Chapter 5
Prion Conversion and Deformed Templating

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Abstract The transmissible agent of prion disease consists of a prion protein in its abnormal, β-sheet-rich state (PrP^{Sc}), which replicates itself according to the template-assisted mechanism. According to this mechanism, the folding pattern of a newly recruited polypeptide chain accurately reproduces that of a PrP^{Sc} template. This chapter introduces an alternative mechanism of prion replication designated as deformed templating that constitutes a switching of the cross-β folding pattern into an alternative pattern. The chapter discusses experimental evidence in support of deformed templating including the work on synthetic prions and illustrations that folding pattern switches within individual amyloid fibrils. The role of deformed templating in prion strain mutations and evolution is reviewed. Changes in the replication environment along with the effects of posttranslational modifications are proposed as driving forces behind deformed templating events. The mechanism of deformed templating is important for a better understanding of the etiology of prion and other neurodegenerative diseases.

Keywords Prion protein · Prion diseases · Neurodegenerative diseases · Deformed templating · Cross-β folding · Amyloid fibrils · Posttranslational modifications · N-linked glycans

5.1 Introduction

Prion diseases, or transmissible spongiform encephalopathies, are fatal neurodegenerative disorders that can be sporadic, inherited, or infectious in origin. Misfolding and aggregation of the normal, cellular form of the prion protein (PrP^{C}) into an

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abnormal β-sheet-rich, disease-related conformation (PrP^{Sc}) underlie the pathogenic mechanisms of the prion diseases for all three origins (Prusiner 1996). Spontaneous conversion of PrP^{C} into PrP^{Sc} is believed to underlie the sporadic forms of prion diseases (Fig. 5.1a). The low occurrence rate of sporadic prion disease is likely to reflect the extremely low probability of spontaneous conversion of PrP^{C} into PrP^{Sc}. Inherited forms of the disease have been linked to a number of single-point mutations, truncation, or octarepeat expansion mutations in the *PRNP* gene (a gene that encodes prion protein), with more than 30 disease-inducing mutations identified so far (Prusiner and Scott 1997) (Fig. 5.1b). In addition to sporadic and inherited

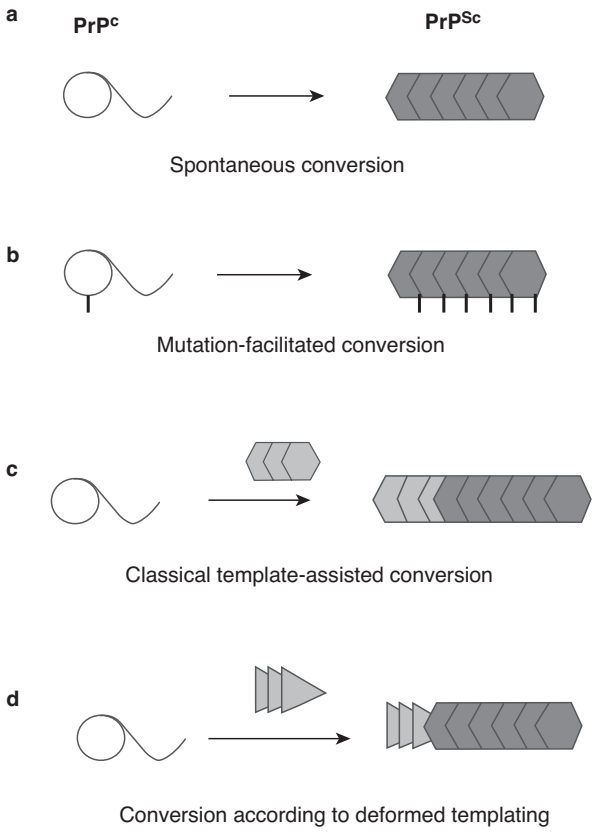

Fig. 5.1 Four mechanisms for PrP^{Sc} formation. (**a**) Spontaneous conversion of PrP^{C} into PrP^{Sc} underlies the sporadic forms of prion diseases. (**b**) Disease-related mutations in prion protein can facilitate the conversion of PrP^{C} into PrP^{Sc}. (**c**) The template-assisted model postulates that PrP^{Sc} replicates its pathogenic structure by recruiting and converting PrP^{C}. According to this model, the folding pattern of a newly recruited polypeptide chain accurately reproduces that of a PrP^{Sc} template. (**d**) The mechanism referred to as deformed templating postulates that the formation of PrP^{Sc} can be seeded by abnormal PrP structures substantially different from that of authentic PrP^{Sc}. A transformation from one cross-β folding pattern to an altered folding pattern occurs during deformed templating

origins, prion diseases can be also acquired via transmission. According to the protein-only hypothesis, the transmissible agent consists of a prion protein in its abnormal, β-sheet-rich, disease-related state (PrP^{Sc}), which propagates its abnormal conformation in an autocatalytic manner by recruiting and converting PrP^{C} into PrP^{Sc} (Prusiner 1982; Griffith 1967). The classical templating mechanism of prion replication postulates that the folding pattern of a newly recruited polypeptide chain accurately replicates that of a PrP^{Sc} template (Fig. 5.1c) (Cohen and Prusiner 1998). As such, the PrP^{Sc}-specific folding pattern replicates endlessly with high fidelity, as far as PrP^{C} molecules are available as a substrate.

