The nature of TSEs

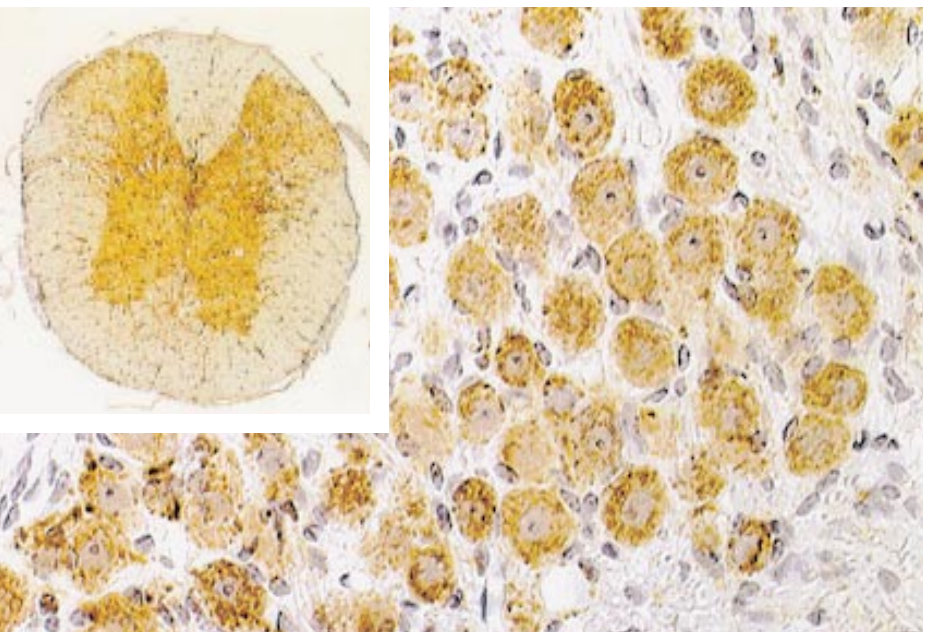
Chris Bostock

Scientists are unsure of the identity of the causative infectious agent of TSEs, but the properties are unlike those of conventional pathogens.

Creutzfeld–Jacob disease (CJD) and bovine spongiform encephalopathy (BSE or mad cow disease) both belong to a group of diseases called transmissible spongiform encephalopathies (TSEs) or prion diseases. BSE and the new variant of CJD (vCJD) have been in the news recently and are new additions to the group, but, as a whole, TSEs have been around for centuries. Scrapie in sheep was first recorded over two hundred years ago and sporadic CJD was first described in humans in the 1920s. Nevertheless, the TSEs remained a rather obscure group of diseases until 1986 when BSE first appeared. In the years since the emergence of BSE, new TSEs have also been found in exotic species of ruminants in UK zoos, exotic and domestic cats and, in 1996, vCJD was described in humans.

The TSEs are fatal once clinical signs appear, but there is a long, usually many years, incubation period between the time of first exposure to the infectious agent and the time of appearance of disease. A normal microbial infection usually elicits an immune response in its target host, but in TSEs there is no conventional immune response, although the immune system plays an important part in the development of the disease, before the infectious agent gets into the central nervous system (CNS). A common feature of all TSEs is degeneration in the brain and spinal cord and part of this process involves a normal host protein, called PrP or prion protein. During a TSE infection this protein is deposited in an abnormal form and in excessive amounts in the brain, spinal cord and many peripheral nerves and tissues (Fig. 1). The causative infectious agent has properties unlike those of conventional pathogens and may be a new class of infectious agent.

BELOW:
Fig. 1. Abnormal prion protein can be detected as brown deposits following staining with antibodies to prion protein in a section through part of the spinal cord taken from a hamster infected orally with the 263K strain of scrapie (inset). The abnormal accumulation of prion protein can be seen at higher magnification in individual neurons in the pathway that connects the ear to the brain.
COURTESY TRICIA McBRIDE



