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Prion Diseases: a dual view of the prion hypothesis as seen from a distance

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This paper is dedicated to Dr D. Carleton Gajdusek on ocacsion of his 80th birthday.

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Abstract. We review the historical background and principles of the prion theory in its current shape. We showed that most of data may be still interpreted dually according to the protein only hypothesis and according to the theory in which additional component is necessary to comprise the infectivity. The enormous impact of structural biological studies is also stressed.

Key words: prion theory, structural biology

INTRODUCTION

The transmissible spongiform encephalopathies (TSEs) or prion diseases are a group of neurodegenerative disorders which include kuru (Fig. 1) (Gajdusek et al. 1996), Creutzfeldt-Jakob disease (CJD) (Gibbs et al. 1968), Gerstmann-Sträussler-Scheinker (GSS) disease (Masters et al. 1981), and fatal familial insomnia (Medori et al. 1992 a, b) in man, natural scrapie in sheep (Fig. 2), goats (Cuille and Chelle 1938, Dickinson 1976) and mufflons (Wood et al. 1991), transmissible mink encephalopathy in ranch-reared mink (Burger and Hartsought 1964), chronic wasting disease of mule deer and elk in the USA (Williams and Young 1980, 1982, Liberski et al. 2002, Williams and Miller 2002, Haigh et al. 2002), bovine spongiform encephalopathy or "mad cow disease" (Wells et al. 1987) and its analogues in several exotic species of antelopes (Jeffrey and Wells 1988, Fletwood and Furley 1990, Kirkwood et al. 1992, Cunningham et al. 1993), wild felids in Zoological gardens (Willoughby et al. 1992) and feline spongiform encephalopathy in domestic cats (Wyatt et al. 1990).

These disorders are caused by a still not completely understood pathogen variously referred to as a "prion" (predominantly), "virus" (now used infrequently and usually with adjectives: slow, unconventional or atypical), "agent", or "virino". Despite wide acceptance for the prion theory and its vindication by a Nobel prize these names still reflect different views on the molecular structure of the pathogen and, by the same token, our ignorance of its nature. The vast majority who prefer to view this pathogen as composed "predominantly or exclusively" of one pathologically folded protein, PrP, use the term "prion" (Prusiner et al. 1983, Prusiner 1984, 1987, 1991, 1998); hence the term "prion diseases".

Fig. 1. A kuru victim who had died a few hours earlier in good nutritional state; here chronic debility is indicated by deep decubitus ulcer below her right hip. She is mourned by her mother. Men and initiated boys rarely participated in the mourning rite around the corpse, and yet more rarely in dissection and preparation of the kuru victim's flesh for ritual cannibalistic consumption. Courtesy of Dr D. Carleton Gajdusek, Paris, France.

Fig. 2. A typical view of scrapie-affected sheep. Courtesy of Dr Richard H. Kimberlin, SARDAS, Edinburgh, Scotland.

The "virino" hypothesis suggests that the pathogen is a molecular chimera composed of a still-to-be-discovered nucleic acid and a shell-protein which is host-encoded (maybe even PrP) (Kimberlin 1982, 1984, 1990, Kimberlin and Hope 1987). The virus hypothesis simply suggests that the pathogen is a yet-to-be-identified unconventional virus (Diringer et al. 1991, Diringer 1993). The "unified theory" of Weissmann (Weissmann 1989, 1991), not totally unlike the virino theory, suggests that the agent is a molecular chimera in which PrP^{sc} confers infectivity and an unidentified oligonucleotide specifies strain characteristics.

HISTORICAL BACKGROUND

Scrapie, a disease of sheep and goats has been known under several names for some 200 years ("rubbers", "rickets", "goggles", "shakings" "shrewcroft" in England, "scratchie", "cuddie trot" in Scotland, "der Trab", "der Traberkrankheit" lub "die Zitterkrankheit" in Germany, "la maladie convulsive", "la maladie follie", "le tremblante" "la prurigo lombaire" in France and "trzęsawka" in Poland). One of the earliest scientific re-

ports on scrapie had been published in the "Agricultural Improvement Society at Bath" (later changed to "Bath and West Society") (anonymous, 1788) and, as a paragraph in the "General View of the Agriculture of Wiltshire" published by Thomas Davies in 1811 (M'Gowan 1914). In 1848, Roche-Lubin claimed that scrapie is caused by sexual overactivity of rams or, alternatively, by the thunderstorms. M'Gowan (1914) himself suggested *sarcosporidium* as the causative agent.

The first who believed that scrapie ("tremblante") is a virus disease was Besnoit in 1899 while the transmissible nature of TSEs had been proved in late thirties by seminal experiments of Cuile and Chelle (1933, 1936, 1938a,b,c, 1939). The contention that scrapie is an infectious disease caused by a filterable agent was accepted with a long-lasting scepticism. In 1938, W.S. Gordon, a deputy director of the famous Moredun Institute in Edinburgh, Scotland, repeated experiments of Cuille and Chelle using 697 animals of which some 200 developed scrapie (Gordon 1966, Pattison 1993). The infectious nature of the scrapie agent was confirmed inadvarently in 1935, when some 7% of 18,000 sheep vaccinated against Louping ill developed scrapie (Gordon et al. 1939, Gordon 1946). Parenthetically, this vaccine was produced from formalin-fixed sheep brains and the findings proved that scrapie infectivity could survive 0.35% of formalin for more than 3 months. The World War II interrupted scrapie research which had been continued practically only by D.R. Wilson (1950). Wilson's research remained largely unpublished as he was reluctant to present data on such an unorthodox pathogen, but the scrapie community had been well aware on unusual properties of the scrapie agent, in particular, its high resistance to formalin and high temperature.

Scrapie was transmitted from sheep to mice by Morris and Gajdusek (1963) and from goats by Chandler (1961, 1963 a,b,c). This enabled a wide-scale laboratory research and the production of many whimsical hypotheses with an average frequency approaching one a year or two. Thus, the infectious agent had been claimed to be a self-replicating membrane (Hunter and Millson 1966, Gibbons and Hunter 1967, Hunter et al. 1968, 1976, Alper 1993) or a subvirus (not well envisaged) linked to a membrane with a "linkage substance" (Adams 1970, Adams and Bell 1976), a viroid (Diener 1972, 1987, Marsh et al. 1974, 1978 a,b, Marsh 1977, Malone et al. 1978, 1979, Diener et al. 1982), a spiroplasma (Bastian 1987) or a retrovirus-like element (Akowitz et al. 1990, 1993, 1994, Taruscio and Manuelidis 1991, Sklaviadis et al. 1992, Manuelidis 1994). Suffice is to say that none of these hypotheses could be subsequently substantiated despite an exhaustive use of all methods of both classical and molecular virology.

The first TSE in humans was kuru (Fig. 1, 3) discovered by Zigas and Gajdusek (1957). The elucidation of Kuru opened a new field in human medicine and initiated 40 years of research which contributed enormously to our understanding of neurodegenerative disorders of the central nervous system including Alzheimer's disease (Lansbury and Caughey 1995, Gajdusek 1996, Liberski and Gajdusek 1997).

Kuru in the Fore language means to shiver or to shake from fever and cold. The Fore used the noun of the Kuru-verb to describe the always fatal disease which principally affected their children (Fig. 1, 3) and adult women. It has been and still is restricted to natives of the

Fig. 3. A preadolescent child, totally incapacitated by kuru in 1957. The child had such severe dysarthria that he could no longer communicated by word, but he was still inteligent and alert. He had spastic strabismus. He could not stand, sit without support, or even roll over; he had been ill for less than six months, and died within a few months of the time of photography. Courtesy of Dr D. Carleton Gajdusek, Paris, France.

Fore linguistic group at Papua New Guinea's Eastern Highlands and those neighbouring linguistic groups which intermarry with Fore.

Ritualistic endocannibalism (eating of the relatives as a part of a mourning ritual but not as an alimentary habit) was practised not only in the Kuru area but in many surrounding Eastern Highland groups which never developed Kuru. In the late 1930's and 1940's, many gold miners, Protestant missionaries, and government officials made contacts with the northern periphery of the Kuru region, and they and later anthropologists became thoroughly familiar with the ritual endocannibalism of Eastern Highland peoples. When the fatal epidemic in the Kuru region was announced, most of the local Caucasians made the obvious assumption it must be spread by the cannibalism practices.

An alternative explanation, favoured by the native population, was that Kuru resulted from sorcery (Lindenbaum 1979). To cause Kuru in a victim, a would-be-sorcerer must obtain a part of the victim's body (nail clippings, hair) or excreta, particularly feces or urine soaked vegetation, saliva, blood, or partially consumed food such as sweet potato peelings, or clothing. These are packed with leaves and made into a "Kuru bundle" and placed into a swamp. Subsequently, the sorcerer shakes the package daily until the sympathetic Kuru tremor is induced in his victim. As a result, kinsmen of a Kuru victim attempted to identify and subsequently kill a suspected sorcerer if they could not bribe or intimidate him to release a victim from a power of Kuru spell. As sorcerers were mostly adult men while Kuru victims were mostly women and children of both sexes, killing of male sorcerers, identified by rites of divination, contributed somewhat to maintaining of sex ratio in the population devastated by the Kuru deaths of their women.

An infectious (transmissible) aetiology, always suspect, was nevertheless not supported by clinical, laboratory, or post-mortem findings (absence of fever, cerebrospinal fluid pleocytosis or elevated protein level, and on autopsy no perivascular cuffings in the brains). However, neither the diverse genetic study nor the search for an environmental toxins resulted in a tenable hypothesis. Attempts to transmit Kuru to small laboratory animals or to isolate a causative micro-organism using tissue cultures or embryonated hen eggs were similarly unsuccessful.

In 1959, Gajdusek, then back in the New Guinea bush, received a letter from the veterinarian William Hadlow (1959), which pointed out the analogies between Kuru and scrapie, a slow neurodegenerative disease of sheep known in United Kingdom since the XVIIIth century and, as already mentioned, experimentally transmitted by the French in 1936. Hadlow enclosed a typescript of a letter to the editor of Lancet (Hadlow 1959). Gajdusek replied that the infectious aetiology of Kuru was being reconsidered; in view of chronic cerebral infections like Iin syphilis, toxoplasmosis, and trypanosomiasis, inoculated laboratory rodents and monkeys were being held for longer observation periods than had been carried out in 1957, and that he was also attempting to obtain better inoculum in the form of autopsied brain tissue (a letter from D.C. Gajdusek dated July 28th, 1959, Gajdusek 1993). Kuru was finally transmitted to chimpanzees in 1965 (Gajdusek et al. 1966), followed by transmission of Creutzfeldt-Jakob disease (Gibbs et al. 1968) and GSS (Masters et al. 1981). The list was apparently closed by a transmission of fatal familial insomnia (Collinge et al. 1995, Tateishi et al. 1995).

Then, in 1987, Gerald A.H.Wells and his colleagues from Central Veterinary Laboratory described a single cow with a novel form of TSE (1987), (parenthetically, the first case of what turned out to be BSE had been reported in a nyala by Jeffrey et al. (1988). The BSE epidemic reached a climax in 1992 with more than 35,000 cases and then their number steadily declined to 1,443 cows in 2000, 1,137 in 2001 and, finally, 438 in 2002 (see, Bradley, this volume). However, in 1996 Will et al. reported on a new variant of CJD (vCJD), most probably resulting from BSE transmission to humans. This epidemiological conjecture was subsequently substantiated by laboratory studies (Collinge et al. 1996, Bruce et al. 1997, Hill et al. 1997, Scott 1999). The number of vCJD cases is increasing and discussion about the extent of the outbreak has become disturbing. On the other hand, the BSE epidemic and appearance of vCJD in humans has accelerated TSE research and changed it from a rather small obscure field into a major scientific endeavour.

PrP, ITS GENE, THE "PRION HYPOTHESIS" AND THE STRAINS OF THE PATHOGEN

PrP (prion protein) is a highly conserved sialoglycoprotein encoded by a cellular gene mapped to chromosome 20 in man and 2 in mouse (Chesebro et al. 1985, Oesch et al. 1985, Basler et al. 1986, Sparkes et al.

1987). The gene is ubiquitous; it has been cloned in numerous mammalian species included marsupials and there are analogues of this gene in birds (Harris et al. 1991, Gabriel et al. 1992), reptiles (Simonic et al. 2000) and amphibians (Strumbo et al. 2001) (Fig. 4). Those in Drosophila and nematodes appeared to be cloning artefacts (Westaway and Prusiner 1986, Prusiner 1986). PrP27-30 was first discovered as a protein co-purifying with infectivity in extracts derived from brains infected with the 263K strain of scrapie agent (Bolton et al. 1982, McKinley et al. 1983) which led to the conclusion that PrP is a part of the infectivity.

The "prion" hypothesis, which is deeply rooted in this association between PrP and infectivity was formulated by S.B. Prusiner in 1982 (Prusiner 1987). The hypothesis postulated that the scrapie agent was a proteinaceous infectious particle (actually it should read "proin" but Prusiner thought that "prion" sounds better than "proin"), because infectivity was dependent on protein but resistant to methods known to inactivate nucleic acids. The idea was not novel which is not that unusual taking into account that practically every class of biologically active molecules had been implicated as being the infectious scrapie agent. A similar proposal was presented by Griffith (1968), Levine (1972) and Gibbons and Hunter (1967) who developed the earlier

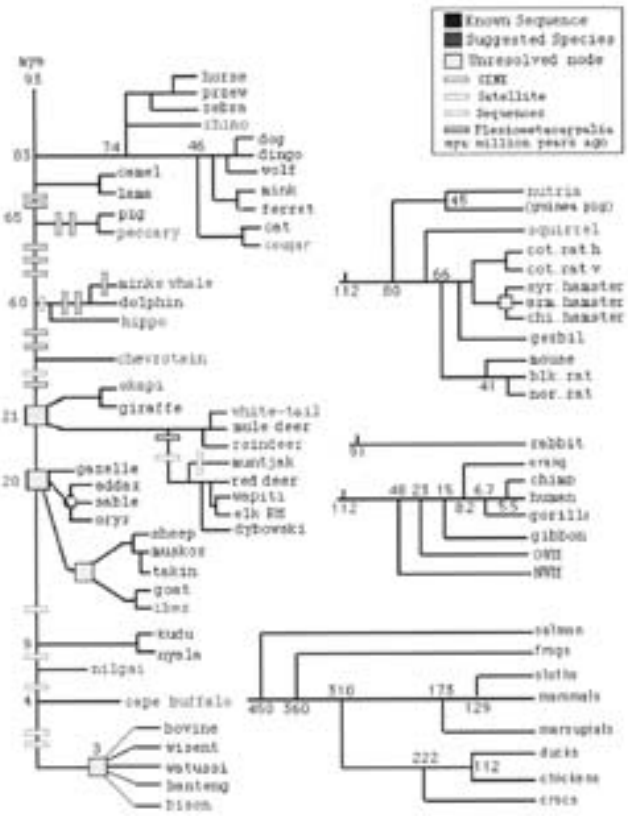

Fig. 4. Prion sequences relative to known phylogeny. Mad-cow.org.

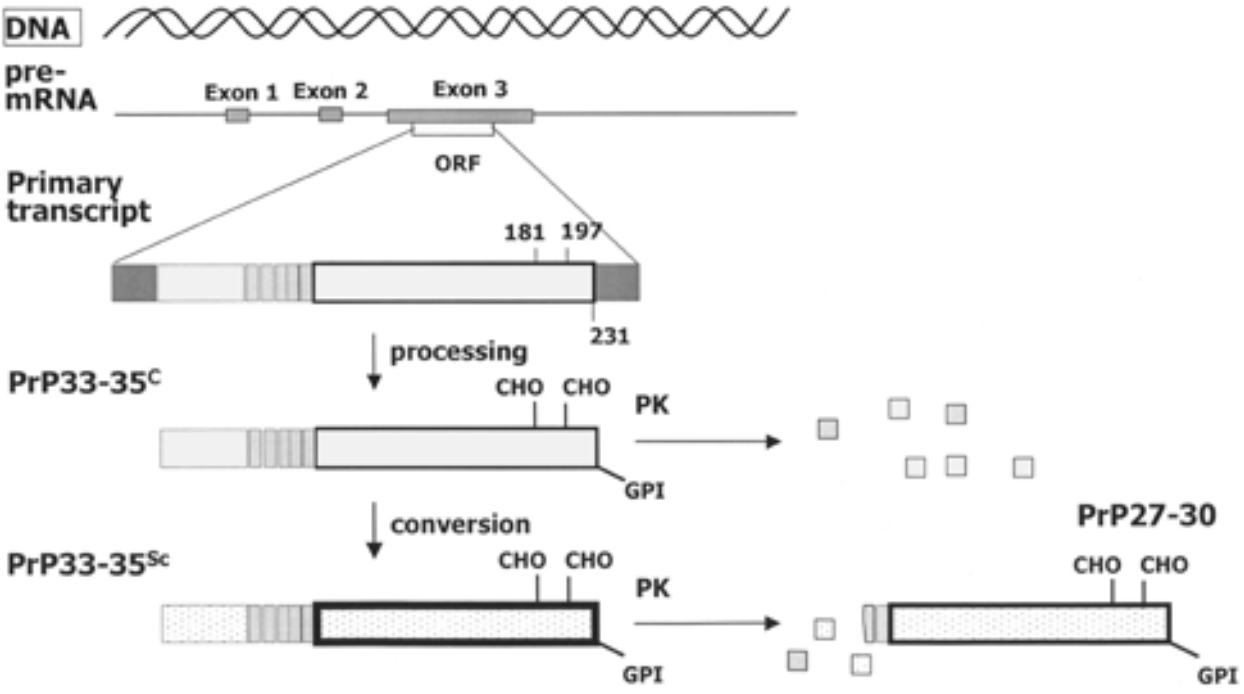

Fig. 5. PrP processing. Courtesy of Dr J. Bratosiewicz, Katowice, Poland.

suggestion of Alper and her co-workers (1967) that scrapie agent was devoid of disease-specific nucleic acid. While the theoretical approach of Alper et al., was seriously criticised by Rohwer (1991), several investigators had found previously that scrapie infectivity was sensitive to proteolytic digestion (Millson et al. 1976, Cho 1980).

Like all amyloid proteins, PrP 27-30 is a proteolytic cleavage product of a precursor protein, PrP 33-35^{sc}. However, PrP 33-35^{sc} is not the *primary* product of the cellular gene (Fig. 5). It has an amino acid sequence and postranslational modifications (like glycosylation and the attachment of GPI, glycophospholipid inositol anchor) identical to those of PrP 33-35^{c} but strikingly different physico-chemical features (Stahl et al. 1987, 1992a,b); in particular, PrP^{c} is completely degraded by a limited proteolysis but PrP^{Sc} is only partially degraded, yielding a core protein (PrP 27-30) which may be visualised by electron microscopy as scrapie-associated fibrils (SAF; Merz et al. 1981, Merz et al. 1984 - see Fig. 6), known as prion rods (Prusiner et al. 1983). The distinction between SAF and prion rods was based on some theoretical considerations and subtle ultrastructural differences between them. To become PrP^{sc}, PrP^{c} must be first transported to the cell surface and then through the endosomal-lysosomal pathway (McKinley et al. 1991, Borchelt et al. 1992).

