

## **Mad Cow and Variant Creutzfeldt-Jacob Disease: "Prions - Proteins which Set a Bad"**



The mad cow, Jean-Philippe Uzan, oil painting 2001

**When it came to infection, doctors used to think they had seen it all. In recent years though, a whole new class of pathogens has appeared. Neither viruses nor bacteria, the prions - responsible for diseases which destroy the nervous systems of humans and other mammals - are none other than proteins.**

Recently, the "mad cow" scare has put diseases caused by prions on the front page. These diseases share a common link to a protein naturally produced by the cells of human beings as well as numerous vertebrate animals. PrP is a small protein - around a hundred times smaller than the smallest virus - and invisible to even the best electron microscope. The largest quantities of this protein are made by neurons, such as the nerve cells of the brain and spinal cord. Like the vast majority of proteins, PrP's existence is limited in time; little by little, as new PrP proteins are produced, the old ones are destroyed by other proteins called "proteases". This keeps them from accumulating in the interior of cells and blocking normal functions. But beyond saying that PrP is associated with the membrane of the cell, we do not know exactly what purpose the protein normally serves.

### ***One Protein. Seven Diseases.***

In humans, this same PrP protein is involved in at least seven rare illnesses, all characterized by memory loss, behavioral problems and disordered movements, followed by a type of dementia which leads rapidly to death. Up until now, the best known was Creutzfeldt-Jacob disease whose three forms each produce slightly different neuropathologies. First there is the sporadic form which affects only one in every million people each year and usually appears around the age of 60. Then there is a genetic form - inherited cases appear when patients are between 45 and 60 years old - due to mutations in the genes coding for the PrP protein. Creutzfeldt-Jacob disease, however, can also be caught from a contaminated source (iatrogenic form). Children have died of Creutzfeldt-Jacob disease after receiving

injections of growth hormones taken from human pituitary glands. Badly sterilized surgical instruments and corneal transplants are other possible routes of infection.

In 1995 there emerged what we now call the variant of Creutzfeldt-Jacob disease (or vCJD). It was characterized by a slower progression - an average of 14 months between the first symptoms and death, compared with just six weeks to six months for the usual form - but also by a shorter incubation period. The variant affected young people (less than 40 years old), who all showed very unusual brain lesions. The first cases were discovered in England, the country most affected by Spongiform Encephalopathy or BSE, a prion disease in cattle. Thus, researchers quickly suspected that vCJD might be the result of people catching the bovine disease from contaminated food.

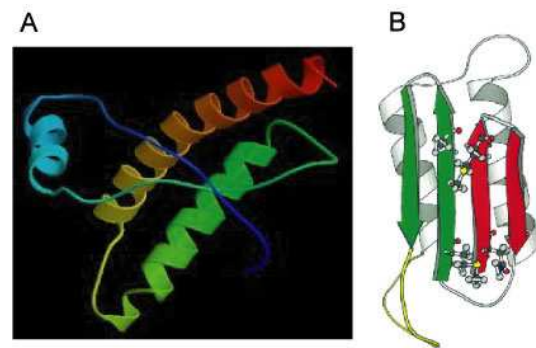
### ***A Revolutionary Hypothesis***

Until recently, scientists assumed that an "infection" was always an invasion of microorganisms which multiplied in the body until they disturbed its normal functions. By 'microorganism' they meant literally a microscopic organism, complete with DNA - in other words its own genetic information - to let it produce its proteins in our bodies and thus multiply there; an organism like a virus, a bacteria, or certain types of fungus. But vCJD is different. All attempts to detect the presence of foreign genetic material which could betray the presence of a microorganism have failed so far. It may well be that the infection is due to eating a humble protein. One of the dogmas of molecular biology is being seriously challenged...

But how could a mere protein become infectious and reproduce within the body (ultimately causing its death) without genetic material? The answer turns out to be a matter of form rather than substance. PrP can actually exist in two forms; healthy (called PrP<sup>C</sup>), or abnormal, called PrP<sup>Sc</sup>. From a chemist's point of view the two are so similar it is nearly impossible to distinguish one from the other. In fact, the only real differences are at the level of their three-dimensional structure.

Imagine a protein as a string of pearls (the amino acids). The order of the pearls and also their number can vary slightly between different species for a given protein. For example, the two forms of the human PrP protein are made up of 253 "pearls", while those of cattle have 264. What is more, this string is neither rigid nor straight. On the contrary, once made, it folds in on itself until it takes on the

particular shape which lets it perform its function within the cell. In fact, between the two forms of the PrP protein there is only one difference in the folds - the abnormal PrP<sup>Sc</sup> which makes up the prion is simply "badly folded", or deformed. Furthermore, and this is the critical part, it looks as though the deformed protein is capable of "molding" a normal PrP<sup>C</sup> protein in its own image so that the latter takes on its twisted shape. Currently, this step is a mystery; it is not understood how the transformation is brought about and it is suspected that an unknown actor present in the cell comes into play. Still, it is certain that the "badly folded" PrP<sup>Sc</sup> proteins play an essential role in setting some kind of bad example for their normal neighbors.



