Review

The prion strain phenomenon: Molecular basis and unprecedented features

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Abstract

Prions are unconventional infectious agents responsible for transmissible spongiform encephalopathies. Compelling evidences indicate that prions are composed exclusively by a misfolded form of the prion protein (PrP^{Sc}) that replicates in the absence of nucleic acids. One of the most challenging problems for the prion hypothesis is the existence of different strains of the infectious agent. Prion strains have been characterized in most of the species. Biochemical characteristics of PrP^{Sc} used to identify each strain include glycosylation profile, electrophoretic mobility, protease resistance, and sedimentation. *In vivo*, prion strains can be differentiated by the clinical signs, incubation period after inoculation and the lesion profiles in the brain of affected animals. Sources of prion strain diversity are the inherent conformational flexibility of the prion protein, the presence of PrP polymorphisms and inter-species transmissibility. The existence of the strain phenomenon is not only a scientific challenge, but it also represents a serious risk for public health. The dynamic nature and inter-relations between strains and the potential for the generation of a large number of new prion strains is the perfect recipe for the emergence of extremely dangerous new infectious agents.

Keywords: Prions; Prion strains; Protein misfolding; Scrapie; Creutzfeldt-Jakob disease

1. Introduction

Transmissible Spongiform Encephalopathies (TSEs), also known as prion disorders, are infectious and fatal neurodegenerative diseases affecting humans and other mammals. In humans, TSEs include Creutzfeldt–Jakob disease (CJD), fatal familial insomnia (FFI), Gertsmann–Straussler–Scheinker Syndrome (GSS) and Kuru [1,2]. In other mammals, bovine spongiform encephalopathy (BSE) is found in cattle, scrapie in sheep and goats and chronic wasting disease (CWD) in elk and deer [1,2]. Although the clinical symptoms vary in distinct diseases, they usually include dementia and/or ataxia with progressive loss of brain function, irreversibly resulting in death [3]. The hallmark of prion diseases is the misfolding of the prion protein observed in the brain of affected individuals [1]. Misfolded proteins have the intrinsic tendency to form large aggregates and fibrillar structures, that may form amyloid deposits in a similar fashion as observed in Alzheimer's, Parkinson's diseases and many other protein misfolding disorders [4].

Although of rare occurrence, prion diseases have drawn considerable attention and led to severe economic and political consequences in Europe and in the United States. The two main reasons of this impact include the unique nature of the infectious agent and the appearance of a new human disease (vCJD) linked to consumption of cattle meat infected by BSE. At present, it is impossible to estimate accurately the number of upcoming cases of vCJD due to the very long incubation time of the disease in humans [5–7]. Prion research has been plagued with the discovery of new and heretic scientific findings that have confronted the most solid paradigms in modern biology. The current evidence suggest that an abnormal form of the prion protein (termed PrP^{Sc}) is the main, and possibly the only, constituent of prion infectious agent [1]. This so-called protein-only hypothesis [1,8] proposes that replication of PrP^{Sc} occurs at expenses of the normal host's version of the prion protein (termed PrP^{C}). PrP^{Sc} has different biochemical characteristics compared to PrP^{C}, for example its insolubility, resistance to denaturation, and its partial resistance to protease degradation

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[9]. PrP^{Sc} treatment with proteases reveal the protease resistant core of the infectious agent (termed $PrP^{27–30}$ according to its molecular weight) [10].

2. Molecular basis of prion strains

Among the unique features that have contributed to place the prion field in the spotlight, one of the most interesting is the prion strain phenomenon. It has been observed that animals affected by prion diseases may develop different pathologies and the clinical and biochemical outcomes could be maintained through several passages in rodents models of prion diseases. In analogy to other infectious agents, these variants have been termed strains. A classical definition of strain makes mention to a genetic variant or subtype of the infectious agent responsible for the disease, but this concept, valid in virology, cannot be extended to prions. In early days, the strain phenomenon was claimed as one of the strongest evidences against the protein-only hypothesis [11,12]. It was assumed that the different phenotypes found in animals were due to differences in the genetic information contained within the TSE causing agent. However, currently it is widely accepted that the main differences between prion strains arise from alternative conformations of PrP^{Sc} that can be stably and faithfully propagated [13,14].

