Transmissible spongiform encephalopathies (TSEs, or prion diseases) such as bovine spongiform encephalopathy (BSE) in cattle, scrapie in sheep and goats, chronic wasting disease (CWD) in deer and elk and Creutzfeldt-Jakob disease (CJD) in man are caused by unusual pathogens termed prions. According to the ‘protein only’ hypothesis prions are made up of a rogue protein (PrPSc or PrPBSE) that arises through a conformational transition from the host-encoded prion protein (PrPC) (for review see [1]).

**Proof for the Prion Achieved?**

All known pathogens harbour nucleic acid to sustain their self-propagation or propagation within a host, except for prions, which are believed to be composed entirely of an abnormally folded conformer of a host protein known as the prion protein [2]. For more than three decades, the protein only hypothesis, formulated for the first time by Griffith in 1967 [3] and extended to its present form by Nobel laureate Stanley Prusiner in 1982 [4], has been resisted its formal proof by way of generating prions from recombinant PrP and demonstrating de novo formed infectivity in an animal model.

**Fig. 1: Experimental evidence for infectious prion proteins.**

A: Recombinant truncated PrP (amino acids 89 – 230) was expressed in E.coli and the α-helical soluble protein was converted in the presence of urea at pH 5 into a β-sheet rich form that polymerized into amyloid fibrils. Inoculation of these fibrils into the brains of transgenic mice that overexpress the same truncated PrP(89-231) at a level 16-fold higher than in normal brains caused a scrapie-like prion disease after an incubation time of 380–660 days. Further passaging of brain tissue from these sick transgenic mice into transgenic mice overexpressing the full-length wild-type PrP at eight times the normal level and into wild-type mice caused disease after 90 and 150 days, respectively.

B: Whether full-length PrP converted into amyloid fibrils causes disease after injection into wild-type and transgenic mice remains to be seen.
A substantial leap forward in providing proof for the prion hypothesis in mammals has recently been reported by Legname and co-workers [5, 6]. They have used an N-terminally truncated version of E. coli derived mouse PrP polymerized into amyloid fibrils which was subsequently inoculated into transgenic mice overexpressing a similar shortened version of mouse PrP. Following an incubation time of 380 to 660 days all mice died with a scrapie-like disease showing typical spongiform changes, astroglisis and deposition of protease resistant PrPSc in their brains (Fig. 1). Further passage of homogenized brain material from diseased transgenic mice to wild-type mice and to transgenic mice over-expressing wild-type PrP, resulted in a dramatic reduction of disease incubation times of 150 and 90 days, respectively, demonstrating that infectious prions were propagated in these latter mice. These results argue for de novo formation of a synthetic prion strain from E. coli derived recombinant PrP and, hence, could provide the crucial evidence for prion proteins as culprits in TSEs; however, there are still some caveats to be considered. In their study, Legname et al. showed that only a truncated form of PrP – which more readily forms amyloid – was able to elicit a prion-like disease in mice, but not the full-length wild-type PrP. A further argument against the notion that prion infectivity was generated from scratch is that the transgenic mice expressed truncated PrP at 16 times the level found in wild-type mice. Since it has been shown that over-expression of PrP by itself can lead to a transmissible neurological disease it would be important to show that brains from transgenic control mice inoculated with PBS do not cause disease upon second passage into wild-type mice.

Modelling Prion Replication

Assuming that the work by Legname et al. shows for the first time de novo generation of prion infectivity from a protein-only substrate, the two hypothetic models for conversion of PrPc into PrPsc should now be revisited in the light of these findings (Fig. 2). In the template assisted heterodimer model, postulated by Prusiner [7], the conformational conversion of PrPc to PrPsc is induced or templated by direct contact of PrPc with monomeric PrPsc. In contrast, the seeded polymerization model which was proposed by Lansbury [8] predicts that PrPsc is stabilized upon binding to an oligomeric form of PrPsc which acts as a seed which rapidly recruits more monomeric PrPsc molecules analogous to the growth phase of nucleated polymerizations. The main difference between the two models is that in the heterodimer model PrPsc aggregates are considered an epiphenomenon whereas in the polymerization model they are required for prion propagation. The results by Legname et al. now suggest that amyloid is a prerequisite for prion infectivity which is consistent with the seeded polymerization model for prion replication.

BSE Diagnostics in the Fast Lane

Since its first recognition in the UK in 1986, BSE has raised great public health concerns because the BSE agent is generally thought to cause variant Creutzfeldt-Jakob Disease (vCJD) in humans. With the introduction of mandatory active surveillance programs in the European Union the development of rapid BSE diagnostic tests has become high priority. Most diagnostic tests approved by the EU authorities exploit the relative protease resistance of PrPsc in brain samples to discriminate between PrPc and PrPsc, in combination with immunological detection of the protease resistant part of PrPsc (PrP27-30). In three evaluation exercises organized by the European Commission between 1999 and 2004 a total of 12 rapid tests have been approved for BSE monitoring in cattle. These rapid screening tests have been used in active surveillance of BSE and have greatly improved the detection
of infected cattle before their entry into the human food chain.

By the end of 2004 more than 40 million rapid tests have been performed in Europe within the mandatory rapid testing of risk animals and of slaughtered cattle above the age of 30 months. In total, about 10 million BSE tests are performed annually in Europe and about 1 million in Japan. The 12 rapid tests that are currently approved for BSE monitoring in cattle are based on different techniques for the detection of the pathological form of the prion protein, PrPSc: One test is based on Western blot technology [9], 10 tests use ELISA technology, and in 2004 an immunochromatographic strip test [10] was approved by the EC for BSE testing [11]. While Western blot-based BSE tests have been shown to be the most reliable and accurate rapid BSE tests giving no false positives – or initial reactivates – ELISA tests have been widely used because they can be automated for high throughput screening.

However, future BSE testing needs will be tackled by rapid BSE tests based on an immunochromatographic principle in which the capture anti-PrP antibody is sprayed as a line perpendicular to the long axis of a nitrocellulose strip (Fig. 3). The detection antibody is coupled to a coloured latex bead. This conjugate is brought into contact with a protease-digested sample in a microplate well. A comb in an 8-strip format is then inserted into the well containing the sample-conjugate mixture. The immune-complexes are forced through the nitrocellulose strip by capillary forces and are captured at the test line. The resulting sandwich appears as a coloured line and can easily be detected by eye or by a dedicated reading device. This detection system tremendously reduces assay time; the whole procedure takes as little as 100 minutes from brain to result.

References