REVIEWS

PRIONS AS ADAPTIVE CONDUITS OF MEMORY AND INHERITANCE

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Abstract | Changes in protein conformation drive most biological processes, but none have seized the imagination of scientists and the public alike as have the self-replicating conformations of prions. Prions transmit lethal neurodegenerative diseases by means of the food chain. However, self-replicating protein conformations can also constitute molecular memories that transmit genetic information. Here, we showcase definitive evidence for the prion hypothesis and discuss examples in which prion-encoded heritable information has been harnessed during evolution to confer selective advantages. We then describe situations in which prion-enciphered events might have essential roles in long-term memory formation, transcriptional memory and genome-wide expression patterns.

PHENOTYPIC SPACE
A multi-dimensional continuum of all possible phenotypes.

ADAPTIVE LANDSCAPE
A graph of the average fitness of a population in relation to the frequencies of genotypes in the population.

CONFORMER
Any of two or more isomers that differ only in their three dimensional conformation.

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How a genotype and its environment interact to yield a phenotype poses a vast epistemological gap. Proteins that exhibit conformational diversity and contingent functional multiplicity increase the dimensions of PHENOTYPIC SPACE encoded by any given genome[1]. Protein folding can therefore radically alter the trajectories that connect genotype and phenotype, modify ADAPTIVE LANDSCAPES and influence evolution. Extraordinary conformational diversity is embodied by prions[2], which are unusual proteins that can traverse between, and exist stably in, many functionally distinct conformations, at least one of which is self-replicating. Prion CONFORMERS operate as a template for other conformers, usually of the same amino-acid sequence, to acquire the prion conformation, and these, in turn, are templates for others, creating a protein-folding chain reaction (FIG. 1a; BOX 1). This self-replication of conformational information enables prions to act as genetic elements with the ability to transmit disease[2], encode heritable phenotypic traits[3] or encrypt molecular memories[4]. That prions are a conduit for the replication of heritable information places them in the CODICAL DOMAIN, more commonly regarded as the territory of DNA or RNA, and potentially empowers prions to operate as units of selection.

Overwhelming evidence now supports the prion hypothesis and most scholars accept the existence of prions[2–8]. However, it is repeatedly suggested that the ultimate vindication of the prion hypothesis will lie in the *de novo* generation of prions *in vitro* using purely recombinant or synthetic polypeptides[5]. Here, we spotlight recent achievements that begin to realize this goal. Furthermore, we describe how information transmitted by prions can be regulated, confer evolutionary advantages and might even underlie long-term memory formation. We then speculate on the potentially widespread incidence of prion-encoded switches in cell behaviour, development and evolution.

Origins of the prion hypothesis

Mammalian prions. Prions underpin several fatal transmissible, genetic and sporadic neurodegenerative diseases that afflict mammals[2,5]. These spongiform encephalopathies include: Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome and kuru in man; chronic wasting disease (CWD) in deer and elk; scrapie in sheep; and bovine spongiform encephalopathy (also called 'mad cow' disease) in cattle[5]. Disturbingly, prion diseases can be transmitted from cattle to humans by means of the food chain[5]. In the United Kingdom, more than 150 people have died of variant CJD, the prion disease transmitted to humans through prion-contaminated beef. In the United States, the horizontal transfer, increasing incidence and broadening geographical range of CWD in

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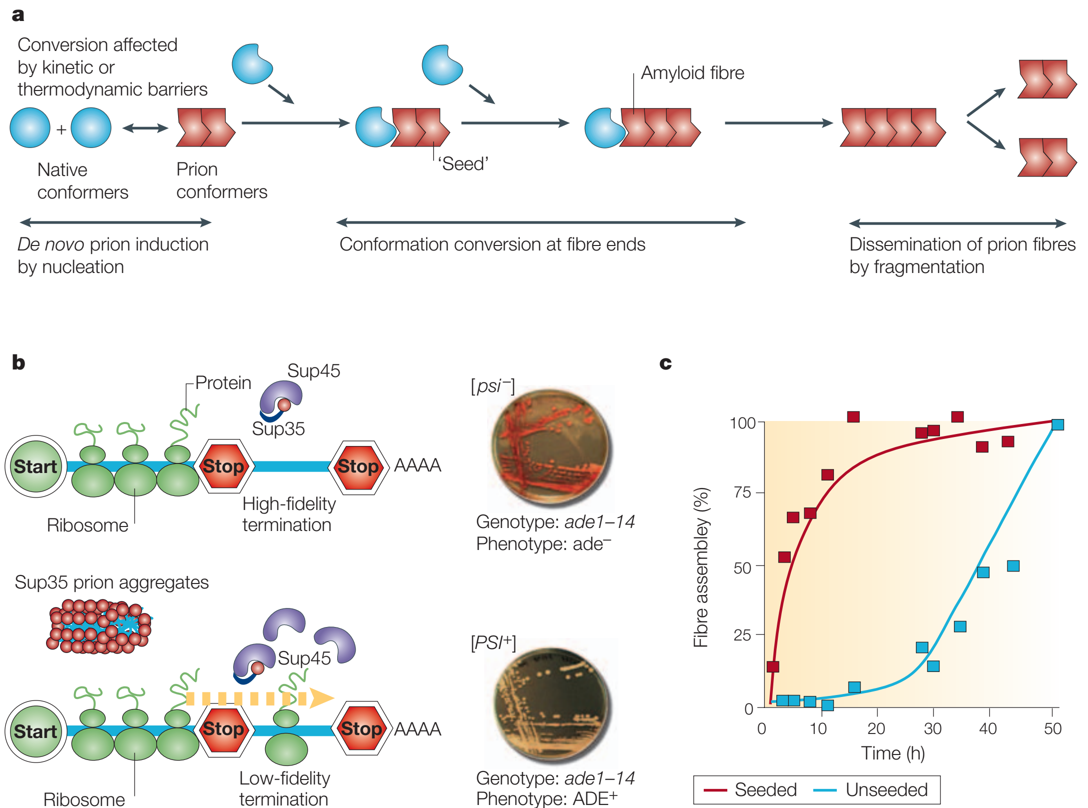

Figure 1 | **The prion hypothesis and the yeast prion [*PSI*+]. a** | A nucleation event stabilizes protein conformers in an altered self-replicating prion conformation. The probability of nucleation is dictated by kinetic and thermodynamic considerations and is exceptionally unlikely for most proteins in physiological settings. Once formed, the nucleus, or 'seed', recruits other conformers (that are probably in a transiently unfolded state) and converts them to the self-replicating conformation. The nucleus then increases in size to become an AMYLOID fibre and continues to convert other conformers to the self-replicating conformation at the fibre ends. Amplification of conformational replication is achieved by the fragmentation of fibres to liberate new ends. Fragmentation also allows the dissemination of infectious material. **b** | In *Saccharomyces cerevisiae* [*psi*−] cells, the translation-termination factor Sup35 functions with Sup45 to recognize stop codons and terminate translation. Cells that carry a premature stop codon in their *ADE1* gene do not make functional Ade1 and accumulate a red metabolite. By contrast, in [*PSI*+] cells most Sup35 protein is sequestered in self-replicating prion fibres, and is unable to participate in translation termination. Consequently, some ribosomes read through the stop codon and functional Ade1 is produced. [*PSI*+] cells therefore produce white colonies and can grow on adenine-deficient medium. The red/white colony assay is convenient and frequently used, but [*PSI*+] can also suppress a wide range of other stop codon mutations. [*PSI*+] increases the efficiency of readthrough by ~0.2–35%, depending on the specific [*PSI*+] variant, yeast strain and stop codon in question[67–69]. Modified with permission from REF. 156 © (2000) Elsevier Science. **c** | Prion fibres form only after a characteristic lag phase in reactions that are not seeded (blue). By contrast, prion fibres form rapidly without a lag phase in reactions seeded with small quantities (2% w/w) of prion fibres (red).

cervids and its potential transmission to livestock and humans, looms as a serious public health threat[5].

The causative agent of these diseases is unlikely to be nucleic-acid based because it is extraordinarily resistant to nucleases and UV radiation[2,5,9]. These observations invoked several diverse hypotheses concerning the composition of the infectious agent, which included the 'protein-only' hypothesis[2,5]. Infectivity is sensitive to agents that inactivate proteins, strongly indicating a protein-based infectious agent (abbreviated to prion)[2,5]. Speculations[10] were found to be prescient once biochemical purification of the infectious agent revealed protein aggregates that were virtually devoid of nucleic acid and were composed principally of one protein[2,5,11]. The conundrum of how a protein could confer infectivity was solved when it was shown to be an endogenous host protein, termed prion protein[2,5,11] (PrP). Infectivity, therefore, only required PrP to encode self-templating conformational information.

PrP is ubiquitously expressed in its normal, α-helical cellular form, termed PrP^C (see TABLE 1 for prion nomenclature), as a glycosylphosphatidylinositol-anchored plasma membrane glycoprotein, the function of which is still obscure[5]. Although spontaneous prion diseases afflict about one in a million people per year, several independent missense mutations in the PrP ORF almost guarantee the development of disease[12]. Conversely, the deletion of the gene that encodes PrP renders animals resistant to prion diseases[13,14]. When linked to infectivity, PrP generally adopts an altered, protease-resistant, β-sheet-rich, polymeric conformation known as PrP^{Sc} (REFS 5,15) (see TABLE 1 for prion nomenclature). PrP^{Sc} propagates by converting PrP^C molecules to the PrP^{Sc} state (FIG. 1a),

CODICAL DOMAIN
The domain of natural selection dealing solely with self-replicating information as opposed to material entities.

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Box 1 | **Related self-perpetuating protein-based phenomena**

Other types of self-perpetuating protein-based phenomena not related to the conformational replication of prions are known. A transcription factor that activates its own transcription could create self-perpetuating heritable phenotypes if introduced into a system in which it is not usually expressed[10]. Examples include MyoD, which directs muscle differentiation and stimulates its own synthesis[103]; and MEC-3, a homeobox protein of *Caenorhabditis elegans* that also directs its own synthesis[104]. An autoactivating zymogen (or any autoactivating enzyme), such as protease B, can be self-perpetuating if the active form is introduced into systems that only contain the inactive form[117]. Self-perpetuating states can also be established in autophosphorylation reactions[101] and are complex, emergent properties of signalling networks[102]. Several non-Mendelian elements in fungi might be attributable to these (or other) types of self-perpetuating phenomenon, or might be due to the conformational replication of prions, such as: [*cif*], a non-chromosomal element of *Schizosaccharomyces pombe* that engenders cell viability in the absence of calnexin (a chaperone that resides in the ER)[118]; [*GR*], a cytoplasmically transmitted element of *Saccharomyces cerevisiae* that confers glucosamine resistance[119]; [*C*+], a cytoplasmically inherited element of *Podospora anserina* that inhibits mycelial growth[120]; [*KIL-d*], an epigenetic factor of *S. cerevisiae* that regulates killer virus gene expression[121]; and [*ISP*+], a nonchromosomal element of *S. cerevisiae* that restores translation-termination fidelity in cells that carry recessive mutations in their *SUP35* gene (which encodes a translation-termination factor)[122].

and this process, once initiated in multiple cell types of the central nervous system (CNS), eventually elicits severe neurodegeneration[13,14].

Fungal prions. Although the putative behaviour of PrP was initially considered highly anomalous, the transmissible character of mammalian prions proved instructive in understanding two non-Mendelian traits in *Saccharomyces cerevisiae*, namely: [*PSI*+] (see TABLE 1 for prion nomenclature), which suppresses nonsense codons[16] (FIG. 1b), and [*URE3*] (see TABLE 1 for prion nomenclature), which inhibits nitrogen catabolite repression[17] . Both traits were discovered ~40 years ago and vexed yeast geneticists for just as long as prion diseases baffled neuropathologists. Attempts to attribute these traits to known non-Mendelian elements — including viruses, episomes or mitochondrial genes — failed. Instead, [*PSI*+] and [*URE3*] are due to self-replicating conformations of proteins encoded by chromosomal genes: Sup35 (a translation-termination factor[6]) in the case of [*PSI*+]; and Ure2 (an antagonist of the transcriptional activators Gln3 and Gat1) in the case of [*URE3*] (REF. 7) (TABLE 1). The fact that these traits are due to self-replicating conformations of endogenous proteins (prions) suddenly resolved a panoply of otherwise irreconcilable data[6,7] (BOX 2).

Since the revelation that [*PSI*+] and [*URE3*] are encoded by prions, another yeast prion, [*RNQ*+], has been elucidated[18]. Similar to [*PSI*+] and [*URE3*], the protein determinant of [*RNQ*+], Rnq1, contains a glutamine (Gln)/asparagine (Asn)-rich prion domain (BOX 2). Other Gln/Asn-rich domains have also been shown to confer prion behaviour[19,20] (TABLE 1). However, not all prions contain a Gln/Asn-rich domain (TABLE 1). For instance, the filamentous fungus *Podospora anserina* harbours the non-Mendelian genetic element [Het-s], which is composed of prion conformers of the HET-s protein, which lacks a Gln/Asn-rich domain[7,21] (see TABLE 1 for prion nomenclature). The [Het-s] prion functions in a genetically programmed cell-death phenomenon, termed HETEROKARYON incompatibility, which occurs when two fungal strains of different genotypes fuse[7,21].

HETEROKARYON
Any cell with more than one nucleus and where the nuclei are not all of the same genetic constitution, or a tissue composed of such cells.

AMYLOID
A general term for protein aggregates that accumulate as fibres of 7–10nm in diameter with common structural features including: β-pleated sheet conformation, resistance to detergents and proteases, and the ability to bind such dyes as Congo red and Thioflavin T and S.

