Cracking the Prion Puzzle: The History and Molecular Pathology of the Heretical Particle

Described as the “strangest thing in all biology”1 the discovery of the Prion was arguably one of the most important scientific breakthroughs of the 20th century. Prion diseases or Transmissible Spongiform Encepthalopathies are a group of rare progressive conditions caused by the accumulation of the abnormally folded cellular prion protein2 (PrPsc) in the brain, damaging the nervous system and brain of the affected individual. What is truly remarkable about prions is the ability of an abnormal prion protein to initiate a conformational change in the normal prion protein causing it to misfold.

The neurodegenerative diseases trigger the physical and mental deterioration of the organism and are transmitted genetically, sporadically or can be acquired.

The Discovery of the Prion

Scrapie is a fatal degenerative disease affecting the central system of goats and sheep and was the first prion disease to be identified in the 1730’s7. The name was derived from one of the clinical signs of the condition where infected animals scrape their hind-quarters raw. It puzzled scientists as the disease spread like a virus but in other ways looked like a genetic disease4, 8 yet produced no detectable immune or inflammatory response within the affected organism. Scrapie was surprisingly resistant to conventional sterilization procedures and had the ability to bind to surfaces of metal and plastic without becoming less infectious9. At the time prions were undiscovered so it was believed that Scrapie was caused by an “unconventional virus with the protein coded by the viral genome”5. Although vaccination was attempted the infectious agent was not inactivated at high temperatures, which led to the conclusion that the disease could not be viral8.

Research into the infectious agent causing Scrapie continued and it was found that polymorphisms in codons 136, 154 and 171 in the prion protein gene (PRNP) 7 leads to the production of an incorrect amino acid sequence, which then causes the formation of the misfolded prion protein- PrPsc leading to accumulation in the brain. The period of time over which replication and accumulation occurs is known as the incubation period. In Scrapie, nervous system dysfunction follows accumulation and results in symptoms such as locomotor incoordination and behavioural changes, eventually leading to death7.

Although Scrapie was identified in the 18th century, the true nature of the infectious agent was not properly understood until recently. It was the discovery and research of Kuru, which was made possible through advancements in laboratory techniques involving electron microscopes and X-ray crystallography that enabled both diseases to be understood1. Kuru is the neurological condition characterised by the loss of coordination and control over muscle movements in the affected individual10. It was prevalent in the 1950s and 60s amongst the Fore people- a tribe in Papua New Guinea as a result of rituals involving the consumption of brain tissue from the recently deceased8- believed to free the spirit of the dead. Between 1957- 1961 the disease reached epidemic status, causing 1000 deaths. However since the ritual practice has stopped, the number of deaths from Kuru has since declined to less than 10 reported cases each year10.

In 1976, Carleton Gajdusek won the Nobel Prize in Medicine for discovering “a completely new type of infectious agent” by demonstrating that Kuru could be transmitted to chimpanzees, carried out by injecting autopsied brain tissue from deceased Creutzfeldt-Jakob disease patients into the cortex of chimpanzees1. Soon after, scientists began to observe brain tissue from those who had suffered from Scrapie, Kuru and Creutzfeldt-Jakob disease and found it strikingly similar under the microscope.

However, it was Stanley Prusiner who was awarded the 1997 Nobel Prize in Physiology for his work on Prions who cracked the prion puzzle and truly made a leap in the field. Prusiner first entered the field of prions after seeing a patient with Creutzfeldt-Jakob disease in a hospital in 1972 and was determined to find the cause of the disease. He was able to purify the scrapie agent to leave behind the molecules solely responsible for the illness. Eventually, he coined the term ‘prion”- an acronym originating from proteinaceous infectious particle. To disprove skeptics, Prusiner synthesized a normal prion protein which he thought would create a prion disease, though was surprised to find that prions unlike other infectious agents were not something infecting the victim from the external world but created by the affected individual themselves. Questions such as “How could a gene cause an infection in its own host” arose and in response he proposed that prions had two forms, the first being the normal prion protein (PrPc) that could be converted into the abnormal type by another infectious prion protein. However objections were raised, this radical idea was completely unproven and no one had seen a protein behave in such a way before, he later conducted experiments that would prove otherwise. Also, by using what was termed as ‘Persuasive experiment’ Prusiner proved that prion diseases did not have as previously thought, a viral counterpart as no such component showed up in purification. He was additionally able to isolate the PRNP gene found on chromosome 20 in humans and proved that it provided instructions for making PrPc. Using this information, researchers were able to find mutations on the PRNP gene which cause various genetic prion diseases.1

