingestion of infected tissue and introduction of the mutant Prion to nervous tissue in the mouth, such as the cranial nerves serving the tongue. It is believed that the mutant Prion is transported within the parenchyma via highly motile microglia. The latent damage from eventual accumulation of mutant Prion is the result of the host's immune response to the protein that involves inflammatory TNF-alpha and IL-1 alpha and beta, among others. Clinical symptoms, however, presented well after the host's immune response resulted in spongiform changes to nervous tissue. Fortunately, there currently exists promising research that seeking to explain natural immunity to TSE and apply it to unaffected individuals.

Les encéphalopathies spongiformes transmissibles (EST) sont signalées chez le bétail depuis des siècles, mais la nature de l'agent causal présumé, une forme mutante contagieuse de protéine encodée par l'hôte, est une découverte très récente qui reste mystérieuse sous bien des rapports. Dans sa conformation normale, le prion serait une protéine messagère dont la vie est courte et qui serait très répandue dans les tissus nerveux. Par contre, si le prion mutant a une structure primaire identique, sa structure tertiaire et sa structure quaternaire sont complètement différentes et lui confèrent une stabilité et une résistance inhabituelles aux réactions post-traductionnelles normales. Et ce qui est plus important, la protéine prion mutante se lie à la protéine normale et modifie sa conformation pour la transformer en protéine mutante. On a observé que la transmission des EST d'un hôte à l'autre s'effectue principalement par l'ingestion de tissus infectés et l'introduction du prion mutant dans les tissus nerveux par voie orale, notamment par les nerfs crâniens au niveau de la langue. On croit que le prion est transporté dans le parenchyme par des microglies très mobiles. Les dommages latents d'une accumulation éventuelle du prion résultent de la réaction immunitaire de l'hôte envers la protéine qui fait intervenir le TNF-alpha et les IL-1 alpha et bêta, entre autres. Les symptômes cliniques, toutefois, qui se présentent longtemps après la réaction immunitaire de l'hôte, consistent en des modifications spongiformes au niveau des tissus nerveux. Heureusement, des recherches prometteuses tentent d'expliquer l'immunité naturelle envers les EST et de l'appliquer aux sujets non atteints.

INTRODUCTION

Creutzfeldt & Jakob first described a constellation of symptoms in humans in the 1920's that were subsequently recognized by other clinicians in their own patients. Although very few of these cases would today be classified as Prion diseases, experiments in 1968 established the existence of a transmissible form of the syndrome that became known as Creutzfeldt-Jakob Disease (CJD) (Johnson & Gibbs 1998). Symptoms of CJD were also seen in some New Guinea tribes and were named kuru. Researchers suspected and eventually showed that cannibalistic ritual meals consisting of the uncooked brains of the dead were responsible for these cases of kuru (Harris 1999).

Clinical presentations in humans include dementia, myoclonus (muscle spasms), cortical defects, sensory abnormalities, and seizures, among others; however, only histological tests of brain tissue in suspect cases can confirm Prion disease by revealing spongiform changes characteristic of the transmissible spongiform encephalopathies (Johnson & Gibbs 1998). Accumulation of the putative disease-causing protein, which occurs well before many of the symptoms manifest themselves, is considered to result in neurodegeneration, plaques, vacuoles, and enlarged ventricles (Lewicki et al. 2003). In animals, symptoms have been described in several species for centuries. These include scrapie in sheep and goats, wasting disease in deer and elk, and bovine spongiform encephalopathy (BSE) in cattle, among others (Johnson & Gibbs 1998).

However, the exact nature of the agent of infection remained elusive for some time. As the field of microbiology evolved, microorganisms such as bacteria and viruses were ruled out. Eventually, Prusiner, a researcher at the University of California, concluded that, because the agent resisted treatments that inactivate nucleic acids but was inactivated by those that denature proteins, the infection could only result from a protein (Johnson & Gibbs 1998).

There have been numerous advances in the field of Prion research since Prusiner's initial discoveries: the Prion has been sequenced and its form determined, its life cycle in the cell has been experimentally observed, and the pathogenesis of its mutant variant has been studied. Nevertheless, it should be understood that the field has many unanswered questions. Additionally, the results of existing research need to be confirmed by other investigators before they are used with confidence. Prion researchers are also pressed by public health officials to solve the danger posed by trans-species infection of variant-CJD through the food supply; although preventive recommendations have so far been sound, more sophisticated research involving immunity to transmissible spongiform encephalopathies have been based primarily

on animal models. The use of these results for human applications needs to be approached with care.