There are several interesting features of PrP. As already mentioned, PrP is a glycoprotein with two Asn-glycosylation sites; thus, PrP may exist as deglycosylated, monoglycosylated and di-glycosylated isoforms of different electophoretic mobilities or glycophorms (Fig.7; Collinge et al. 1996) and the relative abundance of them (glycosylation pattern) correlates well with a phenotypic expression of TSE. In particular, the fourth type of glycosylation pattern are present in both BSE and vCJD (Fig. 7; Collinge et al. 1996, Hill et al. 1997). The glycosylation pattern breeds true - i.e. it is retained under passage through the transgenic mice with a human *PRNP* transgene (Collinge et al. 1996).

A different approach has been used by Parchi et al. (1996, 1999) who explored earlier findings that following proteinase K (PK) treatment, two types of PrP are seen on Western blot - type 2 PrP (19 kDa) and type 1 PrP (21 kDa). These two types of PrP also breed true - the best example is FFI characterised by the presence of type 2 PrP in the brain, which may be retrieved from brains of transgenic animals following the passage of FFI (Telling et al. 1996). The presence of type 1 or 2 of PrP coupled with the presence of Met or Val at the polymorphic 129 site of the *PRNP* gene enabled subclassification of human TSEs into six types (MM1, MV1, MM2, MV2, VV1, VV2) of different but reproducible phenotypic expressions (Parchi et al. 1996, 1999). The problem how to translate the Collinge's and the Parchi's classifications is further complicated by the fact that chelation of the metal ions change both the type 1 and

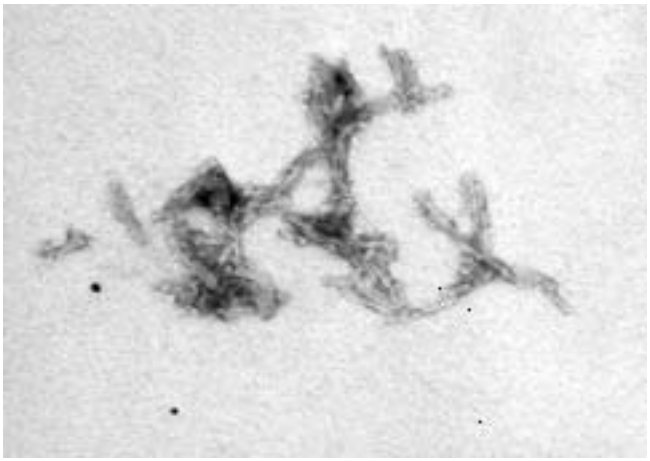

Fig. 6. Fibrillar amyloid structures isolated from CJD-affected mouse brain. Negative staining electron microscopy, magnification, x 27,000.

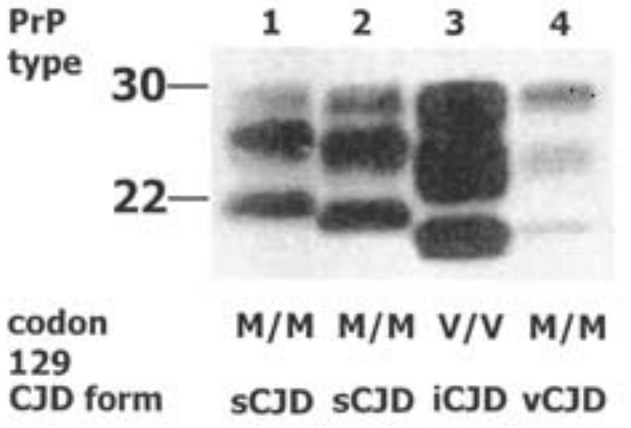

Fig. 7. Glycosylation patterns according to the PrP types and the status of the codon 129 polymorphism. M, methionine; V, valine; sCJD, sporadic form of CJD; iCJD, iatrogenic form of CJD; vCJD, variant CJD. Note the dominance of diglycosylated isoforms of PrP in vCJD; the identical pattern (type IV) is observed in BSE. Courtesy of Dr J. Bratosiewicz, Katowice, Poland.

type 2 glycosylation pattern of Collinge et al. (1996) into so the called type 2^- (Wadsworth et al. 1999). It is thus very probably that the Collinge's and the Parchi's classifications are different because of differences in technologies. The relevance of the glycosylation pattern to the strain problem will be discussed in a separate paragraph.

STRAINS OF THE AGENT

From the early work of Dickinson and his collaborators (Dickinson and Mackay 1964, Dickinson et al. 1968, 1969, 1988, Fraser and Dickinson 1973, Dickinson and Outram 1972, 1977, 1983, 1984, 1988) it was known that scrapie incubation period is tightly linked to the gene designated *Sinc* (in mice; from scrapie incubation) and *SIP* (in sheep; from shorter incubation period) and it was even proposed by Parry (1962) that scrapie is the *bona fide* genetic disorder while its transmissibility is only an epiphenomenon. The discovery of *Sinc* was instrumental in supporting the notion that scrapie agent has an independent genome (Bruce and Dickinson 1987). The existence of biologically different strains of scrapie agent is still the strongest argument against the *proteinaceous only* nature of the scrapie agent. However, the recent advances of structural biology of PrP challenged also this conjecture.

Different strains of the scrapie agent can be identified in terms of their stable biological characteristics (Dickinson et al. 1988, Bruce et al. 1991, 2002). The same strain can be isolated from different hosts and the same host can be infected with different strains. Furthermore, the characteristics of a given strain may sometimes undergo changes to yield a new strain with new characteristics that are stable in subsequent passages. Such changes are consistent with the effects of mutations in the genome of the agent which is presumably an as yet undiscovered disease-specific nucleic acid.

Approximately 20 strains of scrapie agent have been isolated from sheep and goats affected with clinical scrapie (Dickinson and Fraser 1972, Dickinson 1976, Carp et al. 1987). Some isolates from sheep yield a mixture of strains. The best known example is the "scrapie sheep brain pool" (SSBP/1) from which 22A, 22C and 22L strains were isolated (Dickinson 1976, Carp and Callahan 1991). Some sources of sheep scrapie are not transmissible to mice, for example the CH 1641 isolate (Foster and Dickinson 1988); but those that are, can be divided into two groups on the basis of their properties in the two homozygous *Sinc* (*Prn-p*) genotypes of mice. The ME7 group of agents exhibit a short incubation period when passaged through $Sinc^{s7}$ ($Prn\text{-}p^A$) mice (s for short; for example C57Bl mice) and a long incubation period when passaged through $Sinc^{p7}$ ($Prn\text{-}p^B$) mice (p for prolonged; for example VM mice). The 22A group exhibit exactly the opposite characteristics: short incubation period in $Sinc^{p7}$ mice and long incubation period in $Sinc^{s7}$ mice (Dickinson et al. 1972, 1975, 1977, 1984). It has been conclusively demonstrated that the *Sinc* gene is congruent with the *Prn-p* gene; in other words PrP is the product of *Sinc* (Carp et al. 1987, Hunter et al. 1987, 1989, 1991, 1992, 1993, Kimberlin and Hope 1987, Bueler et al. 1992, Hunter 1993, Moore et al. 1998). All scrapie isolates differ from the BSE strain when formally tested, however, spectrum of scrapie strains may have changed over the last 20 years (Bruce et al. 2002).

Passage through a species different from that used for the primary isolation (i.e., across the species barrier) is a useful method to separate mixtures of strains and to isolate (select) new mutant strains (Kimberlin and Walker 1977, 1984, Collinge 2001). One of the best known examples of the isolation of a mutant strain with completely different characteristics from the original isolate is the isolation of the 263K (the same as 237sc) strain of scrapie agent (Kimberlin and Walker 1977, 1978, 1984).

Two additional sets of experiments may be classically interpreted as indicating that scrapie agent must have an independent genome, for which the orthodox candidate has been obviously, a nucleic acid.

First, strains of scrapie agent undergo changes of certain characteristics such as incubation period, lesion profile, and presence and amount of PrP amyloid deposits, which are compatible with mutations of "conventional" pathogens (Bruce and Dickinson 1987). Three classes of strain stability have been established (Bruce and Dickinson 1987). Class I stability strains (ME7, 22C) possess stable characteristics irrespective of the *Sinc* (s7 or p7) (*Prn-p A* or *B*) genotype of mice in which they are passaged. Class II strains (22A, 22F) possess stable characteristics if passaged through mice of the *Sinc* genotype in which they were isolated but change these characteristics gradually over several passages through mice of a different *Sinc* mouse genotype. Class III strains (31A, 51C, 87A, 125A, 138A, 153A) exhibit sudden discontinuous changes of characteristics irrespective of the genotype of mice in which they are

passaged. All six class III strains are characterised by similar incubation periods, the production of large numbers of amyloid plaques (Bruce et al. 1986), and a high frequency of asymmetrical cerebral vacuolation. It is thus conceivable that all six class III isolates represent the same strain of scrapie agent (Bruce and Dickinson 1987).

"Class III breakdown" was defined as a "sudden shortening of an incubation period, in the course of single mouse passage, accompanied by a marked change in neuropathology" (Bruce and Dickinson 1987). This usually occurred at some point between the primary and the 7th passage and yielded an isolate designated 7D. The 7D strain was characterised by a shorter incubation period, a more "generalised" lesion profile, and an approximately 10-fold lower frequency of production of amyloid plaques. All these characteristics are reminiscent of ME7 and it is highly probably that 7D is actually the same as the ME7 strain of scrapie agent. In summary, these data show the selection of mutant strains of scrapie agent in the same host species (the same variant of *Prn-p* gene), indicating that that the genome of scrapie agent is host-independent.

It must be emphasised that the above-mentioned emergence of the new (ME7) strain of scrapie agent is independent of the host although its selection is influenced by the host genotype. Thus, it is misleading to describe two given strains as having "long" and "short" incubation periods because these characteristics are relative and they will depend on the *Sinc* (*Prn-p*) genotype of the mice (Westaway et al. 1987, Weissmann 1989, 1991). Often the relative incubation periods of two given strains can be reversed on changing the mouse strain (Carp and Callahan 1991).

Probably the best known example of a strain of TSE which can be isolated from different host is the strain which caused vCJD and which exhibits the same operational characteristics as the causative strain of BSE (Bruce et al. 1996, Hill et al. 1996, Scott et al. 1999). The BSE/vCJD strain is characterised by the fourth type of glycosylation pattern (Collinge et al. 1996) but the same type is also present in the brains of sheep infected with the CH1641 strain of scrapie (Hope et al. 1999). However, while vCJD/BSE strain is readily transmissible to mice (these mice exhibit a strange behaviour moving backwards), the CH1641 strain is not (Foster and Dickinson 1988). The existence CH1641 strain of scrapie exemplifies a notion that glycosylation pattern may not represent the complete strains characteristics.

Further, different strains of scrapie agent can exhibit competition when inoculated at different times, either intracerebrally (i.c.) (Dickinson et al. 1972) or peripherally (Dickinson et al. 1975). For example, when VM mice ($Sinc^{p7}$) were inoculated i.c. with the 22C (slow) strain a week before a second inoculation of the 22A (fast) strain, the mice were killed by the faster 22A strain, as shown by the short incubation period and the characteristic "lesion profile". In contrast, when the time lapse before the second inoculation was prolonged to 9 weeks, the incubation period of 22A increased by 30 days because of the competition with the earlier inoculation with the slow strain. In another experiment, R III mice ($Sinc^{s7}$), inoculated intraperitoneally with 22A (which now became the slow strain) followed by a second inoculation with the 22C (fast) strain 100 to 300 days later, did not develop disease caused by the 22C strain. The blocking effect of 22A was so complete that the 22C strain did not produce disease in mice which died after the expected incubation period of 22A. Furthermore, Kimberlin and Walker (1984) studied the blocking quantitatively and showed that the blocking agent must be capable of replication (i.e. be infectious). The results were interpreted as showing two different strains competing for a limited number of multimeric "replication sites" - subunits of which are encoded by *Sinc* (PrP itself !) (Dickinson et al. 1972, 1975, 1977, 1984).

The very presence of strains may be readily explained by the existence of strain-specific oligonucleotite or a ubiquitous virus; however, these objects have never been found despite several attempts to detect disease-specific nucleic acids (Meyer et al. 1991, Kellings et al. 1992, Akowitz et al. 1994). Thus, the alternative explanation, in agreement with protein-only hypothesis, came to a turning point.

In 1985 a new epidemic of transmissible mink encephalopathy (TME) broke out in Stetsonville, USA (Marsh et al. 1991) and two strains of TME had been isolated: a "hyper" (HY) strain isolated from minks with hyperactive syndrome and a "drowsy" (DY) one isolated from those of hypoactive, drowsy mink. Both the neuropathological picture and banding pattern of PrP^{Sc} of DY and HY strain differ (Bessen and Marsh 1992a,b, 1994). At that point, the *in vitro* conversion experiments must be introduced. When excessive amount of PrP^{Sc} purified from TSE-affected brain is mixed with S^{35}-labelled PrP^{c}, the S^{35}-labelled-PrP^{Sc} is formed in a cell-free system *in vitro*, implying a nucleation process

where PrP^{Sc} acts as a seed (Kocisko et al. 1994). This experimental system was used to show that the conversion is strain-specific, i.e. HY PrP^{Sc} converted mink S^{35}-PrP^{c} into HY S^{35}-PrP^{Sc} and DY PrP^{Sc} converted the same normal mink isoform of PrP into S^{35}-DY PrP^{Sc} (Bessen et al. 1995). This experiment suggested that strain-specificity is encrypted within the conformation of PrP itself which, in turn, determines the site of proteinase cleavage and strain-specific size of PrP on the Western blot. That the banding pattern (resulted from the actual conformation of PrP) is strain-specific and breeds-true has been subsequently shown in a case of transmission of FFI and CJD to transgenic mice Tg(MHu2M)/Prn-$p^{0/0}$ (Telling et al. 1996). Following deglycosylation, the molecular weight of PrP^{FFI} is 19 kDa while that of PrP^{CJD}, 21 kDa (Monari et al. 1994, Parchi et al. 1996) and the size of PrP^{Sc} was retained following passage into Tg(MHu2M)/Prn-$p^{0/0}$ (Telling et al. 1996). These observations were extended by Collinge et al. (1996) who showed that the glycosylation pattern of PrP also breeds-true and indeed the preservation of the fourth type linked to vCJD/BSE under passage in Tg mice expressing human PrP on the null background was accepted as a compelling evidence that BSE and vCJD is caused by one and the same strain (Hill et al. 1997, Scott et al. 1999).

The above mentioned experiments may be still interpreted dually. According to the protein-only hypothesis, differences in sizes of PrP fragments as seen on Western blot reflect diversity of PrP conformation. As PrP^{Sc} is formed from PrP^{c} in a process of seeding-nucleation (where PrP^{Sc} acts as a seed), the existence of strain-specific PrP's which breed true suggests that strain-specificity is encrypted within the conformation of PrP^{Sc}. However, there are two difficulties with fully acceptance of such a hypothesis. First, it has never been shown that there are more than a few of PrP^{Sc} conformers as it is necessary to encrypt approximately 20 strains of scrapie. To this end, there are only two PrP^{Sc} types (21 kDa type 1 and 19 kDa type 2) and these are responsible for the smorgasbord of human TSEs. In other words, this means that all human TSEs in all astonishing diversity of CJD, GSS and FFI phenotypes are caused by just two strains. Even with more sophisticated scheme of Collinge (Collinge et al. 1996, Collinge 2001), there are just a very few conformational "strains". Secondly, both sizes of PrP may be isolated from different parts of the brain and lymphatic tissues (Parchi et al. 1999). This should mean, that the same host is infected dually with both strains of the agent. While multiple strains have been isolated from sheep brains, it has never been shown that these, in turn, are linked to different PrP conformers and, in particular, to more than 2 conformers. However, this is a testable hypothesis and the presence of several different conformers in lets say, the brain of sheep, may be tested. The last difficulty is the greatest: while generation of new PrP^{Sc} in the conversion *in vitro* experiments should generate new infectivity (an aggregate of PrP^{Sc} is *bona fidae* prion or the infectious agent). On the contrary, it has been shown that no new infectivity is generated (Hill et al. 1999). Thus, either the newly formed PrP^{Sc} is not "true" PrP^{Sc} (in other words, the insolubility and PK-resistance of newly formed PrP^{Sc} are not sufficient to form true a "prion") or something else is necessary to complete the agent.

TRASGENETIC STUDIES AND THE PROBLEM OF SPECIES BARRIER

The most impressive data suggesting close linkage between PrP^{sc} and the agent or, at very least, proving the necessity for *PrP* gene for infection/replication of the agent, stem from the experiments using transgenic and knock-out mice technologies. These experiments were developed because of the strong correlation between familial and sporadic forms of the TSE with specific polymorphisms and mutations in the *Prn-p* gene (the gene which encodes for PrP^{c}; the same as *PRNP* gene in humans).

The 263K (Sc237) strain of scrapie agent is pathogenic for hamsters but not for mice (Kimberlin and Walker 1977, 1978) and such an effect is called a "species barrier". When transgenic (Tg(HaPrP) mice were constructed with a cosmid clone containing the hamster *PrP* gene (Scott et al. 1989), these mice, in contrast to the non-transgenic controls, became susceptible to scrapie after inoculation with the 263K strain. Furthermore, the incubation period in different lines of transgenic animals was inversely proportional to the number of transgene copies and amount of PrP^{c} (Prusiner et al. 1990, Scott et al. 1989). While these studies did "not address the possibility of a putative second component within the prion, such as small nucleic acids" (Scott et al. 1989), they explicitly suggest that the interaction between PrP^{sc} contained in the inoculum and heterologous PrP^{c} (for example, hamster PrP^{sc} and mouse PrP^{c}) is a major factor responsible for "species barrier" effect but that the strain of agent is equally important because the

species barrier is not the same for any pair of donor and recipient strains. It was hypothesised that such protein-protein interaction may result in PrPsc amplification (which thus merely *mimics* replication), not unlike that discovered for the mutant p53 oncoprotein interaction with its wild-type analogue. However, when another set of transgenic mice (Tg(Mo Prn-p^b) was constructed which harbour Prn-p^b variant of *PrP* gene (Prn-p^b is linked to a long and Prn-p^a to a short incubation period following inoculation with the Chandler isolate of scrapie agent) a "paradoxical" shortening of incubation period was observed following challenge with this strain (Westaway et al. 1991). The problem of this experiment lies in the fact that source of scrapie ("Chandler") used may contain at least two strains of agent and these were interacting with different copy number of normal and Tg *PrP* genes. Even if "Chandler" was one strain it could still shorten incubation period depending on the differences of incubation periods in Prn-p^a and Prn-p^b mice, as mentioned below. In Tg(Mo Prn-p^b) mice, the length of the incubation period was also inversely proportional to the number of copies of the Prn-p^b transgene.

