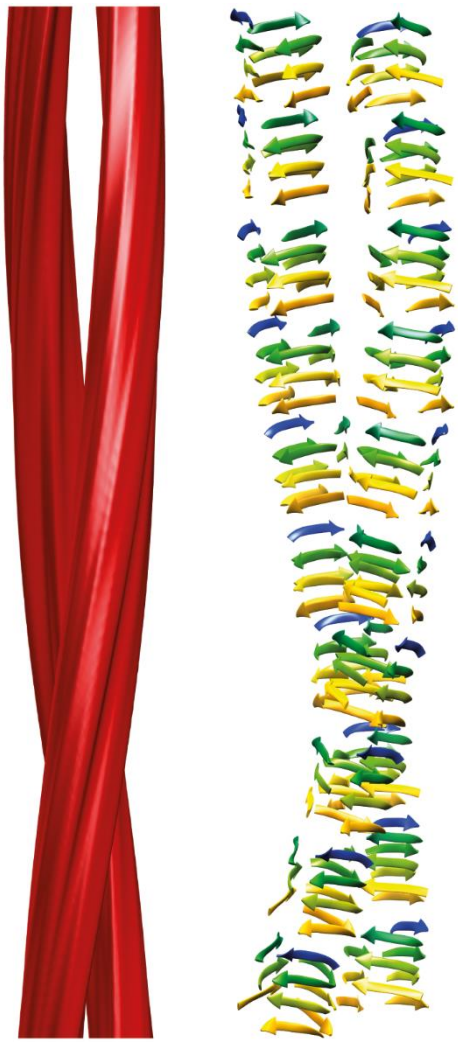
Prion propagation principles

A comparison of theories



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Abstract

Transmissible spongiform encephalitis (TSE) are a group of currently incurable, fatal diseases Unlike any other disease, TSE's can be induced on a genetic, spontaneous or infectious base. It is generally assumed that TSE's are caused by misfolded prion protein. However, this was, and is still, opposed. Furthermore, the exact mechanism of prion infectivity, prion propagation, is still debated. Therefore, the aim of this essay was to compare theories of prion propagation. First, an historical overview of the slow virus and infectious protein theories was given. Overwhelming evidence shows that TSE's are indeed caused by an infectious protein instead of a virus. Second, theories of prion protein misfolding and aggregate formation were discussed. Nucleation-dependent polymerization is the best fitting model for prion protein misfolding and amyloid formation. Third, the protein X and protein-only theory were discussed. Here, the question if co-factors were necessary for prion-propagation was most important. Co-factors were considered to very important, but not essential, for prion propagation. Therefore, the protein X theory was dismissed. These insights can help develop treatment strategies for TSE's.

Introduction

Transmissible spongiform encephalitis

Prion diseases or transmissible spongiform encephalitis (TSE) are a group of neurodegenerative disorders that affect humans and other mammals and are still incurable. TSE causes neurodegeneration, which results in the sponge like appearance of the brain that gives the disease its name. TSE also causes synaptic dysfunction, neuronal loss and amyloid deposits. Furthermore, characteristic for the disease is the long incubation time, which can be decades. TSE's are always fatal, often within weeks to years after onset of disease[1].

These diseases can be induced on a genetic, spontaneous or infectious base. A TSE that can be acquired in all three ways is the human TSE Creutzfeldt-Jacob disease (CJD). In most CJD cases (more than 85%) the cause of disease is unknown as the patients have no risk factors that explains the disease. Moreover, about 5 to 10% of the cases have a genetic basis and 1% of the cases is iatrogenic. These iatrogenic cases are for example caused by hormone-therapy or dura-mater implants originating from infected individuals[2].

Often TSE's are acquired from eating infectious meat. Kuru is one of the best-known examples of a TSE transmitted by eating meat. Kuru was first seen in the first half of the 20th century in a certain population of Papua New Guinea. In this population, consumption of the dead was an important ritual. This was also performed on individuals who died from kuru. Through the consumption of especially brain matter, new individuals were infected. This caused a vicious circle resulting in 200 deaths each year in the late 1950's, after which the ritual was forbidden. As kuru has an incubation time of approximately 40 years, the onset of new cases of kuru was reported at late as the beginning of the 21st century[3].

Another example of an infectious TSE is variant CJD, which affects humans. Fatal familial insomnia (FFI) and Gerstmann–Sträussler–Scheinker syndrome (GSS) are both familial diseases in humans as well. Other known prion diseases in non-human animals are scrapie, chronic wasting disease, bovine spongiform encephalopathy, feline spongiform encephalopathy and transmissible mink encephalopathy[4]. Multiple species are often susceptible to these diseases. However, the transmission of disease from one species to another does require prolonged incubation times and does sometimes not happen at all. This phenomenon is known at the species-barrier[5].

The prion protein

In TSE, the normal form of the host prion protein (PrP^{C}) changes to an infectious protein (PrP^{Sc}). PrP^{Sc} accelerates the conversion of PrP^{C} into PrP^{Sc}. This accumulates in the brain and causes disease. PrP^{C} in humans is expressed in every life stage, but most profoundly during adulthood. The definitive function of the protein has not been found yet, however is it thought to be involved in a large number of processes, including cell signalling and protection against oxidative stress[6]. However, PrP^{c} is also thought to play a role in drug resistance, cancer and Alzheimer's disease[7]. The highest expression of the protein occurs in the neurons of the nervous system[7]. Within cells PrP^{C} is located in the cell membrane and is thought to cluster in caveolae-like domains[8].

