CMLS, Cell. Mol. Life Sci. 58 (2001) 1857–1878
1420-682X/01/131857-22 $ 1.50 + 0.20/0


CMLS Cellular and Molecular Life Sciences

Review

# The protein – only theory and the yeast *Saccharomyces cerevisiae*: the prions and the propagons

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Received 10 April 2001; received after revision 11 June 2001; accepted 15 June 2001

**Abstract.** The yeast prions represent a very attractive and tractable model for investigating the prion world. The more extensively studied yeast prion [PSI] leads to a propagation model that links auto-aggregation in amyloid formation and inactivation of the cellular function of the yeast 'prion protein' Sup35p. The other prion model, [URE3], appears to be similar in some genetic and biochemical properties. The characterisation of both Sup35p and Ure2p, the two 'prion proteins', mainly focusing on their aggregation properties, support this model. However, some important differences still exist that should be examined carefully. In particular, we have shown that Ure2p aggregation in vivo (monitored by fluorescence of Ure2-GFP fusion) does not necessarily give rise to a [URE3] phenotype. Comparisons of these two systems as well as more recent experiments are discussed in this review.

**Key words.** Yeast; prion; amyloid; propagon; [URE3]; [PSI].

## The yeast prions: non-Mendelian elements revisited by the protein – only hypothesis of mammalian prions

The prions represent a new class of infectious agent in mammals that cause spongiform encephalopathies. They also bear an atypical mechanism of transmission of hereditary information, which does not imply DNA modification.

In 1994, Reed Wickner [1] proposed that two non-Mendelian elements, described genetically in the yeast *Saccharomyces cerevisiae* more than 20 years ago, are prions. This hypothesis was first based on genetic criteria, but was further consolidated at biochemical and physicochemical levels, so that the definition of prions diverged from Prusiner's initial model. Nevertheless, the paradigm remains the same: the protein undergoes a modification which leads to a change or a loss of function, and this modification occurs according to an autocatalytic mode, the protein provoking its own conversion.

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## The discovery of [URE*3*]

### Phenotype

François Lacroute discovered [URE3] in 1971 while studying the uracile biosynthesis pathway [2]. A metabolic pathway, involving several enzymes (fig. 1) ensures the synthesis of uridine monophosphate (UMP). The first two steps are under the control of the *URA2* gene product, enacts assures the double function of carbamylphosphate synthetase and aspartate transcarbamylase. It allows the synthesis of ureidosuccinate (USA) from L-aspartate. The ureidosuccinate is then transformed by the enzymes encoded by *URA4*, *URA1* and *URA5* genes, the product of which is orotidine 5′-phos-

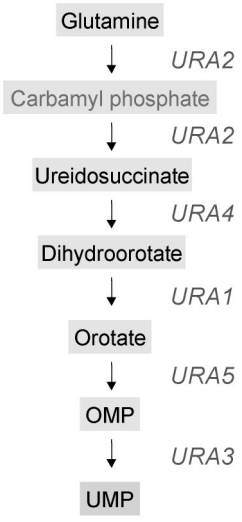


Figure 1. Uracil biosynthesis pathway. OMP, orotidine 5′-monophosphate; UMP, uridine 5′-monophosphate.

phate, which is then transformed into uridine 5′-monophosphate by orotidine-5′-phosphate decarboxylase encoded by the *URA3* gene.

**Two mutants: ure2 and [URE3]**

*ura2* mutants cannot synthesize USA. The uptake of USA is controlled by the allantoate permease encoded by the *DAL5* gene, which is regulated at the transcriptional level by nitrogen catabolic repression (NCR) depending on the nature of the nitrogen source contained in the medium [1, 2]. In the presence of a rich nitrogen source, such as ammonium, the Dal5p permease is not expressed. When the medium contains only a poor nitrogen source, like proline, the *DAL5* gene is transcribed. It allows the uptake of allantoate, which can be used as a nitrogen source. The uptake of USA by this permease is fortuitous, and due to the fact that allantoate and USA share structural resemblance. In the presence of ammonium, allantoate permease is repressed and *ura2* mutants do not allow the uptake of USA and cannot grow (fig. 2). François Lacroute and Michel Aigle searched for *ure* mutants capable of growing on a medium containing ammonium and USA

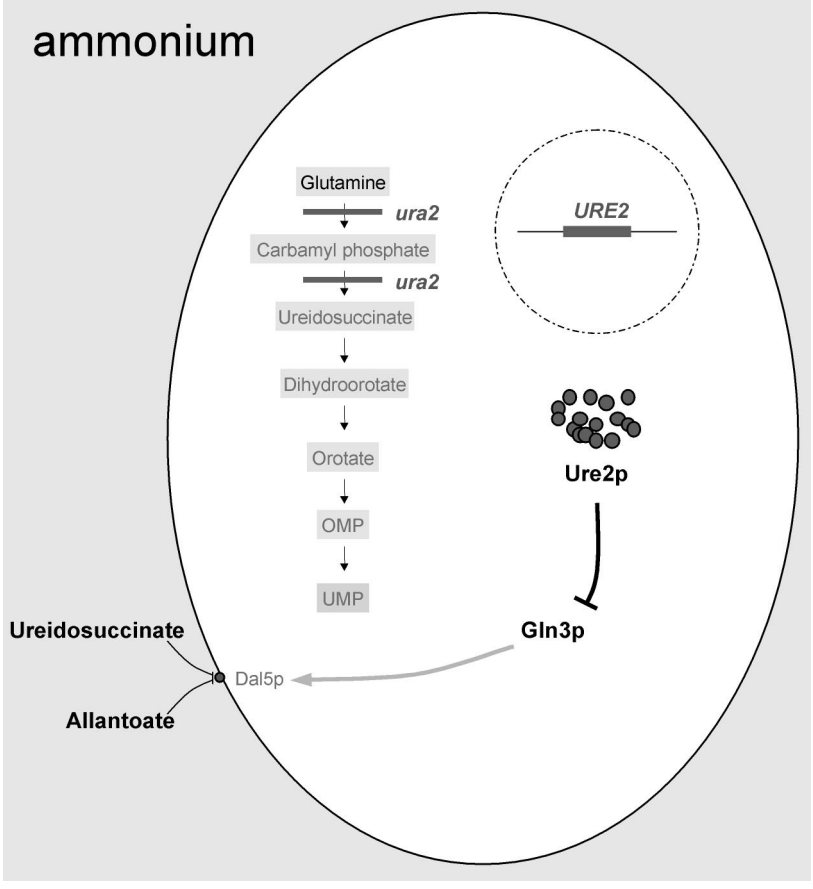


Figure 2. Involvement of Ure2p in nitrogen catabolism repression. In the presence of a rich nitrogen source, like ammonium, Ure2p in the cytoplasm blocks the transcription factor Gln3p which cannot activate expression of the *DAL5* gene, encoding the allantoate permease. Ureidosuccinate cannot be taken up and a *ura2* mutant cannot synthesise UMP. A dashed line schematises the nucleus.

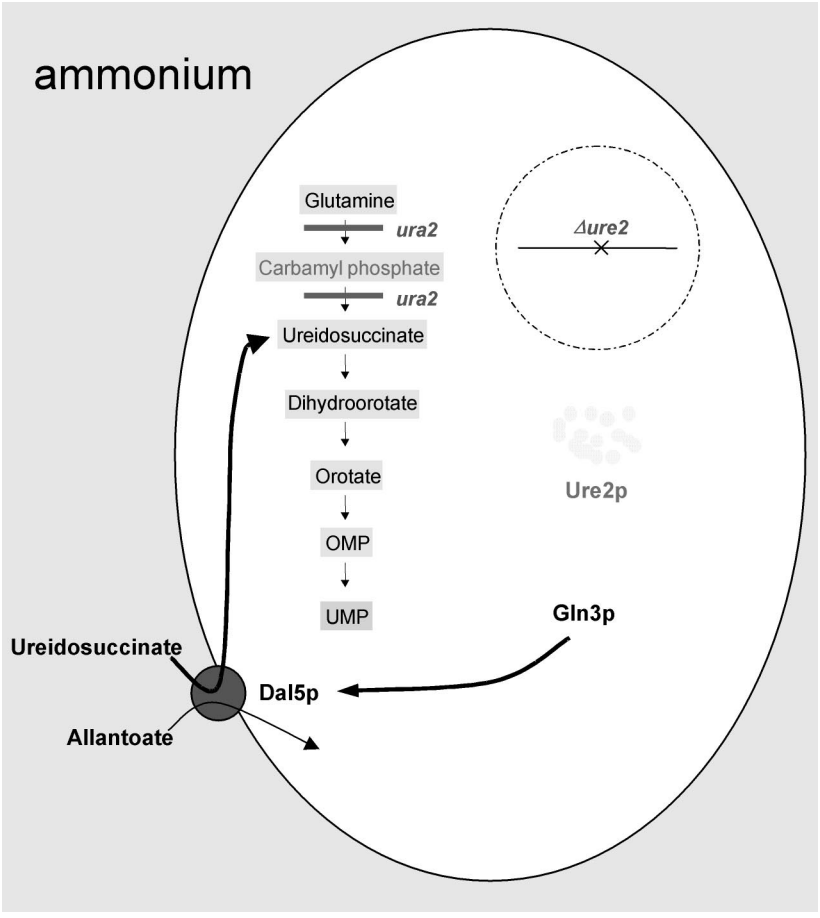


Figure 3. In the absence of functional Ure2 protein, Gln3p constitutively triggers the expression of Dal5p permease, allowing the uptake of allantoate and ureidosuccinate. The biosynthesis of UMP is then restored.

[3]. Among these mutants, they found classic Mendelian mutants. The ure2 mutant is recessive, and its 2:2 transmission corresponds to a loss of function of the chromosome gene *URE2* (fig. 3).

A second category of mutants exists in addition to this complementation group. The Usa+ phenotype is dominant, and segregates in a non-Mendelian manner. Most tetrads have four Usa+ spores. Furthermore, this mutant phenotype can be transmitted by cytoduction, corresponding to a transfer of cytoplasm. These criteria allowed this element to be classivied in the group of non-chromosomal genetic elements. Such non-chromosomal elements such as [PSI+] [4] had already been described in yeast. This new genetic element was called [URE3].

**[URE3]: genetic properties**

1) Dominance and segregation. The crossing of a wild-type strain by a [URE3] strain produces [URE3] diploid cells [2]. After sporulation, the four separated spores present the [URE3] phenotype. This phenotype is, then, not conferred by a recessive mutation of a gene, which would present a classic 2:2 segregation.

2) Cytoduction. [URE3] can be transmitted by cytoduction [3], a technique in which cytoplasms can be merged without provoking fusion of nuclei.

