The PSI' genetic element was first described by Brian Cox more than 30 years ago as a suppressor of all three nonsense mutations in nutritional markers in some strains of *Saccharomyces cerevisiae* [1]. Phenotypically, strains harbouring PSI' behave like yeast strains that harbour other omnipotent suppressors (for review see [2]). However, the inheritance of PSI' exhibits several features which set this element apart: (i) mating and cytoduction experiments suggest that PSI' is inherited as a dominant, cytoplasmic factor [3]; (ii) exhaustive efforts to associate PSI' with a cytoplasmic nucleic acid were inconclusive; (iii) the PSI factor is metastable in that interconversion of strains from psi- to PSI' and vice versa occurs at a low spontaneous frequency [4].

PSI' has a special relationship with the SUP35 gene which encodes a multi-domain protein that is one subunit of the translation termination factor in yeast (Figure 1). Certain mutations in SUP35 confer a phenotype similar to that of PSI' and these map to an essential C-terminal domain of SUP35 which has homology with the eukaryotic release factor eRF3.

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**Amyloid fibres of Sup35 support a prion-like mechanism of inheritance in yeast**


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Other alleles of SUP35 do not cause loss of translational fidelity but are incompatible with PSI* [5]. These alleles encode Sup35 with substitutions within the N-terminal domain. This domain is dispensable for growth but essential for the propagation of PSI* [6,7]. In either case, SUP35 alleles segregate in a normal Mendelian fashion. Most remarkably, transient overexpression of Sup35 or segments of Sup35 containing at least the N-terminal 114 amino acids, is sufficient to efficiently induce the heritable conversion of cells from psi- to PSI* [8].

In 1994 Reed Wickner suggested that the numerous perplexing features of the inheritance of PSI* and another genetic factor URE3, could be explained by a prion-like phenomenon [9]. Wickner hypothesized that the translation termination factor encoded by Sup35 could exist in a normal and abnormal conformation with an altered capacity to function in translation termination. In a process that parallels the conversion of normal PrP to the protease-resistant abnormal PrPsc form, an abnormal form of Sup35 might serve as a template for conversion of normal Sup35 into the abnormal form. During mating, sporulation and vegetative budding events, cytosolic transmission of the abnormal form could be invoked to explain the inheritance patterns of PSI* previously observed.

The discovery that Hsp104 is a modulator of the PSI factor produced strong support for Wickner’s hypothesis [10]. Both transformation with an additional plasmid-borne copy of HSP104, as well as deletion of HSP104 by homologous recombination, result in the restoration of faithful termination and the loss of the heritable PSI* element. When the normal gene dosage of HSP104 is restored by the loss of the extra plasmid copy, PSI* can be reinstated by Sup35 overexpression. HSP104 deletion strains, however, remain refractory to the reintroduction of PSI*.

Why should Hsp104 be such a potent modulator of PSI*? Hsp104 is a member of the Hsp100/Clp family of oligomeric ATPases [11]. These proteins function in a variety of cellular processes but seem to have a common biochemical mechanism. *Escherichia coli* ClpA is capable of activating RepA by disassembling inactive dimers and releasing active monomers [12,13]. ClpX, another *E. coli* family member, is capable of disassembling the Mu transposase [14] and aggregates of λO protein [15]. In experiments comparing the survival of wild-type and Hsp104 mutant yeast during exposure to severe stress, Hsp104 enhanced the chances of survival by two to three orders of magnitude [16]. *In vivo* analysis of the role of Hsp104 indicate that the protein promotes the resolubilization and reactivation of heat-damaged proteins [17]. Thus, the Hsp100/Clp family of proteins constitute a new class of molecular machines which specialize in the remodelling of protein aggregates or assemblies.

Because PSI* is influenced strongly by Hsp104, a protein that helps partition proteins between aggregated and soluble fractions, we decided to investigate whether the aggregation state Sup35 could be readily distinguished in strains differing only in their PSI* status. By differential sedimentation of cell-free lysates derived from PSI* or psi- yeast strains, we determined that Sup35 was largely sedimentable only when PSI* was present [18]. Curing of PSI* by either Hsp104 over-expression or deletion resulted in the recovery of Sup35 solubility. In PSI* cells, expression of Hsp104 derivatives with defective ATP-binding sites [19] resulted in increased Sup35 solubility and restoration of translational fidelity. However, translational fidelity was dependent on continuous expression of the mutant Hsp104 and under experimental conditions where plasmid loss occurred, PSI* reappeared, presumably a process initiated by incompletely resolved Sup35 prions. From these experiments we could conclude that in PSI* cells, conformationally altered Sup35 is sequestered in an insoluble form. The concomitant
depletion of functional termination factor is the most likely reason why ribosomes sometimes read through nonsense mutations in PSI* cells (Figure 2).