Unifying features of mammalian and fungal prions. A crucial unifying facet of prions is a high propensity to assemble into self-propagating AMYLOID fibres under physiological conditions, both *in vitro*[18,22–25] (BOX 2) and *in vivo*[11,26–33]. The amyloid conformation confers characteristics of aggregation, protease-resistance, sodium dodecyl sulfate (SDS)-insolubility, Congo-red and Thioflavin-T binding, and β-sheet-rich structure[22–25,34,35]. For the known yeast prions, amyloidogenesis is driven by their Gln/Asn-rich domains[18,23,24] (BOX 2). *In vitro*, prions assemble into amyloid fibres after a characteristic lag phase, which is eliminated by the addition of preformed fibres (seeding)[18,22–25,29] (FIG. 1c). This seeded catalysis of polymerization is crucial for prion infectivity and conformational replication. Furthermore, prions assemble into an ensemble of related, but structurally distinct, transmissible states[15,22,33,36,37]. Electron-microscopy studies have shown that Ure2 and NM (the N-terminal prion and middle domains of Sup35) fibre populations contain a diverse range of self-perpetuating fibres that have distinct morphologies[23,24,38,39]. Such differences in structure might help to explain[23] the confusing prion 'strain' or 'variant' phenomena (BOX 3).

Definitive evidence for the prion hypothesis

Recent advances validate the prion hypothesis and explain prion-strain phenomena. Definitive evidence for the prion hypothesis requires the nascence of prion conformers *in vitro,* using purely recombinant protein, to infect or transform cells that do not have the prion[5,25,33,36,37,40]. The failure to accomplish this with recombinant PrP and wild-type animals endures as the largest concern for the mammalian prion hypothesis. However it has been actualized for [Het-s] (REF. 25) and [*PSI*+] (REF. 33,36), and this leaves no doubt that amyloid fibres alone can harbour prion infectivity.

The feat was first accomplished for the [Het-s] prion. Transformation of prion-free *P. anserina* with amyloid fibres formed from recombinant HET-s protein (see TABLE 1 for prion nomenclature) induced [Het-s] with ~100% efficiency[25]. Importantly, neither soluble HET-s nor amorphous HET-s aggregates (formed by heat or acid denaturation) induced the prion, nor did amyloid conformers of other proteins[25]. Limited proteolysis of HET-s fibres produces fibres composed of the C-terminal fibre core (amino acids 218–289), which also induce [Het-s], confirming that the amyloid core drives conformational replication[25].

Transformation of [*psi*–] yeast with amyloid fibres composed of the prion domain of Sup35 efficiently induces [*PSI*+] (REFS 33,36). Soluble NM does not induce [*PSI*+]. Importantly, [*PSI*+] is induced by NM fibres with equal efficiency in [*pin*–] and [*PIN*+] cells[36] (BOX 4), showing that [*PSI*+] is induced by prion conformers of NM and not by simply increasing intracellular

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Table 1 | **Protein determinants of known and potential prions and their phenotypes**

Species	Protein determinant	Non-prion state‡	Prion state§	Gln/Asn-rich prion domain	Oligopeptide repeats	Intrinsically unstructured regions ≥50 amino acids (predicted)‖	Requires Hsp104?	Cellular function	Prion-encoded phenotype
Mammals	PrP, ~209 amino acids in mature form	PrP^C	PrP^{Sc}	No	Yes, amino acids 51–91	Yes, amino acids 1–103	No	Unknown	Severe neurodegeneration and inexorable death
S. cerevisiae	Sup35, 685 amino acids	*[psi⁻]*	*[PSI⁺]*	Yes, amino acids 1–124	Yes, amino acids 41–97	Yes, amino acids 1–253 (NM), amino acids 291–340	Yes	Translation termination	Increase in the readthrough of stop codons (nonsense suppression)
S. cerevisiae	Ure2, 354 amino acids	*[ure-o]*	*[URE3]*	Yes, amino acids 1–80	No	Yes, amino acids 1–103	Yes	Transcriptional repressor	Uptake of poor nitrogen sources# in the presence of a good nitrogen source**
S. cerevisiae	Rnq1, 405 amino acids	*[rnq⁻]*	*[RNQ⁺]* (usually acts as *[PIN⁺]*)	Yes, amino acids 153–405	Several degenerate repeat sequences	Yes, amino acids 201–405	Yes	Unknown	Allows *de novo* induction of *[PSI⁺]* by Sup35 overexpression
P. anserina	HET-s, 289 amino acids	[Het-s*]	[Het-s]	No	No	No¶	?	Unclear, but involved in heterokaryon incompatibility	Induces cell death in the presence of the HET-S protein
S. cerevisiae	New1, a putative prion, 1,196 amino acids	—	Can act as *[PIN⁺]*	Yes, amino acids 1–153	Yes, amino acids 50–100	Yes, amino acids 1–118, amino acids 878–984, amino acids 1118–1196	?	Translation elongation	Gln/Asn-rich domain can functionally replace the Sup35 Gln/Asn domain and support *[PSI⁺]*. Overexpression allows *de novo* induction of *[PSI⁺]* by Sup35 overexpression
A. californica	ApCPEB1, a putative prion, 687 amino acids	—	—	Yes, amino acids 1–150	Yes, amino acids 84–138	Yes, amino acids 1–150	No	Regulates translation of specific mRNAs	Regulates protein synthesis at activated synapses and stabilizes long-term facilitation

‡Protein determinant adopts non-replicating conformation. §Protein determinant adopts self-replicating conformation. ‖Predicted using FoldIndex© (see Online links box). ¶Note that the prion domain of HET-s (the C-terminal 72 amino acids) has been shown experimentally to be intrinsically unstructured[29]. #For example ureidosuccinate. **For example ammonium ions. *A. californica*, *Aplysia californica*; NM, the N-terminal prion and middle domains of Sup35; *P. anserina*, *Podospora anserina*; *S. cerevisiae*, *Saccharomyces cerevisiae*.

NM concentration[36]. Furthermore, this transformation procedure has allowed the direct assessment of whether NM fibres of different conformations encode distinct prion variants[33,36] (BOX 3).

Exciting progress has also been made in generating synthetic PrP conformers that induce disease in transgenic mice[37,41,42] (and see Note added in proof). Inoculating amyloid conformers of a synthetic PrP peptide (comprising amino acids 89–143 (PrP 89–143) with the disease-associated P101L mutation) into the brains of mice expressing the PrP P101L mutant allele induces more rapid disease onset[41]. Non-amyloid conformers have no such effect[41]. Furthermore, recombinant mouse PrP 89–230 amyloid fibres induce neurodegenerative disease when they are injected intracerebrally into transgenic mice that overexpress PrP 89–231 (REFS 37,42,43). Intriguingly, seeded and unseeded fibres might encode different prion strains[37].

The generation of synthetic prions will facilitate the precise definition of which protein conformations confer prion status. For example, are prions always amyloids (BOX 5)?

Primordial amyloid?

Clearly, amyloid conformers can embody prions, at least in yeast. Amyloid conformers of specific proteins are also connected with several devastating degenerative disorders including Alzheimer disease (AD), Parkinson disease (PD), Huntington disease, systemic amyloidoses and type II diabetes[35]. However, the amyloids connected with these disorders are not considered prions[8,44]. What distinguishes them (BOX 5)?

Remarkably, amyloidogenesis is an intrinsic property of polypeptides. Even archetypal globular proteins, such as myoglobin, can adopt this form[35]. However, extremes of pH, temperature, or incubation

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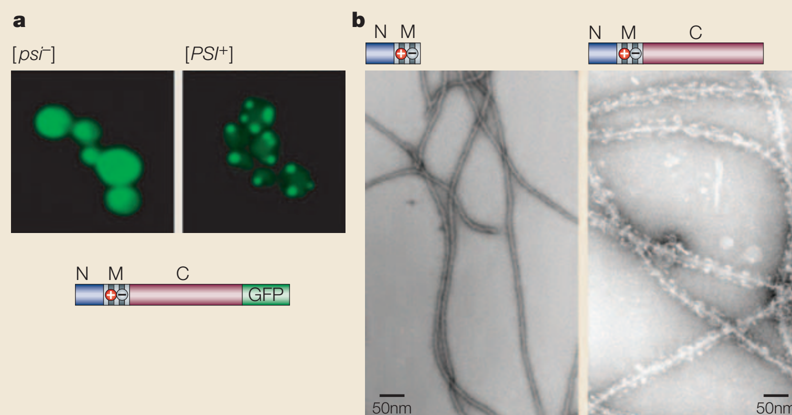
Box 2 | **Characteristics of the [*PSI*+] and [*URE3*] prions**



When cells that carry [*PSI*+] or [*URE3*] are mated to cells that do not carry the traits ([*psi*–] or [*ure-o*]), the diploid displays the trait and, when sporulated, all progeny do as well[6,7]. Both traits are metastable — they are reversibly gained and lost at a low spontaneous rate (10^{-5} to 10^{-7}) (REFS 3,6,7,16,17). Crucially, the transient overexpression of Sup35 or Ure2 increases the frequency of [*PSI*+] or [*URE3*] appearance by several orders of magnitude[3,123] and induces self-perpetuating, dysfunctional, protease-resistant, aggregated conformations of Sup35 (REFS 26,27) and Ure2 (REF 28), respectively. Furthermore, loss of [*PSI*+] or [*URE3*] is accompanied by a return of Sup35 or Ure2 to functional, protease-sensitive, soluble conformations[26–28]. These alternative conformational states can be tracked by tagging Sup35 or Ure2 with GFP. In [*psi*–] cells, Sup35-GFP fluorescence is diffusely distributed throughout the cell (see figure, part **a**). By contrast, in [*PSI*+] cells, Sup35-GFP fluorescence is concentrated at a set of distinct foci (see figure, part **a**). [*PSI*+] and [*URE3*] inheritance is governed by the protein-remodelling factor Hsp104 (BOX 6). Transient overexpression of Hsp104 eliminates [*PSI*+] (REFS 26,59) and deletion of Hsp104 eliminates [*PSI*+] and [*URE3*] (REFS 26,59,60). This enhanced sensitivity of [*PSI*+] to Hsp104 concentration might reflect the need to more tightly regulate the functional depletion of an essential protein, such as Sup35, by its prion conformation. By contrast, Ure2 (and Rnq1) are non-essential proteins and so their depletion by prion conformers might be more readily tolerated.

Sup35 and Ure2 possess unusual N-terminal domains that are enriched in uncharged polar residues, especially glutamine and/or asparagine (TABLE 1). These domains are not required for normal protein function. Instead, they confer prion behaviour and are referred to as prion domains. Deletion of this domain in Sup35 or Ure2 eliminates the corresponding prion[28,124], and the transient overexpression of this domain induces the corresponding prion[26,28,123]. Between the N-terminal prion domain (labelled N in the figure) and the C-terminal functional domain (labelled C in the figure) of Sup35, there is a conserved, highly charged middle domain (labelled M). The middle domain helps to keep Sup35 soluble, enabling it to switch between the prion and non-prion states[125]. Appending the N-terminal prion and middle domains (NM) of Sup35 to unrelated proteins is sufficient to confer all aspects of prion behaviour[109].

In vitro, the Gln/Asn-rich domains of Sup35 and Ure2 drive their assembly into amyloid fibres. Electron microscopy of amyloid fibres formed by NM and full-length Sup35 are shown (see figure, part **b**). Note the smooth appearance of NM fibres and the protrusions emanating from the surface of fibres formed by full-length Sup35 (see figure, part **b**), which correspond to the C-terminal domain. The EM images in part b of the figure are reproduced with permission from REF. 23 © (1997) Elsevier Science.

with organic solvents, denaturants or CHAOTROPES are often required to generate amyloid[35]. This generic amyloidogenicity probably derives from the 'cross-β' structure of amyloid fibres, in which the β-sheet strands run perpendicular to the fibre axis. This architecture is stabilized primarily by hydrogen bonds that emanate from the polypeptide backbone or main chain[35], although side-chain interactions can also contribute[45]. By contrast, natural selection has operated such that, for most proteins, the main chain is unavailable for amyloidogenesis and is inaccessibly contorted within a mass of tightly packed side-chain interactions. This globular architecture provides greater malleability and has facilitated the functional diversification of proteins. However, amyloidogenesis endures as an inherent attribute of polypeptide chemistry. Amyloid fibres might even represent a primordial polypeptide conformation that was enforced by harsh prebiotic conditions[35]. The self-perpetuating conformational states of modern prions might even be a vestige of a mechanism for preserving self-replicating functional states in early life forms.

Amyloid conformers have since been disfavoured by natural selection for most proteins. However, humans now live long past reproductive age; this is a time frame during which natural selection acts less powerfully, so 'late-expressing' harmful mutations can accumulate[46]. Therefore, some types of naturally occurring amyloid — that is, those associated with disease — are more likely to appear in post-reproductive age groups, as is seen for various late-onset amyloidoses, such as AD or PD.

Some proteins (for example, Sup35, Rnq1, Ure2) can switch between a soluble conformation and an amyloid conformation that is not disease-associated. These switchable states might have been selected because they were beneficial and in some cases have been conserved for hundreds of millions of years (REFS 47–50 and L. Li and S.L., unpublished observations). We strongly suspect

CHAOTROPE
Any substance (usually ions) that increases the transfer of apolar groups to water by decreasing the 'ordered' structure of water. Chaotropes alter secondary, tertiary and quaternary protein structure.

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Box 3 | Prion 'strain' or 'variant' phenomena

The prion 'strain' concept originates from the multifarious distinct transmissible prion diseases that can be passaged in the same inbred mouse lines, which, of course, have identical prion protein (PrP)-encoding genes[5]. These strains have distinct neuropathologies and rates of disease progression, a feature common to viruses, and have therefore persistently been cited as evidence for an exogenous nucleic-acid genome that drives prion propagation. However, no clear evidence of such an agent has ever emerged[5]. Alternatively, self-perpetuating conformational diversity of PrP^{Sc} (see TABLE 1 for prion nomenclature) might cause these phenotypically diverse transmissible states[5,15]. Different strains yield characteristically different patterns of proteolytic-cleavage products and equilibrium unfolding profiles, strongly indicating a difference in PrP folding[5,15]. Differences in PrP glycoform ratios and PrP^{Sc} accumulation are also observed[5]. However, the inability to induce disease in wild-type animals (see Note added in proof) with PrP^{Sc} generated *de novo* from recombinant PrP has fuelled lingering doubts concerning the cause and effect of PrP^{Sc} strain diversity.

