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The Mutagen Hypothesis

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Abstract
In 1997, Stanley Prusiner was awarded the Nobel Prize for his prion hypothesis, which is still used today to describe the propagation of some of the deadliest diseases that affect the central nervous system. However, the prion hypothesis remains controversial, as it does not adequately describe the mechanism by which infection occurs, inheritance of diseases such as CJD, or variations among the infected proteins (i.e. "prions"). I propose a counter argument to the prion hypothesis, the "mutagen hypothesis," to explain the uncertain aspects of pathogenesis of these diseases. This new hypothesis is supported by and explains findings from previous experiments performed by other scientists – findings that the prion hypothesis failed to explain.

The Mutagen Hypothesis

Since the 1960s, scientists have searched, without success, for the main cause of a group of similar diseases, which includes Creutzfeldt-Jacob Disease (CJD) among the general population, "kuru" among the Fore tribe of New Guinea, "scrapie" among sheep, and "mad cow disease." In the 1980s, one scientist believed he had found the cause – a small proteinaceous infectious particle, which he called a "prion" (Prusiner 1991). The idea that a protein could serve as an infectious agent challenged the long-standing view that only nucleic acids could spread disease (JYI 2008). Although there is hardly any doubt that the infectious agent is not a nucleic acid, there are many reasons to suspect that a prion is not the *main* cause of transmissible spongiform encephalopathy (TSE), but may just be acting as a sort of "accomplice." My hypothesis is that the real cause of TSE is a mutagen (an agent which can cause mutations in a person's DNA) that attaches to prion proteins and causes them to misfold.

Normal individuals possess a PrP gene that encodes a protein called PrP^{c}. This protein has an unknown function, but it may be involved with the transport of copper ions, which are bound by the N-terminal end of the protein (Riesner 2003). According to the Prion Hypothesis, an abnormal form of the PrP^{c} protein, called a "prion" and designated PrP^{sc} (c = cellular, sc = scrapie), interacts with normal PrP^{c}, forming more and more PrP^{sc} in a chain reaction. Unlike PrP^{c}, PrP^{sc} is insoluble, cannot be destroyed by high temperatures, and is usually resistant to degradation by protease K (Riesner 2003). Experiments have shown that mice without a PrP gene are not susceptible to infection by PrP^{sc} while those with the gene are (Prusiner 1991). No nucleic acid has been found associated with PrP^{sc}, and it was therefore concluded that the protein must be the infectious agent (Prusiner 1991).

However, the prion hypothesis does not attempt to explain how TSEs can be inherited. It assumes that vCJD, sporadic CJD, and familial CJD are all different diseases (Prusiner 1991). It is believed that familial CJD is caused by a mutation in the PrP gene, and that individuals who develop sporadic CJD might have also undergone mutations at various times (Prusiner 1991). However, it is possible that the variants of CJD are actually the same disease, and that PrP^{c} and PrP^{sc} are the same protein, produced by the host's own cells. The discovery of a mutagen that can attach to the PrP^{c} protein would explain how diseases such as scrapie, BSE, and CJD can be inherited – as the mutagen would not only react with the normal prion protein, but also cause mutations in the PrP gene (as shown in Fig. 1).

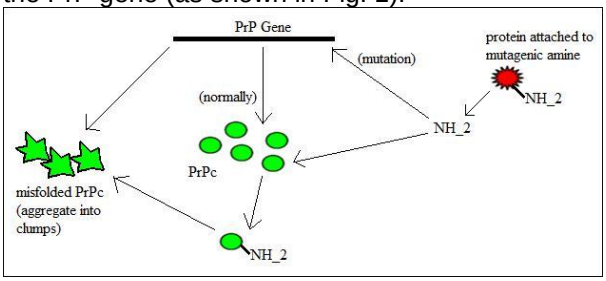

Figure 1. Green circles = PrP^{c}, Red circle = protein attached to mutagenic amine, Green stars = misfolded PrP^{c}.

A study has shown that heterocyclic mutagenic amines such as Trp-P-1, Trp-P-2, and Glu-P-1 can bind to proteins such as α-lactalbumin and β-lactoglobulin found in cow milk (Yoshida et al. 1991). Mutagenic amines were isolated from cooked beef and fried hamburger, and were shown to cause mutations in mice and rats (Yoshida et al. 1991). In addition, the mutagenic amines would bind more readily at a higher temperature and lower pH. Interestingly, experiments in which PrP^{c} was converted to the

supposed "prion" PrP^{sc} were carried out at an acidic pH of 4.0, and showed that "the transition to the PrP^{sc}-like conformation was induced by slightly denaturing conditions" (Riesner 2003). Thus, it is likely that a mutagen causes PrP^{c} to denature (into so-called PrP^{sc}) and that the denatured form is not the infectious agent but rather a result of it. Moreover, it has been found that PrP^{sc} can be reconverted into PrP^{c} by adding 0.3% sodium-dodecylsulphate (Riesner 2003), a detergent that possibly "washes out" the mutagenic amine attached to PrP^{sc}/PrP^{c}, allowing the protein to refold into its normal conformation.