The hypothesis of protein-protein (PrP^c-PrP^{sc}) interaction underlying the pathogenesis of TSE (Prusiner 1998) gained much support from the studies of chimeric PrP proteins (PrPs) and respective transgenic mice (Scott et al. 1992, 1993). When scrapie infected--murine neuroblastoma cell lines were transfected with several chimeric *PrP* genes, which consisted of various combinations of four different segments derived from either hamster or mouse *PrP* gene (these genes differ at 16 amino acid positions), only those chimeric proteins (MHM2 PrP and M4) which were recognised as "murine" were converted into truncated proteinase K-resistant PrP27-30 and thus recognised by hamster- and human-specific 3F4 antibodies. In contrast, HaPrP (H4) and H3MPrP were not recognised as "murine" PrP and not converted into PrP27-30. Transgenic mice were also constructed with these chimeric transgenes along with *MH2M* transgene (Scott et al. 1992, 1993). The latter may represent a gene which is "intermediate" between mouse and hamster *PrP* genes. Analogously to *in vitro* studies, Tg(MH2M) mice were susceptible to 263K strain of scrapie agent (and thus behave like Tg(HaPrP) mice or hamsters) while Tg(MHM2) mice are resistant to this strain (because MHM2 is recognised as a murine protein) and thus behaves like non-transgenic mice for which the 263K strain of scrapie is non-pathogenic. It is noteworthy, that MH2M and *MHM2* differ at only two codons. Of note, Tg(MHM2)294 mice which contained the highest number of copies of chimeric *PrP* transgene became susceptible to infection with 263K agent. The latter results are basically analogous to the paradoxical shortening of incubation time in Tg(Mo Prn-p^b) mice where the number of copies of the Prn-p^b transgene overcame the "inherent" to that allele prolongation of incubation time (Westaway et al. 1991). The presence of *MH2M* transgene influence the incubation period. When the 263K strain was passaged through Tg(MH2M) mice and then inoculated into Syrian hamsters, the incubation period increased. By the same token, the same strain was able to infect mice which are otherwise resistant to the 263K strain. All these data suggest that PrP^c-PrP^{sc} interaction is a major factor which determine at least some of the properties of the given strain. Indeed, the direct evidence for such an interaction was found only recently (Kocisko et al. 1994). In the cited experiment, a convertion of PrP^c into truncated proteinase K-resistant PrP fragments as a result of addition of PrP^{sc} was seen in *vitro*. This observation is consistent with the view already suggested by Gajdusek (1988, 1990, 1991) that PrP^{sc} is produced by the mechanism of "seeded" polymerization but the problem of *de novo* generation of infectivity could not be addressed in this experiment because large excess of PrP^{sc} precluded detection of infectivity hypothetically generated by such a conversion (Beyreuther and Masters 1994).

Results with transgenic mice expressing huPrP are somehow conflicting. In contrast to Tg(HaPrP) which became susceptible to a hamster isolate of scrapie, Tg(HuPrP) become only partially susceptible (Telling et al. 1995). The construction of Tg(HuPrP) on the null background increased this susceptibility while the chimeras Tg(MHu2MPrP) being analogous to chimeric Tg(MH2PrP) mice became highly susceptible to CJD. Collectively, the interpretation of these data, favoured by prion hypothesis suggest that never cloned and thus purely hypothetical "protein X" interacts with mouse PrP more readily than with human PrP and thus blocks the interaction between $huPrP^{Sc}$ containing in the inoculum and $huPrP^c$ encoded by a transgene. While the surface to interact with protein X on the globular C-terminus of PrP has already been mapped (Kaneko et al. 1997), the protein itself remains, as many things in the TSE research, pure mystery.

At this point, the "replication site hypothesis" formulated by Dickinson and Outram (Dickinson et al. 1975,

1977, 1984) may be worthy to be recalled. These investigators hypothesised that scrapie agent is replicated on a limited number of putative replication sites (agent receptors) which are heteromeric products of *Sinc* (congruent with the *PrP* gene). The removal of replication sites, for instance by splenectomy (Fraser and Dickinson 1973, 1978) or in genetically asplenic mice (Dickinson and Meikle 1969) (the agent is replicated in the spleen before it approaches the brain from the periphery; Kimberlin and Walker 1989) prolongs the incubation period. While the transgenic addition of replication sites could not be accomplished in late seventies, it could be predicted that such an addition (*via* acquisition of extra copies of the *PrP* transgene) would tend to *shorten* the incubation period, and such an effect was indeed observed in experiments using transgenic mice (Westaway et al. 1991, Scott et al. 1989, Prusiner et al. 1990). The hypothetical shortening of incubation time would only occur, however, with a single strain if the extent of over-expression overcame the transgene addition of the "slow" allele for the strain of agent injected.

In the next step, knock-out mice in which the *PrP* gene was disrupted ($PrP^{o/o}$ or $PrP^{-/-}$) were constructed (Bueler et al.1992, Prusiner et al. 1993). Surprisingly, these mice first did not show any harmful effect (Brenner et al. 1992) but it was subsequently reported that hippocampal brain slices from $PrP^{o/o}$ mice have impaired GABA receptor-mediated fast inhibition and long-term potentiation (Collinge et al. 1994). Thus, PrP is probably involved in the synaptic function. However, the major finding is that $PrP^{o/o}$ did not develop scrapie after inoculation with acarpie agent (Bueler et al. 1993, Prusiner et al. 1993). Furthermore, even heterozygous mice ($Prp^{+/o}$) showed prolonged incubation period after scrapie inoculation. The results of these experiments with PrP knock-out mice are in principle analogous to those with asplenic mice (Dickinson and Meikle 1969) but the number of "replication sites" in the $Pr^{o/o}$ knock-out mice is apparently reduced to zero. Thus, while such experiments did not solve the problem of the structure of the scrapie agent, it has been cocclusively proven that *PrP* gene is indispensable in scrapie pathogenesis (and *eo ipso* in the development of clinical disease) and not *only* a passive pathological product. Possible reasons include PrP^{c} being a necessary receptor for agent replication and/or being a constituent (virino) or sole component (prion) of the infectious agent.

The only finding which is still difficult to interpret within the framework of the "virino" and "virus" hypotheses is the development of spontaneous degenerative disease in transgenic mice expressing a *PrP* gene mutation at codon 101 (Tg(GSS MoPrP), which is analogous to the codon 102 mutation associated with GSS in man (*vide infra*; Hsiao et al. 1990, 1991). Interestingly, Tg(GSS MoPrP) mice were originally reported to be devoid of PrP^{sc} on Western blot (Hsiao et al. 1990). This last suggestion has been replaced by a report that Tg(GSS MoPrP) mice produced PrP-immunoreactive plaques but little or no PrP^{Sc} (Hsiao et al. 1994, Prusiner 1998). Furthermore, the report of the transmission of scrapie-like disease from the brains of Tg(GSS MoPrP) mice (Hsiao et al. 1994) was followed by a report of the analogous transmission from the brains of transgenic mice constructed with non-mutated (normal or wild) hamster and sheep *PrP* gene (Prusiner et al. 1993). In these wtTg(Ha PrP) mice, the spontaneous neuorodegenerative disorder in the form of necrotizing myelopathy and demyelinating polyneuropathy developed after a prolonged time (Westaway et al. 1994). These mice do not produce PrP^{Sc} but they apparently transmit the disease to Syrian hamsters, analogously to Tg(GSS PrP) mice (Hsiao et al. 1994). The latter finding may suggest that the number of the *PrP* transgenes and not merely presence of mutation at the codon 101, is responsible for the development of neurodegeneration. Indeed, when analogous mice were constructed by means of reciprocal recombination (thus, without extra copies of the transgene) (Manson et al. 1999, 2000), neither "spontaneous neurodegenera- tion" nor "transmission of the disease" have been observed. However, these Tg mice were very susceptible to infection with the GSS inoculum, a disease otherwise difficult to transmit. It has been noticed recently that 20 kDa fragment derived from the transmembrane PrP (Ctm PrP) accumulates on the surface of cells expressing $PrP102^{Leu}$ (Mishra et al. 2002). The authors suggest that the latter phenomenon instead of $PrP102^{Leu}$ *per se* may mediate altered susceptibility of $Tg101^{Leu}$ mice to GSS.

Thus, these transgenic mice may represent true "prion protein disorders" in which alterations (amplification) of the *PrP* gene cause "spontaneous neurodegeneration". However, the absence of transmission from Tg mice without overexpression of the transgene clearly suggest that overexpression itself and not the "genetic construction" of prion is responsible for the "spontaneous neueodegeneration". In conclusion, the Hsiao et al. (1990) experiment, regarded as the most convincing support of the prion theory seems much less convincing

in the light of the reciprocal recombination experiments (Manson et al. 1999, 2000). It is also true, however, that the nature of transmission from brains of Tg(GSS MoPrP) remains an enigma.

PrP GENE, ITS MUTATIONS AND PHENOTYPIC EXPRESSION OF THE TSE

Specific changes in the *PrP* gene sequence are tightly linked with numerous phenotypic expressions of the TSE. As already mentioned, scrapie incubation period both in mice and in sheep is controlled by *Prn-p* (classical names for this gene is *Sinc* and *SIP*, respectively, in mice and sheep). The early evidence for this linkage (Dickinson et al. 1968, 1969, 1988, Dickinson and Outram 1983) was followed by the discovery of polymorphisms within the *Prn-p* gene which are linked to allelic differences affecting the length of the incubation period (Carlson et al. 1986, 1988, 1994, Westaway et al. 1987). Thus, Leu at codon 108 in $Prn\text{-}p^{a}$ (short incubation period, identical with $Sinc^{s7}$) is substituted with Phe in $Prn\text{-}p^{b}$ mice (long incubation period, identical with $Sinc^{p7}$) and Thr at codon 189 becomes Val, respectively (Westaway et al. 1987). Equivalent linkages were soon discovered in sheep (Hunter et al. 1987, 1989, 1991, 1992 a, b, 1993, Hunter 1993). For instance, Cheviot sheep with Val at codon 136 are susceptible for inoculation with SSBP/1 while those with Ala at this codon are not (Hunter 1993). However, the linkage between haplotypes of PrP (*SIP*) gene in sheep and susceptibility to other strains is extremely complex but scrapie in sheep is not merely *genetic* disease as sheep in Australia, where there is no scrapie, exhibit the same "susceptible" haplotypes as those elsewhere (Hunter et al. 1997).

In man, the obvious candidate for such a linkage analysis was Gerstmann-Sträussler-Scheinker (GSS) syndrome and other familial forms of CJD and it was soon discovered that the occurrence of GSS is linked to a mutation (substitution of Pro with Leu) at the codon 102 of the *PRNP* gene (Collinge et al. 1989, Hsiao et al. 1989a,b, 1990, Doh-Ura et al. 1990, Brown et al. 1991, Kertzschmar et al. 1991, 1992, Goldhammer et al. 1993). Several other mutations followed. Two GSS families (from Indiana, USA and from Sweden), characterised by the occurrence of microtubule-associated protein (MAP)-τ-positive neurofibrillary tangles not unlike of those of Alzheimer's disease (Tagliavini et al. 1991, Giaccone et al. 1992), are linked to mutations at codons 198 (Phe to Ser) and 217 (Gln to Arg), respectively (Dlouhy et al. 1992, Hsiao et al. 1992). Familial CJD cases from all three known world clusters in Slovakia ("Oravske kuru") (Goldgaber et al. 1989, Goldfarb et al. 1990a,b, 1991, Mitrova et al. 1991), Israel (the mutation of the "wandering Jews of the Diaspora") (Hsiao et al. 1991, Goldfarb et al. 1990a) and Chile (Brown et al. 1992) are linked to the codon 200 Glu to Lys mutation. However, 200^{Lys} is not only associated with CJD of "wandering Jew of the Diaspora" (Gajdusek 1990, Goldfarb et al. 1990, Brown et al. 1991 a, b, Bertoni et al. 1992) as it was recently discovered in a Japanese family (Inoue et al. 1994). Other CJD families are linked to the codon 178 Asp to Asn (Goldfarb et al. 1991, 1992, Brown et al. 1991, Fink et al. 1991, Nieto et al. 1991, Bertoni et al. 1992, Laplanche et al. 1992). The same mutation was discovered in fatal familial insomnia (Goldfarb et al. 1992, Monari et al. 1994), which has been known in classic descriptions as thalamic dementias.

A few mutations have been better characterised: mutation of codon 117 (Ala→Val) was discovered in the "telencephalic" variant of GSS (Doh-Ura. et al. 1989, Hsiao et al. 1991, Mastrianni et al. 1996); STOP ("Amber") mutation of the codon 145 was described in a sporadic case of spasitc paraparesis and GSS neuropathology in the brain (Kitamoto et al. (1993); mutation in the codon 183 (Thr→Val) is linked to phenotype od long-lasting dementia and parkinsonism (Nitrini et al. 1997); two other mutations, 200 (Asp→Asn) and 212 (Gln→Pro) are linked to a GSS phenotype (Ghetti et al. 1998, Piccardo et al. 1998). The 105^{Leu} codon mutation is linked to a phenotype of familial spastic paraparesis with abundant plaques in motor cortex but sparse plaques in the cerebellum (Kitamoto et al. 1993). A 210^{Ile} mutation was reported from Italy (Pocchiari et al. 1993) and France (Ripoll et al. 1993). Although this mutation is linked to "classic" familial CJD, it was also noted in five apparently sporadic CJD cases from these two countries. The latter notion should be taken, however, with a caution as the consangunity may be obvious only a few centuries before the present apparent absence of it (Mitrova et al. 1991). Last but not least, the double mutations at codon 180 (Val to Ile) and 232 (Met to Arg) were reported in a sporadic CJD case characterized by late age of onset (84 years) and absence of typical EEG pattern (Hitoschi et al. 1993). At one of those codons

(Met232Thr), we reported a mutation in a GSS case from Poland (Liberski et al. 1998, 2000).

Several inserts in the *PrP* region (codons 51 through 91) which normally contain five octapeptide repeats (R1, R2, R2, R3, R4 of the following unit sequence: 51 Pro-(His/Gln)-Gly-Gly-Gly-(-/Gly)-Trp-Gly-Gln) which bind copper ions (Burns et al. 2002) were linked to the occurrence of familial CJD or GSS (Goldfarb et al. 1992, 1993, Owen et al. 1991a,b, 1992, Brown et al. 1991) or of dementia "without characteristic neuropathology" (Collinge et al. 1990). The first mutation in the octapeptide region was described in several related families (Owen et al. 1991, 1992, Collinge et al. 1990, Poulter et al. 1992). The sequence of mutations was as follows: R1, R2, R2, R2, R3, R2, R3g, R2, R2, R3, R4. In another family with six inserts their sequence was different (R1, R2, R2, R3, R2, R3g, R2, R3g, R2, R3, R4, where the sequence of R3g is as follows: Pro-His-Gly-Gly-Gly-Trp-Gly-Gln) (Nicholl et al. 1995). In a Japanese family with six additional inserts (Oda et al. 1995), the sequence is still different (R1, R2, R2, R3g, R2, R2, R3g, R2, R2, R3, R4). Still another Bascqe family was described by Capelliari et al. (1997) with the sequence: R1, R2, R2 , R2, R2, R2, R2, R2, R2, R3, R4. Goldfarb et al. (1992) reported on a French family with eight additional inserts (R2, R2, R2, R2, R2, R2, R2, R2a between repeats R3 and R4(the sequence of insert R2a is as follows: Pro - His - Gly - Gly - Gly - Trp - Gly - Gln). An analogous family was described by Van Gool et al. (1995) and Jansen et al. (1997) (R1, R2, R2, R3', R3, R2, R2, R2, R2, R2, R2, R3, R4) while Brown et al. (1992) reported on a family with seven additional inserts (R1, R2, R2, R2', R3, R2, R3, R2, R3, R2, R2'', R3, R4 with insert R' and R'' of amonoacid sequence: Pro-His-Gly-Gly-Gly-Trp-Gly-Gln but different nucleotide sequence). Krasemann et al. (1995) described a family with nine additional inserts (R1, R2, R2, R3, R2, R3, R3g, R2, R2a, R2, R3, R2, R3, R4) while Goldfarb et al. (1993) with two additional R2a inserts. The last was a CJD case of Campbell et al. (1996) with 4 additional inserts (R1, R2, R2, R2, R2, R2, R2, R3, R4) and a family described by Cochran et al. (1996) with five additional inserts (R1, R2, R2, R3, R2, R2, R2, R2, R3, R4). All known mutations are shown in Table I.

Polymorphisms at codon 129 need a special comment. Codon 129 encodes Met in 62.5% and Val in 37.5% of normal, mostly heterozygous, Caucasian population (Owen et al. 1989 a, b, Bratosiewicz et al. 2001). However, in both sporadic and iatrogenic CJD, there is marked overrepresentation of Met Met and Val Val homozygotes over Met Val heterozygotes (Brown et al. 1990, 1991, Collinge et al. 1991, 1993. Deslys et al. 1994, Miyazono et al. 1992, Palmer et al. 1991). The codon 129 polymorphism exerts modifying effect on a phenotypic expression of a given *PRNP* mutation, for instance - 129^{Val} occurs coupled with 198^{Ser} or 217^{Arg} in GSS (Hsiao et al. 1992, Dlouhy et al. 1992) and with 178^{Asn} in familial CJD (Goldfarb et al. 1992). The presence of 129^{Val} 178^{Asn} is linked to a CJD phenotype while 129^{Met} 178^{Asn} is phenotypically associated with fatal familial insomnia (Goldfarb et al. 1992). Indeed, PrP proteins purified from familial CJD with 178^{Asn} and fatal familial insomnia are different and these differences are probably conformational (Monari et al. 1994). Furthermore, the Fore people in Papua New Guinea exhibit more than 50% of Val Val homozygosity (less than 10% in Caucasians) which may help to explain the devastating spreading of the kuru epidemic within this susceptible population (Cervenakova et al. 1989).

Recently Prusiner and Hsiao (1994) suggested a classification of familial spongiform encephalopathies according to the type of mutation they are linked to. For example, "prion disease(P102L)" would denote GSS with the 102^{Leu} mutation. However, some investigators suggest that different alterations in the *PRNP* gene rather specify phenotypic expression of the same disease (Brown et al. 1991) than different diseases not unlike situation in familial amyloidotic polyneuropathy in which different phenotypes are linked to various alterations of the transthyretin gene (Gajdusek 1988, 1990). Thus, to stress well known phenotypic expressions, classical historical names like Creutzfeldt-Jakob disease or Gerstmann-Sträussler- Scheinker syndrome should be retained.

The existence of familial TSEs (CJD, GSS and FFI) caused by a mutations in the *PRNP* gene may also be dually interpreted. According to the prion theory, the mere presence of any given mutation change the energy barrier for a conversion PrP^{c} to PrP^{Sc} and thus causes a disease. According to virus theory, the mutation merely changes a susceptibility for an ubiquitous virus (Caughey, Chesebro 1997). There is a precedence for such a mechanism: a mutated gene responsible for sickle cell disease or thalassaemias increases susceptibily for B19 parvovirus. It is of note that one of the insert mutations in transgenic mice leads to the development of neurodegeneration but not to the appearance of an infections agent (Chiesa et al. 2001).