Pioneering work by Susan Liebman and colleagues demonstrated that [*PSI*+] also exists in distinct strains[123], termed 'variants' to distinguish them from different yeast genetic backgrounds, also known as strains[68]. [*URE3*] and [*RNQ*+] variants have also been elucidated[86,126,127]. [*PSI*+] variants produce intermediate colours in cells that carry a premature stop codon in the *ADE1* gene and have different growth rates on adenine-deficient media (FIG. 1B). They arise spontaneously or after Sup35 overexpression, and are attributable to an epigenetic process[123]. Weak [*PSI*+] variants have greater mitotic instability and higher translation-termination fidelity than strong [*PSI*+] variants[68,123]. This difference reflects distinct prions with different efficiencies of Sup35 conversion, which leaves different quantities of soluble Sup35 available for translation termination[68,123]. Sup35 fibres purified from weak [*PSI*+] cells are up to 20-fold less effective at seeding recombinant NM (N-terminal prion and middle domains of Sup35) as are those from strong [*PSI*+] cells, indicating a conformational difference between Sup35 fibres of weak and strong [*PSI*+] variants[68].

NM fibres assembled at different temperatures have distinct conformations[36]. At 4°C, NM fibres assemble more rapidly[34], possess different protease sensitivities, and have a lower melting temperature in sodium dodecyl sulfate (SDS) than those generated at 37°C (REF. 36). Remarkably, the transformation of [*psi*−] cells with NM fibres that were assembled at 4°C induced predominantly strong [*PSI*+] variants, whereas NM fibres assembled at 37°C induced predominantly weak [*PSI*+] variants[36]. Therefore, Sup35 folds into several independent self-replicating conformations that induce distinct [*PSI*+] variants. The length of the N domain that is incorporated into the cooperatively folded amyloid core and the specific residues that form intermolecular contacts differentiates these prion variants[96]. Crosslinking the protein to favour distinct contacts is sufficient to nucleate fibre formation and to drive the production of distinct strains[96].

Box 4 | The [*PIN*+] prion

[*PIN*+] stands for [*PSI*+] inducibility. It was originally described as a non-Mendelian factor of unknown origin[84] but was later established to be the prion form, [*RNQ*+], of the Rnq1 protein in *Saccharomyces cerevisiae*[85]. Rnq1 was identified independently in a search for prion-like proteins[18]. [*PIN*+] is required for [*PSI*+] induction by the overexpression of Sup35 or NM (N-terminal prion and middle domains of Sup35)[84,85]. Other Gln/Asn-rich proteins, including New1 and Ure2 can function as [*PIN*+] when expressed at high levels[85,128]. Moreover, aggregation-prone polyglutamine tracts (such as Q72 or Q103) can also function as [*PIN*+], indicating that it is not necessary to be a prion to function as [*PIN*+] (REF. 129).

The most likely explanation for the influence of these proteins on [*PSI*+] induction is a low level of cross-seeding activity. Indeed, Rnq1 fibres can inefficiently seed the assembly of NM fibres *in vitro*[129]. However, once [*PSI*+] is formed the self-seeding activity of Sup35 is strong enough for [*PIN*+] to be dispensable for [*PSI*+] propagation[130]. Indeed, [*PSI*+] can actually impede the inheritance of some [*PIN*+] variants[131]. Furthermore, specific [*PIN*+] variants can disrupt the inheritance of weak [*PSI*+] variants, but not strong [*PSI*+] variants[131]. Delineating the underlying mechanisms for these complementary and antagonistic prion interactions will be fascinating.

BIOFILM
A proliferation on an inert surface of aggregated microbial colonies that have increased resistance to antimicrobial therapies. Biofilms contribute to many human infections.

that self-replicating prion conformations will emerge in many functional aspects of biology[51]. Amyloid fibres also have tightly regulated structural roles in melanosome biogenesis[52] and in the colonization of inert surfaces and BIOFILM formation by *Neurospora crassa*[53] and *Escherichia coli*[54]. Furthermore, amyloid conformers of fibrin-derived peptides activate tissue-type plasminogen activator, which cleaves the zymogen plasminogen to generate plasmin, a protease that degrades fibrin and dissolves blood clots[55]. Amyloid conformers of other proteolytic fragments, such as endostatin (a fragment of collagen, type XVIII), might exert antiangiogenic activity that is important in cancer therapeutics[56]. These broadly distributed functions of amyloid strengthen the possibility that prions might also function in specific settings. If so, a need would also have arisen to evolve systems to control switching to and from the prion conformation.

How are prions regulated?

Reduced toxicity of aggregation pathways. To be a functional prion (or amyloid), rather than a disease-inducing amyloid, several mechanisms must be established. One is specificity. Disease-related amyloids, such as those formed by polyglutamine proteins, often co-precipitate or selectively deplete essential proteins to the detriment of cell physiology[57]. By contrast, the Gln/Asn-rich yeast prion domains are highly selective in their interactions. Mechanisms would also be

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Box 5 | **When is a prion an amyloid and when is an amyloid a prion?**

Not all amyloids are prions, but what differentiates them? It is not an inability to self-propagate, as most amyloid fibres can seed their own assembly *in vitro*[35]. Furthermore, injecting animals with amyloid conformers of specific proteins induces large and disseminated amyloidoses[132–134].

Lack of transmissibility might occur for many reasons. Amyloid conformers might simply not encounter convertible substrates. Ingestion of PrP^Sc (see TABLE 1 for prion nomenclature) induces prion disease only because the prion form is so exceptionally protease-resistant that it survives the gut[5], and because dendritic cells bring it into contact with sympathetic nerves, eventually allowing access to the central nervous system[5,135]. Amino-acid-sequence differences can also preclude inter-species transmission, a phenomenon known as the 'species barrier'[5,8]. Species barriers might only be breached by specific amyloid structures that are compatible with different amino-acid sequences[8,136].

Transmissible amyloids must continuously replicate their structures and disseminate their self-propagating activity. Once the specific activity for replication falls below a certain threshold, transmissibility is lost. For the [*PSI*^+], [*URE3*], and [*RNQ*^+] prions, growth and division must keep pace with mitosis, such that progeny inherit sufficient prion seeds. Furthermore, if the self-propagation rate diminishes below the lifetime of the soluble protein then any prion-encoded phenotype will be eliminated. Because amyloid fibres polymerize and replicate their conformation at their ends, prions must be more readily fragmented to continuously liberate new self-replicating fibre ends. Fragmentation might be inherent to specific amyloid conformers, or might be catalyzed by other factors, such as Hsp104 (REFS 26,27,59,137). Alternatively, if nucleation predominates over polymerization, a population of short fibres would also ensue[138]. Mathematical simulations indicate that short, rapidly fragmenting fibres grow the fastest[139], and strong [*PSI*^+] variants consist of shorter polymers than weak variants[32]. Curing cells of [*PSI*^+] and [*RNQ*^+] with guanidium chloride (see also BOX 6) increases mean polymer length[32,126]. Therefore, conversion from prion to non-transmissible amyloid can involve increasing polymer lengths and consequent reductions in the specific activity of conformational replication.

Another parameter that might dictate prion status is the speed of conformational replication at fibre ends. Fibre ends with higher rates of conformational replication might allow longer fibres to retain transmissibility, whereas fibre ends with lower rates of conformational replication might allow only shorter fibres to retain transmissibility[126].

Non-amyloid prions might also exist. Other self-replicating protein conformations certainly exist, for example, certain coiled-coil peptides[140–142]. Similarly, amyloidogenic oligomers — intermediates that form before fibres[34,35,58] and that have a conformation that is distinct from amyloid fibres[34,35,58] — can initiate fibre assembly[137]. Evidence from PrP also indicates the existence of non-amyloid prions. First, PrP^Sc does not seem to form amyloid fibres unless treated with proteinase K[11,143]. Second, prion fibres derived from PrP^Sc by proteinase K digestion are disrupted by 1,1,1-trifluoro-2-propanol, but retain infectivity[144]. Third, preliminary data indicate that an octameric oligomer of PrP 89–230 (comprising amino acids 89–230), an off-pathway, non-amyloid β-sheet-rich conformer[22], might transmit prion disease[37].

needed to minimize the toxicity or half-life of toxic oligomeric intermediates that accumulate before fibre formation[35,58]. Conversely, toxicity might even have been selected for if a particular type of cell death was beneficial, as might be the case for the [Het-s] prion and heterokaryon incompatibility[7].

Protein-remodelling factors and molecular chaperones. Prion-folding transitions can also be tightly regulated by protein-remodelling factors and molecular chaperones. Hsp104, a protein-remodelling factor, governs the inheritance of [*PSI*^+], [*URE3*] and [*RNQ*^+] (REFS 18,59,60) (BOX 6). Other molecular chaperones modulate yeast prion inheritance, although none do so as stringently as Hsp104 (REFS 6,7). For example, deletion of the glycine (Gly)/phenylalanine (Phe)-rich domain of Sis1, a member of the Hsp40 family, eliminates [*RNQ*^+] (REF. 61). Moreover, Sis1 is incorporated into Rnq1 prion aggregates in equimolar quantities[62]. The precise molecular details and consequences of chaperone-prion interactions will prove to be extremely interesting.

Transcription and translation. Regulation might also occur at the levels of transcription or translation. In principle, transiently silencing the gene that encodes a prion protein might allow the clearance of prion conformers before the gene is reactivated. In mice, neuronal depletion of PrP after infection with PrP^Sc prevents disease and reverses SPONGIOSIS[63]. The advent of small interfering RNAs as therapeutics, and successes in their delivery to the CNS[64,65], raise the possibility of transiently depleting PrP as a realistic therapeutic possibility for prion diseases.

In yeast, [*URE3*] is regulated by directing ribosomes to an internal AUG codon downstream of both the conventional 5′ start codon and the prion domain[66]. The resulting Ure2 product, comprising Ure2 amino acids 94–354, lacks the prion domain and cannot be captured by prion conformers. Therefore, the balance between Ure2 and Ure2 94–354 determines the degree of the [*URE3*] phenotype. This system might have evolved to alleviate or reinforce the [*URE3*] phenotype in response to environmental cues[66]. For example, downregulation of the translation-initiation factor eIF4E during the stationary phase yields more Ure2 94–354 and might facilitate a nutrient-sensitive switch to invasive filamentous growth[66].

Given that at least some fungal prions seem to be both tightly regulated and highly conserved, we will now consider their biological significance.

SPONGIOSIS
A form of neurodegeneration involving the formation of large fluid-filled spaces (vacuoles) in the brain, which if widespread, induces a sponge-like appearance of the brain. This spongiform change is a general (but not universal) pathological hallmark of prion diseases.

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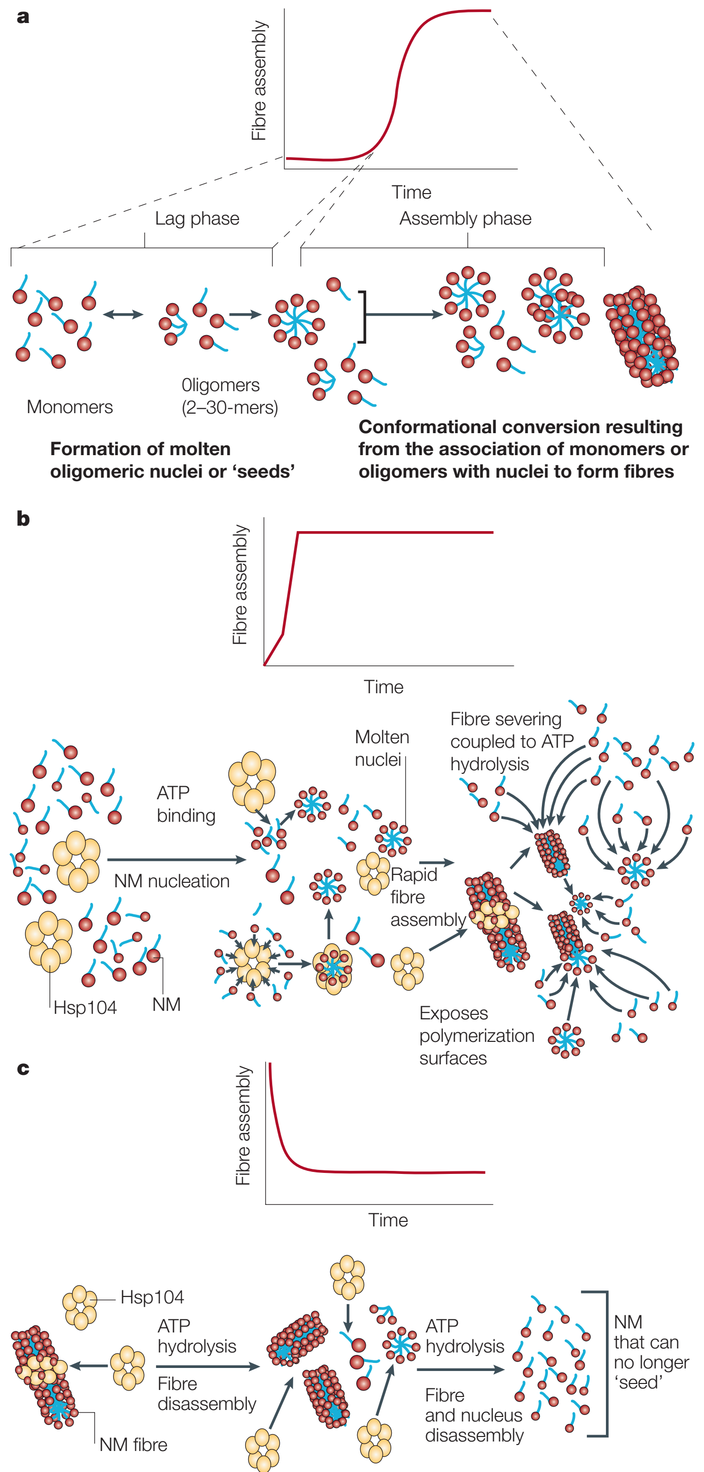
Box 6 | **Control of yeast prion conformers by Hsp104**

Hsp104 is a hexameric protein that has two AAA+ (ATPases associated with diverse activities) ATPase domains per monomer. Hsp104 promotes cell survival after exposure to environmental stress, such as heat shock or high ethanol concentrations, by dissolving denatured protein aggregates and, together with Hsp70 and Hsp40, reactivating them[145,146]. Deletion of Hsp104 cures cells of [*PSI*+], [*URE3*] and [*RNQ*+] (REFS 18,59,60). Strikingly, even transient overexpression of Hsp104 cures [*PSI*+], but not [*URE3*] and [*RNQ*+] (REFS 18,59,60). Inactivating point mutations in the AAA+ domains of Hsp104 eliminate [*PSI*+] (REFS 26,147–149), as does growth on guanidium chloride (GdmCl), an uncompetitive inhibitor of Hsp104 ATPase activity[146,147,150]. Growth on GdmCl also cures cells of [*RNQ*+] and [*URE3*], presumably also because of Hsp104 inactivation[84,151].