In addition, the prion hypothesis does not adequately explain why the so-called infectious PrP^{sc} protein differs from host to host. It is believed that there are different "strains" of the PrP^{sc} prion (Prusiner 1991). However, it could just be that these are not different "strains," but normal variations in PrP^{c} that occur across species and even among organisms of the same species. The infectious agent is nevertheless the same – a mutagen. Even so, there could be several different mutagens that produce the same effect.

The prion hypothesis also fails to explain the immune response seen in people with CJD (JYI 2008). Opponents of the prion hypothesis believe the immune response would not occur "if the infectious agent was the body's own protein" (JYI 2008). If a mutagenic amine that came from outside the host was the infectious agent, this could indeed explain the immune response. Moreover, it could be that the immune response resulted from an entirely different molecule. A study by scientists at the University of California has found that subjects who consumed red meat produced antibodies to N-glycolylneuraminic acid (Neu5Gc), a type of sugar in meat, providing evidence that molecules found in meat can cause an immune response in humans (Tangvoranuntakul et al. 2003). Since CJD is suspected to have been passed from cattle to humans through meat consumption, the immune response might be unrelated to the disease. Moreover, people who do not eat meat but have CJD could have acquired it from a mutagen in the environment. Similarly, these people could have an immune response from another agent acquired from the environment.

Additionally, it has been stated that "infectious prions from BSE and vCJD may accumulate in the lymph nodes (which produce white blood cells), the spleen, and the tonsils" (NIH 2009). Interestingly, more than 20 years ago, a study on the distribution of heterocyclic mutagenic amines in mice found that one of the areas where the mutagenic amines accumulated was lymphomyeloid tissue, which is made up of the spleen, lymph nodes, bone marrow, and thymus (Bergman 1985). Thus, when the mutagenic amine is bound to PrP^{c}, it could cause the bound PrP^{c} (i.e. PrP^{sc} or "prion") to accumulate in these tissues along with it.

It has also been found that the "resistant form" of PrP^{c} – PrP^{res} (i.e. PrP^{sc}) – "interacts with high affinity with nucleic acids, especially RNA" (Soto & Castilla 2004). This would support my hypothesis that the "infectious agent" is a mutagen, as mutagens do interact with nucleic acids (DNA and RNA) and can cause mutations in them. The mutagens may interact with equal affinity with both DNA and RNA, but may cause mutations in RNA more readily because RNA does not have as advanced "proof-reading" mechanisms (to correct mistakes in replication) as DNA does.

The mechanism by which the so-called prions "infect" normal PrP^{c} protein has not been described, as it is still unknown. Perhaps a mutagen attaches to the N-terminus of the PrP^{c} protein, since experiments have shown that "preparations with PrP^{Sc} molecules lacking N-terminal residues did not transmit disease" (Supattapone et al. 2001), which could mean that the mutagen was unable to attach to the PrP^{c} protein and change its conformation.

Even proponents of the prion hypothesis agree that the infectious agent may contain bound elements, such as "peptides, oligosaccharides, fatty acids, sterols, or inorganic compounds" (Prusiner 1991). A mutagen could be disguising itself as a rather harmless-looking bound cofactor. Experiments have shown that "the thermodynamically stable state is the PrPSc-like state" (Riesner 2003). Thus, the mutagen could confer added stability onto the misfolded PrP^{c} protein when bound to it.

In addition, it has been stated that "rather than a self-replicating protein, an infection-induced synthesis of a host protein might be the basis for prion amplification" (Riesner 2003). Rather than "infection-induced," perhaps it is "mutation-induced." Furthermore, in vitro synthesis of PrP^{c} and conversion to PrP^{sc} supports the idea that PrP^{sc} may really just be a misfolded PrP^{c} with a mutagenic amine attached. The mutagenic amine could dislodge from one PrP^{c} molecule and attach to another, causing it to change conformation. This could continue until all of the normal PrP^{c} has bound to the mutagen and become misfolded, similar to the "chain reaction" described by the prion hypothesis. Thus, researchers should not

focus on the protein as the infectious particle, nor look for different "strains" of the protein (unless they are simply studying variation among organisms/species). Instead, they should look for a molecule that attaches specifically to PrP^{c} proteins and can affect the PrP gene.

It is important to note that people with other neurodegenerative disorders, such as Alzheimer's, exhibit strikingly similar symptoms as people suffering from CJD (such as behavioral changes, sudden onset of psychiatric illness, insomnia, dementia, etc.). In fact, in a 1989 study, autopsies revealed that 13% of Alzheimer's patients were actually suffering from CJD (Mitchell 2003). It could be that both Alzheimer's and CJD are caused by the same or a similar mutagen, and in theory they could be the same disease. One scientist (Manuelidis) has stated that "what people call Alzheimer's now is more broad than what people used to call it, and that has the possibility of encompassing more diseases -- including CJD" (Mitchell 2003). If a mutagen is causing these diseases, it would explain their variety of manifestations, and finding the mutagen(s) could bring us a step closer to understanding many neurodegenerative disorders.

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