Table I

Known *PRNP* mutations (Mad-cow.org, modified). The references from the table are not all cited in the reference list

Color key:	causative*	stop causative*	neutral*	silent	* based on consensus opinion
.	22	2	6	12	total: 42 known point mutations, 24 causative

Initial DNA	Final DNA	Initial AA	Final AA	Codon	Reference
cagCCTcat	CCC	Pro	Pro	P68P	Windl (1999) Hum. Gen. 105: 244
aagCCGagt	CTG	Pro	Leu	P102L	Hsiao et al. (1989) Nature 338: 342
aagCCAaaa	CTA	Pro	Leu	P105L	Kitamoto (1993) BBRC 191: 709
aagCCAaaa	ACA	Pro	Thr	P105T	Mad-cow.org
gcaGCAagc	GTA	Ala	Val	A117V	Doh-Ura (1989) BBRC 163: 974
GcaGCAagc	GCG	Ala	Ala	A117A	Hsiao (1989) Nature 338: 342
GggGGCctt	GGG	Gly	Gly	G124G	[Prusiner (1997) Science 278: 245]
tacATGctg	GTG	Met	Val	M129V	Doh-Ura (1989) BBRC 163: 974
cccATC------ata	ATG	Ile	Met	I138M	Laplanche (2000) pers. comm.
ttcG-------Gccag	AGC	Gly	Ser	G142S	Laplanche (2000) pers. comm.
gacTATgag	TAG	Tyr	Stop	Y145s	Ghetti (1996) PNAS 93: 744
aacCAAgtg	TAA	Gln	Stop	Q160s	Finckh U (2000) Am. J. Hum. Genet. 66: 110
caaGTGtac	GTA?	Val	Val	V161V	[Prusiner (1997) Science 278: 245]
agcAACcag	AGC	Asn	Ser	N171S	Samaia (1997) Nature 390: 241
cagAACaac	AAT	Asn	Asn	N173N	Laplanche (2000) pers. comm.
gtgCACgac	CAT	His	His	H177H	Ripoll (1993) Neurology 43: 1934
cacGACtgc[1y]	AAC	Asp	Asn	D178N	Goldfarb et al. (1991) Lancet 337: 425
TgcGTCaat	ATC	Val	Ile	V180I	Kitamoto (1993) BBRTC 191: 709
AtcACAatc	GCA	Thr	Ala	T183A[19]	Nitrini (1997) Ann. Neurol. 42: 138
cagCACacg	CGC	His	Arg	H187R	Cervenakova (1999) Am. J. Med. Genet. 88(6): 653
CacACGgtc	AGG	Thr	Arg	T188R	Windl (1999) Hum. Gen. 105. 244
CacACGgtc	AAG	Thr	Lys	T188K	Finckh U (2000) Am J Hum Genet 66: 110
CacACGgtc	GAG	Thr	Ala	T188A	Collins S (2000) Arch. Neurol 57: 1058
CacACGgtc	ACA	Thr	Thr	T188T	Laplanche (2000) pers. comm. To mad-cow.org
gggGAGaac	AAG	Glu	Lys	E196K	Peoc'h (2000) Human Mutation #323
aacTTCacc	TCC	Phe	Ser	F198S	Hsiao (1992) Nature Genet.1: 68
AccGAGacc	AAG	Glu	Lys	E200K	Goldgaber (1989) Exp. Neurol.106: 204
AccGACgtt	AAC	Asp	Asn	D202N[8]	Piccardo (1998) J. N. Exp. Neur. 57: 979
accGACgtt	GAT	Asp	Asp	D202D	Laplanche (2000) pers. comm. To mad-cow.org
GacGTTaag	ATT	Val	Ile	V203I	Peoc'h (2000) Human Mutation #323
GagCGCgtg	CAG	Arg	His	R208H	Mastrianni (1996) Neurol.47: 1305
gagCGCgtg	CGT	Arg	Arg	R208R	Laplanche (2000) pers. comm. To mad-cow.org
gtgGTTgag	ATT	Val	Ile	V210I	Pocchiari (1993) Ann. Neurol. 34: 802
gttGAGcag	CAG	Glu	Gln	E211Q	Peoc'h (2000) Human Mutation #323
gagCAGatg	CGG	Gln	Pro	Q212P	Piccardo (1998) J. N. Exp. Neur. 57: 979
gagCAGatg	CAA	Gln	Gln	Q212Q	Windl (1999) Hum. Gen. 105.244
accCAGtac	CGG	Gln	Arg	Q217R	Hsiao (1992) Nature Genet. 1: 68
TacGAGagg	AAG	Glu	Lys	E219K	Barbanti (1996) Neurobiology 47: 734
cagAGAgga	AGG	Arg	Arg	R228R	Windl 1999 Hum. Gen. 105.244
GgaTCGagc	TCA	Ser	Ser	S230S	Windl (1999) Hum. Gen. 105.244
AgcATGgtc	AGG	Met	Arg	M232R	Kitamoto (1993) BBRC 191: 709
tctCCAcct	TCA	Pro	Ser	P238S[4]	Windl (1999) Hum. Gen. 105.244

PrP AS AMYLOID: THE CONCEPT OF THE TRANSMISSIBLE CEREBRAL AMYLOIDOSES

The amyloid plaque has long been recognised as a hallmark of the neuropathology of some of TSE, notable kuru (Klatzo and Gajdusek 1959). Because kuru-affected brains were loaded with such amyloid deposits (Fig. 8), kuru was "facetiously" called the "galloping senescence of the juvenile" (Gajdusek 1990, Liberski and Gajdusek 1997). More than three decades later, it was established that the amyloid plaque of TSE contained PrP^{sc} and thus PrP^{sc}, by definition, is the amyloid, irrespective whether it *is* or it *is not* a part of the agent. As a result Gajdusek even suggested calling PrP 27-30 the "scrapie amyloid" (Gajdusek 1988, 1990, Safar et al. 1993 a, b, c, 1994) and already twenty-five years ago proposed that conversion of PrP^{c} to PrP^{sc} may become "autocatalytic when the baby crystal continues the pattern-determining nucleation process" (Gajdusek 1990). Moreover, fibrillar structures isolated from TSE-affected brains (SAF or prion rods) are morphologically very similar to but distinguishable from other amyloid fibrils when visualised by negative-staining electron microscopy (Merz et al.1981, 1984, Prusiner et al. 1983). Direct evidence for a high β-structure content of PrP^{sc} came only in the last few years due to technical problems with the insolubility of PrP fibrils and the difficulty in obtaining enough pure protein to perform crystalographic studies. Using Fourier-transform IR spectroscopy, witch permits correlation of the infrared spectrum with the secondary structure of proteins and does not require the protein to be in solution, Caughey et al. (1991) were the first to demonstrate that conformation of PrP 27-30 (PrP-res in nomenclature used by these investigators) contains high proportion of β-sheet while the α-helical content was much lower that commonly found in other proteins. Subsequently, Gasset et al. (1992,

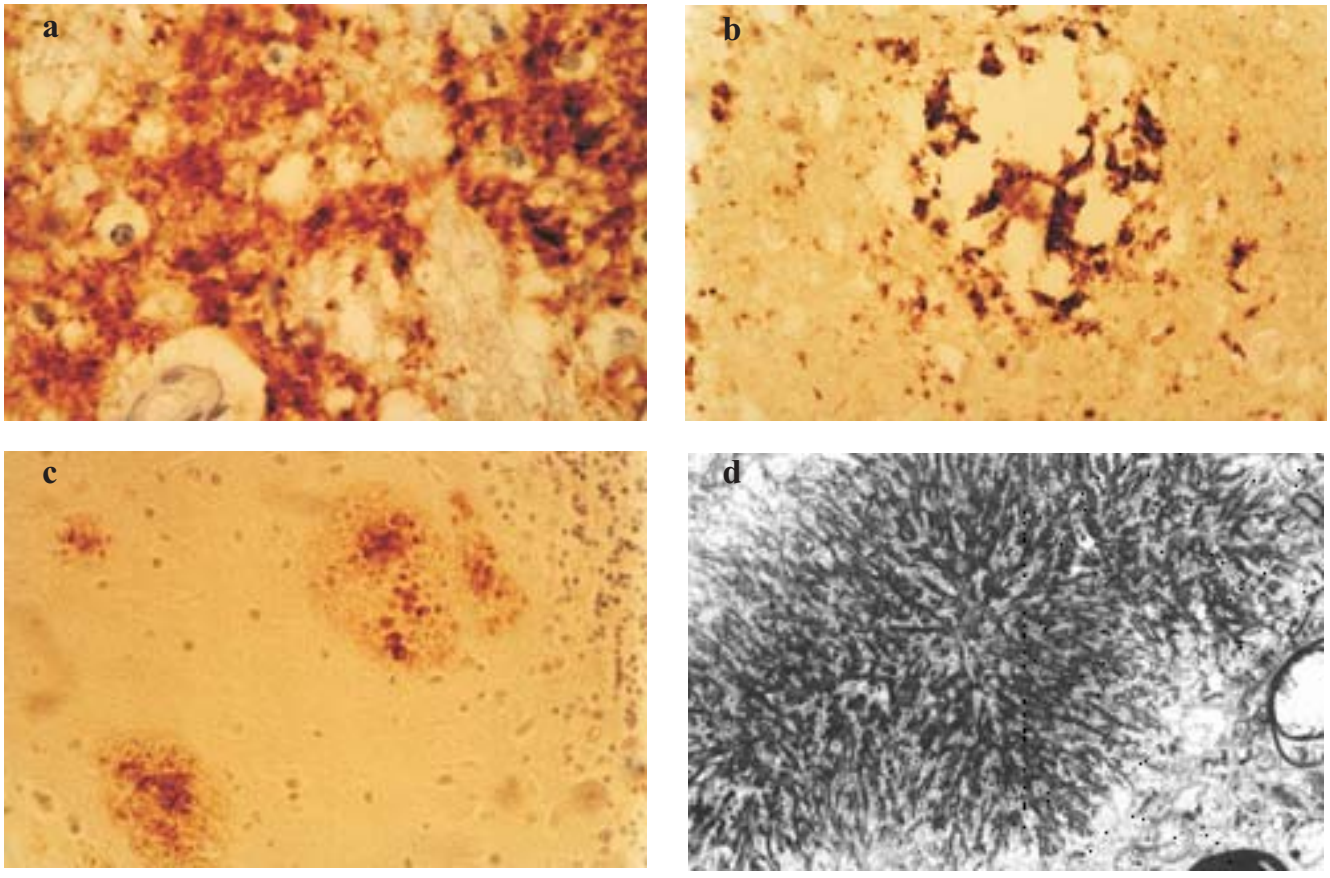

Fig. 8. Different forms of PrP^{Sc} deposits in the affected brains. a, "synaptic" type of PrP; b, perivacuolar deposits; c, multicentric plaques in GSS as detected by hydrated autoclaving and antibies against PrP (3F4, DAKO); d, multicentric plaque from a GSS case as seen by transmission electron microscopy, lead citrate and uranyl acetate, x 12,000.

1993) could confirm a high proportion (54%) of β-structure in the PrP^{Sc} conformation. Following denaturation with SDS (a treatment which reduces infectivity, (Brown et al. 1990) the content of β-structure has decreased. Analogously, basic pH reduced both scrapie infectivity and β-sheet content.

Based on a protein sequence deduced from the known *PRNP* sequence, predictions for the conformation of PrP^{c} have suggested four α-helices ((H1-H4) and tree β-strands (Huang et al. 1994, 1995) while the conformation of PrP^{Sc} is belived to have increased β content. However, the conformation of synthetic peptides derived from regions of predicted secondary structures of PrP had dual nature. Gasset et al. (1992) demonstrated that H1 *in vitro* is in a β-type conformation; H2 may form either an α-helix, β-strand or a β-turn; while H3 i H4, have a β-strand structure. Furthermore, Heller et al. (1996) showed that H1 may have a conformation of either α-helix or β-strand. Collectively, these studies suggested that PrP is able to form either α-helices or β-sheets and conversion from α-helix to β-sheet may underly the formation of prions. Indeed, using hamster recombinant protein, (r)PrP (90-231), Mehlhorn et al. (1996) demonstrated that the same peptide may form stable α-helices or β-sheets with several intermediates.

ABOUT THE CONFORMATION OF PrP^{Sc}

The structure of the soluble form of the prion protein, PrP^{C}, is known from NMR spectroscopic studies in solution. Riek et al. (1996, 1997) and Billeter et al. (1997) found out that the structure of moPrP(121-231) consists of three α-helices and two antiparallel β-strands at the globular C-terminus, while the N-terminus is largely unstructured. The conformation of a longer recombinant fragment, PrP(23-231), was largely the same (Riek et al. 1997), as was the conformation of hamster (James et al. 1997, Liu et al. 1999), bovine (Garcia et al. 2000, Lopez-Garcia et al. 2000), and human (Zahn et al. 2000, Calzolai et al. 2000) PrP^{C} (Fig. 9). A comparison of human, mouse and bovine PrP demonstrated that human and bovine PrPs exhibit virtually identical conformations (Garcia et al. 2000; Fig. 10). Taking into account that the species barrier is encrypted in the sequence that determines the conformation of PrP, the inevitable conclusion is that human is the species „of choice" for transmission from BSE in cattle.

Owing to its insolubility, experimental data on the three-dimensional structure of the PrP^{Sc} isoform are much more difficult to obtain. Recently, an attractive possibility has been offered by the discovery that amyloid-forming proteins are capable of oligomerization through a mechanism known as 3D domain swapping (Bennett et al. 1994), whereby two or more subunits exchange identical structural elements, leading to the recreation of the monomeric fold but from chain fragments contributed by different subunits. Oligomerization via the mechanism of 3D domain swapping requires partial unfolding of the monomeric structure, followed by re-folding in an aberrant way. The possibility that 3D domain swapping might be a mechanism of amyloid

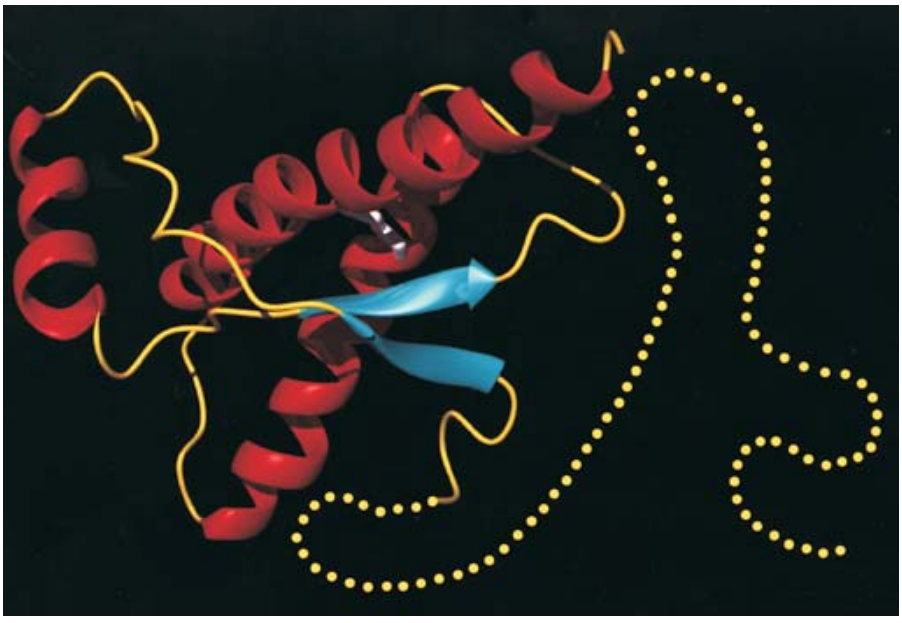

Fig. 9. Human PrP^{c} structure as revealed by NMR spectroscopy. Note the presence of 3 α-helices and 2 β-strands. Courtesy of Prof. Kurt Wuthrich, Institute of Molecular Biology and Biophysics, ETH Honggerberg, Zurich, Switzerland.

formation had been considered earlier (Klafki et al. 1993; Bennett et al. 1995; Cohen and Prusiner 1998), but the first case of 3D domain swapping in an amyloidogenic protein has been described for human cystatin C by Janowski et al. in 2001. This inhibitor of cysteine proteases is often found in pathological deposits in the brain arteries at advanced age (Grubb 2000), and in a mutated form is responsible for massive amyloidosis in HCCAA (hereditary cystatin C amyloid angiopathy) leading to death at young adulthood (Olafsson and Grubb 2002). The crystal structure of oligomeric cystatin C suggests an aggregation mode (Jaskolski, 2001) that would be consistent with the so-called β-sheet helix (Sunde and Blake, 1998), which in turn is compatible with the cross-β structure (Glenner, 1980a,b), believed to dominate the molecular architecture of amyloid fibrils. An analogous antiparallel β-sheet was proposed earlier to explain PrP^{Sc} aggregation (Huang et al. 1995). In the cross-β structure, the individual β-strands are perpendicular, and the β-sheet face - parallel to the fiber axis. Following the discovery of 3D domain swapping in human cystatin C, Knaus et al. (2001) have reproted that also the human prion protein is capable of dimerization via 3D domain swapping. Those dimers, which have the helix 3 swapped, are unusual in that their formation proceeds via disruption of an intramolecular disulfide bridge, which is then re-formed between helices 2-3 in an intermolecular fashion. However, based on hydrogen-exchange NMR studies, Nicholson et al. (2002) argue that the cross-linked helix 2-3 motif is the most stable element and thus unlikely to be disrupted during the process of domain swapping. The connection between 3D domain swapping and amyloid aggregation is supported by an increasing number of observations, but the evidence is still indirect, except for the reports by Liu et al. (2002) who have shown that RNase A can form open-ended aggregates by exchanging two different domains, and by Ogihara et al. (2001) who used protein engineering to force a three-helix bundle molecule to form fibrous aggregates.