This chapter discusses an alternative mechanism of PrP^{Sc} replication designated as deformed templating. Deformed templating involves switching from one cross-β folding pattern present in a template to an altered folding pattern (Fig. 5.1d). Experimental data accumulated in the field over the past decade including the results on synthetic prions provide strong support for this mechanism. The concept of deformed templating offers a new perspective on the genesis, evolution, and adaptation of transmissible prion structures.

5.2 Switching Between Alternative Folding Patterns Within Individual Amyloid Fibrils

According to the prevailing view, multiple amyloid structures could be produced within the same amino acid sequence (Petkova et al. 2005; Makarava and Baskakov 2008). However, the folding pattern within individual amyloid fibrils or PrP^{Sc} particles is believed to be uniform. In amyloid fibrils or PrP^{Sc} particles, β-strands are arranged perpendicularly to the axis of the cross-β spine (Wille et al. 2009; Ostapchenko et al. 2010), and their strain-specific folding pattern provides a template for recruiting and converting a monomeric precursor at the growing edge. Faithful templating of cross-β structures is based on the self-complementation of polypeptide chains involved in cross-β assembly (Eisenberg et al. 2006). Self-complementation can be achieved through several mechanisms including tight complementarity of amino acid side chains in the steric zippers of the cross-β spine; the stacking of side chains in so-called polar zippers, where the side chain hydrogen bonds are formed between β-strands along the fibrillar axis; or domain swapping (Eisenberg et al. 2006).

Our studies that employed single-fibril fluorescence microscopy combined with atomic force microscopy imaging and supplemented with Fourier-transform infrared spectroscopy (FTIR) spectroscopy revealed that the cross-β folding pattern does not always maintain uniform structure upon elongation of individual fibrils (Makarava et al. 2007, 2009) (Fig. 5.2). The cross-seeding reactions, where hamster recombinant PrP (rPrP) fibrils were used to seed fibrillization of mouse rPrP, produced hybrid fibrils consisted of two segments: one composed of hamster and another mouse rPrP (Fig. 5.2b,c). (Makarava et al. 2009). Remarkably, as judged from immunoconformational microscopy assay that probes exposure of PrP epitopes within fibrils (Fig. 5.2a), the folding pattern switched from hamster- to

