The Ninth Datta Lecture

Molecular biology of transmissible spongiform encephalopathies

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Abstract **The prion, the transmissible agent that causes spongiform encephalopathies such as scrapie, bovine spongiform encephalopathy (BSE) and Creutzfeldt–Jakob disease, is believed to be devoid of nucleic acid and identical with PrP^{Sc}, a modified form of the normal host protein PrP^{C} which is encoded by the single copy gene *Prnp*. The *'protein only'* hypothesis proposes that PrP^{Sc}, when introduced into a normal host, causes the conversion of PrP^{C} into PrP^{Sc}; it therefore predicts that an animal devoid of PrP^{C} should be resistant to prion diseases. We generated homozygous $Prnp^{o/o}$ ('PrP knockout') mice and showed that, after inoculation with prions, they remained free of scrapie for at least 2 years while wild-type controls all died within 6 months. There was no propagation of prions in the $Prnp^{o/o}$ animals. Surprisingly, heterozygous $Prnp^{o/+}$ mice, which express PrP^{C} at about half the normal level, also showed enhanced resistance to scrapie disease despite high levels of infectious agent and PrP^{Sc} in the brain early on. After introduction of murine PrP transgenes $Prnp^{o/o}$ mice became highly susceptible to mouse but not to hamster prions, while the insertion of Syrian hamster PrP transgenes rendered them susceptible to hamster but to a much lesser extent to mouse prions. These complementation experiments paved the way to the application of reverse genetics. We have prepared animals transgenic for genes encoding PrP with amino terminal deletions of various lengths and have found that PrP lacking 48 amino proximal amino acids, which comprise four of the five octa repeats of PrP, is still biologically active.**

Key words: Prions; PrP; Knockout mice; Transgenes; Scrapie; BSE; Transmissible spongiform encephalopathies

1. Introduction

Scrapie was described some 250 years ago as a sheep disease presenting with excitability, itching, ataxia and finally paralysis and death. In the past decades it has been studied as the prototype of what has proved to be a group of diseases affecting not only animals but also humans, the transmissible spongiform encephalopathies (TSEs) or prion diseases. It was early on recognized that the transmissible agent had quite extraordinary properties, such as unusually long incubation periods, measured in months to years and uncommon resistance to high temperature, formaldehyde treatment and UV irradiation. The agent was later designated 'prion' to distinguish it from conventional pathogens such as bacteria and viruses [1].

In a separate development, a number of slow degenerative human diseases of the central nervous system were recognized, namely kuru, Creutzfeldt–Jakob disease (CJD), Gerstmann–Sträussler–Scheinker disease (GSS) and fatal familial insomnia (FFI). Although CJD, GSS and FFI are rare diseases, found only once per 10^6–10^7 individuals per year, kuru assumed epidemic proportions in the first decades of this century in Papua New Guinea. Inoculation studies by Gajdusek and his colleagues resulted in the transmission of kuru to chimpanzees [2,3] and by now all human prion diseases have been transmitted to experimental animals, including the mouse. It is believed that kuru was propagated by ritual cannibalism [4,5] and may have originated with the consumption of the remains of a CJD sufferer.

In recent years a new form of prion disease emerged in Great Britain, and to a lesser extent in other European countries, namely bovine spongiform encephalopathy (BSE) or mad cow disease, which has been attributed to the consumption by cattle of feed supplements derived from scrapie-contaminated sheep and later from cattle offal [6]. In my view, it is however equally possible that BSE originated as a sporadic case in cattle and was then spread by contaminated cattle offal. Because there has quite recently been a cumulation in Great Britain of CJD-like disease with novel neuropathological features in humans below the age of 35 [7], there is reason to believe that BSE may have been transmitted to man via the consumption of contaminated bovine offal. The use of bovine offal for consumption by humans and ruminants has been interdicted in Great Britain since 1989.

2. Some characteristics of prion diseases

Prion diseases are unusual because they may arise spontaneously in the population at large without any apparent cause, so-called sporadic forms. They can be familial, tightly linked to certain mutations of the *Prnp* gene or acquired by transplantation, injection and possibly ingestion of contaminated products; in all case the disease can usually be experimentally transmitted to mice by intracerebral inoculation.

Although incubation times are measured in years or decades, once the disease becomes clinically evident, progression to death may take as little as a few months. In man, the first symptoms are usually loss of memory or motor disturbances, leading to dementia and death. The pathological changes in the brain vary in location and intensity; characteristically, extensive vacuolation, neuronal cell death and gliosis, singly or in combination are evident. Accumulation of PrP^{Sc} is the major pathognomonic feature; formation of amyloid plaques consisting mainly of PrP^{Sc} is a typical albeit not invariable

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finding. Neither inflammatory nor immunological responses are observed.

3. Hypotheses about the nature of the scrapie agent

The unusual properties of the scrapie agent early on gave rise to speculations that it might be devoid of nucleic acid [8]. Currently, the most widely accepted proposal is the *'protein only' hypothesis*, first outlined in general terms by Griffith [9] and enunciated in its updated and detailed form by Prusiner [10,11]. The *virino hypothesis* holds that the infectious agent consists of a scrapie-specific nucleic acid genome and host-derived PrP^{Sc}, which is recruited as some sort of coat [12]. Finally, some still believe that the scrapie agent is a *conventional virus* with unusual properties. However, no evidence for the scrapie-specific nucleic acid postulated by the virus or virino theories has been found [13].