The protein is elegantly described by Atkinson *et al.*, (2016)[7]. Briefly, the immature PrP^{C} protein has a length of approximately 253 amino acid residues. Its C-terminal domains consists of 3 α-helices, a β-sheet and a signal sequence. The N-terminal domain contains a hydrophobic region, an octarepeat and a signal sequence as well (Fig. 1A). The protein undergoes several posttranslational modifications

when it is attached to the cell membrane, including the removal of both signal peptides. Furthermore, in the brain and cultured cells, the protein also undergoes a cleavage event. This produces two fragments that remain in the cell membrane (Fig. 1B). A second cleavage event can be mediated by reactive oxygen species (Fig. 1C). A third cleavage can occur very close to the membrane, which causes almost the whole protein to be released into the extracellular medium (Fig 1D)[7].

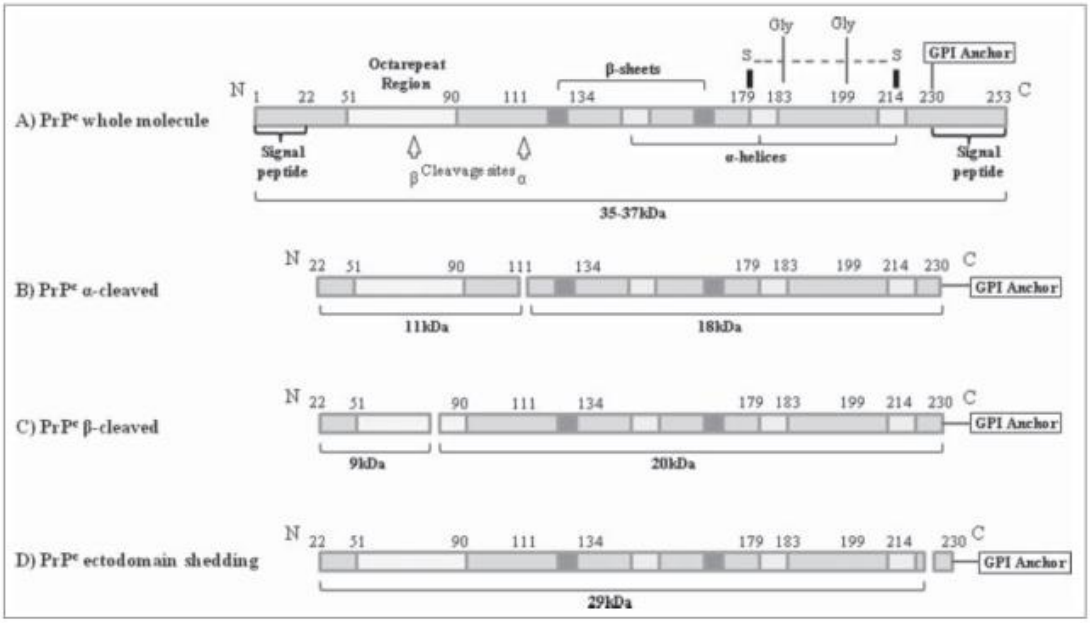

Figure 1. Reprinted from Atkinson *et al.*, (2016)[7]: *"Schematic representation of the protein structure and the proteolytic processing of the PrPC in human cells. (A) Whole PrPC molecule. Post-translational modification is initiated by the removal of N-terminal and C-terminal signal peptides. (B) Normal constitutive cleavage occurring in brain and culture cells. (C) b-cleavage mediated by reactive oxygen species (ROS). (D) Ectodomain Shecking of the PrPC where nearly whole PrPc molecule is released from the cellular membrane."*

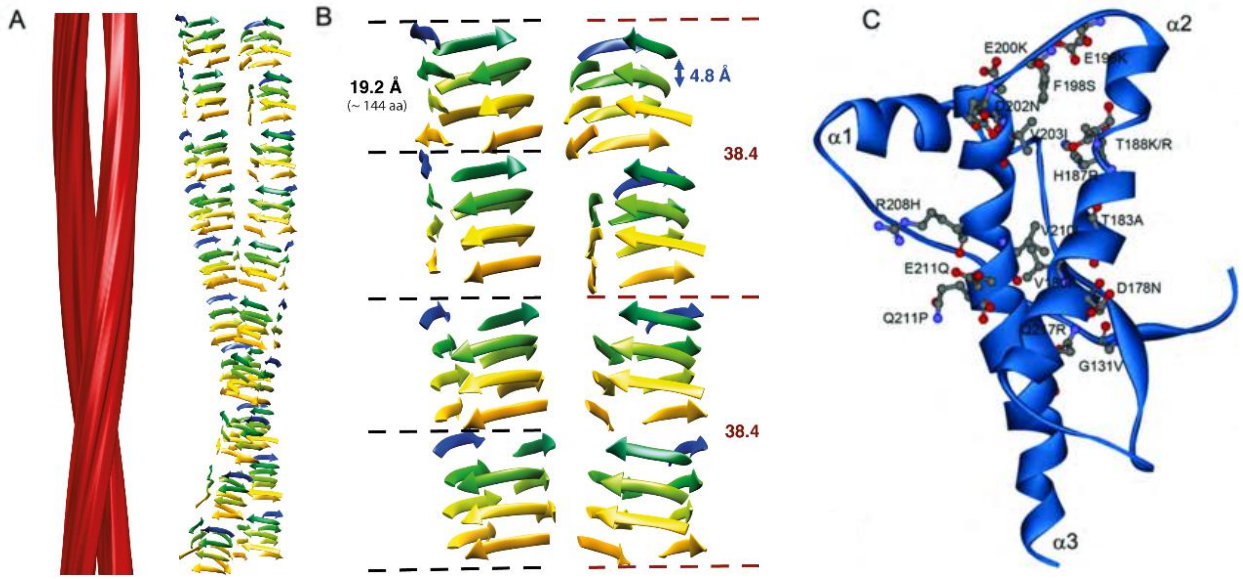

Figure 2. Structure of PrP^{Sc} amyloid fibrils. (A) Cartoon of the amyloid structure. (B) Close up view of the intertwined fibrils. Different colours represent different β- sheets. (C) The structure of normal human PrP. Reprinted from Vázquez-Fernández *et al.,* (2016)[11] and adapted from Zhou and Xiao (2013)[49]