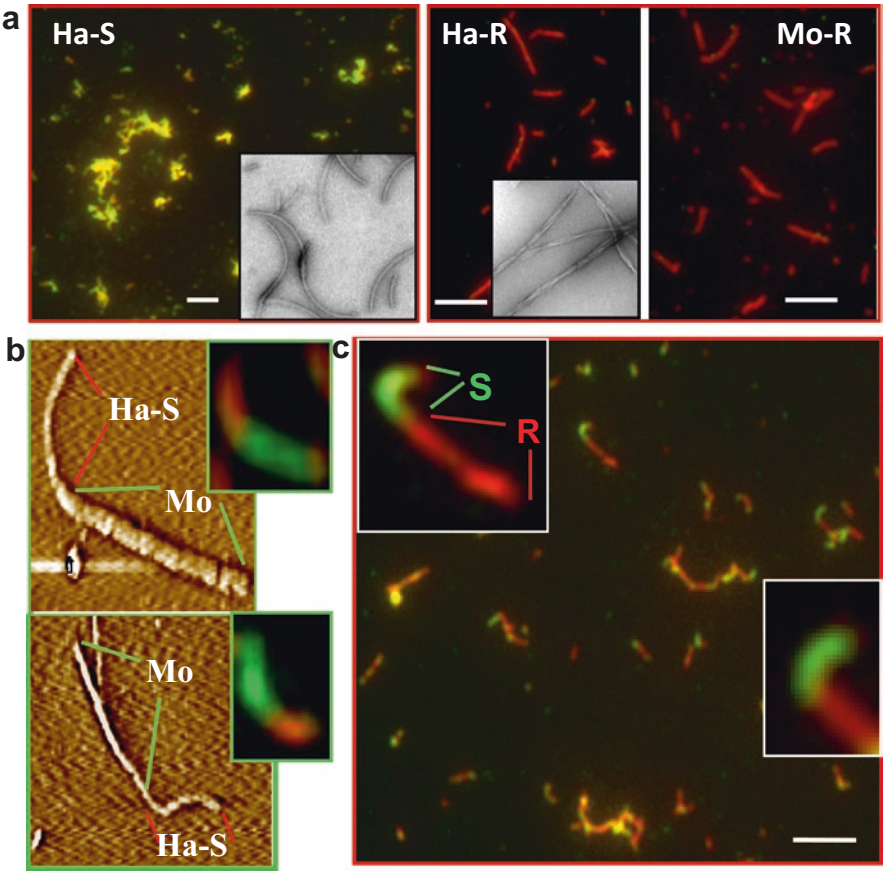

Fig. 5.2 Switching between alternative folding patterns within individual fibril (adapted from Makarava et al. 2009). (**a**) Immunoconformational microscopy imaging assay was designed to probe strain-specific differences in the exposure of epitopes. Examples that immunoconformational assay distinguishes two conformations of rPrP fibrils generated in vitro and designated as S-fibrils (yellow or green) and R-fibrils (red): hamster rPrP S-fibrils (*Ha-S*; *left*), hamster rPrP R-fibrils (*Ha-R*; *center*), and mouse R-fibrils (*Mo-R*; *right*). Insets show electron microscopy images of Ha-S fibrils and Ha-R fibrils. *Scale bars*, 5 μm. (***b, c***). Seeding of mouse rPrP with hamster S-fibrils leads to hybrid mouse-hamster fibrils that show switches in folding patterns from S- to R-specific patterns. (***b***) Atomic Force Fluorescence Microscopy images demonstrate that individual hybrid fibrils consisted of two sections: the sections made of hamster PrP, as detected by hamster-specific anti-PrP antibody (red fluorescence), had a curvy S-like shape, whereas the sections made of mouse PrP, as detected by mouse-specific anti-PrP antibody (green fluorescence), had a straight R-like shape. (***c***) *I*mmunoconformational microscopy imaging assay of hybrid hamster-mouse fibrils showing a switch from S-specific pattern (green) to R-specific pattern (red). Scale bars, 5 μm

mouse-specific within individual hybrid fibrils (Makarava et al. 2009) (Fig. 5.2c). We proposed that for hybrid structures to maintain integrity, alternative folding patterns have to share a common motif (Baskakov 2009).

The observation of a conformational switch within individual fibrils provides a direct illustration of the deformed templating mechanism and highlights the high adaptation potential for amyloid structures. Adaptive conformational switching via deformed templating permits the recruitment of homologous PrP sequences which otherwise are not compatible with the templating structures. Adaptive conformational switching within individual fibrils may provide a mechanistic explanation for strain mutation or modification, phenomena that have been frequently observed upon transmission of prions across species (Peretz et al. 2002; Castilla et al. 2008; Green et al. 2008).

5.3 Generating Transmissible Prion Diseases De Novo

The last two decades witnessed a number of studies, where transmissible prion diseases were generated in animals de novo by inoculating prion material produced in vitro (Legname et al. 2004; Colby et al. 2009, 2010; Makarava et al. 2010, 2011, 2012a, 2015, 2016; Barria et al. 2009; Deleault et al. 2007, 2012a, b; Wang et al. 2010). All studies on generating prion infectivity could be divided into two large groups, where the material for inoculating animals was produced either using (1) serial protein misfolding cyclic amplification (sPMCA) (Barria et al. 2009; Deleault et al. 2007, 2012a, b; Wang et al. 2010) or (2) in vitro fibrillation protocols that utilized rPrP (Legname et al. 2004; Colby et al. 2009, 2010; Makarava et al. 2010, 2011, 2012a, 2015, 2016).

In the studies that employed the first approach, the application of sPMCA accomplished two purposes (1) generating PrP^{Sc} particles de novo and (2) amplification of newly formed PrP^{Sc} to the amounts that can effectively produce clinical disease in wild-type animals with 100% success rate (Barria et al. 2009; Deleault et al. 2007; Wang et al. 2010).

The second approach involved the conversion of rPrP into amyloid fibrils in vitro without the application of sPMCA (Legname et al. 2004; Colby et al. 2009, 2010; Makarava et al. 2010, 2011, 2012a, 2015, 2016). In these studies, transmissible diseases were generated either in transgenic animals with high levels of PrP^{C} expression or in wild-type animals. In transgenic animals, the disease was produced with a 100% success rate in the first passage, although after a relatively long incubation time (Legname et al. 2004; Colby et al. 2009, 2010). In wild-type animals, two or even three serial passages were required for the appearance of clinical prion disease (Makarava et al. 2010, 2011, 2012a, 2015, 2016). Critical concerns that rPrP amyloid fibrils did not induce the disease de novo but only accelerated an ongoing pathogenic process have been raised regarding the studies performed on transgenic mice (Caughey et al. 2009; Caughey and Baron 2006; Soto 2011). Indeed, the transgenic mice that overexpress PrP^{C} were found to develop a neurological disorder that

was accompanied by PrP aggregation, although these disorders were not transmissible in serial passages (Colby et al. 2010). In contrast to the sporadic formation of non-transmissible PrP aggregates, inoculation of rPrP fibrils triggered the formation of authentic PrP^{Sc} that can transmit disease, a process that appears to compete with aggregation of non-transmissible PrP.

Our experiments conducted using Syrian hamsters demonstrated that rPrP fibrils induce transmissible prion disease de novo in wild-type animals (Makarava et al. 2010, 2011, 2012a, 2015, 2016). However, when triggered by rPrP fibrils, only a small fraction of animals showed signs of infection. Furthermore, the clinical disease was observed only at the second or third serial passages (Makarava et al. 2010, 2011, 2012a). Less than a 100% success rate along with a long clinically silent stage raised a number of questions regarding the molecular mechanism underlying the genesis of transmissible prions de novo.