We recapitulated the direct effects of Hsp104 on Sup35 prion conformers[137]. In the absence of Hsp104, Sup35 assembles into prion fibres by nucleated conformational conversion[34]. During the lag phase (see figure, part a), the NM (N-terminal prion and middle domains of Sup35) is natively unstructured and assembles into molten oligomeric structures, a subset of which become obligate, on-pathway intermediates[34,137]. These oligomers allow segments of the N domain to sample intermolecular interactions. When specific juxtapositions occur, they nucleate conversion to an amyloidogenic state[96]. During the assembly phase (see figure, part a), natively unfolded NM conformers are recruited to the nuclei and templated to form amyloid fibres (NM fibrillization)[34,152].

At low concentrations, Hsp104 promotes NM fibre assembly in two ways. First, on binding ATP, Hsp104 catalyzes the formation of amyloidogenic oligomers that rapidly nucleate NM fibrillization, thereby eliminating the lag phase[137] (see figure, part **a**, **b**). Second, Hsp104 couples ATP hydrolysis to the severing of nascent NM fibres to generate extra surfaces for conformational replication, thereby accelerating assembly phase[137] (see figure, part **b**). GdmCl cures cells of [*PSI*+] and inhibits Hsp104-driven fibre severing[153,154]. At high concentrations, Hsp104 couples ATPase activity to the disassembly of amyloidogenic oligomers and the disassembly and severing of NM fibres[137] (see figure, part **c**). Therefore, Hsp104 might minimize the half-life of amyloidogenic oligomers that might be toxic intermediates in amyloidogenesis[58].

Together, these activities explain the otherwise baffling dosage relationship between Hsp104 and [*PSI*+] inheritance[59]. Similar activities might explain the essential role of Hsp104 in [*RNQ*+] and [*URE3*] inheritance, except that in these cases high levels of Hsp104 would not completely eliminate prion conformers, perhaps owing to different conformations of Rnq1 and Ure2 fibres. Notably, by contrast to chemically denatured aggregates[146], Hsp104 does not require Hsp70 or Hsp40 to make NM fibres soluble[137]. This might reflect the extremely different architecture of amyloid fibres, and the fact that soluble NM is intrinsically unstructured and so might not require Hsp70 and Hsp40 to refold[155].



Conservation of [*PSI*+] and its biological impact

Cells employ a myriad of mechanisms to ensure translational fidelity. Therefore, it seems implausible that [*PSI*+]-mediated reductions in translation-termination fidelity (~0.2–35% readthrough[67–69]) (FIG. 1b) could be beneficial. However, the Sup35 prion domain has been conserved in both size and in its unusual composition of uncharged polar amino acids for at least several hundred million years (REFS 47,48,50 and L. Li and S.L., unpublished observations). Moreover, the capacity of this domain to switch into the [*PSI*+] state has been conserved for a similar period[48]. By a common measure of selective pressure, Ka/Ks (the normalized ratio of amino-acid-altering substitutions to silent

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substitutions), the Sup35 prion domain scores close (REF. 50 and L. Li and S.L., unpublished observations) to the average for all functional genes in *S. cerevisiae*[70]. This implies that the Sup35 prion domain is subject to strong PURIFYING SELECTION (REF. 50 and L. Li and S.L., unpublished observations). Over large evolutionary distances, specific amino-acid biases have been maintained, rather than specific amino-acid sequences (L. Li and S.L., unpublished observations). This unusual mode of evolution is compatible with amyloidogenesis but not with the maintenance of specific globular structures. Because the only known function of prion domains is in prion induction and propagation, their maintenance by purifying selection implies that the ability to form prions might confer some selective advantage.

Selective advantage of [PSI⁺]. What could this beneficial function be? Subtle [*PSI*⁺]-mediated alterations in translation-termination fidelity can generate diverse, often beneficial, heritable phenotypes[67,71]. This phenotypic diversity stems from the exposure of CRYPTIC GENETIC VARIATION (CGV) induced by ribosomes reading into regions that have not recently been subject to selective pressures and that are divergent in different yeast genetic backgrounds[67,71,72].

The fitness of ISOGENIC [*PSI*⁺] cells and [*psi*⁻] cells from seven distinct genetic backgrounds was assessed in ~150 diverse growth conditions, including many that are likely to be encountered by yeast in their natural environments[67]. In ~25% of these conditions, [*PSI*⁺] increased the fitness of cells in at least one genetic background, and in another ~25%, [*PSI*⁺] decreased fitness[67]. Occasionally, [*PSI*⁺] induced profound alterations in colony morphology[67] or stress tolerance[72]. In competition experiments, fitness advantages conferred by [*PSI*⁺] or [*psi*⁻] led to a rapid change in population composition to the advantageous state[71] (FIG. 2a). Most [*PSI*⁺]-induced phenotypes tested were elicited solely by increased readthrough[71].

[*PSI*⁺] allows CGV to be sampled on a genome-wide scale, and all of the [*PSI*⁺]-induced phenotypes tested were multigenic in character[71]. Outcrossing experiments revealed that CGV accessed by [*PSI*⁺] was readily fixed and maintained in subsequent generations even after cells were purged of the prion[71]. This genetic assimilation was not due to the appearance of a new global nonsense suppressor[71]. Instead, it most probably entailed the reassortment of genetic polymorphisms and/or mutations that change stop codons to sense codons or modulate mRNA stability[71].

Because [*PSI*⁺] is epigenetic, it produces new phenotypes without any permanent commitment or immediate fixation (in contrast to phenotypic variants produced by mutator alleles). This logically provides a survival advantage in the fluctuating environments that yeast occupy. Moreover, should [*PSI*⁺]-induced phenotypes prove advantageous, the number of cells that harbour those phenotypes would increase, raising the probability of genetic assimilation through new mutations, until eventually the trait becomes [*PSI*⁺]-independent. Fixation allows yeast to maintain the trait and restore normal levels of translation-termination fidelity (which is surely the preferred state). The metastability of [*PSI*⁺] means that large populations will probably harbour both [*PSI*⁺] and [*psi*⁻] individuals, each of which is predisposed to thrive in specific settings, and so potential VALLEY-CROSSING ability is maintained (FIG. 2a). The low rate of switching to and from the [*PSI*⁺] and [*psi*⁻] states (10^{-5}–10^{-7}) ensures that the spontaneous appearance of variants that are not adaptive will have no significant effect on fitness.

[*PSI*⁺] can be conceptualized as an epigenetic switch that confers both phenotypic plasticity and evolvability by unleashing CGV. This allows individuals to access complex multigenic traits in a single step and consequently to rapidly traverse valleys to new ADAPTIVE PEAKS in response to altered selective pressures enforced by environmental fluctuations. Fixation of these traits is consistent with 'adaptive walks' that proceed by the stepwise fixation of new mutations, where the probability of fixation is proportional to the probability that the mutation is adaptive[73].

These proposals[67] were originally greeted with scepticism[74,75]. One argument is that causality appears to demand that evolvability *per se* cannot be an object of natural selection because of its apparently anticipatory nature[74]. However, evolvability patently exists[76]. Moreover, evolutionary simulations that have conservative assumptions indicate that [*PSI*⁺] is more likely to have been fixed due to its evolvability characteristics than due to chance alone, provided that environmental fluctuations make readthrough adaptive once every million years[77]. Other simulations indicate that rapid or drastic environmental alterations create selection pressures for increased evolvability[78]. Because yeast frequently occupy fluctuating environments, [*PSI*⁺] might well be maintained to ensure evolvability, as well as to provide a mechanism to survive these environmental fluctuations.

Another argument is that [*PSI*⁺] has not been observed in natural yeast isolates[7,47,79], some of which contain deletions in the Sup35 prion domain that preclude [*PSI*⁺] propagation[79]. However, given that high rates of translation-termination fidelity have obvious long-term selective value, we predict that [*PSI*⁺] might be a transient state that predominates only under conditions in which it confers a selective advantage[67,71]. In the long term, mutations that cause the trait to become [*PSI*⁺]-independent would be favoured and would allow cells to become [*psi*⁻] and restore normal translation-termination fidelity without incurring any selective disadvantage.

On another level, it does not matter whether [*PSI*⁺] has been maintained because of the phenotypic plasticity and evolvability it confers[80]. Even if it were conserved for some other, as yet completely unknown, reason, the fact that it affects the relationship between genotype and phenotype so profoundly, and often in beneficial ways, indicates that it must affect survival and adaptation in the wild.

PURIFYING SELECTION
A mode of natural selection that preserves the adapted condition, and is observed as a large excess of synonymous substitutions over non-synonymous substitutions in functionally important genes.

CRYPTIC GENETIC VARIATION
Existing genetic variation that makes no contribution to the normal range of phenotypes, but which can modify phenotypes in response to environmental change or the introduction of novel genetic elements.

ISOGENIC
Describing two or more individuals that possess exactly the same genotype.

VALLEY CROSSING
The process of moving from one adaptive peak to another on an adaptive landscape by crossing a valley. Valleys correspond to genotypic frequencies at which average fitness is low.

ADAPTIVE PEAK
A region in an adaptive landscape corresponding to genotypic frequencies at which the average fitness is high.

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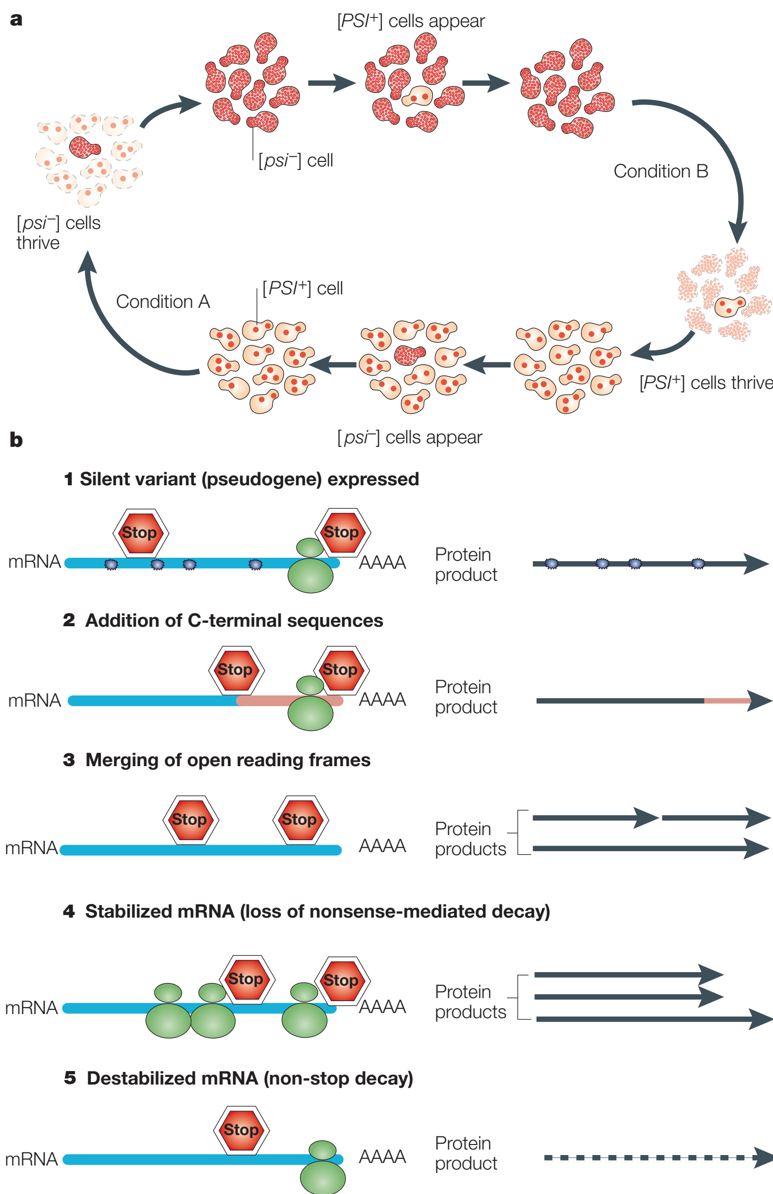

Figure 2 | **[*PSI*⁺] reveals hidden genetic variation and can confer selective advantages.** **a** | Starting from top, [*PSI*⁺] individuals (white cells with red dots) appear spontaneously in a population of [*psi*⁻] cells (red cells), as determined by the low intrinsic rate at which Sup35 acquires the prion conformation *de novo*. If the environment gives [*PSI*⁺] individuals a selective advantage (condition B), they will begin to dominate the population. [*psi*⁻] individuals will appear in this population of [*PSI*⁺] cells owing to the metastability of [*PSI*⁺] inheritance (10^{-5} to 10^{-7}). If the environment changes so that the prion state is not favoured, but [*psi*⁻] cells are (condition A), the prion-containing cells will die and [*psi*⁻] individuals will then begin to increase in frequency. However, if the trait conferred by [*PSI*⁺] has a long-term selective advantage, cells will accumulate mutations that fix the trait and allow cells to return to normal levels of translation-termination fidelity. Certain environmental conditions might specifically induce the [*psi*⁻] individuals to become [*PSI*⁺] or vice versa. **b** | There are several mechanisms by which readthrough enhances the expression of the usually silent genetic information that exists beyond stop codons and might create new traits: (1) Restoring the expression of ordinarily silent pseudogenes that contain premature stop codons and, in their silent state, have accumulated mutations that alter function. (2) Producing C-terminal extensions on polypeptides, perhaps altering protein function. (3) Merging two open reading frames to yield new hybrid proteins[81]. (4) Stabilize mRNAs by repressing nonsense-mediated decay pathways[157]. (5) Destabilize mRNAs by inducing non-stop decay, which occurs when the ribosome fails to recognize any stop codons[158]. Therefore, not only will a distinct set of polypeptides be expressed in the [*PSI*⁺] state, but the expression levels of proteins will also be altered owing to differential mRNA stability. Modified with permission from REF. 71 © (2004) Macmillan Magazines Ltd.

