Journal of NeuroVirology, 8(suppl. 2): 8–20, 2002
 ISSN 1355–0284/02 $12.00+.00
DOI: 10.1080/13550280290101120

Taylor & Francis
Taylor & Francis Group

Review

Molecular basis of prion diseases

Detlev Riesner

Institut für Physikalische Biologie, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany

Journal of NeuroVirology (2002) **8(suppl. 2),** 8–20.

Keywords: diagnosis; epidemiology; pathology; prions; replication; structure

Introduction to the history of prion diseases

Fatal neurodegenerative diseases have been known for more than 300 years. As early as 1759, the scrapie disease of sheep was mentioned as a lethal and transmissible disease in a german veterinary textbook by Leopoldt (1759). Experimental transmission was shown in 1936 by Cuillé (1938). Not knowing about the similarity to the sheep disease, the neurologists Alfons Jakob (1921) and Hans Creutzfeldt (1920) described cases of a progressive neurodegeneration of human patients between 1920 and 1922. The disease, which was later named Creutzfeldt-Jakob disease (CJD), was recognized worldwide with a very low incidence. More spectacular attention was paid to the so-called Kuru disease of the Fore people on Papua Neuguinea. In 1956/57, V. Zigas (1990) and C. Gajdusek (1977) studied many cases, and it was found that the disease was transmitted by ritualistic cannibalism; the brain of the bodies was prepared and consumed or brought into close skin contact with other members of the families. Gajdusek found out that the time from infection to the appearance of symptoms could be as long as 40 years. The agent of the disease was called a slow virus, although a virus had not been identified. The bovine spongiforme encephalopathy (BSE) was recognized in 1985/86 in the United Kingdom as a new cattle disease (Wells *et al*, 1987), and it led to an epidemic with a peak in 1992 and unforseen political and economic impact. It was found that insufficiently decontaminated meat and bone meal from sheep or cattle or both was the source of infection. In 1996, about 10 patients showed a variant form of CJD (see below), which led to the suspicion of a cattle-to-man transmission.

Neuropathological investigations of the brains of animal and human victims led to the conclusion that the different diseases mentioned obove had a very similar molecular and cellular basis. First for Kuru and Scrapie (Hadlow, 1959), and later also for CJD and BSE, typical spongiforme degenerations were detected (Figure 1) in the grey matter of the brains. It was strongly suggested that one uniform class of diseases was described, which were named transmissible spongiforme encephalopathies (TSEs).

Address correspondence to Detlev Riesner, Institut für Physikalische Biologie, Heinrich-Heine-Universität Düsseldorf, Universitätsstrasse 1, 40225 Düsseldorf, Germany. E-mail: riesner@biophys.uni-duesseldorf.de

The author thanks his coworkers, C Dumpitak, H Gruber, K Jansen, O Schäfer, and Dr. J Schell, for their help in preparing the manuscript.

Received 18 September 2002; accepted 20 September 2002.

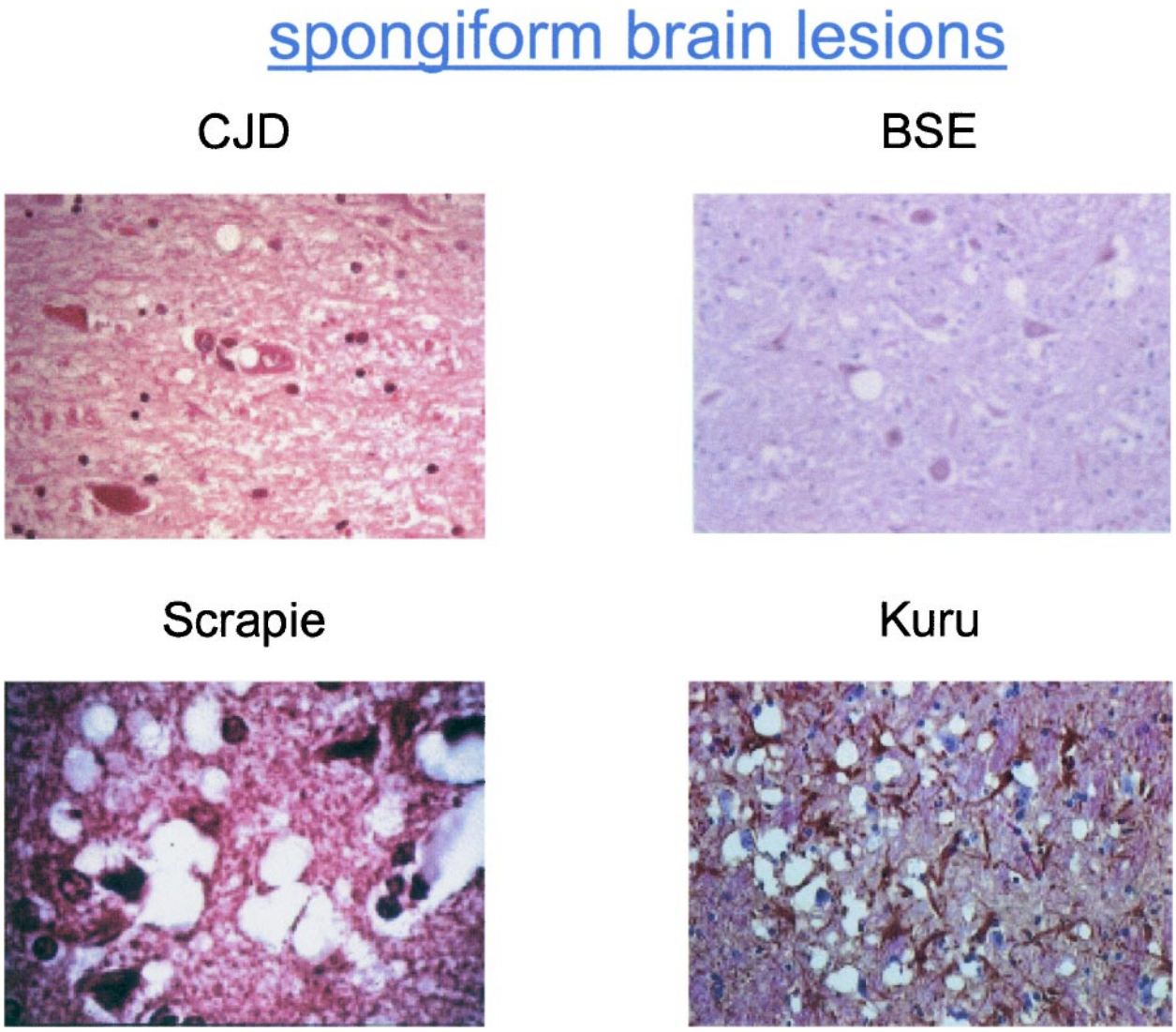

Figure 1

First indication that the agent of scrapie was not a virus came from T. Alper's radiation experiments in the late 1960s. She concluded that the target size of the agent was closer to that of a protein than that of a virus (Alper *et al*, 1966). Stanley Prusiner took up those experiments and extended them over several years by a series of biophysical and biochemical studies. The results were summarized as follows:

1. Chemical and physical methods, which modify or destroy nucleic acids (i.e., those of viruses, bacteria, etc.), do not inactivate the agent.
2. Chemical and physical methods, which modify or destroy proteins, do inactivate the agent.

From that he concluded: The scrapie agent must be a novel type of agent: A **pro**teinaceous **in**fectious particle, called **PRION** (Prusiner, 1982).

Structure and properties of prions

In the following years, the protein component of the agent was purified and cloned in cooperation with the laboratories of L. Hood and Ch. Weissmann (Oesch *et al*, 1985). The dominant if not only protein of the prion was a host-encoded protein called prion protein PrP. Because PrP could be isolated from the noninfected host and from the infectious material as a protein of the same sequence and post-translational modification, it was suggested that PrP has to be present in two isoforms: the cellular isoform PrP^{C} in the normal cell, and the pathogenic isoform PrP^{Sc} (Sc from scrapie) as the dominant component of the infectious material. Indeed, structural and biochemical differences were identified. Electron microscopic studies show that PrP^{C} form monomeric or oligomeric bulky particles, but PrP^{Sc} form typically fibrillar aggregates (Figure 2; Riesner *et al*, 1996).