The first evidence about the existence of prion strains was described in goats affected by scrapie by Pattison and Millson in 1961 [15]. In this report, goats infected by the same batch of infectious scrapie agent developed two different clinical phenotypes, termed by the authors “scratching” and “drowsy”, according to disease’s manifestation. The differences between these infectious agents were alleged to be the consequence of differences in the genetic background of the host. The current evidence supports this hypothesis. In some cases, clinical signs could be very useful to differentiate between prion strains [15–17]. Each prion strain has the capability to affect specific brain areas producing differences in clinical signs. In the case of scrapie in sheep and goats, after identification and isolation of the prion protein gene (*prnp*) several polymorphic differences were recognized when numerous sheep flocks were compared [18].

Prion strains can be classified by different parameters. Incubation periods, profile of histological damage and clinical signs are the main *in vivo* characteristics which can be used to differentiate between prion strains [16,19,20]. The most commonly used is incubation period which corresponds to the time elapsed between experimental inoculation of the infectious agent and clinical onset of the disease. Intra-species inoculation of prions is usually very reproducible [19]. Inoculation of different prion strains preparations usually results in different and reproducible incubation times [19,21]. Histological studies have also shown substantial differences when animals were inoculated with distinct strains. The differences are mainly on the distribution and characteristics of PrP^{Sc} deposition and the degree of vacuolation in specific brain regions [22–25]. In order to quantify this aspect, a well-standardized procedure for vacuolization scoring (lesion profile) in mice [25] has been described; six gray matter and three white matter brain areas are analyzed and scored according to the magnitude of the damage. Using this approach, prion strains having similar incubation times were differentiated, such as ME7 and 79A [25]. In a similar way, PrP^{Sc} accumulation profile has been useful to track the origin of the infectious material. For example, in the case of the transmission of BSE into humans, originating vCJD, similar neuropathological signatures were produce [26,27]. Finally, the clinical signs are also a characteristic that can be very useful to differentiate strains. For example, in human prion diseases, motor incoordination, dementia, ataxia, depression, and insomnia are just few from a much larger list of clinical symptoms that can appear with more or less intensity depending on the strain of the agent [3]. In other animals, such as the case of hamsters, the clinical features can be diametrically opposed. That is the case of the Drowsy (DY) and Hyper (HY) prion strains [16]. Unfortunately, clinical signs cannot always be applied to differentiate and classify prion strains. In mouse for example, several prion strains have the same rough signs, which include ataxia, rough coat, and hunch [28,29]. However, studies using more detailed tests have identified dissimilar behavioral deficits when different prion strains are administrated to mice [30]. Since different brain lesion patterns appear to be responsible for the variation in clinical signs, behavioral studies could give us more specific information about the type of brain damage produced by different prion isolates [30,31].

In addition to the *in vivo* differences, each prion strain has a particular group of biochemical characteristics in the infectious protein that could be specifically associated to them. Among them, the most important are the electrophoretical mobility after proteinase K (PK) digestion [32–34], glycosylation pattern [33–35], extent of PK resistance [32], sedimentation [32] and resistance to denaturation by chaotropic agents [32,36]. Recently, differences in the binding affinity for copper among strains have been described [37]. As illustrated in Fig. 1, the biochemical features of PrP^{Sc} in various forms of CJD are different. The western blot profile of different sources of human PrP^{Sc} shows diversity in terms of glycosylation pattern and electrophoretical mobility after PK digestion [32–34]. Fourier Transform Infrared Spectroscopy (FTIR) studies involving

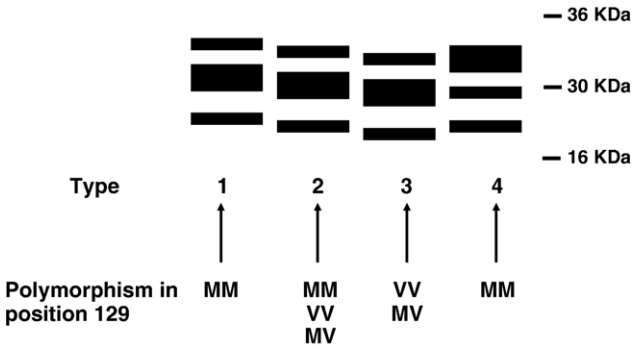


Fig. 1. PrP^{Sc} western blot profiles associated to different strains of human prions. Schematic representation of human PrP^{Sc} types after PK digestion. Particular polymorphic groups in position 129 are associated with specific PrP^{Sc} patterns. Types 1 and 2 are associated with sCJD, type 3 is mostly associated with iCJD and type 4 is found exclusively in vCJD.

different prion strains [36,38,39], conformation dependent immunoassays [36,40] and atomic force microscopy of synthetic prion protein polymers [41] confirm the hypothesis that differences between prion strains lies in the diversity of structures that PrP^{Sc} can acquire. However, the definitive proof for the structural nature of the differences between prion strains is still missing.

