

# Understanding the diversity of prions

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**The protein-only hypothesis proposes that prions propagate by imparting specific folds onto cellular proteins. This hypothesis has met with resistance, partly because of the observation that many phenotypically distinct prion 'strains' are known to exist in yeast and mammals. Recent work on yeast prions may help reconcile the occurrence of prion strains with the prion hypothesis.**

Prions were originally defined as the infectious agents that cause a diverse group of mammalian-transmissible spongiform encephalopathies (TSEs), including 'mad cow disease' and Creutzfeldt-Jakob disease<sup>1</sup>. The prion, or protein-only, hypothesis suggested that the mammalian prion is identical to PrP<sup>Sc</sup>, a protease-resistant variant of the cellular protein PrP<sup>C</sup>. Prusiner proposed that PrP<sup>Sc</sup> propagates by promoting conversion of endogenous PrP<sup>C</sup> into further PrP<sup>Sc</sup> proteins. This concept was then extended by Reed Wickner to include yeast genetic traits whose inheritance could not be explained by transmission through nucleic acids<sup>2</sup>. One yeast protein with self-perpetuating, alternate conformations has been studied in great detail: *PSI<sup>+</sup>*, the prion form of the translational termination factor Sup35. In *PSI<sup>+</sup>* cells, Sup35 is inactivated by aggregation, causing the same phenotypes as hypomorphic mutations of the *SUP35* gene. Now, studies on *PSI<sup>+</sup>* provide further support for the prion hypothesis<sup>3,4</sup>.

Despite much supporting evidence, the protein-only hypothesis is still meeting with less-than-universal consensus<sup>5</sup>. Two major objections have been frequently emphasized by the sceptics. For one thing, although recombinant PrP can be converted into a physical state similar to PrP<sup>Sc</sup>, *de novo* generation of infectious prions under cell-free conditions has never succeeded. Another issue is the counterintuitive existence of prion 'strains'. The idea of prion strains emerged from the finding that distinct versions of prion diseases which differ at the symptomatic and biochemical level can occur within the same mammalian species, even though the *PrP* gene is identical in these animals<sup>6</sup>. One explanation is that the strain-specific properties of prions may be encoded by a nucleic acid genome<sup>7</sup>; however, no evidence for this hypothesis has been forthcoming. Alternatively, if the prion really consists only

of protein, PrP<sup>Sc</sup> must exist in a variety of distinct pathological conformations, each one of which can faithfully impart its own conformation onto PrP<sup>C</sup> (Fig. 1a)<sup>8</sup>, resulting in distinct pathologies. Even well-meaning protein chemists have long considered the latter idea to be outlandish. However, circumstantial evidence is accumulating to suggest that strain phenotypes may be encoded within different conformations of PrP<sup>Sc</sup> which have distinct stabilities against chaotropic salts<sup>9</sup> and heat, although formal proof that conformational variants of PrP<sup>Sc</sup> represent the biological basis of prion strains is still lacking.

To make matters worse, certain aspects of mammalian prion strains seem harder than ever to understand. Some strains are much more stable than one could reasonably expect. Other strains display aberrant behaviour and may mutate to unexpected biochemical phenotypes<sup>10</sup>. These observations are crucially important for public health. Although the prion strain associated with sheep scrapie may be relatively harmless to humans, mad cow (BSE) prions are promiscuous in their species tropism<sup>1</sup> and may maintain their strain characteristics when passaged to other hosts. Thus, if BSE entered the sheep population, the presence of TSE in sheep may pose a much larger threat to human health than previously thought. In addition, the sporadic and variant form of Creutzfeldt-Jakob disease differs in crucial strain-specific properties: the former leads to prion accumulation in skeletal muscle<sup>11</sup>, whereas the latter seems to be transmissible through blood transfusions<sup>12</sup>.

