Protein-only inheritance in yeast: something to get [*PSI*$^+$]-ched about

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Recent work suggests that two unrelated phenotypes, [PSI$^+$] and [URE3], in the yeast Saccharomyces cerevisiae are transmitted by non-covalent changes in the physical states of their protein determinants, Sup35p and Ure2p, rather than by changes in the genes that encode these proteins. The mechanism by which alternative protein states are self-propagating is the key to understanding how proteins function as elements of epigenetic inheritance. Here, we focus on recent molecular-genetic analysis of the inheritance of the [PSI$^+$] factor of S. cerevisiae. Insights into this process might be extendable to a group of mammalian diseases (the amyloidoses), which are also believed to be a manifestation of self-perpetuating changes in protein conformation.

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[*PSI*$^+$] was originally described in 1965 by Cox as a translation infidelity factor (Fig. 1)[1]. In [*PSI*$^+$] strains, a weak suppressor tRNA produced detectable nonsense suppression (stop-codon readthrough; see Box 1), whereas in [*psi*$^-$] strains the same tRNA suppressor appeared to be inactive[1,2]. Later experiments revealed that [*PSI*$^+$] was not required for suppressor tRNA function; rather, it increased the efficiency of suppression to a readily detectable level[3,4]. This enhancement of nonsense suppression (allosuppression) by [*PSI*$^+$] was not restricted to a single suppressor tRNA; suppression by other tRNAs as well as by mutations in ribosomal proteins was affected similarly. Thus, [*PSI*$^+$] functioned as a general or omnipotent allosuppressor[2,5]. Moreover, [*PSI*$^+$] can direct nonsense suppression on its own in strains lacking known genetic suppressors[2,5].

The phenomenon of allosuppression was well characterized in prokaryotes by the time [*PSI*$^+$] was described. However, [*PSI*$^+$] is distinct from these factors in that its pattern of inheritance is unusual. [*PSI*$^+$] is dominant in genetic crosses: if [*PSI*$^+$] and [*psi*$^-$] haploid strains are mated, the resulting diploid has the [*PSI*$^+$] phenotype (nonsense suppression)[1]. By classical genetics predictions, such diploids are presumed to be heterozygous for [*PSI*$^+$]. Surprisingly, however, the nonsense suppressor phenotype segregates to all four meiotic progeny[1]; that is, [*PSI*$^+$] is transmitted as a dominant, non-mendelian trait (hence the capital letters and brackets in its name). This pattern of inheritance was later explained by definitive localization of the [*PSI*$^+$] factor to the cytoplasm: [*PSI*$^+$] could be transmitted by cytoduction – that is, abortive matings in which cytoplasmic mixing occurs in the absence of nuclear fusion[6].

Through a series of experiments, [*PSI*$^+$] was distinguished from the known cytoplasmic nucleic acids, including the mitochondrial genome, killer virus and the sporulation-associated 20S RNA, as well as the extrachromosomal 2μ and 3μ DNAs[2]. To date, transmission of [*PSI*$^+$] has not been linked to the propagation of a novel or altered nucleic acid.

In addition to its unusual mode of inheritance, the [*PSI*$^+$] factor is distinguished from conventional genetic elements by its metastability. The [*PSI*$^+$] and [*psi*$^-$] states are not absolute: [*PSI*$^+$] strains convert to [*psi*$^-$] at low but measurable frequencies, and new [*PSI*$^+$] elements appear spontaneously in [*psi*$^-$] strains at a similar rate[1,7]. Perplexingly, the frequency of [*PSI*$^+$] $\rightarrow$[*psi*$^-$] conversion (i.e. [*PSI*$^+$] curing) is increased dramatically by treatment with low concentrations of agents that are non-mutagenic to nucleic acids, such as high salt[60], guanidine hydrochloride and methanol[8]. Once lost, [*PSI*$^+$] can reappear spontaneously in cured strains[7,9]. Another peculiar aspect of [*PSI*$^+$] is that it can exist in a cryptic state. That is, in specific crosses, the phenotype will reproducibly disappear and then reappear in a predictable way in meiotic progeny[1,10,11]. The spontaneous loss and reappearance of [*PSI*$^+$] in yeast strains (reversible curing) as well as the ability of [*PSI*$^+$] to exist in a cryptic state are difficult to reconcile with the idea of a nucleic acid determinant.

