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RESEARCH ARTICLES

Prions: an evolutionary perspective

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Summary Studies in both prion-due diseases in mammals and some non-Mendelian hereditary processes in yeasts have demonstrated that certain proteins are able to transmit structural information and self-replication. This induces the corresponding conformational changes in other proteins with identical or similar sequences. This ability of proteins may have been very useful during prebiotic chemical evolution, prior to the establishment of the genetic code. During this stage, proteins (proteinoids) must have molded and selected their structural folding units through direct interaction with the environment. The proteinoids that acquired the ability to propagate their conformations (which we refer to as conformons) would have acted as reservoirs and transmitters of a given structural information and hence could have acted as selectors for conformational changes. Despite the great advantage that arose from the establishment of the genetic code, the ability to propagate conformational changes did not necessarily disappear. Depending on the degree of involvement of this capacity in biological evolution, we propose two not mutually exclusive hypotheses: (i) extant prions could be an atavism of ancestral conformons, which would have co-evolved with cells, and (ii) the evolution of conformons would have produced cellular proteins, able to transmit structural information, and, in some cases, participating in certain processes of regulation and epigenesis. Therefore, prions could also be seen as conformons of a conventional infectious agent (or one that co-evolved with it independently) that, after a longer or shorter adaptive period, would have interacted with conformons from the host cells.

Key words Prion · Conformon · Protein evolution · Conformational change · "Yeast prions"

Introduction

According to Stanley B. Prusiner, prions belong to an unprecedented class of infectious agents because they are devoid of nucleic acid, and seem to be composed exclusively of a modified mammalian protein [18, 38].

Prion-due diseases are usually lethal and are commonly termed transmissible subacute spongiform encephalopathies (TSE) owing to the sponge-like aspect of the brains of infected individuals brought about by extensive vacuolation in brain tissues. Among the best known TSE are sheep scrapie and bovine spongiform encephalopathy (BSE), in animals, and Creutzfeldt-Jacob disease (CJD), in humans [36] (Table 1).

Until now, most cases of CJD have occurred sporadically (only one case per 1 million individuals per year). About 10% of cases are considered to be of genetic origin. The inherited form has been detected in about one hundred families. Also, all cases of CJD initially described as being of infectious origin are iatrogenic. Following the crisis of mad cow disease, it is believed that bovine prions, responsible for BSE, may have been passed to humans resulting in a new form of CJD or variant CJD (vCJD). Both in these and in other TSE, the common feature is the accumulation of the modified form of a normal cell protein in affected brains [38].

On investigating sheep scrapie, Prusiner isolated a purified extract of the infectious agent. Seeing that it resisted inactivation by procedures that modify nucleic acids, he concluded that he was essentially dealing with a hydrophobic protein and therefore termed it Prion (proteinaceous infectious particle) [35]. The scrapie agent contains a principal protein, the so-called prion protein (PrP) [36].

Later, it was discovered that prion protein is host encoded. In humans, PrP is encoded by the PrnP gene. Thus, this gene—which has been identified in all mammals studied and which is highly conserved—encodes a normal cellular protein of unknown function. PrP occurs in two different forms; the normal cellular form, or PrP^{C}, and the infectious form, or prion, called PrP^{SC} (from

scrapie) or PrP^{res} (from protease-resistant). PrP^{C} and PrP^{SC} have the same amino acid sequence but differ in their three-dimensional conformations. They are therefore conformational isomers [36].

Table 1 The prion diseases [see Ref. 38]

Disease	Mechanism of pathogenesis
Human diseases	
Kuru (Fore people, Papua New Guinea)	Infection through ritualistic cannibalism
Iatrogenic Creutzfeldt-Jakob disease	Infection from prion-contaminated HGH,* dura mater grafts, and so forth
Variant Creutzfeldt-Jakob disease	Infection from bovine prions?
Familial Creutzfeldt-Jakob disease	Germline mutations in PrP gene
Gerstmann-Sträussler-Scheinker disease	Germline mutations in PrP gene
Fatal familial insomnia	Germline mutations in PrP gene (D178N and M129)
Sporadic Creutzfeldt-Jakob disease	Somatic mutation or spontaneus conversion of PrP^{c} into PrP^{sc} (?)
Animal diseases	
Scrapie (sheep)	Infection in genetically susceptible sheep
Bovine spongiform encephalopathy (cattle)	Infection with prion-contamined MBM**
Transmissible mink encephalopathy (mink)	Infection with prions from sheep or cattle
Chronic wasting disease (mule deer, elk)	Unknown
Feline spongiform encephalopaty (cats)	Infection with prion-contaminated MBM
Exotic ungulate encephalopaty (greater kudu, nyala, oryx)	Infection with prion-contaminated MBM

* HGH: Human Growth Hormone.
** MBM: Meat and Bone Meal.

According to the protein-only hypothesis, PrP^{C} undergoes a conformational change, induced by direct interaction with PrP^{SC} (transient formation of a heterodimer) and is then converted into PrP^{SC}. In turn, this infectious form of PrP is able to propagate its new conformation to other PrP^{C} molecules. PrP^{C} contains much more α-helix than β strand, i.e. 42% α versus 3% β strand. The conformational change, involves the stretching of some portion of these helixes and their conversion into β strands. However, PrP^{SC} preserves a large part of α helix (30% versus 43% β strand) [9, 31]. These results suggest that at least a large part of the β pleated sheet domains are originated from the amino-terminal region of PrP^{C}, which is less structured [40, 41]. Recently, it has been reported that this region undergoes a profound conformational change located at residues 90 to 112, during the transformation of PrP^{C} into PrP^{SC} [34].