The 'protein only' hypothesis proposes that the prion contains no nucleic acid and is identical with PrP^{Sc}, a modified form of PrP^{C} [10]. PrP^{C} is a normal host protein [14–16] found predominantly on the outer surface of neurons (Fig. 1). PrP^{Sc} is defined as a form of PrP^{C} that readily forms protease-resistant aggregates after treatment with detergents [14,17]. Prusiner proposed that PrP^{Sc}, when introduced into a normal cell, causes the conversion of PrP^{C} or its precursor into PrP^{Sc} (Figs. 1–3). The exact nature of the conversion is unknown but it is currently ascribed to conformational modification [18]; it has been determined that the β-sheet content of PrP^{Sc} is high while that of PrP^{C} is low [19,20]. No chemical differences have so far been found between PrP^{C} and PrP^{Sc} [21]. However, because the ratio of infectious units to PrP^{Sc} molecules is only about 1:100 000 [22], the structure of the PrP molecule actually associated with infectivity cannot be definitively inferred. For this reason and because specific infectivity can vary considerably, the PrP species responsible for infectivity is presently better designated as PrP* [23]; it may or may not be identical with PrP^{Sc}, the major species that has been characterized chemically and physicochemically. If it is identical, the low specific activity could be due to a low efficiency of infection or to the infectious unit being an aggregate of a large number of PrP^{Sc} molecules. The conclusion that some form of PrP is the essential, perhaps only constituent of the infectious agent is based on biochemical and genetic evidence, as outlined below.

4. Biosynthesis of PrP^{C} and PrP^{Sc}

The entire PrP coding sequence is contained within one exon of the singular *Prnp* gene [16]. As shown in Fig. 3, an N-terminal signal sequence of 22 amino acids is cleaved off the primary translation product, glycosylation occurs at two Asn residues and 23 C-terminal amino acids are removed when a glycosyl phosphatidylinositol (GPI) residue is attached to Ser231. Mature PrP^{C} is anchored to the outer surface of the plasma membrane [24] and undergoes endocytosis [25,26] and recycling [27]. The highest levels of PrP^{C} are found in brain, particularly in the hippocampus, but substantial amounts are also found in heart and skeletal muscle [28] and lesser levels in most other organs except for liver and pancreas.

No non-allelic *Prnp*-related genes have been identified, no differences between PrP^{C} and PrP^{Sc} have been revealed by protein sequencing, and the amino acid sequences of both agree with that deduced from cloned genomic and cDNA

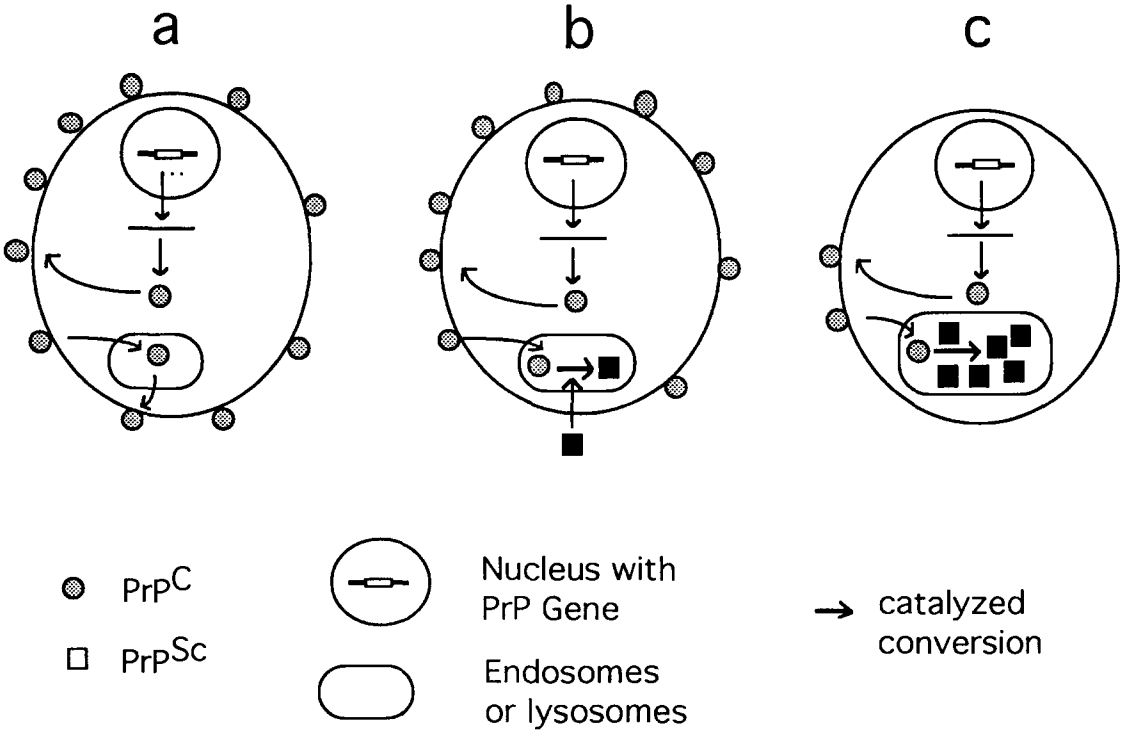

Fig. 1. Models for the propagation of the prion. (a) In the normal cell PrP^{C} is synthesized, transported to the cell surface and recycled (b) The 'protein only' model assumes that the prion is identical with PrP^{Sc}. Exogenous prions cause the conversion of the normal cellular protein PrP^{C} into PrP^{Sc}, either at the cell surface or after internalization. (c) PrP^{Sc} accumulates intracellularly, in late endosomes or lysosomes and the cell surface is depleted of PrP^{C}. PrP^{C} is also released into the extracellular space.