The role of the normal Prion protein is subtle, and does not seem to be vital as many examples of individuals in several species exist that do not express any Prion protein at all (Zulianello et al. 2000). The mechanisms of the mutant form of the Prion, however, are not only devastating, but unique in that the mutant protein is itself transmissible (Bartz et al. 2003). Important avenues of research, therefore, include study of the mechanism of host-to-host transmission and of infection within the host, as well as the study of various methods of natural immunity in certain individuals.

THE NATURE OF THE PRION PROTEIN

The normal conformation of the Prion protein, PrP, is ubiquitous throughout the nervous system tissue, though it has been detected in other tissues. It is found in cerebral and spinal neurons and glia, but is concentrated primarily in the neocortex, hippocampus, cerebellar Purkinje cells, and spinal motor neurons (Harris 1999). It is hypothesized that PrP are involved in cellular recognition, signaling, and adhesion because of their location on cell surfaces; however, other experimenters have concluded that PrP facilitates copper or ligand uptake and metabolism (Harris 1999).

Biochemistry of the Prion

An approximately 250 amino acid PrP is synthesized in the rough endoplasmic reticulum (RER), before going through several structural modifications that include the addition of a glycosyl-phosphatidylinositol (GPI) anchor that allows the final protein to attach itself to the cell membrane (Harris 1999). The normal PrP precursor is then cleaved once with a cellular phospholipase to release a signal polypeptide that is attached to the GPI anchor. The PrP is then proteolytically cleaved within its central hydrophobic segment before it is transported to its destination on the cell surface (mostly to the plasma membrane) where it is attached solely by its GPI anchor (Harris 1999). Studies have shown that PrP continuously cycles between its location on the plasma membrane and an endosome (Harris 1999). This cycling is consistent with suggestions that PrP is an uptake protein.

PATHOGENIC PRP

PrP diseases occur in three forms: infectious, sporadic, or familial. In each of these, some mechanism causes the protein to alter its conformation from one that is mostly alpha-helical to one that is mostly

made of beta-sheets (Johnson & Gibbs 1998). Infectious PrP diseases result from the introduction of a conformational isomer of the PrP (denoted here-in and in most literature as PrP^{Sc}) from an outside source, which catalyzes the formation of altered PrP in a self-propagating way. Historically, most PrP infections were iatrogenic, resulting from infected dura mater graft tissue, human pituitary growth hormone, or, more rarely, from improperly sterilized surgical equipment (Johnson & Gibbs 1998). Genetic studies have shown that familiar PrP diseases result from point, line, or insertional mutations on the PrP gene located on chromosome 20, resulting in spontaneous folding into the PrP^{Sc} conformation (Harris 1999). The sporadic PrP diseases, accounting for 80%-90% of all cases in humans, have no apparent etiology to date (Johnson & Gibbs 1998).

Biochemistry of PrP^{Sc}

The methods currently used to determine subcellular location of molecules have tentatively revealed that PrP^{Sc} are extensively distributed within the cell. The plasma membrane-attached PrP^{Sc} are also thought to attach with the GPI anchor, but they cannot be cleaved from the membrane by phosphotidylinositol lipase (PI-PLC), as PrP can (Harris 1999). GPI extracted from PrP^{Sc} was not found to be altered in a way that would confer PI-PLC resistance, and so it is believed that the GPI-anchors on the PrP^{Sc} become sterically inaccessible to the lipase (Harris 1999). The second property that PrP^{Sc} acquires following PI-PLC resistance is its insolubility in detergents; this is thought to represent PrP^{Sc} aggregation of up to 30 PrP^{Sc} units (Harris 1999). The third property of PrP^{Sc} is its protease resistance, thought to be due to PrP^{Sc} polymerization (Harris 1999). These three properties are conferred in distinct and sequential stages. The first, PI-PLC resistance, occurring within minutes of PrP^{Sc} synthesis, is most likely acquired in the RER. Detergent insolubility, which maximizes after one hour of synthesis, and protease resistance, which is seen several hours later, both develop when the PrP^{Sc} are on the plasma membrane (Harris 1999). It has been determined that PrP^{Sc}, having half-lives of 24-48 hours, are much more stable proteins than PrP, which denature in only 4-6 hours. That PrP^{Sc} is formed in the RER is reasonable as that organelle plays a principal role in assisting protein folding. An important consequence of postulating that PrP^{Sc} begin their synthesis in the RER is that, in the infectious cases, the mutant protein must first be carried there to catalyze future conformational changes. Therefore, ER chaperones would act as cellular cofactors to control PrP^{Sc} synthesis. In general, these chaperones attach to their substrates to prevent certain unfavourable intermediary conformations.