How Prions Cause Disease

The normal PrPc prion protein is found anchored to cell surfaces and is found in high concentrations in the central nervous system and non-brain cells such as immune cells20 . Although there is still uncertainty over the role of the normal prion protein in the human body, it is believed to help maintain myelin – a fatty substance made up of phospholipids and proteins, which is found wrapped around nerve fibres to increase the speed of electrical transmission between neurons17. As well as this it is considered to be involved in cell survival or cell adhesion20. The glycoprotein consists of two functionally distinct parts- a globular sphere and a long unsaturated tail, which is thought to be entirely responsible for maintaining myelin function13 .The secondary structure of PrPc is 43% α -helices and 0% β sheets24. Each α helix is made up of a spiral conformation where a N-H group donates a hydrogen bond to the C=O amino acid group.18 There is also one disulphide bridge created from a single covalent bond formed between two cysteine amino acid molecules19.

Although the PrPsc isoform has the same polypeptide chain as the PrPc form differences are seen in their secondary structure, in PrPsc the α-helical region of the secondary structure is destabilised 21 and converted into β-sheets and it is thought that it is made up of 20% α -helices and 34% β sheets24. This new secondary structure makes PrPsc insoluble in non-ionic detergents and therefore resilient to denaturation by chemical and physical agents as well as making it particularly resistant to digestion by proteinase K- an enzyme that digests proteins22. Once formed, the PrPsc prion protein will replicate and many copies of the abnormal protein will clump together creating insoluble amyloid aggregates- made from atoms bonding across the frame of damaged proteins1. Aggregation occurs as protein intermediates search for a more thermodynamically favourable state23. The aggregates formed are stable and are deposited extracellularly in infected tissue causing damage and destruction to neurons. The loss of these neuron cells leads to microscopic sponge-like holes in the brain, leading to the fatal but rapid cognitive decline of the individual. Though, some researchers argue that amyloid itself does not damage cells but instead protects them by isolating potentially damaging proteins by strongly bonding with them.1

How Prions Misfold

Normal protein folding is the process by which an unfolded polypeptide chain is arranged into a characteristic 3-dimentional structure that determines its function. In protein synthesis, mRNA is transcribed in the nucleus from DNA and is then translated into a polypeptide chain made up of amino acids18. Folding can then either occur as translation is happening (co-translationally) or immediately after the synthesis of the polypeptide26. The native state of a protein where it is in the assembled state required for function25, is largely determined by the protein’s primary structure as the position of specific amino acids determines the extent that different parts of the protein will fold together to give its final conformation. The varying tiers of structure- primary, secondary, tertiary and quaternary are used to improve the overall stability of the protein structure, which is needed to maximise function. Examples of interactions used to improve stability include intermolecular hydrogen bonding used in α- helices and β-pleated sheets, which can lead to amphipathic properties such as that of a phospholipid molecule. Other interactions include ionic bonds, involving the attraction of oppositely charged amino-acid chain groups in the tertiary structure18.

Normal prion proteins misfold either because of mutations in the PRNP gene which cause destabilisation of the structure of the correctly folded state and/or because of the stabilisation of the misfolded state. When considering why a protein misfolds it is important to consider the pressures in vivo that must be overcome in order for successful folding to take place. Firstly, the highly crowded nature of the cell contents means that proteins are constantly being attacked by high- energy collisions with neighbouring proteins molecules. Furthermore, protein folding in eukaryotic cells occurs in various organelles such as the endoplasmic reticulum or the Golgi apparatus. The chemical nature of each organelle is incredibly varied in order to maximise efficiency for individual function but proteins must go through and be able to withstand each condition, increasing the likelihood for error.27

Prion Replication

Many different hypothesis exist offering explanations as to how prion replicate, although there is no substantial scientific proof for any hypothesis, the Templating Mechanism is the most widely accepted. There is also uncertainty over whether or not prion replication requires a cellular co-factor. It is also thought that there is another unknown protein coined Protein X by Prusiner that assists in the change of PrPc to PrPsc however there is no proven scientific data for this1

The Heterodimer Model, initially proposed by Prusiner states that when a single abnormal prion protein (PrPsc) binds to a single precursor normal prion (PrPc) a catalytic reaction will occur, converting PrPc to PrPsc 29. The two molecules then unbind and go on to replicate further. However it is generally disregarded because of insufficient data and properties of the prion that the model fails to explain.