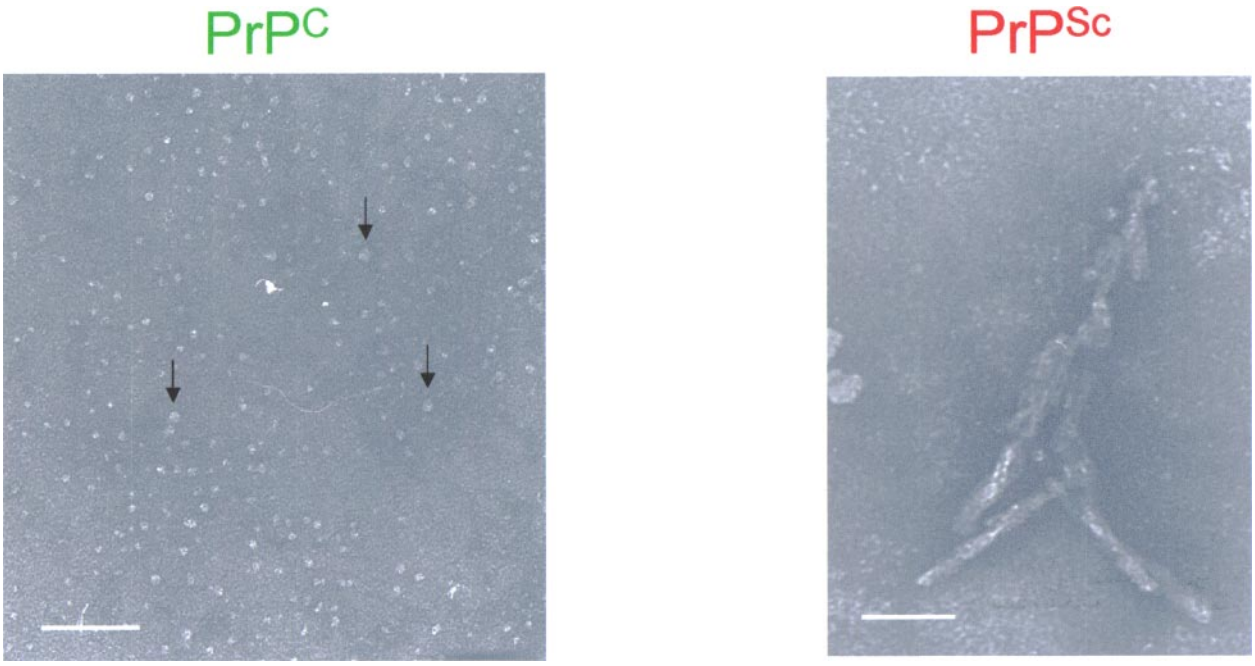

Figure 2

Depending upon the preparation method, diffuse deposits, amyloidic fibres, or condensed plaques are visible. The high tendency to aggregate correlates with a PrP^{Sc}-specific resistance to digestion with proteinase K (PK). In Figure 3, the Western blot of a sodium dodecyl sulfate (SDS)–gel electrophoresis of PrP^{C} and PrP^{Sc}, with and without PK digestion, respectively, is depicted. The characteristic three bands of PrP, i.e., without, with one, and with two glycosyl groups, are visible; they disappear completely after PK digestion of PrP^{C}. On the other hand, the three bands for PrP^{Sc} are still present, although

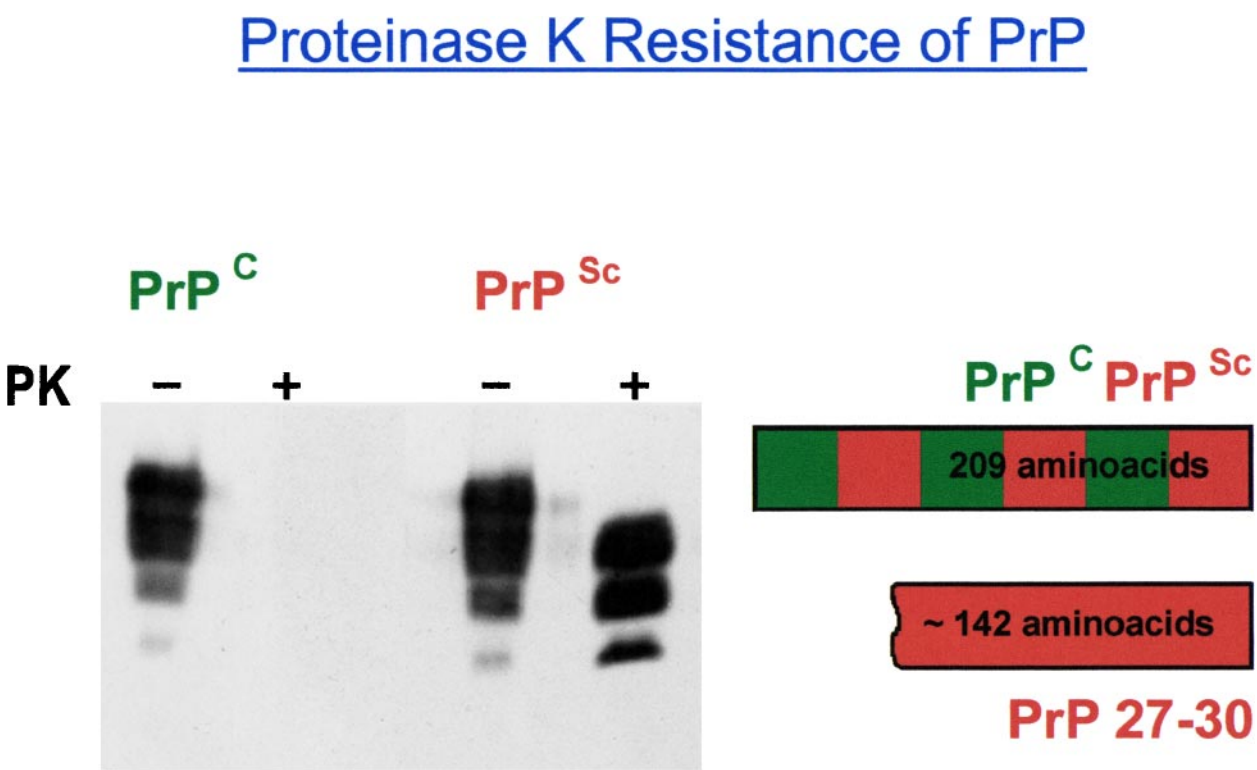

Figure 3

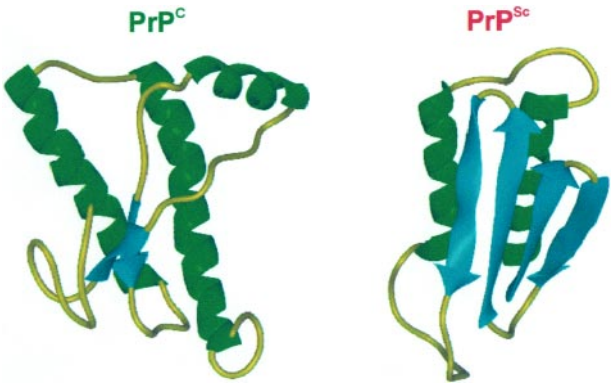

Figure 4

shifted to lower M_r, representing the N-terminally truncated forms of PrP^{Sc}, called PrP 27–30. It is notewortly that PrP 27–30 is fully infectious. Furthermore, it should be mentioned that all commercially available routine tests for BSE and scrapie are based on the PK resistance of PrP 27–30. The molecular structure of PrP^C was elucidated by NMR analysis carried out first by K. Wüthrich's group (Figure 4, *left*; Riek *et al*, 1996). The C-terminal part (amino acids 120–231) assumes a structure with three α-helices and a small β-sheet. For PrP^{Sc}, a structure of comparable accuracy is not known because of the failure of biophysical structure analysis on unsoluble proteins. However, spectroscopic data suggest that at least one α-helix is converted to a β-sheet structure and also the N-terminus is structured, probably due to intermolecular interaction (Figure 4, *right*; Cohen and Prusiner, 1998).

Having in mind the biological and structural features of PrP^C and PrP^{Sc}, a hypothetical model for prion amplification is proposed in Figure 5. PrP^C is expressed from a nuclear gene and presented on the outer membrane. Due to direct or indirect contact of PrP^C with invading PrP^{Sc}, a conformational transition in PrP^C is induced and thereby new PrP^{Sc} is formed. Dissociation of PrP^{Sc} dimer, forming of a heterodimer, and aggregation to PrP^{Sc} multimers are shown in Figure 5 (*right*), but those details should be considered purely hypothetical.