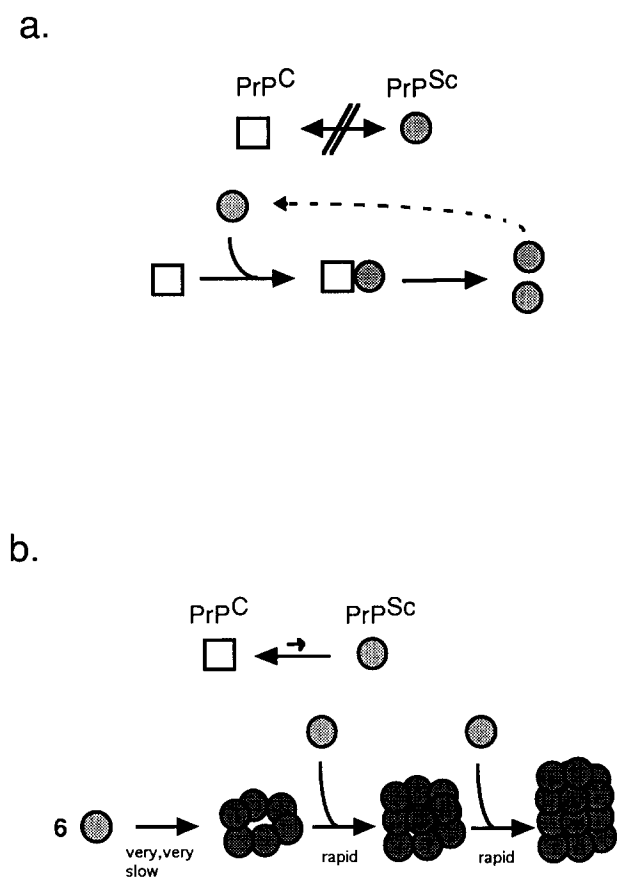

Fig. 2. Models for the conformational conversion of PrP^C to PrP^{Sc}. (a). The 'refolding' model. The conformational change is thermodynamically controlled: a high activation energy barrier prevents spontaneous conversion at detectable rates. As a result of an interaction with exogenously introduced PrP^{Sc}, PrP^C undergoes an induced conformational change to form PrP^{Sc}. This reaction may involve extensive unfolding and refolding of the protein to explain the postulated high energy barrier and could be dependent on an enzyme or chaperone. The process leads to an exponential conversion cascade. In the case of certain mutations in PrP^C, spontaneous conversion to PrP^{Sc} may occur as a rare event, explaining why familial CJD or GSS arise spontaneously, albeit late in life. Sporadic CJD may come about when an extremely rare event (occurring in one among a million individuals per year) leads to spontaneous conversion of PrP^C to PrP^{Sc} and gives rise to a conversion cascade [10,35]. (b). The 'seeding model'. The conformational change is kinetically controlled: the conformational change between PrP^C and PrP^{Sc} or a PrP^{Sc}-like molecule is reversible. PrP^{Sc} is only stabilized when it adds onto a crystal-like seed or aggregate of PrP^{Sc}. Seed formation is kinetically controlled and extremely slow; once a seed is present, monomer addition can ensue at a rapid rate [58,59].

[16,21,29]. Pulse–chase experiments in scrapie-infected neuroblastoma cells suggest that PrP^C is converted to PrP^{Sc} either at the cell surface or following endocytosis [30]. In brain, PrP^{Sc} accumulates to a level up to 100 times higher than that of PrP^C, however PrP mRNA levels are the same in normal and scrapie-infected tissue.

5. Physical linkage of PrP^{Sc} and prions

Purification of scrapie infectivity led to preparations containing PrP^{Sc} as major protein component [31]. Conversely, immunoaffinity purification of scrapie-infected hamster brain extracts using antibodies against PrP (there are currently no antibodies discriminating between PrP^C and PrP^{Sc}) led to enrichment of infectivity [32]. These experiments show that the infectious agent is physically associated with PrP^{Sc} or a molecule very similar to it, but do not preclude the association of the PrP-derived molecule with another component. It has, however, been shown that highly purified prion preparations contain less than one molecule of nucleic acid larger than about 100 nucleotides [33].

6. Genetic evidence linking the PrP gene with prion disease

Prions are transmitted from one species to another much less inefficiently, if at all, than within the same species and only after prolonged incubation times. In the case of prion transmission from hamsters to mice, this so-called species barrier was overcome by introducing hamster *Prnp* transgenes into recipient wild-type mice [34,35]. Importantly, the properties of the prions produced in these transgenic mice corresponded to the prion species used for inoculation [35], that is, infection with hamster prions led to production of hamster prions but infection with mouse prions gave rise to mouse prions. Within the framework of the 'protein only' hypothesis this means that hamster PrP^C but not murine PrP^C (which differs from the former by 10 amino acids), is a suitable substrate for conversion to hamster PrP^{Sc} by hamster prions and vice versa.

Most, if not all familial forms of human spongiform encephalopathies are linked to one of a number of mutations in the PrP gene [36]; for reviews see [37,38]. Prusiner [10,39] proposed that the mutations allow spontaneous conversion of PrP^C into PrP^{Sc} with a frequency sufficient to allow expression of the disease within the lifetime of the individual. Sporadic CJD could be attributed to rare instances of spontaneous conversion of PrP^C into PrP^{Sc} or rare somatic mutations in the *Prnp* gene. In both cases the initial conversion is thought to be followed by autocatalytic propagation. Hsiao et al. [36] showed that mice overexpressing a murine PrP transgene with a mutation corresponding to the human GSS mutation Pro102→Leu spontaneously contract a lethal scrapie-like disease. The brains of these animals contain low levels of infectious prions which can be detected in indicator mice expressing the same mutant transgene but at lower levels which do not lead to spontaneous disease [40].

