

## De novo prions

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*1000 Biology Reports* 2010, **2**:46 (doi:10.3410/B2-46)

The electronic version of this article is the complete one and can be found at: <http://1000.com/reports/biology/content/2/46>

### Abstract

Prions are responsible for a heterogeneous group of fatal neurodegenerative diseases. They occur in three forms – sporadic, genetic, or acquired – and involve non-covalent post-translational modifications of the cellular prion protein (PrP<sup>C</sup>). Prions (PrP<sup>Sc</sup>) are characterized by their infectious properties and intrinsic ability to act as a template, converting the normal, physiological PrP<sup>C</sup> into the pathological form, PrP<sup>Sc</sup>. The ‘protein-only’ hypothesis, postulated by Stanley B Prusiner, implies that the generation of *de novo* prions is possible. Exciting recent work, *in vivo* and *in vitro*, has further strengthened this postulate.

### Introduction and context

Prions, proteinaceous infectious particles, or PrP<sup>Sc</sup>, are responsible for a heterogeneous group of fatal neurodegenerative diseases [1]. Prion diseases manifest as sporadic, genetic, or acquired disorders, involving non-covalent, post-translational modifications of the normal cellular prion protein (PrP<sup>C</sup>, where the superscript C stands for cellular) into PrP<sup>Sc</sup> (where the superscript Sc stands for scrapie, the prion disease that affects sheep) [2]. Prions are characterized by their infectious properties and by the intrinsic ability of their secondary, tertiary, and quaternary structures to act as a template and convert the normal physiological PrP<sup>C</sup> into the pathological, disease-causing form, PrP<sup>Sc</sup> [3].

### Major recent advances

#### *In vitro* support for de novo prions

The ‘protein-only’ hypothesis for prion propagation implies that it should be possible to generate prions *in vitro* from highly purified recombinant PrP (recPrP). Several attempts have been made to confirm this hypothesis, but the resulting prions had little or no infectivity, as well as minimal or no protease digestion resistance – two elements necessary to prove the ‘protein-

only’ hypothesis [4,5]. In recent years, the most striking work supporting the ‘protein-only’ postulate has shown that pure recPrP can be converted *in vitro* into a structural form similar to prions [6-9]. When PrP<sup>Sc</sup> is treated with proteinase K (PK) and further processed, the N-terminal region is removed, resulting in a PK-resistant core with an apparent molecular mass of 27-30 kDa, called PrP27-30 [10]. Legname *et al.* [6] produced truncated recombinant mouse PrP (MoPrP), corresponding to PrP27-30, and then converted this into amyloid fibrils. Amyloid fibrils were intracerebrally inoculated into transgenic (Tg) mice over-expressing the N-terminally truncated form of MoPrP, MoPrP(89-231). These mice, called Tg9949, express MoPrP(89-231) at levels 16 times higher than the normal PrP<sup>C</sup> expressed in wild-type mice [11]. These inoculated fibrils caused neurological defects between 380 and 660 days post-inoculation (DPI), depending on the biochemical protocol applied [6]. Brain homogenates of mice inoculated with the amyloid preparations exhibited the PK-resistant form of PrP<sup>Sc</sup>. Serial transmission of these brain homogenates caused disease in transgenic and wild-type mice. Thus, the synthetic prions generated using truncated recombinant MoPrP demonstrated that PrP is necessary and sufficient

for infectivity [6]. This seminal work has further contributed to our understanding of the biochemical and biophysical characteristics of prions, indicating the existence of an inverse correlation between the stability and infectivity of prion isolates [8]. Colby and colleagues [9] took this work a step further; they produced an array of PrP amyloids with different conformational stabilities and found that the inverse relationship between stability and incubation time was still valid. The most stable prion strains exhibited the longest incubation times, whereas more labile amyloids revealed shorter incubation times [9]. More recently, Colby and colleagues [12] isolated protease-sensitive synthetic prion (sPrP<sup>Sc</sup>). Often, PrP<sup>Sc</sup> is protease-resistant but sPrP<sup>Sc</sup> has been isolated in humans and other animals. Colby *et al.* reported that sPrP<sup>Sc</sup> was generated *in vitro* during polymerization of recPrP into amyloid fibers. In 22 independent experiments, recPrP amyloid preparations, but not recPrP monomers or oligomers, transmitted disease to Tg9949 mice (n = 164). In addition, they inoculated three synthetic prion isolates into Tg4053 mice that overexpress full-length MoPrP. Tg4053 mice are not prone to developing spontaneous neurological dysfunction [13]. The resulting synthetic prion isolates caused disease in 600-750 DPI in Tg4053 mice, which exhibited sPrP<sup>Sc</sup>. These novel synthetic prions demonstrate that conformational changes in wild-type PrP can produce prions composed exclusively of sPrP<sup>Sc</sup> [12].

