Histol Histopathol (1997) 12: 583-594

Histology and Histopathology

Invited Review

Molecular clues to pathogenesis in prion diseases

M. Laurent and G. Johannin

Service d' Imagerie Cellulaire, Université Paris-Sud, Orsay Cedex, France

Summary. The infectious agent of the transmissible spongiform encephalopathies (TSE) resembles a virus in that it propagates *in vivo* and has distinct strains. However, compelling evidence strongly suggests that a posttranslational structural alteration in a glycoprotein PrP^{C} (the normal, cellular isoform of the so-called prion protein) is responsible for pathogenesis of these diseases. According to this hypothesis - now close to being generally accepted -, iatrogen, sporadic and familial forms of TSE would have the same molecular mechanism: the conversion of PrP^{C} into a protease-resistant isoform PrP^{Sc} kinetically behaves as an autocatalytic process which, combined with the high turnover rate of the normal isoform, may endow the system with bistability properties and subsequent threshold behavior between normal and pathogenic steady-states. Normal prion protein seems to be necessary for long-term survival of Purkinje neurons, regulation of circadian rhythms and, more controversially, for normal synaptic function. At least part of the pathology might be due to the unavailability of normal isoform rather than to the accumulation of PrP^{Sc}. NMR structure of the normal mouse prion protein reveals a short, unexpected ß-sheet which might be a nucleation site for the conformational transition between PrP^{C} and PrP^{Sc}. Prion diseases may challenge the edged distinction that we use to make between informational (DNA) and functional (proteins) macromolecules. Pathogenic mechanism of prions might also be involved in other proteins to achieve and pass on their conformation. Hence, structural inheritance at the molecular level might be the missing link for the understanding of the structural inheritance processes featured at the cellular level. Moreover, evolutionary paradigm postulating a primitive RNA world is weakened by the mechanism of prion diseases.

Key words: Prion diseases, Prion protein, Spongiform encephalopathies, Bistability, Structural inheritance

Offprint requests to: Dr. Michel Laurent, Service d'Imagerie Cellulaire, URA 116 CNRS, Bât 440, Université Paris-Sud, 91405 Orsay Cedex, France

Introduction

Although recent and public developments about 'mad cow disease' and Creutzfeldt-Jakob disease go far beyond purely scientific concern, it would be wrong to believe that prion diseases - which is the generic name for all these transmissible neurodegenerative diseases - have been only recently discovered. The first case of a sheep which presented all the symptoms of scrapie (excitability, itching, ataxia and finally paralysis and death) was recognized in the middle of the 18th century (Reibel, 1994; Weissmann, 1994a). Of course, other unidentified cases might have occurred long before. Scrapie is the prototype of what has proved to be a group of lethal diseases affecting animals such as sheep, cows, minks, moufflons, ostriches, cheetahs, cats and also humans (Reibel, 1994). In 1936, Cuillé and Chelle showed that scrapie was due to an agent found in the brain and in the spinal chord of diseased animals and that the disease could be transmitted to sheep and goats by inoculating them with the lombard cord of affected animals (Weissmann, 1994a). This agent was shown early on to be non-conventional because it has particular properties such as uncommon resistance to treatments that usually destroy nucleic acids found in viruses and bacteria. Moreover, incubation periods for the disease are extraordinarily long (measured in months in animals and years in man). In 1982, Prusiner proposed naming this agent as 'prion' to distinguish it from conventional pathogenic agents such as bacteria and viruses (Prusiner, 1982).

In humans, four slow degenerative diseases of the central nervous system have been described: kuru; Creutzfeldt-Jakob disease (CJD); Gerstmann-Sträussler-Scheinker disease (GSS); and fatal familial insomnia (FFI). Kuru, historically contracted at the beginning of this century in Papua New Guinea's Fore highlanders, may have originated from the consumption of the remains of a CJD sufferer (Dormont and Bursaux, 1996) and was propagated through ritual cannibalism. Inoculation studies by Gajdusek's group (Gajdusek et al., 1966) had demonstrated the infectious nature of kuru and then of CJD and GSS by transmitting the disease to chimpanzees through the injection of diseased brain

tissue. The infectious nature of FFI was shown much more recently (Tateishi et al., 1995). Although incubation periods are particularly long, once the first clinical symptoms such as loss of memory or motor disturbances appear, progression to death may take only a few months. The pathological changes vary in location and intensity but the brain tissue typically develops spongelike holes as neurons die while glia proliferate. No inflammatory nor immunological counterpart is observed.

The current epizooty of bovine spongiform encephalopathy (BSE or 'mad-cow disease') which principally occurs in Great Britain, is believed to result from a change in the process of making feed supplements given to cattle. These supplements coming from cattle (50%) and sheep (15%) offal were probably contaminated by a scrapie-like agent. In 1980, the stopping of the fat-extracting process by organic solvents probably increased contamination of the feed supplements. Hence, at least in the corresponding species, the agent responsible for ESB and kuru can diffuse through the digestive tract. Kuru has almost disappeared with the cessation of ritual cannibalism, strongly suggesting that this disease was transmitted orally, as proposed for BSE. Iatrogen forms of CJD have been observed as a result of the use of contaminated surgical material, cornea or eardrum transplants or therapeutic use of hypophyse-derivated products from human origin. However, prion diseases may also arise spontaneously without any apparent cause (so-called sporadic) or be genetic (5 to 15% of CJD are thought to be familial). Whatever its iatrogen, or sporadic or familial origin, the disease can usually be transmitted to animals by intracerebral inoculation. The possibility of a vertical transmission (from the cow to the calf for instance) is as yet hypothetical, although such an occurrence has been reported in the case of captive mule deer and elk in zoological gardens (Reibel, 1994). However, much of the current upheavals come from a recent report (Will et al., 1996) indicating that ten cases of CJD were detected in Great Britain whose clinical and pathological features seem significantly distinct from those of the common (although rare) disease, especially concerning the fact that the patients were unusually young. Moreover, clinical symptoms (with more ataxia than dementia) and histo-immunochemical features seem to place these new cases closer to kuru or iatrogen CJD rather than sporadic CJD. Correlatively to the current epizooty of BSE and the long incubation periods of prion diseases, these data make us wonder if these human cases might be linked to eating BSE-tainted beef. In any case, many intriguing questions about the molecular mechanism of prion diseases remain to be solved before an answer can be unambiguously given concerning the problem of the species barrier betweeen animals and humans. Beyond the important problems of public health, spongiform encephalopathies are of fundamental interest for biologists because they might come to challenge the current dogma of molecular biology concerning the edged distinction that we make between informational and functional macromolecules, namely DNA and proteins respectively.

Nature of the transmissible agent

The unsual properties of prion diseases gave rise early to speculations about the nature of the transmissible agent responsible for neurodegenerative diseases. In the 60s, the leading theory supposed that an unidentified, slow-acting virus was the real culprit. Although viruses can be very hard to find, no-scrapie-associated virus or nucleic acid has ever turned up, in spite of many attempts to find traces of it, during the last 30 years (Kellings et al., 1992). However, Alper's experiments (Alper et al., 1966, 1967) weakened this theory by showing that scrapie infectivity resists inactivation by both ultraviolet and ionizing radiations, which usually destroy nucleic acids. The possibility that the scrapie agent might be devoid of nucleic acid was reinforced by Prusiner's group who later showed that reagents - such as nucleases, psoralens, hydroxylamine and Zn^{2+} ions - that specifically modify or damage nucleic acids, do not alter scrapie infectivity in homogenates or partially purified preparations (Diener et al., 1982; McKinley et al., 1983; Gabizon et al., 1987). Although it is known that some viruses can resist treatments that normally destroy nucleic acids, two additional observations go against the viral hypothesis. Modern subtraction hybridization experiments were unable to reveal the presence of DNA or RNA sequences in infected brain tissues which would be absent in normal ones (Mestel, 1996). Moreover, the occurrence of some familial prion diseases makes it much more unlikely to be a virus, without definitively excluding such a possibility. One would have to imagine that although the virus would infect all the members of the family, only those who have a putative mutation would develop the disease.