Table 1
Prion titers in brain and spleen of $Prnp^{+/+}$ and $Prnp^{o/o}$ mice

Time after inoculation	Log LD_{50} units/ml			
	Brain		Spleen	
	$Prnp^{+/+}$	$Prnp^{o/o}$	$Prnp^{+/+}$	$Prnp^{o/o}$
4 days	<1.5	2.0	5.7±0.9	2.3
2 weeks	<1.5	<1.5	6.2±0.8	<1.5
8 weeks	5.4	<1.5	6.9±1.0	<1.5
12 weeks	6.8	<1.5	5.9±0.6	<1.5
20 weeks	8.6	<1.5	6.9±0.6	<1.5
23/25 weeks	8.1±0.8	<1.5	n.d.	<1.5

Mice with the genotype indicated were inoculated intracerebrally with mouse prions. Titers were determined by end point titration on homogenates of pooled organs from 4 mice after heating for 20 min at 80C. Data from [41].

7. Resistance to scrapie of mice devoid of PrP^C

The 'protein only' hypothesis predicts that in the absence of PrP^C mice should be resistant to scrapie and fail to propagate the infectious agent.

To generate mice devoid of PrP, we disrupted one *Prnp* allele of murine embryonic stem (ES) cells by homologous recombination with a recombinant DNA fragment in which two thirds of the 254-codon open reading frame were replaced by extraneous DNA. The ES cells were introduced into blastocysts, from which chimeric mice were generated. Appropriate breeding gave rise to offspring homozygous for the disrupted *Prnp* gene ($Prnp^{o/o}$). PrP was undetectable in $Prnp^{o/o}$ brains and present at about half the normal level in the brains of heterozygous ($Prnp^{o/+}$) mice [41]. No abnormalities were noted in $Prnp^{o/o}$ mice at the macroscopic, microscopic or behavioral levels [41]. The suggestion that there may be a synaptic deficiency in $Prnp^{o/o}$ mice [42,43] has not been confirmed [44]. The claim that aged mice (with a mixed genetic background) develop ataxia and suffer a loss of Purkinje cells [45] as a consequence of PrP gene disruption is not consistent with previous investigations on independently generated $Prnp^{o/o}$ mouse lines [41,46]. Because the phenotype might be due to the undefined, mixed genetic background of the knockout mice [47], it is necessary to show that complementation with a PrP transgene restores the normal phenotype.

When challenged with mouse prions, mice devoid of PrP were completely protected against scrapie disease (Fig. 4A). Prions were not propagated in brains of $Prnp^{o/o}$ mice at detectable levels, while in scrapie-inoculated $Prnp^{+/+}$ animals infectious agent was absent up to 2 weeks after inoculation (p.i.) but was present at 8 weeks and increased to about 8.6 log LD_{50} units/ml by 20 weeks p.i. (Table 1) [48,49]. As opposed to brain, spleen of $Prnp^{+/+}$ animals contained infectivity at the earliest time point tested, namely 2 days p.i. and increased thereafter to a level of about 7 log LD_{50} units/ml. In contrast, spleen of knockout animals showed only a low prion level at 4 days p.i., which thereafter became undetectable, suggesting that prions are initially transported from the intracerebral injection site to the spleen, where they are soon degraded. It had previously not been clear whether infectivity in spleen of wild-type animals, particularly at early times, was due to transport from the site of inoculation or whether it was synthesized in the spleen itself. The fact that in spleen of wild-type animals the prion titer is high at 2 weeks, when no infectivity is found in the brain, coupled with the fact that in knockout animals inoculum-derived infectivity has disappeared by that time, strongly suggests that in wild-type animals prions are in fact synthesized in the spleen. It is, incidentally, quite puzzling that following intracerebral injection prion synthesis occurs so early in spleen and only after a long delay in brain.

Interestingly, even heterozygous $Prnp^{o/+}$ mice were partially protected, inasmuch as they showed prolonged incubation times of about 290 days as compared to about 180 days in the case of the wild-type controls. Moreover, the disease progressed much more slowly in $Prnp^{o/+}$ mice than in $Prnp^{+/+}$ mice, the interval between first symptoms and death being about 13 days in the case of $Prnp^{+/+}$ mice and 150 or more days in $Prnp^{o/+}$ mice (Fig. 5) [75]. These and other findings (see below and [35]) show that susceptibility to scrapie is a function of PrP^C levels in the host. It is evident that mice can carry high levels of scrapie infectivity and PrP^{Sc} in their brain without showing clinical disease; the same might be true for humans and other animals.

When a modified phenotype is observed following a targeted mutation or gene disruption, it is important to show that the original phenotype –in this case, susceptibility to scrapie– can be recovered by restoring wild-type function. We therefore introduced murine *Prnp* transgenes into $Prnp^{o/o}$ mice and obtained several lines with varying expression levels of PrP^C. As shown in Fig. 4B, knockout mice expressing *Prnp* transgenes became susceptible to mouse prions; in fact, the higher the PrP^C content of the brain, the shorter the incuba-