3) Curability. [URE3] can be eliminated on a medium containing 5 mM guanidium chloride (M. Aigle, cited in ref. [5]). When the strain is cured, [URE3] clones can be selected de novo. The frequency of spontaneous appearance of [URE3] is about $10^{-6}$, and is unchanged after the cure [1]. If [URE3] were dependent on a replicative autonomous element constituted by a nucleic acid, it could not disappear and then reappear spontaneously.

4) Dependence of the *URE2* gene. Another original characteristic is the relationship, between the *URE2* gene and [URE3]. Although they present the same phenotype [6], [URE3] cannot appear and remain in a *ure2* mutant [3]. Thus, the expression of the wild-type *URE2* gene is necessary for the maintenance and transmission of [URE3]. To explain the epistatic relationship between the presence of [URE3] and the *URE2* gene, Michel Aigle suggested that [URE3] could inactivate the product of the *URE2* gene [3].

5) A new element. This element was demonstrated as different from other known non-Mendelian elements, such

as the mitochondria, double-stranded RNA viruses of yeast, the plasmide 2 $\mu$ and [PSI] [7].
[URE3] was thus described as being a new non-Mendelian genetic element, different from all such previously described elements.

**[URE3] possesses the characteristics of a prion**

In 1994, Reed Wickner [1] suggested the behavior of this element fulfilled the criteria for a prion. The product of the *URE2* gene would be the normal form of the protein, Ure2p, and the [URE3] phenotype would be due to the conversion of the protein Ure2p into its inactive prion form (Ure2p $^{[URE3]}$; fig. 4). The autocatalytic distribution of the prion form of Ure2p would then explain the genetic characteristics of a prion expressed in a unicellular organism such as yeast. If [URE3] is an infectious protein, its genetic properties can be predicted.
1) The prions of yeast are non-Mendelian elements. An infectious protein would present transmission properties similar to those of viruses, plasmids and other infectious agents of yeast. The phenotype conferred by the presence of such an element is dominant. Furthermore, meiotic segregation produces four daughter cells which receive the infectious protein in an unpredictable way. The segregation of the phenotype will also be unpredictable, presenting a segregation of 4:0, 3:1, 2:2, 1:3 or 0:4 (fig. 5).
Because [URE3] is a cytoplasmic element, it can be transmitted by cytoduction.
On the other hand, many other non-Mendelian elements, which are not prions, are known in yeast. For a non-Mendelian element to be a prion it must fulfill other criteria.
2) The phenotype induced by the presence of the prion can be reversibly eliminated. Some non-Mendelian genetic elements can be eliminated, using chemical substances specific to every element. Ethidium bromide, for example, is used to eliminate mitochondria, generating respiratory-deficient cells.
A cell that presents the prion phenotype can also lose this phenotype, either naturally, or under the effect of specific chemical substances (the 'cure'). The important criterion here is the r*eversibility* of the cure (fig. 6). In contrast to viruses or mitochondria, the prion phenotype can reappear spontaneously, at the same initial frequency, without

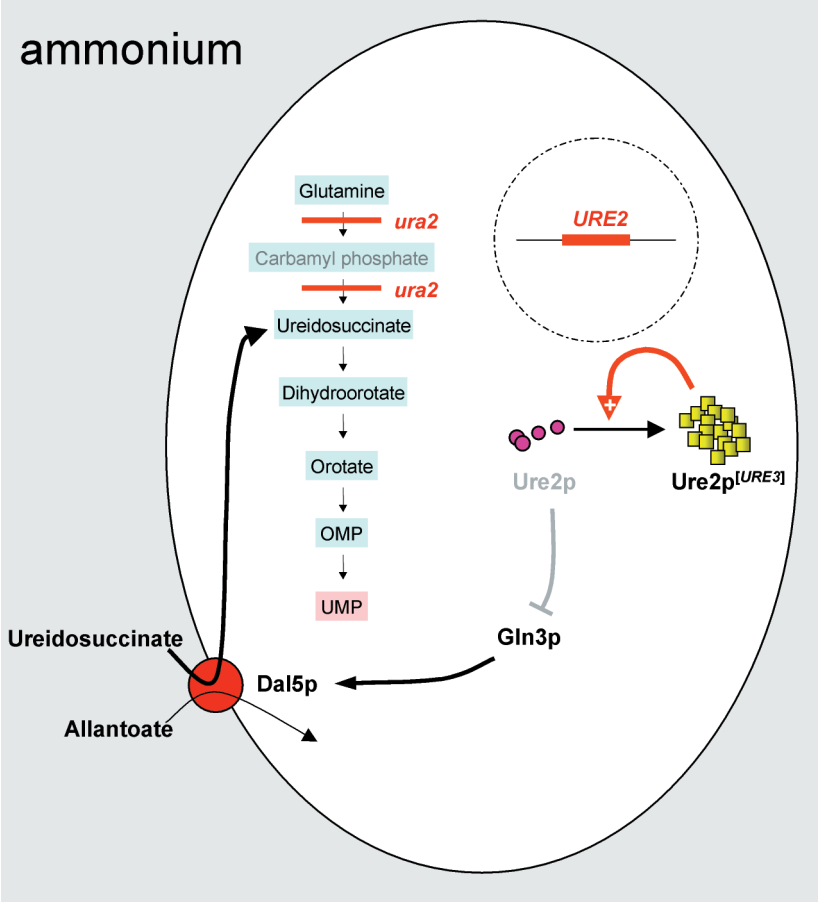


Figure 4. In its prion form, the Ure2 protein (Ure2p$^{[URE3]}$) no longer blocks Gln3p. Moreover, the functional protein is converted by an autocatalytic mechanism that explains the transmission properties of the [URE3] element.

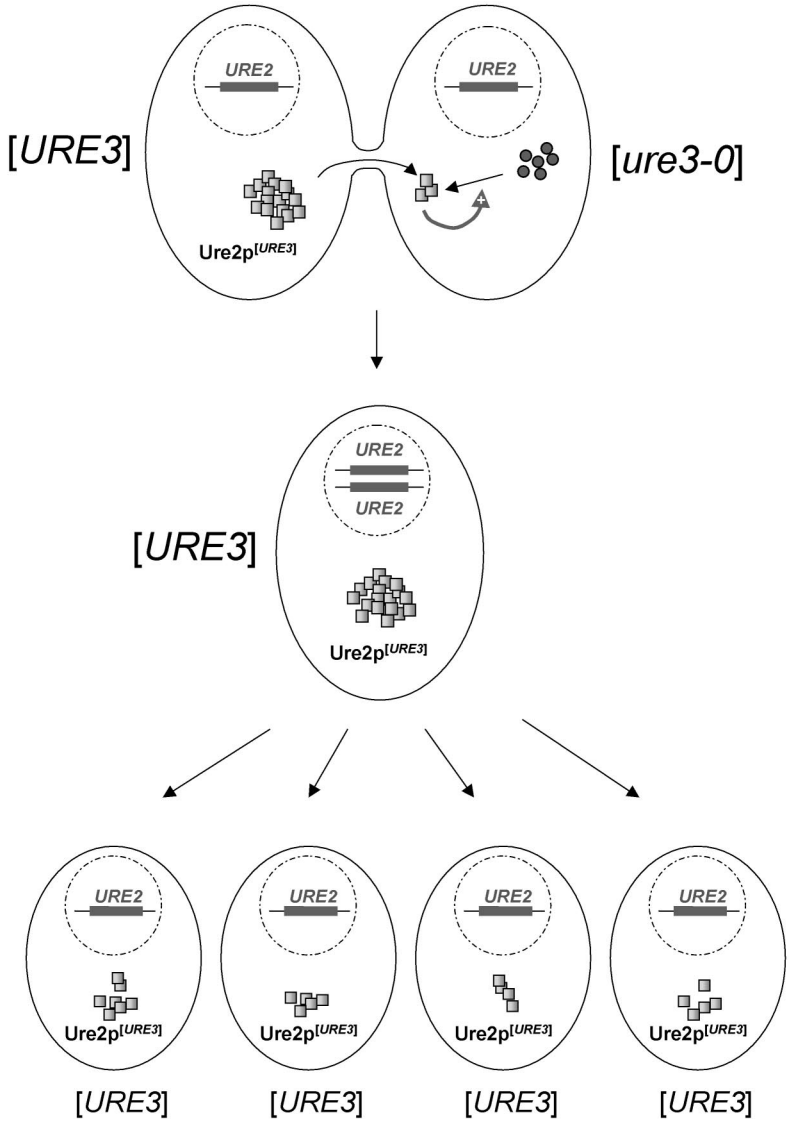


Figure 5. Dominance and non-Mendelian transmission of the infectious protein Ure2p[URE3]). Here, the [URE3] phenotype is transmitted to all spores, but its transmission can also be incomplete, leading to either 3 : 1, 2 : 2, 1 : 3 or 0 : 4 segregation.

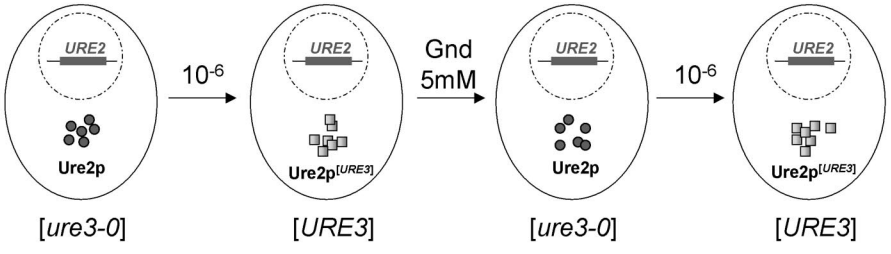


Figure 6. [URE3] can be reversibly eliminated by treatment with guanidine hydrochloride. The [URE3] phenotype can then reappear de novo at the same frequency.

reintroduction of the element by mating or cytoduction. In the case of the viruses of yeast, such as L-A and killer, or mitochondrial genes, their loss is irreversible and the phenotype may be reverted only by reintroduction of these elements into the cell.

3) Dependence between a chromosome gene and prion. The prion is a protein that propagates by conversion of the normal form to the prion form. The absence of expression of the normal protein in the cell prevents prion replication, leading to its loss after several generations. This experiment was realized using a Δ*URE2* strain (fig. 7) [1]. In a strain in which the *URE2* gene was deleted, the *URE2* gene was expressed on a plasmid. After selection of [URE3] clones, the plasmid expressing the Ure2 protein was chased. This plasmid was then reintroduced into these cells, and the [URE3] phenotype was lost, indicating that the persistence of [URE3] requires the continuous expression of Ure2p.

4) Overexpression of the gene encoding the normal protein increases the frequency of appearance of the prion phenotype. Overexpression of the gene encoding the normal protein increases in the cell the quantity of proteins capable of being converted into the prion form. As a result, the frequency of appearance of the prion phenotype increases. This was shown for [URE3] (fig. 8). Overexpression of the *URE2* gene increases by a factor of about 100, the appearance of the prion phenotype [1].