Prior to the discussion of molecular mechanisms for triggering transmissible prion diseases, it is useful to briefly review the data on the structure of rPrP fibrils and PrP^{Sc}. Several studies presented strong evidence that the structures of rPrP amyloid fibrils are different from those of authentic PrP^{Sc} whether isolated from scrapie-infected animals or produced via sPMCA in vitro (Wille et al. 2009; Ostapchenko et al. 2010; Piro et al. 2011; Wang et al. 2020; Kraus et al. 2021). X-ray diffraction experiments revealed substantial differences in equatorial diffraction patterns collected from rPrP fibrils and PrP^{Sc} purified from scrapie brains, suggesting that they have different folding patterns (Wille et al. 2009; Ostapchenko et al. 2010). The results of the X-ray analysis were consistent with the FTIR data, which also pointed to differences between conformations of PrP^{Sc} and rPrP fibrils (Spassov et al. 2006; Makarava and Baskakov 2008). The maxima of the β-sheet absorption collected for PrP^{Sc} isolates varied between 1625 and 1637 cm^{-1} depending on specific PrP^{Sc} strain (Spassov et al. 2006), whereas the maxima of β-sheet absorption for rPrP fibrils was found to be at 1614 and 1626/28 cm^{-1} under the same solvent conditions (Makarava and Baskakov 2008; Ostapchenko et al. 2010). Finally, according to recent cryo-EM studies, both rPrP fibrils and PrP^{Sc} consist of parallel in register β-sheet structure, however, their folding patterns were found to be profoundly different (Wang et al. 2020; Kraus et al. 2021). If rPrP fibrils and PrP^{Sc} have different structures, how can the first template be the last one?

Bearing in mind the results of structural studies, two alternative mechanisms can be considered. According to one mechanism, the preparations of rPrP fibrils contained very small amounts of PrP^{Sc} or particles with a structure similar to authentic PrP^{Sc} (Fig. 5.3a). If this is the case, the low success rate in infecting the animals and the long clinically silent stage should be attributed to the miniscule amounts of PrP^{Sc} in preparation of the fibrils. The second mechanism designated as deformed templating proposes that the formation of PrP^{Sc} and transmissible prion diseases in wild-type animals are triggered by rPrP seeding material that lacks PrP^{Sc} (Fig. 5.3b). According to this mechanism, rPrP fibrils trigger the formation of PrP^{Sc} despite substantial differences in their folding patterns. The low rate of infection in the first passage is due to the stochasticity of the deformed templating process. Moreover, the transformation of rPrP amyloid structure into PrP^{Sc} might involve several steps before authentic PrP^{Sc} emerges (Fig. 5.3b).

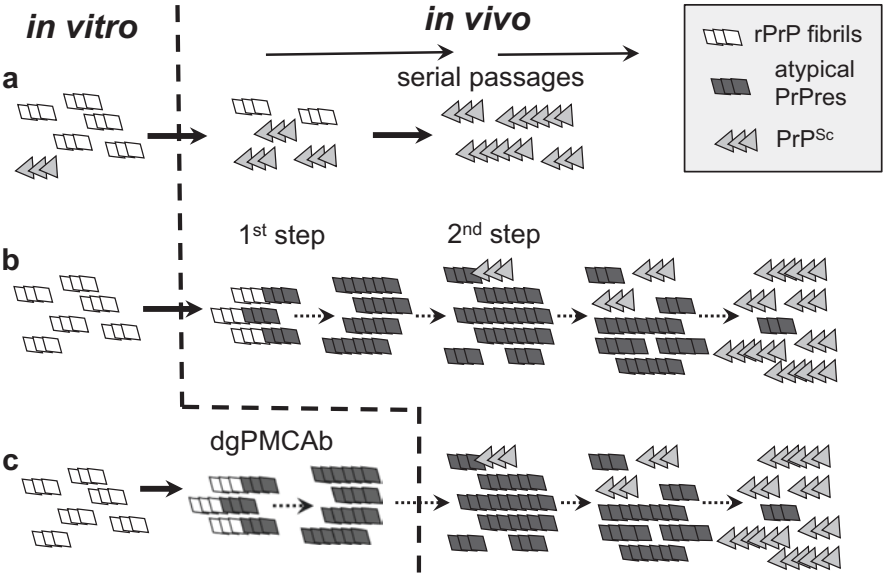

Fig. 5.3 Schematic representation of the mechanisms responsible for generating transmissible prion diseases de novo using rPrP fibrils prepared in vitro. According to the first mechanism, (**a**) the preparations of rPrP amyloid fibrils (schematically shown as *white parallelograms*) contain very small amounts of PrP^{Sc} (shown as *triangles*). The silent stage of the disease is attributed to the long time required for the amplification of this extremely small amount of PrP^{Sc}. (**b**) A second mechanism referred to as deformed templating postulates that there are no PrP^{Sc} particles in the preparations of amyloid fibrils. Instead, when inoculated into animals, amyloid fibrils seed conversion of PrP^{C} into PrP^{Sc}-like structures, although with low efficiency. The process of transformation of rPrP fibrils into PrP^{Sc} involves two steps. In the first step, rPrP fibrils seed atypical PrPres (shown as *dark parallelograms)*, a transmissible form of PrP that replicates silently without causing clinical disease. In the second step, atypical PrPres produces PrP^{Sc} in rare and stochastic seeding events that are described by a deformed templating mechanism. PrP^{Sc} replicates faster than atypical PrPres and eventually replaces it during serial passages. (**c**) An alternative pathway for producing transmissible prion diseases de novo involves the generation of atypical PrPres in dgPMCAb reactions that employ partially deglycosylated PrP^{C} as a substrate upon seeding with rPrP fibrils. Serial transmission of dgPMCAb-derived atypical PrPres in animals leads to the formation of PrP^{Sc} via deformed templating and prion disease (Makarava et al. 2015)