Conversion of PrP to PrP^{Sc}

At some point in the pathogenesis of infection from the PrP^{Sc}, an interaction of high physical specificity between the mutant and wild types is believed to take place (Harris 1999). Experimental evidence to support this comes partly from studies of genetically modified mice that do not express any form of PrP and are found to be immune to PrP^{Sc} infection (Harris 1999). Although different strains of PrP^{Sc} exist where each strain is characterized by differences in primary, secondary, or tertiary structures, the most critical location for infection is within the central hydrophobic core between amino acids 94 and 188, which is why interspecies infections are rare (Vorberg et al. 2001). These residues form one alpha-helix and two antiparallel beta-sheets where the alpha-helix binds the mutant PrP^{Sc}, while the beta-sheets precipitate the conformational change (Vorberg et al. 2001). This was seen by engineering PrP that lacked any combination of these three secondary structures, and observing that the conversion of the modified PrP to PrP^{Sc} was greatly diminished (Vorberg et al. 2001). In addition, it was noted that deletion of only the first alpha-helix and the second beta-sheet in this core region affected the cycling of the PrP through the cellular organelles. Predictive models have shown that the first beta-sheet would be responsible for stabilizing an intermediate conformation of PrP^{Sc} (Vorberg et al. 2001). It is this central hydrophobic region of the PrP that is most specific to the species that synthesizes the protein. In fact, even a point mutation in this region can prevent infection. In the cases in which interspecies infection is observed, it is because this region of the PrP is similar enough to allow the necessary interaction (though other regions may not be) (Harris 1999).

The seeded-polymerization model of PrP^{Sc} propagation, which is considered the most likely pathway, requires a seed of aggregate PrP^{Sc} to catalyze new PrP^{Sc} (Vorberg et al. 2001). There is no evidence that PrP^{Sc} covalently differs from PrP because they both have the same amino acid sequence, and mostly similar post-synthesis additions. The most noticeable conformational difference between the two is a drastic increase in beta-sheet, especially in the N-terminus half, at the expense of alpha-helix structures (Harris 1999). Specifically, the PrP tertiary structure has a substantial tail at the N-terminus that is folded, along with a neighbouring alpha-helix, into a beta-sheet.

The hypothesis that different symptoms of Prion diseases represent different conformations of PrP^{Sc} was suggested by comparisons of the resistance to protease-K by a strain that caused hyperactivity (HY), and another that caused drowsiness (DY) in rodents (Scott et al. 1997). However, the diversity of PrP^{Sc} conformations is probably highly limited due to the low variability in primary structure Scott et al. 1997). Figures 1 and 2 show a computer-generated model of the normal and variant forms of the Prion protein (Jackson et al. 1999). Note the unwinding of the three major helices.

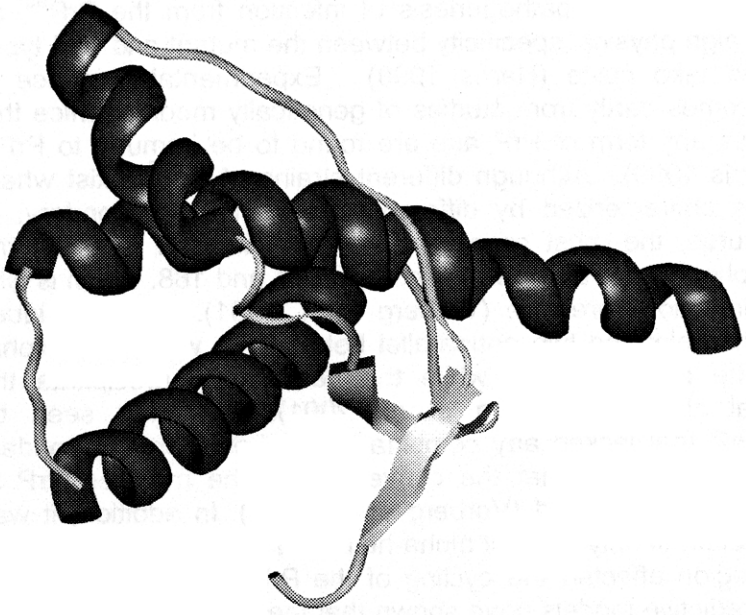

Figure 1 - Normal Prion Structure

Image Credit: http://www.cmpharm.ucsf.edu/cohen/ (used with permission)