3. Species barrier and generation of new prion strains

The principal source of strain diversity arise from interspecies infection [23,42–45]. One of the characteristics of the agent responsible for prion diseases is its ability to infect some species and not others. This phenomenon is known as "species barrier" and is manifested as the prolongation in the incubation periods when prions from one species are used to infect a different one [46,47]. Differences in the sequence of prion protein could lead to different conformations, explaining both, species barrier and diversity of PrP^{Sc} conformations [41,48–50]. In some species, PrP^{C} conformation does not permit conversion by prions coming from other species. A clear example of this is found in rabbit, an animal that has been unable to be infected by various sources of prions. In these cases, it is considered that the species barrier is absolute.

Interspecies prion transmission from cattle to human is probably the most relevant problem in terms of public health [51–53]. It is widely accepted that consumption of BSE infected material is the cause of vCJD in humans [27,54]. Strikingly, vCJD presents many different features compared to the previously known human strains, arisen sporadically. Differences between vCJD and sCJD include the clinical manifestation of the disease, the profile of brain damage and the biochemical features of PrP^{Sc} [55]. The BSE epidemic in the United Kingdom demonstrated how dangerous prions could be. So far, BSE is the only non-human prion described to be transmissible to humans. Despite the fact that people have consumed for centuries sheep potentially affected by scrapie, no correlation has been found between patients suffering by CJD and sheep consumption. Scrapie transmissibility experiments using transgenic animal models expressing chimeric human/mouse PrP support this assumption [56].

BSE has not only been transmitted to humans. The extensive use of cow-derived material for feeding other animals led to the generation of new diseases in exotic felines such as tiger and cheetah, non human primates, and domestic cats [52,57–60]. The transmission of BSE into these different species could create many new prion strains, each one of them with particular biological and biochemical characteristics and thus a potentially new hazard for human health. Successful transmission of BSE in pigs has been described [61,62] and also in transgenic mice expressing pig PrP [63]. Porcine derivates are widely consumed and the hypothetic case of "mad pigs" could increase the events of zoonotic transmission of prions to humans. Fortunately, transmission of BSE to pigs is possible only in very drastic experimental conditions, not likely to be occurring naturally [62,63]. More frightening is perhaps the possibility that BSE has been passed into sheep and goats. Studies have already shown that this transmission is possible and actually relatively easy and worrisomely produces a disease clinically similar to scrapie [64]. The cattle origin of this new scrapie makes possible that the new strain may be transmissible to humans. Transmission experiments of BSE infected sheep brain homogenate into transgenic animal models expressing human PrP are currently ongoing in several laboratories. It is important to note that all materials generated by transmission of BSE in experimental and natural cases show similar biochemical behavior compared to the original inoculum [65], suggesting that these new generated infectious agents might be hazardous for humans. The origin of BSE is still a mystery. Abundant evidence supports the hypothesis that BSE was produced by cattle feeding with scrapie derivated material [66,67], indicating that bovine PrP^{Sc} might be a "conformational intermediary" between ovine PrP^{Sc} and human PrP^{C}.