PrP^{Sc} is characterized by a resistance to protease digestion, a high percentage of β- sheets and its insolubility. Moreover, it has two domains that are important for the conversion of PrP^{C} into PrP^{Sc} [9]. PrP^{Sc} accumulates to form amyloid fibrils, what is thought to contribute to the disease pathology[10]. Due to these aggregates it is hard to obtain a high-resolution image of the protein[2]. Recently images made with electron cryomicroscopy showed that these amyloids consist of two intertwined fibrils that consist of β- sheets, a very different secondary structure than normal human PrP[11] (Fig. 2).

Research aim

Unique about prion diseases is that they can develop spontaneous or genetically but still can be infectious. This has not been seen in any other disease. The infectious agent that causes TSE and the exact way this propagation occurs have been and still are a matter of debate in which many different theories have been proposed. Therefore, the aim of this essay is to compare theories of prion propagation. I will provide a historical overview of the slow virus versus infectious protein debate. Furthermore, prion protein misfolding and aggregation according to the nucleation polymerisation model and the template assistance model will be compared. Lastly, the need for an accessory element for the conversion of PrP^{C} into PrP^{Sc} will be discussed by comparing the protein-only theory with the protein X theory.

The infectious agent in TSE

Although scrapie was already described in the mid-18th century, it took more than a century to find the agent responsible for scrapie and other TSE's[10]. The unusual characteristics of TSE's did not fit known infectious agents. Instead, TSE's were thought to be caused by a slow virus or and infectious protein. Only relatively recently, the conclusive proof for one of these theories was provided[7].

Slow virus hypothesis

At first, TSE's were thought to be caused by classical infectious agents, such as bacteria, parasites and viruses[12]. Especially the theory that TSE's are caused by a virus was popular and is still advocated by some groups today. In this theory, PrP might act as a viral receptor for this unknown virus[13,14].

The existence of a virus as causative agent of TSE would fit the transmission of TSE. First, viruses are infectious. Viral diseases can be acquired from eating infectious meat, as is the case with most human TSE infections. For example, Hepatitis E virus and influenza virus can be transmitted through the consumption of meat[15].

Second, endogenous retroviruses explain how the genetical and spontaneous arise of a TSE might happen[16]. A large part of the human genome consists of endogenous retroviruses. These retroviruses have entered the germ-line tens of millions of years ago, and have since often re-infected the host. Endogenous retroviruses can no longer produce viral particles in humans. However, endogenous retroviruses are found to produce viral particles and cause disease in some non-human animals. In humans, endogenous retroviruses are proposed as the cause for several cancers and autoimmune diseases, although no conclusive evidence has been found. Furthermore, *ex vivo*

experiments have shown that, through recombination, human endogenous retroviruses are capable of producing infectious viral particles[17,18].

Therefore, human endogenous retroviruses might arise spontaneously, just as TSE. In theory, human endogenous retroviruses would have been capable of infectious, genetical and spontaneous induction of disease. Lastly, the *in vivo* spread of the causative agent of TSE is classically viral, as agent uses lymphocytes to get to the lymph nodes before entering the central nervous system[14,19]. This shows that a virus might be capable of the propagation of TSE.

The theory that a virus is responsible for TSE was first proposed by Jean Cuille and Paul-Louis Chelle in 1938[20]. The term 'slow viruses' was then introduced by Björn Sigurdsson in 1954 as he noticed the long incubation time of the TSE inducing agent[21]. Furthermore, he noticed the ability of this 'virus' to survive outside the body while remaining infectious[21]. Around this time, it was also discovered that viral nucleic acids encode genetic information and are infectious on their own[22]. This caused growing support for the slow virus theory. However, no virus that adhered to the Koch postulates was ever found[9]. One of the first signs that there might not be a virus causing TSE, was that scrapie infectious material remained infectious when treated with high ionizing radiation and UV, as well as with other techniques that destroy DNA[23,24]. Moreover, the agents causing scrapie were too small to contain any nucleic acid[25]. Furthermore, in humans and other mammals, viruses are recognised by pattern recognition receptors. This causes an immune response that can be recognised by cytokines and chemokine excretion[26]. No such immune reaction has been found for TSE[19].

The virus hypothesis is currently only advocated by a few groups and is supported with relatively old papers[13,14]. These papers are mostly formed around nucleic acid-protein complexes originating from infected animals. These complexes were highly infectious and resembled a viral core. Anti-PrP antibodies did not attach to these core-like structures. If the nucleic acids found in this fraction were destroyed, almost all infectivity was lost as well. Moreover, isolated abnormal PrP was mostly not included in this fraction and was barely infectious[27]. To conclude, infectious material containing core-like structures were found. Infectivity was thought to be depended on nucleic acid and not on Prp[14,28]. A more extensive overview of the slow virus theory can be found in a review by Laura Manuelidis (2007)[14]. However, not all references found in this review contain the information which they are used as a reference for. Furthermore, current reviews supporting the slow virus hypothesis often have a high number of self-citations. Considering the large amount of effort made to find a virus that causes TSE and the current technical abilities to characterize a virus, it is very likely that there is no such virus.