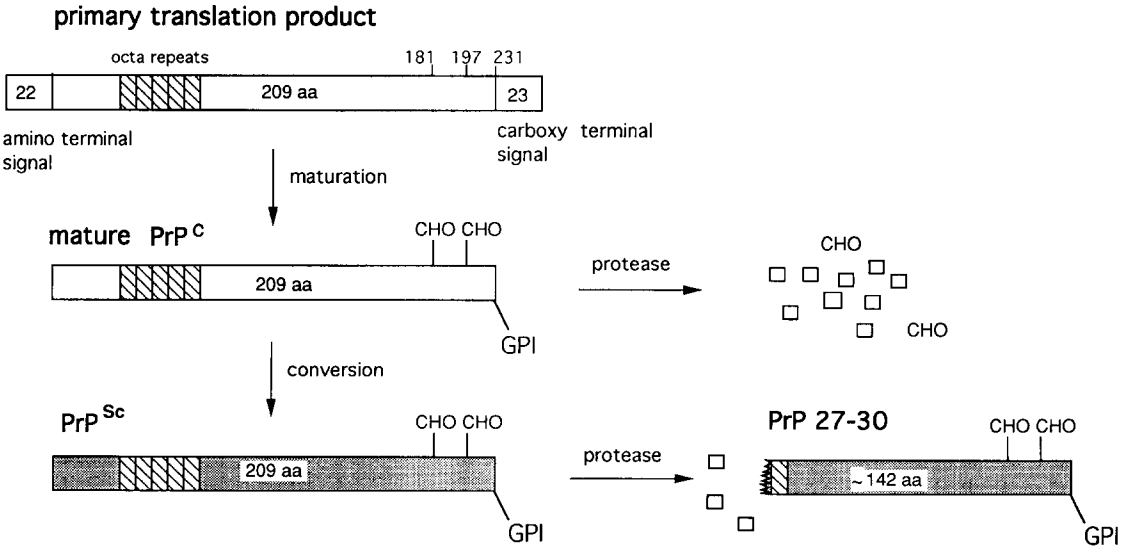

Fig. 3. Biosynthesis of PrP^C and PrP^{Sc}. Maturation of the PrP precursor protein involves cleavage of the signal sequence, removal of 23 C-terminal amino acids, attachment of a glycosyl phosphatidylinositol (GPI) anchor at Ser231 and glycosylation (CHO) at Asn181 and Asn197. Mature PrP^C is anchored at the outer surface of the plasma membrane and is sensitive to proteinase K. Conversion of PrP^C to PrP^{Sc} occurs after exposure of the host to scrapie prions. PrP^{Sc} is partially resistant to proteinase K and yields PrP27–30 after digestion; the N-terminus is frayed and ranges from residues 73 to 90. 'Octa repeats' are repeats of almost identical sequences of 8 amino acids.