The specific details of the [*PSI*⁺]-induced phenotypes remain mysterious. How might [*PSI*⁺]-mediated readthrough generate phenotypic diversity (FIG. 2b)? There are some hints as to which genes might contribute to the various phenotypes in individual strains[81,82], but the combinatorial effects of [*PSI*⁺] are likely to be specific to individual genetic backgrounds.

Beneficial roles for [Het-s], [*RNQ*⁺] and [*URE3*]?

[Het-s] functions in heterokaryon incompatibility, and is often suggested to be a beneficial prion[7,25,29]. However, the biological relevance of heterokaryon incompatibility is unclear, but might reduce the transmission of viruses or other infectious agents[7]. Curiously, the *het-s* allele (which encodes the HET-s protein) is a MEIOTIC DRIVE element[83]. Therefore, *het-s* might be maintained even if [Het-s] is detrimental[7].

The function of the [*RNQ*⁺] prion and Rnq1 protein are unknown. Deletion produces no obvious phenotype except for a reduction in the *de novo* appearance of [*PSI*⁺] and other prions[84–86]. However, the Ka/Ks value of the prion-determining region indicates that it is subject to strong purifying selection (L. Li, S.L., unpublished observations). Moreover, in contrast to [*PSI*⁺], [*RNQ*⁺] is commonly found in natural yeast isolates and various laboratory strains[79]. One possibility is that [*RNQ*⁺] exists to facilitate protein-folding transitions that include, but are not limited to, those that regulate [*PSI*⁺] induction (BOX 4).

The potential benefits of [*URE3*] are less obvious, but, similar to [*RNQ*⁺], [*URE3*] can function as [*PIN*⁺] (REF. 85) (BOX 4). Unlike [*PSI*⁺] and [*psi*⁻] cells, which grow equally well under standard growth conditions[67,71,72], [*URE3*] cells have a slight disadvantage compared with [*ure-o*] cells[17]. [*URE3*] uncouples nitrogen-supply signals from downstream transcription. This can be cost ineffective if poor nitrogen sources are used despite the availability of good nitrogen sources. However, some *ure2*, and perhaps [*URE3*] strains, proliferate more rapidly than wild-type strains in grape juices[87] or during salt stress[88]. Therefore, the use of poor and good nitrogen sources simultaneously might confer fitness advantages in specific settings[87,88].

Prions as evolutionary capacitors

Most, if not all, genes expose some phenotypic variation when they are functionally compromised, and loss-of-function mutations might accelerate evolution to new optimum phenotypes[89]. Therefore, any prion that mimics a loss-of-function mutation in its protein determinant might function in the same way. Documentation of the effects of deleting each yeast gene on growth rates and the expression of all other yeast genes[90], indicates that deletions tend to increase variation in the expression of other genes, even when those genes are not regulatory targets of the deleted gene[90]. Furthermore, this variation in gene expression is linked to phenotypic variation, as deletions with lower fitness tend to be those with the greatest variation in gene expression[89]. In evolutionary simulations, loss-of-function mutations that reveal phenotypic

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variation facilitate more rapid evolution to new adaptive peaks[89]. Therefore, EVOLUTIONARY CAPACITORS might accelerate adaptation to new phenotypic optima. A key tenet of these simulations is that a gene that is inactivated would, at a low rate, be restored to function[89]. This feature echoes the characteristic metastability of prion inheritance. Therefore, prions that mimic loss-of-function mutants can potentially function as evolutionary capacitors similar to Hsp90 (REF. 91), although they might or might not depend on the environment[89].

Characteristics of prion domains

Gln/Asn-rich domains. Prokaryotic and eukaryotic proteomes have been scoured for prion domains using search algorithms (REFS 18,19,92,93 and L. Li and S.L., unpublished observations). An initial scan for 80-amino-acid stretches that contain 30 or more glutamines and asparagines revealed a paucity of this type of domain in prokaryotes[92]. However, 1–3.5% of proteins that have diverse functions have this type of domain in *S. cerevisiae, Caenorhabditis elegans,* and *Drosophila melanogaster*, indicating that prion-type switches might be more widespread[92]. Similar domains have been found in ~200 proteins in *Arabidopsis thaliana* and humans[93]. Candidate yeast prion domains have been appended to MC (the middle and C-terminal domains of Sup35) in place of the N (the N-terminal prion domain of Sup35). The Rnq1 and New1 Gln/Asn-rich domains could replace N and support a [*PSI*+]-like prion, whereas the Gln/Asn-rich domain of Pan1 could not[18,19,92]. Therefore, Gln/Asn-richness is insufficient to confer prion activity. The Sup35, Rnq1, and New1 prion domains also have a negative bias for glutamate, aspartate, arginine, and lysine and a positive bias for glycine, tyrosine and serine[93].

The Sup35, Rnq1, and New1 prion domains also contain oligopeptide repeat sequences (TABLE 1). Residues 41–97 of Sup35 comprise five imperfect repeats (R1–R5) and one partial repeat (R6) of the nonapeptide PQGGYQQYN. This is the only sequence similarity between Sup35 and PrP. PrP has five imperfect copies of an octapeptide PHGGGWGQ repeat in its N-terminal domain. Increasing the number of PrP repeats induces spontaneous prion disease[12], whereas repeat deletion retards disease and diminishes PrP^{Sc} formation[94], although some inherited prion diseases are associated with PrP-encoding alleles with repeat deletions[12]. Deletion of two or more Sup35 repeats eliminates [*PSI*+] (REFS 20,95), whereas two extra copies of R2 increase the spontaneous appearance of [*PSI*+] 5,000-fold (REF. 95). *In vitro*, two extra copies of R2 cause NM to fibrillize with an abbreviated lag phase, whereas deletion of R2–R5 extends the lag phase[95,96]. Therefore, the repeats might facilitate the correct alignment of intermolecular contacts between NM molecules that drive conformational replication[96]. Although many repeat deletion mutants cannot maintain [*PSI*+], they can form amyloid fibres *in vitro*, aggregate *in vivo*, induce [*PSI*+], and join pre-existing Sup35 fibres[20,95]. Repeat deletion mutants might therefore be defective in their dissemination to progeny, possibly owing to altered interactions with Hsp104 (REF. 20). Corroboratively, appending a polyglutamine tract (Q62) to MC does not support [*PSI*+], but appending Q62 plus the Sup35 repeats does support [*PSI*+], although extensive aggregates form in both cases[20]. This reinforces that amyloid fibres are not necessarily prions (BOX 5). Therefore, an important challenge is to distinguish putative prion domains that encode self-replicating conformations from those that represent simple aggregation modules.

Curiously, the Ure2 prion domain lacks oligopeptide repeats, but does contain two poly-Asn tracts[6,7]. The Ure2 prion domain can be scrambled and still form amyloid fibres *in vitro* and support [*URE3*] induction and propagation, albeit with varying efficiency and stability[97]. Therefore, the specific amino acid content and biases of the Ure2 prion domain are essential for prion inheritance, rather than the precise amino-acid sequence, as indicated by the purifying selection acting on this domain (L. Li, S.L., unpublished observations).

Other prion domains. Notably, the PrP and [Het-s] prions lack a Gln/Asn rich domain, indicating that other prion domains might exist and that prion phenomena might be even more widespread. The prion domain of HET-s comprises its C-terminal 72 amino acids, a region that forms amyloid fibres *in vitro*, and which, similar to the prion domains of Sup35 and Ure2 (REFS 23,39), is unstructured before fibre assembly[29]. Similarly, the N-terminal domain of PrP is also unstructured, but does not comprise part of the proteinase-K-resistant amyloid core of PrP^{Sc} (REF. 5). A census of particularly amyloidogenic proteins revealed that many contain intrinsically unfolded portions, and this flexibility — that is, the absence of side-chain interactions — might facilitate the key backbone interactions that drive fibrillization[98].

It is obvious that we are only beginning to understand and recognize prion domains. Applying these often highly conserved biases to refine algorithms might help pinpoint new prions[93]. However, the knowledge accrued has allowed the identification of a novel prion domain that might function in long-term memory formation[4,99].

Prions in long-term memory formation

Any molecular basis for long-term memory formation must explain its endurance (for many years) despite the continuous turnover (every few hours) of the proteins that might encode them[100]. Information storage despite molecular turnover is traditionally explained by autophosphorylation loops[101], self-sustaining feedback loops in complex signalling networks[102], or transcription factors that stimulate their own synthesis once they cross a threshold concentration[103,104] (BOX 1). The conformational replication of prions provides another durable form of molecular memory[51]. Moreover, as is clear from studies in yeast, prion conformations are not usually toxic[6]. Incredibly, prion-based mechanisms might operate in individual neuronal synapses to maintain their growth and contingent LONG-TERM FACILITATION[4,99].

MEIOTIC DRIVE
Any process that causes some alleles to be over-represented in gametes formed during meiosis.

EVOLUTIONARY CAPACITOR
An entity (for example, Hsp90) that buffers genotypic variation under neutral conditions, thereby allowing the accumulation of hidden polymorphisms.

LONG-TERM FACILITATION
The long-lasting increase in synaptic activity that contributes to long-term memory and results from prolonged or iterated exposure of synapses to neurotransmitters, which induce the synthesis of new proteins leading to the stabilization of new synaptic connections.

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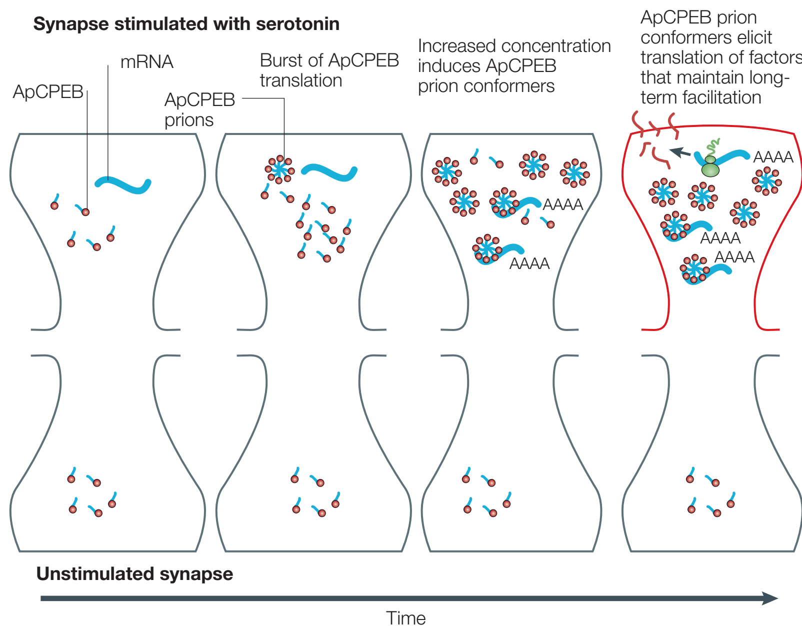

Figure 3 | **Maintenance of long-term facilitation by the ApCPEB prion.** CPEBs are RNA-binding proteins that bind short 3′ RNA motifs termed cytoplasmic polyadenylation elements (CPEs). Just as yeast prions are induced by the overexpression of their protein determinants[3,26,28,123], localized bursts of translation of a neuronal CPEB in *Aplysia californica* at specific synapses in response to neurotransmitter cues induces ApCPEB prion conformers. Therefore, ApCPEB prions might partially constitute the engram (or memory trace) of neuronal tissues. In serotonin-stimulated neurites, ApCPEB adopts a punctate distribution, perhaps representing aggregates[99]. Once formed, ApCPEB prions trigger the translation of otherwise dormant CPE-containing mRNAs that are essential for long-term facilitation exclusively at specific stimulated synapses. This active ApCPEB prion state is self-perpetuating, transcends many generations of molecules, and persists without any further extraneous signals. The stability and Gln-rich character of prion fibres means that they are less likely to be turned-over than soluble proteins[159,160]. Therefore, the prion switch is particularly perdurant and less easily reversed than more traditional molecular memories that require continuous kinase and/or phosphatase activity. The aggregated nature of ApCPEB prion conformers restricts them to activated synapses, such that unstimulated synapses of the same neuron would not become activated. Furthermore, ApCPEB prion strains might contribute to the strength and/or durability of long-term facilitation at different synapses, with weak strains perhaps being reversed more readily than strong strains. The huge conformational diversity characteristic of prion conformers (‘strains’ or ‘variants’) might permit a corresponding diversity in the strength of long-term facilitation events at different synapses.

A neuronal isoform of* Aplysia californica *CPEB behaves like a prion. CPEBs are highly conserved, sequence-specific RNA-binding proteins that bind short 3’ RNA motifs termed cytoplasmic polyadenylation elements (CPEs) and that regulate the activation of translationally dormant mRNAs by modulating polyadenylation as well as affecting mRNA localization[105]. In the California sea hare, *A. californica*, a neuronal form of CPEB, ApCPEB, elicits the localized translation of otherwise dormant CPE-containing mRNAs that encode structural proteins (for example, N-actin) and regulatory proteins (for example, ephrins) that maintain long-term synaptic growth and plasticity[99,100]. Crucially, ApCPEB translation is induced by the neurotransmitter serotonin, and selectively inhibiting this burst of ApCPEB translation abolishes long-term facilitation[99].