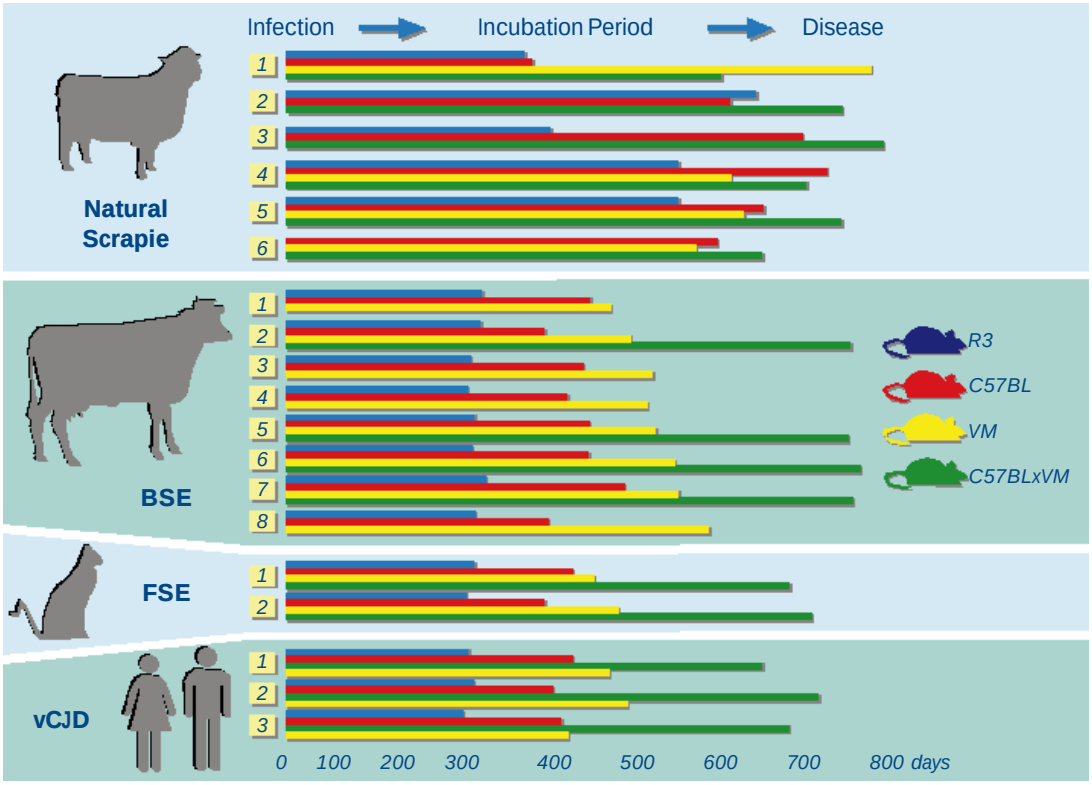
The nature of the infectious agent

There are currently three theories. One says that the infectious agent is somewhat like a small conventional virus, with genetic material coding for its own proteins. At present there is little evidence to support this theory. The second, and now most widely held theory states that it is the altered physical state of normal host prion protein, which is able to propagate itself by inducing other normal prion protein molecules to adopt the abnormal conformation. This is commonly referred to as the prion hypothesis. The third idea, called the virino hypothesis, is most easily thought of as a hybrid of these hypotheses. It suggests that there is a very small piece of genetic material, which encodes only information for its own survival and replication through interaction with a host protein, perhaps the prion protein, using it also as a protective coat. The essential, but very important difference between the prion and virino hypotheses is that in the prion hypothesis, all the information to determine the properties of the infectious agent is carried in the abnormal conformational state of the prion protein, whereas in the virino hypothesis, the information is carried in an independent 'genetic' molecule. As yet no-one has defined the precise molecular state(s) of the normal and infectious forms of the prion protein, nor produced infectious 'prions' *in vitro* from normal prion protein, as would be predicted by the prion hypothesis. But neither has anyone yet found any molecule that might fit the role of the 'virino'.

Prion protein genes

As with conventional microbes, there are different strains of TSEs, and the characteristics of an infection are determined by an interaction between a particular strain of infectious agent and a gene (or genes) in the host animal. The host gene that has the biggest effect in determining the outcome of an exposure to an infectious agent is the same gene that codes for the normal prion protein. In humans there are two common versions of the prion protein that differ in a single amino acid (whether it has valine or methionine at coding position 129), but there are also many rare mutant forms, most of which are associated with inherited susceptibility to prion disease. Sheep also have many different forms of the prion protein, none of which have so far been linked to 'inherited' scrapie and only some of which appear to affect the outcome of a scrapie infection. Two versions of the prion protein have been found in cattle but these do not appear to differ in their association with susceptibility of animals to BSE.