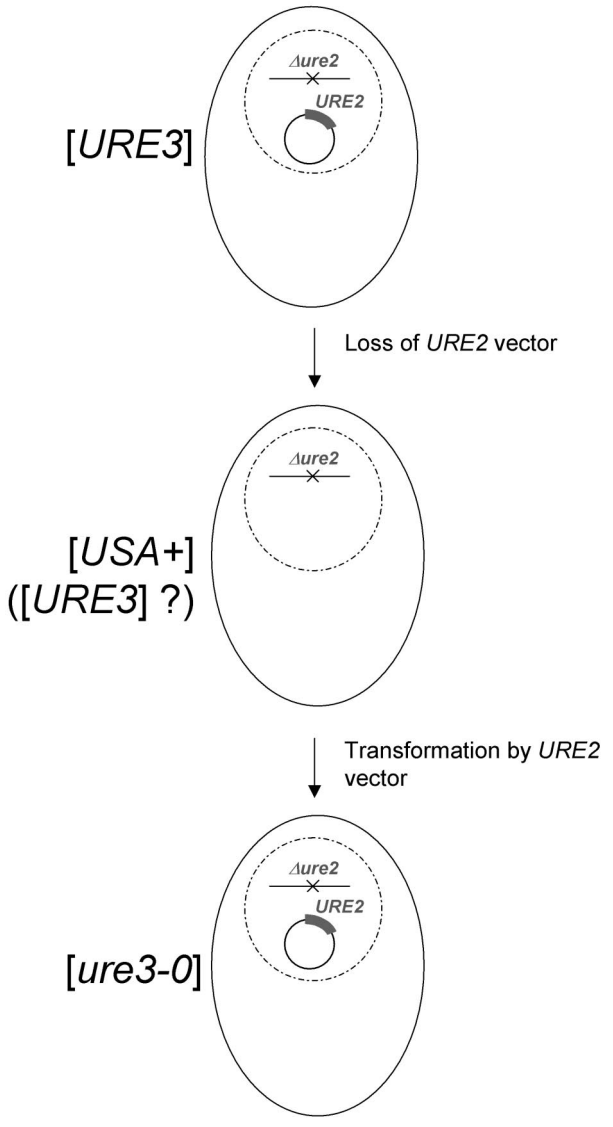


Figure 7. [URE3] cannot be maintained in the absence of expression of the Ure2 protein. A transitory elimination of the wild-type *URE2* gene leads to the elimination of the [URE3] prion.

**Another prion in yeast: [PSI]**

The element [PSI], like [URE3], was discovered prior to the prion hypothesis. [PSI] was described by Brian Cox in 1965 [4]. The [PSI] element was initially discovered because of its capacity to increase the effect of a weak suppressor tRNA specific for the stop codon ochre (UAA), *SUQ5* [5]. An *ade2-1* strain carries an *ADE2* gene interrupted by a nonsense (ochre) mutation in the open reading frame (fig. 9). In the *SUQ5* [psi−] context, the level of suppression is not sufficient, and the adenine biosynthesis pathway is interrupted at the step assured by the product of the *ADE2* gene. A red pigment upstream of this chain accumulates and colonies appear red on a rich medium. However, in a [PSI] context, these same cells remain white, because the level of suppression is sufficient to restore the expression of the *ADE2* gene. Moreover, this element is also capable of suppressing three stop codons, weakly, even in the absence of a tRNA suppressor [8, 9], giving rise to its designation as a 'ubiquitous' suppressor [5, 10].

The genetic properties of [PSI] are identical to those of [URE3]. [PSI] is dominant, and presents a non-Mendelian segregation [4]. [PSI] can be also transmitted by cytoduction [5]. This element was shown to be independent of the other non-Mendelian elements: the mitochondria, the 2 $\mu$ plasmid, and the viruses L-A and M of yeast [reviewed in ref. 5]. Involvement of other elements in the appearance of [PSI], like the 3 $\mu$ DNA, which is an autonomous and extra-chromosomal replicon of genes of ribosomal RNA, was tested, but no direct proof was obtained [11].

[PSI] fulfills the criteria as a non-Mendelian element of yeast, and like [URE3], [PSI] presents characteristics expected for an infectious protein: [PSI] can be lost following treatment with 1–5 mM guanidium chloride [12] and also by osmotic stress [13]. After the cure, [PSI] can reappear at the same frequency as initially [14], at least in the strain used for this study.

[PSI] requires the expression of the wild-type gene *SUP35*. The protein Sup35p is one of the subunits of the

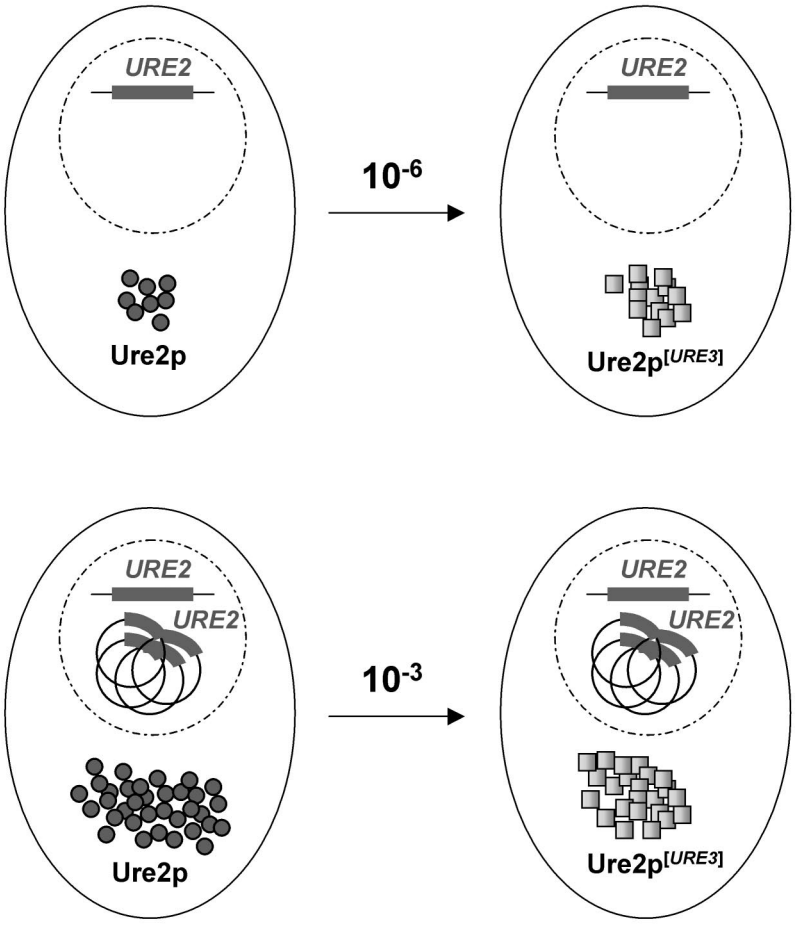


Figure 8. Overexpression of the *URE2* gene produces more 'normal' protein that can be converted to the prion form, increasing the [URE3] frequency.

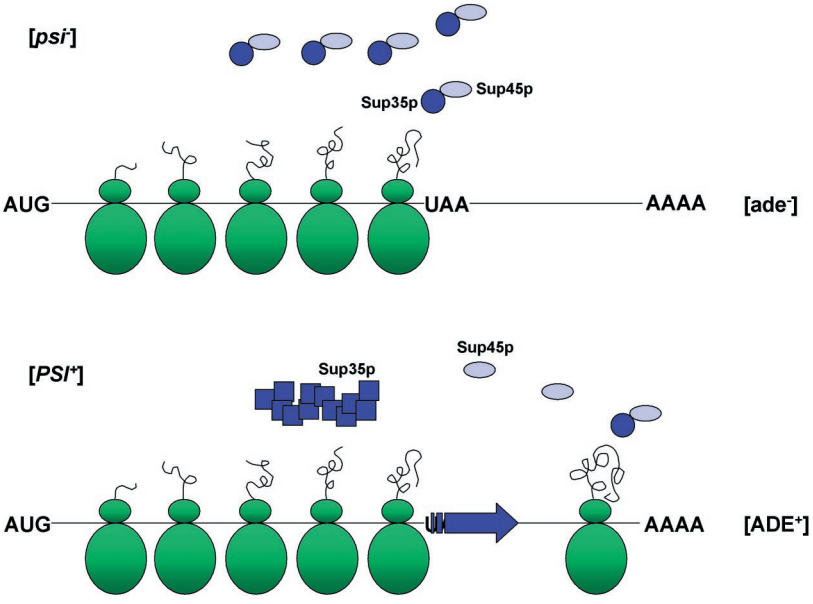


Figure 9. The *ade2-1* allele contains a nonsense mutation that interrupts the coding frame of the *ADE2* gene. In a [psi–] context, Sup35p and sup45p allow efficient translation termination that provokes adenin auxotrophy. However, in a [PSI+] context, Sup35p is in its prion shape and translation termination is not so efficient. Partial suppression of the stop codon restores a function *ADE2* gene.

termination factor of translation in eukaryotes, which corresponds to eRF3 [15, 16]. In yeast, it is a 79-kDa protein consisting of 685 amino acids. It forms, with Sup45p, the termination factor eRF1 [17], a complex responsible for the recognition of stop codons and allows the cleavage of the complete peptide at the end of translation. As the *SUP35* gene is indispensable to viability [18–21], the preservation of [PSI] in a cell cannot be tested in the absence of Sup35p expression. On the other hand, [PSI] is incompatible with some mutations of the *SUP35* gene. By genetic analysis, some mutants called PNM, for '[PSI] No-More', were isolated. They prevent the appearance and transmission of [PSI] introduced by mating or by cytoduction [5]. These mutations are intra- and extragenic. In the same way that a mutant *ure2* shares the same phenotype as [*URE3*] cells, the mutant *sal3-4*, which corresponds to a mutation in the C-terminal domain of Sup35p that affects translation fidelity [22], and [PSI] present the same phenotype of suppression.

Overexpression of the *SUP35* gene increases the appearance of [PSI] [14] about 100-fold, another argument in favour of the prion nature of [PSI], just as the effect of *URE2* gene overexpression increases the frequency of appearance of [URE3].

## Molecular analysis of the prions of yeast

### Ure2p: a two-domain protein

The Ure2p protein was analyzed by a molecular approach, by overexpressing various fragments of the protein, deleted in either the N- or C-terminal extremities, or inside the gene [23, 24]. These various deletion mutants were overexpressed in a *URE2*-deleted strain, and in a wild-type strain, to test both the functional activity and the effect on the appearance of [URE3] (fig. 10).

