

# Prions: in[PSI]<sup>+</sup> from yeast

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## Summary

Prions, responsible for such neurodegenerative diseases as mad-cow disease and scrapie, are proteins that transmit a trait without the use of conventional genetic material (DNA or RNA). Prions' infectivity arises from their tendency to adopt an altered conformation that induces the normally folded prion protein to change its shape as well. My lab started out studying yeast protein chaperones, which help proteins fold properly or, in the event of environmental stress, refold properly. One chaperone, Hsp104, has the unique role of breaking up protein aggregates. The discovery that Hsp104 mediates a yeast phenotype, called [PSI]<sup>+</sup>, that shows a prion-like pattern of inheritance altered the course of my work dramatically. My lab has since found that [PSI]<sup>+</sup> arises from the altered conformation of a translation termination factor, Sup35, resulting in insoluble aggregates. Paradoxically, either deletion or overexpression of Hsp104 abolishes [PSI]<sup>+</sup>. One of our more astounding findings was that whereas mammalian prions are harmful, yeast prions can be beneficial. In one fungus, prions are even essential for survival during part of its life cycle. Thus, in addition to providing a simple system in which to study prion genetics, yeast has broadened our view of prion function overall.

## Introduction

Prions were first discovered as the infectious agents responsible for such neurodegenerative diseases as scrapie, mad cow disease, and Creutzfeldt-Jakob disease<sup>1</sup>. They incited immediate interest and controversy since they appeared to pass on the disease phenotype without the use of DNA or RNA, henceforth considered the only molecules capable of passing on a trait.

The prion hypothesis, set forth by Stanley Prusiner, states that prions propagate themselves via a protein-only mechanism in which prion protein of an altered conformation induces normally folded proteins to adopt the prion form<sup>1</sup>. The prion protein in mammals is called PrP and appears to be solely responsible for a host of diseases with differing pathologies<sup>1</sup>.

Protein chaperones are proteins that help other proteins acquire their proper conformation<sup>2</sup>. Many chaperones are upregulated in response to such environmental stressors as heat, metals, and ethanol<sup>3</sup>, hence the common prefix Hsp (heat-shock protein). Several families of protein chaperones have been identified, including hsp110, hsp100, hsp90, hsp70, hsp60, hsp40, hsp10, and small hsp's<sup>3</sup>. Hsp70, with the help of Hsp40, binds to proteins as they are being made by the ribosome to prevent inappropriate folding before complete protein synthesis<sup>2</sup>. Hsp60 is a barrel-shaped structure that acts to sequester misfolded proteins from other proteins, giving them a chance to refold without risk of aggregation<sup>2</sup>.

This review charts the course of my work over the past 13 years, and the remarkable turn it took when two seemingly unrelated fields collided.

## A novel chaperone, a novel function

My lab started out attempting to identify and characterize protein chaperones in yeast. One question we addressed was that of thermotolerance. Although yeast prefer to grow at 25°C, they are able to withstand long periods at significantly higher temperatures. Because chaperones help cells survive exposure to stressors such as heat, it seemed logical that a chaperone would be responsible for this thermotolerance.

We sought out the protein responsible for this thermotolerance by comparing the proteins present in yeast cells grown at 25°C and cells that had been heat shocked by growing at 39°C. In addition to a few known chaperones, one previously unidentified 104-kilodalton (kDa) protein was upregulated<sup>4</sup>. We named this protein Hsp104<sup>4</sup>.