The second hypothesis named the Fibril Model states that the abnormal prion protein (PrPsc) exists only as fibrils where the normal prion protein (PrPc) binds to the fibril end and is converted into PrPsc leading to an increase in fibril lengthhowever the fibrils eventually break, increasing the quantity of infectious particles and PrPsc in the infected tissue. The length of the incubation period is determined by the exponential growth of both PrPsc and the quantity of infective particles29.

The final hypothesis named the Templating Mechanism occurs the incorrectly folded protein- PrPsc influences the normal ubiquitous protein- PrPc to convert into the abnormal form. Research suggests PrPsc monomers themselves are not toxic but become destructive when forming oligomers of 6-12 monomers28. However the exact cause behind the toxicity of abnormal prion proteins is still unknown.

Transmission of Prion Diseases

Different strains of the infectious isoform PrPsc give rise to the diversity of prion diseases which have different phenotypes and pathological states30 known as the ‘Prion Strain Phenomenon”.Techniques such as incubation periods, profile of histological damage and clinical signs in vivo can be used to differentiate between the prion strains as each have specific and therefore distinguishable biochemical characteristics31. These different strains give rise to the various prion diseases, which can be transmitted genetically, sporadically or can be acquired.

Firstly, it should be noted that there are two types of mutation- loss-of-function where the protein is unable to perform its specific role and gain-of-function where a protein does something unrequired32. In the case of familial forms of prion disease, the mutation is gain-of-function where one copy of the altered PRNP gene is inherited from an affected parent in an autosomal dominant pattern. This is because the PRNP gene is not found on a sex chromosome so both sexes are equally affected by genetic prion disease33**.** However in 60% of genetic prion diseases there is no family history of the disease34, it is instead caused by a new mutation in the gene occurring during the formation of a parent’s sex cells or during early embryonic development. The majority of genetic prion diseases are completely penetrant so everyone who has the mutation will acquire the disease however only 15% of all human prion diseases are genetic35. Examples of genetic prion diseases include Gerstmann-Straussler-Scheinker Disease and Fatal Familial Insomnia35- each which have a slightly different set of symptoms, which characterise the illness. Over 40 genetic mutations exist and typically different mutations tend to give a different age of disease onset and duration of disease although common features regarding symptoms do exist.36

Sporadic diseases are the most common, responsible for 85% of all human prion diseases35. They result from a spontaneous mutation in the PRPN gene which leads to protein misfolding which then sets off a chain reaction1. There are three hypothesises as to why this is, firstly that there are sufficient abnormal prion protein molecules to trigger a self- sustaining process in the brain38. Secondly that the normal prion protein spontaneously changes into the prion form and finally that the PRNP gene somatically mutates into the faulty gene that ends up producing faulty prions. However, these somatic mutations cannot be passed on38. The disease tends to affect individuals between the ages of 45-75 and although the duration of the illness varies it tends to last less than a year with an insidious onset followed by a rapid decline38. Examples of sporadic prion diseases include sporadic fatal insomnia and sporadic Creutzfeldt-Jakob disease35

Acquired prion diseases occur through exposure but are incredibly rare accountable for less than 1% of all prion diseases35. One famous example of an acquired prion disease is bovine spongiform encephalopathy, which can be passed on from cows to humans through contaminated food and was responsible for the nation-wide food crisis in the U.K. in the 1980’s and 90’s infecting at least 800,000 cattle and 160 humans1. However, prion diseases can also be transmitted through contaminated surgical instruments, human growth supplements and dura mater transplants, causing iatrogenic infections39.

The concept that a misfolded protein was the infectious agent responsible for Transmissible Spongiform Encephalopathies once seemed incredulous to say the least, Prusiner’s discovery of the prion “introduced a new biological principal of infection into science” as stated by the Swedish academy1 and gave scientists the opportunity to challenge scientific fundamentals and change the way we look at disease. Although there is much uncertainty in this field, further research into how exactly prions cause disease and how replication can be prevented, will help to eliminate the threat of these ‘protein-only’ infectious diseases.

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