Two types of inducing and repressing domains of the prion phenotype can be distinguished:

(i) domains acting in *trans:* they modify the frequency of [URE3] appearance in a wild-type strain; it is their *presence* which has an effect on the conversion of the prion,

(ii) domains acting in *cis:* their deletion modifies the inductive properties of the protein on which they are located; the effect of these domains is then observed indi-

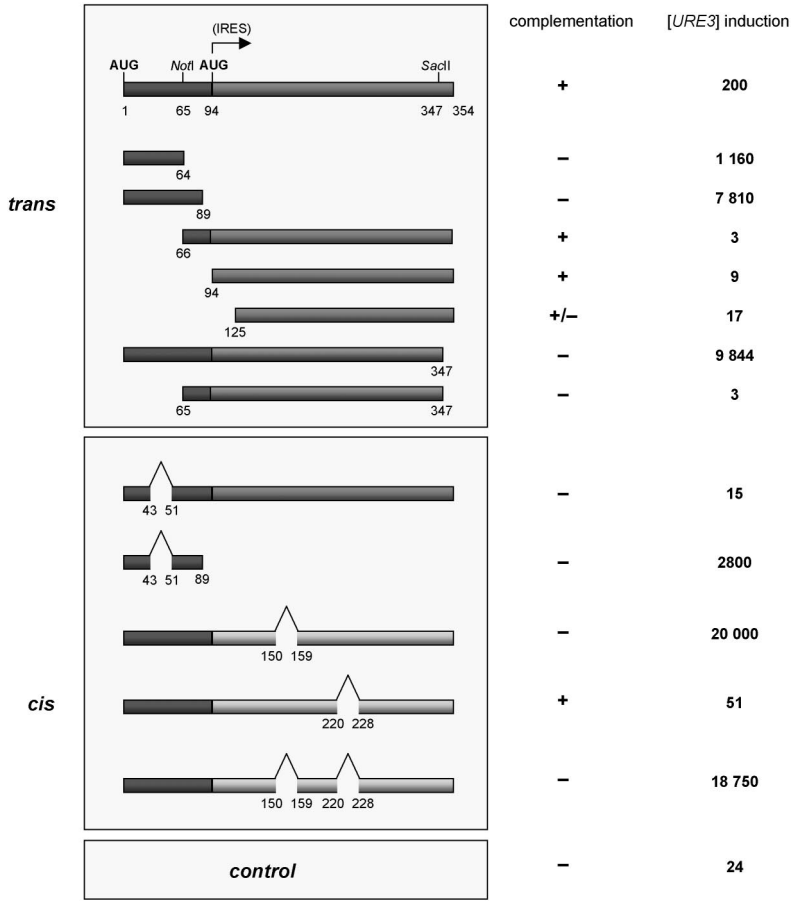


Figure 10. Ure2p contains two domains: a prion domain (red box) which induces the appearance of the [URE3] prion phenotype and a catalytic domain (green box). Different deletion mutants were tested for their ability to induce [URE3] (relative values) and their functionality. The inducing effect was recalculated from Masison and Wickner [24] with wild-type *URE2* as internal reference.

rectly, because it is their *absence* that modifies the frequency of conversion.

**Effect of deleted forms in the appearance of [URE3] de novo**

1) In *trans.* When the N-terminal fragment extending from the 1st to the 65th amino acid is overexpressed in a strain wild-type for the *URE2* gene, it provokes a 50- to 100-fold increase in the appearance of the [URE3] phenotype [24]. This fragment is not able to complement a *ΔURE2* strain. On the other hand, the overexpression of the complementary C-terminal fragment, extending from the 66th to the last amino acid, restores the *URE2* function in a ΔURE2 strain. Thus, the N-terminal domain of *URE2* is responsible for the induction of the prion phenotype, while the C-terminal domain carries the catalytic function. Reed Wickner named the N-terminal domain including the first 65 residues the 'prion-forming domain' (PFD). This definition of the PFD was arbitrary because it was based on the presence of a *NotI* site located at the 65th amino acid of Ure2p. In fact, when a fragment extending from the 1st to the 80th amino acid is overexpressed, the inducing effect is even stronger [23].

Overexpression of the catalytic part decreases the spontaneous frequency of [URE3] appearance [24]. The C-terminal domain carries not only the function of *URE2*, but also represses [URE3] induction. This domain shares homology with glutathione-S-transferases. Furthermore, a very short deletion from base pair 348 to 354 abolishes its function. This region represses the induction of the prion phenotype, because its deletion allows the construction of an inductive gene.

2) In *cis.*

(i) The role of asparagine repetitions. One of the remarkable molecular characteristics of the PFD of URE2p is its abundance of asparagine, glutamine and serine residues. There are two almost continuous stretches of asparagines located between residues 44–50 to 62. To determine if these series of asparagines could play a role in the induction of the prion phenotype, various deletions in the N-terminal domain were studied systematically [23]. Deletion of the regions rich in asparagines abolishes the inductive effect of the complete protein Ure2p. Their presence is not, however, absolutely necessary for induction, because their deletion in various fragments deleted in the C-terminal, which are very inductive, reduces the inductive properties only twofold.

(ii) The other internal deletions. Deletion of a region located in the C-terminal part of the protein, between residues 221–227, in the complete Ure2p protein, leads to an abolishment of the capacity of [URE3] induction, without provoking the loss of the catalytic function [23]. Deletion of a region situated between residues 151 and 158 increases the inductive capacity of the complete protein. Simultaneous deletion of the two regions (from 151 to 158, and from 221 to 227) in the complete Ure2p protein increases the inductive capacity.

The repression or induction mechanism of these Ure2p domains is unknown. Unfortunately, the effect of these domains expressed independently was not tested in *trans*. For example, the eight amino acids, 151–158, which are inhibitory, were not expressed in a wild-type strain to confirm their negative effect on [URE3] frequency. Their effect was deduced only on the basis of their absence. Wickner suggests that the catalytic domain of Ure2p stabilizes the N-terminal inducing domain through a set of interactions between the N terminus and the catalytic parts. These interactions would involve the previous domain and their absence would destabilize the protein, which would be more easily converted into the prion form. Nevertheless, these deletions can completely destabilize the protein without having any direct inducing or repressing effect on the conversion into the prion form. As a result the protein could be unfolded, and as the PFD of Ure2p would be accessible, the prion properties would be due to this fact, just as when the PFD expressed alone in *trans* can induce [URE3]. In that case, these deleted domains would not correspond to secondary prion-inducing domains. Conversely, deletion from 221 to 227, which does not abolish function but prevents the fragment from inducing [URE3], could mask the N-terminal domain, which would not be able to induce a wild-type Ure2p protein. In that case also, it is not the prion mechanism which would be inhibited, but a simple conformational effect.

None of the inductive constructions analyzed in these experiments were functional. Induction could, therefore, also be due to the absence of catalytic function. Only recently, a point mutant has been described that retains the catalytic activity of Ure2p while at the same inducing a rise in [URE3]. The experiment characterised an inducing allele of the *URE2* gene, which induced [URE3] appearance 1000-fold when expressed at a physiological level [25]. This allele contains several mutations in the PFD and in the catalytic domain of Ure2p. Moreover, the expression of a chimeric allele containing the mutated prion domain and a wild-type catalytic domain led to the generation of the first allele that is both inducing and functional. The effect of these mutations could be due to intramolecular interactions involving electrostatic forces.

**Effect of the expression of Ure2p domains in the pre-existing [URE3] phenotype**

If overexpression of *URE2* provokes [URE3] induction, its behaviour toward a preexisting [URE3] is different and contradictory. Wickner and colleagues [26] studied the effect of the expression of the *URE2* gene or various truncated forms in a [URE3] strain. When overexpressed – on a multicopy plasmid under the control of the strong

promoter of the alcohol dehydrogenase, *ADH1*, gene [26] – or expressed at a physiological level – on a plasmid monocopy under the control of the *URE2* gene promoter – a full-length *URE2* does not affect a pre-existing [URE3]. When overexpressed, the catalytic domain eliminates [URE3], while regular expression does not cure it. [URE3] and the catalytic domain of Ure2p may therefore co-exist in the same cell if the full-length Ure2p is also produced.

When overexpressed in yeast cells, the PFD eliminate a pre-existing [URE3] although it should facilitate its rising! When expressed at 'physiological levels', more than 50% of the cells (10 of 18 colonies tested) were found to be found [usa–], indicating loss of [URE3].

**Sup35p contains a prion domain**

The *SUP35* gene is essential, in contrast to *URE2*. Like *URE2*, it contains several domains (fig. 11). The C terminus domain is the catalytic domain, homologous to EF1$\alpha$. This domain is necessary to ensure translation termination. The replacement of the wild-type *SUP35* gene in a haploid strain by the C-terminal domain beginning at the 254th amino acid does not allow propagation and maintenance of [PSI]. However, overexpression of the N-terminal domain of 253 amino acids in a [psi–] strain induces the appearance of [PSI]. An N-terminal fragment of 123 amino acids is sufficient to induce and to maintain [PSI]. Interestingly, overproduction of the Sup35 PFD alone induces prion formation even more efficiently than overproduction of the complete protein [27].

The protein has been described as a three-domain protein: an N-terminal domain of 123 amino acids responsible for the prion phenotype, a catalytic domain in the C terminal of amino acids 254 to 685; and between them, an optional domain M, the so-called 'hinge domain' (fig. 11).

This division is very similar to that of the *URE2* gene. Furthermore, the N-terminal domain of Sup35p is also rich in asparagine, glutamine, glycine and tyrosine. It contains five imperfect repetitions of a nonapeptide, QGGYQ(Q)QYNP.

**Intragenic modifiers of [PSI]**

1) *PNM2:* an intragenic suppressor of [PSI]. Mutations in the *SUP35* gene affecting [PSI] generation have been sought. A mutant, called *PNM2*, corresponds to the substitution of a glycine by a glutamic acid in position 58, in the prion domain of Sup35p, in the second nonapeptidic repetition [22]. The expression of *PNM2* leads to the loss of [PSI]. *PNM2* can have variable effects on [PSI], depending on the prion variant in the cell [28]. *PNM2* does not abolish the properties of the Sup35 prion, but modifies them.

2) *ASU* mutants and the importance of the repeated motives of Sup35p. Another class of intragenic mutants of Sup35p has been discovered: antisuppressor mutants (*ASU*), While *PNM* mutations cure a pre-existing [PSI] element, *ASU* mutants restore a functional SUP35 phenotype, but do not eliminate the [PSI] prion [29]. This phenotype is epistatic to the [PSI] suppressor.

The various *ASU* and *PNM* mutants present substitutions in the prion domain, most often replacement of a glutamine by an arginine. But besides the nature of the amino acids, the number of nonapeptidic repeats is also important. Mutants with reduced repeat number behave as *ASU* mutants [30]. On the other hand, a mutant with two supplementary repeats leads to the high-frequency appearance of [PSI] colonies, by directly destabilizing Sup35p and facilitating its conversion into the prion form. The repeats in Sup35p are similar than those of the prion protein (PrP). Some human transmissible spongiform encephalopathies are associated with extensions of repeats in PrP, while some mutant PrPs with a reduced number of repeats are less susceptible to prion diseases. These alterations may affect the thermodynamic stability of the prion protein, and modify the energy quantities necessary for the conversion of the normal to the prion conformation [31–33], although this is not true for all the mutant proteins.