The model is in good accordance—but is not proven—with the finding that transgenic mice missing the PrP gene neither get sick nor can transmit the disease (Bueler *et al*, 1993). The so-called strain phenomenon, however, cannot be explained satisfactorily; different scrapie isolates from sheep can be transmitted to hamster as an experimental animal and exhibit different incubation times and lead to different lesion pattern in the brain (Fraser and Dickinson, 1968). In further passages, characteristic

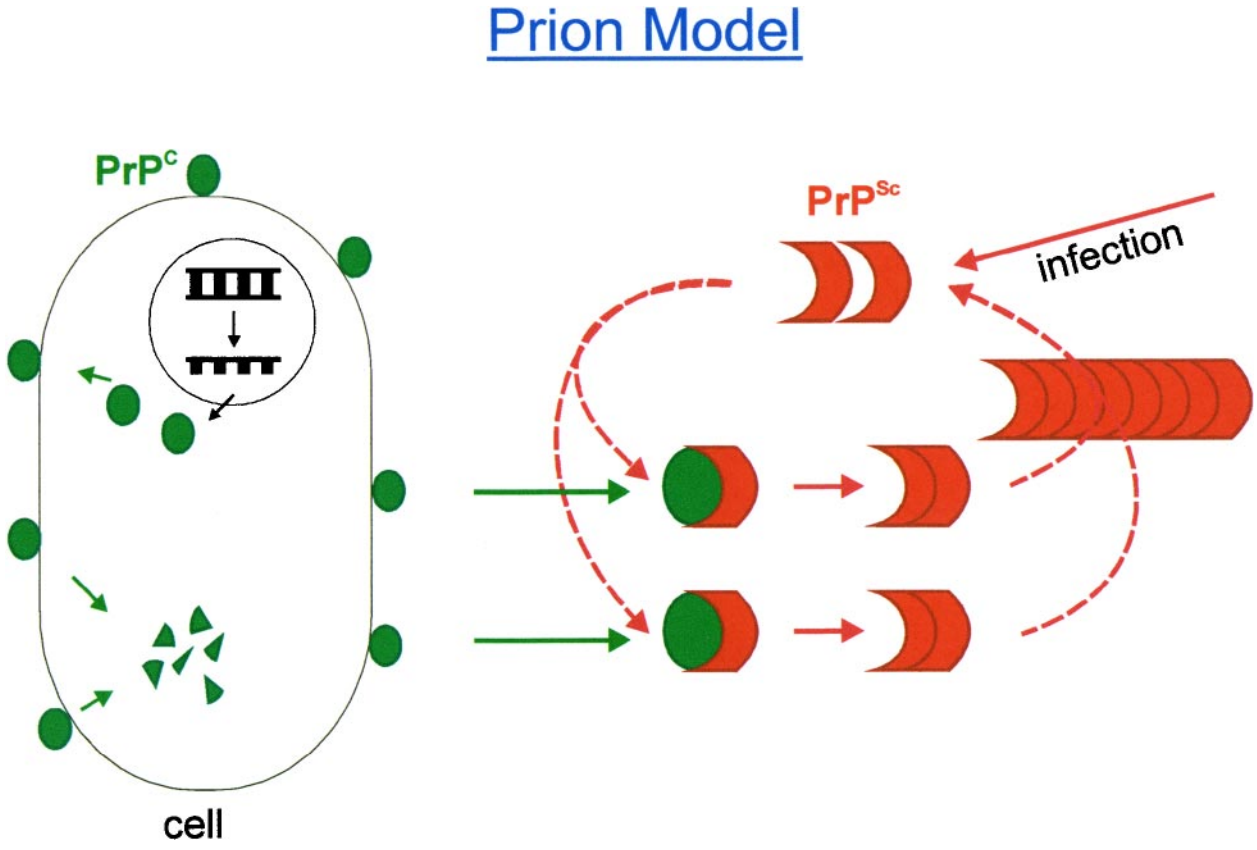

Figure 5

Table 1 Human prion diseases

Manifestation	*Disease*	*Mechanism*
Infectious	Kuru latrogenic CJD	Transmission of PrP^{Sc}
Sporadic	CJD	Somatic mutation or spontaneous $PrP^{C} \rightarrow PrP^{Sc}$
Genetic	GSS Familiar CJD Familiar fatal insomnia	Germline mutation

strain feature are maintained, although the same PrP genotype from the hamster is transmitted. Different phenotypes of the strain with identical PrP sequences and posttranslational modifications are difficult to explain on the basis of the model in Figure 5. The repeated claim of an undiscovered nucleic acid, coding at least for the strain features, could be disproven by a direct and quantitative nucleic acid analysis in which nucleic acids with more than 50 to 100 nucleotides in length could be excluded as being essential for scrapie infectivity (Meyer *et al*, 1991; Riesner, 1992).

It was found during the last 20 years that human prion diseases are manifest as infectious as well as sporadic and genetic diseases. The prion model was able to deliver the molecular basis of the different etiologies of the same disease. In Table 1, the different diseases with their molecular mechanisms are listed. Among the different manifestations, the sporadic cases with around 1 case/1 million per year is the most frequent etiology; several thousand kuru cases and several hundred iatrogenic, i.e., transmitted, cases from contaminated surgical instruments, corea and dura mater transplants, and growth hormone were documented (cf. Prusiner, 1993). The fact that different etiologies can be explained may be regarded as an outstanding achievement of the model.

In vitro conversion and models of prion replication

The interpretation of different etiologies on the basis of the prion model in Figure 5 is a convincing demonstration of the very close vicinity of basic research and medicinal application of prion diseases. Thus, a closer look into the basics of $PrP^{C} \leftrightarrow PrP^{Sc}$ conversion appears attractive. The biophysical studies are difficult because of the insolubility of PrP^{Sc}. In Figure 6, a particular biophysical approach is sketched (Riesner *et al*, 1996; Post *et al*, 1998); the aggregated state represents the PrP^{Sc}-like conformation, the disaggregated state the PrP^{C}-like conformation. Transitions are induced by detergents and the states characterized in terms of size, PK resistance, structure, and infectivity. Among many detergents tested, SDS in low concentrations ($\leq$0.2%) at room temperature was found effective. It represents a membrane-like environment. Thus, the mono- or oligomeric state is present in the membrane-like environment, the aggregated, PrP^{Sc}-like state in the waterphase. Table 2 summarizes the biophysical and biochemical methods that had been applied for the analysis of the conformational transitions.

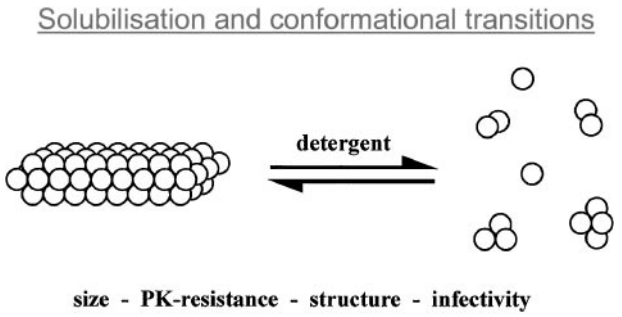

Figure 6

In Figure 7, an example of the biophysical analysis is presented (Jansen *et al*, 2001). The diffusion time as determined by the fluorescence correlation spectroscopy (FCS) and the ellipticity ratio $\theta_{218}/\theta_{207}$ from the circular dichroism were measured as a function of decreasing SDS concentration. Ellipticity ratio provides information on the secondary structure: with an α-helical structure at values around 0.8 (>0.05% SDS) and a β-sheet–rich structure around 2.0 (<0.03% SDS). Thus, a narrow transition from the PrP^{C}-like, α-helical structure into the PrP^{Sc}-like, β-sheet–rich structure was induced when lowering the SDS concentration from 0.04% to 0.02% SDS. Because increasing diffusion time is a measure of increasing aggregation, it can be seen from Figure 7, that drastic aggregation occurs below 0.01% SDS. Consequently the shift in secondary structure could be well separated from forming large insoluble aggregates; in this case, a soluble, β-sheet–rich conformation of hamster PrP (90–231) was established in the range between 0.04% and 0.02% SDS, which is very useful for further structural studies on the β-sheet–rich conformations.

Structural intermediates identified so far are depicted in Figure 8 (Jansen *et al*, 2001). The systematic study was carried out with recombinant PrP (90–231), which has the same sequence as hamster

Table 2 Conformational transitions and aggregation

Methods	*Prion properties*
Gelelectrophoresis	Protein degradation
Spectroscopy	Secondary structure
Differential centrifugation	Solubility
Crosslinking and masspectrometry	Intermolecular interactions
Electronmicroscopy	Structure of aggregates (plaques, fibrilles)
Fluorescence-Correlation Spectroscopy	Mechanism of aggregation
Bioassay	Infectivity

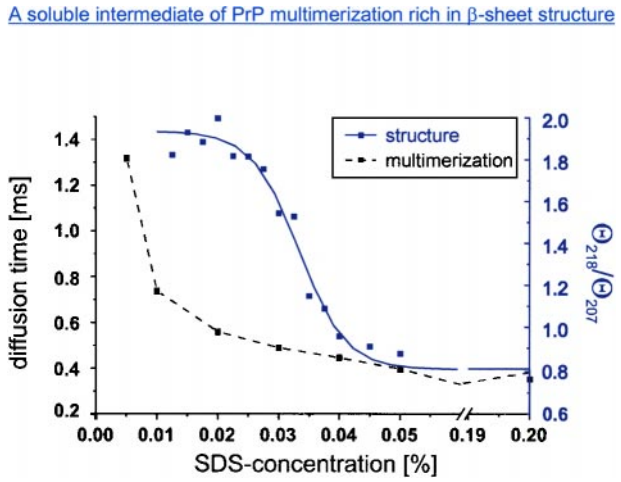

Figure 7

PrP 27–30. A concentration of 0.2% SDS induces a monomeric, partially denatured α-helical structure (α/R). Below 0.1% SDS, renaturation to an α-helical, PrP^{C}-like structure, concommitant with dimerization, was observed. In a cooperative transition, 5 to 10 SDS molecules are released and an oligomeric, PrP^{Sc}-like structure, which is rich in β-sheets, is formed. Further release of SDS leads to large, insoluble, β-sheeted aggregates. From the influence of SDS, it can be concluded that the aggregates of PrP are formed mostly by hydrophobic interactions, because the interacting parts of the surface are covered by SDS in 0.2% SDS and become uncovered step by step from SDS and accessible for PrP-PrP interactions during removal of SDS.