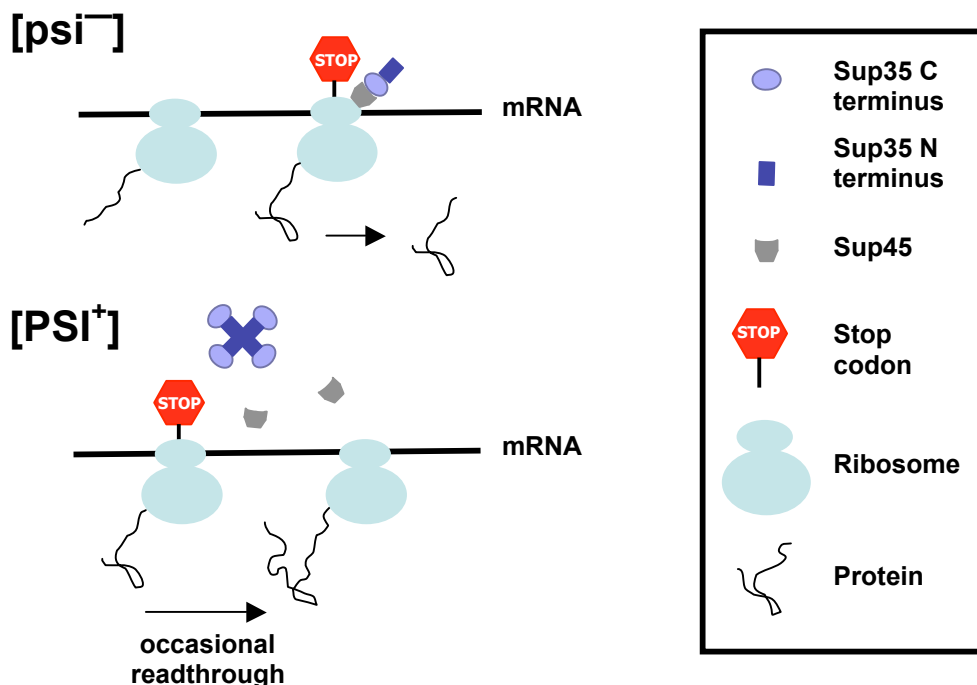
Hsp104 is part of the highly conserved Hsp100 gene family<sup>5</sup>, homologues of which are present in bacteria, trypanosomes, plants, fungi, and mammals<sup>5,6</sup>. Its two ATP-binding sites show high similarity to the bacterial ClpA/ClpB proteases and are both essential for proper function<sup>5</sup>.

Studies on hsp104 null mutants demonstrated the many key roles that this protein plays. Hsp104 is responsible for endowing spores with their ability to withstand extreme heat<sup>7</sup> and is essential in growing cells for what is known as induced thermotolerance. Induced thermotolerance results when cells are gradually shifted to high temperatures, versus basal thermotolerance, which is measured by shifting from normal temperatures to high temperatures directly<sup>4</sup>. Hsp104 is also upregulated in response to ethanol and certain metals, bringing with it the predicted tolerance to high heat<sup>7</sup>.

Compared to other known chaperones, the mechanism of Hsp104 is unique. Rather than preventing improper folding of newly made or denatured proteins, Hsp104 breaks up protein aggregates that may form when proteins denature at high heat<sup>8</sup>.

An exquisite example of form meets function, Hsp104 becomes arranged into ring-shaped hexamers in the presence of ATP<sup>5</sup>. This shape immediately brings to mind the barrel shapes of Hsp60 and proteasomes, both of which engulf other proteins to either help them fold or digest them<sup>9</sup>.

\* This paper was written for BIO324 Molecular Neuroscience. In this assignment, Jennifer McGuire role-played a noted cell biologist, Susan L. Lindquist, and wrote a state-of-the-art review article on Dr. Lindquist's research field, as is she were Dr. Lindquist herself. She then presented a PowerPoint seminar as Dr. Lindquist in an annual public student research conference "NeuroFrontiers" held at Lake Forest College.



**Figure 1. The [PSI<sup>+</sup>] phenotype**

In [psi<sup>-</sup>] (normal) cells, Sup35 works in conjunction with Sup45 to terminate protein translation. In [PSI<sup>+</sup>] cells, the N-termini of Sup35 aggregate, meaning there is less Sup35 available for translation termination and the ribosome reads through some stop codons. Adapted from Uptain and Lindquist (2002).

#### The prion connection

Two yeast factors discovered in the 60's and 70's, [PSI<sup>+</sup>] and [URE3], showed a strange pattern of inheritance that could not be explained until recently<sup>9</sup>. Instead of the 3:1 ratio expected from Mendelian inheritance, these factors showed a 1:1:1:1 inheritance; that is, if the parent cell displayed either of these phenotypes, all four progeny did too. One possible explanation for the [PSI<sup>+</sup>]/[URE3] phenomenon was put forth in 1994 when Reed Wickner made the brilliant suggestion that perhaps, akin to the infectivity seen in mammalian prions, these factors followed a protein-only pattern of inheritance<sup>11</sup>.

Several lines of evidence supported this hypothesis. For one, overexpression of Sup35 results in [PSI<sup>+</sup>] cells<sup>12</sup>, just as overexpression of PrP results in prion disease in mice<sup>13</sup>. For another, both of these factors are metastable, spontaneously disappearing at rates higher than expected from random mutations<sup>10</sup>. Finally, cells can be "cured" of these factors through low concentrations of guanidine hydrochloride, a protein denaturant<sup>10</sup>.