An important new experimental data on the structure of the PrP^{Sc} isoform have been recently provided by the observation of isomorphous two-dimensional crystals in the preparations of PrP 27-30 and its internal-deletion analogue $PrP^{Sc}106$ ("miniprion"), the shortest form of PrP that still supports infectivity (Wille et al. 2002). An analysis of these 2D lattices using electron crystallography has revealed structural information to 7 Å resolution. Coupling these data with other experimental constraints has led to a molecular model of PrP^{Sc} featuring a parallel β-helix. A parallel β-helix is formed by a progression of β-strands winding in a (right- or

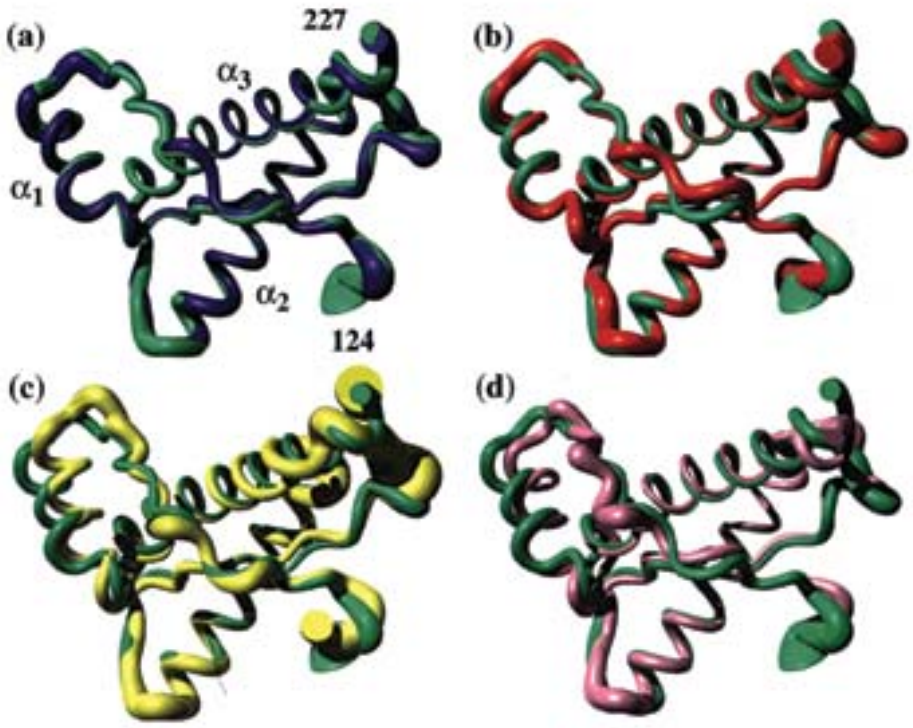

Fig. 10. (*a*) Superposition of the mean NMR structures of the polypeptide segment 124-227 in bovine PrP(23-230) (violet) and bovinePrP(121-230) (green). (*b-d*) Superposition of the segment 125-227 in bovine PrP(121-230) (green) with the corresponding residues in human PrP(121-230) (*b,* orange), mouse PrP(121-231) (*c,* yellow), and sheep PrP(90-231) (*d,* pink), respectively (Garcia et al. 2000). Courtesy of Prof. Kurt Wuthrich, Institute of Molecular Biology and Biophysics, ETH Honggerberg, Zurich, Switzerland.

left-handed) helical fashion, and is different from the β-sheet helix. The authors conclude that the conversion of PrP^{C} to PrP^{Sc} could be understood as a stabilization of a proto-β-helical element by neighboring PrP^{Sc} molecules, followed by an extension of the growing β-helix.

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REFERENCES

Alper T., Cramp W.A., Haig D.A., Clarke M.C. (1967) Does the agent of scrapie replicate without nucleic acid ? Nature 214: 764-776.

Akowitz A., Sklaviadis T., Manuelidis L. (1994) Endogenous viral complexes with long RNA cosediment with the agent of Creutzfeldt-Jakob disease. Nucleic Acids Res. 22: 1101-1107.

Basler K., Oesch B., Scott M., Westeway D., Walchli M., Groth D.F., McKinley M.P., Prusiner S.B., Weissmann C. (1986) Scrapie and cellular PrP isoforms are encoded by the same chromosomal gene. Cell 46: 417-428.

Bessen R.A., Kocisko D.A., Raymond G.J., Nandan S., Lansbury P.T., Caughey B. (1995) Non-genetic propagation of strain-specific properties of scrapie prion protein. Nature 375: 698-700.

Bessen R.A., Marsh R.F. (1992a) Identification of two biologically distinct strains of transmissible mink encephalopathy in hamsters. J. Gen. Virol. 73: 329-334.

Bessen R.A., Marsh R.F. (1992b) Biochemical and physical properties of the prion protein from two strains of the transmissible mink encephalopathy agent. J. Virol. 66: 2096-2101.

Bastian F.O. (1987) Spiroplasma-like inclusions in Creutzfeldt-Jakob disease. Arch. Pathol. Lab. Med. 103: 665-669.

Bennett M.J., Choe S., Eisenberg D.S. (1994) Domain swapping: Entangling alliances between proteins. Proc. Natl. Acad. Sci. USA 91: 3127-3131.

Bennett M.J., Schlunegger M.P., Eisenberg D. (1995) 3D Domain swapping: A mechanism for oligomer assembly. Protein Sci. 4: 2455-2468.

Bertoni J.M., Brown P., Goldfarb L.G., Rubenstein R., Gajdusek D.C. (1992) Familial Creutzfeldt-Jakob disease (codon 200 mutation) with supranuclear palsy. JAMA 268: 2413-2415.

Besnoit C. (1899) La tremblante ou nevrite peripherique enzootique du mouton. Rev. Vet. (Tolouse) 24: 265-277.

Beyreuther K., Masters C.L. (1994) Catching the culprit prion. Nature 370: 419-420.

Billeter M., Riek R., Wider G., Hornemann S., Glockshuber R., Wuthrich K. (1997) Prion protein NMR structure and species barrier for prion diseases. Proc. Natl. Acad. Sci. USA 94: 7281-7285.

Bolton D.C., McKinley M.P., Prusiner S.B. (1982) Identification of a protein that purifies with the scrapie prion. Science 218: 1309-1311.

Borchelt D.R., Taraboulos A., Prusiner S.B. (1992) Evidence for synthesis of scrapie prion proteins in the endocytic pathway. J. Biol. Chem. 267: 16188-16199.

Braig H., Diringer H. (1985) Scrapie: a concept of virus-induced amyloidosis of the brain. EMBO J. 4: 2309-2312.

Bratosiewicz J., Liberski P.P., Kulczycki J., Kordek R. (2001) Codon 129 polymorphism of the *PRNP* gene in normal Polish population and in Creutzfeldt-Jakob disease, and the search for new mutations in *PRNP* gene. Acta Neurobiol. Exp. 61: 151-156.

Brenner H.R., Herczeg A., Oesch B. (1992) Normal development of nerve-muscle synapses in mice lacking the prion protein gene. Proc. R. Soc. Lond. B 250: 151-155.

Brown P., Cervenkova L., Goldfarb L.G., McCombie W.R., Rubenstein R., Will R.G., Pocchiari M., Martinez-Lage J.F., Scalici C., Masullo C., Graupera G., Ligan J., Gajdusek D.C. (1994) Iatrogenic Creutzfeldt-Jakob disease: an example of the interplay between ancient genes and modern medicine. Neurology 44: 291-293.

Brown P., Galvez S., Goldfarb L.G., Nieto A., Cartier L., Gibbs C.J. Jr, Gajdusek D.C. (1992) Familial Creutzfeldt-Jakob disease in Chile is associated with the codon 200 mutation of the PRNP amyloid precursor gene on chromosome 20. J. Neurol. Sci. 112: 65-67.

Brown P., Goldfarb L.G., Brown W.T., Goldgaber D., Rubenstein R., Kascak R.J., Guiroy D.C., Piccardo P., Boellaard J.W., Gajdusek D.C. (1991) Clinical and molecular genetic study of a large German kindred with Gerstmann-Straussler-Scheinker syndrome. Neurology 41: 375-379.

Brown P., Goldfarb L.G., Cathala F., Vbrovska A., Sulima M., Nieto A., Gibbs C.J. Jr, Gajdusek D.C. (1991) The molecular genetics of familial Creutzfeldt-Jakob disease in France. J. Neurol. Sci. 105: 240-246.

Brown P., Goldfarb L.G., Gajdusek D.C. (1991) The new biology of spongiform encephalopathy: infectious amyloidoses with a genetic twist. Lancet 337: 1019-1022.

Brown P., Goldfarb L.G., Gibbs C.J. Jr, Gajdusek D.C. (1991) The phenotypic expression of different mutations in trans-

missible familial Creutzfeldt-Jakob disease. Eur. J. Epidemiol. 7: 469-476.

Brown P., Goldfarb L.G., Kovanen J., Haltia M., Cathala F., Sulima M., Gibbs C.J. Jr, Gajdusek D.C. (1991) Phenotypic expression of familial Creutzfeldt-Jakob disease associated with the codon 178Asn *PRNP* mutation. Ann. Neurol. 31: 282-285.

Brown P., Goldfarb L.G., McCombie W.R., Nieto A., Trapp S., Squillacote D., Shermata W., Godec M., Gibbs C.J. Jr, Gajdusek D.C. (1991) Atypical Creutzfeldt-Jakob disease in an American family with an insert mutation in the *PRNP* amyloid precursor gene. Neurology 42: 422-427.

Brown P., Liberski P.P., Wolff A., Gajdusek D.C. (1990) Conservation of infectivity in purified fibrillary extracts of scrapie-infected hamster brain after sequential enzymatic digestion or polyacrylamide gel electrophoresis. Proc. Natl. Acad. Sci. USA 87: 7240-7244.

Bruce M.E., Boyle A., Cousens S., McConnell I., Foster J., Goldmann W., Fraser H. (2002) Strain characterization of natural sheep scrapie and comparison with BSE. J. Gen. Virol. 83: 695-704.

Bruce M.E., Dickinson A.G. (1987) Biological evidence that scrapie agent has an independent genome. J. Gen. Virol. 68: 79-89.

Bruce M.E., McBride P.A., Farquhar C.F. (1986) Precise targeting of the pathology of the sialoglycoprotein, PrP, and vacuolar degeneration in mouse scrapie. Neurosci. Lett. 102: 1-6.

Bruce M.E., McConnell I., Fraser H., Dickinson A.G. (1991) The disease characteristics of different strains of scrapie in *Sinc* congenic mouse lines: implications for the nature of the agent and host control of pathogenesis. J. Gen.Virol. 72: 595-603.

Bruce M.E., Will R.G., Ironside J.W., McConnell I., Drummond D., Suttie A., McCardle L., Chree A., Hope J., Birkett C., Cousens S., Fraser H., Bostock C. (1997) Transmission of mice indicate that "new variant" CJD is caused by the BSE agent. Nature 389: 498-501.

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

Bueler H., Fischer M., Lang Y., Bluthmann H., Lipp H.P., DeArmond S.J., Prusiner S.B., Aguet M., Weissmann C. (1992) Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. Nature 356: 577-582.

Burger D., Hartsough G.R. (1964) A scrapie-like disease of mink. In: Report of a scrapie seminar. United States Department of Agriculture. paper 27, p. 225-227.

Burns C.S., Aronoff-Spencer E., Dunham C.M., Lario ZP., Avdievich N.I., Antholine W.E., Olmstead M.M., Vrielink A., Gerfen G.J., Peisach J., Scott W.G., Millhauser G.L. (2002) Molecular features of copper binding sites in the octarepeat domain of the prion protein. Biochemistry 41: 3999-4001.

Calzolai L., Lysek D.A, Guntert P., von Schroetter C., Riek R., Zahn R., Wuthrich K (2000) NMR structures of three single-residues variants of the human prion protein. Proc. Natl. Acad. Sci. USA 97: 8340-8345.

Campbell T.A., Palmer M.S., Will R.G., Gibb W.R.G., Luthert P.J., Collinge J. (1996) A prion disease with a novel 96-base pair insertional mutation in the prion protein gene. Neurology 46: 761-766.

Capellari S., Vital C., Parchi P., Petersen R.B., Ferrer X., Jarnier D., Pegoraro E., Gambett P., Julien J. (1997) Familial prion disease with a novel 144 bp-insertion in the prion protein gene in a Basque family. Neurology 49: 133-141.

Carlson G.A., Ebeling C., Yang S.-L., Telling G., Torchia M., Groth D., Westeway D., DeArmond S.J., Prusiner S.B. (1994) Prion isolate specified allotypic interaction between the cellular and scrapie prion proteins in congenic and transgenic mice. Proc. Natl. Acad. Sci. USA 91: 5690-5694.

Carlson G.A., Goodman P.A., Lovett M., Taylor B.A., Marshall S.T., Peterson-Torchia M., Westaway D., Prusiner S.B. (1988) Genetics and polymorphism of the mouse prion gene complex: control of scrapie incubation time. Mol. Cell. Biol. 8: 5528-5540.

Carlson G.A., Kingsbury D.T., Goodman P.A., Coleman S., Marshall S.T., DeArmond S.J., Westaway D., Prusiner S.B. (1986) Linkage of prion protein and scrapie incubation time genes. Cell 46: 503-511.

Carp R.I., Callahan M. (1991) Variation in the characteristics of 10 mouse-passaged scrapie lines derived from five scrapie-positive sheep. J. Gen. Virol. 72: 293-298.

Carp R.I., Moretz R.C., Natelli M., Dickinson A.G. (1987) Genetic control of scrapie: incubation period and plaque formation in I mice. J. Gen. Virol. 68: 401-407.

Caughey B., Chesebro B. (1997) Prion protein and transmissible spongiform encephalopathies. Trends in Cell Biology 7: 56-62.

Caughey B., Dong A., Bhat K.S., Ernst D., Hayes S.F., Caughey W.S. (1991) Secondary structure analysis of the scrapie-associated protein PrP 27-30 in water by infrared spectroscopy. Biochemistry 30: 7672-7680.

Cervenakova L., Goldfarb L.G., Brown P., Kenney K., Cochran E.J., Bennett D.A., Ros R., Gajdusek D.C. (1995) Three new PRNP genotypes associated with familial Creutzfeldt-Jakob disease. Am. J. Hum. Genet. 57: A209 (Abstr.).

Cervenakova L., Goldfarb L.G., Garruto R., Lee H.-S., Gajdusek D.C. (1998) Phenotype-genotype studies in kuru: Implications for new variant Creutzfeldt-Jakob disease. Proc. Natl. Acad. Sci. USA 95: 13239-13241.

Chesebro B., Race R., Wehrly K., Nishio J., Bloom M., Lechner D., Bergstrom S., Robbins K., Mayer L., Keith J.M., Garon C., Haase A. (1985) Identification of scrapie prion protein-specific mRNA in scrapie-infected and uninfected brain. Nature 315: 331-333.

Chiesa R., Pestronk A., Schmidt R.E., Tourtellotte W.G., Ghetti B., Picacrdo P., Harris D. (2001) Primary myopathy and accumulation of PrPSc-like molecules in peripheral tissues of transgenic mice expressing a prion protein insertional mutation. Neurobiol. Dis. 8: 279-288.

Cho H.J. (1980) Requirement of a protein component for scrapie infectivity. Intervirology 14: 213-216.

Cochran E.J., Bennett D.A., Cervenakova L., Kenney K., Bernard B., Foster N.L., Benson D.F., Goldfarb L.G., Brown P. (1996) Familial Creutzfeldt-Jakob disease with a five-repeat octapeptide insert mutation. Neurolog. 47: 727-733.

Cohen F.E. (2000) Prions, peptides and protein misfolding. Mol. Med. Today 6, 292-293.

Cohen, F.E., Prusiner, S.B. (1998) Pathologic conformations of prion proteins. Annu. Rev. Biochem. 67: 793-819.

Collinge J. (2001) Prion diseases of humans and animals: their causes and molecular basis. Annu. Rev. Neurosci. 24: 519-550.

Collinge J., Harding A.E., Owen F., Poulter M., Lofthouse R., Boughey Am, Shah T., Crow T.J. (1989) Diagnosis of Gerstmann-Straussler syndrome in familial dementia with prion protein gene analysis. Lancet 2: 15-17.

Collinge J., Owen F., Poulter M., Leach M., Crow T.J., Rossor M.N., Hardy J., Mullan M.J., Janota I., Lantos P.L. (1990) Prion dementia without characteristic pathology. Lancet 336: 7-9.

Collinge J., Palmer M.S., Dryden A.J. (1991) Genetic predisposition to iatrogenic Creutzfeldt-Jakob disease. Lancet 337: 1441-1442.

Collinge J., Palmer M.S., Rossor M.N., Janota J., Lantos P.L. (1993) "Prion dementia". Lancet 341: 627.

Collinge J., Palmer M.S., Sidle K.C.L., Gowland I., Medori R., Ironside J., Lantos P. (1995) Transmission of fatal familial insomnia to laboratory animals. Lancet 346: 569-570.

Collinge J., Sidle K.C.L., Meads J., Ironside J., Hill A.F. (1996) Molecular analysis of prion strain variation and the aetiology of "new variant" CJD. Nature 383: 685-670.

Collinge J., Whittington M.A., Sidle K.C.L., Smith C.J., Palmer M.S., Clarke A.R., Jeffreys G.R. (1994) Prion protein is necessary for normal synaptic function. Nature 370: 295-297.

Cuille J., Chelle P-L. (1938) La tremblante du mouton est bien inoculable. CR Acad. Sci. 206: 1687-1688.

Cunningham A.A., Wells G.A.H., Scott A.C., Kirkwood J.K., Barnett J.E.F. (1993) Transmissible spongiform encephalopathy in greater kudu (*Tragelaphus strepsiceros*). Vet. Rec. 132: 68.

Deslys J.-P., Marce D., Dormont D. (1994) Similar genetic susceptibility in iatrogenic and sporadic Creutzfeldt-Jakob disease. J. Gen. Virol. 75: 23-27.

Dickinson A.G. (1976) Scrapie in sheep and goats. In: Kimberlin RH, Ed, slow virus diseases of animals and man. North-Holland Publ. Comp., Amsterdam, p. 209-241.

Dickinson A.G., Bruce M.E., Outram G.W., Kimberlin R.H. (1984) Scrapie strain differences: the implications of stability and mutation. In: Proceedings of workshop on slow virus transmissible diseases (Ed. J. Tateishi) Res. Comm on Slow Virus Infection, the Japanesse Ministry of Health and Welfare. Takekashi-Kaikan p. 105-118.

Dickinson A.G., Fraser H. (1972) Scrapie: effect of Dh gene on incubation period of extraneurally injected agent. Heredity 29: 91-93.

Dickinson A.G., Fraser H., McConnell, Outram G.W., Sales D.I., Taylor D.M. (1975) Extraneural competition between different scrapie agent leading to loss of infectivity. Nature 253: 556.

Dickinson A.G., Fraser H., Meikle V.M.H., Outram G.W. (1972) Competition between different scrapie agents in mice. Nature 237: 244-245.

Dickinson A.G., Mackay J.M.K. (1964) Genetical control of the incubation period in mice of the neurological disease, scrapie. Heredity 19: 279-288.

Dickinson A.G., Meikle V.M.H. (1969) A comparison of some biological characteristics of the mouse-passaged scrapie agents, 22A and ME7. Genet. Res. (Camb) 13: 213-225.

Dickinson A.G., Meikle V.M.H., Fraser H. (1968) Identification of a gene which controls the incubation period of some strains of scrapie agent in mice. J. Comp. Pathol. 78: 293-299.

Dickinson A.G., Meikle V.M.H., Fraser H. (1969) Genetical control of the concentration of ME7 scrapie agent in brain of mice. J. Comp. Pathol. 79: 15-22.

Dickinson A.G., Outram G.W. (1979) The scrapie replication-site hypothesis and its implications for pathogenesis. In: Slow transmissible diseases of the nervous system (Eds. S.B. Prusiner and W.J. Hadlow). Vol 2, Academic Press, New York, p. 13-31.

Dickinson A.G., Outram G.W. (1983) Operational limitations in the characterisation of the infective units of scrapie. In: Virus non conventionnels et affections du systeme nerveaux central (Eds. L.A. Court and F. Cathala). Masson, Paris p. 3-16.

Dickinson A.G., Outram G.W. (1988) Genetic aspects of unconventional virus infections: the basis of the virino hypothesis. In: Novel infectious agents and the central nervous system (Eds. G. Bock, J. Marsh and J. Wiley & Sons). Chichister p. 63-77.