As a summary of the studies reported above, one has to conclude that the properties of β-sheeted structure, insolubility, and PK resistance are found in prions but are not strictly correlated with infectivity. PK resistance could be induced by several methods without acquiring infectivity. PK resistance is correlated with aggregation and aggregation with β-sheet structure, but these features are not sufficient for infectivity. At present, it cannot be decided whether the right conditions for inducing infectivity have not been found; a second, not yet identified component is still missing, or a principal feature of the $PrP^{C} \rightarrow PrP^{Sc}$ transition is not yet understood. It should be noted that a rigid chemical analysis of highly purified prion rods showed small amounts of specific lipids (Klein *et al*, 1998) and significant amounts, i.e., above 10% of a polyglucose scaffold (Appel *et al*, 1999). It is, however, not clear whether these components are essential for infectivity.

The structural transitions are the basis of mechanistic models for prion replication. As depicted in

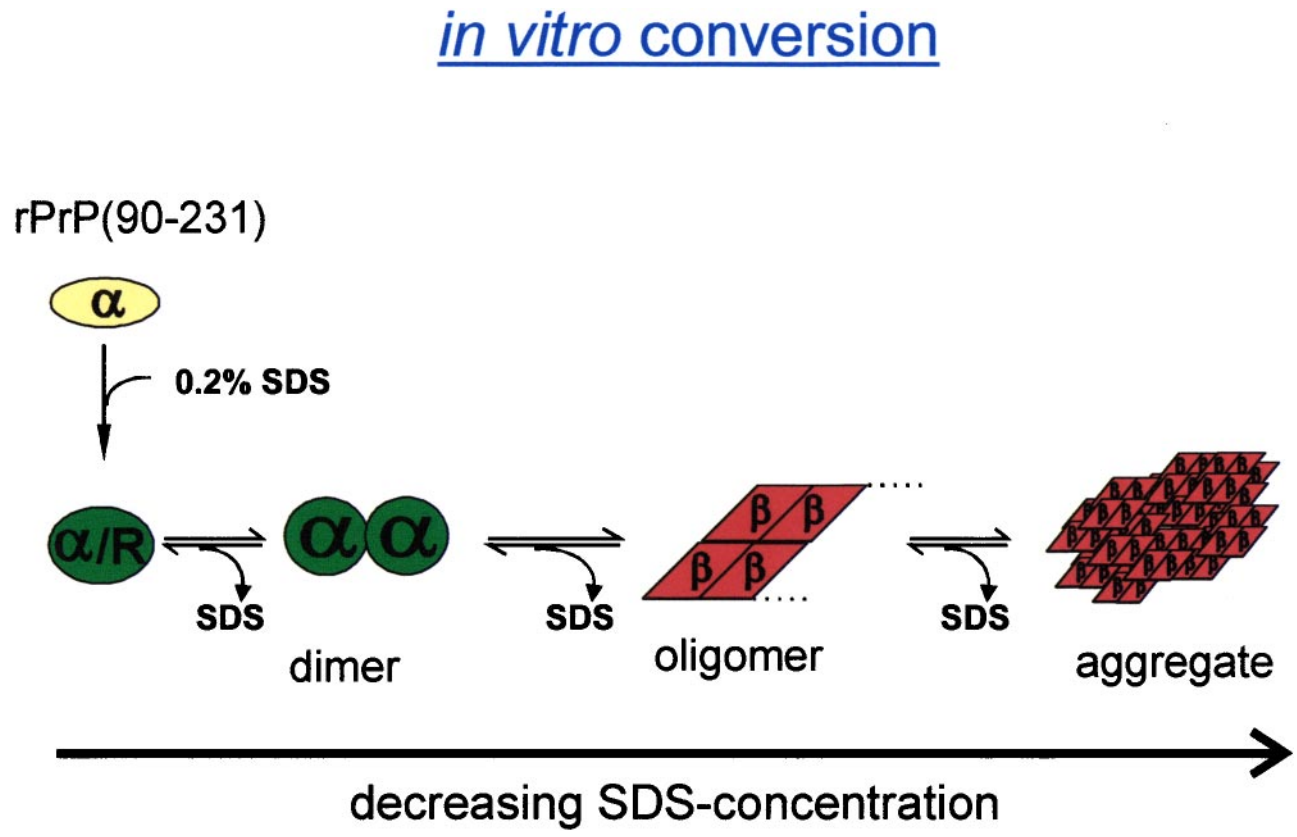

Figure 8

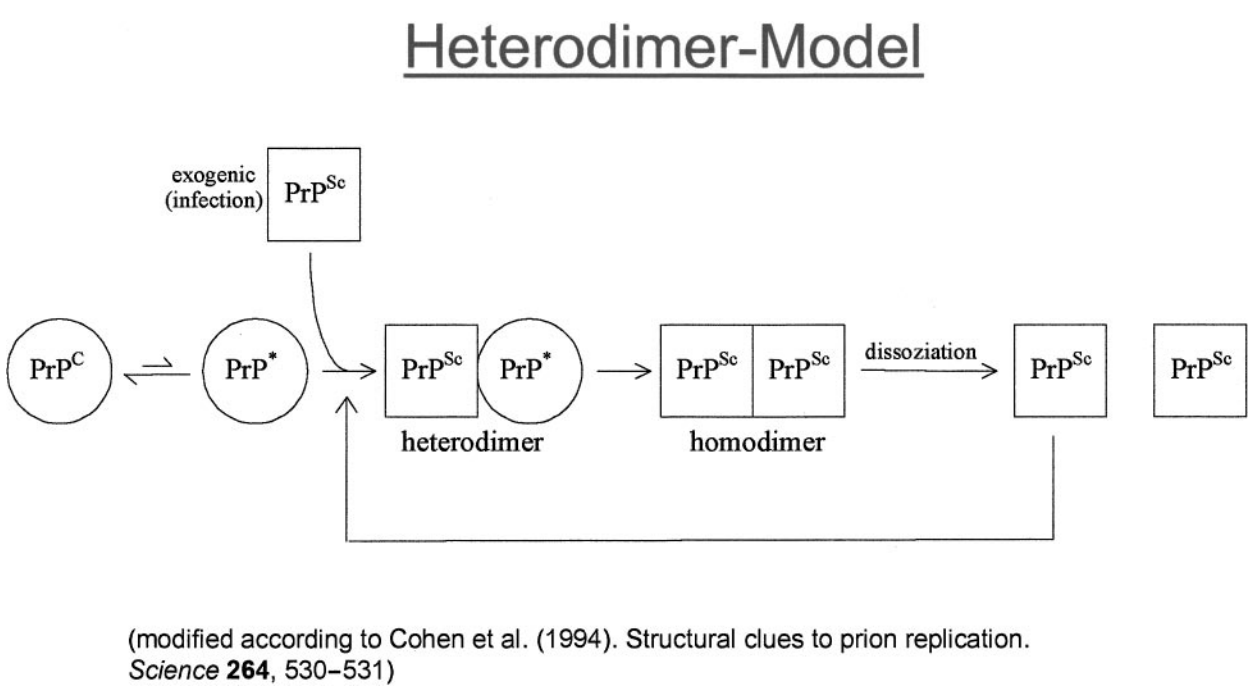

Figure 9

Figure 9, Prusiner and his group proposed the "heterodimer model." A complex of PrP^C and PrP^{Sc} is formed, in which PrP^C is transformed into PrP^{Sc}, similar to the enzymatic mechanism of an induced fit. More recently, the model was specified in that a protein X has to be involved in the complex (Telling *et al*, 1995). Regarding the equilibrium $PrP^C \leftrightarrow PrP^{Sc}$, PrP^{Sc} would be the favored state, otherwise there would be no driving force for the catalytic turnover.

Another mechanism was proposed by Lansbury and his colleagues (Come *et al*, 1993), in which formation of fibrils—as known for actin or β-amyloid and called "linear crystals"—and generation of infectivity are closely connected. The model is depicted in Figure 10. Monomeric PrP^C is in fast equilibrium with a PrP^{Sc}-like conformation, with PrP^C being the favorable state. A number of PrP^{Sc}-like molecules can form aggregates with decreasing concentrations, down to a nucleus of *n* PrP^{Sc}-like molecules. If the nucleus has formed, growth of the aggregates is faster than dissociation, and increasingly larger aggregates will be formed. In that mechanism—and in contrast with the heterodimer model—the first stable aggregate corresponding to a functional PrP^{Sc} will be the nucleus of *n* PrP^{Sc}-like molecules. Both models assume conformation transition of PrP occurs freely in solution.