Link to *SUP35* and Sup35p

An unexpected and at first baffling connection between [*PSI*$^+$] and a nuclear gene, *SUP35*, was revealed in experiments from different research groups using distinct approaches. First, partial loss-of-function mutations in *SUP35* were shown to have a nonsense-suppression phenotype that mimics the effects of [*PSI*$^+$][12–14]. Unlike [*PSI*$^+$], however, these mutations were nuclear and segregated 2:2 in the meiotic progeny.

A nonsense-suppression phenotype could also be induced in a wild-type yeast strain by episomal plasmids carrying the *SUP35* gene[15], and, surprisingly, this suppressor phenotype persisted even after the *SUP35*-containing plasmid was lost[16]. In later experiments, the suppression induced by the extra copy of *SUP35* was eliminated by treatment with guanidine hydrochloride and was thus equated with [*PSI*$^+$][16]. Furthermore, increased levels of the Sup35 protein (Sup35p) rather than the DNA or mRNA were

0962-8924/00/$ – see front matter
PII: S0962-8924(99)01711-0

BOX 1 – GLOSSARY

Allosuppression – enhancement of nonsense suppression.

Antisuppression – restoration of translational fidelity (e.g. translational termination occurs at all nonsense codons).

Cytoduction – abortive matings that allow cytoplasmic mixing in the absence of nuclear fusion. One of the mating partners must contain a nuclear karyogamy mutation, usually at the *KAR1* locus.

Metastable – a heritable phenotypic state that changes to an alternative heritable phenotypic state at a rate higher than expected for the loss or gain of a novel nucleic acid.

Nonsense suppression – phenotypic suppression of a nonsense mutation without reversion of the mutation. Examples of nonsense suppressors include mutant tRNAs capable of decoding UAA, UAG or UGA, mutations in ribosomal proteins and mutations in translational termination factors (*SUP35* or *SUP45*) and [*PSI*$^+$].

PNM/pnm – [*PSI*$^+$]-no-more mutations. These nucleic acid changes interfere with the propagation of [*PSI*$^+$], either dominantly (*PNM*) or recessively (*pnm*).

Prion – a protein that can exist in at least two alternative physical states that are stable, self-perpetuating and associated with distinct phenotypes.

[*PSI*$^+$] strains – yeast strains with identical genomes that have different levels of nonsense suppression due to alternative forms of [*PSI*$^+$]. These are believed to derive from alternative conformations or packing of Sup35p protein. Unlike genetically distinct strains, [*PSI*$^+$] strains can interconvert without nucleic acid alterations.

Reversible curing – successive conversions from [*PSI*$^+$] to [*psi*$^-$] to [*PSI*$^+$] . Unlike phenotypic traits that are based on nucleic acid determinants, the [*PSI*$^+$] and [*psi*$^-$] states are readily interconverted either spontaneously or by treatments that are non-mutagenic to nucleic acids.