Dickinson A.G., Outram G.W., Taylor D.M., Foster J.D. (1988) Further evidence that scrapie agent has an independent genome. In: Unconventional virus diseases of the central nervous system (Eds. L.A Court, D. Dormont, P. Brown and D.T. Kingsbury). Commissariat a' l'Energie Atomique, Paris p. 446-460.

Dickinson A.G., Stamp J.T., Renwick C.C., Rennie J.C. (1977) Some factors controlling the incidence of scrapie in Cheviot sheep injected with a Cheviot-passaged scrapie agent. J. Comp. Pathol. 78: 313-321.

Diringer H. (1993) Hidden amyloidoses. Exp. Clin. Immunogenet. 9: 212-229.

Diringer H., Blode H., Oberdieck U. (1991) Virus-induced amyloidosis in scrapie involves a change in covalent linkages in the preamyloid. Arch. Virol. 118: 127-131.

Dlouhy S.R., Hsiao K., Farlow M.R., Foroud T., Conneally P.M., Johnson P., Prusiner S.B., Hodes M.E., Ghetti B. (1992) Linkage of the Indiana kindred of Gerstmann-Straussler-Scheinker disease to the prion protein gene. Nature Genetics 1: 64-67.

Doh-Ura K., Tateishi J., Kitamoto T., Sasaki H., Sakaki Y. (1990) Creutzfeldt-Jakob disease patients with congophilic kuru plaques have the missense variant prion protein common to Gerstmann-Straussler syndrome. Ann. Neurol. 27: 121-126.

Fink J.K., Warren J.T., Drury I., Murman D., Peacock M.L. (1991) Allele-specific sequencing confirms novel prion gene polymorphism in Creutzfeldt-Jakob disease. Neurology 41: 1647-1650.

Fletwood A.J., Furley C.W. (1990) Spongiform encephalopathy in an eland. Vet. Rec. 126: 215-222.

Foster J.D., Dickinson A.G. (1988) The unusual properties of CH1641, a sheep-passaged isolate of scrapie. Vet. Rec. 123: 5-8.

Fraser H., Dickinson A.G. (1970) Pathogenesis of scrapie in the mouse: the role of the spleen. Nature 226: 462-463.

Fraser H., Dickinson A.G. (1973) Scrapie in mice. Agent-strain differences in the distribution and intensity of grey matter vacuolation. J. Comp. Pathol. 83: 29-40.

Fraser H., Dickinson A.G. (1978) Studies of the lymphoreticular system in the pathogenesis of scrapie: the role of spleen and thymus. J. Comp. Pathol. 88: 563-573.

Gabriel J.-M., Oeach B., Kretzschmar H., Scott M., Prusiner S.B. (1992) Molecular cloning of a candidate chicken prion protein. Proc. Natl. Acad. Sci. USA 89: 9097-9101.

Gajdusek D.C. (1977) Unconventional viruses and the origin and disappearance of kuru. Science 197: 943-960.

Gajdusek D.C. (1988) Transmissible and nontransmissible dementias: distinction between primary cause and pathogenetic mechanisms in Alzheimer's disease and aging. Mount Sinai J. Med. 55: 3-5.

Gajdusek D.C. (1990) Subacute spongiform encephalopathies: transmissible cerebral amyloidoses caused by unconventional viruses. In: Fields Virology (Eds. B.N. Fields and D.M. Knipe). Vol. 2. 2nd ed. Raven Press, New York p. 2289-2324.

Gajdusek D.C. (1991) Transmissible amyloidoses: genetical control of spontaneous generation of infectious amyloid proteins by nucleation of configurational change in host precursors: kuru-CJD-GSS-scrapie-BSE. Eur. J. Epidemiol. 7: 567-578.

Gajdusek D.C. (1993) Kuru and scrapie. [In:] Prion diseases of humans and animals (Eds. S.B., Prusiner , J. Collinge, J. Powell, B. Anderton) Ellis Horwood, New York, London, Toronto, Sydney, Singapore p. 47-52

Gajdusek D.C. (1996) Kuru in childhood: implications for the problem of whether bovine spongiform encephalopathy affects humans, In: Transmissible subacute spongiform encephalopathies: prion disease. IIIrd International Symposium on transmissible subacute spongiform encephalopathies: prion disease (Eds. L. Court and B. Dodet) Val-de-Grace, Paris, France, Elsevier, Amsterdam, Oxford, Paris, p. 15-26.

Gajdusek D.C., Gibbs C.J. Jr (1990) Brain amyloidoses. Precursor proteins and the amyloids of transmissible and non transmissible dementias: scrapie-kuru-CJD viruses as infectious polypeptides or amyloid enhancing factors. In: Biomedical advances in aging (Ed. A.L. Goldstein). Plenum Publ. Corp. p. 3-24.

Gajdusek D.C., Gibbs C.J., Alpers M.P. (1966) Experimental transmission of a kuru-like syndrome to chimpanzees. Nature 209: 794-796.

Garcia F.L., Zahn R., Riek R., Wuthrich K. (2000) NMR structure of bovine prion protein. Proc. Natl. Acad. Sci. 97: 8334-8339.

Gasset M., Baldwin M.A., Fletterick R.J., Prusiner S.B. (1993) Perturbation of the secondary structure of the scrapie prion protein under conditions that alter infectivity. Proc. Natl. Acad. Sci. USA 90: 1-5.

Gasset M., Baldwin M.A., Lloyd D.H., Gabriel J.-M., Holtzman D.M., Cohen F., Fletterick R.J., Prusiner S.B. (1992) Predicted a-helical region of the prion protein when synthesized as peptides from amyloid. Proc. Natl. Acad. Sci. USA 89: 10940-10944.

Ghetti B., Piccardo P., Lievens P.M.J., Young K., Pocchiari M., Bugiani O., Tagliavini F., Dlouhy S.R. (1998) Phenotypic and prion protein (PrP) heterogeneity in Gerstmann-Sträussler-Scheinker disease (GSS) with a proline to a leucine mutation at *PRNP* residue 102. The 6th International Conference on Alzheimer's Disease and Related Disorders, Amsterdam, Neurobiol. Aging 19, p. 298.

Giaccone G., Verga L., Bugiani O., Frangione B., Serban D., Prusiner S.B., Farlow M.F., Ghetti B., Tagliavini F. (1992) Prion protein preamyloid and amyloid deposits in Gerstmann-Straussler-Scheinker disease, Indiana kindred. Proc. Natl. Acad. Sci. USA 89: 9349-9353.

Gibbons R.A., Hunter G.D. (1967) Nature of the scrapie agent. Nature 215: 1041-1043.

Gibbs C.J. Jr, Gajdusek D.C., Asher D.M., Alpers M.P., Beck E., Daniel P.M., Matthews W.B. (1968) Creutzfeldt-Jakob disease (spongiform encephalopathy): transmission to chimpanzee. Science 161: 388-389.

Glenner G.G. (1980a). Amyloid deposits and amyloidosis – the β-fibrilloses 1. N. Eng. J. Med. 302: 1283-1292.

Glenner G.G. (1980b). Amyloid deposits and amyloidosis – the β-fibrilloses 2. N. Eng. J. Med. 302: 1333-1343.

Glenner G.G., Eanes E.D., Page D.L. (1972) The relation of properties of Congo-red stained amyloid fibrills to the β-conformation. J. Histochem. Cytochem. 20: 821-826.

Glenner G.G., Wong C.W. (1984) Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem. Biophys. Res. Commun. 120: 885-890.

Goldfarb L., Brown P., Goldgaber D., Garruto R.M., Yanagihara R., Asher D.M., Gajdusek D.C. (1990) Identical mutation in unrelated patients with Creutzfeldt-Jakob disease. Lancet 336: 174-175.

Goldfarb L., Brown P., Little B.W., Cervenakova L., Kenney K., Gibbs C.J. Jr, Gajdusek D.C. (1993) A new (two-repeat) octapeptide coding insert mutation in Creutzfeldt-Jakob disease. Neurology 43: 2392-2394.

Goldfarb L., Brown P., McCombie W.R., Goldgaber D., Swergold G.D., Wills P.R., Cervenakova L., Baron H., Gibbs C.J. Jr, Gajdusek D.C. (1991) Transmissible familial Creutzfeldt-Jakob disease associated with five, seven, and eight extra octapeptide coding repeats in the PRNP gene. Proc. Natl. Acad. Sci. USA 88: 10926-10930.

Goldfarb L., Brown P., Mitrova E., Cervenakova L., Goldin L., Korczyn A.D., Chapman J., Galvez S., Cartier L., Rubenstein R., Gajdusek D.C. (1991) Creutzfeldt-Jakob disease associated with *PRNP* codon 200Lys mutation: an analysis of 45 families. Eur. J. Epidemiol. 7: 477-486.

Goldfarb L., Brown P., Vrbovska A., Baron H., McCombie W.R., Cathala F., Gibbs C.J. Jr, Gajdusek D.C. (1992) An insert mutation in the chromosome 20 amyloid precursor gene in a Gerstmann-Straussler-Scheinker syndrome. J. Neurol. Sci. 111: 189-194.

Goldfarb L.G., Brown P., Haltia M., Cathala F., McCombie W.R., Kovanen J., Cervenkova L., Goldin L., Nieto A., Godec M., Asher D.M., Gajdusek D.C. (1992) Creutzfeldt-Jakob disease cosegregates with codon 178Asn *PRNP* mutation in families of European origin. Ann. Neurol. 31: 274-281.

Goldfarb L.G., Haltia M., Brown P., Nieto A., Kovanen J., McCombie W.R., Trapp S., Gajdusek D.C. (1991) New mutation in scrapie amyloid precursor gene (at codon 178) in Finish Creutzfeldt-Jakob disease. Lancet 337: 425.

Goldfarb L.G., Korczyn A.D., Brown P., Chapman J., Gajdusek D.C. (1990a) Mutation on codon 200 of scrapie amyloid protein gene linked to Creutzfeldt-Jakob disease in Sephardic Jews of Libyan origin and non-Libyan origin. Lancet 336: 637.

Goldfarb L.G., Mitrova E., Brown P., Toh B.H., Gajdusek D.C. (1990b) Mutation in codon 200 of scrapie amyloid protein gene in two clusters of Creutzfeldt-Jakob disease in Slovakia. Lancet 336: 514.

Goldfarb L.G., Petersen R.B., Tabaton M., Brown P., LeBlanc A.C., Montagna P., Cortelli P., Jullien J., Vital C., Pendelbury W.W., Haltia M., Wills P.R., Hauw J.J., McKeever P.E., Monari L., Schrank B., Swergold G.D., Autillo-Gambetti L., Gajdusek D.C., Lugaresi E., Gambetti P. (1992) Fatal familial insomnia and familial Creutzfeldt-Jakob disease: disease phenotype determined by a DNA polymorphism. Science 258: 806-808.

Goldgaber D., Goldfarb L., Brown P., Asher D., Brown T., Lin S., Teener J.W., Feinstone S.M., Rubenstein R., Kascak R., Bollaard J.W., Gajdusek D.C. (1989) Mutations in familial Creutzfeldt-Jakob disease and Gerstmann-Straussler-Scheinker syndrome. Exp. Neurol. 106: 204-206.

Goldhammer Y., Gabizon R., Meiner Z., Sadeh M. (1993) An Israeli family with Gerstmann-Straussler-Scheinker disease manifesting the codon 102 mutation in the prion protein gene. Neurology 43: 2718-2720.

Gordon W.S. (1946) Louping ill, tickborne fever and scrapie. Vet. Rec. 47: 516-520.

Gordon W.S. (1946) Review of work on scrapie at Compton, England, 1953-1964. Report of scrapie seminar, (Washington, D.C., January 27-30, 1964), US Dept. Agric, p. 19–40.

Gordon W.S., Brownlee A., Wilson D.R. (1939) Studies in the louping-ill, tick-born fevera nd scrapie. In: Proc. 3rd Int. Congress of Microbiologists, New York, p. 130-131.

Griffith J.S. (1968) Self-replication and scrapie. Nature 215: 1043-1044.

Grubb A. (2000). Cystatin C – properties and use as diagnostic marker. Adv. Clin. Chem. 35: 63-99.

Hadlow W.J. (1959) Scrapie and kuru. Lancet 2, 289-290.

Haigh J.C., Mackintosh C., Griffin F. (2002) Viral, parasitic and prion diseases of farmed deer and bison. Rev. Sci. Tech. 21: 219-248.

Harris D.A., Falls D.L., Johnson F.A., Fischbach G.D. (1991) A prion-like protein from chicken brain copurifies with an acetylcholine receptor-inducing activity. Proc. Natl. Acad. Sci. USA 88: 7664-7668.

Heller J., Kolbert A.C., Larsen R., Ernst M., Bekker T., Baldwin M., Prusiner S.B., Pines A., Wemmer D.E., (1996) Solid-state NMR studies of the prion proteinH1 fragment. Protein Sci. 5: 1655-1661.

Hill A.F., Antoniou M., Collinge J. (1999) Protease-resistant prion protein produced *in vitro* lacks detectable infectivity J. Gen. Virol. 80: 11-14.

Hill A.F., Desbruslais M., Joiner S., Sidle K.C.L., Gowland I., Collinge J., Doey L.J., Lantos P. (1997) The same prion strain causes vCJD and BSE. Nature 389: 448-450.

Hitoschi S., Nagura H., Yamanouchi H., Kitamoto T. (1993) Double mutations at codon 180 and 232 of the *PRNP* gene in an apparently sporadic case of Creutzfeldt-Jakob disease. J. Neurol. Sci. 120: 208-212.

Hope J., Wood S.C., Birkett C.R., Chong A., Bruce M.E., Cairns D., Goldmann W., Hunter N., Bostock C.J. (1999) Molecular analysis of ovine prion protein identifies similarities between BSE and an experimental isolate of natural scrapie, CH 1641. J. Gen. Virol. 80: 1-4.

Hsiao K., Baker H.F., Crow T.J., Poulter M., Owen F., Terwilliger J.D., Westaway D., Ott J., Prusiner S.B. (1989a) Linkage of a prion protein missense variant to Gerstmann-Straussler syndrome. Nature 338: 342-345.

Hsiao K., Dlouhy S.R., Farlow M.R., Cass C., Da Costa M., Conneally P.M., Hodes M.E., Ghett B., Prusiner S.B. (1992) Mutant prion proteins in Gerstmann-Straussler--Scheinker disease with neurofibrillary tangles. Nature Genetics 1: 68-71.

Hsiao K., Doh-Ura K., Kitamoto T., Tateishi J., Prusiner S.B. (1989b) A prion protein amino acid substitution in ataxic Gerstmann-Straussler syndrome. Ann. Neurol. 26: 106.

Hsiao K.K., Groth D., Scott M., Yang K.K., Serban H., Rapp D., Foster D., Torchia M., DeArmond S.J., Prusiner S.B. (1994) Serial transmission in rodents of neurodegeneration from transgenic mice expressing mutant prion protein. Proc. Natl. Acad. Sci., USA 91: 9126-9130.

Hsiao K., Meiner Z., Kahana E., Cass B.A., Kahana I., Avrahami D., Scarlato G., Abramsky O., Prusiner S.B., Gabizon R. (1991) Mutation of the prion protein in Libyan Jews with Creutzfeldt-Jakob disease. N. Engl. J. Med. 324: 1091-1097.

Hsiao K., Scott M., Foster D., Groth D.F., DeArmond S.J., Prusiner S.B. (1990) Spontaneous neurodegeneration in transgenic mice with mutant prion protein. Science 250: 1587-1590.

Hsiao K.K., Groth D., Scott M., Yang S-L., Serban A., Rapp D., Foster D., Torchia M., DeArmond S.J., Prusiner S.B. (1991) Neurologic disease of transgenic mice which express GSS mutant prion proteins is transmissible to inoculated recipient animals. In: Abstracts of the prion diseases in humans and animals. September 2-4, 1991.

Huang Z., Gabriel J.-M., Baldwin M.A., Fletterick R.J., Prusiner S.B., Cohen F. (1994) Proposed three-dimensional structure for the cellular prion protein. Proc. Natl. Acad. Sci. USA 91: 7139-7143.

Huang Z., Prusiner S.B., Cohen F. (1995) Scrapie prions: a three-dimensional model of an infectious fragment. Folding & Design 1: 13-19.

Hunter N. (1993) Genetic control of scrapie incidence in sheep and its relevance for bovine spongiform encephalopathy in cattle. Rev. Med. Virol. 3: 195-200.

Hunter N., Cairns D., Foster J.D., Smith G., Goldmann W., Donnelly K. (1997) Is scrapie solely a genetic diseases ? Nature 386: 137.

Hunter N., Foster J.D., Benson G., Hope J. (1991) Restriction fragment length polymorphisms of scrapie-associated fibril protein (PrP) gene and their association with susceptibility to natural scrapie in British sheep. J. Gen. Virol. 72: 1287-1292.

Hunter N., Foster J.D., Dickinson A.G., Hope J. (1989) Linkage of the gene for the scrapie-associated fibril protein (PrP) to the SIP gene in Cheviot sheep. Vet. Rec. 124: 364-366.

Hunter N., Foster J.D., Hope J. (1992) Natural scrapie in British sheep: breeds, ages and prP gene polymorphisms. Vet. Res. 130: 389-392.

Hunter N., Goldman W., Benson G., Foster J.D., Hope J. (1993) Swaledale sheep affected by natural scrapie differ significantly in PrP genotype frequencies from healthy sheep and those selected for reduced incidence of scrapie. J. Gen. Virol. 74: 1025-1031.

Hunter N., Hope J., McConnell I., Dickinson A.G. (1987) Linkage of the scrapie-associated fibril protein (PrP) gene and Sinc using congenic mice and restriction fragment length polymorphism analysis. J. Gen. Virol. 68: 2711-2716.

Hunter N., Dann J.C., Bennett A.D., Somerville A., McConnell I., Hope J. (1992) Are Sinc and the PrP gene congruent? Evidence from PrP gene analysis in Sinc congenic mice. J. Gen. Virol. 73: 2751-2755.

Inoue I., Kitamoto T., Doh-Ura K., Shii H., Goto I., Tateishi J. (1994) Japanese family with Creutzfeldt-Jakob disease with codon 200 point mutation of the prion protein gene. Neurology 44: 299-301.

James T.L., Liu H., Ulyanov N.B., Farr-Jones S., Zhang H., Donne D.G., Kaneko K., Groth D., Mehlhorn I., Prusiner S.B., Cohen F.E. (1997) Solution structure of a 142-residue recombinant prion protein corresponding to the infectious fragment of the scrapie isoform. Proc. Natl. Acad. Sci. USA 94: 10086-10091.

Janowski R. Kozak M., Jankowska E., Grzonka Z., Grubb A., Abrahamson M., Jaskólski M. (2001) Human cystatin C, an amyloidogenic protein, dimerizes through three-dimensional domain swapping. Nature Struct. Biol. 8: 316-320.

Jansen G.H., Wesseling P., VanGool W.A., Steenbergen H. (1997b) Histopathology and immunohistochemistry of a Gerstmann-Sträussler-Scheinker family with a new octa repeat insertion. Brain Pathol. 7: 1380.