The so-called two-phase model (Figure 11; Riesner, 2001) takes into account that PrP^C is mormally dispersed due to its glycolipid anchor on the outer cellular suface and thereby is prevented from aggregation. If it is released in sufficient amounts from the surface, then the conformational transitions as depicted in Figure 8 can occur in the water phase. Spontaneous occurrence of PrP^{Sc}-like states in the water phase is possible but improbable because of the low concentration of PrP. If, however, a prion particle as exogenic seed is present, the newly transformed PrP can be bound quickly and stabilized in the β-sheet form, thus leading to an amplification of PrP in the PrP^{Sc} state.

Cellular and pathological functions of PrP

The cellular and pathological functions of PrP might be interrelated closely. Is the pathological function of PrP a loss of the cellular function of PrP^C, in the sense that PrP^C is converted to an inactive form, i.e., PrP^{Sc} aggregates? For this and other purposes, transgenic mice were constructed with the PrP gene ablated, the so-called "PrP-knockout mice"(Bueler *et al*, 1993). They showed normal development and reproduction, demonstrating that PrP^C is not essential for those features. Depending upon the details of the transgenic construct, the knockout mice showed, however, symptoms as age progresses; these symptoms were lowered long-term potentiation (LTP) (Collinge *et al*, 1994), lowered Ca^{2+}-activated K^+ conductivity (Collinge *et al*, 1996), slightly distorted day-night rhythm (Tobler *et al*, 1996), progressive ataxia, and loss of Purkinje cells (Sakaguchi *et al*, 1996). Thus, loss of PrP^C activity might lead to symptoms.

The symptoms and in particular the histopathology is more expressed in nontransgenic, but PrP^{Sc}-infected animals. Cellular dysregulations, such as spongiosis, neuronal loss, astrocytic gliosis, and

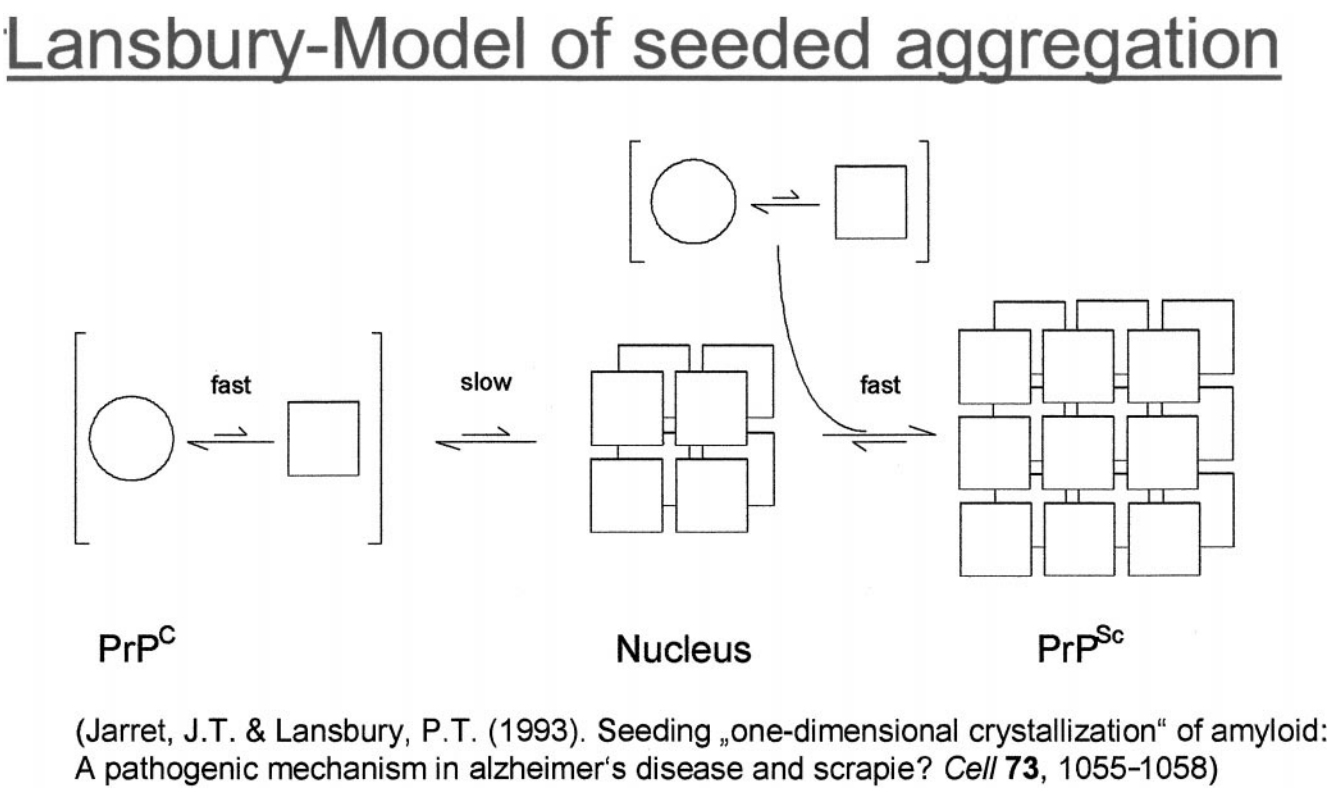

Figure 10

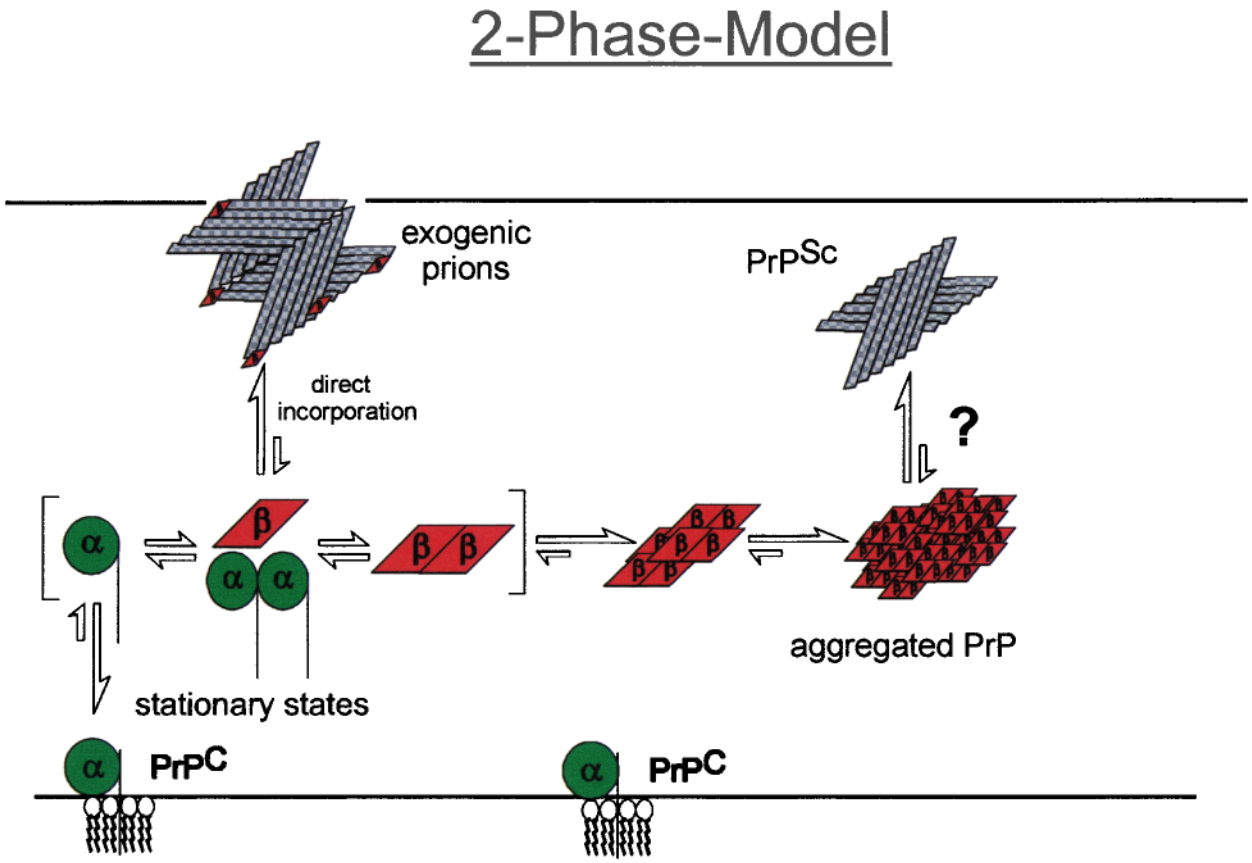

Figure 11

microglia activation, have been found. Deposits, in the form of dense "Kuru" plaques, amyloids, or diffuse depositions, all containing PrP^{Sc}, are characteristic of the disease. Muscle pathology was detected so far only in transgenic mice that overexpressed PrP (for review, see Kretzschmar, 1993). Pathological effects, which appeared in cell cultures, were dependent upon PrP expression. The cell death followed the apoptotic mode. The pathological function of PrP^{Sc} in cultured cells is reversibly dependent upon the structure of PrP^{Sc}.