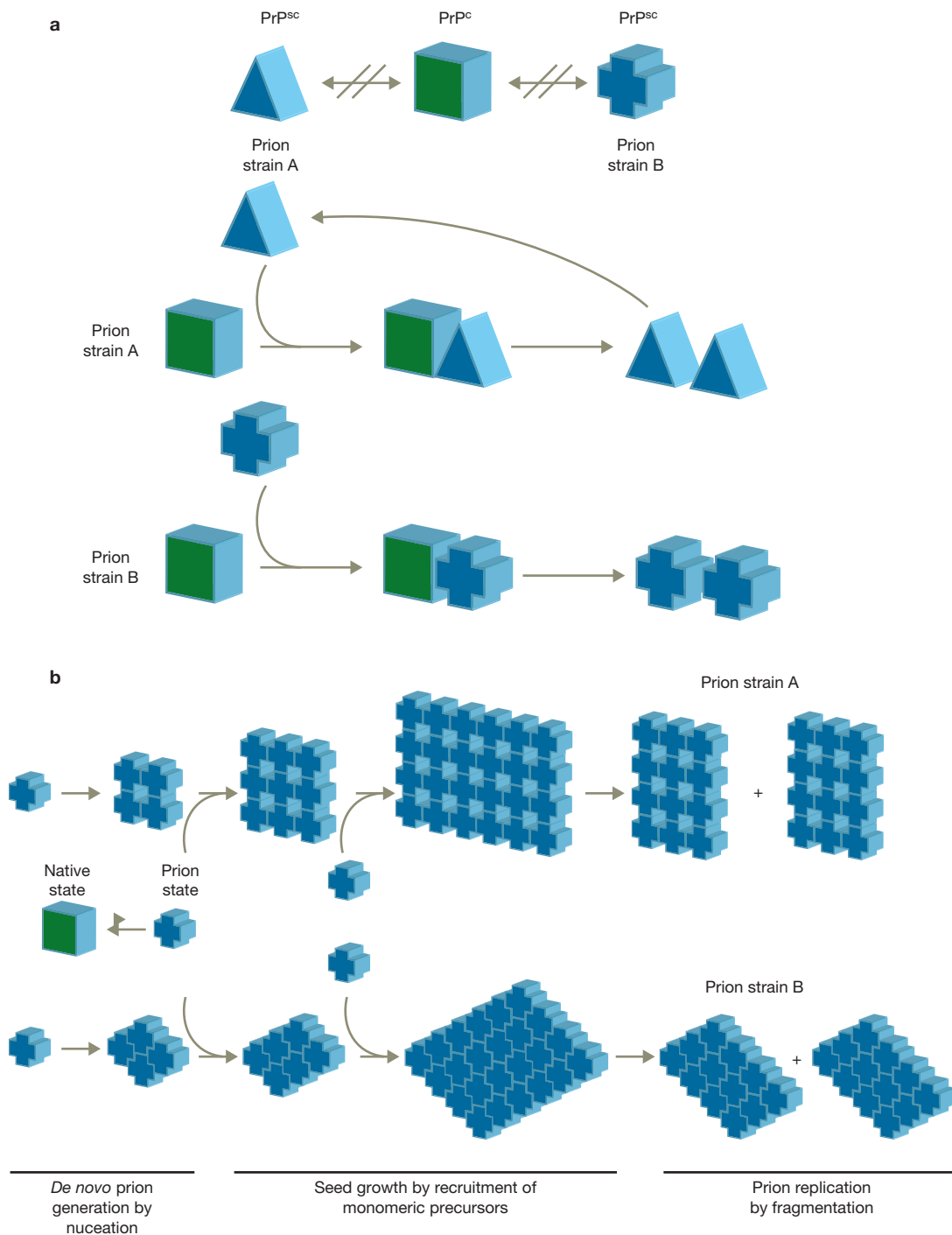
Not unexpectedly, while their colleagues were struggling with recalcitrant mammalian systems, yeast prionologists have made swift progress. Yeast prion strains with distinct heritable phenotypes have been isolated from genetically identical cells<sup>13</sup>, and *in vitro* generation of Sup35 fibres with distinct conformations suggestive of different *PSI<sup>+</sup>* strains have also been described<sup>14,15</sup>. Earlier work from Jonathan Weissman's laboratory<sup>16</sup> had shown that a purified Sup35 prion domain, introduced into yeast cells by lipofection, induced a *PSI<sup>+</sup>* phenotype in 1–2% of transformants. This impressive achievement comes close to the final

proof of the protein-only hypothesis, at least in the case of Sup35. However, as aggregated Sup35 — the presumed prion form of Sup35 — could not be directly loaded into liposomes, these experiments could not exclude the possibility that *PSI<sup>+</sup>* prions may have appeared *de novo* in the cell as a result of a high local concentration of non-infectious Sup35.