On the other hand, there is now a wealth of biochemical and genetic evidence supporting the contention that a protein (so-called prion protein or PrP) is the key for infectivity. Prusiner first reported the existence in scrapie brains of a protein that was required for infectivity and that did not seem to be present in healthy brains (Prusiner et al., 1981; Prusiner, 1982). According to a scenario originally outlined by Griffith (1967), this protein (so-called PrP^{Sc}, Sc for scrapie) might direct its own replication. The fact that the prion protein was encoded by a chromosome gene (and not by a virus) was suggested by the observation that the level of PrP messenger RNA remained unchanged throughout the course of scrapie infection (Oesch et al., 1985; Basler et al., 1986). This result led to the identification of the normal gene product, a protein designated PrP^{C}, and also to the determination of its primary structure, in hamsters and mice (and later in other mammal species) by sequencing of molecular clones recovered from cDNA libraries (Chesebro et al., 1985; Oesch et al.,

1985; Basler et al., 1986; Locht et al., 1986). The amino acid sequences of both the normal PrP^{C} and pathological PrP^{Sc} proteins were found to be identical. The direct implication of the PrP protein in infectivity was demonstrated by several groups, using distinct types of evidence: Weissman showed that "knockout" mice lacking the *PrP* gene are resistant to scrapie and fail to propagate the infectious agent when they are inoculated with diseased brain tissue from the same species (Büeler et al., 1992). Moreover, they showed that susceptibility to scrapie is a function of PrP^{C} levels in the host (Büeler et al., 1993). Hsiao et al. (1990) showed that transgene mice overexpressing the PrP gene with a mutation corresponding to that found in the familial form of human GSS, spontaneously contract a lethal scrapie-like disease. Finally, Bessen et al. (1995) took advantage of the fact that two hamster-adapted mink strains give rise to two distinguishable PrP^{Sc} molecular species (so-called strain-specific species). They found that in a cell-free system, PrP^{Sc} from the two strains could convert the same PrP^{C} protein into two distinct sets of products that exhibit the same physico-chemical properties as those of natural PrP^{Sc} molecules associated with the two strains. In the same vein, it has been shown that synthetic peptides which mimic some of the conformational features of PrP^{Sc}, are able to induce cellular PrP to acquire properties of the scrapie isoform (Kaneko et al., 1995). Even if it has not yet been established that the *in vitro* converted form of the protein can also generate infectivity, these results support the idea that the pathogenic isoform PrP^{Sc} of the prion protein may impose its conformation upon the native protein PrP^{C}. Today, although some authors still believe that the infectious agent is a specific nucleic acid associated with or packaged in some host-derived protein (Manuelidis et al., 1987; Xi et al., 1992; Mestel, 1996), the most accepted theory in the field - known as 'the protein-only' hypothesis - postulates that the conversion of the constitutive host protein PrP^{C} to a pathogenic, isomeric form PrP^{Sc} constitutes the molecular basis of prion diseases.

PrP gene and protein

The entire open reading frame of *PrP* gene is contained within one exon (Basler et al., 1986), localized in the short arm of human chromosome 20 and the homologous region of mouse chromosome 2. This suggests that the *PrP* gene probably exists in the common ancestor of mammals and its appearance thus precedes the speciation of mammals. Accordingly, DNA sequences related to a PrP cDNA clone have been detected in invertebrates including nematode, drosophila and possibly yeast (Westaway and Prusiner, 1986). Phylogenetic relationships among the *PrP* gene of vertebrates (Krakauer et al., 1996) show that the ancestor of the hominoids (excluding the orangutan) and of cattle (*Bos taurus*) uniquely shared (among 33 species of vertebrates so far analyzed) two pairs of derived substitutions (Tyr to His at site 155 and Asn to Ser at site 143). The fact that this event is the only one of its kind in the gene tree might suggest that these changes could also have predisposed humans towards a prion strain present in cattle. However, since correlation is not causation, this observation does not demonstrate that BSE can be transmitted to humans.

The PrP protein is synthetized as a precursor of 254 amino acids in Syrian hamsters and mice or 253 amino acids in humans. An N-terminal signal sequence of 22 amino acids is cleaved in the endoplasmic reticulum (Hope et al., 1986; Turk et al., 1988; Safar et al., 1990) and 23 amino acids are removed from the COOH terminus on addition of a glycosyl-phosphatidylinositol (GPI) anchor at Ser 231 (Stahl et al., 1987, 1990a). Two additional glycosylations occur at Asn 181 and Asn 197 respectively and a disulphide bond is formed between Cys 179 and Cys 197 (Fig. 1). NMR three-dimensional structure of the mouse PrP domain 121-131 (Riek et al., 1996) reveals that the protein contains three α-helices and a two-stranded antiparallel β-sheet. These results disagree with previous model calculations (Huang et al., 1994) that predicted a four helix bundle model without any β-structured element in the corresponding domain of the normal prion protein. The second and third α-helix are linked by the single disulphide bond in the protein and their twisted V-shape forms the scaffold onto which the first helix and the short β-sheet are anchored (Fig. 2). Since it has been proposed (Pan et al., 1993; Huang et al., 1996) that the transition from PrP^{C} to PrP^{Sc} is

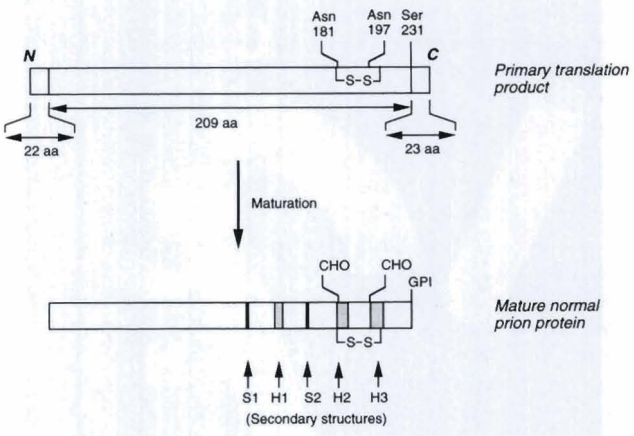

Fig. 1. Biosynthesis and maturation of the normal prion protein. Maturation of the precursor of the prion protein involves three distinct events: cleavage of the sequence signal at the N-terminus, glycosylation (CHO) at Asn 181 and Asn 197 and removal of 23-C-terminal aminoacids on addition of a glycosyl-phosphatidylinositol (GPI) anchor at Ser 231. A disulphide bond is formed between Cys 179 and Cys 214. Positions of the regular secondary structures (H, α-helice and S, b-strand), are shown for the mature mouse prion protein: S1 (128-131), S2 (161-164), H1 (144-154), H2 (179, 193) and H3 (200-217). Calculations have not yielded conclusive results (Huang et al., 1994) for the segment 23-108, which contains the prion-characteristic octapeptide

accompanied by an increase in the β-sheet content of the protein, it seems tempting to speculate that the short β-sheet found in the normal protein might be a nucleation site for a conformational transition that could include the loop connecting the β-sheet to the first helix which is essentially hydrophobic.