This structural modification is accompanied by significant differences in the chemical properties of both isoforms; unlike the cellular form, PrP^{SC} is not soluble in non-denaturing detergents and it also displays a certain degree of resistance to protease digestion, which is not seen in PrP^{C} [36]. Other experiments, whose results support the protein-only hypothesis, seem to prove the in vitro conversion of PrP^{C} mediated by PrP^{SC}, although the resulting material is not infectious [2].

As well as the foregoing, the protein-only hypothesis—which is currently favoured by most researchers in the field—receives support from other observations, mainly genetic. Knock-out (KO) transgenic mice, which do not express the PrP gene, are viable [4]. In fact, despite its ubiquitous localization—mainly in the brain, but also in other organs—the function of PrP^{C} is unknown. Nevertheless, the gene encoding PrP^{C} has been found to be an essential component for the development of all pathological forms. In humans, non-conservative mutations in the PrP gene have been detected in all hereditary forms of TSE [38]. Prusiner believes that prions may be generated de novo through mutations in PrP. Thus, in experiments conducted on transgenic mice, in which a mutated gene of PrP had been introduced, this gene was seen to govern the period of incubation of the disease; the greater the number of copies, the earlier the onset of the disease [4, 19, 20, 45, 46]. In these experiments, it was observed that the molecules of mutant PrP were able to adopt the infectious conformation since (i) transgenic mice that produce large amounts of mutant PrP developed all the symptoms of the disease, and (ii) inoculation of brain tissue from mice suffering from TSE to healthy mice, which produce the same mutant PrP, at low level, transferred the disease to the latter animals. In turn, the brain tissue of these latter was able to propagate the disease to other identical healthy mice. It would seem that the requirement of the PrP gene for the disease to appear and the nature of modified PrP as a transmissible infectious agent have been demonstrated. In this sense, KO mice were found to be resistant to infection with prions.

The species barrier is not identical for all species. Essentially, it depends on the greater or lesser identity in the amino acid sequence of the PrP of the species (donor and receptor) considered (above all that of certain regions of the protein) [37, 39]. Transgenic mice that express the PrP gene from the Syrian hamster may develop the disease when they receive hamster prions while normal mice do not [43]. Thus the greater the similarity in the PrP, prion and cellular sequences of the host, the greater the probability of disease transmission. Furthermore, other factors also contribute to the species barrier; namely, the strain of the prion and the species specificity of the so-called protein X [38]. Infectious material from different sources can produce distinct and reproducible patterns of incubation times, distribution of Central Nervous System (CNS) involvement and even the patterns of proteolytic cleavage of PrP^{SC}. The concept of prion "strains" lies on such differences of the same converted PrP^{C} (reviewed in [18]). For Prusiner, each prion strain seems to be identified by a given conformation of the many conformations that a PrP^{SC} species may adopt (identified by their sequence) [46]. These conformations propagate by inducing the corresponding conformational change in PrP^{C} with appropriate sequences (that is, that they do not display differences that might act as a "species barrier"). Protein X

has been proposed to act as a molecular chaperone, binding to the terminal COOH region of the PrP^{C}, of its same species, and facilitating its conversion into PrP^{SC} [22, 45].

As well as the species barrier, other phenomena suggest a certain degree of variability among prions and also the existence of different strains. As mentioned, Prusiner explains this variability by assuming that PrP^{C} are able to adopt different conformations [2, 5, 30]. The preservation of in vitro strain specificity has been reported [2]. In this sense, it has been observed that a given PrP^{C}, incubated with different strains of prions, develops into the PrP^{SC} strain corresponding to the strain used as template. Thus, new strains of prions may also be generated during the passage of a given PrP^{SC} through animals with different PrP genes [44].

Prions and prebiotic evolution

The capacity shown by prions to propagate their protein conformation may have been very useful during the stage of chemical evolution prior to the appearance of life.

Crystallographic studies of the structure of the proteins suggest that they only use a low number of folding conformations. This limited number of tertiary structures is, however, much higher than the number of secondary and supersecondary structures [8].

By contrast, enormous diversity is seen on analyzing the sequences of the proteins. Despite this, examination of the patterns of variability of different protein families reveals strong structural constraints on such variability [3]. These restrictions mainly affect hydrophobic residues (which essentially form the hydrophobic core of the proteins), which therefore contribute most of the structural information. Conversely, the variability seen in the polar residues of the surface of the proteins is generally much greater. In this sense, there is considerable evidence to suggest that the gene segments (exons) on which the mechanisms of diversity generation act basically correspond to the protein structural units selected along evolution [14].

In our opinion, all these data about the structure of the proteins suggest that they may have evolved in two main stages: (i) an initial, prebiotic, conformational evolutionary stage, in which a small number of conformations was selected from randomly-formed polypeptide sequences (probably all possible conformations, which are those currently found in all proteins); and (ii) a second stage of sequential evolution, in which—from already genetically encoded polypeptides, and using the mechanisms of diversity generation developed along biological evolution—enormous variability in the protein sequences became accumulated, although always conserving the conformation selected during the previous step.

During the long prebiotic stage of chemical evolution, the structure of the proteins (proteinoids) must have become conformed and selected through direct interaction with the environment. After the establishment of the genetic code (origin of the first cells and the beginning of evolution through natural selection), all the adaptive modifications of the proteins (i.e. the structural units selected during the prebiotic stage) occurred through the "new pathway" which, paradoxically, implied both stability and conservation as a source of variability DNA → RNA → Proteins. In this sense, we believe that during the transition from prebiotic to biological evolution through natural selection the environment may have changed from having a direct instructive role to a fundamentally selective role for the genetically encoded protein variability.