Recently progress was achieved in elucidating the cellular function of PrP^{C} in the healthy, nontransgenic organism. In the brain, PrP^{C} is located predominantly in the synaptic membrane. Because PrP can bind cooperatively five to six Cu^{2+} ions, it was suggested that PrP^{C} might regulate the Cu^{2+} concentration in the synaptic cleft (Brown *et al*, 1997). Furthermore, binding to the laminin receptor (Gauczynski *et al*, 2001) and Src kinase (Mouillet-Richard *et al*, 2000) was reported, inferring an involvement in signal transduction, and similarities to cell adhesion proteins such as N-CAM suggested a role in neural plasticity (Schmitt-Ulms *et al*, 2001).

New developments in diagnosis and therapy

Much research concentrates presently on diagnosis and therapy of prion diseases. Diagnosis is required particularly for animal diseases such as BSE and scrapie in order to ensure safety of food products and to eradicate the diseases in the future. Therapy, on the other hand, is of utmost importance for the human diseases. Diagnosis of human prion disease will become more important as soon as first therapeutic successes are available. Quite different methods are available or at present under development for diagnosis:

Established methods, which are available as diagnostic kits, are based on the PK resistance of PrP^{Sc}. Their sensitivity is limited because they detect only the PK-resistant material, which is possibly a late form during the disease.

Surrogate markers are cellular products, the expression of which is up- or down-regulated in close correlation with the disease. Under development are tests based on 14.3.3 protein, PrP^{C}, laminin receptor, γ-interferon, erythroid factor and RNA profiling. Sensitivity and specificity can be tested only empirically in large experimental series of cases.

Test for protein-aggregates have attracted special attention recently. They are based on the presence of PrP^{Sc} aggregates but without the PK resistence as a prerequisite, so that hopefully an earlier form of infectious material might be detected. The so-called conformation-dependent immunoassay (CDI), the analysis by fluorescence correlation spectrocopy (FCS), and the test by binding of specific ligands, should be mentioned. CDI and FCS are most advanced (Pitschke *et al*, 1998; Safar *et al*, 1998; Bieschke *et al*, 2000).

Amplification procedures have been proposed recently for improving the sensitivity (Saborio *et al*, 2001; Eigen, 2001). In a cyclic mode, PrP^{C} is added to PrP^{Sc}, transformed under particular conditions into PrP^{Sc}, the newly formed aggregates broken by ultrasound, and the addition of PrP^{C} in the next round leads to a further amplification of the original PrP^{Sc}. The method is not yet ready for routine application.

Bioassays for BSE utilize transgenic mice with several bovine PrP genes on mouse PrP-knockout background; minimal incubation times around 200 days were achieved (Scott *et al*, 2000).

Prevention of prion diseases could interfere with uptake and multiplication of prions at quite different steps. In Figure 12, the steps that are known at present are shown; it does not mean, however, that the chain in Figure 12 is complete. An effective therapy has to interfere with prion formation in the brain or even revert the $PrP^{C} \rightarrow PrP^{Sc}$ transition. Because *in vitro* conversion systems are available, chemical compounds as well as specific antibodies are tested presently for their ability to prevent or revert the formation of PrP^{Sc}. So far, quinacrine was the most promising compound, which was applied even to human patients (Korth *et al*, 2001).

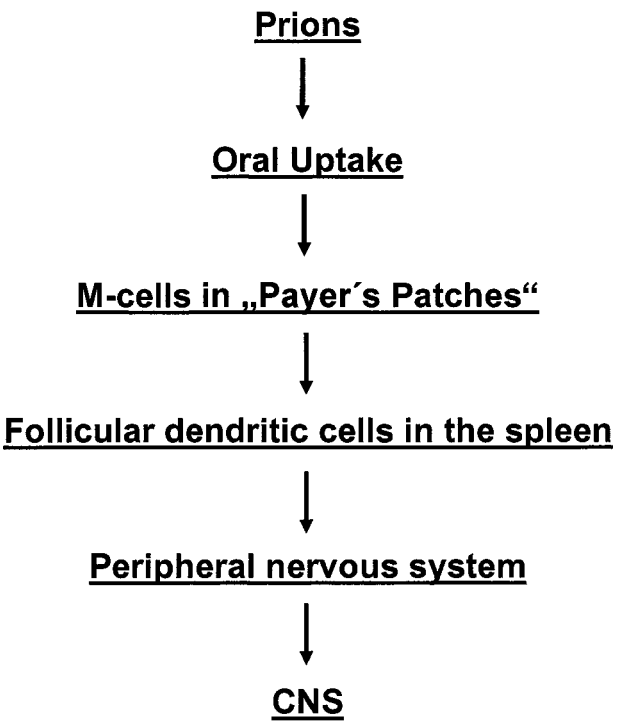

Figure 12

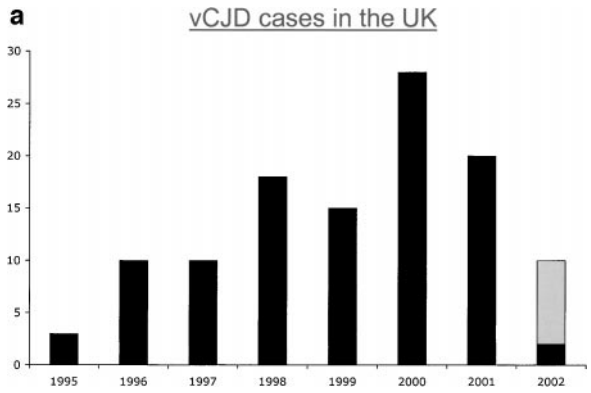

Figure 13a

Variant CJD: human variant of BSE?

The appearance of the variant form of CJD (vCJD) in 1996 was the first indication that humans can be afflicted by BSE. In the meantime, the evidence for the transmissibility of BSE to humans is overwhelming. The experimental evidence reaches from the epidemiology to the biochemical properties of the infectious material.

Epidemiology. Except for one case in Eire and three cases in France, all cases were located in the UK where the BSE epidemic was dominant.

Symptoms. The symptoms of vCJD are clearly different from those of the CJD known so far (also called conventional CJD), indicating a new disease. The most obvious difference is that all patients of vCJD are young, i.e., below or around 30, whereas the known form of CJD afflicted humans of an average age over 60 years.

Transmission. BSE could be transmitted experimentally to the primates maquaques, and the prion plaques in their brain were of florid shape, very similar to those in the brains of vCJD victims.

Lesion pattern. BSE, vCJD, and conventional CJD was transmitted to mice. The lesion pattern in the brain of the mice was very similar, when infected with BSE or vCJD, but clearly different from those infected with conventional CJD.

Biochemical properties. PrP^{Sc}, prepared either from BSE or vCJD cases, exhibited similar or idential cleavage sites after PK digestion and similar distribution of PrP molecules without, with one, or with two glocosylgroups, but the corresponding properties of PrP^{Sc} from conventional CJD are clearly different. Also, a particular genetic disposition seems to be needed for vCJD. All patients were homozygous Met/Met at codon 129, but only 35% of the human population are Met/Met homozygous. Conventional CJD is not restricted to this homozygosity.