5.4 Experimental Evidence Supporting the Mechanism of Deformed Templating

Several lines of experimental evidence support the idea that synthetic prion strains emerged via the mechanism of deformed templating. First, no PrP^{Sc} could be detected in the preparations of rPrP amyloid fibrils using a highly sensitive sPMCA with beads (sPMCAb) format that detects single PrP^{Sc} particles (Makarava et al. 2011). If one assumes that the first model is correct, the amount of infectivity should be equivalent to approximately 0.5 infectious doses to account for the less than

100% infection rate in the first passage. This amount of infectivity is equivalent to ~10,000–100,000 PrP molecules or to ~100–1000 PrP^{Sc} particles, assuming that an average PrP^{Sc} particle consists of ~100 PrP molecules (Saa et al. 2006; Makarava et al. 2012b). This amount of PrP^{Sc} was well above the detection limits of sPMCAb and should have been easily detected if present in preparations of rPrP fibrils.

Second, the experimental protocol used for producing rPrP amyloid fibrils employs denaturants (a mixture of 1 M GdnHCl and 3 M urea) – the solvent conditions that denature PrP^{Sc}. Using denaturing conditions for fibril formation is possible because rPrP fibrils are much more conformationally stable than PrP^{Sc} (Makarava et al. 2010; Peretz et al. 2001; Sun et al. 2007). Furthermore, the formation of authentic PrP^{Sc} in vitro requires RNA and lipids (Deleault et al. 2007, 2012a, b; Wang et al. 2010), whereas rPrP amyloid fibrils were formed in the absence of these cellular cofactors. Therefore, it is unlikely that authentic PrP^{Sc} could be formed in the preparation of rPrP fibrils conducted in the absence of cofactors essential for authentic PrP^{Sc} structures and under solvent conditions that promote PrP^{Sc} denaturation.

Third, in studies on synthetic prions, a strong correlation between the conformational stability of rPrP amyloid fibrils, the stability of PrP^{Sc} produced in animals upon inoculating rPrP fibrils, and the incubation time to disease were described (Colby et al. 2009). If a minuscule fraction in the preparation of rPrP fibrils is responsible for the disease, the correlation between the stability of rPrP amyloid, which is a bulk property of fibril preparation, and the incubation times would be challenging to explain. Again, these results are consistent with the second model.

Fourth, when transmissible prion disease is triggered by rPrP amyloid fibrils, a decrease in PrP^{Sc} conformational stability was observed during serial passages of synthetic prions (Legname et al. 2005; Makarava et al. 2010; Colby et al. 2009). Similar dynamics in PrP^{Sc} conformational stability were found regardless of whether transgenic mice or Syrian hamsters were inoculated with rPrP fibrils, suggesting that a common pathway in the genesis and evolution of infectious structures might exist (Legname et al. 2005; Makarava et al. 2010; Colby et al. 2009). Observed changes in physical properties illustrate that the PrP^{Sc} structure undergoes a transformation during serial transmission, again providing support to the second model.

Fifth, as judged from the clinical and neuropathological features, the synthetic prion strains generated by rPrP fibrils were remarkably different from prion strains of natural origin or synthetic strains generated via sPMCA (Deleault et al. 2007; Barria et al. 2009; Wang et al. 2010; Makarava et al. 2010, 2011, 2012a, 2020, 2021; Jeffrey et al. 2014). The fact that rPrP fibrils produced a disease phenotype remarkably different from the phenotype expressed by strains generated in sPMCA or strains of natural origin is consistent with the hypothesis that rPrP fibrils gave rise to PrP^{Sc} with a unique structure.

5.5 Deformed Templating In Vivo

How did amyloid fibrils structurally different from authentic PrP^{Sc} give rise to PrP^{Sc} and transmissible disease? An exhaustive search for minuscule amounts of PrP^{Sc} in the preparations of rPrP fibrils yielded negative results (Makarava et al. 2011, 2012a). Instead, a search for intermediate products on a pathway toward PrP^{Sc} revealed that the first product of PrP^{C} misfolding triggered by rPrP fibrils in animals was a new self-replicating PrP state referred to as atypical PrPres (Makarava et al. 2011, 2012a) (Fig. 5.3b). Atypical PrPres displayed an abnormally short, C-terminal proteinase K (PK)-resistant core that was similar to the PK-resistant core of rPrP fibrils with respect to its size and position (Bocharova et al. 2005; Makarava et al. 2011, 2012a). Unlike authentic PrP^{Sc}, atypical PrPres preferentially recruited un- and mono-glycosylated PrP^{C}, while its amplification was RNA-independent arguing that it is structurally different from PrP^{Sc}. Accumulation of atypical PrPres in animal brains did not lead to any notable clinical signs of prion diseases and was associated only with minor lesions (Kovacs et al. 2013). Despite replication and accumulation in the brain, atypical PrPres was a clinically silent state. Over the course of several serial passages, atypical PrPres gave rise to PrP^{Sc} (Makarava et al. 2011, 2012a, 2015, 2016) (Fig. 5.3b). The appearance of PrP^{Sc} was stochastic and always followed the accumulation of atypical PrPres first. The dynamics between the two states suggests that the birth of PrP^{Sc} was a result of a series of deformed templating events and a selection of the most favorable structural variants that were best suited for replication in animal brains (Makarava et al. 2011, 2012a).