Jaskólski M. (2001) 3D Domain swapping, protein oligomerization, and amyloid formation. Acta Biochim. Polon. 48: 807-827.

Jeffrey M., Wells G.A.H. (1988) Spongiform encephalopathy in a Nyala (*Tragelaphus angasi*). Vet. Pathol. 25: 398-399.

Kaneko K., Zulianello K., Scott M., Cooper C., Wallace A.C., James T.L., Cohen F.E., Prusiner S.B. (1997) Evidence for protein X binding to a discontinuous epitope on the cellualr prion protein during scrapie prion propagation. Proc. Natl. Acad. Sci. USA 94: 10069-10074.

Kellings K., Meyer N., Mirenda C., Prusiner S.B., Riesner D. (1992) Further analysis of nucleic acids in purified scrapie prion preparations by improved return refocusing gel electrophoresis. J. Gen. Virol. 73: 1025-1029.

Kimberlin R.H. (1982) Scrapie agent: prions or virinos? Nature 297: 107-108.

Kimberlin R.H. (1984) Scrapie: the disease and the infectious agent. Trends Neurosci. 7: 312-316.

Kimberlin R.H. (1990) Scrapie and possible relationships with viroids. Sem. Virol. 1: 153-162.

Kimberlin R.H., Hope J. (1987) Genes and genomes in scrapie. Trends Genetics 3: 117-118.

Kimberlin R.H., Walker C.A. (1977) Characteristics of a short incubation model of scrapie in the Golden hamsters. J. Gen. Virol. 34: 295-304.

Kimberlin R.H., Walker C.A. (1978) Evidence that the transmission of one source of scrapie agent to hamsters involve separation of agent strains from a mixture. J. Gen. Virol. 39: 487-496.

Kimberlin R.H., Walker C.A. (1984) Competition between strains of scrapie depends on the blocking agent being infectious. Intervirology 23: 74-81.

Kimberlin R.H., Walker C.A. (1989) The role of the spleen in the neuroinvasion of scrapie in mice. Virus. Res. 12: 201-212.

Kirkwood J.K., Wells G.A.H., Cunningham A.A., Jackson S.I., Scott A.C., Dawson M., Wilesmith (1992) Scrapie-like encephalopathy in a greater kudu (*Tragelaphus strepsiceros*) which had not beed fed ruminant-derived protein. Vet. Res. 130: 365-367.

Kitamoto M., Amano N., Terao Y., Nakazato Y., Isshiki T., Mizutani T., Tateishi J. (1993) A new inherited prion disease (PrP P105L mutation) showing spastic paraparesis. Ann. Neurol. 34: 808-813.

Kitamoto T., Iizuka R., Tateishi J. (1993) An amber mutation of prion protein in Gerstmann-Sträussler syndrome with mutant PrP plaques. Bioch. Biophys. Res. Comm. 192: 525-531.

Kitamoto T., Ohta M., Doh-Ura K., Hitoshi S., Terao Y., Tateishi J. (1993c) Novel missense variants of prion protein in Creutzfeldt-Jakob disease or Gerstmann-Sträussler--Scheinker syndrome. Bioch. Bioph. Res. Comm. 191: 709-714.

Klafki H., Abramowski D., Swoboda R., Paganetti P.A., Staufenbiel M. (1996) The cabroxyl termini of beta-amyloid peptides 1-40 and 1-42 are generated by distinct gamma-secretase activities. J. Biol. Chem. 271: 28655-28659.

Klafki H.-W., Pick A.I., Pardowitz I., Cole T., Awni L.A., Barnikol H.V., Mayer F., Kratzin H.D., Hilschmann N. (1993) Reduction of disulfide bonds in an amyloidogenic Bence Jones protein leads to formation of "amyloid-like" fibrils *in vitro*. Biol. Chem., Hoppe-Seyler, 372: 1117-1122.

Klatzo I., Gajusek D.C. (1959) Pathology of kuru. Lab. Invest. 8: 799-847.

Knaus K.J., Morillas M., Swietnicki W., Malone M., Surowicz W.K., Yee V. C. (2001) Crystal structure of the human prion protein reveals a mechanism for oligomerization. Nature Struct. Biol. 8: 770-774.

Kocisko D.A., Come J.H., Priola S.A., Chesebro B., Raymond G.J., Lansbury P.T., Caughey B. (1994) Cell-free formation of protease-resistant prion protein. Nature 370: 471-474.

Kraseman S., Zerr I., Weber T., Poser S., Kretzschmar H., Hunsmann G., Bodemer W. (1995) Prion disease associated with a novel nine octapeptide repeat insertion in the PRNP gene. J. Neurol. Sci. 34: 173-176.

Kretzschmar H.A. (1993) Human prion diseases (spongiform encephalopathies). Arch. Virol. Suppl. 7: 261-293.

Kretzschmar H.A., Honold G., Seitelberger F., Feucht M., Wessely P., Mehraein P., Budka H. (1991) Prion protein mutation in family first reported by Gerstmann, Straussler, and Scheinker. Lancet 337: 1160.

Kretzschmar H.A., Kufer P., Riethmuller G., DeArmond S., Prusiner S.B., Schiffer D. (1992) Prion protein mutation at codon 102 in an Italian family with Gerstmann-Straussler--Scheinker syndrome. Neurology 42: 809-810.

Laplanche J-P., Chatelain J., Thomas S., Launay J.M., Gaultier C., Derouesne C. (1992) Uncommon phenotype for a codon 178 mutation of the human PrP gene. Ann. Neurol. 345.

Levine P. (1972) Scrapie: an infective polypeptide? Lancet 1: 748.

Liberski P.P. (1993) The enigma of slow viruses: facts and artefacts. Arch. Virol. Suppl. 6: 1-263.

Liberski P.P., Barcikowska M., Cervenakova L., Bratosiewicz J., Marczewska M., Brown P., Gajdusek D.C. (1998) A case of sporadic Creutzfeldt-Jakob disease with a Gerstmann--Sträussler-Scheinker phenotype but no alterations in the PRNP gene. Acta Neuropathol. 96: 425-430.

Liberski P.P., Bratosiewicz J., Barcikowska M., Cervenakova L., Marczewska M., Brown P., Gajdusek D.C. (2000) A case of sporadic Creutzfeldt-Jakob disease with a Gerstmann--Sträussler-Scheinker phenotype but no alterations in the *PRNP* gene. Acta Neuropathol. (Berl.) 100: 233-234.

Liberski P.P., Gajdusek D.C. (1997) Kuru: forty years later. Brain Pathol. 7: 555-560.

Liberski P.P., Guiroy D.C., Williams E.S., Walis A., Budka H. (2001) Deposition patterns of disease-associated prion protein in captive mule deer brains with chronic wasting disease. Acta Neuropathol. (Berl.) 102: 496-500.

Lindenbaum S. (1979) Kuru sorcery. Disease and danger in the New Guinea Highlands. Mayfield Publishing Company, Palo Alto.

Liu H., Farr-Jones S., Ulyanov N.B., LlinasM., Marquesee S., Groth D., Cohen F.E., Prusiner S.B., James T.L. (1999) Solution structure of syrian hamster prion protein rPrP(90-231). Biochem. 38: 5362-5377.

Lopez Garcia F., Zahn R., Riek R., Wuthrich K. (2000) NMR structure of the bovine prion protein. Proc. Natl. Acd. Sci. USA 97: 83334-8339.

Manson J.C., Jamieson E., Baybutt H., Tuzi N.L., Barron R., McConnell I., Somerville R., Ironside J., Will R., Sy M.S., Melton D.W., Hope J., Bostock C. (1999) A single aminoacid alteration (101L) introduced into murine PrP dramatically alters incubation time of tarnsmissible spongiform encephalopathy. EMBO J. 18: 6855-6864.

Manson J.E., Barron R., Jamieson E., Baybutt H., Tuzi N., McConnell I., Melton D., Hope J., Bostock C. (2000) A single aminoacid alteration in murine PrP dramatically alters TSE incubation time. Arch. Virol. Suppl. 16: 95-102.

Marsh R.F., Bessen R.A., Lehmann S., Hartsough G.R. (1991) Epidemiological and experimental studies on a new incident of transmissible mink encephalopathy. J. Gen. Virol. 72: 589-594.

Marsh R.F., Hartsough G.R. (1985) Is there a scrapie-like diseases in cattle ? In: Proc of the 89th Annu. Meet. of the US Animal Health Ass. p. 8-9.

Masters C.L., Gajdusek D.C., Gibbs C.J. Jr (1981) Creutzfeldt-Jakob disease virus isolations from the Gerstmann-Straussler syndrome. With an analysis of the various forms of amyloid plaque deposition in the virus induced spongiform encephalopathies. Brain 104: 559-588.

Mastrianni J.A., Curtis M.T., Oberholtzer J.C., Prusiner S.B., Garberg J.Y. (1994) Ataxic Gerstmann-Sträussler--Scheinker disease caused by a prion protein gene mutation at codon 117. Neurology 44: A260.

McKinley M.P., Bolton D.C., Prusiner S.B. (1983) A protease resistant protein is a structural component of the scrapie prion. Cell 35: 57-62.

McKinley M.P., Taraboulos A., Kenaga L., Serban D., Stieber A., DeArmond S.J., Prusiner S.B., Gonatas N. (1991) Ultrastructural localization of scrapie prion proteins in cytoplasmmic vesicles of infected cultured cells. Lab. Invest. 65: 622-630.

Medori R., Montagna P., Tritschler H-J., LeBlanc A., Cordelli P., Tinuper P., Lugaresi E., Gambetti P. (1992) Fatal familial insomnia: a second kindred with mutation of prion protein gene at codon 178. Neurology 42: 669-671.

Medori R., Tritschler H-J., LeBlanc A., Villare F., Manetto V., Chen H.Y., Xue R., Leal S., Montagna P., Cortelli P., Tinuper P., Avoni P., Mochi M., Baruzzi A., Hauw J.J., Ott J., Lugaresi L., Autilio-Gambetti L., Gambetti P. (1992) Fatal familial insomnia, a prion disease with a mutation at codon 178 of the prion protein gene. N. Engl. J. Med. 326: 444-449.

Mehlhorn I., Groth D., Stockel J., Moffat B., Reilly D., Yansura D., Willett W.S., Baldwin M., Fletterick R., Cohen F.E., Vandlen R., Henner D., Prusiner S.B. (1996) High-level and characterization of purified 142-residue polypeptide of the prion protein. Biochemistry 35: 5528-5537.

Merz P.A., Rohwer R.G., Kascak R., Wisniewski H.M., Somerville R.A., Gibbs C.J. Jr, Gajdusek D.C. (1984) Infection specific particle from the unconventional slow virus diseases. Science 225: 437-476.

Merz P.A., Somerville R.A., Wisniewski H.M., Iqbal J. (1981) Abnormal fibrils from scrapie-infected brain. Acta Neuropathol. (Berl) 60: 63-74.

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

M'Gowan J.P. (1914) Investigation into the disease of sheep called "scrapie" (Traberkrankheit, La tremblante). With especial reference to its association with Sarcosporidiosis. William Blackwood and Sons, Edinburgh.

Millson G.C., Hunter G.D., Kimberlin R.H. (1976) The physico-chemical nature of the scrapie agent. In: Slow virus diseases of animals and man (Ed. R.H. Kimberlin). North-Holland Publ. Comp., Amsterdam, p. 243-266.

Mishra R.S., Gu Y., Bose S., Verghese S., Kalepu S., Singh N. (2002) Cell surface accumulation of a truncated transmembrane prion protein in GSS P102L. J. Biol. Chem. 2002 (in press).

Mitrova E., Brown P., Hroncova D., Tatra M., Zilak J. (1991) Focal accumulation of CJD in Slovakia: retrospective investigation of a new rural familial cluster. Eur. J. Epidemiol. 7: 487-489.

Miyazono M., Kitamoto T., Doh-Ura K., Iwaki T., Tateishi J. (1992) Creutzfeldt-Jakob disease with codon 129 polymorphism (valine): a comparative study of patients with codon 102 point mutation or whithout mutations. Acta Neuropathol. (Berl) 84: 349-354.

Monari L., Chen S.G., Brown P., Parchi P., Petersen R., Mikol J., Gray F., Cortelli P., Montagna P., Ghetti B., Goldfarb L.G., Gajdusek D.C., Lugaresi E., Gambetti P., Autilio-Gambetti L. (1994) Fatal familial insomnia and familial Creutzfeldt-Jakob disease: different prion proteins determined by a DNA polymorphism. Proc. Natl. Acad. Sci. USA 91: 2839-2842.

Nicholl D., Windl O., de Silva R., Sawcer S., Dempster M., Ironside J.W., Estibeiro J.P., Yuill G.M., Lathe R., Will R.G. (1995) Inherited Creutzfeldt-Jakob disease in a British family associated with a novel 144 base pair insertion of the prion protein gene. J. Neurol. Neurosurg. Psychiatr. 58: 65-69.

Nicholson E.M., Mo H., Prusiner S.B., Cohen F.E., Marqusse S. (2002) Differences between the prion protein and its homolog Doppel: a partially structured state with implications for scrapie formation. J. Mol. Biol. 316:807-15.

Nieto A., Goldfarb L.P., Brown P., McCombie W.R., Trapp S., Asher D.M., Gajdusek D.C. (1991) Codon 178 mutation in ethically diverse Creutzfeldt-Jakob disease. Lancet 337: 662-663.

Nitrini R., Rosemberg S., Passos-Bueno M.R., Teixeira de Silva L.S, Iughetti P., Papadopoulos M., Carrilho P.M.,Caramelli P., Albrecht S., Zatz M., LeBlanc A.

(1997) Familial spongiform encephalopathy asociated with a novel prion protein gene mutation. Ann. Neurol. 42: 138-146.

Oda T., Kitamoto T., Tateishi J., Mitsuhashi T., Iwabuchi K., Haga I., Oguni E., Kato Y., Tominaga I., Yanai K., Kashima H., Kogure T., Hori K., Ogino K. (1995) Prion disease with 144 base pair insertion in a japanese family line. Acta Neuropathol. (Berl.) 90: 80-86.

Oesch B., Westaway D., Walchlii M., McKinley M.P., Kent S.B.H., Aebersold R., Barry R.A., Tempst P., Teplow D.B., Hood L.E., Prusiner S.B., Weissmann C. (1985) A cellular gene encodes scrapie PrP 27-30 protein. Cell 40: 735-746.

Ogihara N.L., Ghirlanda G., Bryson J.W., Gingery M., DeGardo W.F., Eisenberg D. (2001) Design of three-dimensional domain-swapped dimers and fibrous oligomers. Proc. Natl. Acad. Sci. USA 98: 1404-1409.

Olafsson I., Grubb A. (2000) Hereditary cystatin C amyloid angiopathy. Amyloid 7: 70-79.

Owen F., Poulter M., Collinge J., Crow T. (1989) A codon 129 polymorphism in the PrP gene. Nucl. Acid. Res. 18: 3103.

Owen F., Poulter M., Collinge J., Crow T. (1990) Codon 129 changes in the prion protein gene in caucasians. Am. J. Hum. Genet. 46: 1215-1216.

Owen F., Poulter M., Collinge J., Leach M., Shah T., Lofthouse R., Chen Y., Crow T.J., Harding A.E., Hardy J., Rossor M.N. (1991a) Insertions in the prion protein gene in atypical dementia. Exp. Neurol. 112: 240-242.

Owen F., Poulter M., Lofthouse R., Collinge J., Crow T.J., Risby D., Baker H.F., Ridley R.M., Hsiao K., Prusiner S.B. (1989) Insertion in prion protein gene in familial Creutzfeldt-Jakob disease. Lancet 1: 51-52.

Owen F., Poulter M., Shah T., Collinge J., Lofthouse R., Baker H., Ridley R., McVey J., Crow T.J. (1991b) An in-frame insertion in the prion protein gene in familial Creutzfeldt-Jakob disease. Brain Res. Mol. Brain Res. 7: 273-276.

Palmer M.S., Dryden A.J., Hughes J.T., Collinge J. (1991) Homozygous prion protein genotype predisposes to sporadic Creutzfeldt-Jakob disease. Nature 352: 340-342.

Pan K.-M., Baldwin M., Nguyen J., Gasset M., Serban A., Groth D., Mehlhorn I., Huang Z., Fletterick R.J., Cohen F.E,. Prusiner S.B. (1993) Conversion of alpha-helices into beta-sheets features in the formation of the scrapie prion proteins. Proc. Natl. Acad. Sci. USA 90: 10962-10966.

Parchi P., Castellani R., Capellari S., Ghetti B., Young K., Chen S.G., Farlow M., Dickson D.W., Sima A.A., Trojanowski J.Q., Petersen R.B., Gambetti P. (1996) Molecular basis of phenotypic variability in sporadic Creutzfeldt-Jakob disease. Ann. Neurol. 39: 767-778.

Parchi P., Giese A., Capellari S., Brown P., Schulz-Schaeffer W., Windl O., Zerr I., Budka H., Kopp N., Piccardo P., Poser S., Rojiani A., Streichemberger N., Julien J., Vital C., Ghetti B., Gambetti P., Kretzschmar H. (1999) Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. Ann. Neurol. 46: 224-233.

Parry H.B. (1962) Scrapie: a transmissible and hereditary disease of sheep. Heredity 17: 75-105.

Piccardo P., Dlouhy S.R., Lievens P.M , Young K., Bird T.D., Nochlin D., Dickson D.W., Vinters H.V., Zimmerman T.R., Mackenzie I.R., Kish S.J., Ang L.C. ,De Carli C., Pocchiari M., Brown P., Gibbs C.J. Jr., Gajdusek D.C., Bugiani O., Ironside J., Tagliavini F., Ghetti B. (1998) Phenotypic variability of Gerstmann-Sträussler-Scheinker disease is associated with prion protein heterogeneity. J. Neuropathol. Exp. Neurol. 57: 979-988.

Pocchiari M., Salvatore M., Cutruzzola F., Genuardi M., Travaglini Allocatelli C., Massullo C., Macchi G., Alema G., Galgani S., Xi Y.G., Petraroli R., Silvestrini M.C., Brunori M. (1993) A new point mutation of the prion protein gene in Creutzfeldt-Jakob disease. Ann. Neurol. 34: 802-87.

Poulter M., Baker H.F., Frith C.D., Leach M., Lofthouse R., Ridley R.M., Shah T., Owen F., Collinge J., Brown J., Hardy J., Mullan M.J., Harding A.E., Bennett C., Doshi R., Crow T.J. (1992) Inherited prion disease with 144 base pair gene insertion. 1. Genalogical and molecular studies. Brain 115: 675-685.

Prusiner S.B. (1982) Novel proteinaceous infection particles cause scrapie. Science 216: 136-144.

Prusiner S.B. (1984) Some speculations about prions, amyloid, and Alzheimer's disease. N. Engl. J. Med. 310: 661-663.

Prusiner S.B. (1987) Terminology. In: Prions. Novel infectious pathogens causing scrapie and Creutzfeldt-Jakob Disease (Ed. S.B. Prusiner). McKinley MP, Acad. Press, New York, p. 38-53.

Prusiner S.B. (1991) Molecular biology of prion diseases. Science 252: 1515-1522.