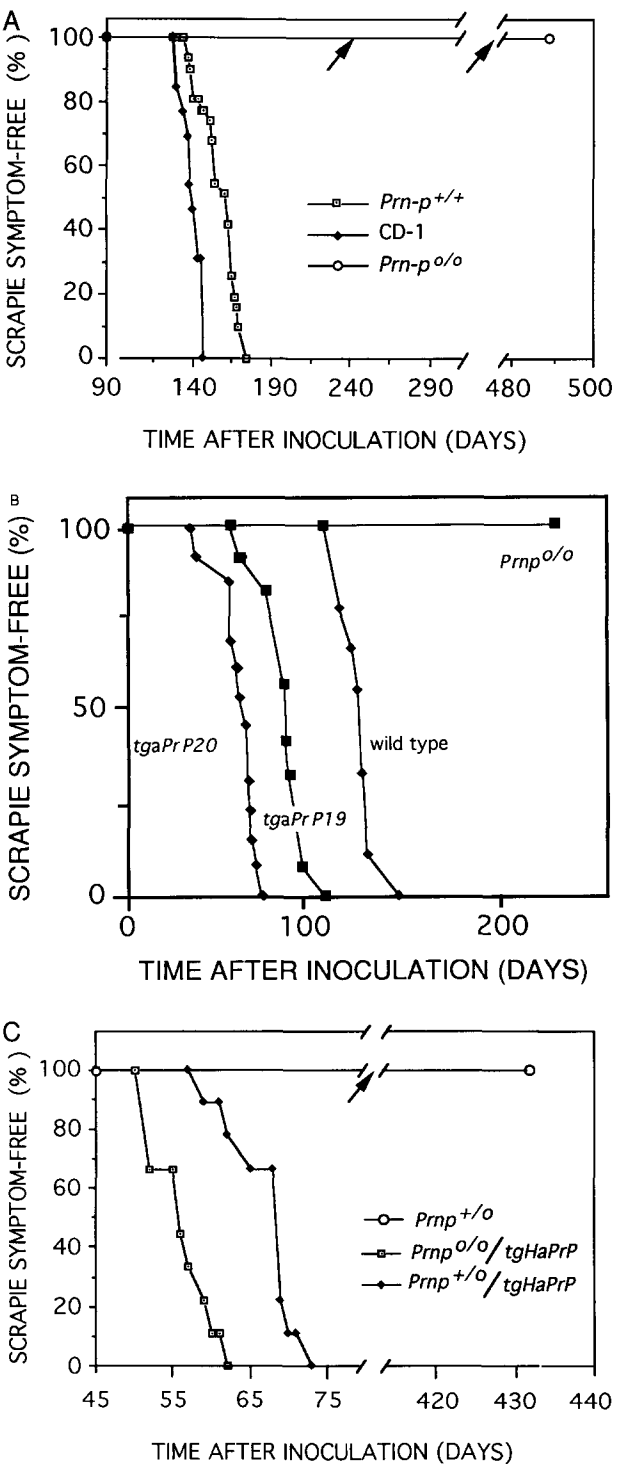

Fig. 4. Scrapie resistance of mice with disrupted PrP genes. (A) *Prnp*$^{o/o}$ mice remain symptom-free after inoculation with mouse scrapie prions. *Prnp*$^{+/+}$ litter mates or wild-type CD-1 mice show scrapie symptoms at the times indicated. Arrows: Five mice were killed at various times; none had scrapie symptoms. Modified from [48]. (B). *Prnp*$^{o/o}$ mice were rendered transgenic for *Prnp* genes. *tga19*/+ mice had 3–4 times the normal PrP^{C} level; *tga20*/+ mice had 6–7 times the normal level. From [50]. (C). *Prnp*$^{o/o}$ and *Prnp*$^{o/+}$ mice with hamster PrP transgenes at different times after inoculation with hamster scrapie prions. Groups of 9–11 mice of each genotype were inoculated with the Sc237 isolate of hamster prions. Arrow: One animal died spontaneously without scrapie symptoms and one was killed because of a tumor. Modified from [48].

tion times [50]. Even more interestingly, introduction of multiple hamster *Prnp* transgenes into *Prnp*$^{o/o}$ mice rendered them very susceptible to hamster-derived prions (56 days incubation time) (Fig. 4C) but much less so to mouse-derived prions (303 days incubation time) [48], demonstrating the requirement for a homotypic relationship between incoming prion and resident PrP protein for optimal prion propagation and development of pathology, as foreshadowed by the results of Prusiner et al. [35] described above.

8. Mechanism of pathogenesis

The mechanism of pathogenesis, i.e. the events leading to vacuolization and neuronal death are not yet well understood. Because neurons (and astrocytes) may be depleted of PrP^{C}, it has been speculated that this may be the cause of cell damage [42]. The fact that PrP knockout animals show no scrapie-like symptoms and appear quite healthy would argue against this hypothesis, unless one postulates that chronic deprivation of PrP allows for the recruitment of compensatory mechanisms while acute depletion, as may occur in scrapie, does not. Alternatively, exposure to PrP^{Sc} may lead to toxic effects. Brandner et al. [51] introduced PrP-overproducing neuroectodermal grafts into brains of knockout mice; contralateral inoculation with scrapie prions led to pronounced scrapie pathology in the graft but not in the surrounding tissue. Moreover, PrP^{Sc} was released by the infected graft, transported to distant sites, presumably by diffusion within the extracellular space [52] and deposited in form of granules without causing any apparent damage to surrounding cells. It would seem that damage is only caused to cells expressing PrP^{C}, perhaps because this leads to infection and intracellular processes, including accumulation of PrP^{Sc}. Interestingly, PrP-derived peptides cause toxic damage to PrP^{C}-expressing cortical cell cultures, but not to PrP$^{o/o}$ cultures [53,54].

9. Reverse genetics

The demonstration that disruption of the PrP gene confers resistance to scrapie and reintroduction of a PrP-encoding transgene restores susceptibility to the disease opens up the possibility of practising reverse genetics on PrP, that is, introducing deletions or mutations into the *Prnp* gene and determining the capacity of the modified gene to confer susceptibility to scrapie to a PrP knockout mouse.

As mentioned above, protease treatment of prion preparations cleaves off about 60 amino terminal residues of PrP^{Sc} [55] but does not abrogate infectivity [56]. We introduced into PrP knockout mice transgenes encoding wild-type PrP or PrP lacking 26 or 49 amino proximal amino acids which are protease-susceptible in PrP^{Sc}. Inoculation with prions led to fatal disease (Fig. 6), prion propagation and accumulation of PrP^{Sc} both in mice expressing wild-type and truncated PrPs [50]. Within the framework of the 'protein only' hypothesis this means that the amino proximal segment of PrP^{C}, which contains 3.5 of its 5 octa repeats, is required neither for its susceptibility to conversion into the pathogenic, infectious form of PrP nor for the generation of PrP^{Sc}.