Throughout the prebiotic stage, some ability to propagate protein conformations, similar to that currently displayed by prions, may have been fundamental in order to advance towards biological evolution (establishment of the genetic code). From an evolutionary point of view, proteinoids that acquired this ability (as it will be explained later, we propose the terms "conformers" or "conformons" to refer to them) would have been protein structures that were essentially selected for their ability to induce conformational changes, depending on their structure and function (propagation capacity), in certain polypeptides that showed sequences compatible with the change. They would therefore have acted as selectors for favourable changes (permissive changes in specificity with the essential structural units), facilitating their propagation. In other words, of all the polypeptides—formed randomly in the "primeval soup" and compatible with the capacity to propagate their conformations—shown by conformons, those bearing the most suitable active sites to

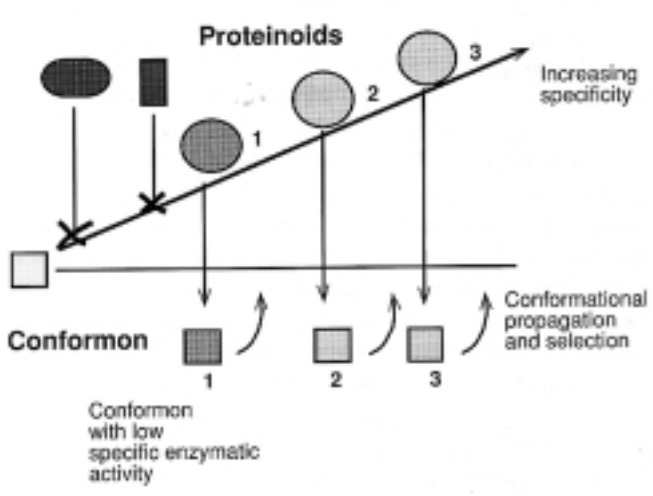

Fig. 1 Conformons and prebiotic evolution. During the prebiotic stage, proteinoids with the capacity to propagate their conformations (like current prions) would have been selected by: (i) their structural stability and their capacity for structural propagation, and (ii) some somewhat non-specific enzymatic capacity (sensu latu). As far as our knowledge about the species barrier phenomenon goes, the newly formed conformon would be able to select other proteinoids more similar to it rather than the previous one, implying the beginning of a new line in evolutionary progression

carry out increasingly more specific activities would have been positively selected. However, although efficient and lasting, in this function they would not have had rapid and precise genetic mechanisms of inheritance for the transference of these finely-tuned changes (Fig. 1). In this stage—in the prebiosphere—these more efficient protein structures would have accumulated and associated, forming protobionts with elementary metabolism and reproductive capacity.

Prions and cellular evolution: the case of yeast

Despite the enormous evolutionary advantage implied by the appearance of a genetic code, the property of the induction of conformational changes did not necessarily have to disappear. Here we propose two possible theoretical frameworks, which are not necessarily mutually exclusive, that depend on the degree of participation of prions/conformons in biological evolution: (i) prions as an evolutionary atavism of ancestral conformons that, as independent entities, would have coevolved with cells, and (ii) conformons as cellular proteins that would have participated in certain regulatory and epigenetic processes. As indicated above, this second possibility does not exclude the possible existence of prions as relatively independent entities.

In the first case, therefore, prions could be an atavism from the prebiotic stage that, owing to their peculiar characteristics, would have persisted up to the present, propagating their structure through conformational changes in certain genetically synthesized proteins. The biological evolution of cells would have been accompanied by the coevolution of a kind of "germinal" prion line that would have operated across the generations on proteins that changed genetically. Prions would have propagated their conformations, but not their sequences, in proteins with sequences compatible with such conformations. Thus, prions would have established a conformational line whose sequences would have varied with cellular evolution (Fig. 2).

As we have seen, certain strains of prion induce certain conformational changes in some sequences rather than in others. A given form of prion can propagate as such in a given PrP^C with an appropriate sequence for such propagation, while in another PrP^C it would induce a different form. As prions (as independent entities) infected different cell types along the course of evolution, new prion species may have become selected, defined by the amino acid sequence of the cellular protein that was most suitable for the transformation. The species barrier would only appear in PrP^C sequences incompatible with the conformational change. Generally, this barrier would become stronger as the species diverge in evolutionary terms. However, it would always be possible to find "bridge" species between two species showing the barrier effect (Fig. 3). As discussed below, if there were prions in bacteria, the probabilities of bridging the barrier among species, by horizontal transmission mechanisms, would increase considerably.

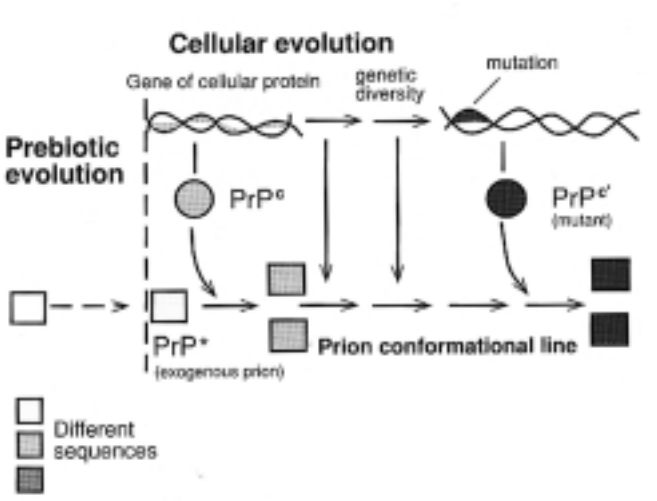

Fig. 2 Coevolution of a prion conformational line throughout cellular evolution. Parallel to cellular evolution, a coevolution of prions would have occurred, in which positively-selected conformations would have been propagated and modified by the most suitable cellular proteins of the host cell (PrP^C)