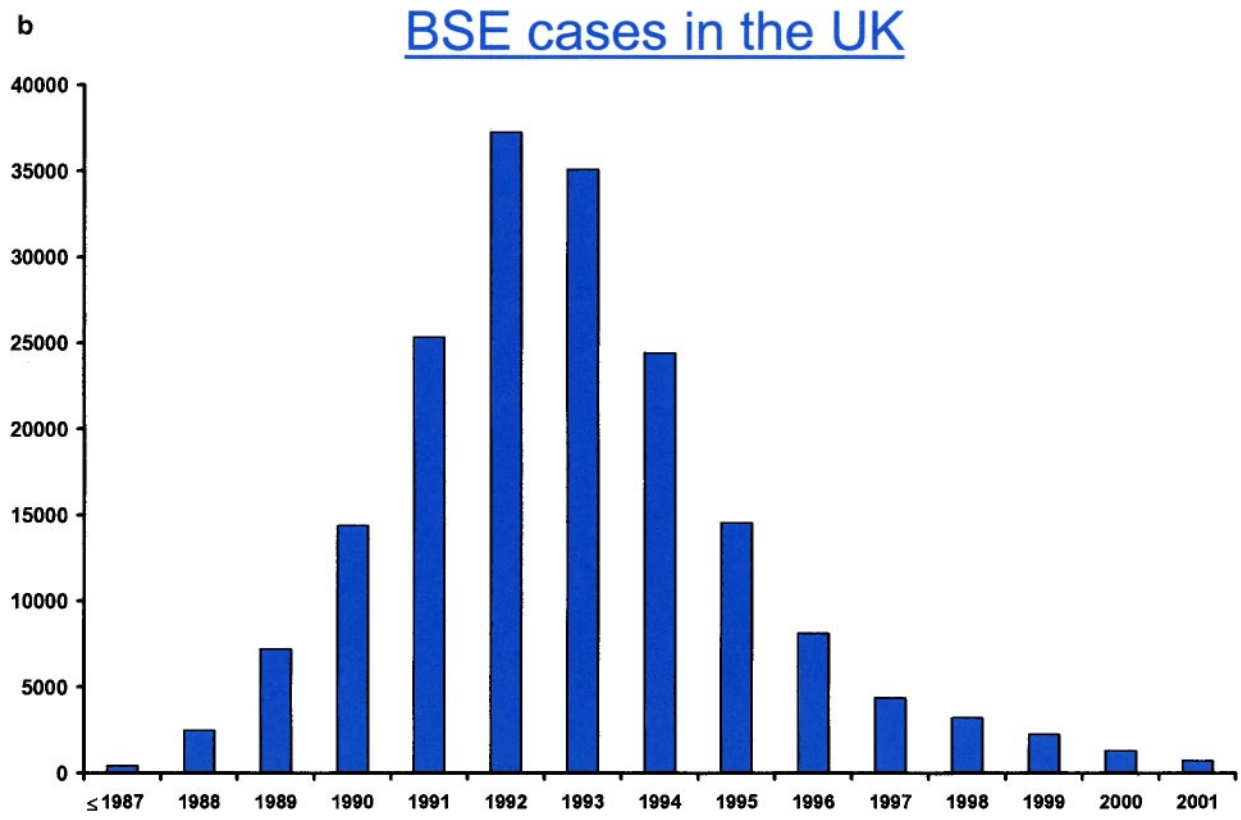

Figure 13b

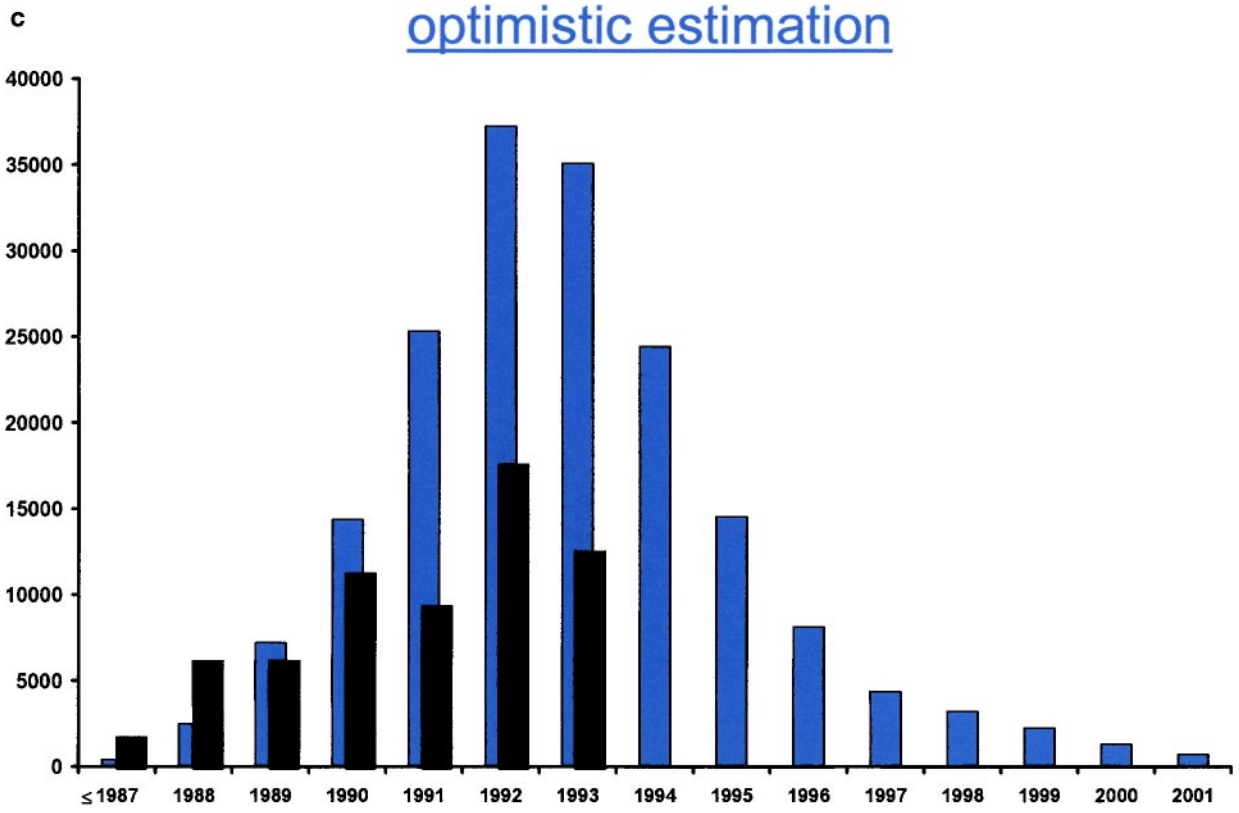

Figure 13c

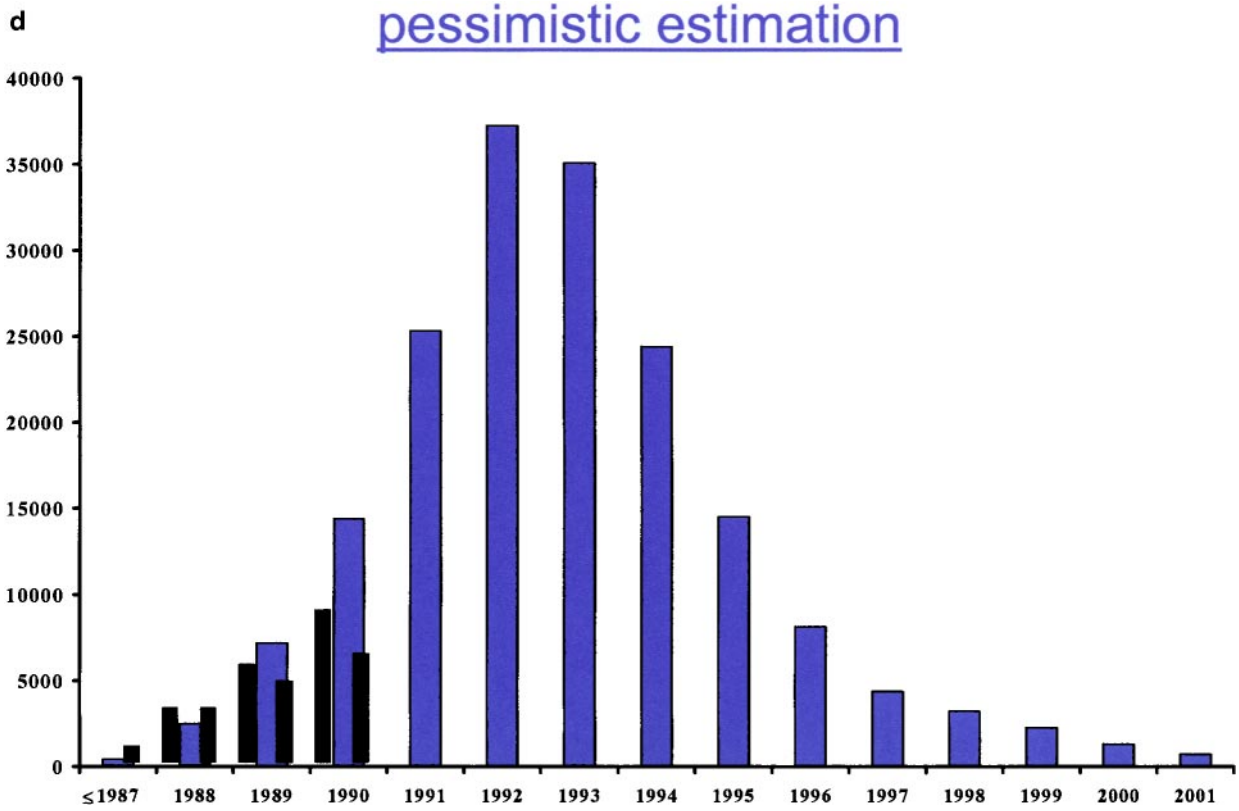

Figure 13d

An urgent question is the forecast of the human vCJD cases. In Figure 13, the numbers of vCJD-cases per year (**a**) and those of BSE (**b**) are presented, respectively. Whereas the BSE epidemic in the UK is in its final stage, a forecast for the vCJD case is very difficult. The numbers were increasing up to the year 2000; within the statistical error it cannot be stated whether they stay nearly constant, level off already, or would increase again. If the vCJD cases are projected into the BSE epidemic (Figure 13**c**), one might come to the optimistic conclusion that the peak has been reached already. But this might be too optimistic because not much is known about the width of the vCJD curve. More pessimistically, one might assume that the width is double that of the BSE curve, because the incubation time is much longer and more heterogeneous. In that case, the forecest might be estimated as in Figure 13**d**. In summary, an optimistic estimation would lead to a total number of vCJD cases between 200 and 300, whereas a pessimistic estimation would place those between 1000 and 3000. In any case, estimations of several hundred thousand cases as given a few years ago are not realistic on the basis of the present numbers.