Primary sequences of normal PrP^{C} and infectious PrP^{Sc} isoforms of the prion protein are indistinguishable but the two forms differ in their physicochemical properties and in their secondary structure (Pan et al., 1993; Stahl et al., 1993; Cohen et al., 1994), although the NMR or cristallographic three-dimensional structure of the PrP^{Sc} isoform has not yet been observed. In particular, PrP^{Sc} is partially resistant to proteinase K under conditions where PrP^{C} is readily cleaved and solubilized (Meyer et al., 1986). More precisely, proteinase K only removes 23 amino acids from the NH_2 terminus of the PrP^{Sc} isoform, leading to a 27-30 kD fragment which posseses all the characteristics of infectivity. Moreover, PrP^{Sc}, unlike PrP^{C}, gives rise to cerebral amyloid formation, a highly ordered protease-resistant aggregate characterized by its insolubility and fibrillar structure (Alper et al., 1967; Jarrett and Lansbury, 1993). The resistance of PrP^{Sc} to proteinase K treatment was found for all the forms of transmissible spongiform encephalopathies. Although no chemical difference has been detected so far between PrP^{C} and PrP^{Sc}, its possible occurrence cannot be completely excluded because the ratio of infectious units to PrP^{Sc} molecules is about 1:100000 (Bolton et al., 1991),

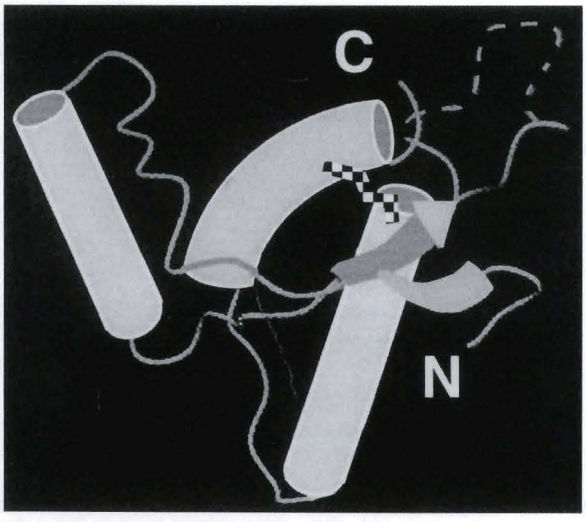

Fig. 2. Three-dimensional structure of the mouse prion domain PrP(121-231) as determined by NMR. Ribon diagram indicating the positions of the three α-helices (cylinders) and the antiparallel two-stranded ß-sheet (arrows). The second and third helices are linked by a disulphide bond (checkered pattern). Dashed lines indicate structures that are not well defined (adapted from Riek et al., 1996). The first α-helice might be part of a single binding site for the pathogenic isoform PrP^{Sc}, and sequence differences between species in this part of the structure might be involved in the barrier of prion disease transmission between species.

making undetectable a putative difference between PrP^{C} and any infectious subspecies of PrP^{Sc}.

Is it the depletion of PrP^{C} or the accumulation of PrP^{Sc} that is responsible for neurotoxicity in prion diseases?

Normal prion protein is constitutively expressed at the outer surface of the plasma membrane, where it is anchored by its GPI moiety (Stahl et al., 1987, 1990a,b). The physiological function of the normal host protein PrP^{C} has long been obscure and still remains controversial. Although substantial amounts of normal PrP protein are found not only in brain but also in heart and skeletal muscle (Büeler et al., 1992) and, to a lesser extent, in most other organs except liver and pancreas (Weissmann, 1994a), it is quite justified to search for the putative function of the normal prion protein in neuron cells since prion diseases are characterized principally by neurodegenerative disorders, although pathological changes in peripheral organs have also been reported in scrapie-infected animals (Ye and Carp, 1995).

Uninoculated old mice harboring high copy numbers of wild-type PrP transgenes were found to develop truncal ataxia, hindlimb paralysis, and tremors (Westaway et al., 1994). The normal PrP protein was claimed to be necessary for normal synaptic function (Collinge et al., 1994), although other findings report that the loss of the normal form of prion protein does not alter neuronal excitability and synaptic transmission in the hippocampus in mice (Lledo et al., 1996). Tobler et al. (1996) showed that mice devoid of normal prion protein have altered circadian activity rythms and sleep. Hence, one can wonder if FFI, one of the inherited prion diseases in human for which similar alterations in sleep and circadian rythms of many hormones are observed, might be related to the normal function of the prion protein rather than to a dysfunction resulting from the accumulation of the PrP^{Sc} isoform. Furthermore, mice homozygous for a disrupted *PrP* gene grew normally after birth (histology of the cerebellum is still normal at 18 weeks and may be so later) but they began to show, at about 70 weeks of age, progressive symptoms of ataxia and motor disturbances correlated with an extensive loss of Purkinje neurons (Sakaguchi et al., 1996). These results disagree with previous studies performed with other PrP null-mice (Büeler et al., 1992, 1993) which exhibit no ataxic phenotype at up to 93 weeks. However, differences related to breeding conditions or to the embryonic stem cell lines used or to the transfection process may explain that in some transgenic mice, susceptibility to prion is abrogated, but that some physiological functions of normal PrP protein are retained. Hence, if the results of Sakaguchi et al. (1996) are confirmed, they would indicate that the loss of PrP and not the accumulation of the PrP^{Sc} isoform should be the primary cause of the death of Purkinje cells, at least in PrP null-mice. In any case, pathogenic effects resulting from the absence of the normal isoform of the

protein should appear only after a very prolonged time delay. However, one fundamental question would remain unexplained in such a case: why incubation time is much shorter in scrapie-infected non-transgenic mice than in PrP null-mice. If PrP^{Sc} is pathogenic only because it promotes conversion of PrP^{C} to PrP^{Sc} and subsequently diminishes the level of available normal PrP^{C} isoform, delayed onset of disease would have to be observed in scrapie-infected mice compared to PrP null-mice. Hence, even if the loss of PrP^{C} may contribute to the scrapie histopathology and disease, it is probable that PrP^{Sc} also has, directly or by acting synergically in a complex with PrP^{C}, a route of pathogenicity that is distinct from that resulting from the loss of the normal function of the PrP protein. According to this possibility, it was shown (Westaway et al., 1994) that overexpression of wild-type PrP^{C} in uninoculated transgenic mice is pathogenic, but that the novel neurological syndrome observed in these cases (necrotizing myopathy involving skeletal muscle, demyelinating polyneuropathy and focal vacuolation of the central nervous system) widens the spectrum of prion disease pathogenesis.

The role, in scrapie pathology, of the loss of the normal function of the PrP^{C} protein was also studied by grafting experiments recently performed by Weissmann and Aguzzi's groups (Brandner et al., 1996; Isenmann et al., 1996). They grafted neural tissue overexpressing PrP^{C} into brains of knockout mice for the PrP gene, i.e. in mice which are resistant to scrapie and do not propagate infectivity. The mice were then infected with scrapie prions by intracerebral inoculation. The grafted tissue accumulated high levels of PrP^{Sc} associated with typical histopathological changes of scrapie and it became infectious. However, although important amounts of PrP^{Sc} migrated from the grafted tissue to the host cerebral tissue, no pathological change was observed in this host tissue 16 months after inoculation. Hence, brain tissue devoid of normal PrP^{C} protein, in addition to being resistant to scrapie infection, is not damaged by exogenous PrP^{Sc}. Once again, these results disagree with those of Sakaguchi et al. (1996) on the particular point concerning the long-term survival of Purkinje neurons in PrP-null mice. Most importantly, they seem to indicate that it is not the accumulation of PrP^{Sc} but rather the unavailability of normal PrP^{C} isoform, for some unknown intracellular process that is directly linked to spongiosis and neural death. However, one cannot exclude that graft-derivated PrP^{Sc} which have migrated in the host brain cannot be internalized in PrP null-cells, especially if PrP^{Sc} can be endocytosed only by way of association with PrP^{C}. Therefore, it is not possible to conclude at this time that PrP^{Sc} is inherently non-toxic, particularly in mice that are not devoid of the PrP gene.

The 'protein only' hypothesis and its dynamic consequences

Prusiner, Weissmann and Caughey conducted many of the crucial experiments which make the 'protein only' hypothesis, originally outlined by Griffith (1967), a more and more solid hypothesis for prion diseases. The agent responsible for the disease would be the modified form PrP^{Sc} of the normal host prion protein PrP^{C}, the pathogenic form PrP^{Sc} multiplying by converting the normal form into a copy of itself (Griffith, 1967). Normal host protein PrP^{C} and modified form PrP^{Sc} are believed to be conformational isomers. According to the above discussion, we do not know yet if PrP^{Sc} is intrinsically pathogenic or whether its pathogenicity results from the fact that the conversion mechanism renders the normal host isoform PrP^{C} unavailable, or whether accumulation of PrP^{Sc} and loss of PrP^{C} both contribute, in kinetically distinct ways, to pathogenecity.