The second theoretical scenario finds support in the adaptive fitness of prion-like molecular mechanisms in lower eukaryotes. Since 1994 much evidence has been gathered in favour of the existence of physiological molecular mechanisms in yeasts and in the fungus *Podospora anserina* that would imply the propagation of conformational changes in proteins, similar to what has been proposed in the protein-only hypothesis for prion replication [11, 49, 50]. In this sense, according to S. Lindquist "yeast prions" would behave as inheritable genetic elements. Thus, "in both mammalian and yeast prions, protein structure acts in a manner previously thought to be the unique province of nucleic acids, in the one case as transmissible agents of disease, and in the other as heritable determinants of phenotype" [27].

In the yeast *Saccharomyces cerevisiae* there are two phenotypes, [PSI+] and [URE3], that are dominant and inherited in a non-Mendelian manner. In the [PSI+] phenotype, the chromosomically encoded protein Sup35 (a subunit of the translation termination factor) may accumulate in a non-functional aggregate through a process similar to that of prions. This process produces the suppression of nonsense codons which, if this did not occur, would be lethal. Likewise, in the [URE3] phenotype, the Ure2 protein can exist in an inactive aggregated form. Under certain environmental conditions, this form may provide a certain selective advantage to the [URE3] phenotype [6, 12, 28, 32, 49]. Among other interesting observations, in these latter studies it was observed that Sup35 and Ure2 do not have homologous sequences; however, whereas their normal biological functions are located in their carboxy-terminal domains, the amino-terminal domains are dispensable and have an unusual amino acid composition. Likewise, deleting the amino terminus suppresses these phenotypes. By contrast, over-expression of Sup35 or Ure2 (or of their NH_2–terminal domains) may induce the non-Mendelian elements de novo.

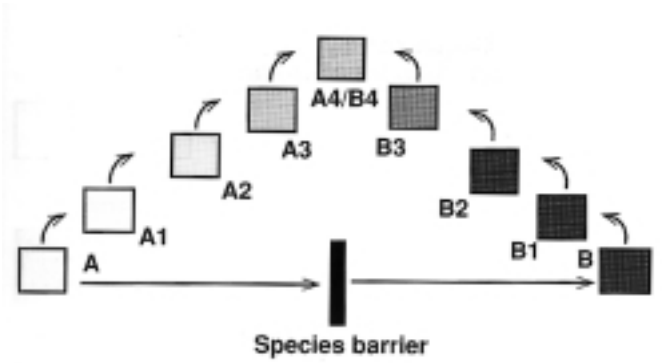

Fig. 3 Species barrier and bridge prions. Bridge species would have intermediate sequences between two species (here A and B) showing the barrier effect. Species A1 would have a sequence very similar to that of species A but would be closer to B than A. Species A2 would be very similar to A1 but would be closer than it to B. The same follows for B1, B2, etc. In this figure, A4 is identical to B4. Thus, the prions could propagate from A to B by conformational changes through bridge species

As happens with mammalian prions, in yeasts it is assumed that the amino-terminal domains of these proteins undergo aggregation-dependent structural alterations that somehow affect the corresponding functional domains. The N-terminal domain of Sup35 or Ure2 acquires an altered conformation that can interact with other molecules of the same type, causing them to adopt the same form.

Another similarity with mammalian prions lies in the involvement of a chaperone (the proposed protein X) in yeasts. Thus chaperone HSP104 (heat-shock protein 104) plays an essential role in the inheritance of the [PSI+] phenotype; maintenance of this phenotype requires an intermediate concentration of HSP104 and the phenotype may be lost through both an excess and a deficit in HSP104 [6, 32].

Continuing with the similarities with mammals, Sup35 is more resistant to digestion in cells having the [PSI+] phenotype and Ure2 is more resistant in cells with the [URE3] phenotype [28, 32]. Similarly, Sup35 is found in the form of large aggregates in [PSI+] cells but is soluble in cells with the [psi–] phenotype. Direct visualization of the conversion process with GFP (green fluorescent protein) shows that when cells contain aggregates, these are transmitted to the daughter cells [32].

Differences in [PSI+] phenotypes attributable to different conformational variants of Sup35 have been detected. This phenomenon would also be similar to that of prion strains in mammals [12].

Thus, the two "protein only" phenomena—where apparently mammalian PrP acts like a virus, and yeast Sup35 acts like a gene—may share a common mechanism involving a nucleation- or seed-dependent polymerization process [16, 25]. In this sense, in vitro both Sup35 and its amino-terminal domains form amyloid fibrils through a nucleated polymerization mechanism [15, 23, 33]. Likewise, cytoplasm derived from PSI+ cells is able to induce the conversion of 400-fold excess of the normal monomeric form of Sup35 [33].

For Lindquist, the [PSI+] and [URE3] phenotypes help yeast populations to respond rapidly to changes in their environment: in [PSI+] strains, the pattern of gene expression may be altered. It is possible that [PSI+] could afford a mechanism through which individuals with identical genomes are able to adapt to different selective niches. Rather than a mechanism of environmental adaptation, [PSI+] would represent a mechanism for accumulating genetic variants that are suppressible under certain natural conditions. Finally, since [PSI+] is regulated by HSP104, and since HSP104 is induced by environmental stress, this phenomenon affords the first plausible molecular mechanism for a cell to respond to its environment with an inheritable phenotypical change. For Lindquist, [PSI+] and [URE3] offer a convincing argument that phenotypical inheritance may sometimes be based on the inheritance of different conformations of proteins rather than on the inheritance of nucleic acids [27].