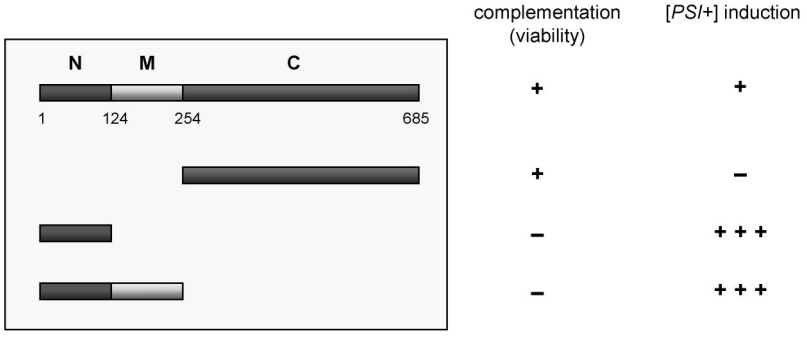


Figure 11. Sup35p contains three domains: a prion-inducing domain (red box), a functional domain (green) and a hinge domain (yellow box).

## Cellular and physicochemical analysis

### Proteinase K resistance

Ure2p presents differential resistance to proteinase K (PK) digestion in a wild-type and a [URE3] strain [24]. However, this resistance is not comparable to the resistance acquired by the mammalian prion [24, 34, 35]. Partially digested PrPSc presents a characteristic degradation product called PrP 27–30, which can resist the action of protease for several hours, a treatment that would completely degrade the Ure2 protein present in a [URE3] extract (table 1).

The other yeast prion [PSI] has also been investigated for differential PK resistance. PK resistance of Sup35p$^{[PSI+]}$ is higher than that of Sup35p$^{[psi-]}$ [35]. The resistant core of Sup35p corresponds to the N-terminal domain of the protein, which contains the octarepeats [36]. However, this resistance is, as for Ure2p, much less than for PrP$^{Sc}$. The digestion leads to a general degradation of the Sup35p protein, with less efficiency in the case of Sup35p$^{[PSI+]}$. This resistance could be acquired by the aggregation of the protein, making it less accessible to the protease.

### Sup35p of differential solubility

Centrifugation assays of cellular extracts of [psi–] and [PSI] strains have demonstrated that Sup35p$^{[PSI+]}$ is less soluble than Sup35p$^{[psi-]}$ (fig. 12) [35, 37]. The [PSI] phenotype leads to the aggregation of the Sup35p protein, which can form high-molecular-weight complexes, composed essentially of the Sup35p protein.

### In vitro propagation of [PSI]

The prion hypothesis proposes that the protein converted into the prion form can convert the normal protein by an autocatalytic process. Using the insolubility properties of the Sup35p protein, which differentiates the wild-type protein from the prion form, this conversion process can be observed in vitro (fig. 13).

Table 1. PK resistance of mammalian and yeast prion proteins [24, 34, 35]

| | Ure2p | PrP |
|---|---|---|
| Quantity of proteins | crude extract; 150 µg total proteins/60 µl; mix 2 µg PK + 110 µg crude extract in 60 µl | brain homogenate of Syrian hamster; 1 mg/ml of total protein |
| PK concentration | 33.3 µg/ml | 100 µg/ml |
| Wild-type protein | degraded completely within 1 min | |
| Prion protein form | degraded partially after 1 min (intermediate fragment of 30 kDa), completely between 15 and 30 min | fragments of 27–30 kD a (PrP 27–30) resistant for more than 4 h |

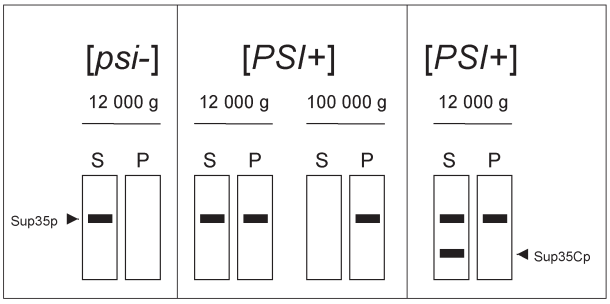


Figure 12. Sup35p solubility in protein extracts from [psi–] and [PSI+] strains. Immunodetection of Sup35 protein in centrifuged protein extracts demonstrates that the protein is soluble in a [psi–] strain extract and precipitates in a [PSI+] strain extract. However, the prion phenotype does not affect the solubility of a prion domain-deleted Sup35 protein. S, supernatant; P. pellet.

By mixing a protein extract of a [psi–] strain with a protein extract from a [PSI] strain which expresses a fragment of Sup35p corresponding to the NM domains, the full-length Sup35p protein becomes gradually insoluble. Moreover, the conversion efficiency depends on the concentration of [PSI] protein extract added, there being a threshold level of [PSI] extract necessary to convert the entire wild-type Sup35p protein.

Moreover, this in vitro conversion process can be propagated. The Sup35p protein, which has been converted into the insoluble form, can be added to a new protein extract from a [psi–] strain and convert the soluble Sup35p protein to the insoluble form. This conversion can be repeated several times with the new insoluble form of Sup35p. This conversion process is highly efficient. In contrast to the in vitro conversion of PrP, this conversion can occur in the presence of a large (about sevenfold) excess of the soluble protein.

### In vivo observations of chimeric green fluorescent protein fusions

1) SUP35 green fluorescent protein (GFP) fusions. The expression of the Sup35p protein fused to GFP, or the N domain of Sup35p fused to GFP leads to formation of foci in a [PSI] strain, whereas this fluorescence is diffuse in a [psi–] strain (fig. 14) [37]. These foci can be transmitted in the buds of dividing cells. However, an N domain-truncated Sup35p protein presents a diffuse fluorescence in both contexts, [psi–] and [PSI]. Thus, the in vivo aggregation of the protein depends on the presence of the prion domain.

2) URE2-GFP fusions. Cellular localization of the Ure2p protein has been performed by expressing a *URE2-GFP* fusion mutant gene in wild-type and [URE3] cells [26]. This gene was expressed under physiological conditions, under control of the *URE2* gene promoter and on a single-

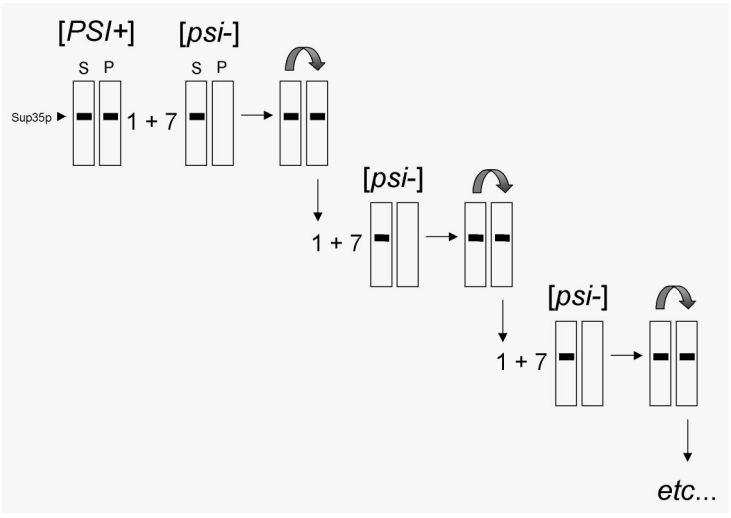


Figure 13. In vitro conversion of the Sup35$^{[psi–]}$ protein. By mixing a large excess of a protein extract from a [psi–] strain with a protein extract of a [PSI+] strain, the soluble protein can be converted to an insoluble form. That insoluble form can then be used to again convert the soluble protein. S, supernatant; P. pellet.

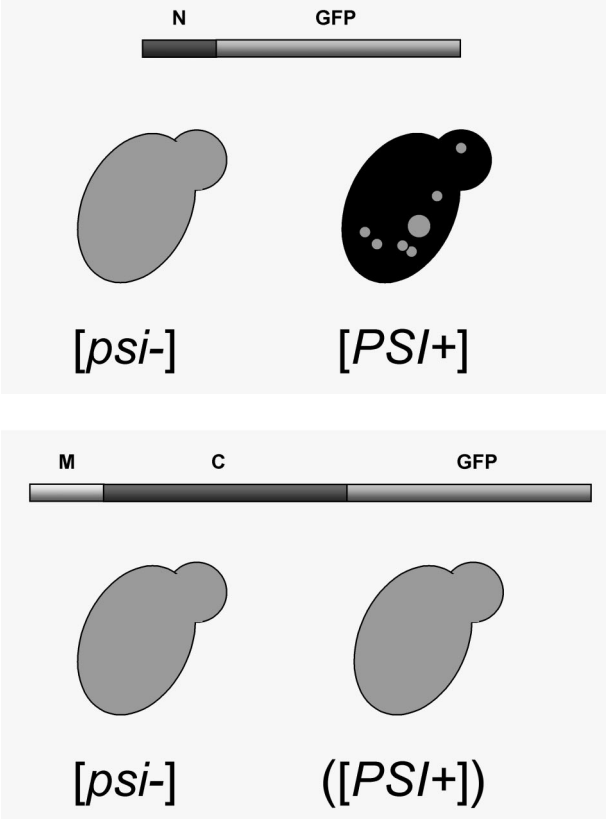


Figure 14. In vivo aggregation of Sup35p. A fusion protein of the N domain of Sup35p fused to GFP presents foci in a [PSI+] strain and is diffuse in a [psi–] strain. The expression of the Sup35MC domains fused to GFP is diffuse, whatever the phenotype of the transformed strain.

copy plasmid. In a wild-type strain, fluorescence is diffuse and localised in the cytoplasm. However, fluorescent foci of variable number and size could be observed in [URE3] cells. Curing cells on guanidium chloride the fluorescence pattern becomes diffuse again and foci are eliminated.

Expression of the PFD alone fused to GFP leads to the observation of these foci in [URE3] cells. On the other hand, expression of the C-terminal domain alone fused to GFP never leads to this aggregation pattern, whether the strain has a wild-type or [URE3] phenotype, demonstrating that the prion domain is necessary for in vivo aggregation.

A model has been proposed in which Ure2p is aggregated specifically in cells presenting the prion phenotype [38]. This protein aggregation which needs the PFD would lead to a loss of function of the protein.

However, some contradictory results have been published, because a [URE3] bearing a *URE2-GFP* allele does not present any clumps although the fluorescent protein is in the prion conformation [25].

3) The prion model of yeast: auto-catalytic aggregation of a protein. Cellular studies have provided clues to establish a mechanism of propagation of the prion. The properties of protease resistance and insolubility demonstrate that the prion protein is less acessible to protease through a polymer assembly mechanism that could be associated with a modified tertiary structure of the protein. Moreover, curing by guanidine has been related to resolubilisation of the protein. The prion form of the protein would then be an aggregated form of the normal cellular protein, which could not accomplish its function.