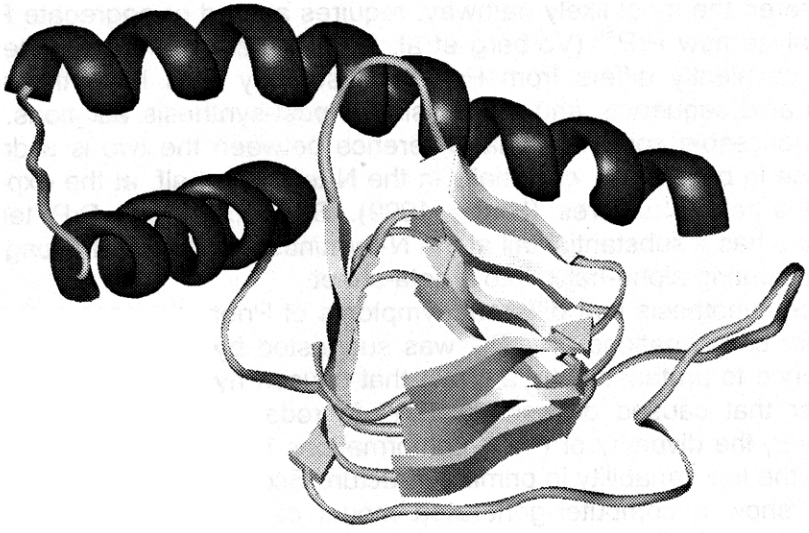

Figure 2 – Mutant Prion Structure

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PrP^{Sc} Infection Pathways

Some forms of PrP^{Sc} disease are easily understood: familial Prion disease is genetic and iatrogenic infection occurs following invasive surgeries using infected equipment and materials. Other pathways of infection, such as in kuru, variant CJD, and BSE, have been somewhat more mysterious. This is because these pathways all involve ingesting tissue infected with PrP^{Sc}, sometimes from the same species and sometimes not. The PrP^{Sc} protein will experience not only the very low pH of the stomach, but will also face destruction from gastric pepsin. Recent studies using suitable animal parallels have shown, however, that the primary path of infection is through the cranial nerves serving the tongue, the enteric nervous system, and the abdominal lymphatic system (Bartz et al. 2003). In cases of ingestion of tissue infected with PrP^{Sc}, the most effective path of infection starts from the skeletal muscle in the tongue, proceeds up the hypoglossal nerve (CN XII), transsynaptically spreads to the vagus nerve (CN X), and finally infects the brain stem from the dorsal motor nucleus of the vagus nerve (Bartz et al. 2003). Because PrP are found in subsynaptic areas on the nerve cell, entry is most likely to occur at the neuromuscular junction on the tongue. The presence of terminal nerve endings exposed by other infections, cuts, or scrapes in the mouth and on the tongue increases the rate of propagation and likelihood of infection of PrP^{Sc} (Bartz et al. 2003). It has been shown that PrP^{Sc} can replicate and accumulate in individual axons on the tongue during early stages of infection. As the infection progresses, the PrP^{Sc} are observed in the nucleus of the vagus nerve (CN X), and in the motoneurons of CN XII (Bartz et al. 2003). Additionally, it has been observed that intracranial infection will spread peripherally along the same pathways and end up in the tongue, both on the nerves and on the skeletal muscle (Bartz et al. 2003). Secondary infection can spread through the lymphoreticular system, and concentrate in lymph organs such as the spleen (Bartz et al. 2003). Alternate pathways from oral ingestion are through the trigeminal nerve (CN V) and the glossopharyngeal nerve (CN IX) or through the facial nerve (CN VII) and the glossopharyngeal nerve (CN IX) (Bartz et al. 2003).