Two alternative mechanisms have been proposed for the conversion of PrP^{C} to PrP^{Sc} in prion strains. The 'nucleation model' (Gajdusek, 1988; Jarret and Lansbury, 1993; Kocisko et al., 1994, 1995) supposes that several PrP^{Sc} monomers polymerize to form a nucleus. Once the nucleus has been formed, further accretions occur through the binding of PrP^{C} to the polymer, a conformational rearrangement (PrP^{C}-PrP^{Sc}) taking place to adjust the incoming molecule PrP^{C} to the template. The trapping of PrP^{C} would be an essentially irreversible aggregation process which would drive the bulk conversion reaction. The alternative model known as the 'refolding model' (Prusiner, 1991) proposes that constitutive PrP^{C} monomers would be unfolded to some extent and subsequently refolded under the influence of PrP^{Sc} molecules (Fig. 3). As an example, Liautard suggested that prions could be misfolded molecular chaperones (Liautard, 1991, 1992). In both models, the pathogenic, conformational isomer PrP^{Sc} would thereby impose its conformation upon the native protein PrP^{C} in a globally autocatalytic process requiring the presence of preexisting PrP^{Sc} (Weissmann, 1995). According to Come et al. (1993), the heterodimer PrP^{C}-PrP^{Sc} would act as a catalyst for the conversion.

Most, if not all, inherited prion diseases in humans (which are autosomal dominant disorders) are linked to one of a number of mutations in the PrP gene. For Prusiner (1991), the mutations increase the frequency of the spontaneous conversion of PrP^{C} into PrP^{Sc}, allowing the expression of the disease to occur within the lifetime of the individual. NMR structure of the prion protein (Riek et al., 1996) reveals that all six residues of the domain PrP(121-231) for which mutation is believed to be associated with familial prion diseases (or predisposition to prion diseases for those who think that the PrP protein is not the true infectious agent) are located in (or close to) regular secondary structure elements (except in the first helix). Mutation of one of these residues might therefore destabilize the three-dimensional structure of the protein or modify its ligand-binding properties. Sporadic CJD would also arise from the spontaneous conversion of prion protein from the normal to the pathogenic isoform, due either to a *PrP* gene somatic mutation or to rare instances involving

modification of wild-type PrP^C protein. In both cases the initial conversion is thought to be followed by autocatalytic propagation (Weissmann, 1994a).

To understand how the prion invasion accords with the 'protein only' hypothesis, two linked phenomena have to be considered: 1) The $PrP^C \rightarrow PrP^{Sc}$ conversion is an autocatalytic process. 2) In a cell, the normal prion protein PrP^C turns over whereas PrP^{Sc} isoform does not (Fig. 4). This is precisely one of the important differences that was evidenced between the two isoforms of the protein. Normal PrP^C undergoes endocytosis (Caughey et al., 1989; Borchelt et al., 1990) and possibly recycling (Harris et al., 1993). The synthesis and degradation of PrP^C are rapid with half-life estimated to 0.1 and 5 hours, respectively (Caughey et al., 1989; Borchelt et al., 1990). In contrast, no degradation pathway is known for the PrP^{Sc} isoform and that is probably why this molecular species accumulates in cells when it is formed.

Theoretical kinetic analysis of the dynamic process including the turnover of the normal prion protein (Laurent, 1996a,b) shows that the system exhibits bistability properties, indicating that the very slow accumulation of abnormal form of the protein in the brain would in fact be the consequence and not the cause of the disorders. The cause would be a transition between two alternate, stable steady states of the system. The two alternate steady states are associated, respectively, with low (normal steady state) and high (pathogenic steady state) stationary concentrations of the pathogenic isoform PrP^{Sc} of the prion protein (Fig. 5). Dynamics of prion infection differs from those of virus replication, healthy organisms being able to eliminate spontaneously infrathreshold amounts of foreign PrP^{Sc} protein. Under controlled conditions, addition of suprathreshold quantities of PrP^{Sc} or also, paradoxically, of the normal PrP^C protein, provokes a transition towards the pathogenic steady-state. Hence, the possible presence of a small amount of the PrP^{Sc} protein in lymphocytes (which was reported as prevalent in the human population (Manuelidis and Manuelidis, 1993) does not necessarily constitute an indication of a non-symptomatic but infectious pathogenic state. Moreover,

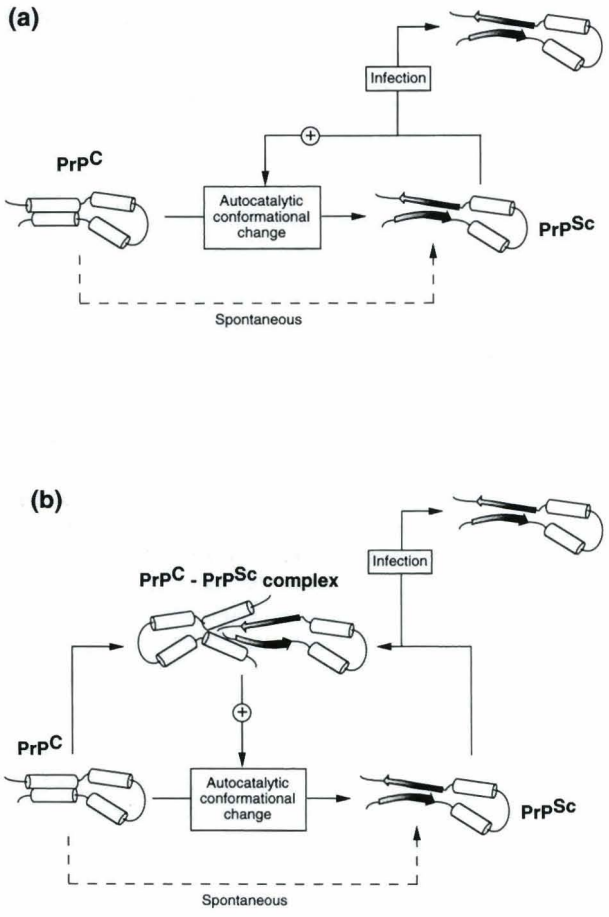

Fig. 3. Models for the catalysed conformational conversion of PrP^C to PrP^{Sc}. **a.** The simplest model of Prusiner (1991) in which the native protein PrP^C binds to endogenous or exogenous PrP^{Sc} which acts as a catalyst in the PrP^C-PrP^{Sc} conversion. **b.** In this slightly different model, PrP^{Sc} isoform forms a complex with normal PrP^C protein and the heterodimer PrP^C-PrP^{Sc} is the catalyst for the PrP^C-PrP^{Sc} conversion. In both cases, the initial conversion is followed by autocatalytic propagation. Sporadic form of prion diseases are thought to result from the occurrence of the spontaneous conversion of PrP^C to PrP^{Sc} which would be a very rare event (but see Laurent, 1996a,b). In the case of certain mutations in the *PrP* gene, spontaneous conversion may occur about a million times more frequently than in the case of the wild-type protein (Weissmann, 1994).