10. Conversion of PrP^{C} to PrP^{Sc}

Conversion of PrP^{C} to PrP^{Sc} in scrapie-infected cells is a

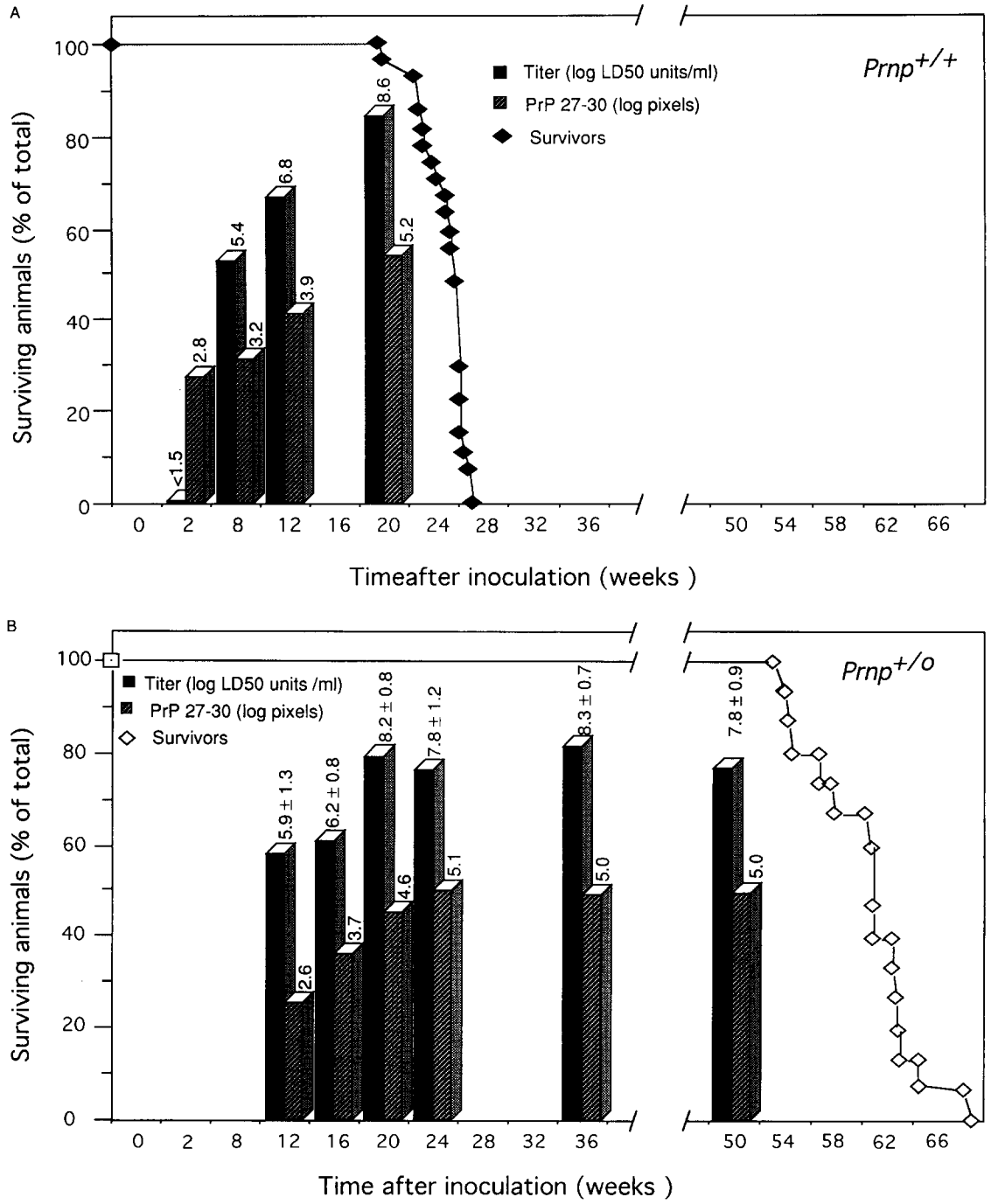

Fig. 5. Survival, prion titers and PrP^{Sc} in brains of (A) wild type and (B) *Prnp*$^{o/+}$ mice at various times after inoculation with mouse prions. From [75].

late post-translational process, occurring after PrP^{C} has reached its normal extracellular location or thereafter [30] Spontaneous conversion of PrP^{C} is obviously – and luckily – an extremely rare event. Why is this so, and how does scrapie infection promote conversion? The 'refolding model' (Fig. 2a) proposes that conversion requires unfolding PrP^{C} to some extent and refolding it under the influence of a PrP^{Sc} molecule [39], a process which would have to overcome a high activation energy barrier and might require a chaperone and an energy source. The 'nucleation model' (Fig. 2b) proposes that PrP^{C} is in equilibrium with PrP^{Sc} (or a precursor thereof) and that PrP^{Sc} is only stabilized when it adds onto a crystal-like seed or aggregate of PrP^{Sc}. If a stable aggregate needs to consist of a minimum of n PrP^{Sc} molecules, then the spontaneous formation of such aggregates would proceed at a rate proportional to the nth power of the monomer concentration, which would explain why spontaneous disease is so rare. Once a seed is present, monomer addition can ensue at a rapid rate [57–59]. Trapping of PrP by an essentially irreversible aggregation reaction would drive the bulk conversion process. The proposed process is akin to the assembly of, for example, (protease-sensitive) flagellin to (protease-resistant) flagellar filaments [60]. Interestingly, the same flagellin molecule can assemble into two types of flagella, depending on the prove-

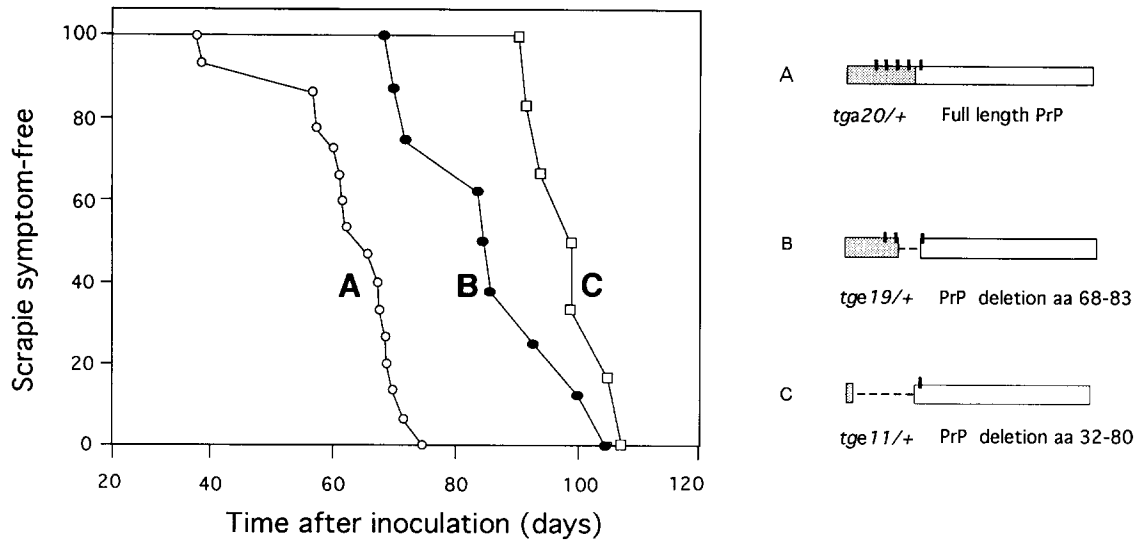

Fig. 6. Susceptibility to scrapie of *Prnp* knockout mice carrying *Prnp* transgenes with amino proximal deletions. *Prnp* transgenes with the structures indicated in the right half of the panel were introduced into $Prnp^{o/o}$ mice. *tga20*/+ (A, wild-type), *tga19*/+ (B, a deletion removing 2 octa repeats) and *tga11*/+ (C, a deletion removing 3.5 octa repeats) expressed PrP at levels 6–7, 3–4 and 6 times, respectively, those of wild-type. Mice were inoculated intracerebrally with mouse prions. Data from [50].