More recently, Makarava *et al.* [14] showed additional experimental evidence that synthetic prions can be generated when amyloid fibrils produced from full-length recombinant Syrian hamster (SHa) PrP were intracerebrally inoculated in SHa animals.

Castilla and colleagues generated infectious scrapie prions [15] using the protein misfolding cyclic amplification (PMCA) *in vitro* system [16]. PMCA is a procedure involving cyclic amplification of PrP misfolding that allows rapid conversion of large excess PrP<sup>C</sup> into a protease-resistant, prion-like form in the presence of low quantities of PrP<sup>Sc</sup> template [16]. In the experiment of Castilla *et al.*, 263K-infected (SHa-adapted scrapie form of prion) SHa brain homogenates were subjected to 20 PMCA cycles in the presence of PrP<sup>C</sup>. Although no molecules of the original scrapie brain homogenate were present in samples, biochemical and structural properties of the *in vitro*-amplified samples still revealed the same features of 263K prions. In particular, the amplified sample was PK-resistant, highly insoluble in non-denaturant detergent (such as 10% sarkosyl), and enriched in  $\beta$ -sheet content. The PK-resistant-inoculated animals died at around 170 DPI showing the typical

signs of scrapie. To verify the PK-resistant *in vitro*-generated stability over time, serial transmissions were carried out; animals inoculated with this material exhibited neurological dysfunction and died after a mean of 136.5 DPI.

To investigate the mechanism of prion formation, Deleault *et al.* [17] conducted a series of experiments using the PMCA *in vitro* system in the presence of co-purified lipids and polyanions. They generated PrP<sup>Sc</sup> *de novo* from the simple combination of PrP<sup>C</sup>, co-purified lipids, and simple polyanions [17,18], although some questions were raised of whether generation of *de novo* prions from standard PMCA methodology might be due to cross-contamination from existing prions [4].

The PMCA *in vitro* system has also been used to propagate prion strains [19-21]. Numerous PMCA amplifications were carried out using, as a seed, different mouse-adapted (RML [Rocky Mountain Laboratory], ME7, 139A and 79A), human (vCJD [variant Creutzfeldt-Jakob disease]), bovine (301C), SHa (263K), and cervid (CWD [chronic wasting disease]) prion strains. Biochemical analyses – PK digestion, glycosylation ratio, and electrophoresis mobility – of end products revealed the same strain features as the original seeds, suggesting that strains are dependent on the properties of PrP<sup>Sc</sup> [19-21]. PMCA was also employed with prions from various species in combination with transgenic mice to study, and even overcome, the prion species barrier [19,21]. *De novo in vitro* generation of PrP<sup>Sc</sup> from PrP<sup>C</sup> and poly(A)RNA using the PMCA system has opened up new ways to investigate the molecular mechanisms underlying the spontaneous misfolding of the PrP and its propagation. To convert *de novo* PrP<sup>C</sup> from healthy SHa and mouse brain homogenates into its infectious isoform, Barria and colleagues [4] modified the PMCA conditions, increasing the number of PMCA cycles. Animals inoculated with this PK-resistant PrP showed abnormalities after an average incubation period of 112.6 days and revealed spongiform degeneration, astroglyosis, and PrP accumulation. Even more recently, Wang *et al.* [22] employed PMCA under standard conditions (cycles) and produced prions from bacterially expressed recombinant MoPrP(23-230) in the presence of the synthetic anionic phospholipid 1-palmitoyl-2-oleoylphosphatidylglycerol and RNA (as a polyanion). These prions were protease-resistant. When injected into wild-type mice, this new aggregated and pathogenic PrP isoform induced neurological signs after about 130 DPI, with the terminal phase occurring around 150 DPI [22]. Nevertheless, upon second passage the newly discovered prion isolate did not lead to abbreviation or stabilization

of incubation times in mice, casting doubts about the identification of novel synthetic prions.