## Extragenic modificators of [PSI] and role of the chaperone proteins

### Role of a molecular chaperone: Hsp104

The *HSP104* gene has been described as playing a major role in the maintenance of the [PSI] element [39]. This effect can be definitive (curing) or reversible and depends on the expression level of the *HSP104* gene in the cell. A moderate increase in *HSP104* gene expression in a [PSI] strain restores a [psi–] phenotype. This effect is reversible: if the level of expression of the *HSP104* gene becomes normal again, the [PSI] phenotype reappears. However, overexpression of the *HSP104* gene eliminates the [PSI] phenotype. Deletion of the *HSP104* gene or the expression of a mutant allele of *HSP104* mutated at both ATP-binding sites also cures the [PSI] phenotype, even when overexpressing the *SUP35* gene. A role for *HSP104* in [URE3] maintenance has been also described more recently [40].

Maintenance and propagation of the [PSI] phenotype depend on a balance between the levels of Sup35p and Hsp104 proteins. Hsp104p belongs to a thermal shock protein family. This family, called Hsp100 or Clp, groups proteins of molecular weight 100–110 kDa [41]. Hsp104 has been highly conserved during evolution [42]. Genes homologous to *HSP104* exist in other eukaryotes, such as *Schizosaccharomyces pombe* and *Arabidopsis thaliana*, and also prokaryotes, such as *Escherichia coli* (ClpA) [43].

In *S. cerevisiae*, Hsp104 is a non-essential protein, induced by a stress, especially a thermal shock [44]. At a molecular level, Hsp104p is responsible for the resolubilisation of aggregated proteins after a thermal shock [45]. This protein is essentially expressed at the end of the exponential growth phase, during the stationary phase and during sporulation [44]. It has been characterised genetically as a chaperone protein [46], being involved in the folding of other proteins.

### Hsp104 resolubilises Sup35p protein aggregated in a [PSI] strain

1) Influence of Hsp104 on solubility. The aggregation of the protein Sup35p is correlated with the existence of the [PSI] element, and this aggregation depends on two parameters: the presence of the N domain of Sup35p, in *cis*, and normal expression of *HSP104*, in *trans.* This state of aggregation would then provoke, by a sequestration effect, a decrease in translation termination activity by the protein Sup35p. The activity of Sup35p is indispensable, thus in [PSI] cells, either the inactivation is not complete, or there is sufficient soluble protein in the cell to ensure translation termination activity.

An increase or the deletion of *HSP104* in a [PSI] strain leads to resolubilisation of the Sup35p protein, which cannot be pelleted by centrifugation assay, and the disaggregation of Sup35-GFP foci in a [PSI+] cell (fig. 15). Resolubilisation of Sup35p in protein extracts of a [PSI] strain by Hsp104 is thus correlated with the loss of the [PSI] element, leading to a reversion to the [psi–] phenotype.

Solubilisation of aggregated proteins by Hsp104 provides a good explanation for the cure of the [PSI] phenotype: Hsp104p would solubilise Sup35p aggregates and the protein would become active again. However, the curing effect is also provoked by the elimination of *HSP104*. Two models have been proposed to explain the necessity from minimal expression level of *HSP104* for the preservation of the [PSI] element.

The first model suggests that Hsp104p would allow Sup35p to acquire a transitional unstable state necessary for forming the prion convormation [37] (fig. 16A). In this state, Sup35p can bind a pre-existing [PSI] element, corresponding to a seed of aggregated Sup35 proteins. In the absence of such a seed, the protein would return to the normal state. In an *HSP104*-deleted strain, Sup35p would never achieve this state and could not bind any [PSI] ele-

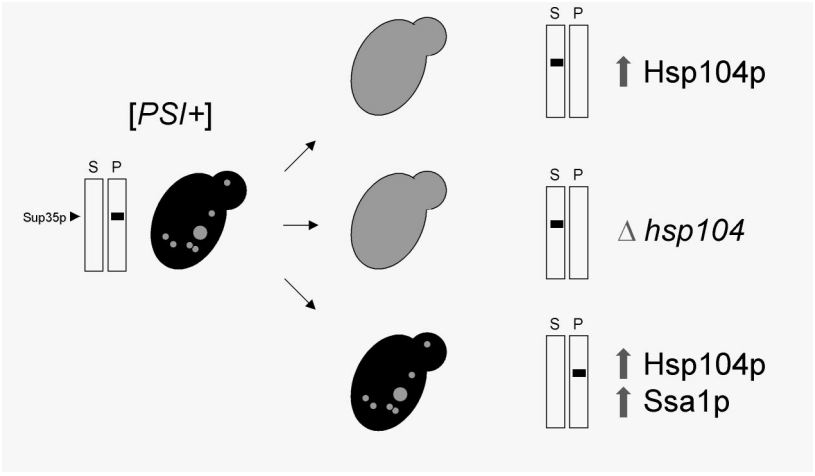


Figure 15. Effect of the overexpression or the elimination of Hsp104p in a [PSI+] strain, and of the simultaneous expression of Ssa1p (Hsp70p) and Hsp104p. S, supernatant; P, pellet.

ment (fig. 16C). In contrast, overexpression of *HSP104* would destabilise the stoichiometry necessary for the assembly of Sup35p aggregates, leading to the elimination of [PSI] (fig. 16B).

The second model stipulates that Hsp104 is not involved in the conversion of Sup35p. However, constitutive expression of Hsp104p would be necessary for the partial dissociation of Sup35p aggregates [35], producing new extremities capable of binding neo-synthesised Sup35 proteins (fig. 17A). These numerous aggregates could then be transmitted to daughter cells. In the absence of *HSP104*, [PSI] aggregates would be lost by dilution in the cellular population (fig. 17C). However, overexpression of *HSP104* would lead to complete resolubilisation of Sup35p (fig. 17B).

**Another extragenic modifier of [PSI]: Hsp70p**

Other heat shock proteins are involved in the aggregation of proteins, such as Hsp70p [47]. Hsp70p is an essential protein expressed at all temperatures, in contrast to Hsp104p, which is expressed after a thermal shock [48]. These two proteins play a role in the dissolution of protein aggregates, but they act at different levels of aggregate formation. While Hsp104p solubilises proteins that are already aggregated (curative role), Hsp70p acts by preventing aggregate formation. Hsp70p alone cannot reactivate proteins that are already aggregated. *HSP70* corresponds to several multigenic subfamilies: *Ssa* and *Ssb*.

**The subfamily Ssa**

In yeast, Ssa is formed by four homologous genes. These genes are very similar but have different expression levels [49–51]. *Ssa1*, *3* and *4* are, like *HSP104*, involved in the thermal shock response [52]. Simultaneous deletion of the four *Ssa* genes is lethal, but the expression of only one gene is sufficient for viability.

In strains lacking *Ssa1*, *Ssa2* or both, the stability of [PSI] is not affected [53]. The overexpression of Ssa1p itself has no effect on [PSI], but interferes with the effect of the overexpression of *HSP104*, by abolishing or attenuating its curing effect (fig. 15) [54]. *HSP104* is epistatic to *Ssa1*: elimination of *HSP104* cures [PSI], whatever the level of *Ssa1* expression. Ssa1 has then an antagonistic effect on *HSP104* in the maintenance of [PSI], and also on the protection by *HSP104* against thermal shocks. The molecular mechanism is not very well understood and seems to be very complex, depending on the genetic background of the cells.

**The subfamily Ssb**

The subfamily *Ssb* of *HSP70* genes is composed of two identical proteins: Ssb1p and Ssb2p [55]. In contrast to *Ssa1*, Ssb proteins are not indispensable, and are not induced by a thermal increase. Ssb is involved in protein folding during the translation process [55, 56] and in protein degradation [57]. *Ssa* and *Ssb* have diverged functionally during evolution [58]. The role played by Ssb on [PSI] is antagonistic to that of Ssa1 [59]: overexpression of Ssb increases twofold the curing effect of [PSI] by *HSP104* overexpression. As with Ssa, overexpression of Ssb has no effect on [PSI] by itself. However, its deletion leads to an increase in the appearance of the [PSI] phenotype. Ssb is associated with the ribosome, and so could interfere with the readthrough effect associated with [PSI], or could facilitate the elimination of proteins mistranslated through a stop codon.

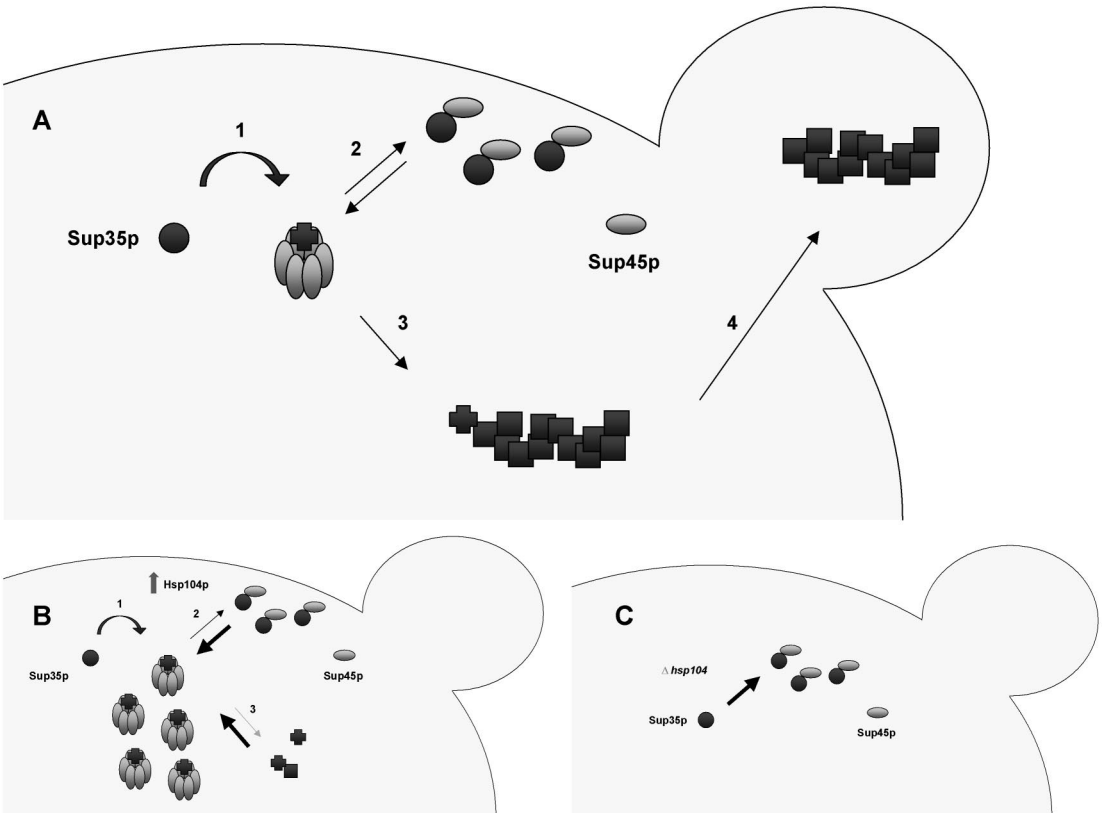


Figure 16. Transitional-state model of the action of Hsp104p. (*A*) Hsp104p interacts with Sup35p, to create an intermediary and unstable conformational state (1). In that state, Sup35p can either return to an active form and ensure biological function (2) or be assembled in the pre-existing sup35p aggregates (3). Aggregates would then be transmitted to daughter cells. (*B*) In a cell overexpressing Hsp104p, cure of the prion phenotype would be due to a stoichiometric destabilisation allowing the assembly of Sup35P (2 and 3) or to a resolubilisation of the aggregates. (*C*) In a cell no longer expressing Hsp104p, the transitional state is never reached, preventing aggregate formation.