The [PSI<sup>+</sup>] phenotype results in some stop codons being read through (Fig. 1). It is the product of the Sup35 protein<sup>10</sup>, which, along with Sup45, makes up the translation termination factor<sup>14</sup> (Fig. 1). The [URE3] phenotype causes cells to take up catabolites during nitrogen abundance that it normally takes up only during nitrogen starvation<sup>11</sup>. It is associated with the protein Ure2, which prevents transcription of a certain catabolite transporter when nitrogen is abundant<sup>10</sup>.

As described above, prions were first defined as proteins capable of a protein-only model of infectivity. Wickner's proposal broadened this definition

to include a protein-only model of inheritance while challenging the dogma that only nucleic acids can act as genetic elements.

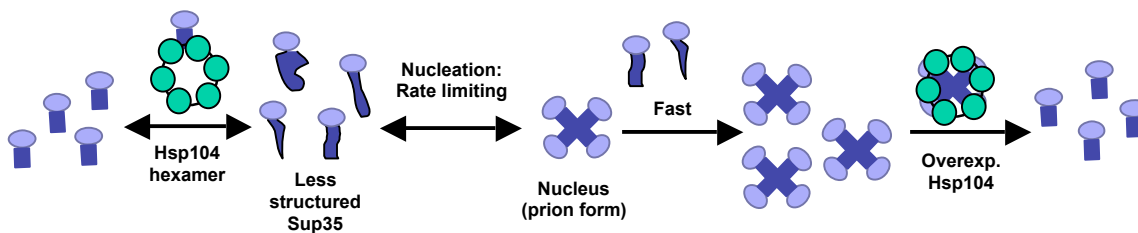
#### Hsp104 and [PSI<sup>+</sup>]

The burgeoning field of yeast prions abruptly became of the utmost interest to my lab when its relationship with a now familiar member of my work, Hsp104, was revealed. The connection was made when Yury Chernoff, working in association with Bun-ichiro Ono, Sergei Inge-Vechtomov, and Susan W. Liebman, screened an *S. cerevisiae* genomic library for genes that modified [PSI<sup>+</sup>]. Just one clone from this library suppressed the [PSI<sup>+</sup>] phenotype: Hsp104<sup>15</sup>.

As it turns out, Hsp104's relationship with [PSI<sup>+</sup>] is rather paradoxical: while overexpression of this chaperone makes [PSI<sup>+</sup>] cells [psi<sup>-</sup>], so does removing Hsp104 altogether<sup>15</sup>. In fact, once the plasmid bearing Hsp104 is lost, cells are unable to become [PSI<sup>+</sup>]<sup>15</sup>, demonstrating the essential role of Hsp104 in maintaining this phenotype.

Investigation of yeast prions immediately lent support for the prion hypothesis that proteins can act as genetic elements. The solubility of Sup35 correlates to whether a cell is [PSI<sup>+</sup>] or [psi<sup>-</sup>]. In [psi<sup>-</sup>] cells, most Sup35 is soluble while in [PSI<sup>+</sup>] cells, a substantial portion of the Sup35 is insoluble<sup>16</sup>. [PSI<sup>+</sup>] cells contain Sup35 aggregates in the cytosol detectable by GFP<sup>15</sup>.

That [PSI<sup>+</sup>] is caused by protein aggregates immediately makes clear the role of Hsp104 in mediating this phenotype, or at least in abrogating it upon overexpression. The need for Hsp104 in maintaining the [PSI<sup>+</sup>] is less easy to explain. One possibility is that this chaperone may convert normal Sup35 protein to a form that forms aggregates (Figure 2



**Figure 2. A model for Sup35 conversion and assembly**

In this model, an intermediate amount of Hsp104 converts soluble Sup35 to a less structured form that nucleates over time. This nucleus then rapidly seeds further conversion. Overexpression of Hsp104 resolves Sup35, eliminating [PSI<sup>+</sup>].

and below). The insolubility of these prions bears a striking resemblance to the scrapie form of PrP<sup>1</sup>, as does their resistance to proteolysis<sup>1,16</sup>.