**Fig.1** *A: Three-dimensional structure of the human PrP<sup>C</sup> protein, B: Possible model of the three-dimensional structure of the PrP<sup>Sc</sup> protein.*

The change in the protein's shape has multiple consequences. First, the change in shape causes a chain reaction, since each new "badly folded" protein can in turn cause another normal protein to change its shape, and so on. But since both shapes are after all the same protein, the immune system - whose task is to defend the organism against infections - does not set off any alarms and makes no attempt to destroy the now dangerously misshapen proteins. Second, due to its twisted shape, PrP<sup>Sc</sup> aggregates in clumps called "amyloids" or "amyloid plaques" inside and around neurons, thus preventing the latter from functioning correctly. To top it off, in this compact form, PrP<sup>Sc</sup> is totally resistant to proteases, the proteins responsible for destroying "spoiled" or abnormal proteins.

Since cells cannot eliminate the deformed prions, they just keep accumulating indefinitely. At a certain point, this situation drives neurons to autodestruct in a kind of collective cellular suicide, thus freeing the abnormal proteins which in turn infect neighboring cells. This leads rapidly to the

mass death of neurons and creates actual holes in the brain, giving it the characteristic appearance of a sponge. However, the extent of damage observed in patients' brains suggests that the accumulation of abnormal protein is not the only cause. It is likely that the abnormal PrP<sup>Sc</sup> protein interacts with other proteins which also participate directly in the destruction of neurons.

### *From Cattle to People*

Long discredited by the scientific community, the revolutionary idea of an infectious protein - advanced for the first time by the American biologist Stanley Prusiner in 1982 - has gradually won general acceptance. But the discovery of the existence of different strains of prions nearly dealt the theory a fatal blow.

The notion of strains of prions rests on several observations. First, on the differing lapse of time between infection and first symptoms (incubation period), then on the different kinds of lesions observed - for example different strains destroy different parts of the brain - and finally, on certain differences in the physical structure of the protein which produce greater or lesser resistance to proteases.

But contrary to what we might assume, these strains do not necessarily correspond to different species. In fact, several strains of prion can coexist within the same species. In these cases, the proteins have exactly the same sequence (order and number of "pearls" on the "string"). For example, the sporadic and the iatrogenic forms of Creutzfeldt-Jacob disease are due to two different human strains.

The bad news is that each strain is capable of "transmitting" its characteristics to normal PrP<sup>C</sup> proteins. Take for example a hamster infected by a particular strain of hamster prions. From its own normal PrP<sup>C</sup> proteins, it will start to make new PrP<sup>Sc</sup> prions with the same characteristics as the strain which infected it. Furthermore, when injected into yet another hamster, the latter's new prions will produce the same type of lesion after the same incubation period as well as have the same biochemical properties as the strain from the first hamster! A puzzler for researchers: how to explain that the same sequence of "pearls" can produce diverse abnormal proteins, each presenting different properties transmissible to normal PrP proteins? For certain researchers, that was self-evident proof that the infection is after all due to a microorganism with its own DNA, probably a virus. Indeed, the DNA

of viruses *does* evolve very quickly, meaning that it easily undergoes mutations which change the information it carries. This could explain perfectly the existence of the different strains observed.

Prusiner's retort is a firm "no". He still believes that the solution is hidden within the structure of the protein; that each strain corresponds to a different physical configuration ("conformation") of PrP<sup>Sc</sup>. Still, how to explain that the same protein can adopt as many different shapes as there are strains? A key piece of information is that once produced, a protein undergoes a number of further modifications which do not change its sequence but which can, among other effects, influence its shape. Recent results do seem to show that some of these modifications vary according to the strain. This would explain how the same protein, PrP, could give birth to several different infectious agents, or prions. But even if this hypothesis proves correct, it does not explain how an abnormal PrP<sup>Sc</sup> protein can "transmit" its own modifications to the normal PrP proteins it comes in contact with. This crucial mechanism remains totally obscure.

Today, only one strain of prion can be identified from the brains of cows struck down with BSE. An intriguing point is that all the cases of human vCJD observed are due to a specific strain which provokes brain lesions similar to those seen in animals naturally or experimentally infected with BSE. The incubation period is also identical. Both of the above suggest a bovine origin for vCJD. Taken with other biochemical and biological similarities between the two infectious agents, these observations have managed to convince the medical community that the cow prion is capable of infecting humans.

### *The Emergence of a "Super" Prion*

Injecting a prion from one species into another is far less effective at transmitting the disease than a transfer within the same species. This resistance to infection illustrates what we call the "species barrier". The small differences in the sequence (order and number of pearls on the string) between the normal PrP<sup>C</sup> proteins of different mammals give rise to variations in the "normal" three-dimensional structures of those proteins. Such variations certainly play a central role in this resistance to infection. In the laboratory, it is possible to overcome this barrier by using highly elevated doses of the infectious agent and a direct path of infection (injecting the prions directly into the brains of animals); even so the incubation period is much longer.