In view of the physiological role of both the change and propagation of the conformations of these proteins in healthy cells, we believe it is appropriate to use an unequivocal term for the proteins that, using these mechanisms, do not have an infectious role: (i) in their possible prebiotic origin, (ii) in their cellular evolution, and (iii) in their physiology. We thus propose the term *conformon* to refer to the cellular protein agent able to propagate one of the possible conformations (through direct contact) in other proteins compatible with such changes. The protein undergoing this conformational change may be a different conformation of the same protein or another closely related one, in both structural and functional terms, within the framework of a physiological response. A prion (infectious protein particle) would be a conformon of a conventional infectious agent (or one that evolves in it) that would interact with the conformons of the host.

Some obscure points

Within the set of PrP transformed into the pathological form the ratio of infectious units to protease-resistant molecules is about 1:100,000 or less. This fact suggests that the infectious units would be a smaller and different fraction of a mixture of different PrP [51]. Currently, it is unknown whether the in vitro "conversion" of PrP^{C} gives rise to infectivity and not only to a protease-resistant isoform [2]. "Interestingly, pretreatment of PrP^{SC} with 3 M guanidine HCl, which produces a reversible unfolding of PrP^{SC}, increases the extent of conversion, suggesting that PrP^{SC} itself may also need to undergo a conformational change for conversion to proceed, potentially accounting for the high particle:infectivity ratio" [18]. However, it is known that prion-due diseases may occur in the absence of detectable levels of PrP^{res} [10]. Non-infectious PrP^{res} may also be generated [42].

In view of this, Weismann has adopted a different terminology from that of Prusiner to refer to prions. Weismann used the term PrP* for the infectious form, which "may coincide or not with the protease-resistant form PrP^{res}, or PrP^{SC}" [1, 47, 48].

Other authors, who favour the viral hypothesis (they propose that the agent of TSE would be an unusual virus), distinguish between PrP^{res} and infectivity [26]. In a study of the interspecies transmission of BSE to mice, although all mice injected showed neurological symptoms and neuronal death, it was observed that PrP^{res} could not be detected in more than 55%. During serial passages, PrP^{res} appeared (together with neuronal vacuolation and astrocytosis) after the agent had become adapted to its new host. These authors suggested that PrP^{res} could be involved in species adaptation but that another unidentified agent able to transmit BSE must exist. Despite this, currently very little is known about how brain damage occurs in TSE and it would be unwise to draw hasty conclusions. Nevertheless, there is evidence to suggest that neuronal death could be due to a deficit in PrP^{C} rather than to the production of PrP^{SC} [1, 18].

Additionally, those favouring the viral hypothesis find another weak point in the conformational hypothesis advanced by Prusiner to explain the polymorphism of prions. They base their argument on the difficulty for a single protein to adopt a large variety of different conformations, and are rather inclined to the notion of different strains of another transmissible agent, perhaps a rare virus [7, 13, 26]. Other authors have also doubted the exclusively genetic interpretation of certain types of TSE and propose the existence of an infectious agent: "Scrapie is not solely a genetic disease, as scrapie-associated PrP alleles are present in sheep from Australia and New Zealand, both countries that are entirely free of scrapie" [21].

We believe that one could be dealing with ancestral conformons/prions and/or cellular conformons/prions that, by "riding" conventional infectious agents, have hopped from one species to another throughout evolution. These agents would mainly be cellular, although the possibility of some virus bearing prions, or the gene of a prion, cannot be ruled out. In any case, the prion would act as postulated in the protein-only hypothesis. The polymorphism would be difficult to detect since sequence differences would only appear in infecting prions (which could be low in number). These sequence differences would correspond to greater or lesser conformational differences. Each of the different possible conformations could propagate on the unique substrate of the PrP^{C} of infected cells. In this way, a single PrP^{C} sequence could adopt several different conformations, induced by the different conformations of the infecting prions. In this case, one would be confronting a high number of PrP with identical sequences and different conformations together with a lower number of exogenous infectious PrP (PrP*, in the terminology of Weismann) (Fig. 4).

As we have seen, these PrP* may arise from bacteria or other infectious organisms. Bacteria are able to act very well as "bridge species" owing to their greater probability of mutation, the capacity for horizontal genetic transmission and their ability to incorporate human genes [17]. If the adaptation involved modification capacity, in bacteria, prions would be able to considerably increase their variability in a way similar to that shown in Fig. 2. At the same time, bacteria can acquire

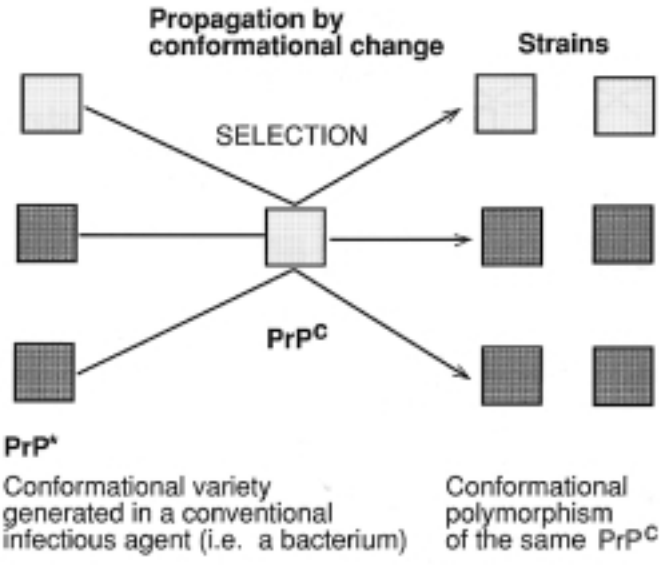

Fig. 4 Generation of polymorphism in prions species. The different conformations of infecting species (PrP*) are selected by PrP^{C}, depending on its capacity for conformational change. Thus, PrP^{C} would undergo a conformational change when challenged by some species but not others. Each of the different conformations adopted by PrP^{C} would constitute a strain of the PrP^{C} species (defined by its sequence)