There is currently no mean to predict which will be the conformation of a newly generated strain and how this new PrP^{Sc} conformation could affect other species. One interesting new prion disease is CWD, a disease affecting farm and wild species of cervids [68,69]. The origin of CWD and its potential to transmit to humans are currently unknown. This is worrisome, considering that CWD has became endemic in some parts of USA and the number of cases continues to increase [69]. It is presumed that a large number of hunters in the US have been in contact or consumed CWD-infected meat [70]. CWD transmissibility studies have been performed in many species in order to predict how this disease could be spread by consumption of CWD meat [71–73]. In these studies, a special attention has been done to scavenging animals [74], which are presumed to be exposed to high concentration of cervid prions, resulting in the putative generation of many new forms of TSEs. Fortunately negative results were obtained in experiments done in raccoons infected with CWD [74]. Transmission of CWD to humans cannot be ruled out at present and a similar infective episode to BSE involving CWD could result in catastrophic events, spreading the disease in a very dangerous way through the human population. No clinical evidence linking CWD exposed humans and CJD patients have been found [70], but experimental inoculation of CWD prions into squirrel monkeys propagated the disease [71]. Nevertheless, the species barrier between humans and cervids appears to be greater than with cattle, as judged by experiments with transgenic mice models [75]. Finally, it is important to be aware about CWD transmissibility to other species in which a "conformational intermediary" could be formed, facilitating human infection.

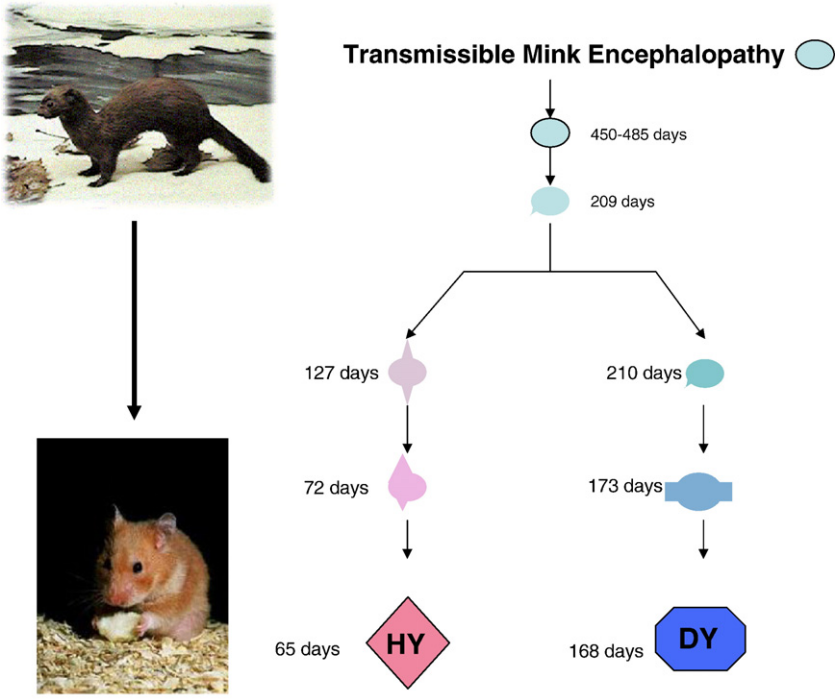
4. Use of experimental animals to study prion strains

Probably the best way to study the strain phenomenon is using experimental animals for the generation of diverse strains through inoculation with prion infectious material coming from different species. Among the experimental models, perhaps mouse is the most useful one, in which more than 20 phenotypically distinct strains have been isolated [19]. Many of these strains have their origin in the transmission of different

sources of scrapie from goat and sheep, BSE derived material from cattle [23,24,76] and human sources as sCJD and GSS [44,77,78]. Serial passage of infectious prions in one species, with constant biological background is necessary to stabilize and define a prion strain.

PrP^{Sc} obtained from mouse adapted scrapie prion strains such as RML, ME7, 139A and 79A show similar electrophoretical characteristics after PK digestion. PrP^{Sc} coming from these strains show an electrophoretical mobility of ~21 kDa for the unglycosylated band and a similar glycosylation pattern, with the monoglycosylated form as the most abundant [79–83]. Despite the lack of biochemical differences, these strains can be differentiated when inoculated in mice by measuring the incubation time or the profile of brain lesions [25,28,82,84]. Other mouse strains have been generated by inoculation of animals with BSE and sCJD prions, leading to strains termed 301C and Fukuoka, respectively [42,43,85]. The transmission of BSE into mice generated two different phenotypes: one presenting PK resistant isoform of PrP^{Sc}, and another lacking this characteristic [76]. This phenotype is maintained after two serial passages in mice, but finally only the PK resistance phenotype remains. The presence of a PK sensitive infectious material (termed $sPrP^{Sc}$) has been also described in some cases of human prion diseases [86,87].