### In vivo de novo prions

The existence of human genetic prion diseases, in which patients with mutations in the open-reading frame of the human PrP gene, *PRNP* (*PRioN Protein*), spontaneously develop prion disease (transmissible in most forms), strongly supports the 'protein-only' hypothesis [23,24]. Additionally, many transgenic mice with mutations homologous to human prion diseases also develop spontaneous disease that is often transmissible [25]. Transgenic mice carrying mutations and over-expressing PrP mutants spontaneously develop neurological symptoms similar to those of prion disorders, but their brain homogenates are infectious only for mice carrying the same mutation, and not for wild-type mice [5,26,27]. Therefore, it has been argued in the prion field as to whether this represents true *de novo* prion generation, or merely an acceleration of disease [28].

Recently, Sigurdson and coworkers [29] developed a transgenic mouse over-expressing two novel mutations in the MoPrP gene, *Prnp*; these change two amino acids into those normally present in the elk PrP primary sequence that confer a rigid structure to the  $\beta 2$ - $\alpha 2$  loop in the protein. Although they created this transgenic mouse to study the role of the rigid-loop structure, surprisingly they found that these mice, called transgenic rigid loop (RL)-PrP mice, developed spontaneous prion disease at variable time points, with a 50% incidence by 364 DPI. Sick RL-PrP mice showed ataxia, weight loss, lethargy, kyphosis, and hind limb paralysis, while their brains showed PrP deposits in the stratum lacunosum-moleculare of the hippocampus, within the corpus callosum, and in the cingulum. Due to a transmission barrier, RL-PrP mice inoculated with wild-type prion strains exhibited prolonged incubation time before developing prion disease. Therefore, the authors did not attempt to transmit transgenic RL-PrP prions directly to wild-type mice; rather, they stabilized the prion isolate from RL-PrP mice by passaging it in mice over-expressing wild-type PrP, called Tga20 mice [30]. Tga20 mice inoculated with brain homogenates from sick RL-PrP mice developed neurologic signs by 481 DPI. Serial passages into Tga20 mice of brain homogenates from RL-PrP-infected Tga20 mice caused similar symptoms with a shorter incubation period. Moreover, both PK resistance and conformational stability increased after each passage. After serial passaging in Tga20 mice, these *de novo* generated prions were transmissible to wild-type mice [29]. This is the first time that prions generated spontaneously in mice models are able to infect mice carrying a wild-type sequence.

### Future directions

In order to define a prion, one needs to fulfill four criteria: transmissibility into recipient animals, neuropathological features (e.g., gliosis, vacuolation, immunopositive PrP deposits) [31], biochemical features, such as PK resistance [32], and stability (defined by resistance to denaturation with chaotropic agents, such as guanidium HCl) [33]. The strain of a prion is determined by these characteristics. Now, we can design synthetic prions with defined biophysical characteristics and predict their infectivity [9]. A major frontier in prion research is to determine the precise structural properties of the prion at the molecular level, such as differences in secondary, tertiary, and quaternary structures among different strains that confer defined prion strain characteristics.

### Abbreviations

DPI, days post-inoculation; MoPrP, mouse PrP; PK, proteinase K; PMCA, protein misfolding cyclic amplification; PrP, prion protein; PrP<sup>C</sup>, 'cellular' PrP or normal form of PrP; PrP<sup>Sc</sup>, 'scrapie' PrP or pathological form of PrP; recPrP, recombinant PrP; RL, rigid loop; SHa, Syrian hamster; sPrP<sup>Sc</sup>, sensitive PrP<sup>Sc</sup>; Tg, transgenic.

### Competing interests

The authors declare that they have no competing interests.

### Acknowledgments

MDG was funded by the Michael J Homer Family fund and the National Institutes of Health National Institute on Aging (NIH/NIA) K23 AG021989 and R01-AG031189. GL has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement number 222887 - the PRIORITY project.

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