Now, work from the laboratories of Chih-Yen King and Jonathan Weissman<sup>3,4</sup> uses ingenious protein transduction protocols to overcome the limitations of the earlier approach. Weissman and colleagues<sup>4</sup> generated infectious Sup35 prions by assembling a bacterially expressed Sup35 prion domain into ordered fibrillar structures called 'amyloid seeds'. In addition to confirming their earlier work, the authors found that multimerization of Sup35 at 4 °C, 23 °C or 37 °C results in the formation of strikingly distinct amyloid fibres. Hence, the same protein can give rise to several unique quaternary assemblies. Utterly surprising, however, is the finding that these distinct amyloids propagated with different and stable phenotypic traits when transduced to yeast cells. These results demonstrate that not only can prions be generated *de novo* from homogenous, bacterially expressed protein, but that such spontaneous generation can occur in various heritable flavours, thus proving that the prion strain phenomenon is, in principle, compatible with the protein-only hypothesis.

In a parallel study, King and Diaz-Avalos<sup>3</sup> introduced a tagged protein consisting of the Sup35 'prionization' domain fused to green fluorescent protein (GFP) into *PSI<sup>+</sup>* yeast cells that had been previously infected with various Sup35 variants, and Sup35–GFP aggregates were recovered by affinity chromatography. Incubation with bacterially expressed Sup35–GFP, sonication and dilution resulted in prion amplification, essentially reproducing the cycle hypothesized in Fig. 1b. These studies provide further evidence that *PSI<sup>+</sup>* prions consist exclusively of aggregated Sup35 protein, and that in this model system the seeded nucleation hypothesis is much more plausible than the template-directed refolding hypothesis (Fig. 1a, b). In addition, the work

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**Figure 1** Hypothetical models for prion-encoded strain phenotypes. **(a)** The template-directed refolding hypothesis postulates that a monomeric prion protein adopts a multitude of infectious tertiary structures (symbolized here as prisms and crosses), each one of which imparts its conformation onto its native counterpart. The experimental evidence supporting this hypothesis is slim and indirect at best.

**(b)** Alternatively, as proposed by the seeded nucleation hypothesis, yeast *PSI<sup>+</sup>* prion strains may be specified by the structure of Sup35 aggregates. In this scenario, a single batch of purified Sup35 protein may give rise to distinct quaternary structures (symbolized here as grids of different shapes), each one of which self-assembles *in vitro* and sustains itself after transduction into yeast cells.

of Weissman and colleagues Iso suggests that differences in *PSI<sup>+</sup>* strains may be determined by the higher-order structure of Sup35 fibrils.

These discoveries are pivotal to understanding the prion strain enigma, but several

unanswered questions remain. For one, the structural nature of *PSI<sup>+</sup>* prion strains is still murky. Does the supramolecular structure of prion fibrils determine the various phenotypes of prion strain, or do the monomeric

precursors assume different tertiary structures as well? High-resolution structures of the various fibrils would be a necessary precondition to understanding the molecular details of the prion formation and replication process.

Perhaps the most important unanswered question concerns just how universal this mechanism is for generating prion diversity. The work discussed above provides strong support for the contention that, in the yeast Sup35 prion model, the same protein may build aggregates with different structures that then correlate with different strain-specific phenotypes. But might distinct quaternary structures of PrP<sup>Sc</sup> (Fig. 1b) underlie the strain properties of the prototypic prions that cause mammalian prion diseases? While prion biology draws closer to complete elucidation in the yeast paradigm, the situation in mammals

is much less clear. Given the importance of understanding prion variability for human health, it can only be hoped that mammalian prionologists will catch up fast with their yeast colleagues in elucidating the biophysical underpinnings of the strain phenomenon. □

1. Aguzzi, A. & Polymenidou, M. *Cell* **116**, 313–327 (2004).
2. Wickner, R. B. *Science* **264**, 566–569 (1994).
3. King, C. Y. & Diaz-Avalos, R. *Nature* **428**, 319–323 (2004).
4. Tanaka, M., Chien, P., Naber, N., Cooke, R. & Weissman, J. S. *Nature* **428**, 323–328 (2004).
5. Aguzzi, A. & Heikenwalder, M. *Nature* **423**, 127–129 (2003).
6. Dickinson, A. G. & Meikle, V. M. *Mol. Gen. Genet.*