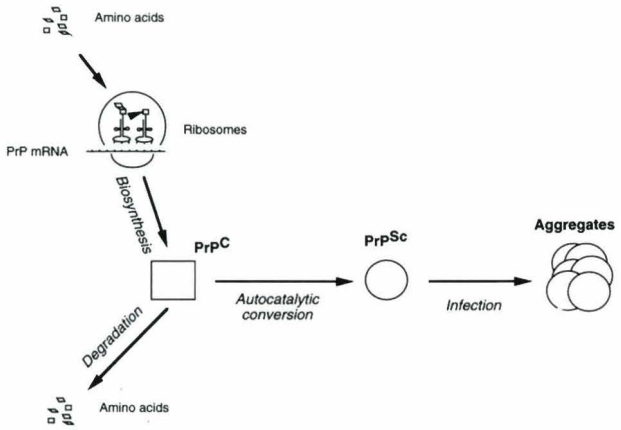

Fig. 4. Normal and pathogenic pathways for the prion protein. PrP mRNA is constitutively expressed in the brains of adult animals and its concentration remains unchanged in the brain of scrapie-infected animals (compared to normal tissue). The synthesis and degradation of PrP^C are rapid although the accumulation of PrP^{Sc} is slow. PrP^{Sc} formation is a posttranslational, autocatalytic process (see Fig. 3) and no degradation pathway is known for this protease-resistant isoform. Accumulation of PrP^{Sc} is the major pathognomonic feature of prion diseases, with frequent formation of amyloid plaques consisting mainly of aggregates of PrP^{Sc}.

infectious prion particles should not be seen as necessarily composed of the abnormal isoform of the protein, as usually stated. Theoretical analysis shows that particles containing overproduced normal PrP protein might also be pathogen, as was experimentally reported (Westaway et al., 1994; Mestel, 1996). However, mice expressing high levels of PrP transgenes do not seem to accumulate high levels of protease resistant PrP protein (Westaway et al., 1994). Convergent data (Büeler et al., 1992, 1993; Sakaguchi et al., 1995) also demonstrate that the disease progresses much more slowly in heterozygous mice (*Prn-p*$^{0/+}$ mice having one normal allele for the *PrP* gene and one allele in which the *PrP* gene is disrupted) than in wild-type controls (*Prn-p*$^{+/+}$). The difference in incubation times between transgenic and wild-type mice is about a factor of 2 in the case of the mouse-adapted CJD agent (Sakaguchi et al., 1995) and exceeds a factor of 10 in the case of the scrapie agent (Büeler et al., 1992, 1993). If we compare incubation times for *Prn-p*$^{0/0}$ (homozygous for the disrupted PrP gene) *Prn-p*$^{0/+}$ and *Prn-p*$^{+/+}$ mice, one can conclude that normal PrP^C afford some kind of partial protection against scrapie or CJD disease. However, in the framework of the 'protein only' hypothesis, these data indicate that the susceptibility to scrapie or CJD is a function of the level of normal PrP^C in the host. This observation agrees fairly well with the dynamic analysis.

Within the limits of the accuracy of these conclusions which are those of the 'protein only' hypothesis, compounds that would act on the turnover rate of the normal PrP^C protein appear as a possible therapeutic strategy against prion diseases.

Prion strains and species barrier

The weak point of the 'protein only' hypothesis has long been the phenomenon of scrapie strains. About 20 different prion strains have been isolated so far in mice, depending on how rapidly infection takes place in different mouse strains and also how the patterns of symptoms and brain lesions are observed (DeArmond et al., 1994). Obviously, the problem of prion strains is closely related to the so-called species barrier, which hinders (at least under some circumstances) prion transmission from one host species to another.

According to the 'protein only' hypothesis which assumes that the difference between PrP^C and PrP^{Sc} is solely conformational, strain differences would correspond to different conformational states of the pathogenic PrP^{Sc} isoform. Hence, each stable PrP^{Sc} strain would convert host PrP^C into a copy of itself. Although recent experiments of Bessen et al. (1995) and Collinge et al. (1996) agree with such a possibility, the authors assumed (but do not demonstrate) that the relative electrophoretic mobility on western blots or strain-specific susceptibilty to hydrolysis of PrP^C by proteinase K that they observed for different prion strains, was due to differences in conformation.

Usually, transmission of prions from one species to another is very inefficient (if at all) and this phenomenon occurs only after prolonged incubation times. For instance, Prusiner (1987) and Telling et al. (1994) reported that only 10% of mice intracerebrally inoculated with human prions developed some dysfunction of the central nervous system after incubation times exceeding 500 days. The species barrier between mice and Syrian hamsters for prion

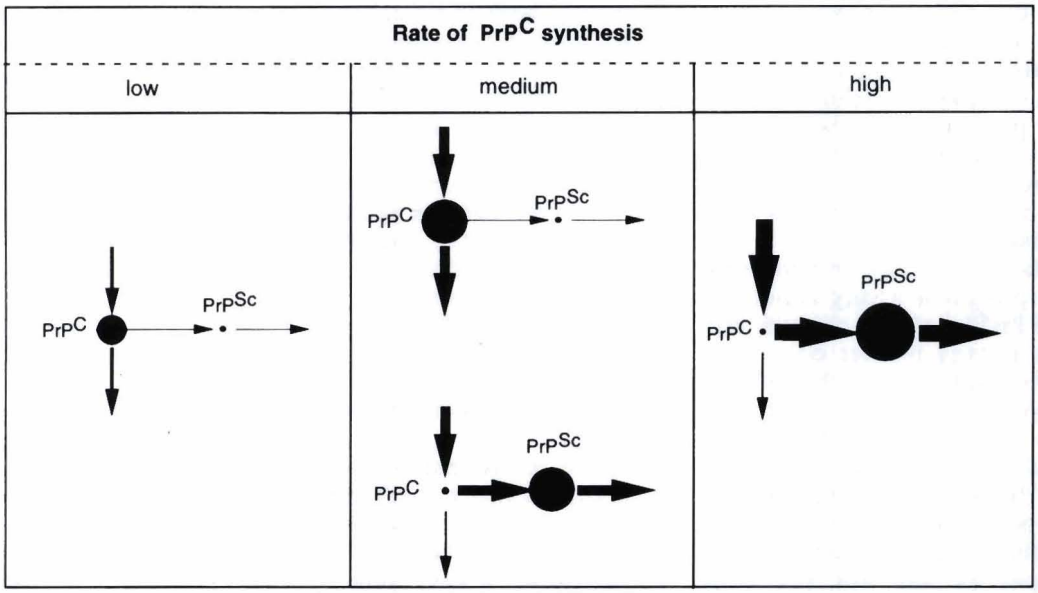

Fig. 5. Dynamic process of prion propagation including the turnover of the normal prion protein. Size of arrows and circles respectively reflect the flux intensities and the relative stationary concentration of the PrP^C and PrP^{Sc} species. For low rates of PrP^C biosynthesis, normal form PrP^C is dominant and the flux merely represents the flux of degradation of that species. On the other hand, for a high rate of PrP^C biosynthesis, the metabolic flux is preponderantly oriented toward the formation of the isoform PrP^{Sc}, the stationary rate of which is high. For intermediate rates, two distinct stationary state may exist, one being normal, the other being pathogenic and the transitions between these states exhibit threshold features (Laurent, 1996a,b). The same results are obtained when the rate of degradation of the normal form of prion protein is allowed to vary.

transmission is overcome by introducing hamster PrP transgene into the recipient mice (Prusiner et al., 1989; Scott et al., 1989). In these experiments, the transgenic mice produced prion protein corresponding to that used for inoculation. This agrees with other results (Büeler et al., 1993) showing that introduction of multiple hamster *PrP* transgenes into mice homozygous for the disrupted *PrP* gene rendered them highly susceptible to hamster prions (56 days incubation time) but much less to mouse prions (303 days incubation time). Both results indicate that optimal prion propagation and development of pathology make a homotypic relationship between exogenous prion and host PrP protein necessary: hamster PrP^C is largely a better substrate than murine PrP^C for conversion to hamster PrP^{Sc} by hamster prions and, reciprocally, mouse PrP^C is much more easily converted than hamster PrP^C into mouse PrP^{Sc} by mouse prions.