Strikingly, ApCPEB possesses an N-terminal domain (residues 1–150) that is rich in Gln (~48%) and uncharged polar residues (~63%), and that contains six imperfect Gln-Gln-Gln-Leu (leucine) repeats[4]. Many characteristics of the ApCPEB prion-like domain are conserved in neuronal CPEBs of other metazoa. Some mammalian (for example, CPEB3) and *D. melanogaster* (for example, CG5735-PA) homologues of neuronal ApCPEB also contain a Gln-rich N-terminal domain and are also enriched in serine, another uncharged polar amino acid[4]. Furthermore, these domains are predicted to be natively unstructured, a key trait of prion domains and one which does not necessitate being Gln-rich[106]. Intriguingly, long-term potentiation of mouse hippocampal neurons is induced by dopamine[107], which also induces CPEB3 expression[108].

ApCPEB behaves as a prion in yeast. Appending the ApCPEB prion domain to a constitutively active glucocorticoid receptor (GR) allows GR to behave as a yeast prion[4,109]. Full-length ApCPEB also adopts several heritable, functional states in yeast associated with distinct conformational states of ApCPEB[4]. Intriguingly, ApCPEB that lacks its prion domain can also exist in alternative active states, although these are much less stable[4]. This indicates that the CPEB domain has an intrinsic propensity to exist in alternative states, which might be stabilized by the prion domain[4].

Although ApCPEB has not yet been shown to form a prion in neurons, the fact that it does so in yeast leaves little doubt that it could also do so in specific synapses of *A. californica*[4] (FIG. 3). Remarkably, ApCPEB prion conformers bind and activate otherwise dormant CPE-containing mRNAs (for example, N-actin and ephrins), whereas unpolymerized ApCPEB does not[4]. Therefore, in contrast to [*PSI*$^+$] and [*URE3*], ApCPEB prion conformers are the biochemically active species. This is not unprecedented because other proteins appended to a prion domain, such as GFP or glutathione S-transferase, retain activity in assembled fibres[26,39]. This is because only the prion domain is sequestered in the amyloid core and the appended domains project outwards on the fibre surface[23,39] (see figure in BOX 2). Therefore, steric effects imposed by their fibrous states might compromise diffusion-limited Sup35 and Ure2 functions[39]. In ApCPEB, the CPE-binding site might be orientated optimally for RNA binding only on the exterior of prion fibres. Additionally, soluble ApCPEB might only weakly bind CPEs, and the localized concentration of several ApCPEBs in the context of a fibre might ensure RNA binding and/or assembly with cofactors into a larger cooperatively functioning complex (FIG. 3). However, how would prion formation be initiated (FIG. 3)?

Long-term memories can be forgotten, but how could this apply to prion-based memories? Yeast prions provide several clues. For example, *A. californica* neurons might express a second CPEB isoform that lacks the prion domain and antagonizes the prion phenotype, as in the case of yeast prions[28,66,110]. ApCPEB fibre ends might become inactive for conformational replication or the number of actively replicating fibre ends might become too low to sustain the prion. Unlike [*PSI*$^+$], ApCPEB prion propagation in yeast does not require Hsp104 (REF. 4), indicating that ApCPEB fibres might readily fragment and disseminate to progeny

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or that ApCPEB has a high frequency of spontaneous nucleation. However, Hsp104 overexpression eliminates the ApCPEB prion, perhaps by disassembling it[4]. Similarly, disassembly of ApCPEB fibres at synapses might reverse long-term facilitation. Hsp104 has no obvious metazoan homologues, but other AAA+ (ATPases associated with diverse activities) proteins or chaperones could perform an analogous function, and might even modulate ApCPEB prion conformers in response to stress.

Widespread prion-based molecular memories?

The widespread occurrence of Gln/Asn-rich domains in eukaryotes[92,93] raises the possibility that similar mechanisms to those outlined for ApCPEB could operate in settings as diverse as transcription or development in which physiological or environmental cues might trigger self-perpetuating prion states[51]. Cell-fate decisions are frequently made at the transcriptional level in response to developmental stimuli. Furthermore, several key regulatory proteins are expressed only transiently, but the gene expression patterns that they establish persist. Such transient bursts of expression could allow the nucleation of prion conformers, which would then be stably inherited through developmental lineages.

Polycomb- and trithorax-group proteins. For example, the expression patterns of homeotic genes in *D. melanogaster* must be precisely maintained throughout development, but the gap, pair-rule and segment-polarity transcriptional modulators that establish these patterns disappear after a few hours of embryogenesis[111]. The Polycomb and trithorax groups of proteins subsequently maintain these established states[111]. Once Polycomb- or trithorax-induced patterns of repressed or activated transcription are established, they frequently spread over large areas of the genome and persist through many rounds of cell division, a phenomenon termed 'transcriptional memory'[111]. This is achieved through complex multistep mechanisms that involve several dynamic multiprotein complexes that assemble at specific DNA sequences and coordinate nucleosome modification, chromatin remodelling and interactions with transcription factors[111]. Several Polycomb- and trithorax-group proteins contain Gln/Asn-rich domains and/or unstructured regions (for example, the proteins brahma, trithorax-like, zeste, polyhomeotic, Polycomb and trithorax) and we wonder whether self-replicating prion conformations that can be dynamically regulated might also participate in the construction of transcriptional memory. The gap, pair-rule and segment-polarity gene products recruit Polycomb- and trithorax-group proteins, and, by concentrating them locally, might drive a conformational change to a self-perpetuating form. Intriguingly, transcriptional repressors such as anterior open (also known as yan), an ETS family member, and polyhomeotic (a Polycomb-group protein) contain a sterile α-motif (SAM) domain that folds into a small five-helix bundle and assembles these proteins into linear helical polymers, which might facilitate the spreading of repressional complexes to both local and distant sites along the chromosome[112,113]. Oligomerization is often crucial for the function of Polycomb-group proteins and is not always mediated by SAM domains[112,113]. Perhaps analogous mechanisms exist, driven not only by SAM domains but also by prion-like domains.

Snf5 and Swi1. Two very interesting yeast proteins with putative Gln/Asn-rich prion domains are Snf5 and Swi1 (REF. 92), members of the evolutionarily conserved, multi-subunit SWI/SNF complex that mobilizes nucleosomes and remodels chromatin[114]. Approximately 6% of all yeast genes are transcriptionally regulated by SWI/SNF (REF. 114), and SWI/SNF can repress or activate gene expression[115]. The inactivation of Snf5 or Swi1 through a self-replicating prion conformation would have extensive repercussions for transcription on a genome-wide scale. Alternatively, the assembly of Swi1 or Snf5 prion conformers on chromatin might potentiate SWI/SNF remodelling.

TIA1. TIA1 is a metazoan RNA binding protein that contains a Gln/Asn-rich, putative prion domain[116]. This domain mediates its appearance in discrete cytoplasmic and nuclear aggregates, termed stress granules, which sequester stalled translation-initiation complexes in the cytoplasm following environmental stress[116]. It is unclear whether this Gln/Asn-rich domain confers a self-replicating conformation or represents a simple aggregation module[20]. However, the prion domain of Sup35 can replace the Gln/Asn-rich domain of TIA1 to promote stress granule formation[116]. Stress granules might protect mRNAs during cell stress by preventing their unproductive translation and/or splicing[116]. Here it might seem that ordered aggregation and general stability are the key functional features of the prion-like domain, rather than any need for a long-term self-perpetuating structure. The fact that the functional alterations of TIA1 are governed by environmental conditions is just one case of what might prove to be many, in which prion-like states are dynamically regulated.

Conclusions

A multitude of proteins that harbour putative prion domains are positioned at crucial regulatory nodes and could exert important effects on the connection between genotype and phenotype. We propose that prions and the molecular memories that they convey will continue to be revealed in more and more beneficial and crucial junctures of information storage, in cell and developmental biology and evolution[51]. The challenge is to recognize, decode and comprehend this information.

Note added in proof

Castilla *et al.*[161] recently succeeded in inducing prion disease in wild-type animals using a prion generated *in vitro* using a PCR-like amplification system. These findings bring us ever closer to a definitive proof for the mammalian prion hypothesis[162].