The characteristics of mouse strains generated from scrapie or from BSE are quite different. For example, intracerebral inoculation of RML strain into mice present an onset of ~150 days post inoculation (dpi), while 301C preparations cause the disease at ~200 dpi [19,88]. Intraperitoneal inoculation of RML and 301C material in the same animals shows a larger difference in the incubation periods: 200 and 300 dpi, respectively [24,84]. There are also differences in the



Clinical signs	Hyperexcitability	Lethargy
PrP^{Sc} deposition profile	Mostly diffuse deposits	Amyloid plaques
PrP^{Sc} electrophoretical mobility	21kDa unglycosylated band	19kDa unglycosylated band
PrP^{Sc} PK resistance	Highly resistant	Slightly resistant
Peripheral infectivity	Yes	No
Infectious to mink (memory)	No	Yes

Fig. 2. Origin and properties of the HY and DY prion strains in hamsters. The Hyper (HY) and Drowsy (DY) scrapie strains were generated upon serial passage of transmissible mink encephalopathy (TME) infectious material in Syrian hamsters. The initial passage resulted in a very large incubation period that was upon successive passage stabilized in two different strains exhibiting strikingly different clinical, neuropathological, biochemical and infectious properties. The HY and DY hamster strains represent a prototype example of strain diversity without changes in amino acid sequence of the prion protein.

brain affected areas by both mouse adapted prions (Castilla J., Morales R., Saá P., and Soto C.; unpublished data). In addition, it is also possible to find biochemical differences between RML and 301C strains. As mentioned above, PK digestion pattern of RML shows an electrophoretical mobility of ~21 kDa for the unglycosylated band and is rich in the monoglycosylated isoform of prion protein. In contrast, 301C prion strain show a different electrophoretical pattern compared to RML. Unglycosylated isoform of BSE adapted mouse strain shows an electrophoretical mobility of ~19 kDa and its glycoform distribution favors the diglycosylated isoform [83].

How different PrP^{Sc} conformations could induce stable conformational changes in the same host protein is still unknown. Even more interesting is the isolation of different prion strains from the same host after inoculation of PrP^{Sc} from a single species. Probably the most representative experience is the isolation of DY and HY strains after inoculation of the agent associated to transmissible mink encephalopathy (TME) in Syrian hamsters (Fig. 2) [13,16]. This interspecies transmission of prions presented the expected behavior of the species barrier phenomenon: a long incubation period in the first passage, but shorter incubation periods after inoculation of serial passages from the resulting infectious material into Syrian hamsters. Incubation periods became stable in two different groups with different clinical signs: the first one with an incubation period of ~150 dpi presented lethargy, while a shorter incubation period strain (~60 dpi) presented hyperactivity. These strains were called Drowsy (DY) and Hyper (HY) respectively according to their clinical signs [16]. Histopathological analysis of animal groups infected with both TME hamster adapted agents show differences in the vacuolation distribution among different brain regions [16] and also in the PrP^{Sc} deposition areas [89] (Fig. 2).

As 301C and RML in mouse, DY and HY present differences in their electrophoretical mobility after PK treatment. The unglycosylated band of DY has a molecular weight of 19 kDa, while HY show the same band at 21 kDa [32,90]. This is the most direct evidence that suggested conformational differences between both PrP^{Sc} species. Supporting this assumption, structural differences using Fourier transform infrared spectroscopy (FTIR) between both Syrian hamster adapted TME strains were found [38]. Another biochemical difference found between DY and HY lies in their differential resistance to PK digestion, where DY is the most sensitive to digestion compared to HY [32] (Fig. 2). All these biological and biochemical characteristics make DY and HY one of the most intriguing examples of prion strain variation.