Remarkably, atypical PrPres could be generated in vitro via seeding of PMCAb reactions that utilized partially deglycosylated PrP^{C} as a substrate (dgPMCAb) using rPrP fibrils (Makarava et al. 2013, 2015, 2016) (Fig. 5.3c). As far as un- and mono-glycosylated PrP^{C} are available as a substrate, rPrP fibrils give rise to atypical PrPres whether in vivo or in vitro (Makarava et al. 2013, 2015, 2016). However, because di-glycosylated PrP^{C} is not compatible with the structure of atypical PrPres, di-glycosylated PrP^{C} interfered with the replication of atypical PrPres triggering deformed templating events. Upon inoculation into animals, dgPMCAb-generated atypical PrPres gave rise to PrP^{Sc} and prion disease with a phenotype similar to those induced by rPrP fibrils (Makarava et al. 2015) (Fig. 5.3c). These results confirmed that atypical PrPres is an intermediate on the pathway toward PrP^{Sc}, and illustrated that transmissible prion diseases could be produced via two alternative procedures: direct inoculation of rPrP fibrils or in vitro-produced atypical PrPres (Fig. 5.3b,c).

What factors define the rate of deformed templating? In transgenic mice that overexpress hamster PrP^{C}, elevated levels of PrP^{C} expression accelerated the formation of atypical PrPres but did not facilitate the second step, i.e. the transition from atypical PrPres to PrP^{Sc} (Makarava et al. 2016). As deformed templating is believed to be stochastic in nature, the rate of deformed templating does not depend on the concentration of a substrate but is likely to be controlled by the intrinsic rate of conformational errors in templating altered self-propagating states (Makarava et al. 2016).

5.6 Deformed Templating In Vitro

If rPrP fibrils or atypical PrPres can seed authentic PrP^{Sc} via deformed templating, one can assume that the opposite reaction, that is, the seeding of rPrP fibrils by PrP^{Sc}, is possible too. Indeed, while rPrP fibrils and PrP^{Sc} have different structures, they can seed each other upon changes in the replication environment and exposure to an appropriate substrate (Fig. 5.4a,b). In fact, for detecting minuscule amounts of PrP^{Sc}, several assays including quaking and amyloid seeding assays exploited the phenomenon of PrP^{Sc}-seeded conversion of rPrP into amyloid fibrils (Colby et al. 2007; Atarashi et al. 2007). While the amyloid seeding assays are extremely sensitive for detecting minute amounts of PrP^{Sc} seeds (Atarashi et al. 2007), prion infectivity is lost upon PrP^{Sc}-seeded fibrillation of rPrP in vitro arguing that PrP^{Sc}-specific structure is not maintained in seeding assays in vitro (Fig. 5.4a).

5.7 Prion Strain Mutation and Evolution via Deformed Templating

How do prions mutate? What is the origin of strain mutations? The "cloud" hypothesis proposes that pools of PrP^{Sc} particles within individual strains or isolates are intrinsically heterogeneous and that the heterogeneity arises due to spontaneous

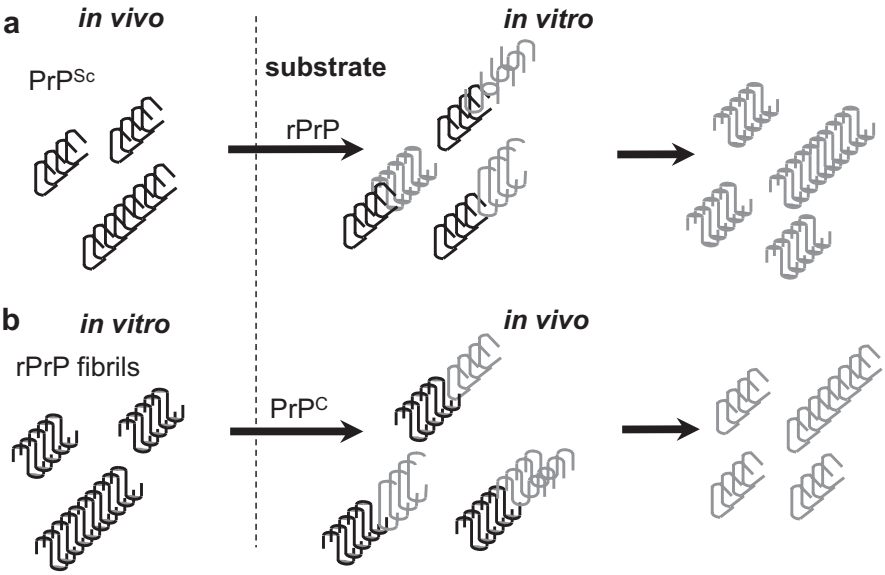

Fig. 5.4 Examples of deformed templating are (**a**) PrP^{Sc}-seeded fibrillation of rPrP in vitro and (**b**) generation of synthetic strains upon serial passaging of rPrP fibrils in animals