### Fiber formation in [PSI<sup>+</sup>]

Several mammalian diseases, such as the transmissible spongiform encephalopathies, Alzheimer's disease, and Huntington's disease are characterized by the presence of amyloid fibers<sup>1</sup>. Amyloid fibers contain high amounts of beta sheet and can be identified by their ability to bind the dye Congo red<sup>17</sup>. The NM region of Sup35 (i.e. the prion-determining region, see below) spontaneously forms amyloid fibers *in vitro*<sup>17</sup>. However, "seeding" the reaction by adding a tiny amount of NM fiber greatly increases the speed with which soluble Sup35 forms fibers<sup>17</sup>. These experiments directly demonstrate the ability of aggregated proteins to "pass on" a trait to normal proteins.

Several possible models can potentially explain the mechanism of fiber formation by Sup35. However, kinetic analysis of fiber assembly supports a model called nucleated conformational conversion. In this model, less structured Sup35 slowly forms nuclei, which then rapidly seed the nucleation of other Sup35 proteins<sup>18</sup>. Hsp104 may aid in the production of less structured forms of Sup35 that are used to form nuclei<sup>18</sup> (Figure 2).

### The Sup35 prion-determining domain

Sup35 contains three domains with distinct properties. The C-terminus (C) is soluble, conserved among species, and is the only domain necessary for translation termination<sup>17</sup>. The middle domain (M) is still not well understood but appears to influence the inheritance pattern of [PSI<sup>+</sup>]<sup>19</sup>. The N-terminus (N) is insoluble, unconserved, and unessential for biological function<sup>17</sup>. Overexpression of this region results in aggregates and makes cells [PSI<sup>+</sup>]; cells with this region deleted can never be [PSI<sup>+</sup>]<sup>10</sup>. In short, N is responsible for endowing Sup35 with all of its prion-like traits.

The Sup35 N-terminus shows similarity to the N-terminus of mammalian PrP in that it contains five imperfect glutamine- and asparagine-rich oligopeptide repeats<sup>17</sup>. Expanded polyglutamine repeats in the protein huntingtin (Ht) are associated with Huntington's disease<sup>20</sup>. Such conservation suggests that these repeats have an inherent tendency to aggregate. Indeed, removing four of the five repeats from Sup35 results in the inability of cells to become [PSI<sup>+</sup>] while inserting two more repeats enhances the [PSI<sup>+</sup>] phenotype<sup>21</sup>. And Sup35 containing mutations in the glutamine/asparagine-rich region greatly diminishes the

prion-forming capacity of this protein, as found by my colleague, Jonathan Weissman<sup>22</sup>.

We extended these findings by conducting similar experiments on Ht in both yeast and *Caenorhabditis elegans*. As predicted, with increasing lengths of the polyglutamine repeat of Ht, the greater the amount of insoluble Ht in the cell<sup>20, 23</sup>. In yeast, deletion of Hsp104 eliminated aggregates while in both systems, overexpression of Hsp104 reduced aggregates<sup>20, 23</sup>.

The aggregation properties of NM are such that we were able to take an unrelated protein, the glucocorticoid receptor, and turn it into a prion simply by fusing it with NM<sup>24</sup>. This experiment demonstrated that NM is sufficient for inducing a prion-like state, and provides unequivocal evidence for a protein-only mechanism of inheritance.

### Peculiarities of yeast

In addition to providing strong evidence supportive of the prion hypothesis outlined by Prusiner<sup>1</sup>, yeast prions have suggested solutions to questions that are harder to address in mammals.

One of these questions surrounds the issue of strains. If prion diseases are all caused by a single protein, PrP, what can account for the different pathologies seen in many of them? In yeast, different [PSI<sup>+</sup>] strains can be distinguished based on their differing degrees of stop codon suppression. The basis for these strains lies in the efficiency with which they convert Sup35 to the prion form. Weaker strains convert Sup35 less efficiently, resulting in lower ratios of soluble to insoluble Sup35 and less stop codon suppression<sup>25</sup>.

In mammals, prions are harmful and so were at first assumed to be the consequence of an aberrant if not defective protein. In yeast, it appears that this is not always the case. [PSI<sup>+</sup>] and [psi<sup>-</sup>] yeast grown in a variety of conditions show different amounts of survival that varies according to the strain and the conditions<sup>26</sup>. For some strain-conditions combinations [PSI<sup>+</sup>] cells survive more; for others, [psi<sup>-</sup>] cells survive more; and for still others, [PSI<sup>+</sup>] state has no effect. Thus, [PSI<sup>+</sup>] can spontaneously "turn on" different phenotypes, aiding the survival of some cells and increasing the overall survival of the population in changing environments.