**Mechanism of curing by guanidine**

[URE3] and [PSI] can be cured in the presence of 1–5 mM of guanidine chloride. The molecular mechanism is not, however, well understood. One of the hypotheses is a direct action of guanidinium on Sup35p aggregates inside the cell [12]. Guanidine is a chaotropic agent commonly used as an in vitro denaturating agent of proteins. However, guanidine concentrations effective at curing are much lower than those usually used in vitro.

Another hypothesis suggests that guanidine could activate genes in the stress response, such as *HSP104*, whose overexpression is capable of eliminating the [PSI] element.

Curing of [PSI] needs cellular division, and begins after six generations [60]. A segregational model of seeds has been proposed [60]. The guanidine would act by blocking the replication of the prion element (fig. 18). In the cell, the 'seeds' of Sup35p aggregates would be responsible for transmission of [PSI] to daughter cells. During cellular division, seeds are transmitted to daughter cells. New seeds generated in these cells would then propagate the [PSI] phenotype to the following generations. The guanidinium would block the replication step to the seeds. The seeds would then be 'diluted' in the cell culture and disappear after several generations. This model corresponds to experimental observations. Mathematically, the initial average number of seeds in a [PSI] cell would be 61 [60], but this number can vary depending on the mathematical model used.

**Factors affecting [URE3]**

The NCR regulator Mks1p has a strong effect on prion initiation [61]. Overexpression of the protein increases the frequency of [URE3] whereas deletion of *MKS1* prevents [URE3] initiation. When introduced by cytoduction, [URE3] may propagate in a mutant *mks1* strain. Mks1p is implicated in the RAS-cAMP pathway. A direct interaction between Mks1p and Ure2p remains to be established. As previously mentioned, *HSP104* also plays a major role in the propagation of [URE3], although its overexpression has no effect on it. Another chaperone, Ydj1, which is mainly involved in the import of proteins

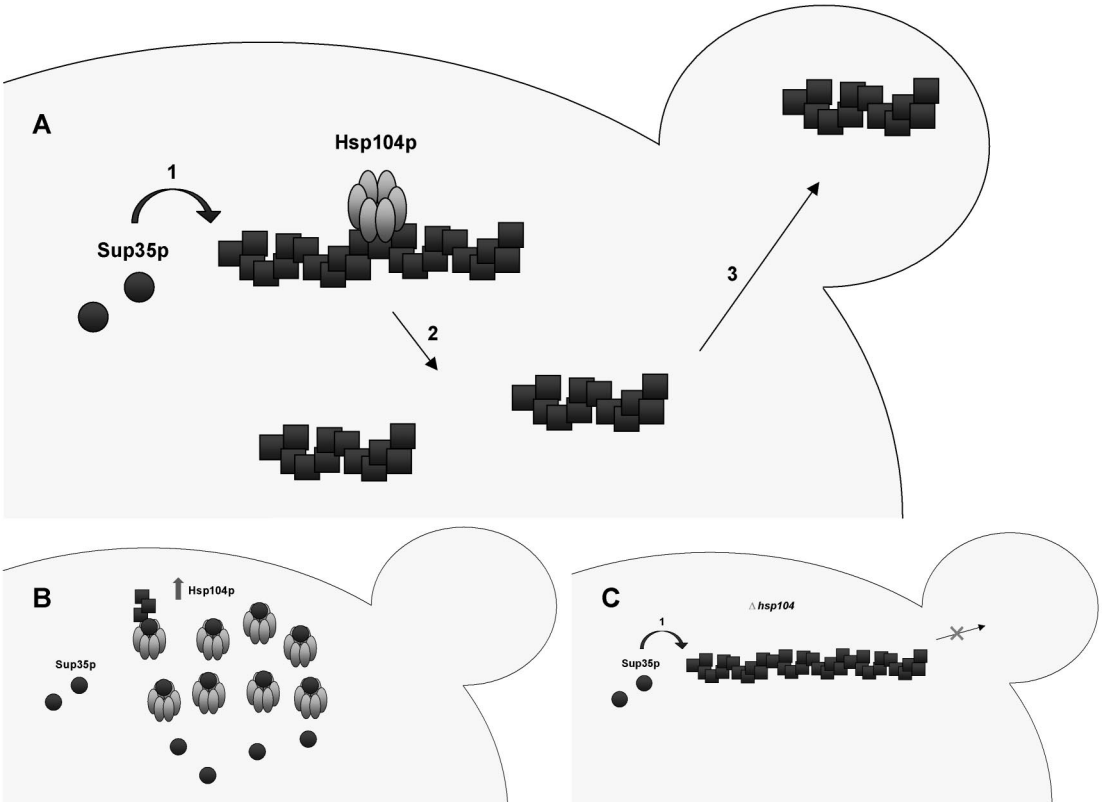


Figure 17. Partial dissociation of aggregates model. (*A*) Hsp104p would only have a disaggregating role (1), allowing the generation of smaller particles (2), efficiently transmitted to daughter cells (3). (*B*) An increase in the level of expression of Hsp104p leads to the complete resolubilisation of Sup35p aggregates. (*C*) In a cell that does not express Hsp104p, Sup35p aggregates are not fractionated and cannot be transmitted to daughter cells.

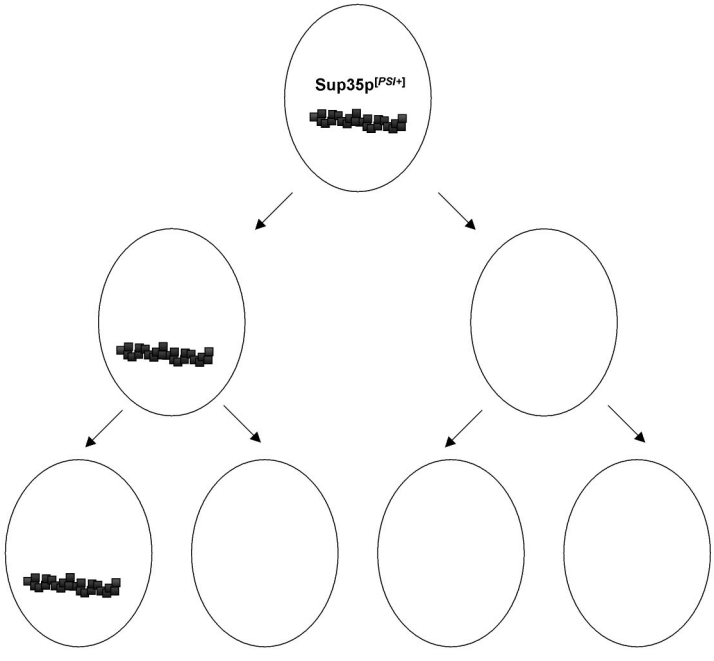


Figure 18. Segregational model of [PSI+] transmission. Guanidinium would block a replication step of Sup35p aggregation, and these aggregates would be lost by a dilution effect after several generations.

into mitochondria, destabilises [URE3] when overexpressed [40]. However, no such effect on [PSI+] has been reported.

These observations demonstrate that if some factors, like Hsp104, play a major role in [URE3] and [PSI] prion propagation in yeast, some other factors can be prion specific. This suggests that cellular control of prion replication can depend on common mechanisms but also have distinct subtle regulation.

## Biochemical analysis

### Sup35p

The Sup35 protein fused to a poly-histidine tag was purified as a soluble protein [62]. That protein could then self-assemble into amyloid fibres, after a long incubation time (about 60 days). Depending on the buffer composition, these fibres presented diameters ranging from 10 to 17 mm.

The different domains of Sup35p have also been studied biochemically; the C domain of Sup35p, which inhibits [PSI] by blocking aggregation [18, 35, 37] and the M domain, which does not induce [PSI], are soluble. The C domain can give rise to amorphous aggregates in some buffers [62]. However, the N or the NM domains can form proteases-resistant amyloid fibres. These fibres can assemble laterally.

### Is the aggregated form the prion form?

Do these amyloid fibres correspond to the prion form of Sup35p? The in vitro characterisation of different alleles of *SUP35* provides some information. In vitro, the Sup35$^{PNM2}$ would cure [PSI] not by destabilising aggregates, but by bounding these aggregates and blocking their propagation (fig. 19, left). In a protein extract of a [PSI] strain expressing an *ASU* mutant, this protein remains in the soluble fraction. GFP fusions to *ASU* mutants confirmed the homogenous distribution of such protein inside the cell. So, an *ASU* mutant protein is propsed to be unable to bind the Sup35p aggregates and would remain soluble in the cell, ensuring Sup35p function (fig. 19, right), The suppressor phenotype is transitorily masked, and the loss of the Sup35 *ASU* mutant restores the [PSI] phenotype. In vitro, *ASU* mutant protein has a slower kinetics of amyloid fibre formation than the wild-type protein.

The kinetics of amyloid fibre formation in vitro of these mutants is also affected by a modification in the number of repeats: mutants with increased repeat numbers form amyloid fibres faster than does the wild-type protein. A mutant with less repeats forms these fibres very slowly, and never reaches the maximum level obsserved with the wild-type Sup35p [30].