Prion hypothesis

The prion hypothesis states that a protein is the infectious particle that causes TSE. As this was the first time a protein was found to be infectious, this theory remained controversial for years. The theory that a protein could be infectious and also replicating in the body was first proposed by Griffith in 1967[29]. In this time, the central dogma that replication, and therefore infectivity, can only occur through DNA was just firmly established. Therefore, it was not until 1982 that this prion theory became more popular when Prusiner[30] showed the likeliness of this theory with several experiments and named this *in*fectious *pro*tein ''Prion".

Prusiner and his group proceeded to isolate PrP^{Sc} from infectious material. They showed that this purified PrP^{Sc} was infectious and that this infectivity was correlated to the concentration of PrP^{Sc}.

Infectivity only decreased when antibodies against PrP^{Sc} were used or if the protein was destroyed[31,32]. After isolation of PrP, *PRNP* was discovered[33]. *PRNP* was found to be a host gene and was thus also present in uninfected animals[33]. This strengthens the theory that a protein, and not DNA, was the infectious agent. Furthermore, mRNA transcribed from this gene was found in both uninfected and infected brain tissue[34]. Chemically, PrP^{C} and PrP^{Sc} were found to be identical[35]. Instead, PrP^{Sc} was found to differ in structure from PrP^{C} due to a post-transcriptional modification as described before. Therefore, the two proteins must have a conformational difference[36].

Weissmann and colleagues (1993) proved that PrP was essential for the development of TSE by infecting *PRNP* knockout-mice with mouse scrapie prions[37]. The mice did not develop scrapie and could also not infect other animals. Mice that were heterologous for *PRNP* showed enhanced resistance but did develop scrapie. When hamster *PRNP* genes were introduced in the knockout mice, the mice were very susceptible to hamster scrapie, but had decreased susceptibility to mouse scrapie. These experiments did not only show that PrP is essential for the development to scrapie, but also showed that PrP is needed for the propagation of scrapie. The infectivity of PrP^{Sc} was also shown on a cellular level. Neuroblastoma cells were infected using brain homogenate that included PrP^{Sc} and maintained in this state for several months[38].

A milestone for the prion hypothesis was achieved when Caughey and colleagues showed the conversion of PrP^{C} into PrP^{Sc} in a cell-free medium with the use of a small amount of PrP^{Sc} as template[39]. However, the best proof for the prion hypothesis is spontaneous prion formation without the use of any PrP^{Sc}. This was achieved by Soto and colleagues in 2009[40]. They used brain homogenate of healthy, wildtype mice and hamsters, as well as frozen human brain homogenate for PMCA (Protein Misfolding Cyclic Amplification). This technique mimics the autocatalytic replication of PrP^{Sc} *in vitro* by using ultrasound waves and has been demonstrated to maintain the replication of PrP^{Sc} indefinite. They showed spontaneous conversion of PrP^{C} into PrP^{Sc} in the mice and hamster samples[40]. In conclusion, conversion of PrP^{C} into PrP^{Sc} in shown to cause TSE.

Further prove that TSE was not caused by a conventional infectious agent was provided when GSS was linked to a mutation in *PRNP*[41]. Other familial forms of TSE have also been linked to mutations in *PRNP*[7,42]. Lindquist and colleagues introduced the FFI-associated mutation of human *PRNP* in mice. These mice then developed a disease very similar to FFI[43]. To summarize, mutations in *PRNP* were found to be responsible for familial TSE.

To conclude, PrP^{Sc} is the agent that causes TSE. This occurs through the conversion of host protein PrP^{C} into PrP^{Sc}. Mutations in the PrP gene *PRNP* were proven to cause familial TSE. Furthermore, spontaneous conversion of PrP^{C} into PrP^{Sc} was shown in hamsters and mice, strongly indicating that this conversion causes spontaneous cases of TSE.

However, there are also several critiques on the prion hypothesis. TSE's occur in different strains with different incubation times, neuropathology and other characteristics. Excluding familiar TSE's, the host PrP^{C} remains the same. In infectious diseases, mutations in infectious agents cause different strains, but this is not the case here. It has been shown that different strains of PrP^{sc} have different secondary structures and that the transformed host PrP will have the same conformation[44,45]. These different secondary structures might catalyse the conversion of PrP^{C} into PrP^{sc}, thereby changing the incubation time of the disease. Moreover, different secondary structures can also cause the protein to target different brain regions, thereby causing different neuropathology. However, it is still unknown if this is the cause of result of different strains[46].

Many other neurological diseases associated with misfolded proteins, such as Alzheimer's disease, are also being considered as prion diseases. In these diseases, the misfolded proteins can also cause the formation of new misfolded proteins. However, these diseases have been proven to be non-infectious, which shows that the mere ability of a misfolded protein to produce more misfolded proteins does not guarantee infectivity[1,46]. Therefore, the question arises: why are prions infectious and other similar proteins not? Moreover, although PrP^{Sc} is thought to be the source of infectivity in TSE, there have been reports of infectivity without PrP^{Sc} and samples being not infective despite an abundancy of PrP^{Sc} [47,48].

PrP misfolding and aggregation

As said previously, it is generally believed that the misfolding of PrP^{C} into PrP^{Sc} causes TSE. For the development of prevention and treatment strategies, it is highly important that the exact cause and mechanism of this conversion, and therefore of prion propagation, are known. There are two major theories regarding the exact mechanism of prion conversion: the nucleation polymerization model and the template assistance model. Briefly: in de nucleation polymerization model PrP^{C} proteins are aggregated to form a nucleus of PrP^{Sc}, after which the conversion and addition of new PrP^{Sc} proteins is energetically favourable. In the template assistance model, PrP^{Sc} is a stable monomer that binds PrP^{C} and causes the conformational change[49].