mutations in PrP^{Sc} structure (Collinge 2010; Li et al. 2010) (Fig. 5.5a). Upon changes in the replication environment, minor variants that fit best to replicate in the new environment receive selective advantages. Consistent with this view, several studies highlighted the fact that prion strains exhibit high levels of conformational plasticity and are subject to transformation when exposed to new replication environments. For instance, drug-resistant prions emerged in cultured cells following treatment with prion inhibitors swainsonine or quinacrine (Ghaemmaghami et al. 2009; Li et al. 2010). Accumulation of PrP^{Sc} variants in cloned prion material was attributed to ongoing processes of spontaneous 'mutations' of PrP^{Sc} structure (Li et al. 2010). According to the "cloud" hypothesis, changes in the replication environment give a selective advantage to minor PrP^{Sc} variants that are already present in the PrP^{Sc} pool. The "cloud" hypothesis does not explain how minor variants are generated or what is their origin (Fig. 5.5a).

Unlike the "cloud" hypothesis, the deformed templating mechanism proposes that changes in the replication environment play an active role in generating new PrP^{Sc} variants, in addition to its role in imposing a selective pressure (Fig. 5.5b) (Makarava and Baskakov 2013). Under circumstances the PrP^{Sc} template does not fit into a new environment, it can still seed new PrP^{Sc} variants via deformed templating. While the majority of the newly generated variants might not replicate effectively, a variant that fits well to the new environment will eventually emerge through

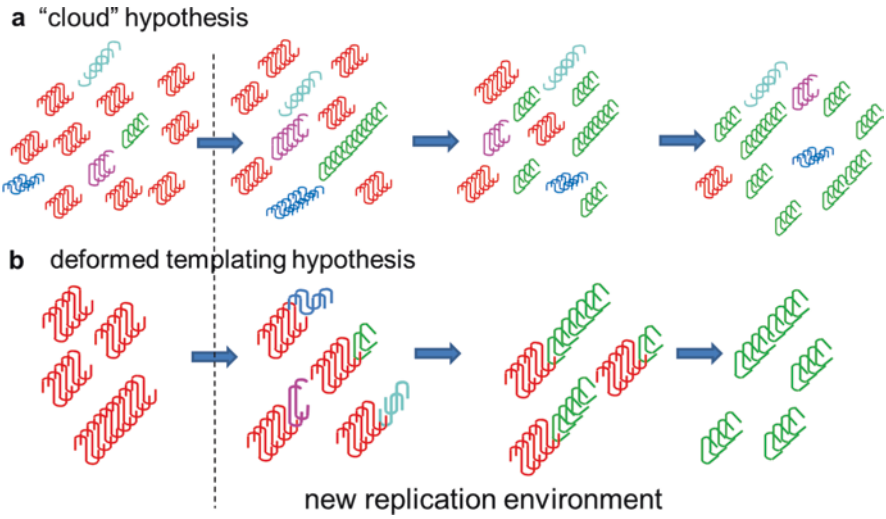

Fig. 5.5 Two hypotheses on the origin of prion strain mutations. (**a**) The "cloud" hypothesis proposes that prion isolates are intrinsically heterogeneous and consist of major (red) and minor (various colors) PrP^{Sc} variants. Changes in the replication environment provide selective advantages for the replication of a minor variant leading to transformations in the composition of PrP^{Sc} variants. (**b**) The deformed templating mechanism postulates that diverse structural variants are generated as a result of changes in the replication environment via numerous trial-and-error deformed templating events. A newly generated variant that fits better than parent PrP^{Sc} to an altered environment replaces the original PrP^{Sc} variant

multiple trial and error seeding events. Therefore, the change in the replication environment boosts the conformational diversity of the PrP^{Sc} pool and selects the variant that is the best fit for that environment.

The two models are not mutually exclusive, and both are likely to be involved in prion evolution. While deformed templating does not argue against structural heterogeneity of a PrP^{Sc} population of natural or synthetic origin, it helps to explain observations that would be difficult to understand solely based on the "cloud" hypothesis. The fundamental difference between the two models is in the origin of altered PrP^{Sc} states. In contrast to the "cloud" hypothesis, the deformed templating proposes that changes in the replication environment play an active role in expanding the pool of altered PrP^{Sc} variants. While new variants emerge with a help of a template they do not faithfully reproduce the parent state.