5. Polymorphisms and prion strains

Polymorphisms in the prion protein and their effects in the prion strain phenomenon were indirectly described a long time before the prion hypothesis was developed [15]. Differences in prion pathology were found and extensively described in sheep and mice [17–19,25]. The drowsy and scratchy phenotypes found in sheep were attributed to polymorphic differences in the host [17]. The identification of "scrapie incubation period gene" (*sinc*) and its polymorphic differences was a very big hit in the study of prion strains [91]. In mouse, two polymorphic animal groups were originally described: $sinc^{s7}$ and $sinc^{p7}$. Later, it was discovered that the *sinc* gene was indeed the gene encoding PrP and the polymorphisms resulted in differences in the prion protein at positions 108 and 189 [92]. The transmission of infectious agents from sheep, goats and cattle to both mice groups resulted in the emergence of a wide diversity of prion strains [19,28]. When incubation periods of mouse adapted prions were stabilized in each group, new generated infectious agent could be assayed in the other animal group. It was found that the presence of the polymorphism produced a prolongation in the incubation period in a similar way as observed in the species barrier phenomenon [19]. It was postulated that prion strains in mouse could be differentiated inoculating infectious material in both animals' types and identifying the short and long incubation period animal cluster [19]. Interestingly, when a mouse prion strain is inoculated in *sinc* heterozygous animals, either intermediate or longer incubation periods are observed [28]. Inter-polymorphic transmissions can lead to the generation of new prion strains [19], which implies new vacuolation, infectivity and/or dominance characteristics, among others. All this information suggests that polymorphisms in the prion protein are able to favor strain diversity. Table 1 shows mice and prion strains corresponding to each polymorphic group.

After the isolation of prion protein gene [93], it was described that *sinc* and *prnp* genes were congruent [94]. Analysis of long and short incubation period animal groups revealed the expected polymorphic differences in the prion protein gene [21]. These findings strongly supports the prion hypothesis, because as observed in the species barrier phenomenon, differences in the sequence of the prion protein affect extensively the transmission and strain characteristics of the infectious agent. According to a new nomenclature generated, $sinc^{s7}$ animals are re-baptized as $prnp^{a}$, while $sinc^{p7}$ as $prnp^{b}$ [21]. Recently a new group of mice have been identified and named $prnp^{c}$ (Table 1) [88].

PrP polymorphisms are not unique of mouse. Indeed, polymorphisms in the prion protein have been described in most of the species. In sheep, several inter-bred crosses have been performed in order to optimize the quality and productivity

Table 1
Polymorphisms associated to prion diversity in mouse

Mouse prnp genotype	Mouse strain	Prion strains	Associated polymorphisms
$prnp^{a}/sinc^{s7}$	C57	RML-ME7-	Leu-108
	RIII	139A-301C-	Thr-189
	Swiss	22C-79A-	
	NZW	87A-	
	SJL		
$prnp^{b}/sinc^{p7}$	VM	301V-22A-	Phe-108
	IM	87V-79V-	Val-189
$prnp^{c}$	Mai/Pas		Phe-108
	C57 MAI-Prnp		Thr-189

The table shows different mouse strain and prion strains isolated in each polymorphic group.

of these animals, producing a wide range of polymorphic variants for *prnp* [18]. However, only five alleles of the PrP gene are significantly present giving a total of 15 possible PrP genotypes, each likely to favor or disfavor the selection of different scrapie strains [18]. These five common polymorphic alleles are ARQ, ARR, AHQ, ARH and VRQ. Polymorphic changes are present principally in codons 136, 154 and 171, but in order to simplify the nomenclature they are designated by the amino acid present in each position. In a recent revision by Baylis and Goldman [18] it is documented that sheep carrying the VRQ/VRQ, ARH/VRQ and ARQ/VRQ alleles are most susceptible to develop scrapie, whereas the less vulnerable are animals having ARR/ARR, ARR/ARH and AHQ/ARH alleles. Therefore, it is generally agreed that the VRQ allele promotes susceptibility to scrapie, whereas ARR diminishes the manifestation of the disease. Interestingly, other alleles such as ARH alone appear to favor the development of the disease while in combination with other alleles appear to confer resistance. In the same study, a correlation was established between incubation periods and the type of polymorphism. A linear relationship between age of death and five polymorphic groups was observed. Many prion strains have been described for scrapie. Each strain is associated to a particular allelic group, and allelic groups are associated to a particular breed of animals [42,95]. However, as previously described, prion protein diversity could exist with the same sequence in the prion protein and sheep is not the exception. CH1641, a prion strain with clear biochemical differences compared to other scrapie strains was isolated from a natural case of scrapie in Cheviot sheep [96]. Recently a new scrapie strain designated Nor98 has been described [95], mostly in animals having AHQ/AHQ and AHQ/ARQ genotypes (a variation relatively resistant to scrapie). In this case, "classically susceptible" alleles seem to be resistant to this class of prions [18,97]. All this information arise questions about how natural cases of scrapie are developed.