nance of the seed [61], thereby providing an analogy for conformationally determined prion strain specificity (see below).

Whatever the nature of the conversion process, an important experimental advance [62] has been the demonstration that incubation of ^{35}S-labeled biosynthetic hamster PrP^{C} with about 50 fold excess of PrP^{Sc} from scrapie-infected hamster brain resulted in the conversion of some labeled protein into the partly proteinase K-resistant state characteristic for hamster PrP^{Sc}. To achieve this conversion, ^{35}S-labeled PrP^{C} and PrP^{Sc} were partly denatured with 3M guanidinium chloride prior to incubation in a lower, critical concentration of the denaturant. Because of the denaturation step, the experiment is compatible with both the 'refolding' and the 'nucleation' model. The large amount of infectious agent associated with the PrP^{Sc} added, the modest extent of conversion and the imprecision of the infectivity assay currently preclude attempts to search for an increase in infectivity. The specificity of the reaction was documented by showing that murine PrP^{C} was converted by murine PrP^{Sc} but not by hamster PrP^{Sc}, as would be expected from the species barrier for in vivo transmission from hamster to mouse, however in the converse reaction conversion was observed, perhaps reflecting the less stringent barrier from mouse to hamster [63].

11. Prion strains

Dickinson and his colleagues [64,65] showed that many distinct strains of scrapie prions can be derived from sheep isolates. These strains differ by their incubation times in various inbred mouse lines and by the lesion patterns they occasion in the affected brains. Interestingly, different strains can be propagated in one inbred mouse strain (homozygous with regard to its PrP gene) [65]. Within the framework of the 'protein only' hypothesis this is at first blush puzzling, because it means that one and the same polypeptide chain is able to mediate different strain phenotypes. At least two explanations can be considered in this connection. The *conformational hypothesis* postulates that each strain is associated with a different conformation of PrP^{Sc} (or PrP^{*}) and that each of these can convert the PrP^{C} of its host into a likeness of itself. Bessen et al. [66] took advantage of the finding that two hamster-adapted scrapie strains, HY and DY, give rise to PrP^{Sc} molecules which are cleaved to products of different length by proteinase K [67]. In the in vitro system described above, ^{35}S-labeled hamster PrP^{C} incubated with DY PrP^{Sc} gave a radioactive proteolytic product shorter by about 1 kDa than that obtained after incubation with HY PrP^{Sc}, the same difference exhibited by the protease-resistant moieties of natural PrP^{Sc} molecules of the two strains. These remarkable results, if extended to show that conversion also results in generation of infectivity, will definitively confirm the 'protein only' model, allow structural characterization of the prion and solve the strain problem.

The *targeting hypothesis* (K.H. Meyer, pers. commun. 1991) [68,69] proposes that PrP^{Sc} carries a modification, for example carbohydrate residues, which varies from strain to strain and which targets it to a particular subset of cells. These cells would impart the same modification to the newly formed PrP^{Sc} molecules. Different strains would thus be targeted to different subsets of cells and retain their specific modification. This hypothesis is supported by the observation that different hamster prion strains [68] or mouse prion strains [70] give rise to different patterns of PrP^{Sc} deposition in the brain.

12. Implications and outlook

While each individual piece of evidence described above could be explained in several ways, the conjunction of data strongly supports the proposal that the prion is composed partly or entirely of a PrP-derived molecule (PrP^{*} or PrP^{Sc}), and that protein-encoding nucleic acid is not an essential component. Probably the closest one could come to irrefutable proof for the 'protein only' hypothesis would be the demonstration that biosynthetic, pure PrP^{C} can be converted not only into a protease-resistant form, but to infectious scrapie agent under defined conditions in vitro.

Because mice with disrupted *Prnp* genes are viable and resistant, it should be possible to breed sheep or cattle that are resistant to this disease; knockout methodology is in principle available for sheep [71] but not yet for cattle. While it is hardly practical to consider complete replacement of conventional animals by PrP knockout counterparts, herds of BSE-resistant cattle would be useful as a source for products required for pharmaceutical purposes. Moreover, the fact that $Prnp^{o/+}$ heterozygous mice show prolonged scrapie incubation times argues that an (as yet conjectural) drug leading to moderate reduction of PrP^{C} synthesis or one retarding the conversion of PrP^{C} to PrP^{Sc} [72] might substantially mitigate disease progression in incipient cases of human spongiform encephalopathies.

Finally one may raise the question whether prion-like agents cause other diseases or appear in non-vertebrate organisms. Although several human diseases accompanied by amyloid formation are known, none of them have been reproducibly transmitted. Interestingly, two yeast phenotypes are ascribed to 'heritable protein conversion', namely the [URE3] and the [psi^{+}] systems [73,74], opening new perspectives for the elucidation of this phenomenon.

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