The nature of the species barrier was further studied by Prusiner's group in mice expressing human and chimeric *PrP* transgenes (Telling et al., 1995). A paradoxical result was reported: although transgenic mice expressed 4- to 8-fold higher levels of human PrP^C than those of endogenous mouse PrP^C, they failed to develop neurological dysfunction more frequently than nontransgenic controls, upon inoculation with human prions. However, these transgenic mice became susceptible to human prions upon disruption of the mouse *PrP* gene. This observation suggests that endogenous mouse PrP^C inhibits the conversion of human PrP^C into PrP^{Sc}. To explore the origin of the resistance of these transgenic mice to human prions, Telling et al. (1995) constructed mice expressing a chimeric PrP transgene having the central domain of human PrP and the N and C-terminus of mouse PrP. Mice expressing low levels of the chimeric transgene were found to be susceptible to human prions and exhibited only a modest decrease in incubation times upon endogenous mouse PrP gene disruption. These results were interpreted as a demonstration of the possibility that prion propagation needs the interaction of host PrP^C protein with a yet unknown cellular protein. This putative additional protein would be species-specific and might act as a chaperone in the formation of PrP^{Sc}. Differential binding properties of PrP^C to this additional species-specific protein would explain species barrier for prion transmission.

The possible intervention of another protein in the $PrP^C \rightarrow PrP^{Sc}$ conversion process is a very reasonable hypothesis. Since PrP^C and PrP^{Sc} isoforms are both very stable, a high activation energy barrier does exist between the two states. This explains why the sporadic conformational change from PrP^C to PrP^{Sc} is very rare and why it needs catalysis (by the complex PrP^C-PrP^{Sc} itself or maybe with the assistance of another protein) to occur more frequently. However, evidence for the existence of the additional protein are yet very weak and quite indirect. Moreover, the proteineous nature of the supposed additional species has not yet been demonstrated. Above all, no stuctural data (except its primary sequence) were provided for the chimeric PrP protein. Does the folding of the chimeric protein lead to a three-dimensional structure which resembles the structure of the native protein from mice or humans, the functional properties of which are simply the sum of the properties inferred from the local primary sequences? Results of previous conformational calculations (Huang et al., 1994) have revealed large discrepancies between the calculated structure and the real three-dimensional shape of the mouse PrP protein, as determined recently by NMR studies (Riek et al, 1996).

Much of the problem of species barrier is not yet clearly understood and different hypotheses are opened (Weissmann, 1994a; Kocisko et al., 1995). Of particular concern is whether or not various strains of transmissible bovine spongiform encephalopathies can be transmitted from cattle to humans (Collinge et al., 1996), as recent intracerebral inoculation studies seem to indicate for the transmission of BSE to macaques (Lasmézas et al., 1996). Simpler features will have probably to be explained before this issue can be solved. Just to mention a few crucial questions, 1) why has it not been possible to achieve conversion *in vitro* between PrP^C and PrP^{Sc} in mixing experiments (Raeber et al., 1992), in spite of the fact that the abnormal form of the prion protein can propagate in cell-free systems (Kocisko et al., 1994)? 2) Why has it been possible to create, from various human mutant PrP in *E. Coli* or mammalian cells, a material which was protease resistant and rich in β structure but devoid of any infectious properties (Mestel, 1996), although that 'strain' properties seem to be faithfully reproduced in *in vitro* assays (Bessen et al., 1995)? 3) Why does endogenous mouse PrP^C seem to inhibit the conversion of human PrP^C into PrP^{Sc} in transgenic mice (Telling et al., 1995)? Today, the 'protein only' hypothesis appears as a valuable concept that numerous experiments and compelling evidence is making more and more solid. However, it is likely that the purest form of this elegant and provocative hypothesis is too simple to give a complete explanation for all the observations reported so far about prion diseases. Further insights into the molecular mechanism of prion propagation may be expected from the use of the cell culture system that Lehman and Harris recently described (Lehmann and Harris, 1996) as a promising *in vitro* model for the study of familial diseases. Understanding these neurodegenerative diseases could advance our knowledge of the processes by which neurons function for decades and suddenly turn to senescence or apoptose.

Other proteins might behave as the prion protein

Since the leading explanation for prion diseases postulates that a malfolded protein can flip correctly-folded protein of the same type, a major point to elucidate is whether the prion protein behaviour constitutes a very unusual, pathogenic mechanism specifically bound to this particular protein or illustrates

a mechanism which might also be involved in other normal proteins to achieve and pass on their functional conformation. As Weissmann (1994b) noted: "*Phenomena discovered in higher eucaryotes acquire additional respectability if they are also found in yeast*". This criterium seems to be satisfied for prions insofar as other examples pertaining to yeast seem to indicate that such a process might be less exceptional than it appeared at first glance. Yeast has a variety of examples of extrachromosomic inheritance controlling phenotypic characters via a non-mendelian way. Two determinants, namely URE3 and psi, seem relevant to a process similar to prions. URE3 corresponds to the spontaneous development of an unusual nitrogen metabolism properties in yeast cells. The URE3 determinant is associated to a non-mitochondrial cytoplasmic element, and the URE3 phenotype would be best explained as if the protein called Ure3 had flipped into an abnormal configuration (Wickner, 1994). The strains possessing the URE3 determinant show exactly the same deregulation features as the nuclear mutants *ure2*. The strains ure2 are unable to propagate the URE3 phenotype, as transgenic mice deleted of the gene PrP cannot be infected by the prion protein. Conversely, the surexpression of the Ure2 protein leads to a large increase of the intracellular rate of the URE3 determinant and the Ure3 protein from URE3 cells is much more resistant to a protease than is the normal protein (Masison and Wickner, 1994). The protein determinant of the psi phenotype has been recently characterized as the product of the SUP35 gene, which has structural analogies with the PrP protein (Cox, 1994). As a working hypothesis, these results are in favour of considering URE3 or psi as submitted to the mechanism involved in prion diseases, although it has not yet been shown that the abnormal proteins can actually infect normal yeast cells.

A very puzzling observation has been reported about the p53 protein. This protein is a multifunctional protein which plays a central role in modulating gene transcription, policing cell cycle check-points, activating apoptosis, controlling DNA replication and repair and maintaining genomic stability (Elledge and Lee, 1995). Under certain circumstances, the mutant protein can drive wild type into a mutant conformation (Milner and Medcalf, 1991). This effect is observed when *p53* mutant and wild-type are cotranslated. The cotranslational effect of mutant *p53* upon wild-type conformation was attributed to some interaction between nascent polypeptides and oligomerization of the full-length proteins. Oligomers of p53 proteins can be induced to conformational change in a cooperative manner. The mechanism herein evoked strongly reminds one of the 'chaperone' hypothesis, previously quoted about the prion protein (Liautard, 1991, 1992). By the way, we should remark that it has also been shown that molecular chaperones can control their own assembly (Cheng et al., 1990; Lissin et al., 1990).

Structural inheritance and the primitive world

From a molecular point of view, the examples evoked above rely on a mechanism in which a protein passes its structural information to another protein molecule without any direct involvement of the genomic material. In other words, it means that a protein could have the property of bearing both functional and informational roles. Such a process might be connected to the phenomena of structural inheritance observed at the cellular level, especially in ciliates (Beisson and Sonneborn, 1965; Fleury and Laurent, 1994).

A set of experiments, first carried out on *Paramecium* (Beisson and Sonneborn, 1965), and extended to miscellaneous ciliate species, has shown that the polarity inversion of a row of cilia was a phenotypic character transmitted from one generation to the next without any modification of the genoma. Although the cellular material which is present at the periphery of the cell (the cortex) is not the support of any genetic material, it seems to contain the necessary information for the clonal transmission of this inversion. The origin of this inversion, -thereafter named 'structural inheritance' in opposition to genomic inheritance- must be searched for at the level of some organization coupled to structural determinants rather than at the level of a molecular code, as genetics used to teach it. At the present time, our knowledge about the molecular basis of this mechanism is rather scarce. However, while the concept of structural inheritance is one of the major contributions to contemporary biology made by the study of ciliates, it does not necessarily constitute a simple atypical curiosity about protozoa. In effect, such mechanisms have been invoked at the intracellular level, for bacteria (Hedges, 1985; Shapiro, 1993) as well as mammals (form of the cytoskeleton (Solomon, 1981; Theurkauf, 1994), form of the cells (Albrecht-Buehler, 1977), number of microtubule organizing centres (Shay et al., 1978)). Moreover, with regard to some pluricellular species which, like *Stenostomum*, duplicate by elongation followed by division, some processes of structural inheritance have been clearly established (Frankel, 1989). Thus, a process of non-nucleic inheritance can concern not only a specific protein, but also the organization of complex macromolecular structures such as cilia, centrioles etc. Hence, structural inheritance at the molecular level might be the missing link for the understanding of the structural inheritance processes featured at the cellular level.