Although Prions are primarily associated with neural cells, the infectious PrP^{Sc} have been identified in circulating leukocytes that have never directly contacted infected neurons. Therefore, it was postulated and shown that PrP^{Sc} can be carried by microglia (Baker et al. 2003). Microglia are the macrophages of the nervous system, and are myeloid in nature. Damage to the brain, through diseases such as Alzheimer's and CJD, or injury, will activate microglia, causing them to alter their morphology. Experimental limitations have not permitted researchers to determine if microglia support PrP^{Sc} replication, but mathematical models that account for their actual level of infectivity suggest that they do

(Baker et al. 2003). Further, it is postulated that microglia are only one type of cell in a network that are responsible for the widespread distribution of the infection (Baker et al. 2003). PrP^{Sc} may be able to take advantage of the high motility of microglia within the parenchyma to move from cells with lower replication to those with higher potential. In this respect, PrP^{Sc} mimic viral pathways of infection that invade myeloid cells and remain dormant until they accumulate in numbers significant enough to cause structural brain damage (Baker et al. 2003).

Immune Response to PrP^{Sc}

As the amounts of PrP^{Sc} increase in the brain and central nervous system, there is an immune response that involves CD4 and CD8 T-lymphocytes, major histocompatibility complex (MHC) I and II molecules, and chemokines (Lewicki et al. 2003). As the PrP^{Sc} progress, the genes that activate tumor necrosis factor alpha (TNF-alpha), interleukin-1 alpha and beta are expressed in the central nervous system (Lewicki et al. 2003). TNF-alpha is an inflammatory mediator that has many functions including increasing the expression of MHC class I receptors on endothelial cells and causing fever; IL-1 alpha and beta are also pyrogens, affect sleep, and cause anorexia, inflammation, and lymphocyte activation, among other functions. Chemokines, which induce chemotaxis of T-cells and natural killer cells, are produced from infected astrocytes and neurons. These responses of the immune system were always observed to occur well before clinical symptoms of PrP^{Sc} appeared, both in animal experiments and in human subjects (Lewicki et al. 2003). The infection outside the central nervous system is thought to activate the T-cells, which can cross the blood-brain barrier only in this form. Perhaps simultaneously, chemokines of CNS origin attract the activated T-cells. In certain studies that measured the effect of CD4 and CD8 T-cells on the course of PrP^{Sc} disease, it was found that removal of either had no effect at all, but removal of both drastically delayed the onset of symptoms (Lewicki et al. 2003).

An unknown protein denoted in the literature as Protein X assists conformational change between PrP and PrP^{Sc}; changes in the primary structure of Protein X will hinder the onset of PrP^{Sc} disease (Zulianello et al. 2003). Four critical residues, 167, 171, 214, and 218, have been identified on the PrP that prevent its conversion to PrP^{Sc}, should any of them be changed to a basic amino acid. These changes, which cause PrP to bind more tightly to Protein X, are effective only in certain combinations, and were found to have reduced inhibition of PrP^{Sc} formation if they are compounded (Zulianello et al. 2003). In animals and humans that are naturally resistant to PrP^{Sc} diseases, the phenotype that expresses this modified PrP has been found to be responsible. It was determined that protection from PrP^{Sc} diseases is the result of dominant-negative inhibition where those with a single allele that encodes for PrP have immunity (Zulianello et al. 2003). The knowledge of the mechanism

of this natural immunity as it relates to the interaction of PrP with Protein X suggests that pharmacological treatment for PrP^{Sc} may lie in its successful imitation.

CONCLUDING REMARKS

The Prion protein, when it acquires its correct tertiary and quaternary structure in the RER, performs such a subtle role within the cell that it goes entirely unnoticed: even a definitive consensus of its main function remains elusive. It is only when the Prion is misfolded that its effects become felt. Herders of certain breeds, such as cows, goats, and sheep, have left for posterity their descriptions of inexplicable blights upon their livestock that we can now retrospectively guess were transmissible spongiform encephalopathies. People have also been infected through surgeries, grafts, and cannibalism, and suffered the consequent neurodysfunction. The work of Prusiner to identify the agent of this disease, not as a virus or a bacterium, but as a protein, resulted in a series of questions about the Prion that are still being investigated, and recently with added vigour as the economic and public health issues surrounding BSE and variant CJD become more pressing. Despite its pathogenicity, this author believes that PrP^{Sc} exhibits wondrous elegance in its ability to catalyze its own self-replication *in vivo* using healthy PrP as a source.

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