PrP genes from mammals (including humans), which could also generate new mutant prions. These mutants would be selected by the PrP^{C} of the host; those overcoming the species barrier would be selected positively and those unable to overcome it would be selected negatively. In support of the possible involvement of bacteria or other conventional infectious agents, in prion-due diseases it has been reported that the antibiotic amphotericin B can retard both the clinical symptoms and the appearance of protease-resistant PrP (PrP^{res}) aggregates in diseased brain tissue, without affecting the levels of the agent replication [51]. Despite the role that we propose for conventional infectious agents in TSE, in certain cases of experimental, iatrogenic or alimentary infections, infection due to prions alone cannot be ruled out. In any case, owing to the low frequency of natural cases, the infection process must be long and complex. In infection, apart from the amount and diversity of PrP*, the following may also vary: the route of entry, the amount of PrP^{C} produced by the host's cells, the greater or lesser similarity between the infectious strains and the cellular isoform, the presence of possible vectors harbouring the prion strains (bacteria, protozoa, fungi, etc.), the presence of other proteins that influence pathogenesis (chaperones), etc. In this sense, it is interesting that differentiated B lymphocytes play an important role in prion neuroinvasion, perhaps by acting as physical carriers of the prions [24]. In our opinion, these and other leukocytes could equally transport conventional infectious agents bearing prions. The immunitary system could serve as a reservoir where prions would be selected, silently, during the incubation phase of TSE [1].

This possible multifactorial nature of prion-due diseases would

explain why the incubation period of CJD ranges between a few months and 10 years [38]. Certain bacteria might bear the genes of the prions and/or prions, more or less suitable for causing the conformational change of the PrP^{C} of a given host, while others would have to pass through a varying period of adaptation.

Induction of the structural change

Two distinct models for the conversion of PrP^{C} to PrP^{SC} have been proposed. In the "seeding" model, the formation of PrP^{SC} is a nucleation-dependent polymerization process. In the template-directed, or refolding, model, PrP^{SC} could promote conversion by catalyzing the rearrangement of a molecule of PrP^{C}, or of a partially destabilized intermediate, to the more stable PrP^{SC} conformation (reviewed in [18]).

In yeasts, in vitro conversion assays support the nucleation-polymerization model [15, 23, 33]. However, this model could be compatible with the template-directed model and both mechanisms could be involved [18, 29]. In this sense, in the case of tubulin polymerization, the polymerizing monomers can assume the conformation of even heterologous seed material, reflecting a "templating" behaviour [18].

We believe that this question could be explained within the framework of our hypothesis. Thus, we propose that in the large majority of cases, if not in all, the conversion of PrP^{C} is mediated by exogenous PrP*. In vivo, PrP* would be a mixture of different strains. The different exogenous strains can be distinguished by their ability to induce different conformational changes in PrP^{C}, generating the corresponding PrP^{C} strains (different conformations of the same sequence). Each of these new strains would exhibit a different kind of behaviour, characterized by its greater or lesser tendency to be infectious (i.e., to induce new conformational changes in PrP^{C}) or to form only aggregates. Under certain conditions, at least part of the PrP* could form aggregates.

As proposed, protein X could be a chaperone. Perhaps there might be more than one chaperone; one of them might participate in the conformational change process, whereas the other might accumulate misfolded PrP (perhaps only the non-infectious type). In this sense, we believe that the participation of chaperones could be different in yeasts, where one is dealing with a physiological process, from the situation in mammals, where one is dealing with a pathological process. In mammals, transformed PrP could aggregate with the help of a chaperone in a non-infectious, protease-resistant form (PrP^{SC} or PrP^{res}) (Fig. 5).

The main genetic determinant of TSE lies in the fact that if no PrP gene is present, there is no substrate to be changed. Furthermore, certain mutations of PrP may favour infection by certain prion strains.

In our hypothesis, the common element is the entry, in greater or lesser amounts, and selection of an exogenous infectious protein (PrP*) into a host cell, which then produces the corresponding PrP^{C} compatible with the conformational change. The prion and/or its genes could enter the host by "riding" conventional infectious agents.

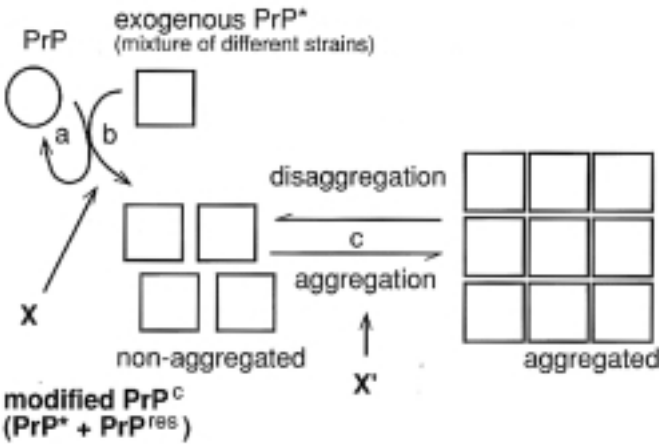

Fig. 5 Model of prion replication. Model for the conformational conversion of PrP^{C}. (a) Non-effective interactions due to incompatibility between PrP* and PrP^{C} for the conformational change. (b) After a more or less prolonged period of adaptation to the PrP^{C} by the different exogenous strains (PrP*), modified PrP^{C} begins to be accumulated. These new strains of modified PrP^{C} would differentiate owing to: (i) their greater or lesser capacity to induce new conformational changes in PrP^{C} (they would form the fraction of new infectious PrP*), or (ii) owing to their tendency to form aggregates only, regardless of their infective capacity (this fraction would mainly be protease-resistant, PrP^{res}). It should be stressed that at least part of the PrP* fraction could form aggregates. The process of conformational change could involve the proposed chaperone X. (c) Polymerization-depolymerization equilibrium. Another chaperone, X', could facilitate the formation of aggregates, accumulating misfolded protein