Even though the hypothesis about the ability of a protein to impose its own conformation to a distinct one is not definitively demonstrated, strong arguments favor such a possibility for prion protein and other proteins as well. Therefore, what seems possible nowadays could have been realized in the past, and it seems quite conceivable that a protein could have been able to mediate a process of structural inheritance at the very beginning of the evolution of living organisms. By showing that a protein can support a process of structural

heritage, the prion proteins might be able to drive the current paradigm of a primitive RNA world to a radical reappraisal. In this sense, the remark of Griffith (1967), the precursor of the 'protein only' hypothesis, that "*there is no reason to fear that the existence of a protein agent would cause the whole theoretical structure of molecular biology to come tumbling down*" might not be completely justified.

Acknowledgements. We thank Phil Luboff for careful reading of the manuscript. This work was supported by grants from the Centre National de la Recherche Scientifique (URA 1116) and the Université Paris-Sud.

References

Albrecht-Buehler G. (1977). Daughter 3T3 cells: are they mirror images of each other? J. Cell Biol. 72, 595-603.

Alper T., Haig A. and Clarke M.C. (1966). The exceptionally small size of the scrapie agent. Biochem. Biophys. Res. Commun. 22, 278-284.

Alper T., Cramp W.A., Haig A. and Clarke M.C. (1967). Does the agent of scrapie replicate without nucleic acid? Nature 214, 764-766.

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

Beisson J. and Sonneborn T.M. (1965). Cytoplasmic inheritance of the organization of the cell cortex in *Paramecium aurelia*. Proc. Natl. Acad. Sci. USA 53, 275-282.

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

Bolton D.C., Rudelli R.D., Currie J.R. and Bendheim P.E. (1991). Copurification of Sp33-37 and scrapie agent from hamster brain prior to detectable histopathology and clinical disease. J. Gen. Virol. 72, 2905-2913.

Borchelt D.R., Scott M., Taraboulos A., Stahl N. and Prusiner S.B. (1990). Scrapie and cellular prion proteins differ in their kinetics of synthesis and topology in cultured cells. J. Cell Biol. 110, 743-752.

Brandner S., Isenmann S., Raeber A., Fisher M., Sailer A., Kobayashi Y., Marino S., Weissmann C. and Aguzzi A. (1996). Normal host prion protein necessary for scrapie-induced neurotoxicity. Nature 379, 339-343.

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

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

Caughey B., Race R.E., Ernst D., Buchmeier M.J. and Chesebro B. (1989). Prion protein biosynthesis in scrapie-infected and uninfected neuroblastoma cells. J. Virol. 63, 175-181.

Cheng M.Y., Hartl F.U. and Horwich A.L. (1990). The mitochondrial chaperonin hsp60 is required for its own assembly. Nature 348, 455-458.

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

Cohen F.E., Pan K.M., Huang Z., Baldwin M., Fletterick R.J. and Prusiner S.B. (1994). Structural clues to prion replication. Science 264, 530-531.

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

Collinge J., Sidle K.C.L., Meads J., Ironside J. and Hill A.F. (1996). Molecular analysis of prion strain variation and the aetiology of 'new variant' CJD. Nature 383, 685-690.

Come J.H., Fraser P.E. and Lansbury P.T. (1993). A kinetic model for amyloid formation in the prion diseases: importance of seeding. Proc. Natl. Acad. Sci. USA 90, 5959-5963.

Cox B. (1994) Prion-like factors in yeast. Cur. Biol. 4, 744-748.

DeArmond S.J., Yang S.L., Cayenato C.J., Groth D. and Prusiner S.B. (1994). The neuropathological phenotype in transgenic mice expressing different prion protein constructs. Philos. Trans. R. Soc. Lond. B Biol. Sci. 343, 415-423.

Diener T.O., McKinley M.P. and Prusiner S.B. (1982). Viroids and prions. Proc. Natl. Acad. Sci. USA 79, 5220-5224.

Dormont D. and Bursaux E. (1996). L'encéphalopathie spongiforme bovine: commentaires sur un cyclone. Médecine/sciences 12, 673-675.

Elledge R.M. and Lee W.H. (1995). Life and death by p53. BioEssays 17, 923-930.

Fleury A. and Laurent M. (1994). Transmission of surface pattern through a dedifferentiated stage in the ciliate *Paraurostyla*. Evidence from the analysis of microtubule and basal body deployment. J. Euk. Microbiol. 41, 276-291.

Frankel J. (1989). Pattern formation. Ciliate studies and models. Oxford University Press. New York.

Gabizon R., McKinley M.P. and Prusiner S.B. (1987). Purified prion proteins and scrapie infectivity copartition into liposomes. Proc. Natl. Acad. Sci. USA 84, 4017-4021.

Gajdusek D.C. (1988). Transmissible and non-transmissible amyloidoses: autocatalytic post-translational conversion of host precursor proteins to beta-pleated sheet configurations. J. Neuroimmunology 20, 95-110.

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

Griffith J.S. (1967). Self-replication and scrapie. Nature 215, 1043-1044.

Harris D.A., Huber M.T., Van Dijken P., Shyng S.L., Chait B.T. and Wang R. (1993). Processing of a cellular prion protein: identification of N- and C-terminal cleavage sites. Biochemistry 32, 1009-1016.

Hedges R.W. (1985). Inheritance of magnetosome polarity in magnetotropic bacteria. J. Theoret. Biol. 112, 607-608.

Hope J., Morton L.J., Farquhar C.F., Multhaup G., Beyreuther K. and Kimberlin R.H. (1986). The major polypeptide of scrapie-associated fibrils (SAF) has the same size, charge distribution and N-terminal protein sequence as predicted for the normal brain protein (PrP). EMBO J. 5, 2591-2597.

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

Huang Z., Gabriel J.M., Baldwin M.A., Fletterick R.J., Prusiner S.B. and

Cohen F.E. (1994). Proposed three-dimensional structure for the cellular prion protein. Proc. Natl. Acad. Sci. USA 91, 7139-7143.

Huang Z., Prusiner S.B. and Cohen F.E. (1996). Structures of prion proteins and conformational models for prion diseases. Curr. Top. Microb. Immunol. 207, 49-67.

Isenmann S., Brandner S. and Aguzzi A. (1996). Neuroectodermal grafting: A new tool for the study of neurodegenerative diseases. Histol. Histopathol. 11, 1063-1073.

Jarrett J.T. and Lansbury P.T. (1993). Seeding "one-dimensional crystallization" of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie? Cell 73, 1055-1058.

Kaneko K., Peretz D., Pan K.M., Blochberger T.C., Wille H., Gabizon R., Griffith O.H., Cohen F.E., Baldwin M.A. and Prusiner S.B. (1995). Prion protein (PrP) synthetic peptides induce cellular PrP to acquire properties of the scrapie isoform. Proc. Natl. Acad. Sci. USA 92, 11160-11164.

Kellings K., Meyer N., Mirenda C., Prusiner S.B. and 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.

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

Kocisko D.A., Priola S.A., Raymond G.J., Chesebro B., Lansbury P.T. and Caughey B. (1995). Species specificity in the cell-free conversion of prion protein to protease-resistant forms: a model for the scrapie species barrier. Proc. Natl. Acad. Sci. USA 92, 3923-3927.

Krakauer D.C., Pagel M., Southwood T.R.E. and Zanotto P.M.A. (1996). Phylogenesis of prion protein. Nature 380, 675.

Lasmézas C.I., Deslys J.P., Demaimay R., Adjou K.T., Lamoury F., Dormont D., Robain O., Ironside J. and Haw J.J. (1996). BSE transmission to macaques. Nature 381, 743-744.