This is why at first it was thought that a "badly folded" protein could only influence the shape of a protein of the same species; in other words that a sheep protein could only infect another sheep, a cow prion another cow, and a human prion another human. As a general rule this is true. However, laboratory studies have confirmed something already suspected from a number of observations - that although the disease initially known in sheep under the name "scrapie" was not capable of infecting humans, it could well have been transmitted to cattle through animal-based fodder. In contrast, cattle seem to be quite capable of transmitting it to humans as well as to numerous other species. Today we think that the change in methods of preparing animal-based fodder towards the end of the 1970s may have led to the emergence of a "super" prion which cheerfully leaped the species barrier.

### ***Should We be Scared of Eating Beef?***

If you eat food contaminated with prions, the abnormal PrPSc protein - which has become resistant to proteases - stays intact while the normal PrPC protein is totally broken down by digestive enzymes. A mechanism still poorly understood then permits the deformed protein to pass from the intestine to the nervous system, and in particular the brain. Despite the confusion which reigns over this question, it is important to grasp that the skeletal muscles of a cow - in other words the meat or a steak - do not contain prions. In fact, it seems that the normal PrPC protein in the cells of this tissue somehow resists the transformation into the abnormal PrPSc variant. This has been demonstrated by laboratory tests, though the reasons remain unclear. On the other hand, the infectious agent *is* present in the peripheral nerves and the blood of a

contaminated animal, and these cannot be eliminated when preparing the meat. Still, the risk of infection by this route is probably very low, though it will be difficult to estimate more precisely until the minimum infectious dose for humans is known, and whether the latter is cumulative or must be eaten at one time.

With prion diseases, researchers are confronted with a radically new concept. Two priorities of current research are to track the development of the disease through epidemiological studies and to develop fast, sensitive tests which would make it possible to detect the infection in a living patient. In the therapeutic domain everything remains to be done since there is currently no treatment. Still, the recent progress in understanding how prions propagate is encouraging. Much hope has been placed in the elucidation of the three-dimensional structure of the abnormal PrPSc protein. Once obtained this could be compared with the structure of the normal protein, which is already known. With the help of bioinformatics, molecular modeling should one day allow us to design molecules capable of preventing or even reversing the fatal change in the protein's shape. For now we can only hope that intensive efforts will bring success in containing and eventually eradicating this disease.

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### ***Further Reading in French***

- "Vache folle : transmission expérimentale". La Recherche : n°332 (juin 2000), p46.
- "La fonction du prion". Pour la Science : n°278 (déc. 2000), p21.
- "Maladie de Creutzfeld-Jacob et autres maladies à prions". Pierre Beauvais et Thierry Billette de Villemeur, Médecine-Science Flammarion.

### ***In depth (and in English):***

- Zahn S. et al., "NMR solution structure of the human prion protein", Proc. Natl. Acad. Sci. USA. 97:145-150(2000) PMID: 10618385
- Huang Z. et al., "Scrapie prion : a three-dimensional model of an infectious agent", Fold. Des. 1:13-19(1996) PMID: 9079359
- Prusiner S.B., "Prion Diseases and the BSE Crisis", Science 278:245-251(1997) PMID: 9323196

**On the Web:**

- Mohammed Moudjou, Unité de Virologie et Immunologie Moléculaires, INRA  
"Bref survol des connaissances, des hypothèses et des incertitudes sur les maladies à prions" , "A brief survey of current knowledge, hypotheses and uncertainties in prion diseases":  
<http://www.expasy.org/prolune/pdf/moudjou.pdf>
- Fondation pour la Recherche médicale :  
[http://www.frm.org/informez/info\\_ressources\\_dossiers\\_article\\_sommaire.php?id=5&type=10&listedossier=5](http://www.frm.org/informez/info_ressources_dossiers_article_sommaire.php?id=5&type=10&listedossier=5)
- Site in English: [www.mad-cow.org/](http://www.mad-cow.org/)
- The National Creutzfeldt-Jakob Disease Surveillance Unit: <http://www.cjd.ed.ac.uk/>

**Illustrations:**

- Heading illustration, Source: Jean-Philippe Uzan, [http://qcd.th.u-psud.fr/page\\_perso/Uzan/index.html](http://qcd.th.u-psud.fr/page_perso/Uzan/index.html)
- Fig1A, Source: PDB ID: 1QLX, Zahn, R., Liu, A., Luhrs, T., Riek, R., von Schroetter, C., Lopez Garcia, F., Billeter, M., Calzolari, L., Wider, G., Wuthrich, K., NMR solution structure of the human prion protein. *Proc.Natl.Acad.Sci.USA* **97** pp.145 (2000)
- Fig1B, Source: Reprinted from *Folding and Design*, Vol 1, Huang Z., Prusiner S.B., Cohen F.E., "Scrapie prion : a three-dimensional model of an infectious agent", 13-19, Copyright (1996), with permission from Elsevier

**At UniProtKB/Swiss-Prot:**

- Major prion protein, Homo sapiens (humain): P04156
- Major prion protein 1, Bos taurus (bovin): P10279
- Major prion protein, Mus musculus (souris): P04925

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