References

1. Aguzzi A, Weissmann C (1997) Prion research: the next frontiers. Nature 389:795–798
2. Bessen RA, Kocisko DA, Raymond GJ, Nandan S, Lansbury PT, Caughey B (1995) Non-genetic propagation of strain-specific properties of scrapie prion protein. Nature 375:698–700
3. Bowie JU, Reidhaar-Olson JF, Lim WA, Sauer RT (1990) Deciphering the message in protein sequences: Tolerance to amino acid substitutions. Science 247:1306–1310
4. Büeler H, Fischer M, Lang Y, Bluethmann H, Lipp H-P, DeArmond SJ, Prusiner SB, Aguet M, Weissmann C (1992) Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. Nature 356:577–582
5. Carlson GA, Ebeling C, Yang S-L, Telling G, Torchia M, Groth D, Westaway D, DeArmond SJ, Prusiner SB (1994) Prion isolate specified allotypic interactions between the cellular and scrapie prion proteins in congenic and transgenic mice. Proc Natl Acad Sci USA 91:5690–5694
6. Chernoff YO, Lindquist SL, Ono B, Inge-Vechtomov SG, Liebman SW (1995) Role of the chaperone protein Hsp104 in propagation of the yeast prion-like factor [psi+]. Science 268:880–883
7. Chesebro B (1998) BSE and prions: uncertainties about the agent. Science 279:42–43
8. Chothia C, Finkelstein AV (1990) The classification and origins of protein folding patterns. Annu Rev Biochem 59:1007–1039
9. Cohen FE, Pan K-M, Huang Z, Baldwin M, Fletterick RJ, Prusiner SB (1994) Structural clues to prion replication. Science 264:530–531
10. Collinge J, Palmer MS, Sidle KC, Gowland I, Medori R, Ironside J, Lantos