Laurent M. (1996a). Les maladies à prions: l'hypothèse de la 'protéine seule' et ses conséquences dynamiques. Médecine/sciences 12, 774-785.

Laurent M. (1996b). Prion diseases and the 'protein only' hypothesis: a theoretical dynamic study. Biochem. J. 318, 35-39.

Lehmann S. and Harris D.A. (1996). Mutant and infectious prion proteins display common biochemical properties in cultured cells. J. Biol. Chem. 271, 1633-1637.

Liautard J.P. (1991). Are prions misfolded molecular chaperones? FEBS Lett. 294, 155-157.

Liautard J.P. (1992). Les prions sont-ils des protéines chaperonnes mal repliées? Medecine/sciences 8, 55-57.

Lissin N.M., Venyaminoy S.Y. and Girshovich A.S. (1990). (Mg-ATP)-dependent self assembly of molecular chaperone GroEL. Nature 348, 339-342.

Lledo P.M., Tremblay P., DeArmond S.J., Prusiner S.B. and Nicoll R.A. (1996). Mice deficient for prion protein exhibit normal neuronal excitability and synaptic transmission in the hippocampus. Proc. Natl. Acad. Sci. USA 93, 2403-2407.

Locht C., Chesebro B., Race R. and Keith J.M. (1986). Molecular cloning and complete sequence of prion protein cDNA from mouse brain infected with the scrapie agent. Proc. Natl. Acad. Sci. USA 83, 6372-6376.

Manuelidis E.E. and Manuelidis L. (1993). A transmissible Creutzfeldt-Jakob disease-like agent is prevalent in the human population. Proc. Natl. Acad. Sci. USA 90, 7724-7728.

Manuelidis L., Sklaviadis T. and Manuelidis E.E. (1987). Evidence suggesting that PrP is not the infectious agent in Creutzfeldt-Jakob disease. EMBO J. 6, 341-347.

Masison D. and Wickner R.B. (1995). Prion-inducing domain of yeast Ure2p and protease resistance of Ure2p in prion-containing cells. Science 270, 93-95.

McKinley M.P., Marsiarz F.R., Isaacs S.T., Hearst J.E. and Prusiner S.B. (1983). Resistance of the scrapie agent to inactivation by psoralens. Photochem. Photobiol. 37, 539-545.

Mestel R. (1996). Putting prions to the test. Science 273, 184-189.

Meyer R.K., McKinley M.P., Bowman K.A., Braunfeld M.B., Barry R.A. and Prusiner S.B. (1986). Separation and properties of cellular and scrapie prion proteins. Proc. Natl. Acad. Sci. USA 83, 2310-2314.

Milner J. and Medcalf E.A. (1991). Cotranslation of activated mutant p53 with wild type drives the wild-type p53 protein into the mutant conformation. Cell 65, 765-774.

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

Pan K.M., Baldwin M., Nguyen J., Gasset M., Serban A., Groth D., Mehlhorn I., Huang Z., Fletterick R.J., Cohen F.E. and 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.

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

Prusiner S.B. (1987) The biology of prion transmission and replication. In: Prions: Novel infectious pathogens causing scrapie and Creutzfeldt-Jakob disease. Prusiner S.B. and McKinley M.P. (eds). Academic Press. New York. pp 83-112.

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

Prusiner S.B., McKinley M.P., Groth D.F., Bowman K.A., Mock N.I., Cochran S.P. and Masiarz F.R. (1981). Scrapie agent contains a hydrophobic protein. Proc. Natl. Acad. Sci. USA 78, 6675-6679.

Prusiner S.B., Scott M., Foster D., Pan K.M., Groth D., Mirenda C., Torchia M., Yang S.L., Serban H., Carlson G.A., Hoppe P.C., Westaway D. and DeArmond S.J. (1989). Transgenetic studies implicate interactions between homologous PrP isoforms in scrapie prion replication. Cell 63, 673-686.

Raeber A.J., Borchelt D.R., Scott M. and Prusiner S.B. (1992). Attempts to convert the cellular prion protein into the scrapie isoform in cell-free systems. J. Virol. 66, 6155-6163.

Reibel S. (1994) Encéphalopathie spongiforme bovine. Epidémiologie et implications. Polytechnica. Paris.

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

Safar J., Wang W., Padgett M.P., Ceroni M., Piccardo P., Zopf D., Gajdusek D.C. and Gibbs C.J. (1990). Molecular mass, biochemical composition, and physicochemical behavior of the infectious form of the scrapie precursor protein monomer. Proc. Natl. Acad. Sci. USA 87, 6373-6377.

Sakaguchi S., Katamine S., Shigematsu K., Nakatani A, Moriuchi R., Nishida N., Kurokawa K., Nakaoke R., Sato H., Jishage K., Kuno J., Noda T. and Miyamoto T. (1995). Accumulation of proteinase K-resistant prion protein (PrP) is restricted by the expression level of normal PrP in mice inoculated with a mouse-adapted strain of the

Creutzfeldt-Jakob disease agent. J. Virol. 69, 7586-7592.

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

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

Shapiro L. (1993). Protein localization and asymmetry in the bacterial cell. Cell 73, 841-855.

Shay J.W., Peters T.T. and Fuseler J.W. (1978). Cytoplasmic transfer of microtubule organizing centers in mouse tissue culture cells. Cell 14, 835-842.

Solomon F. (1981). Specification of cell morphology by endogenous determinants. J. Cell Biol. 90, 547-553.

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

Stahl N., Borchelt D.R. and Prusiner S.B. (1990a). Differential release of cellular and scrapie prion proteins from cellular membranes by phosphatidylinositol-specific phospholipase C. Biochemistry 29, 5405-5412.

Stahl N., Baldwin M.A., Burlingame A.L. and Prusiner S.B. (1990b). Identification of glycoinositol phospholipid linked and truncated forms of the scrapie prion protein. Biochemistry 29, 8879-8884.

Stahl N., Baldwin M.A., Teplow D.B., Hood L., Gibson B.W., Burlingame A.L. and Prusiner S.B. (1993). Structural studies of the scrapie prion protein using mass spectrometry and amino acid sequencing. Biochemistry 32, 1991-2002.

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

Telling G.C., Scott M., Hsiao K.K., Foster D., Yang S.L., Torchia M., Sidle K.C.L., Collinge J., DeArmond S.J. and 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., Mastrianni J., Gabizon R., Torchia M., Cohen F.E., DeArmond S.J. and 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.

Theurkauf W.E. (1994). Microtubules and cytoplasm organization during *Drosophila* oogenesis. Dev. Biol. 165, 352-360.

Tobler I., Gaus S.E., Deboer T., Achermann P., Fischer M., Rülicke T., Moser M., Oesch B., McBride P.A. and Manson J.C. (1996). Altered circadian activity rhythms and sleep in mice devoid of prion protein. Nature 380, 639-642

Turk E., Teplow D.B., Hood L.E. and Prusiner S.B. (1988). Purification and properties of the cellular and scrapie hamster prion proteins. Eur. J. Biochem. 176, 21-30.

Weissmann C. (1994a). Molecular biology of prion diseases. Trends Cell Biol. 4, 10-14

Weissmann C. (1994b) The prion connection: now in yeast? Science 264, 528-530.

Weissmann C. (1995). Yielding under the strain. Nature 375, 628-629.

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

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

Wickner R.B. (1994). [URE3] as an altered URE2 protein: evidence for a prion analog in *Saccharomyces cerevisiae*. Science 264, 566-569.

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

Xi Y.G., Ingrosso L., Ladogara A., Masullo C. and Pocchiari M. (1992). Amphotericin B treatment dissociates *in vivo* replication of the scrapie agent from PrP accumulation. Nature 356, 598-601.

Ye X. and Carp R.I. (1995) The pathological changes in peripheral organs of scapie-infected animals. Histol. Histopathol. 10, 995-1021.