### Prions abound

Once yeast prions and the essential features of their prion-determining domain had been identified, it was a simple matter of searching databases for proteins with similar domains. Such searches turned up several other candidate prion proteins in several species of fungus.

We were able to confirm that one of these candidates, [RNQ<sup>+</sup>], is in fact a prion: when the N-terminus of its protein-determinant Rnq1 was substituted for the N-terminus of Sup35, this recombinant Sup35 made cells [PSI<sup>+</sup>]<sup>27</sup>. The protein chaperone Sis1, part of the Hsp40 family, is necessary for maintenance of [RNQ<sup>+</sup>]<sup>28</sup>. Several other putative prions have yet to be well characterized<sup>29</sup>.

Another prion, [PIN<sup>+</sup>] ([PSI<sup>+</sup>] inducibility factor), was first identified not via homology but by its ability to substantially increase the appearance of [PSI<sup>+</sup>]<sup>29</sup>. Overexpression of both Sup35 and Rnq1 makes [pin<sup>+</sup>] cells [PIN<sup>+</sup>]<sup>29</sup>.

Another prion, [HET-s], found in the filamentous fungus *Podospora anserina*<sup>29</sup>, takes the finding that prions are not always harmful even farther. Whereas [PSI<sup>+</sup>] is advantageous to budding yeast under certain conditions, [HET-s] is necessary to prevent lethal fusion of mycelia of incompatible strains<sup>29</sup>.

The widespread presence and diverse roles of prions suggests that the tendency of certain proteins to misfold has frequently been exploited by cells and that the aberrant appearance of mammalian prions may be the exception rather than the rule.

### Findings in mammalian prions

In the past seven years, my lab has spent some time investigating mammalian prions and their relationships to chaperones. The protein that gives rise to mammalian prions, PrP, can exist in two forms: the soluble, protease-sensitive form (PrP<sup>C</sup>), and the insoluble, protease-resistant form responsible for disease (PrP<sup>Sc</sup>, named after one of the first known prion diseases, scrapie)<sup>1</sup>. PrP is known to be a cell-surface glycoprotein that is processed through the cell secretory pathway, but its function remains a mystery<sup>30</sup>. For reasons that are still unknown, PrP<sup>Sc</sup> tends to selectively accumulate in and kill neurons, even though the normal form is ubiquitous<sup>1</sup>.

A series of in vitro experiments elucidated the mechanics of PrP. We found that yeast Hsp104 interacts not only with Sup35 but with hamster PrP<sup>Sc</sup><sup>31</sup>. Interestingly, whereas incubation of Hsp100 proteins with a substrate normally increases their ATPase activity, Sup35, PrP, and other amyloidogenic proteins *decreased* Hsp104 ATPase activity<sup>31</sup>. This phenomenon suggests that Hsp104 reacts with prion-like proteins in a manner that differs from its normal chaperone function. The conversion of PrP to its insoluble state is enhanced by Hsp104 and another chaperone, GroEL<sup>32</sup>. However, in addition to chaperones, a small amount of pre-formed PrP<sup>Sc</sup> is necessary<sup>32</sup>.

Part of the reason for the toxicity of misfolded PrP can be attributed to the inherent tendency of this protein to misfold and accumulate in the cytosol<sup>33</sup>. Proteins that have misfolded beyond repair in the endoplasmic reticulum are often booted out into the cytosol to be digested by proteasomes, a process called retrograde transport<sup>33</sup>.

In strong support of a retrograde transport model, cells treated with proteasome inhibitors display an accumulation of PrP in the cytosol<sup>33</sup>. What's more, the form of PrP that accumulates in the cytosol lacks an N-terminal ER translocation signal peptide and C-terminal GPI sequence, indicative of processing in the ER but not the Golgi<sup>33</sup>. Increasing concentrations of cytosolic PrP increases the formation of PrP<sup>Sc</sup><sup>34</sup> and leads to increasing toxicity in neuroblastoma cells<sup>35</sup>,

suggesting that an overabundance of PrP overwhelms the proteasomes, allowing PrP<sup>Sc</sup> to spontaneously form.

### Conclusion

In summary, yeast has provided a genetically tractable system in which to study the genetics of prions. Our studies have done much to support the prion hypothesis and have even extended it to include a protein-only mechanism of inheritance in addition to the protein-only mechanism of infectivity seen in mammals. Yeast have demonstrated that prions are a universal phenomenon whose functions can differ greatly.

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