P (1995) Transmission of fatal familial insomnia to laboratory animals. Lancet 346:569–570
11. Coustou V, Deleu C, Saupe S, Begueret J (1997) The protein product of the het-s heterokaryon incompatibility gene of the fungus *Podospora anserina* behaves as a prion analog. Proc Natl Acad Sci USA 94:9773–9778
12. Derkatch IL, Chernoff YO, Kushnirov VV, Inge-Vechtomov SG, Liebman SW (1996) Genesis and variability of [PSI] prion factors in *Saccharomyces cerevisiae*. Genetics 144:1375–1386
13. Farquhar CF, Somerville RA, Bruce ME (1998) Straining the prion hypothesis. Nature 391:345–346
14. Gilbert W (1985) Genes-in-pieces revisited. Science 228:823–824
15. Glover JR, Kowal AS, Schirmer EC, Patino MM, Liu JJ, Lindquist S (1997) Self-seeded fibers formed by Sup35, the protein determinant of [PSI+], a heritable prion-like factor of *S. cerevisiae*. Cell 89:811–819
16. Harper JD, Lansbury Jr PT (1997) Models of amyloid seeding in Alzheimer's disease and scrapie: Mechanistic truths and physiological consequences of the time-dependent solubility of amyloid proteins. Annu Rev Biochem 66:385–407
17. Holmgren A, Bränden C-I (1989) Crystal structure of chaperone protein PapD reveals an immunoglobulin fold. Nature 342:248–251
18. Horwich AL, Weissman JS (1997) Deadly conformations-protein misfolding in prion disease. Cell 89:499–510
19. Hsiao KK, Scott M, Foster D, Groth DF, DeArmond SJ, Prusiner SB (1990) Spontaneus neurodegeneration in transgenic mice with mutant prion protein. Science 250:1587–1590
20. Hsiao KK, Groth DF, Scott M, Yang S-L, Serban H, Rapp D, Foster D, Torchia M, DeArmond SJ, Prusiner SB (1994) Serial transmission in rodents of neurodegeneration from transgenic mice expressing mutant prion protein. Proc Natl Acad Sci USA 91:9126–9130
21. Hunter N, Cairns D, Foster JD, Smith G, Goldmann W, Donnelly K (1997) Is scrapie solely a genetic disease? Nature 386:137
22. Kaneko K, Zulianello L, Scott M, Cooper CM, Wallace AC, James TL, Cohen FE, Prusiner SB (1997) Evidence for protein X binding to a discontinuous epitope on the cellular prion protein during scrapie prion propagation. Proc Natl Acad Sci USA 94:10069–10074
23. King C, Tittmann P, Gross H, Gebert R, Aebi M, Wüthrich K (1997) Prion-inducing domain 2–114 of yeast Sup35 protein transforms *in vitro* into amyloid-like filaments. Proc Natl Acad Sci USA 94:6618–6622
24. Klein MA, Frigg R, Flechsig E, Raeber AJ, Kalinke U, Bluethmann H, Bootz F, Suter M, Zinkernagel RM, Aguzzi A (1997) A crucial role for B cells in neuroinvasive scrapie. Nature 390:687–690
25. Lansbury Jr PT (1997) Yeast prions: Inheritance by seeded protein polymerization? Current Biology 7:R617–R619
26. Lasmézas CI, Deslys J-P, Robain O, Jaegly A, Beringue V, Peyrin J-M, Fournier J-G, Hauw J-J, Rossier J, Dormont D (1997) Transmission of the BSE agent to mice in the absence of detectable abnormal prion protein. Science 275:402–405
27. Lindquist S (1997) Mad cows meet Psi-chotic yeast: The expansion of the prion hypothesis. Cell 89:495–498
28. Masison DC, Wickner RB (1995) Prion-inducing domain of yeast Ure2p and protease resistence of Ure2p in prion-containing cells. Science 270:93–95
29. Masters CL, Beyreuther K (1997) Tracking turncoat prion proteins. Nature 388:228–229
30. Monari L, Chen SG, Brown P, Parchi P, Petersen RB, Mikol J, Gray F, Cortelli P, Montagna P, Ghetti B, Goldfarb LV, Gajdusek DC, 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
31. Pan K-M, Baldwin M, Nguyen J, Gasset M, Serban A, Groth D, Mehlhorn I, Huang Z, Fletterick RJ, Cohen FE, Prusiner SB (1993) Conversion of α-helices into β-sheets features in the formation of the scrapie prion proteins. Proc Natl Acad Sci USA 90:10962–10966
32. Patino MM, Liu JJ, Glover JR, Lindquist S (1996) Support for the prion hypothesis for inheritance of a phenotypic trait in yeast. Science 273:622–626
33. Paushkin SV, Kushnirov VV, Smirnov VN, Ter-Avanesyan MD (1997) In vitro propagation of the prion-like state of yeast Sup35 protein. Science 277:381–383
34. Peretz D, Williamson A, Matsunaga Y, Serban H, Pinilla C, Bastidas RB, Rozenshteyn R, James TL, Houghten RA, Cohen FE, Prusiner SB, Burton DR (1997) A conformational transition at the N terminus of the prion protein features in formation of the scrapie isoform. J Mol Biol 273:614–622
35. Prusiner SB (1982) Novel proteinaceus infectious particles cause scrapie. Science 216:136–144
36. Prusiner SB (1989) Scrapie prions. Annu Rev Microbiol 43:345–374
37. Prusiner SB, Scott M, Foster D, Pan K-M, Groth D, Mirenda C, Torchia M, Yang S-L, Serban D, Carlson GA, Hoppe PC, Westaway D, DeArmond SJ (1990) Transgenic studies implicate interactions between homologous PrP isoforms in scrapie prion replication. Cell 63:673–686
38. Prusiner SB (1997) Prion diseases and the BSE crisis. Science 278:245–251
39. Raymond GJ, Hope J, Kocisko DA, Priola SA, Raymond LD, Bossers A, Ironside J, Will RG, Chen SG, Petersen RB, Gambetti P, Rubenstein R, Smits MA, Lansbury PT, Caughey B (1997) Molecular assessment of the potential transmissibilities of BSE and scrapie to humans. Nature 388:285–288
40. Riek R, Hornemann S, Wider G, Billeter M, Glockshuber R, Wüthrich K (1996) NMR structure of the mouse prion protein domain PrP (121–231). Nature 382:180–182
41. Riek R, Hornemann S, Wider G, Glockshuber R, Wüthrich K (1997) NMR characterization of the full-length recombinant murine prion protein, mPrP (23–231). FEBS Letters 413:282–288
42. Riesner D, Kellings K, Post K, Wille H, Serban H, Groth D, Baldwin MA, Prusiner SB (1996) Disruption of prion rods generates 10-nm spherical particles having high α-helical content and lacking scrapie infectivity. J Virol 70:1714–1722
43. Scott M, Foster D, Mirenda C, Serban D, Coufal F, Wälchli M, Torchia M, Groth D, Carlson G, DeArmond SJ, Westaway D, Prusiner SB (1989) Transgenic mice expressing hamster prion protein produce species-specific scrapie infectivity and amyloid plaques. Cell 59:847–857
44. Scott M, Groth D, Tatzelt J, Torchia M, Tremblay P, DeArmond SJ, Prusiner SB (1997) Propagation of prion strains through specific conformers of the prion protein. J Virol 71:9032–9044
45. Telling GC, Scott M, Mastrianni J, Gabizon R, Torchia M, Cohen FE, DeArmond SJ, Prusiner SB (1995) Prion propagation in mice expressing human and chimeric PrP transgenes implicates the interaction of cellular PrP with another protein. Cell 83:79–90
46. Telling GC, Parchi P, DeArmond SJ, Cortelli P, Montagna P, Gabizon R, Mastrianni J, Lugaresi E, Gambetti P, Prusiner SB (1996) Evidence for the conformation of the pathologic isoform of the prion protein enciphering and propagating prion diversity. Science 274:2079–2082
47. Weissmann C (1991) The prion's progress. Nature 349:569–571
48. Weissmann C (1996) Molecular biology of transmissible spongiform encephalopathies. FEBS Letters 389:3–11
49. Wickner RB (1994) [URE3] as an altered URE2 protein: Evidence for prion analog in *Saccharomyces cerevisiae*. Science 264:566–569
50. Wickner RB, Masison DC (1996) Evidence for two prions in yeast: [URE3] and [PSI]. Curr Top Microbiol Immunol 207:147–160
51. Xi YG, Ingrosso L, Ladogana A, Masullo C, Pocchiari M (1992) Amphotericina B treatment dissociates in vivo replication of the scrapie agent from PrP accumulation. Nature 356:598–601