- 112, 73–79 (1971).
7. Weissmann, C. *Nature* **352**, 679–683 (1991).
8. Aguzzi, A. & Weissmann, C. *Nature* **389**, 795–798 (1997).
9. Safar, J. *et al. Nature Med.* **4**, 1157–65 (1998).
10. Asante, E. A. *et al. EMBO J.* **21**, 6358–6366 (2002).
11. Glatzel, M., Abela, E., Maissen, M. & Aguzzi, A. *New Engl. J. Med.* **349**, 1812–1820 (2003).
12. Aguzzi, A. & Glatzel, M. *Lancet* **363**, 411–412 (2004).
13. Derkatch, I. L., Chernoff, Y. O., Kushnirov, V. V., Inge-Vechtomov, S. G. & Liebman, S. W. *Genetics* **144**, 1375–1386 (1996).
14. Chien, P. & Weissman, J. S. *Nature* **410**, 223–227 (2001).
15. King, C. Y. *J Mol Biol* **307**, 1247–1260 (2001).
16. Sparrer, H. E., Santoso, A., Szoka, F. C., Jr. & Weissman, J. S. *Science* **289**, 595–599 (2000).

## Bicarbonate secretion: it takes two to tango

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**New research reveals a reciprocal regulation between the CFTR chloride channel, implicated in cystic fibrosis, and several members of the SLC26 family of chloride-bicarbonate exchangers. These findings provide new insights into the mechanism of epithelial bicarbonate and fluid transport and may lead to better treatments for cystic fibrosis and congenital chloride diarrhoeas.**

Cystic fibrosis is a chronic recessive disease caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), a member of the ABC family of membrane transporters. CFTR functions as an ATP-gated chloride channel that is regulated by PKA phosphorylation and is expressed mainly in the apical plasma membrane of epithelial tissues<sup>1</sup>, where it has a crucial role in regulating fluid secretion in the airways, salivary glands, intestine and genital tract. When CFTR fails to work properly, it produces an imbalance in salt and fluid transport that results in thickened secretions in all these tissues. In the airways there is an accumulation of sticky mucus that eventually results in respiratory failure. Blockages in the gut, pancreas and liver lead to poor digestion and malnutrition, and defects in the genital tract lead to infertility<sup>2</sup>.

The main defect in cystic fibrosis has long been thought to be reduced epithelial chloride transport. However, work published by Muallem and colleagues<sup>3</sup> turned this theory

on its head. They found that the primary defect in cystic fibrosis was not a failure in chloride transport *per se*, but a problem with bicarbonate-driven fluid secretion, caused by the inability of mutant forms of CFTR to activate chloride-bicarbonate exchange<sup>3</sup>. Indeed, we have known for many years that most, if not all, cystic-fibrosis-affected epithelia show aberrant bicarbonate and fluid transport, and this work has provided an explanation for these observations. Subsequent studies from the same group<sup>4</sup> extended the link between CFTR and bicarbonate by demonstrating that CFTR specifically up-regulated the activity of three members of the relatively new SLC26 gene family of multifunctional anion exchangers<sup>5</sup>, although the exact mechanism involved was not determined. Importantly, these findings highlighted the fact that chloride transport and modulation of chloride-bicarbonate exchange were distinct and separable activities of CFTR, thus providing an important framework for understanding the mechanism of aberrant CFTR-dependent bicarbonate secretion in cystic fibrosis<sup>4</sup>. In this issue, Muallem and colleagues provide clear evidence for a direct molecular interaction between the CFTR protein and two SLC26 exchangers<sup>6</sup>, A3 (also known as DRA) and A6 (also known as

PAT1). Binding of the two transporters results in a unique, mutual, up-regulation of their transport activity, a finding that provides a satisfying explanation for the dependence of bicarbonate and fluid secretion on the expression of both proteins.

The authors use a series of elegant molecular, biochemical and electrophysiological experiments to show that the interaction between CFTR and SLC26 members is mediated by binding of the regulatory (R) domain of CFTR to the highly conserved STAS (sulphate transporter and anti-sigma antagonist<sup>7</sup>) domain of SLC26. The interaction is enhanced by phosphorylation of the R-domain by PKA and is modulated by PDZ-binding scaffold proteins (such as EBP50) that tether the two transporters into a multimeric complex with other regulatory proteins (see Fig. 1). An important consequence of this interaction is that SLC26 anion exchange activity is then enhanced when CFTR is activated by phosphorylation. The fact that the stimulatory effect of CFTR is antagonized by excess recombinant STAS domain highlights the requirement for physical association of CFTR with the SLC26 transporter.

The authors also demonstrate a reciprocal regulation, whereby expression of either

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