## Ure2p

### Ure2p can form amyloid fibres

An early study considered the prion domain of Ure2p, necessary for the induction and maintenance of [URE3] [64]. This N-terminal domain of Ure2p, corresponding to the first 65 amino acids, was chemically synthesised [65]. This fragment can spontaneously form amyloid fibres 4.5 nm in diameter. Moreover, these fibres can provoke the formation of amyloid fibres by the native Ure2 pro-

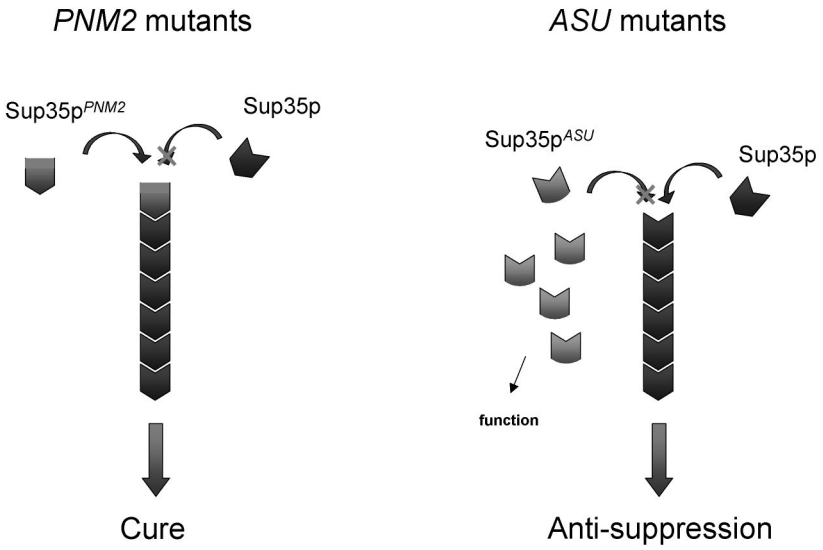


Figure 19. *ASU* and *PNM* mutants. *PNM2* mutants links aggregates and block their assembly. *ASU* mutants cannot link aggregates and ensure the catalytic function. Their expression hides the [PSI+] phenotype but does not eliminate the prion element. It is an antisupressor phenotype.

tein. Digestion of these fibres by PK releases a resistant core of about 10 kDa containing the PFD.

A second study characterised filaments of Ure2 protein expressed in E. coli [66]. That protein was purified in a soluble form. Furthermore, it was found in solution as a monomer, dimer and tetramer, depending on concentration, suggesting that these various forms are in equilibrium. This protein is able to assembly spontaneously as amyloid fibres.

This assembly follows three steps: lag phase of several hours, followed by an exponential phase an a plateau. The lag phase is shortened when the concentration of Ure2 protein increases, suggesting assembly is limited by the nucleation step. If preformed fibres of Ure2p are added to soluble Ure2 protein, assembly occurs without a lag, confirming that the assembly process is nucleated and concentration dependent. To determine if in vitro assembly of Ure2p is associated with a structural modification, soluble Ure2p and fibres of Ure2p were treated with various proteases. The aggregated form of Ure2p resists the action of proteases much longer, but released fragments have the same size as with soluble protein. This demonstrates that aggregation decreases the access of proteases but that no major conformational change is associated with the assembly of Ure2p. However, the intrinsic fluorescence of the protein during its aggregation reveals that the C-terminal part of the protein could undergo a slight structural change. Nevertheless such a change would not be sufficient to modify the action of proteases.

Amyloid fibres were also obtained with the Ure2 protein or with just the PFD fused to glutathione s-transferases (GST) [67]. Interestingly, the assembly of the purified PFD-GST fusion is associated with a change of conformation of GST, which contains more $\beta$ sheets. In all cases, the aggregation of the protein requires specific interactions. Two interacting domains have been characterised by two-hybrid experiments [68]. One domain is located in the first 63 amino acids of Ure2p (the PFD) and another is located in the catalytic domain, between the 152$^{nd}$ amino acid and the end of the protein. The first domain is able to interact with itself and also with the second domain. The N-N interaction has been confirmed with in vitro-translated proteins. However, no functional data are available to establish whether these N-N and N-C interactions are intra- or intermolecular and in the latter case, whether intermolecular interactions are related to the catalytic function or the prion conversion process.

### Structural characteristics of the catalytic domain of Ure2p

The three-dimensional structure of the non-prion domain of Ure2p has been obtained independently in two laboratories [69, 70]. The results are quite similar and demonstrate that the nitrogen-regulating domain is related to the GST superfamily. The absende of GSt activity could be due to the mutations of catalytic residues conserved in the GST superfamily.

## Common aspects of yeast and mamalian prions

### Prion strains

Prion strains were first described in mamals. These strains correspond to different disease expression parameters, e. g. incubation period, location of PrPSc in the brain) as well as different physicochemical characteristics of the PrPSc protein mainly detected by protease digestion [71–73]. Prion strains can be propagated in animals, and the characteristics of the prion strain remain stable after several passages through the brain.

Prion strains also exist in yeast. Strains of [PSI] have been selected, corresponding to different levels of suppression efficiency [27]. These colonies can appear spontaneously or can be selected by guanidinium hydrochloride treatment of a strong [PSI] strain. These weak [PSI] strains are mitotically less stable than strong [PSI] strains, but retain the same suppression efficiency after reintroduction in an isogenic yeast strain, even if the suppression efficiency depends on the genetic background [5].

Another non-Mendelian element of yeast, called [ETA+], is also considered a weak strain of [PSI] [74]. Expression of a fusion *SUP35-GFP* in an [ETA+] strain shows both foci and a diffuse fluorescence, whereas a strong [PSI] cell pressents essentially foci. Moreover, the weakness of the [PSI] prion [ETA+] is correlated with the level of aggregation of the Sup35 protein.

### Species barrier

A species barrier to prion strains was first characterised in mammals, and prevents the infection of one mammal species by a prion from another mammal species. This species barrier relates only to the origin of the *PRNP* gene. A *PRNP* knockout mouse bearing a *PRNP* gene from hamster will be more easily infected by the agent multiplied in a hamster than that from a mouse. Study of the Sup35 protein from different yeast species also revealed a kind of species barrier in the case of [PSI].

The *SUP35* gene is very conserved in eukaryotes. The orthologous *SUP35* genes of different yeast species that have been cloned contain a relatively well-conserved amino-terminal domain [75], rich in asparagine and glutamine (about 40%). The ability of these proteins to behave as a prion has been studied.

The NM domain of the orthologous Sup35 protein of *Pichia methanolica* was exchanged for the corresponding domain of the endogenous Sup35 protein. The chimeric protein thus obtained was functional and could promote a [PSI] phenotype. However, this [PSI] determinant could not been transmitted to a wild-type Sup35 protein. Thus

prion properties are conserved through evolution, but are extremely dependent on the nature of the *SUP35* gene [76]. These properties have also been demonstrated by expressing in *S. cerevisiae* fusions of NM domains of Sup35n protein from *P. methanolica, Kluyveromyces lactis* and *Candida albicans* fused to the catalytic domain of Sup35p of *S. cerevisiae* [75, 77]. Both endogenous and chimeric proteins can be inactivated by a prion mechanism in the cell, and can also aggregate, as observed by GFP experiments, but these prions are independent: the inactivation and aggregation of one chimeric protein does not lead to the inactivation of the other. This also demonstrates that the prion domains of the different Sup35 proteins are responsible for the species barrier.

However, this independence is not absolute. Overexpression of the *SUP35* gene of *P. methanolica* in *S. cerevisiae* can induce the appearance of [PSI] [77].

## Other prions

### PIN+

[PIN+] has been described as a non-Mendelian element of yeast, as a cytoplasmic factor required for de novo induction of [PSI] by Sup35p overexpression [78]. This element is considered as a prion because it presents the characteristics of reversible curability, dependence on the expression of Sup35 protein, and dependence on HSP104 for its maintenance. Genetic studies have suggested that [PIN+] could be a conformational determinant of Sup35p [79], but its complete characterisation remains unclear.

### Asparagine- and glutamine-rich proteins Rnq1 and New1

The research for new prion candidates in yeast has been complemented by the search for open reading frames encoding proteins with high glutamine and asparagine content. Two proteins have been found in yeast, Rnq1 [80] and New1 [75]. The function of these proteins has not been characterised. They exist in the cell in distinct states, soluble and 'pelletable', that can be transmitted by a prion mechanism. They both contain a putative prion domain, with a high asparagine and glutamine content, that, when fused to the catalytic domain of Sup35p, can mimic the phenotypic and genetic properties of [PSI] prion.

The capacity of a prion domain to transform a soluble protein to a prion species has also been demonstrated using a fusion of the prion domain of Sup35p with the glucocorticoid receptor of rat [81].

### Het-s

*Podospora anserina* is a filamentous fungus that crosses both by sexual breeding and in a phenomenon of cellular fusion, which produces hyphae, which are filaments of haploid multinucleated cells. Nine *het* genes control this fusion. One *het* locus, called *het-s*, presents an atypical genetic behaviour [82]. This allele encodes a protein, Het-s, that has been shown to present two forms, and behaves as a prion [83]. The normal form of the protein, pHet-s*, is neutral towards incompatibility. However, it can be converted to the prion form, Het-s, which has the epigenetic characteristics of a prion, such as curability, self-propagation and cytoplasmic transmission, and which is responsible for incompatibility.

## Conclusion

The power of genetic analysis available in *S. cerevisiae* combined with the innocuity of the studied phenomena allowed rapid advances in understanding the molecular mechanisms of prion proteins. The yeast models are based on cellular, biochemical and genetic studies. Cellular studies of both [PSI] and [URE3] were realized using fusion proteins between Sup35p or Ure2p and GFP. The yeast presenting the prion phenotype appears to differ from wild-type yeast by the presence of cytoplasmic aggregates. In a correlated way, after centrifugation of 15,000 g, the Sup35 protein is found in the pellet of a crude extract made with [PSI] yeast. Under the same experimental conditions, the protein is found in the supernatant of a crude extract made with wild-type yeast. The precipitated fraction thus obtained is able to initiate a new cycle of precipitation in an auto-catalytic manner.

Genetic analysis of URE2 and SUP35 revealed the presence in both cases of an amino-terminal domain rich in glutamine and asparagine that is necessary and sufficient to initiate and propagate the two 'prion' phenotypes. These domains, which were named prion domains, were purified or chemically synthesized [65]. Both show a very great propensity to assemble in a particular aggregate structure: the amyloid fibres. Once formed, these structures can also initiate the polymerization of the full-length soluble protein. This mechanism echoes the genetic property of these prion domains that induce in vivo and in trans the appearance of the prion phenotype. Introducing these structures into wild-type yeast promotes the [PSI] phenotype [84]. Although different interpretations can be drawn from this experiment, it is in fact the first direct experiment demonstrating the correctness of the 'protein-only' hypothesis.

These results seem to show that the prion phenotypes are due to polymerisation mechanisms. These structures in the cell have still to be characterised.

In vivo, [PSI] and [URE3] cannot propagate without specific partners. A genetic screen for factors affecting the stability of [PSI] identified *HSP104*, the absence of which, neither [PSI] nor [URE3] can be maintained. We

do not yet know the role played by *HSP104* in the formation of amyloid structures in vitro.

Yeast prions do not behave as pathogenic agents in yeast and should not be compared to infectious and deleterious agent such as mammalian prions. We therefore propose using the term 'propagon' instead of yeast prion. The mammalian prion could be considered as a particular propagon with specific toxicity, but the general paradigm 'protein only' may correspond to a vaster domain: the propagon world.


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