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1. James, L. C. & Tawfik, D. S. Conformational diversity and protein evolution — a 60-year-old hypothesis revisited. *Trends Biochem. Sci.* **28**, 361–368 (2003).
2. Prusiner, S. B. Novel proteinaceous infectious particles cause scrapie. *Science* **216**, 136–144 (1982).
3. Wickner, R. B. [*URE3*] as an altered URE2 protein: evidence for a prion analog in *Saccharomyces cerevisiae*. *Science* **264**, 566–569 (1994).
4. Si, K., Lindquist, S. & Kandel, E. R. A neuronal isoform of the *Aplysia* CPEB has prion-like properties. *Cell* **115**, 879–891 (2003).
This paper shows that ApCPEB can function as a prion in yeast, and that the prion conformation is the most active in stimulating translation of CPEB-regulated mRNA. Together with data from reference 99, the authors propose that the formation of ApCPEB prions in specifically stimulated synapses helps to maintain long-term synaptic changes that are associated with memory storage.
5. Prusiner, S. B. *Prion Biology and Diseases* (Cold Spring Harbor Laboratory Press, New York, 2004).
6. Uptain, S. M. & Lindquist, S. Prions as protein-based genetic elements. *Annu. Rev. Microbiol.* **56**, 703–741 (2002).
7. Wickner, R. B., Liebman, S. W. & Saupe, S. J. in *Prion Biology and Diseases* (ed. Prusiner, S. B.) 305–372 (Cold Spring Harbor Laboratory Press New York, 2004).
8. Chien, P., Weissman, J. S. & DePace, A. H. Emerging principles of conformation-based prion inheritance. *Annu. Rev. Biochem.* **73**, 617–656 (2004).
9. Alper, T., Cramp, W. A., Haig, D. A. & Clarke, M. C. Does the agent of scrapie replicate without nucleic acid? *Nature* **214**, 764–766 (1967).
10. Griffith, J. S. Self-replication and scrapie. *Nature* **215**, 1043–1044 (1967).
11. Prusiner, S. B. *et al.* Scrapie prions aggregate to form amyloid-like birefringent rods. *Cell* **35**, 349–358 (1983).
12. Kong, Q. *et al.* in *Prion Biology and Diseases* (ed. Prusiner, S. B.) 673–775 (Cold Spring Laboratory Press New York, 2004).
13. Bueler, H. *et al.* Mice devoid of PrP are resistant to scrapie. *Cell* **73**, 1339–1347 (1993).
14. Brandner, S. *et al.* Normal host prion protein necessary for scrapie-induced neurotoxicity. *Nature* **379**, 339–343 (1996).
15. Safar, J. *et al.* Eight prion strains have PrP^{Sc} molecules with different conformations. *Nature Med.* **4**, 1157–1165 (1998).
16. Cox, B. S. [PSI], a cytoplasmic suppressor of super-suppression in yeast. *Heredity* **20**, 505–521 (1965).
17. Lacroute, F. Non-Mendelian mutation allowing ureidosuccinic acid uptake in yeast. *J. Bacteriol.* **106**, 519–522 (1971).
18. Sondheimer, N. & Lindquist, S. Rnq1: an epigenetic modifier of protein function in yeast. *Mol. Cell* **5**, 163–172 (2000).
19. Santoso, A., Chien, P., Osherovich, L. Z. & Weissman, J. S. Molecular basis of a yeast prion species barrier. *Cell* **100**, 277–288 (2000).
20. Osherovich, L. Z., Cox, B. S., Tuite, M. F. & Weissman, J. S. Dissection and design of yeast prions. *PLoS Biol.* **2**, E86 (2004).
21. Coustou, V., Deleu, C., Saupe, S. & Begueret, J. The protein product of the *het-s* heterokaryon incompatibility gene of the fungus *Podospora anserina* behaves as a prion analog. *Proc. Natl Acad. Sci. USA* **94**, 9773–9778 (1997).
22. Baskakov, I. V., Legname, G., Baldwin, M. A., Prusiner, S. B. & Cohen, F. E. Pathway complexity of prion protein assembly into amyloid. *J. Biol. Chem.* **277**, 21140–21148 (2002).
23. Glover, J. R. *et al.* Self-seeded fibres formed by Sup35, the protein determinant of [*PSI*$^+$], a heritable prion-like factor of *S. cerevisiae*. *Cell* **89**, 811–819 (1997).
24. Taylor, K. L., Cheng, N., Williams, R. W., Steven, A. C. & Wickner, R. B. Prion domain initiation of amyloid formation *in vitro* from native Ure2p. *Science* **283**, 1339–1343 (1999).
25. Maddelein, M. L., Dos Reis, S., Duvezin-Caubet, S., Coulary-Salin, B. & Saupe, S. J. Amyloid aggregates of the HET-s prion protein are infectious. *Proc. Natl Acad. Sci. USA* **99**, 7402–7407 (2002).
26. Patino, M. M., Liu, J. J., Glover, J. R. & Lindquist, S. Support for the prion hypothesis for inheritance of a phenotypic trait in yeast. *Science* **273**, 622–626 (1996).
27. Paushkin, S. V., Kushnirov, V. V., Smirnov, V. N. & Ter-Avanesyan, M. D. Propagation of the yeast prion-like [*psi*$^+$] determinant is mediated by oligomerization of the SUP35-encoded polypeptide chain release factor. *EMBO J.* **15**, 3127–3134 (1996).
28. Masison, D. C. & Wickner, R. B. Prion-inducing domain of yeast Ure2p and protease resistance of Ure2p in prion-containing cells. *Science* **270**, 93–95 (1995).
29. Balguerie, A. *et al.* Domain organization and structure-function relationship of the HET-s prion protein of *Podospora anserina*. *EMBO J.* **22**, 2071–2081 (2003).
30. Speransky, V. V., Taylor, K. L., Edskes, H. K., Wickner, R. B. & Steven, A. C. Prion filament networks in [*URE3*] cells of *Saccharomyces cerevisiae*. *J. Cell Biol.* **153**, 1327–1336 (2001).
31. Kimura, Y., Koitabashi, S. & Fujita, T. Analysis of yeast prion aggregates with amyloid-staining compound *in vivo*. *Cell Struct. Funct.* **28**, 187–193 (2003).
32. Kryndushkin, D. S., Alexandrov, I. M., Ter-Avanesyan, M. D. & Kushnirov, V. V. Yeast [*PSI*$^+$] prion aggregates are formed by small Sup35 polymers fragmented by Hsp104. *J. Biol. Chem.* **278**, 49636–49643 (2003).
33. King, C. Y. & Diaz-Avalos, R. Protein-only transmission of three yeast prion strains. *Nature* **428**, 319–323 (2004).
34. Serio, T. R. *et al.* Nucleated conformational conversion and the replication of conformational information by a prion determinant. *Science* **289**, 1317–1321 (2000).
35. Dobson, C. M. Protein folding and misfolding. *Nature* **426**, 884–890 (2003).
36. Tanaka, M., Chien, P., Naber, N., Cooke, R. & Weissman, J. S. Conformational variations in an infectious protein determine prion strain differences. *Nature* **428**, 323–328 (2004).
References 33 and 36 provide definitive evidence for the yeast prion hypothesis. They establish beyond doubt that [*PSI*$^+$] is caused by self-replicating conformers of Sup35, and that distinct, stably propagating Sup35 conformations underpin different [*PSI*$^+$] variants.
37. Legname, G. *et al.* Synthetic mammalian prions. *Science* **305**, 673–676 (2004).
38. DePace, A. H. & Weissman, J. S. Origins and kinetic consequences of diversity in Sup35 yeast prion fibres. *Nature Struct. Biol.* **9**, 389–396 (2002).
39. Baxa, U., Speransky, V., Steven, A. C. & Wickner, R. B. Mechanism of inactivation on prion conversion of the *Saccharomyces cerevisiae* Ure2 protein. *Proc. Natl Acad. Sci. USA* **99**, 5253–5260 (2002).
40. Fink, G. R. A transforming principle. *Cell* **120**, 153–154 (2005).
41. Kaneko, K. *et al.* A synthetic peptide initiates Gerstmann-Straussler-Scheinker (GSS) disease in transgenic mice. *J. Mol. Biol.* **295**, 997–1007 (2000).
42. Legname, G. *et al.* Strain-specified characteristics of mouse synthetic prions. *Proc. Natl Acad. Sci. USA* **102**, 2168–2173 (2005).
References 37, 41 and 42 provide the initial foundations of definitive evidence for the mammalian prion hypothesis.
43. Edskes, H. K. & Wickner, R. B. Transmissible spongiform encephalopathies: prion proof in progress. *Nature* **430**, 977–979 (2004).
44. Goudsmit, J. *et al.* Evidence for and against the transmissibility of Alzheimer disease. *Neurology* **30,** 945–950 (1980).
45. West, M. W. *et al. De novo* amyloid proteins from designed combinatorial libraries. *Proc. Natl Acad. Sci. USA* **96**, 11211–11216 (1999).
46. Medawar, P. B. *An Unsolved Problem of Biology* (H. K. Lewis, London, 1952).
47. Chernoff, Y. O. *et al.* Evolutionary conservation of prion-forming abilities of the yeast Sup35 protein. *Mol. Microbiol.* **35**, 865–876 (2000).
48. Nakayashiki, T., Ebihara, K., Bannai, H. & Nakamura, Y. Yeast [*PSI*$^+$] 'prions' that are crosstransmissible and susceptible beyond a species barrier through a quasi-prion state. *Mol. Cell* **7**, 1121–1130 (2001).
49. Baudin-Baillieu, A., Fernandez-Bellot, E., Reine, F., Coissac, E. & Cullin, C. Conservation of the prion properties of Ure2p through evolution. *Mol. Biol. Cell* **14**, 3449–3458 (2003).
50. Jensen, M. A., True, H. L., Chernoff, Y. O. & Lindquist, S. Molecular population genetics and evolution of a prion-like protein in *Saccharomyces cerevisiae*. *Genetics* **159**, 527–535 (2001).
51. Lindquist, S. Mad cows meet psi-chotic yeast: the expansion of the prion hypothesis. *Cell* **89**, 495–498 (1997).
52. Berson, J. F. *et al.* Proprotein convertase cleavage liberates a fibrillogenic fragment of a resident glycoprotein to initiate melanosome biogenesis. *J. Cell Biol.* **161**, 521–533 (2003).
53. Mackay, J. P. *et al.* The hydrophobin EAS is largely unstructured in solution and functions by forming amyloid-like structures. *Structure* **9**, 83–91 (2001).
54. Chapman, M. R. *et al.* Role of *Escherichia coli* curli operons in directing amyloid fiber formation. *Science* **295**, 851–855 (2002).
55. Kranenburg, O. *et al.* Tissue-type plasminogen activator is a multiligand cross-β structure receptor. *Curr. Biol.* **12**, 1833–1839 (2002).
References 52–55 provide fascinating examples of beneficial amyloid conformers.
56. Gebbink, M. F., Voest, E. E. & Reijerkerk, A. Do antiangiogenic protein fragments have amyloid properties? *Blood* **104**, 1601–1605 (2004).
57. Nucifora, F. C. Jr. *et al.* Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity. *Science* **291**, 2423–2428 (2001).
58. Kayed, R. *et al.* Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science* **300**, 486–489 (2003).
59. Chernoff, Y. O., Lindquist, S. L., Ono, B., Inge-Vechtomov, S. G. & Liebman, S. W. Role of the chaperone protein Hsp104 in propagation of the yeast prion-like factor [*psi*$^+$]. *Science* **268**, 880–884 (1995).
60. Moriyama, H., Edskes, H. K. & Wickner, R. B. [URE3] prion propagation in *Saccharomyces cerevisiae*: requirement for chaperone Hsp104 and curing by overexpressed chaperone *Ydj1p*. *Mol. Cell Biol.* **20**, 8916–8922 (2000).
61. Sondheimer, N., Lopez, N., Craig, E. A. & Lindquist, S. The role of Sis1 in the maintenance of the [*RNQ*$^+$] prion. *EMBO J.* **20**, 2435–2442 (2001).
62. Lopez, N., Aron, R. & Craig, E. A. Specificity of class II Hsp40 Sis1 in maintenance of yeast prion [*RNQ*$^+$]. *Mol. Biol. Cell.* **14**, 1172–1181 (2003).
63. Mallucci, G. *et al.* Depleting neuronal PrP in prion infection prevents disease and reverses spongiosis. *Science* **302**, 871–874 (2003).
This remarkable paper shows that prion diseases might be treated post infection by downregulating endogenous PrP.
64. Dorn, G. *et al.* siRNA relieves chronic neuropathic pain. *Nucleic Acids Res.* **32**, e49 (2004).
65. Xia, H. *et al.* RNAi suppresses polyglutamine-induced neurodegeneration in a model of spinocerebellar ataxia. *Nature Med.* **10**, 816–820 (2004).
66. Komar, A. A. *et al.* Internal initiation drives the synthesis of Ure2 protein lacking the prion domain and affects [URE3] propagation in yeast cells. *EMBO J.* **22**, 1199–1209 (2003).
67. True, H. L. & Lindquist, S. L. A yeast prion provides a mechanism for genetic variation and phenotypic diversity. *Nature* **407**, 477–483 (2000).
68. Uptain, S. M., Sawicki, G. J., Caughey, B. & Lindquist, S. Strains of [*PSI*$^+$] are distinguished by their efficiencies of prion-mediated conformational conversion. *EMBO J.* **20**, 6236–6245 (2001).
69. Bidou, L. *et al.* Nonsense-mediated decay mutants do not affect programmed-1 frameshifting. *RNA* **6**, 952–961 (2000).
70. Kellis, M., Patterson, N., Endrizzi, M., Birren, B. & Lander, E. S. Sequencing and comparison of yeast species to identify genes and regulatory elements. *Nature* **423**, 241–254 (2003).
71. True, H. L., Berlin, I. & Lindquist, S. L. Epigenetic regulation of translation reveals hidden genetic variation to produce complex traits. *Nature* **431**, 184–187 (2004).
Together with reference 67, this paper makes a compelling case for [*PSI*$^+$] as a beneficial prion.
72. Eaglestone, S. S., Cox, B. S. & Tuite, M. F. Translation termination efficiency can be regulated in *Saccharomyces cerevisiae* by environmental stress through a prion-mediated mechanism. *EMBO J.* **18**, 1974–1981 (1999).
73. Orr, H. A. The population genetics of adaptation: the distribution of factors fixed during adaptive evolution. *Evolution* **52**, 935–949 (1998).
74. Partridge, L. & Barton, N. H. Evolving evolvability. *Nature* **407**, 457–458 (2000).
75. Brookfield, J. F. Evolution: the evolvability enigma. *Curr. Biol.* **11**, R106– R108 (2001).
76. Kirschner, M. & Gerhart, J. Evolvability. *Proc. Natl Acad. Sci. USA* **95**, 8420–8427 (1998).
77. Masel, J. & Bergman, A. The evolution of the evolvability properties of the yeast prion [*PSI*$^+$]. *Evolution Int. J. Org. Evolution* **57**, 1498–1512 (2003).
A convincing modelling study suggesting that [*PSI*$^+$] has probably been maintained owing to the evolvability properties that it confers.
78. Earl, D. J. & Deem, M. W. Evolvability is a selectable trait. *Proc. Natl Acad. Sci. USA* **101**, 11531–11536 (2004).