Prusiner S.B. (1997) Prion diseases and the BSE crisis. Science 278: 245-251.

Prusiner S.B., Groth D., Serban A., Koehler R., Foster D., Torchia M., Burton D., Yang S-L., DeArmond S.J. (1993) Ablation of the prion protein (PrP) gene in mice prevents scrapie and facilitates of anti-PrP antibodies. Proc. Natl. Acad. Sci. USA 90: 10608-10612.

Prusiner S.B., Hsiao K.K. (1994) Human prion diseases. Ann. Neurol. 35: 385-395.

Prusiner S.B., McKinley M.P., Bowman K.A., Bolton D.C., Bendheim P.E., Groth D.C., Glenner G.G. (1983) Scrapie prions aggregate to form amyloid-like birefringent rods. Cell 35: 349-358.

Prusiner S.B., Scott M., Foster D., P K-M., Groth M., Mirenda C., Torchia M., Yang S-L., Serban D., Carlson G.A., Hoppe P.C., Westeway D., DeArmond S.J. (1990) Transgenic studies implicate interactions between ho-

mologous PrP isoforms in scrapie prion replication. Cell 63: 673-686.

Riek R., Hornemann S., Wider G., Billeter M., Glockshuber R., Wuthrich K. (1996) NMR structure of the mouse prion protein domain PrP (121-231). Nature 382: 180-182.

Riek R., Hornemann S., Wider G., Glockshuber R., Wuthrich K. (1997) NMR characterization of the full-length recombinant murine prion protein mPrP(23-231). FEBS Lett. 413: 282-288.

Ripoll L., Laplanche J-L., Salzmann M., Jouvet A., Planques B., Dussaucy M., Chatelain J., Beaudry P., Launay J-M. (1993) A new mutation in the prion protein gene at codon 210 in Creutzfeldt-Jakob disease. Neurology 43: 1934-1938.

Roche-Lubin (1848) Memoire practique sur la maladie des betes a laine connue sous les noms de prurigo-lombaire, convulsive, trembleuse, tremblante. Recueil. Med. Vet. Pract. 5: 698-714.

Rohwer R. (1986) Estimation of scrapie nucleic acid MW from standard curves for virus sensitivity to ionizing radiation. Nature 320: 381.

Safar J., Roller P., Gajdusek D.C., Gibbs C.J., Jr., (1994) Scrapie amyloid (prion) protein has the conformation characteristics of an aggregated molten globule folding intermediate. Biochemistry 33: 8375-8383.

Safar J., Roller P., Ruben G.C., Gajdusek D.C., Gibbs C.J., Jr. (1993a) Conformational transitions, dissociations and unfolding of scrapie amyloid (prion) protein. J. Biol. Chem. 268: 20276-20284.

Safar J., Roller P., Ruben G.C., Gajdusek D.C., Gibbs C.J., Jr. (1993b) Secondary structure of proteins associated in thin films. Biopolymers 33: 1461-1476.

Safar J., Roller P., Ruben G.C., Gajdusek D.C., Gibbs C.J., Jr. (1993c) Thermal stability and conformational transitions of scrapie amyloid (prion) protein correlate with infectivity. Protein Science 2: 2206-2216.

Scott M., Foster D., Mirenda C., Serban D., Coufal F., Walchli M., DeArmond S.J., Westaway D., Prusiner S.B. (1989) Transgenic mice expressing hamster protein produce species-specific scrapie infectivity and amyloid plaques. Cell 59: 847-857.

Scott M., Groth D., Foster D., Torchia M., Yang S-L., DeArmond S.J., Prusiner S.B. (1993) Propagation of prions with artificial properties in transgenic mice expressing chimeric PrP genes. Cell 73: 979-988.

Scott M., Kohler R., Foster D., Prusiner S.B. (1992) Chimeric prion protein expression in cultured cells and transgenic mice. Protein Sci. 1: 986-997.

Scott M.R., Will R., Ironside J., Nguyen H.O., Tremblay P., DeArmond S.J., Prusiner S.B. (1999) Compelling transgenetic evidence for transmission of bovine spongiform encephalopathy prions to humans. Proc. Natl. Acad. Sci. USA 96: 15137-15142.

Simonic T., Duga S., Strumbo B., Asselta R. (2000) cDNA cloning of turte protein. FEBS Lett. 469: 33-38.

Sparkes R.S., Simon M., Cohn V.H., Fournier R.E.K., Lem J., Klisak I., Heinzmann C., Blatt C., Lucero M., Mohandas T., DeArmond S.J., Westaway D., Prusiner S.B., Weiner L.P. (1987) Assignment of the human and mouse prion protein genes to homologous chromosomes. Proc. Natl. Acad. Sci. USA 83: 7358-7362.

Stahl N., Baldwin M.A., Hecker R., Pan K-M., Burlingame A.L., Prusiner S.B. (1992a) Glycosylinsoitol phospholipid anchors of the scrapie and cellular prion proteins contain sialic acid. Biochemistry 31: 5043-5053.

Stahl N., Borchelt D.R., Hsiao K., Prusiner S.B. (1987) Scrapie prion protein contains a phosphatydylinositol glycolipid. Cell 51: 229-240.

Stahl N., Borchelt D.R., Prusiner S.B. (1992b) Differential release of cellular and scrapie prion proteins from cellular membranes by phosphatidylinositol-specific phospholipase C. Biochemistry 29: 5405-5412.

Strumbo B., Ronchi S., Bolis L.C., Simonic T. (2001) Molecular cloning of the cDNA codong for *Xenopus laevis* prion. FEBS Lett. 508: 170-174.

Sunde M., Serpell L.C., Bartlam M., Fraser P.E., Pepys M.B., Blake C.C.F. (1997) Common core structure of amyloid fibrils by synchrotron X-ray diffraction. J. Mol. Biol. 273: 729-739.

Tagliavini F., Prelli F., Ghiso J., Bugiani O., Serban D., Prusiner S.B., Farlow M.R., Ghett B., Frangione B. (1991) Amyloid protein of Gerstmann-Straussler-Scheinker (Indiana kindred) is an 11 kd fragment of prion protein with an N-terminal glycine at codon 58. EMBO J. 10: 513-519.

Tateishi J., Brown P., Kitamoto T., Hogue ZM, Roos R., Wollman R., Cervenakova L., Gajdusek D.C. (1995) First experimental transmission of fatal familial insomnia. Nature 376: 434-435.

Telling G.C., Haga T., Torchia M., Tremblay P., DeArmond S.J., Prusiner S.B. (1996) Interactions between wild-type and mutant prion proteins modulate neurodegeneration in transgenic mice, Genes Develop., 10: 1736-1750.

Telling G.C., Parchi P., DeArmond S.J., Cortelli P., Montagna P., Gabizon R., Mastrianni J., Lugaresi E., Gambetti P., Prusiner S.B. (1996) Evidence for the conformation of the pathologic isoform of the prion protein enciphering and propagating prion diversity. Science 274: 2079-2082.

Telling G.C., Scott M., Hsiao K.K., Foster D., Yang S.-L., Torchia M., Sidle K.C.L., Collinge J., DeArmond S.J., Prusiner S.B. (1994) Transmission of Creutzfeldt-Jakob disease from humans to transgenic mice expressing chimeric human-mouse prion protein. Proc. Natl. Acad. Sci., USA 91: 9936-9940.

Telling G.C., Scott M., Mastriannni J., Gabizon R., Torchia M., Cohen F.E., DeArmond S.J., Prusiner S.B. (1995) Prion propagation in mice expressing human and chimeric PrP transgenes implicates the interaction of cellular PrP with another protein. Cell, 83 79-90.

Van Gool W.A., Hensels G.W., Hoogerwaard E.M., Wiezer J.H.A, Wesseling P., Bolhuis F.A. (1995) Hypokinesia and presenile dementia in a Dutch family with a novel insertion in the prion protein gene. Brain 118: 1565-1571.

Wadsworth J.D., Hill A.F., Joiner S., Jackson G.S., Clarke A.R., Collinge J. (1999) Strain-specific prion-protein conformation by metal ions. Nature Cell Biol. 1: 55-59.

Weissmann C. (1989) Sheep disease in human clothing. Nature 338: 298-299.

Weissmann C. (1991) A "unified theory" of prion propagation. Nature 352: 679-683.

Wells G.A.H., Scott A.C., Johnson C.T., Gunning R.F., Hancock R.D., Jeffrey M., Dawson M., Bradley R. (1987) A novel progressive spongiform encephalopathy in cattle. Vet. Rec. 121: 419-420.

Westaway D., DeArmond S.J., Cayetano-Canlas J., Groth D., Foster D., Yang S-L., Torchia M., Carlson G.A., Prusiner S.B. (1994) Degeneration of skeletal muscle, peripheral nerves, and the central nervous system in transgenic mice overexpressing wild-type prion protein. Cell 76: 117-129.

Westaway D., Goodman P.A., Mirenda C.A., McKinley M.P., Carlson G.A., Prusiner S.B. (1987) Distinct prion proteins in short and long scrapie incubation period mice. Cell 51: 651-662.

Westaway D., Mirenda C., Foster D., Zebaradjan J., Scott M., Torchia M., Yang S-L., Serban H., DeArmond S.J., Ebeling C., Prusiner S.B., Carlson G.A. (1991) Paradoxical shortening of scrapie incubation times by expression of prion protein transgenes derived from long incubation period mice. Neuron 7: 59-68.

Westaway D., Prusiner S.B. (1986) Conservation of the cellular gene encoding the scrapie prion protein. Nucl. Acid. Res. 14: 2035-2044.

Will R.G., Ironside J.W., Zeidler M., Cousens S.N., Estibeiro K., Alperovitch A., Poser S., Pocchiari M., Hofman A., Smith P.G. (1996) A new variant of Creutzfeldt-Jakob disease in the UK. Lancet 347: 921-925.

Wille H., Michelitsch M.D., Gunebaut V., Supattapone S., Serban A., Cohen F., Agard D.A., Prusiner S.B. (2002) Structural studies of the scrapie prion protein by electron crystallography. Proc. Natl. Acad. Sci. USA 99: 3563-3568.

Williams E.S., Miller M.W. (2002) Chronic wasting disease in deer and elk in North America. Rev. Sci. Tech. 21: 305-316.

Williams E.S., Young S. (1980) Chronic wasting disease of captive mule deer: a spongiform encephalopathy. J. Wildl. Dis. 16: 89-98.

Williams E.S., Young S. (1982) Spongiform encephalopathy in a Rocky Mountain elk. J. Wildl. Dis. 18: 465-471.

Willoughby K., Kelly D.F., Lyeon D.G., Wells G.A.H. (1992) Spongiform encephalopathy in a captive puma (*Felis concolor*). Vet. Res. 131: 431-434.

Wilson D.R., Ruth D., Anderson R.D., Smith W. (1950) Studies in scrapie. J. Comp. Pathol. 60: 267-282.

Wood J.L.N., Lund L.J., Done S.H. (1991) The natural occurrence of scrapie in moufflon. Vet. Rec. 130: 25-27.

Wyatt J.M., Pearson G.R., Smerdon T., Gruffyd-Jones T.J., Wells G.A.H. (1990) Spongiform encephalopathy in a cat. Vet. Rec. 126: 513-132.

Zahn R., Liu A., Luhr T., Riek R., von Schroetter C., Garcia F.L., Billeter M., Calzolai L., Wider G., Wuthrich K. (2000) NMR solution structure of the human prion protein. Proc. Natl. Acad. Sci. 97: 145-150.

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A COMMENTARY ADDED IN PROOF

Is the final proof of the prion hypothesis obtained?

A paper in the last issue of Proc. Natl. Acad. Sci. USA (Maddelein et al. 2002; Liebman 2002) came closer to be accepted as an ultimate proof of prion hypothesis (Weissmann 2002). Did it? These authors used the [Het-s] "prion" element (see Table I) of the filamentous fungus *Podospora anserina* to attempt to proove that during formation of fungal "prion protein" a new "infectivity" is generated *de novo.* As mentioned in a previous review, PrP^{Sc} may be generated *in vitro* (Kocisko et al. 1994) but the new infectivity is not (Hill et al. 1999).

Podospora fungus does not grow as individual cells but as a scyncytium (the *mycelium*), in which cells are connected by cytoplasmic tonques; in other words, anything which arrives in the single cell spreads rapidly through the entire mycelium. Furthermore, the elegant system of microprojectile bombardment (biolistic) was developed to insert the given protein into the cell via tungsten "micromissiles". In this system, recombinant [HET-s]-prion protein was inserted into the mycelium of this fungus and the infectivity was generated *de novo* from r[HET-s]-protein (Fig. 1). Recombinant [HET-s] converted [HET-s^*] into [HET-s] and the [Het-s] prion state was generated w ith 87-99% efficiency. Furthermore, the conversion activity of [HET-s] was associated with its aggregated fibrillar form and this activity is proteinase-resistant.

Is that all? Not really. The clue to the interpretation of these experimental data is encoded within the meaning of the term "infectious". Maddelein et al. used this term to denote the ability to convert normal [HET-s*] into its

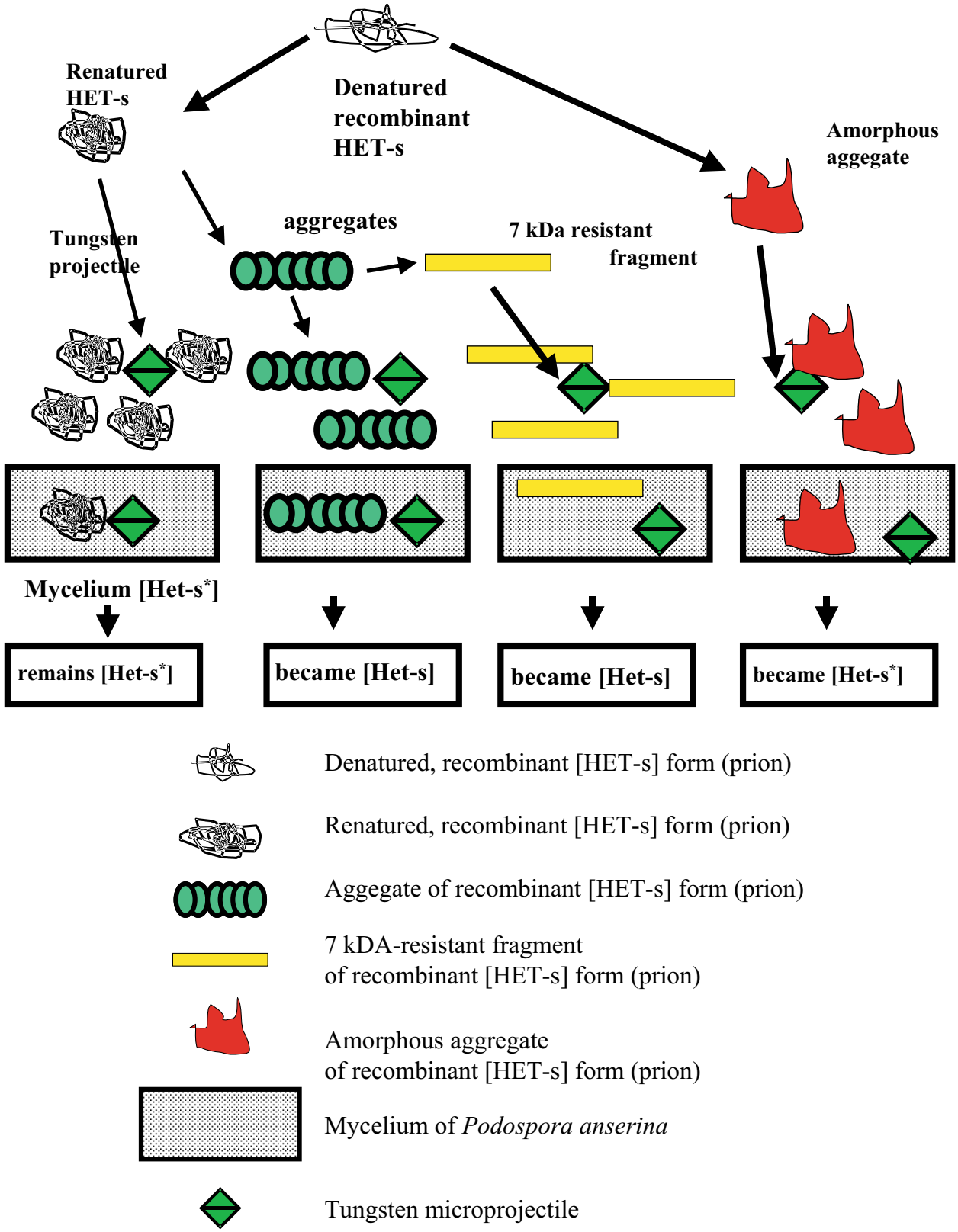

Fig. 1. Schematic view of HET-s* conversion by HET-s into HET-s (prion form). According to Maddelein et al. (2002); modified.

Table I

Nomenclature for the prion element of *Podosporaanserina* (Maddelein et al. 2002)

Alleles		
	Het-s	encodes a protein that can exist in both prion and nonprion state
	Het-S	encodes a protein that cannot exist in the prion state
Proteins		
	HET-s	prion isoform encoded by *Het-s*
	HET-s*	nonprion isoform encoded by *Het-s*
	HET-S	nonprion isoform encoded by *Het-S*
Phenotypes[&]		
	[Het-s]	prion state; converts [Het-s*] to [Het-s] by cytoplasmic mixing; incompatible with [Het-S]
	[Het-s*]	nonprion state; converted to prion state [Het-s] by cytoplasmic mixing with [Het-s]; compatible with both [Het-s] and [Het-S]
	[Het-S]	incompatible with [Het-s]

[&][Het-s] and [Het-s*] phenotypes are defined by the compatibility of cell fusion with the [Het-S] state.

prion form [HET-s] and this effect was indeed demonstrated. It is equivalent to the conversion of PrP^{c} into PrP^{Sc} in a cell-free *in vitro* system (Kocisko et al. 1994). However, the real infectivity (as it is understood in microbiology) could not be demonstrated in the fungal system as there is neither a disease of Podospora anserina caused by the prion form [Het-s] to look at nor an independent measurement o such infectivity analogous to end-point titration or even the transmission to transgenic mice. In conclusion, while Maddelein et al. elegantly demonstrated that conversion indeed takes place in Podospora, demonstration of the generation of infectivity *de novo* is yet to be achieved.

Hill A.F., Antoniou M., Collinge J. (1999) Protease-resistant prion protein produced *in vitro* lacks detectable infectivity. J. Gen. Virol. 80: 11-14.

Kocisko D.A., Come J.H., Priola S.A., Chesebro B., Raymond G.J., Lansbury P.T., Caughey B. (1994) Cell-free formation of protease-resistant prion protein. Nature 370: 471-4.

Liebman S.W. (2002) Progress toward an ukltimate proof of the prion hypothesis. Proc. Natl. Acad. Sci. USA 99: 9098-9100.

Maddelein M-L., Reis S.D., Duvezin-Caubet S., Coulary-Salin B., Saupe S. (2002) Amyloid aggregates of the HET-s prion protein are infectious. Proc. Natl. Acad. Sci. USA 99: 7402-7407.