In humans there is a polymorphism at codon 129, where an ATG or GTG results in either a methionine (Met) or a valine (Val) at that position. A large body of evidence indicates that this polymorphism alone or in conjunction with mutations in the prion gene modulates disease susceptibility and phenotypic expression of human TSE [2,34,98–104]. Both Met and Val homozygous are over-represented, while heterozygous cases are under-represented in sCJD [98,99,105]. About 40% of the normal population is Met-homozygous, however 78%, 50% and 100% of patients affected by the sporadic, iatrogenic and variant forms of CJD are Met-homozygous, respectively [105–109]. These data suggest that the presence of Met at position 129 confers a higher susceptibility for the protein to be converted into the pathogenic isoform. The polymorphism has also been shown to alter the neuropathological pattern of lesions in sporadic CJD, the glycoform profile of protease-resistant PrP^{Sc} and the duration and severity of the disease [34,102,103,110–113]. A study involving 300 patients showed that Met-homozygous develop a more aggressive phenotype characterized by a short duration of disease (4.5 months), while heterozygous and Val-homozygous have a much longer disease duration (14.3 and 16.9 months, respectively) [103]. Val-homozygous seems to cause damage preferentially in the deep gray matter, while Met-homozygous seems to target mainly cortical structures [103]. Codon 129 polymorphism also influences the phenotypic expression of mutations elsewhere in the prion gene [104,114–119]. For example, people with a mutation at codon 178 resulting in a change of aspartic acid to asparagine develop either familial CJD or FFI depending on whether the amino acid at codon 129 is Val or Met, respectively [104].

Despite the clear importance of PrP polymorphism at position 129 in the disease propensity and pathogenesis, the molecular mechanism of this effect is unknown. Experimental and computational modeling studies of the tridimensional structure of PrP have been unable to identify any significant difference between the two isoforms [120]. In addition, no difference was reported on the in vitro thermodynamic stability of recombinant PrP bearing either Met or Val at position 129 [120,121]. Structural studies show evidence for hydrogen bonding between Asp178 and Tyr128, which might provide a structural basis for the influence of the polymorphism on the disease phenotype that segregates with the mutation Asp178Asn [121]. In addition, it has been reported that a slightly different conformation of recombinant Met- or Val-containing PrP isoforms was induced upon copper binding [122]. Using short model peptides, we found that M at position 129 increases the propensity of this region to aggregate into β-sheet rich fibrillar structures [123]. These findings were interpreted to suggest that Met induces a higher local propensity to extend the short β-sheet present in the normal protein into a larger sheet, which results in an increase in the rate of PrP conversion to the pathological isoform [123].

6. Unique features of prion strains

The biological and infectious characteristics of prions are dramatically different to the conventional infectious agents. These differences are manifested in the prion strains phenomenon in unique and unprecedented features, such as for example strain adaptation and memory, the coexistence and competition of prion strains, among others. In this section, some of these interesting phenomena will be briefly described.

6.1. Adaptation of prion strains

Interspecies transmission of prions could result in the emergence of more than one variety of infectious material with different strain characteristics. That is the case of DY and HY prion strains generation [13,16]. When interspecies transmission of prions occurs, serial passages in the new host are needed in order to stabilize the characteristics of new generated infectious material. In the case of TME transmission in hamsters, at least four serial passages in the new species were required for stabilization (Fig. 2) [13]. The first passage was characterized by long incubation periods and a dominance of a 19 kDa fragment when newly obtained PrP^{Sc} was analyzed after PK digestion. In the three first passages, clinical symptoms were not characteristic of the hamster-adapted HY or DY TME

strains. This phenotype was attributed to the combination effects of both strains replicating simultaneously. Thereafter, each of the strains was stabilized in some of the animals and once they are adapted and stabilized, they can be serially propagated *in vivo* and the characteristics are maintained. It is accepted that both strains present differential conversion kinetics *in vitro*, with DY being the slowest and HY the fastest [124]. For this reason, in order to select efficiently this prion strain, limiting dilution experiments must be performed [13]. In that way, the most abundant and less convertible DY is favored against the less abundant but fastest HY strain.