References

Alper T, Haig DA, Clarke MC (1966). The exceptionally small size of the scrapie agent. *Biochem Biophys Res Commun* **22:** 278–284.

Appel TR, Dumpitak C, Matthiesen U, Riesner D (1999). Prion rods contain an inert polysaccharide scaffold. *Biol Chem* **380:** 1295–1306.

Bieschke J, Giese A, Schulz-Schaeffer W, Zerr I, Poser S, Eigen M, Kretzschmar H (2000). Ultrasensitive detection of pathological prion protein aggregates by dual-color scanning for intensely fluorescent targets. *Proc Natl Acad Sci USA* **97:** 5468–5473.

Brown DR, Qin K, Herms JW, Madlung A, Manson J, Strome R, Fraser PE, Kruck T, von Bohlen A, Schulz-Schaeffer W, Giese A, Westaway D, Kretzschmar H (1997). The cellular prion protein binds copper in vivo. *Nature* **390:** 684–687.

Bueler H, Aguzzi A, Sailer A, Greiner RA, Autenried P, Aguet M, Weissmann C (1993). Mice devoid of PrP are resistant to scrapie. *Cell* **73:** 1339–1347.

Cohen FE, Prusiner SB (1998). Pathologic conformations of prion proteins. *Annu Rev Biochem* **67:** 793–819.

Collinge SB, Collinge J, Jefferys JG (1996). Hippocampal slices from prion protein null mice: disrupted Ca(2+)-activated K+ currents. *Neurosci Lett* **209:** 49–52.

Collinge J, Whittington MA, Sidle KC, Smith CJ, Palmer MS, Clarke AR, Jefferys JG (1994). Prion protein is necessary for normal synaptic function. *Nature* **370:** 295–297.

Come JH, Fraser PE, Lansbury PT Jr (1993). A kinetic model for amyloid formation in the prion diseases: importance of seeding. *Proc Natl Acad Sci USA* **90:** 5959–5963.

Creutzfeldt HG (1920). Über eine eigenartige herdförmige Erkrankung des Zentralnervensystems. *Z Gesamte Neurol Psychiatrie* **57:** 1–18.

Cuillé (1938). La tremblante du mounton est bien inoculable. *Acad Sci Paris* **206:** 78–79.

Eigen M (2001). BSE und das Prionen Problem. *Spektrum der Wissenschaften* **April:**

Fraser H, Dickinson AG (1968). The sequential development of the brain lesion of scrapie in three strains of mice. *J Comp Pathol* **78:** 301–311.

Gajdusek DC (1977). Unconventional viruses and the origin and disappearance of kuru. *Science* **197:** 943–960.

Gauczynski S, Peyrin JM, Haik S, Leucht C, Hundt C, Rieger R, Krasemann S, Deslys JP, Dormont D, Lasmezas CI, Weiss S (2001). The 37-kDa/67-kDa laminin receptor acts as the cell-surface receptor for the cellular prion protein. *EMBO J* **20:** 5863–5875.

Hadlow WJ (1959). Scrapie and kuru. *Lancet* **ii:** 289–290.

Jakob A (1921). Über eigenartige Erkrankungen des Zentralnervensystems mit bemerkenswerten anatomischen Befunden (spastische Pseudosklerose—Encephalomyelopathie mit disseminierten Degenerationsherden). *Z Gesamte Neurol Psychiatrie* **64:** 147–228.

Jansen K, Schafer O, Birkmann E, Post K, Serban H, Prusiner SB, Riesner D (2001). Structural intermediates in the putative pathway from the cellular prion protein to the pathogenic form. *Biol Chem* **382:** 683–691.

Klein TR, Kirsch D, Kaufmann R, Riesner D (1998). Prion rods contain small amounts of two host sphingolipids as revealed by thin-layer chromatography and mass spectrometry. *Biol Chem* **379:** 655–666.

Korth C, May BC, Cohen FE, Prusiner SB (2001). Acridine and phenothiazine derivatives as pharmacotherapeutics for prion disease. *Proc Natl Acad Sci USA* **98:** 9836–9841.

Kretzschmar HA (1993). Neuropathology of human prion diseases (spongiform encephalopathies). *Dev Biol Stand* **80:** 71–90.

Leopoldt JG (1759). Nützliche und auf die Erfahrung gegründete Einleitung zur Landwirtschaft **5:** 344–360.

Meyer N, Rosenbaum V, Schmidt B, Gilles K, Mirenda C, Groth D, Prusiner SB, Riesner D (1991). Search for a putative scrapie genome in purified prion fractions reveals a paucity of nucleic acids. *J Gen Virol* **72(Pt 1):** 37–49.

Mouillet-Richard S, Ermonval M, Chebassier C, Laplanche JL, Lehmann S, Launay JM, Kellermann O (2000). Signal transduction through prion protein. *Science* **289:** 1925–1928.

Oesch B, Westaway D, Walchli M, McKinley MP, Kent SB, Aebersold R, Barry RA, Tempst P, Teplow DB, Hood LE (1985). A cellular gene encodes scrapie PrP 27–30 protein. *Cell* **40:** 735–746.

Pitschke M, Prior R, Haupt M, Riesner D (1998). Detection of single amyloid beta-protein aggregates in the cerebrospinal fluid of Alzheimer's patients by fluorescence correlation spectroscopy. *Nat Med* **4:** 832–834.

Post K, Pitschke M, Schafer O, Wille H, Appel TR, Kirsch D, Mehlhorn I, Serban H, Prusiner SB, Riesner D (1998). Rapid acquisition of beta-sheet structure in the prion protein prior to multimer formation. *Biol Chem* **379:** 1307–1317.

Prusiner SB (1982). Novel proteinaceous infectious particles cause scrapie. *Science* **216:** 136–144.

Prusiner SB (1993). Genetic and infectious prion diseases. *Arch Neurol* **50:** 1129–1153.

Riek R, Hornemann S, Wider G, Billeter M, Glockshuber R, Wuthrich K (1996). NMR structure of the mouse prion protein domain PrP(121–321). *Nature* **382:** 180–182.

Riesner D (1992). The search for a nucleic acid component to scrapie infectivity. *SemiVirol* **2:** 215–226.

Riesner D (2001). Die Scrapie-Isoform des Prion-Proteins PrP^{Sc} im Vergleich zur zellulären Isoform PrP^{C}. In: *Prionen und Prionkrankheiten*, Hörnliman B, Riesner D (eds). De Gruyter: Berlin, pp 81–91.

Riesner D, Kellings K, Post K, Wille H, Serban H, Groth D, Baldwin MA, Prusiner SB (1996). Disruption of prion rods generates 10-nm spherical particles having high alpha-helical content and lacking scrapie infectivity. *J Virol* **70:** 1714–1722.

Saborio GP, Permanne B, Soto C (2001). Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. *Nature* **411:** 810–813.

Safar J, Wille H, Itri V, Groth D, Serban H, Torchia M, Cohen FE, Prusiner SB (1998). Eight prion strains have PrP(Sc) molecules with different conformations. *Nat Med* **4:** 1157–1165.

Sakaguchi S, Katamine S, Nishida N, Moriuchi R, Shigematsu K, Sugimoto T, Nakatani A, Kataoka Y, Houtani T, Shirabe S, Okada H, Hasegawa S, Miyamoto T, Noda T (1996). Loss of cerebellar Purkinje cells in aged mice homozygous for a disrupted PrP gene. *Nature* **380:** 528–531.

Schmitt-Ulms G, Legname G, Baldwin MA, Ball HL, Bradon N, Bosque PJ, Crossin KL, Edelman GM, DeArmond SJ, Cohen FE, Prusiner SB (2001). Binding of neural cell adhesion molecules (N-CAMs) to the cellular prion protein. *J Mol Biol* **314:** 1209–1225.

Scott MR, Supattapone S, Nguyen HO, DeArmond SJ, Prusiner SB (2000). Transgenic models of prion disease. *Arch Virol Suppl* 113–124.

Telling GC, Scott M, Mastrianni J, Gabizon R, Torchia M, Cohen FE, DeArmond SJ, Prusiner SB (1995). Prion propagation in mice expressing human and chimeric PrP transgenes implicates the interaction of cellular PrP with another protein. *Cell* **83:** 79–90.

Tobler I, Gaus SE, Deboer T, Achermann P, Fischer M, Rulicke T, Moser M, Oesch B, McBride PA, Manson JC (1996). Altered circadian activity rhythms and sleep in mice devoid of prion protein. *Nature* **380:** 639–642.

Wells GA, Scott AC, Johnson CT, Gunning RF, Hancock RD, Jeffrey M, Dawson M, Bradley R (1987). A novel progressive spongiform encephalopathy in cattle. *Vet Rec* **121:** 419–420.

Zigas V (1990). Laughing death—the untold story of kuru. Human Press: Clifton, NJ.