Nucleation polymerization model

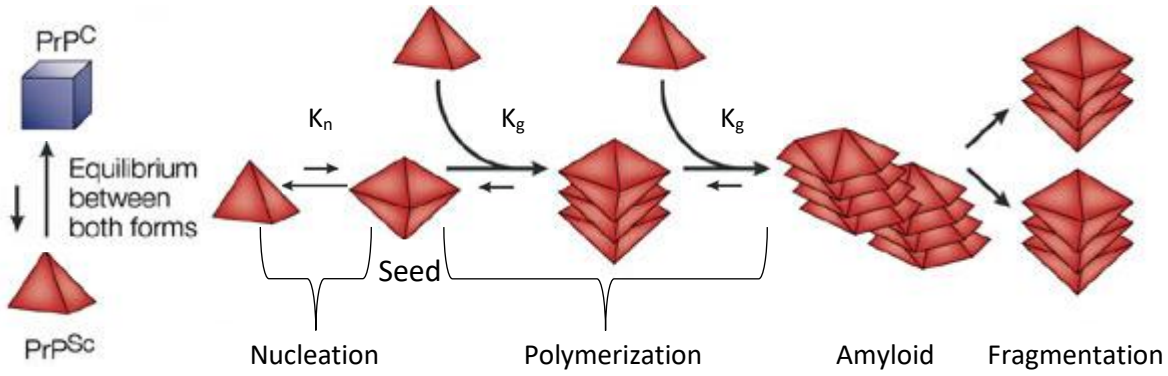

Figure 3. The nucleation polymerization model. The formation of PrP^{C} into PrP^{Sc} occurs in a thermodynamic equilibrium that strongly favours the formation of PrP^{C}. When a PrP^{Sc} monomer is formed, this can act as a seed for the attachment of other PrP^{Sc} monomers. These monomers stabilize the seed, after which other PrP^{Sc} monomers are recruited and amyloid fibrils are formed. Fragmentation of these amyloids increases the number of infectious seeds. Adapted from Aguzzi and Sigurdson (2004)[4].

The first step of the nucleation polymerization model is nucleation (Fig. 3). This includes the formation of PrP^{C} into PrP^{Sc}. Nucleus formation occurs in an equilibrium that strongly favours the formation of PrP^{C} ($K_n << 1$) and is therefore a rate-limiting step. Mutations and cofactors are thought to shift the equilibrium in favour of the conversion of PrP^{Sc} from PrP^{C}. Therefore, individuals with mutations associated with familial TSE have a higher chance of producing an infectious seed.

Elongation or growth of seeds with more PrP^{Sc} monomers is energetically favourable ($K_g >> 1$) as it binds the available PrP^{Sc}. This causes the conversion of more PrP^{C} into PrP^{Sc}, which are used to

form amyloid fibrils. Fragmentation of these amyloids is known to increase infectivity by increasing the number of seeds. Introduction of a seed into a new susceptible individual causes the formation of PrP^{C} into PrP^{Sc} to be energetically favourable as well and therefore causes PrP^{Sc} accumulation and subsequent disease formation in that individual. Furthermore, fragmentation is thought to damage neuronal cells[49,50].

Characteristic for the nucleation-dependent polymerization model is the energetically unfavourable lag phase during nucleation (Fig. 4). During this phase, the PrP^{Sc} is kinetically soluble, meaning that it has not precipitated yet. However, trace amounts of PrP^{Sc} dimers and trimers can be present. Since these intermediate forms are not detected, it's impossible to distinguish between a solution on the beginning and the end of the lag phase. The length of this lag phase is very dependent on PrP^{Sc} concentration[50,51]. Jarrett and Lansbury (1993) gave the following example: 'if a protein requiring an octameric nucleus (n = 8) has a lag time of 1 hr at 10-fold supersaturation, its lag time at 2-fold supersaturation will be ~ 45 yr, and its lag time at 100-fold supersaturation will be ~ 36 μs'[50]. Larger nuclei will increase the influence of protein concentration even more. At the so called 'critical concentration seeds are formed and polymerization occurs[50].

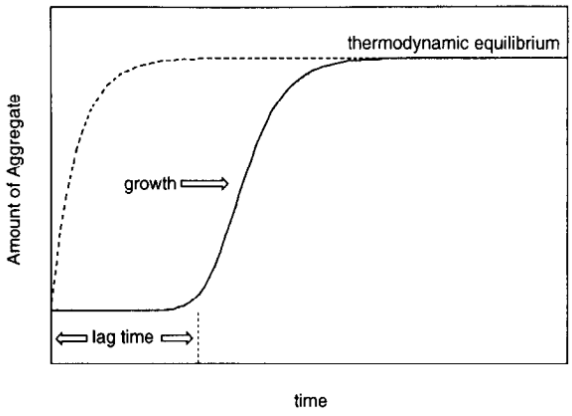

Figure 4. Experimentally observable aggregate formation in nucleation-dependent polymerization. Aggregate formation has a long lag time at low protein concentrations (black line). The lag phase is not observed at high protein concentrations (dashed line). Adapted from Jarrett and Lansbury (1993)[50].

The nucleation-dependent polymerization of prion proteins would explain the different ways one can acquire a TSE. The initial conformation is very thermodynamically unfavourable, which would explain the very low occurrence of spontaneous onset of TSE's. If a mutation shift the equilibrium even a little bit, disease would be much more likely to occur, explaining the familial TSE's. Lastly, acquiring an infectious seed will highly increase the chance of TSE, as the PrP^{Sc} forming reaction would become more energetically favourable[52]. Moreover, nucleation-dependent polymerization is very common in nature, for example in sickle cell anaemia and actin polymerization[53,54]. It would be logical that prion conformation occurs via a similar mechanism. These other cases of nucleation dependent polymerization sometimes have different strains as well. PrP^{Sc} fibril formation in this theory is thought of as a crystallisation-like process. This can cause slightly different fibrils, which explain the different TSE strains[5].