Much of the work that has lead to an understanding of the role of the prion protein gene in TSEs was done in laboratory mice before anything was known about the existence of the prion protein or its gene. Different lines of inbred mice were found to differ in their response to infection with scrapie and a gene, called *Sinc* (for scrapie



*inc*ubation period), was identified that controlled the incubation period. There are two versions of *Sinc*, s7 and p7, which determine, respectively, short and prolonged incubation after infection of mice with a strain of scrapie called ME7. *Sinc* turns out to be the gene that encodes the prion protein. The difference between s7 and p7 *Sinc* genes is now known to lie in the amino acids at two positions in the protein; if it has leucine at position 108 and threonine at 189 it is *Sinc* s7, whereas if it has proline at position 108 and valine at 189 it is *Sinc* p7.

Properties of different strains of infectious agents

In addition to the different versions of the prion protein gene within a species there are many different strains of the infectious agent that can infect that species. This is most clearly demonstrated by infecting the same inbred lines of *Sinc* s7 or *Sinc* p7 mice with different sources of scrapie. Different sources of scrapie can produce widely different incubation periods and result in differing patterns of damage in the brain (called lesion profile). These two features, relative incubation periods in s7 and p7 mice and the lesion profile, are classically used to define the different strains of infectious agent. Several different strains of TSE agent can be propagated in the same inbred line of mouse and thus, if the abnormal conformation of the prion protein determines the properties of the infectious agent, this means that the same normal prion protein must be able to adopt reproducibly several distinct abnormal disease-associated states.

Using the criteria of relative incubation period and lesion profile it has been possible to characterize and compare the infectious agents causing contemporary scrapie, BSE, the spongiform encephalopathies of cats (FSE), kudu and nyala, as well as classical and vCJD of humans (Fig. 2). Sources of scrapie, collected contemporaneously with the BSE epidemic, are heterogeneous, each being distinct and different from the others. This situation contrasts with the homogeneity found in several different sources of BSE sampled at different times during the epidemic and from different geographical locations. The infectious agents causing FSE and disease in exotic ruminants in zoos were found to be indistinguishable from the BSE strain, and, although classical CJD is quite distinct, the transmissible agent causing vCJD has the same strain properties as the BSE agent. This suggests that the agent that causes vCJD is the same as that which causes BSE, but as yet there is no firm evidence to say how humans became infected. From a scientific point of view, one of the interesting observations to come out of this work is that the same strain of agent (BSE) can be propagated by several species, each of which has a slightly different normal prion protein. Thus, if the conformation of the abnormal form of the prion protein determines the biological properties of a strain of infectious TSE agent, it must be independent of the structure of the normal prion protein in these different species. Different prion proteins must be able to adopt the same abnormal conformation even though they have very different structures in their normal states.

Strains of TSEs differ in several other respects in addition to incubation periods and lesion profiles. They show large differences in their resistance to inactivation by heat or chemicals. Primary BSE and a mouse-passaged strain derived from it (called 301V) are the TSE agents most resistant to inactivation by heat yet to be discovered. Nevertheless, resistance to inactivation is a property of the 'strain' and not the primary structure of the prion protein that carries it because the resistance properties of a strain remain the same when it is produced in mice differing in the type of prion protein they make (s7 v. p7).

Different strains of TSEs are associated with different biochemical and biophysical properties of the abnormal prion protein. The prion protein has two sites at which it can be glycosylated, i.e. amino acids at which sugar residues can be attached, but not all molecules of the prion protein are fully glycosylated. Thus, an individual molecule may have no added sugars, sugars added at one or other of the sites or at both sites. The relative ratios of the prion protein in these different states can be quantified and they differ predictably between different strains.