6.2. Co-existence of prion strains

Related to the above, it has been shown that two or more prion strains can co-exist in natural cases of TSE. Co-existence of prion strains has been found in sporadic cases of CJD [113,125]. Analyses of several sCJD tissue showed that different biochemical profiles of PrP^{Sc} could be found in different brain areas from the same patient [113]. Co-existence of prion strains was mainly observed in patient heterozygous for codon 129 [113]. As many as 50% of these patients present different types of PrP^{Sc} in their brains, whereas 9% of MM patients were positive for co-existence of strains. On the other hand, more than one PrP^{Sc} type was not observed in VV patients [113].

The biochemical and structural properties of the protein seem to be the major cause of this differential distribution. This observation may explain why sCJD is so heterogeneous in terms of clinical manifestation [34,126,127]. In a recent publication by Bishop et al. [107], vCJD infected transgenic mice expressing human PrP^{C}, present changes in their PrP^{Sc} and vacuolation patterns in the brain according to their polymorphic classification for codon 129.

6.3. Competition of prion strains

In particular experimental conditions some prion strains can extend their specific incubation period when co-infected with another strain. Long incubation period prions increase the incubation period of "faster" prions. This phenomenon of "competition of prion strains" has been observed in mice and hamster. In mice, competition between 22A and 22C strains was reported in 1975 by Dickinson et al. [128]. In this study, RIII mice (homozygous for $sinc^{s7}$ allele) were used. 22A and 22C showed long and short incubation period (550 and 230 days), respectively. When 22C strain was intraperitoneally inoculated 100, 200 and 300 days after intraperitoneal administration of the 22A agent, all three experimental groups resulted in incubation periods and lesion patterns matching 22A prions, suggesting that 22C prions were degraded or excreted, in animals previously infected by 22A. Similar results were obtained by Kimberlin and Walker in 1985 [129] using a different strain of $sinc^{s7}$ mice. These authors treated mice using 22A and 22C prion strain. Before inoculation, 22A was treated with different chemical and physical agents in order to see if the "competitor" or "blocking" characteristics of 22A were maintained. From all treatments, 12 M urea was shown to almost abolish the blocking properties of 22A agent. This information suggests that infectious properties of long incubation period agent are strictly necessary in order to increase the incubation period of faster prions.

In hamster, similar observations were reported using DY and HY [130]. DY prion strain was inoculated 30 and 60 days prior intraperitoneal inoculation of HY at three different doses. When incubation periods of HY inoculated control group were compared with the animals inoculated at 60 days with DY, significant differences in the incubation periods were found, especially when HY prions were administrated in a higher dose [130]. On the other hand no differences were observed in the case of intranerve inoculation, revealing that competition phenomenon occurs only when peripheral inoculation is performed. These results are surprising considering the fact that DY was reported not to be infectious when intraperitoneally inoculated in hamsters [130]. These data suggest that replication of DY is occurring in peripheral tissues but is not able to reach the central nervous system.

In general, the principal variables that need to be observed for a successful competition are the route of infection, the interval between injections and the particular strains and doses of agent used. Prolongation of incubation periods in TSE are therapeutically beneficial and several strategies are under development to reach this aim, including antibodies, beta-sheet breakers, and other chemical agents [131–133]. The experimental evidence described above suggests that prions could be potentially useful for this purpose. For example, in order to prevent spread of prion disease in cattle or humans, prion strains with incubation periods longer than species' lifespan could be used to slowdown the replication of BSE or vCJD prions.

7. Concluding remarks

The existence of different strains of an infectious agent composed exclusively of a protein has been one of the most puzzling issues in the prion field. If is already difficult to understand how a protein can adopt two stable and different folded structures and that one of them can transform the other one into itself, it is unthinkable that the misfolded form can in turn adopt multiple conformations with distinct properties. Yet, compelling scientific evidence support the idea that PrP can adopt numerous folding patterns that can faithfully replicate and produce different diseases. The existence of the strain phenomenon is not only a scientific challenge, but it also represents a serious risk for public health. The dynamic nature and inter-relations between strains and the potential for the generation of many new prion strains depending on the polymorphisms and the crossing of species barrier is the perfect recipe for the emergence of extremely dangerous new infectious agents. Although, substantial progress has been made in understanding the prion strains phenomenon, there are many open questions that need urgent answers, including: what are the structural basis of prion strains? How are the phenomena of strain adaptation and memory enciphered in the conformation of the prion agent? To what species can a given prion strain be

transmissible? What other cellular factors control the origin and properties of prion strains?

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