However, as nucleation-dependent polymerization is very common in nature, why are other harmful proteins that form via nucleation-dependent polymerization, such as those in Alzheimer's disease, not infectious?

Template assistance model

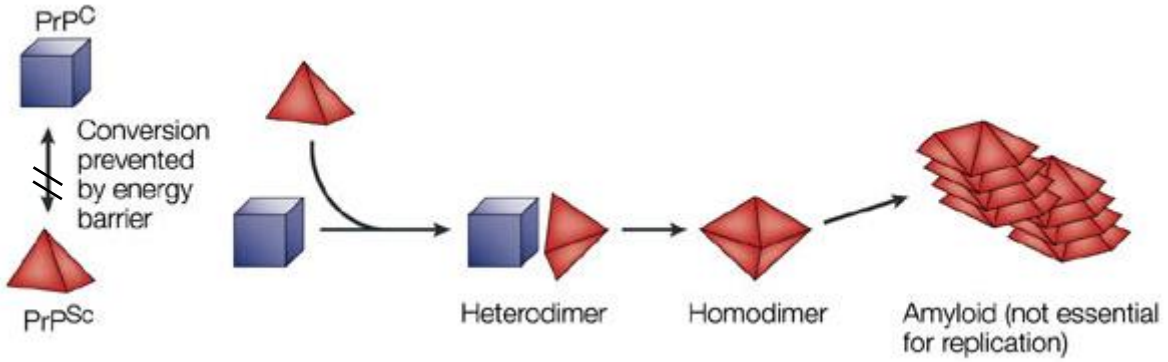

Figure 5. The template assistance model. In this model, PrP^{C} can spontaniously convert into PrP^{Sc}, but this is generally prevented by a high-energy barrier. When PrP^{Sc} is availible, it binds with PrP^{C} to form a heterodimer. Due to this interaction, PrP^{C} is converted into PrP^{Sc} more easily. Adapted from Aguzzi and Sigurdson (2004)[4].

In the template assistance model, or refolding model, the conversion of PrP^{C} into PrP^{Sc} is prevented by a high-energy barrier. As in the nucleation polymerization model, this energy barrier is thought to be lowered by mutations and co-factors. A PrP^{Sc} protein can also enter the body through an infection. PrP^{Sc} than binds to PrP^{C} to form a heterodimer. Through this interaction, the secondary structure of PrP^{C} changes and PrP^{C} converts into PrP^{Sc}. These two proteins now form a homodimer which can form an amyloid fibril (Fig. 5). This amyloid is not necessary for replication, but is thought to play a large role in the pathogenesis of TSE. Individual PrP^{Sc} can continue converting PrP^{C} proteins, either in the host or in another individual if they end up there. Here, the infectious particle is not a seed but the individual PrP^{Sc} [5,49].

The template assistance model would explain the different ways one can acquire a TSE as well. A mutation could lower the energy barrier for the formation of PrP^{Sc}, increasing the change that a PrP^{Sc} infectious particle is made. Furthermore, when one is infected with a PrP^{Sc} particle, this particle will act as a template to produce more PrP^{Sc}. This PrP^{Sc} production causes disease. However, the spontaneous transformation of PrP^{C} into PrP^{Sc} seems very unlikely.

In the nucleation polymerization model, there is a very minimal but stable conversion of PrP^{C} into PrP^{Sc}, which can cause a seed in the perfect, or very unlucky, conditions. In the template assistance model, there is essentially never PrP^{Sc} production unless it is induced by certain co-factors, which will be discussed later in this review. The chance of the last scenario happening seems much more unlikely than de nucleation polymerization scenario. However, many TSE's are extremely rare, only occurring in one or less of every million individuals. Therefore, an extremely rare event seems to fit the disease.

Moreover, the template assistance model would explain the species barrier better that the nucleation polymerization model. In the nucleation polymerization model, host PrP^{Sc} either do or don't fit the foreign seed, there is no in-between state. If the proteins won't fit the seed, no disease will occur. If the proteins do fit the seed, disease will occur with little to no delay as an inter-species infection. In the template assistance model, there is an interaction between the foreign PrP^{Sc} and the host PrP^{C}. PrP^{Sc} might fit PrP^{C}, but conversion might only occur when certain co-factors are available, or the conversion might occur in a much slower rate. Both scenario's will cause no or a slower development of TSE, fitting the species barrier.

The major differences between the nucleation polymerization model and the template assistance model are the existence of a PrP^{C}-PrP^{Sc} dimer and if a single PrP^{Sc} protein is infectious on its

own. Whether a PrP^{Sc} seed is needed or if an individual PrP^{Sc} is enough to cause infectivity can be tested. However, these individual PrP^{Sc} would likely also cluster together. Therefore, this method would never give any conclusive evidence to support one of the two theories. So far, a PrP^{C}-PrP^{Sc} dimer has not been found[49], which suggest that the nucleation polymerization is how prions propagate.

Protein X or truly protein-only

Stanley Prusiner and colleagues explained the species barrier with a so-called 'protein X'. Protein X stands for a protein or other agent that is species specific and essential for the conversion of PrP^{C} into PrP^{Sc} [55,56]. This theory helped the acceptance of the infectious protein theory, as it not only explained the different TSE strains but also left room for the possibility of involvement of other agents than proteins in the induction of TSE's[57]. Opposite of the protein X hypothesis is the protein-only hypothesis. The protein-only hypothesis has had multiple meanings, originally that TSE's are caused by proteins devoid of nucleic acid. In this essay, I will consider the protein-only hypothesis in a stricter sense, namely that PrP conversion can occur without any additional co-factors.