Experimentally, it is difficult to prove whether upon changing the replication environment, new PrP^{Sc} variants appear via selective amplification of pre-existing minor variants or emerge de novo via deformed templating (Mahal et al. 2012; Cancellotti et al. 2013). Nevertheless, changes in the replication environment were found to generate new PrP^{Sc} states (Gonzalez-Montalban et al. 2013; Katorcha et al. 2018). Adaptation of hamster strains 263K or Hyper to RNA-depleted brain homogenates and then re-adaptation to brain homogenates containing RNA in PMCAb was shown to lead to stable changes in PrP^{Sc} properties including PK-resistance, conformational stability, and amplification rates (Gonzalez-Montalban et al. 2013). Remarkably, upon reversible changes in RNA content, the amplification rate of the newly emerged PrP^{Sc} variants (referred to as $263K^{R+}$ or $Hyper^{R+}$) was 10^4-fold higher than that of brain-derived 263K. Moreover, consistent with the deformed templating mechanism, $263K^{R+}$ was absent in the original 263K brain material and emerged de novo as a result of reversible changes in the replication environment (Gonzalez-Montalban et al. 2013).

5.8 Role of Posttranslational Modifications in Driving Deformed Templating

In classical templating, the folding pattern of a newly recruited polypeptide chain accurately reproduces that of a template. In deformed templating, templates provide seeding too, yet newly recruited polypeptide chains acquire new folding patterns which only partially overlap with the folding pattern of a template. What are the driving forces behind deformed templating? Posttranslational modifications (PTMs) in PrP^{C} and, in particular, two N-linked groups along with Glycosylphosphatidylinositol (GPI) anchor are likely to impose spatial constraints limiting the spectrum of folding patterns available to PrP^{C} upon conversion into PrP^{Sc} (Breydo et al. 2007).

In the absence of PTMs, rPrPs acquire fibrillar structures that are thermodynamically and kinetically preferable (Baskakov et al. 2002; Sun et al. 2007), but do not easily accommodate PTMs. Under the circumstances that PTMs impose spatial or

electrostatic constraints not compatible with the rPrP fibrillar structures, rPrP fibrils select only those PrP^C molecules in vivo that can fit into the fibrillar structure. Indeed, as discussed above, the first product of misfolding triggered by rPrP fibrils in vivo was atypical PrPres, which is predominantly un- and monoglycosylated (Makarava et al. 2011, 2012a) (Fig. 5.3c). For accommodating diglycosylated PrP^C molecules, new structures have to emerge. Not only PTMs drive deformed templating, but N-glycans are also important for maintaining high fidelity of PrP^{Sc} replication. Transmission of prions to hosts expressing PrP^C deficient in N-glycans was found to change strain-specific characteristics of the 79A strain (Cancellotti et al. 2013). Loss of prion infectivity and PrP^{Sc}-specific structure upon PrP^{Sc}-seeded fibrillation of rPrP in vitro also argues that N-glycans are important for maintaining high fidelity of replication. Selective recruitment of PrP^C sialoglycoforms, specified by strain-specific structure, has multiple important implications in prion biology and is discussed elsewhere (Katorcha et al. 2015; Baskakov and Katorcha 2016; Baskakov et al. 2018).

5.9 Deformed Templating as a Mechanism of a Cross-Talk Between Amyloidogenic Proteins

The hypothesis that transmissible prion diseases can be triggered by cross-β PrP structures substantially different from that of authentic PrP^{Sc} has important implications for understanding the etiology of prion and other neurodegenerative diseases. A growing number of studies have documented that amyloid forms of several proteins linked to neurodegenerative diseases were capable of seeding their own aggregation in a prion-like manner in a cell and spreading from cell to cell through the nervous system (reviewed in Miller (2009), Frost and Diamond (2010), and Aguzzi and Rajendran (2009)). It is generally assumed that self-perpetuating aggregation requires identity in amino acid sequence between seeds and substrate. Nevertheless, the possibility of cross-talk between non-related amyloidogenic proteins has been illustrated in several studies (Jean et al. 2007; Yan et al. 2007; Morales et al. 2010; Katorcha et al. 2017). In vivo, amyloidosis of one protein was found to be triggered by fibrils of an unrelated protein in a manner similar to cross-seeded polymerization (Jean et al. 2007; Yan et al. 2007; Morales et al. 2010). Cross-talk between several yeast prion proteins provides another example of how direct interactions between newly forming and preexisting heterologous fibrils might take place in a cell (Derkatch et al. 1997, 2001, 2004). Moreover, protein aggregates produced from two different proteins or peptides, including PrP, Aβ, α-synuclein, immunoglobulin light chain λ, and β_2 microglobulin, often colocalize within the same amyloid plaque in a variety of organs or tissues (Haik et al. 2002; Adjou et al. 2007; Takahashi et al. 1996; Miyazono et al. 1992; Galuske et al. 2004). The promiscuous nature of the propagating activity of amyloid structures can lead to devastating consequences for cellular health. For instance, the cross-talk between non-related amyloidogenic

proteins may offer a possible explanation for the development of age-related conformational disorders that are considered to be sporadic in origin. In an effort to identify the spectrum of structures and sequences capable of triggering the PrP^{C} to PrP^{Sc} conversion, we found that α-synuclein aggregates formed in cultured cells or in vitro, but not non-fibrillar α-synuclein or fibrillar Aβ, triggered misfolding of the PrP^{C} into self-replicating PrP states that induced transmissible prion disease in wild type host (Katorcha et al. 2017).

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