We also obtained evidence to suggest that Hsp104 over-expression may have an unrecognized role in the curing PSI* by a variety of stress-evoking treatments. Under non-stress conditions Hsp104 is barely detectable but is apparently required at this low level to maintain PSI*. Both heat-shock- and stress-response elements occur in the upstream region of the HSP104 gene [20], and these confer induction in response to heat and a variety of other stresses. For example, the low concentration of guanidinium chloride (5 mM) used to routinely convert PSI* cells to psi− is unlikely to destroy the conformational determinant of the PSI* state directly. More likely, it is the specific induction of Hsp104 under these conditions which is responsible for the curing [21]. Curiously, other conditions in which Hsp104 expression is up-regulated, such as entry into stationary phase or heat shock, do not efficiently destabilize PSI*. Presumably in these cases, other co-regulated physiological changes, including the accumulation of osmolytes and the expression of many other heat-shock proteins, stabilize PSI* or attenuate its interaction with Hsp104.

To follow the aggregation behaviour of the crucial N-terminal domain of Sup35 in living cells, we constructed a fusion between it and the Green Fluorescent Protein (GFP), and expressed the fusion protein from an inducible promoter. In the presence of pre-existing PSI* elements the fusion protein coalesced immediately upon induction. We interpreted this behaviour as evidence that pre-existing, aggregated Sup35 in PSI* cells indeed served as a seed for the aggregation of newly synthesized protein carrying the prion determinant of Sup35. In psi− cells, the fusion protein was diffusely distributed. After prolonged expression, the GFP fusion protein began to coalesce into intense foci—a change in aggregation behaviour that correlated with the formation of new heritable PSI* elements.

The same GFP fusion protein also aggregated in psi− cells lacking Hsp104 when it was expressed at high levels for a long time. This suggests that the conformational switch from the

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**Figure 2**

**Translation termination in psi− and PSI* cells**

In psi− cells soluble Sup35 forms a heterodimeric translation termination factor which interacts with ribosomes to promote polypeptide release. Open reading frames that are disrupted by non-sense mutations are not fully translated in psi− strains. In PSI* cells, most of the Sup35 protein is sequestered in an aggregated form resulting in reduced efficiency of translation termination. Read-through of all three types of non-sense mutations occurs with sufficient frequency to suppress the phenotypes associated with auxotrophic markers.
soluble to aggregated form of Sup35 could proceed in an Hsp104-independent manner even if, without continued over-expression, propagation of these PSI'-like elements was blocked by the Hsp104 mutation. Indeed, even in the absence of Hsp104, recombinant Sup35 purified from *E. coli* was initially soluble but, after some time, formed extremely long, rigid fibres [22]. The extreme N-terminal domain of Sup35 and a longer segment composed of this and the highly charged middle domain formed similar structures. In addition to the highly uniform, unbranched morphology typical of amyloid fibres formed by other proteins including PrP and Aβ, Sup35 fibres also bound Congo red and were enriched in β-sheet secondary structure.

Using either Congo red binding, CD or sedimentation analysis (Figure 3) to follow fibre assembly in *vitro* we found that spontaneous fibre formation proceeded in undisturbed solutions only after a lengthy lag period. Most significantly, we found that a small amount of fibre

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**Figure 3**

**Aggregation of a Sup35-derived polypeptide containing the prion determinant is a self-seeded process**

A metabolically labeled recombinant polypeptide (NM) corresponding to the N-terminal 254 amino acids of Sup35 was diluted from 8 M urea to a final concentration of 5 μM. Aggregation was determined in undisturbed solutions of NM alone (□) or in the presence of a small amount of preformed NM fibres (■) by measuring radioactivity remaining in the supernatant following centrifugation at 100,000 g. Note that the lag time which precedes aggregation in unseeded reactions is eliminated by the addition of fibres.
parity between the molecular mechanism of amyloid formation in mammals and yeast, we expect that the biological and genetic issues that can be addressed in yeast are likely to find parallels in other systems. The extent to which the capacity of molecular chaperones to influence protein conformation may be used in preventing or curing various forms of amyloidosis in humans remains compelling subject matter for future work.

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