Co-factors

Although the prion protein seems to be the cause and infectious agent of TSE, there are multiple cellular co-factors thought to be either essential or helpful for the propagation of prions. First, a co-factor might increase the biological stability of the infectious protein. PrP^{Sc} is thought to be very stable for a protein. However, PrP^{Sc} is just a protein, without any structures for protection. Propagation of PrP^{Sc} depends for a large part on whether the protein survives inside the body. It has been shown that some substances such as nitrocellulose and soil particles increase the survival and infectivity of PrP^{Sc} [58,59]. For example, a co-factor could improve the resistance of the PrP^{Sc} to gastric acid as gastric juice is thought to at least degrade some of the protein[60]. Furthermore, microglia are thought to degrade PrP^{Sc} through Mfge8-mediated phagocytosis[61]. A co-factor might decrease the affinity of Mfge8 to prions and thereby decrease degradation.

Second, a co-factor might act as a catalyst to improve the conversion of PrP^{C} into PrP^{Sc}. In both models for prion conversion discussed in this review, the spontaneous conversion of PrP^{C} into PrP^{Sc} is energetically costly. It is known that PrP^{Sc} acts as some sort of catalyst in this conversion. However, other substances are also thought to increase the chance of a successful conversion as prion conversion is more easily achieved when extra substances are added[62].

Third, a co-factor could stabilize the PrP^{Sc} polymer. Especially in the nucleation polymerisation model, stability of the PrP^{Sc} is very important for the amount of PrP^{Sc} that is produced. Stabilization of the polymer by co-factors has been shown for Alzheimer's disease[63] and might therefore also occur in TSE. However, extra stability of the PrP^{Sc} polymer might also decrease the amount of fragmentation of the amyloid fibrils. As fragmentation is thought to correlate directly to infectivity, too much stabilisation of the polymer might not benefit prion propagation[64].

Fourth, a co-factor might also increase fragmentation of the amyloid fibrils, thereby causing an increase in infectious units according to the polymerisation nucleation theory. In yeast prions, the chaperone heat shock protein (HSP) 70, HSP100 and HSP104 are thought to increase fragmentation events[65]. It is possible that a similar mechanism also occurs in mammals.

Although a protein is thought to be the sole cause of TSE, DNA and RNA have been reported to aid in the conversion of PrP^{C} into PrP^{Sc}. Furthermore, they are known to induce this conversion. The phenotype of the prion polymer that is created in such a way can differ depending on whether DNA or RNA is used. DNA produced the classical amyloid structures, while RNA caused amorphous amyloid structures[66]. This is likely due to their different binding sites. While DNA binds to the C and N terminus of the prion protein, RNA only binds to the N terminus, which is important for the stability of the prion protein[67]. The inclusion of nucleic acid into the prion structures explains the nucleic acid-PrP complexes that were discussed earlier in this review. Moreover, RNA has been shown to induce PrP^{Sc} aggregation[67]. So, although RNA or DNA is not the cause of TSE, they are thought to be important for prion propagation. However, no single RNA or DNA sequence that specifically binds to PrP has been identified yet[67].

Another co-factor that has been proposed are lipids. Tikvah Alper noticed that the spectrum of UV light that destroys lipids also highly decrease prion infectivity. She thus proposed that certain lipids are crucial for prion propagation[23]. Although lipids are no longer thought to be crucial for prion propagation[40], they are thought to improve infectivity of prions[68]. However, some polar lipids were also shown to inhibit prion amyloid formation[69].

Are co-factors essential for prion propagation?

Cleary, co-factors can improve prion infectivity. The question however, is if these co-factors are essential for the conversion of PrP^{C} into PrP^{Sc} and in a broader sense, essential for prion propagation *in vivo*. The definitive proof for the protein only hypothesis would be the conversion of PrP^{C} into PrP^{Sc} without any co-factors present. This is no easy task. Prion conversion has been shown using brain homogenate[40]. However, many elements that could have acted as co-factors were present in the used material. The cell-free conversion of PrP^{C} into PrP^{Sc} proved that these cellular elements were not essential for prion conversion[39]. Although it was found possible to convert PrP^{C} into PrP^{Sc} without the initial addition of PrP^{Sc}, this conversion was not successful without the addition of other substances[40,70–72]. RNA, other polyanions or lipids were needed for the conversion of the prion protein[40,71,72].

The conversion of PrP^{C} into PrP^{Sc} was performed with the use of a PrP^{Sc} seed without the addition of any co-factors[73]. According to the nucleation polymerisation model, the elongation of seeds is a energetically favourable reaction[50]. Therefore, one could argue that if co-factors where needed for the prion conversion, the seed formation and not the elongation would need a co-factor the most. Furthermore, it is not impossible that some trace amounts of potential co-factors were still present in the PrP^{Sc} seed which originated from brain material[70].

This illustrates the problem with trying to prove the true protein-only hypothesis: it is nearly impossible to prove that something is not present. Moreover, the *de novo* formation of PrP^{Sc} from PrP^{C} is a very rare event in nature. When testing the possibility of converting of PrP^{C} into PrP^{Sc} without co-factors, one must include tricks like PMCA to make sure this rare event happens. Because these tricks are necessary to see prion conversion with no or minimal co-factors, one can't be sure this conversion will work in the same way *in vivo,* as PMCA does not occur *in vivo*. As the presence of potential co-factors cannot be excluded *in vivo* and *in vitro* experiments can't be fully translated to *in vivo* settings, the need of co-factors for the *de novo* formation of PrP^{Sc} from PrP^{C} cannot be fully disproven with today's technology.