© 2005 Nature Publishing Group

79. Resende, C. G., Outeiro, T. F., Sands, L., Lindquist, S. & Tuite, M. F. Prion protein gene polymorphisms in *Saccharomyces cerevisiae*. *Mol. Microbiol.* **49**, 1005–1017 (2003).
80. Lindquist, S. But yeast prion offers clues about evolution. *Nature* **408**, 17–18 (2000).
81. Harrison, P. *et al.* A small reservoir of disabled ORFs in the yeast genome and its implications for the dynamics of proteome evolution. *J. Mol. Biol.* **316**, 409–419 (2002).
82. Namy, O., Duchateau-Nguyen, G. & Rousset, J. P. Translational readthrough of the PDE2 stop codon modulates cAMP levels in *Saccharomyces cerevisiae*. *Mol. Microbiol.* **43**, 641–652 (2002).
83. Dalstra, H. J., Swart, K., Debets, A. J., Saupe, S. J. & Hoekstra, R. F. Sexual transmission of the [Het-s] prion leads to meiotic drive in *Podospora anserina*. *Proc. Natl Acad. Sci. USA* **100**, 6616–6621 (2003).
84 Derkatch, I. L., Bradley, M. E., Zhou, P., Chernoff, Y. O. & Liebman, S. W. Genetic and environmental factors affecting the *de novo* appearance of the [*PSI*+] prion in *Saccharomyces cerevisiae*. *Genetics* **147**, 507–519 (1997).
85. Derkatch, I. L., Bradley, M. E., Hong, J. Y. & Liebman, S. W. Prions affect the appearance of other prions: the story of [*PIN*+]. *Cell* **106**, 171–182 (2001).
86. Bradley, M. E., Edskes, H. K., Hong, J. Y., Wickner, R. B. & Liebman, S. W. Interactions among prions and prion 'strains' in yeast. *Proc. Natl Acad. Sci. USA* **99** (Suppl. 4), 16392–16399 (2002).
87. Salmon, J. M. & Barre, P. Improvement of nitrogen assimilation and fermentation kinetics under enological conditions by derepression of alternative nitrogen-assimilatory pathways in an industrial *Saccharomyces cerevisiae* strain. *Appl. Environ. Microbiol.* **64**, 3831–3837 (1998).
88. Crespo, J. L., Daicho, K., Ushimaru, T. & Hall, M. N. The GATA transcription factors GLN3 and GAT1 link TOR to salt stress in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **276**, 34441–34444 (2001).
89. Bergman, A. & Siegal, M. L. Evolutionary capacitance as a general feature of complex gene networks. *Nature* **424**, 549–552 (2003).
This remarkable study indicates that many, if not all, genes expose phenotypic variation when functionally compromised, and that the availability of loss-of-function mutations expedites adaptation to new phenotypic optima. Therefore, evolutionary capacitors might be more widespread than previously anticipated.
90. Hughes, T. R. *et al.* Functional discovery via a compendium of expression profiles. *Cell* **102**, 109–126 (2000).
91. Sangster, T. A., Lindquist, S. & Queitsch, C. Under cover: causes, effects and implications of Hsp90-mediated genetic capacitance. *Bioessays* **26**, 348–362 (2004).
92. Michelitsch, M. D. & Weissman, J. S. A census of glutamine/asparagine-rich regions: implications for their conserved function and the prediction of novel prions. *Proc. Natl Acad. Sci. USA* **97**, 11910–11915 (2000).
93. Harrison, P. M. & Gerstein, M. A method to assess compositional bias in biological sequences and its application to prion-like glutamine/asparagine-rich domains in eukaryotic proteomes. *Genome Biol.* **4**, R40 (2003).
94. Flechsig, E. *et al.* Prion protein devoid of the octapeptide repeat region restores susceptibility to scrapie in PrP knockout mice. *Neuron* **27**, 399–408 (2000).
95. Liu, J. J. & Lindquist, S. Oligopeptide-repeat expansions modulate 'protein-only' inheritance in yeast. *Nature* **400**, 573–576 (1999).
96. Krishnan, R. & Lindquist, S. L. New structural insights on a yeast prion illuminate nucleation and strain diversity. *Nature* (in the press).
This paper defines which regions of the Sup35 prion domain make intermolecular contacts in assembled prion fibres. It also describes how variations in these intermolecular contacts facilitate the construction of different prion variants.
97. Ross, E. D., Baxa, U. & Wickner, R. B. Scrambled prion domains form prions and amyloid. *Mol. Cell Biol.* **24**, 7206–7213 (2004).
98. Uversky, V. N. & Fink, A. L. Conformational constraints for amyloid fibrillation: the importance of being unfolded. *Biochim. Biophys. Acta* **1698**, 131–153 (2004).
99. Si, K. *et al.* A neuronal isoform of CPEB regulates local protein synthesis and stabilizes synapse-specific long-term facilitation in *Aplysia*. *Cell* **115**, 893–904 (2003).
This paper establishes that neurotransmitter cues upregulate ApCPEB at specific synapses, and that consequent ApCPEB-stimulated translation has a crucial role in the maintenance of synaptic growth associated with long-term facilitation. Together with reference 4, this paper makes a compelling argument that ApCPEB prions function in long-term memory formation.
100. Bailey, C. H., Kandel, E. R. & Si, K. The persistence of long-term memory: a molecular approach to self-sustaining changes in learning-induced synaptic growth. *Neuron* **44**, 49–57 (2004).
101. Lisman, J., Schulman, H. & Cline, H. The molecular basis of CaMKII function in synaptic and behavioural memory. *Nature Rev. Neurosci.* **3**, 175–190 (2002).
102. Bhalla, U. S. & Iyengar, R. Emergent properties of networks of biological signaling pathways. *Science* **283**, 381–387 (1999).
103. Thayer, M. J. *et al.* Positive autoregulation of the myogenic determination gene *MyoD1*. *Cell* **58**, 241–248 (1989).
104. Way, J. C. & Chalfie, M. The mec-3 gene of *Caenorhabditis elegans* requires its own product for maintained expression and is expressed in three neuronal cell types. *Genes Dev.* **3**, 1823–1833 (1989).
105. Mendez, R. & Richter, J. D. Translational control by CPEB: a means to the end. *Nature Rev. Mol. Cell Biol.* **2**, 521–529 (2001).
106. Uversky, V. N., Gillespie, J. R. & Fink, A. L. Why are 'natively unfolded' proteins unstructured under physiologic conditions? *Proteins* **41**, 415–427 (2000).
107. Sajikumar, S. & Frey, J. U. Late-associativity, synaptic tagging, and the role of dopamine during LTP and LTD. *Neurobiol. Learn. Mem.* **82**, 12–25 (2004).
108. Theis, M., Si, K. & Kandel, E. R. Two previously undescribed members of the mouse CPEB family of genes and their inducible expression in the principal cell layers of the hippocampus. *Proc. Natl Acad. Sci. USA* **100**, 9602–9607 (2003).
109. Li, L. & Lindquist, S. Creating a protein-based element of inheritance. *Science* **287**, 661–664 (2000).
110. Ter-Avanesyan, M. D. *et al.* Deletion analysis of the SUP35 gene of the yeast *Saccharomyces cerevisiae* reveals two non-overlapping functional regions in the encoded protein. *Mol. Microbiol.* **7**, 683–692 (1993).
111. Ringrose, L. & Paro, R. Epigenetic regulation of cellular memory by the polycomb and trithorax group proteins. *Annu. Rev. Genet.* **38**, 413–443 (2004).
112. Kim, C. A., Gingery, M., Pilpa, R. M. & Bowie, J. U. The SAM domain of polyhomeotic forms a helical polymer. *Nature Struct. Biol.* **9**, 453–457 (2002).
113. Qiao, F. *et al.* Derepression by depolymerization; structural insights into the regulation of Yan by Mae. *Cell* **118**, 163–173 (2004).
114. Roberts, C. W. & Orkin, S. H. The SWI/SNF complex — chromatin and cancer. *Nature Rev. Cancer* **4**, 133–142 (2004).
115. Sudarsanam, P., Iyer, V. R., Brown, P. O. & Winston, F. Whole-genome expression analysis of *snf/swi* mutants of *Saccharomyces cerevisiae*. *Proc. Natl Acad. Sci. USA* **97**, 3364–3369 (2000).
116. Gilks, N. *et al.* Stress granule assembly is mediated by prion-like aggregation of TIA-1. *Mol. Biol. Cell* **15**, 5383–5398 (2004).
117. Roberts, B. T. & Wickner, R. B. Heritable activity: a prion that propagates by covalent autoactivation. *Genes Dev.* **17**, 2083–2087 (2003).
118. Collin, P., Beauregard, P. B., Elagoz, A. & Rokeach, L. A. A non-chromosomal factor allows viability of *Schizosaccharomyces pombe* lacking the essential chaperone calnexin. *J. Cell Sci.* **117**, 907–918 (2004).
119. Ball, A. J., Wong, D. K. & Elliott, J. J. Glucosamine resistance in yeast. I. A preliminary genetic analysis. *Genetics* **84**, 311–317 (1976).
120. Silar, P., Haedens, V., Rossignol, M. & Lalucque, H. Propagation of a novel cytoplasmic, infectious and deleterious determinant is controlled by translational accuracy in *Podospora anserina*. *Genetics* **151**, 87–95 (1999).
121. Talloczy, Z., Menon, S., Neigeborn, L. & Leibowitz, M. J. The *[KIL-d]* cytoplasmic genetic element of yeast results in epigenetic regulation of viral M double-stranded RNA gene expression. *Genetics* **150**, 21–30 (1998).
122. Volkov, K. V. *et al.* Novel non-Mendelian determinant involved in the control of translation accuracy in *Saccharomyces cerevisiae*. *Genetics* **160**, 25–36 (2002).
123. Derkatch, I. L., Chernoff, Y. O., Kushnirov, V. V., Inge-Vechtomov, S. G. & Liebman, S. W. Genesis and variability of [*PSI*] prion factors in *Saccharomyces cerevisiae*. *Genetics* **144**, 1375–1386 (1996).
124. Ter-Avanesyan, M. D., Dagkesamanskaya, A. R., Kushnirov, V. V. & Smirnov, V. N. The SUP35 omnipotent suppressor gene is involved in the maintenance of the non-Mendelian determinant [*psi*+] in the yeast *Saccharomyces cerevisiae*. *Genetics* **137**, 671–676 (1994).
125. Liu, J. J., Sondheimer, N. & Lindquist, S. L. Changes in the middle region of Sup35 profoundly alter the nature of epigenetic inheritance for the yeast prion [*PSI*+]. *Proc. Natl Acad. Sci. USA* **99** (Suppl. 4), 16446–16453 (2002).
126. Bagriantsev, S. & Liebman, S. W. Specificity of prion assembly *in vivo*. [*PSI*+] and [*PIN*+] form separate structures in yeast. *J. Biol. Chem.* **279**, 51042–51048 (2004).
127. Schlumpberger, M., Prusiner, S. B. & Herskowitz, I. Induction of distinct [URE3] yeast prion strains. *Mol. Cell Biol.* **21**, 7035–7046 (2001).
128. Osherovich, L. Z. & Weissman, J. S. Multiple Gln/Asn-rich prion domains confer susceptibility to induction of the yeast [*PSI*+] prion. *Cell* **106**, 183–194 (2001).
129. Derkatch, I. L. *et al.* Effects of Q/N-rich, polyQ, and non-polyQ amyloids on the *de novo* formation of the [*PSI*+] prion in yeast and aggregation of Sup35 *in vitro*. *Proc. Natl Acad. Sci. USA* **101**, 12934–12939 (2004).
130. Derkatch, I. L. *et al.* Dependence and independence of [*PSI*+] and [*PIN*+]: a two-prion system in yeast? *EMBO J.* **19**, 1942–1952 (2000).
131. Bradley, M. E. & Liebman, S. W. Destabilizing interactions among [*PSI*+] and [*PIN*+] yeast prion variants. *Genetics* **165**, 1675–1685 (2003).
132. Baker, H. F., Ridley, R. M., Duchen, L. W., Crow, T. J. & Bruton, C. J. Induction of β(A4)-amyloid in primates by injection of Alzheimer's disease brain homogenate. Comparison with transmission of spongiform encephalopathy. *Mol. Neurobiol.* **8**, 25–39 (1994).
133. Lundmark, K. *et al.* Transmissibility of systemic amyloidosis by a prion-like mechanism. *Proc. Natl Acad. Sci. USA* **99**, 6979–6984 (2002).
134. Xing, Y. *et al.* Induction of protein conformational change in mouse senile amyloidosis. *J. Biol. Chem.* **277**, 33164–33169 (2002).
135. Prinz, M. *et al.* Positioning of follicular dendritic cells within the spleen controls prion neuroinvasion. *Nature* **425**, 957–962 (2003).
136. Tanaka, M., Chien, P., Yonekura, K. & Weissman, J. S. Mechanism of cross-species prion transmission; an infectious conformation compatible with two highly divergent yeast prion proteins. *Cell* **121**, 49–62 (2005).
137. Shorter, J. & Lindquist, S. Hsp104 catalyzes formation and elimination of self-replicating Sup35 prion conformers. *Science* **304**, 1793–1797 (2004).
A delineation of how Hsp104 directly regulates Sup35 prion conformers.
138. Scheibel, T. *et al.* Conducting nanowires built by controlled self-assembly of amyloid fibres and selective metal deposition. *Proc. Natl Acad. Sci. USA* **100**, 4527–4532 (2003).
139. Masel, J., Jansen, V. A. & Nowak, M. A. Quantifying the kinetic parameters of prion replication. *Biophys. Chem.* **77**, 139–152 (1999).
140. Lee, D. H., Granja, J. R., Martinez, J. A., Severin, K. & Ghadiri, M. R. A self-replicating peptide. *Nature* **382**, 525–528 (1996).
141. Lee, D. H., Severin, K., Yokobayashi, Y. & Ghadiri, M. R. Emergence of symbiosis in peptide self-replication through a hypercyclic network. *Nature* **390**, 591–594 (1997).
142. Saghatelian, A., Yokobayashi, Y., Soltani, K. & Ghadiri, M. R. A chiroselective peptide replicator. *Nature* **409**, 797–801 (2001).
References 140–142 describe a remarkable set of short α-helical peptides that can undergo chemical and conformational replication, and which might even help to explain the origins of homochirality.
143. Pan, K. M. *et al.* Conversion of α-helices into β-sheets features in the formation of the scrapie prion proteins. *Proc. Natl Acad. Sci. USA* **90**, 10962–10966 (1993).
144. Wille, H., Zhang, G. F., Baldwin, M. A., Cohen, F. E. & Prusiner, S. B. Separation of scrapie prion infectivity from PrP amyloid polymers. *J. Mol. Biol.* **259**, 608–621 (1996).
145. Parsell, D. A., Kowal, A. S., Singer, M. A. & Lindquist, S. Protein disaggregation mediated by heat-shock protein Hsp104. *Nature* **372**, 475–478 (1994).
146. Glover, J. R. & Lindquist, S. Hsp104, Hsp70, and Hsp40: a novel chaperone system that rescues previously aggregated proteins. *Cell* **94**, 73–82 (1998).

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147. Wegrzyn, R. D., Bapat, K., Newnam, G. P., Zink, A. D. & Chernoff, Y. O. Mechanism of prion loss after Hsp104 inactivation in yeast. *Mol. Cell Biol.* **21**, 4656–4669 (2001).
148. Hattendorf, D. A. & Lindquist, S. L. Analysis of the AAA sensor-2 motif in the C-terminal ATPase domain of Hsp104 with a site-specific fluorescent probe of nucleotide binding. *Proc. Natl Acad. Sci. USA* **99**, 2732–2737 (2002).
149. Hattendorf, D. A. & Lindquist, S. L. Cooperative kinetics of both Hsp104 ATPase domains and interdomain communication revealed by AAA sensor-1 mutants. *EMBO J.* **21**, 12–21 (2002).
150. Grimminger, V., Richter, K., Imhof, A., Buchner, J. & Walter, S. The prion curing agent guanidinium chloride specifically inhibits ATP hydrolysis by Hsp104. *J. Biol. Chem.* **279**, 7378–7383 (2004).
151. Ripaud, L., Maillet, L. & Cullin, C. The mechanisms of [*URE3*] prion elimination demonstrate that large aggregates of Ure2p are dead-end products. *EMBO J.* **22**, 5251–5259 (2003).
152. Collins, S. R., Douglass, A., Vale, R. D. & Weissman, J. S. Mechanism of prion propagation: amyloid growth occurs by monomer addition. *PLoS Biol.* **2**, e321 (2004).
153. Inoue, Y., Taguchi, H., Kishimoto, A. & Yoshida, M. Hsp104 binds to yeast Sup35 prion fibre but needs other factor(s) to sever it. *J. Biol. Chem.* **279**, 52319–52323 (2004).
154. Ferreira, P. C., Ness, F., Edwards, S. R., Cox, B. S. & Tuite, M. F. The elimination of the yeast [*PSI*$^+$] prion by guanidine hydrochloride is the result of Hsp104 inactivation. *Mol. Microbiol.* **40**, 1357–1369 (2001).
155. Shorter, J. & Lindquist, S. Navigating the ClpB channel to solution. *Nature Struct. Mol. Biol.* **12**, 4–6 (2005).
156. Serio T. R. & Lindquist S. L. Protein-only inheritance in yeast: something to get [*PSI*$^+$]-ched about. *Trends Cell Biol.* **10**, 98–105 (2000).
157. Leeds, P., Peltz, S. W., Jacobson, A. & Culbertson, M. R. The product of the yeast UPF1 gene is required for rapid turnover of mRNAs containing a premature translational termination codon. *Genes Dev.* **5**, 2303–2314 (1991).
158. Frischmeyer, P. A. *et al.* An mRNA surveillance mechanism that eliminates transcripts lacking termination codons. *Science* **295**, 2258–2261 (2002).
159. Bence, N. F., Sampat, R. M. & Kopito, R. R. Impairment of the ubiquitin-proteasome system by protein aggregation. *Science* **292**, 1552–155 (2001).
160. Venkatraman, P., Wetzel, R., Tanaka, M., Nukina, N. & Goldberg, A. L. Eukaryotic proteasomes cannot digest polyglutamine sequences and release them during degradation of polyglutamine-containing proteins. *Mol. Cell* **14**, 95–104 (2004).
161. Castilla, J., Saa, P., Hetz, C. & Soto, C. *In vitro* generation of infectious scrapie prions. *Cell* **121**, 195–206 (2005).
162. Zou, W. Q. & Gambetti, P. From microbes to prions the final proof of the prion hypothesis. *Cell* **121**, 155–157 (2005).

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