When the prion protein adopts the abnormal conformation characteristic of a TSE infection it becomes partially resistant to digestion with enzymes that degrade proteins – called proteinases. This relative resistance of the abnormal form of the prion protein to proteinase digestion is used as a diagnostic feature for infection, but the size of the resistant protein fragment has been found to differ slightly for abnormal prion proteins produced by different strains, indicating that strain-specific

ABOVE:
Fig. 2. Strains of TSE agents differ in the relative lengths of incubation period in different inbred lines of mice. R3 and C57BL are *s7* mice, VM is a *p7* mouse and C57BLxVM is the cross between the two lines. Several sources of BSE from cattle, FSE from domestic cats and vCJD from humans produce very similar patterns, indicating that the same strain of TSE agent is causing the infection. Different sources of scrapie on the other hand produce very different patterns of incubation in the different mouse lines, indicating that they are different from BSE as well as being different from each other.
BASED ON DATA PROVIDED BY MOIRA BRUCE AND COLLEAGUES

conformational differences might change the site which is accessible to the cutting enzyme.

It has been suggested that these two properties, ratios of glycosylated forms and the size of the proteinase-resistant fragment, could be used as a basis for biochemically identifying different strains. If this were possible it would provide several advantages since it would be both quicker and would not involve the use of large numbers of mice to bioassay the infectious agent. In the case of well characterized mouse passaged strains of scrapie it appears that a particular strain/mouse combination produces a characteristic narrow range of ratios of glycosylated forms (Fig. 3). The method has been used successfully to distinguish between the various forms of CJD in humans. Whether this type of biochemical typing can be made precise enough so that each strain of TSE has its own unique 'fingerprint', irrespective of the species or prion protein background, remains to be seen. In the case of natural and experimental sheep scrapie the patterns are numerous and complex and it is likely to take some time before it will be known whether the approach is feasible as a practical diagnostic method.

The observation that strains differ in the degree of glycosylation of the accumulated abnormal prion protein has lead to the hypothesis that the pattern of glycosylation may itself be the biochemical determinant of strain. It is proposed that a certain type of cell in the host might recognize individual patterns of sugars on the prion protein molecule and thus 'permit' the infectious agent to enter and replicate within it. If, in the process of replicating the agent, the cell 'stamped' its own glycosylation signal on the newly formed abnormal prion protein, it would ensure the next generation of 'infectious prions' were of its own kind, thus perpetuating the tropism and properties of the strain. This idea has been tested by infecting a single cell type with two different strains of agent. If the strain properties derive from a cell-imparted glycosylation pattern, the two originally different strains would be expected to emerge with the same strain characteristics and glycosylation patterns. Alternatively, if the determinant of strain is independent of glycosylation pattern of prion protein and the cell type in which it is replicating, the two strains should retain their original characteristics. Experiments using cells infected in culture indicate that the latter is the case and suggest that differences in glycosylation result from strain differences, and are not the cause of them.

Conclusion

Returning finally to the question of the nature of the agent. Work on strain properties of TSE agents has shown that if the prion protein is the sole component of the infectious agent, the conformational determinant of infectiousness and strain must be independent of prion protein primary structure (sequence of amino acids). A single prion protein must be capable of adopting and propagating several different stable abnormal conformations while several different prion proteins must be able to adopt a common abnormal conformation. A full understanding of how this is achieved will have to await a complete molecular description of the various forms of the prion protein.

Much of this article is based on the work of my colleagues at the Institute for Animal Health, whom I thank for providing the material that has been incorporated in the figures.

Professor Christopher J. Bostock is Director of Research at the Institute for Animal Health, Compton Laboratory, Compton, Newbury, Berkshire RG20 7NN. Tel. 01635 577238; Fax 01635 577237; email chris.bostock@bbsrc.ac.uk

BELOW:
Fig. 3. The prion protein can have sugars added at one or other or both of two sites, the effect being to increase the size of those molecules with added sugars. These size differences can be used to separate and quantify the various forms. The percentage of the total abnormal prion protein with sugars added at two sites (diglycosylated) relative to the percentage of those molecules with sugar added at only a single site (monoglycosylated) can be constant for a particular strain of TSE agent, but differ between strains of agent, replicated in a single inbred line of mouse. The ratio can change when a strain is propagated in an inbred line of mouse with a different prion protein gene.
BASED ON DATA PROVIDED BY ROBERT SOMERVILLE AND JAMES HOPE