Summarizing discussion

Conclusion

TSE's are diseases unlike any other disease found. They develop spontaneous or genetically but are still infectious. Therefore, the nature and propagation of these TSE agents has and is the subject of many debates and theories. The aim of this essay was to compare theories of prion propagation. I first provided a historical overview of the slow virus versus infectious protein debate. While at first TSE's were thought to be caused by a virus, this is not generally believed anymore. Even with modern techniques, no such virus that adhered to the Koch postulates was ever found[9]. Furthermore, no immune reaction to the TSE causing agent has been measured[19]. Lastly, when infectious material was subjected to methods that destroy DNA, the material remained infectious[23,24]. Therefore, TSE's cannot be caused by a virus. Instead TSE's are caused by the infectious protein PrP, when the normal host PrP^{C} is converted into the toxic PrP^{Sc}. Knock-out experiments have shown that PrP is essential for the development of TSE[37], PrP^{Sc} formation could be shown in a cell-free medium[39] and without the initial addition of PrP^{Sc} [40]. Lastly, all familial forms of TSE have been linked to mutations in *PRNP*[42].

Moreover, the prion protein misfolding according to the nucleation polymerization model and the template assistance model were compared. An essential difference between the two models is that in the template assistance model a PrP^{C}-PrP^{Sc} dimer is formed. This dimer has never been found[49]. Nucleation-dependent polymerization is a common mechanism in mammals[53,54] and explains the different prion strains[5]. Therefore, it is likely that prion propagation occurs through nucleation-dependent polymerization. However, some questions remain. The nucleation polymerization model does not explain the species barrier. Furthermore, it is still unknown why prions are infectious while other proteins that are misfolded via nucleation-dependent polymerization are not infectious.

Lastly, the need for an accessory element for the conversion of PrP^{C} into PrP^{Sc} was discussed. It is clear that the protein PrP is the cause of TSE. However, the need for a co-factor in this process is still questioned. No single co-factor has been shown to be essential for prion propagation. However, many different co-factors have been proven to be beneficial for the conversion of PrP^{C} into PrP^{Sc} [40,58,59,62,66–68,70–72]. I think that there is not one co-factor essential for prion formation, but that there are many that aid in prion propagation. For example, by stabilizing PrP^{Sc} and increasing amyloid fragmentation. It is very likely that without these factors, prions would also propagate but at a much slower rate. Thus, I consider the protein only hypothesis to be true.

Implications

I first and foremost advocate the continuation and expansion of fundamental research into prions. Although, the prion hypothesis has been proven, and there are strong indications on how prions cause and spread disease, there is no definitive prove for the exact mechanisms of this. Knowing the exact propagation of prions is essential for the development of treatment strategies. This was already shown by Gordon in 1946. He believed scrapie was caused by a virus and treated scrapie infectious material with formalin to inactivate these viruses. This was used to vaccinate animals against scrapie. As prions were resistant to the formalin, all animals died of scrapie[20]. Fundamental research can help avoid such mistakes in current times.

Although it has been established that TSE's are caused by an infectious protein and not by a virus, viral treatment strategies can be adjusted to work on prion proteins as well. For example, one could target the pion protein with immunotherapy. These efforts have so far yielded little result as PrP^{Sc} is seen as a self-protein by the immune system[74]. However, recently the difference between PrP^{C} and PrP^{Sc}, its structure, has been determined[11]. PrP^{Sc} might have disease specific epitopes on the surface of the protein, that cannot be reached in PrP^{C}. These epitopes can then be synthesised as small peptides and used to produce antibodies for passive immunization. However, a large problem in both active and passive immunization remains crossing the blood-brain barrier, which has been challenging so far[75]. Prion immunotherapy research are described in more detail by Burchell and Panegyres (2016)[74].

The realization that prion propagation likely occurs through nucleation-dependent polymerization can help design a cure strategy as well. Aggregation is a key component in nucleation-dependent polymerisation. One could try to synthesize small molecules to bind to PrP^{Sc}. Such a molecule would cap the aggregate and thereby inhibit any further growth of the amyloids. An example of such a molecule is Anle138b, which stopped the aggregation of prion strains in mice. The drug has been tested with and worked on human prion strains, but has not been tested in humans yet[76]. Drugs could also be designed to target other parts of nucleation-dependent polymerization, such as the aggregation. For example, one could aim to cross-link PrP aggregates specifically to stop further aggregation and prevent fragmentation. Preventing or slowing down prion aggregation would greatly benefit patients, as the aggregates themselves are also thought to be toxic[10]. Furthermore, the amyloid seeds might be used as biomarkers to improve diagnostics. These and other ways to target protein aggregation are elegantly described by Eisele *et al.* (2015)[77].

Lastly, co-factors were described. As co-factors are thought to be important but not essential for prion propagation and as multiple co-factors are thought to contribute to prion propagation and infectivity, it would not be advisable to focus on these co-factors during research into a TSE cure. Prohibiting the interaction of one co-factor with PrP would likely not help, as other co-factors can help in prion propagation just as well. Furthermore, as these co-factors are part of the host, drugs against multiple of these co-factors will probably cause major side-effects. Instead cure research should focus on PrP itself. However, I strongly believe that fundamental research into the relation of PrP with co-factors should continue. Co-factors are thought to play an important role in prion propagation. Research of these interaction might, for example, lead to a common pathway for interaction of PrP with co-factors. With such a discovery, targeting co-factors in patients with TSE would be a more achievable option.

To conclude. TSE's are caused by the infectious prion protein. Prion propagation is thought to occur through nucleation-dependent polymerization and can likely happen without any co-factors, although co-factors highly increase infectivity of prions. This information can be of great help in developing treatment strategies against infectious prion proteins.

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