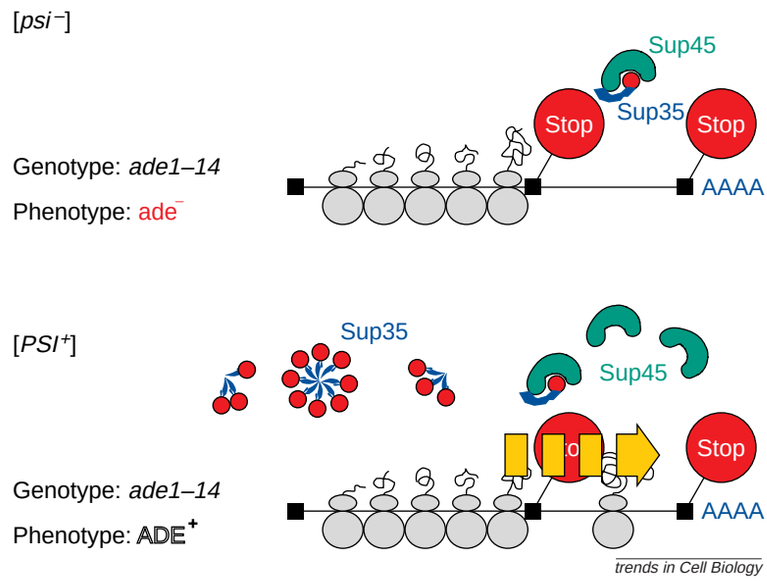

FIGURE 1

The [*PSI*$^+$] phenotype. The *ade1–14* allele is a UGA nonsense mutation in the *ADE1* gene. In [*psi*$^-$] strains, polysomes (grey spheres) translate the *ade1–14* mRNA until they reach the UGA mutation, where Sup35p–Sup45p complexes efficiently terminate translation. Because a full-length *ADE1* protein was not synthesized, the *ade1–14* [*psi*$^-$] strain requires exogenous adenine for growth (i.e. this strain is auxotrophic for adenine or ade$^-$). In [*PSI*$^+$] strains, polysomes proceed through the UGA mutation in the *ade1–14* mRNA at a reduced rate until the natural stop codon is reached. The amount of *ADE1* protein produced is below wild-type levels but is sufficient to confer adenine prototrophy to the strain (growth occurs in the absence of exogenous adenine). Based on our current model for the [*PSI*$^+$] phenotype, most of the Sup35p (red and blue particles) in [*PSI*$^+$] cells self-assembles into large complexes where it cannot bind to Sup45p (green crescents) or function in translation termination. A small fraction of the total cellular Sup35p, however, still complexes with Sup45p and directs termination most frequently at stop codons placed in their natural context at the end of open reading frames.

responsible for the *de novo* induction of [*PSI*$^+$][17]. Thus, transient overexpression of Sup35p was sufficient to induce a heritable change in phenotype in yeast – a remarkable and surprising phenomenon.

SUP35 is an essential gene and is now known to be the yeast homologue of the eukaryotic release factor 3 (eRF3). It functions together with Sup45p (eRF1)[18] to bring about the faithful termination of translation at all three nonsense codons[19,20]. The Sup35p sequence has been divided into three regions based on its unusual amino acid (aa) composition and its homology to other proteins (Fig. 2)[21–23]. The N-terminal 123 residues (N) are rich in glutamine, asparagine, glycine and tyrosine residues. Five imperfect repeats of the nonapeptide QGGYQ(Q)QYNP are present in the N region. The middle region (M; aa 124–253) is highly charged, with its residues strongly skewed to lysine (18.5%) and glutamate (17.7%). NM (aa 1–253) is not required for viability[24], and the primary sequence of these regions is not evolutionarily conserved, although all Sup35 proteins cloned to date contain N-terminal extensions of variable lengths[2]. The C-terminal region (C; aa 254–685) is the only region of the protein whose sequence is conserved from yeast to man[2]. This region is homologous to the translation elongation factor EF-1α; it contains four putative GTP-binding sites and functions in translation termination *in vitro*[19,25].

In a series of elegant experiments, work from three groups linked both *de novo* induction and propagation of the [*PSI*$^+$] phenotype to the N-terminal 114 residues (Fig. 2)[17,24,26,27]. Overexpression of this fragment of Sup35p alone was sufficient to induce new [*PSI*$^+$] elements in [*psi*$^-$] strains, and deletion of

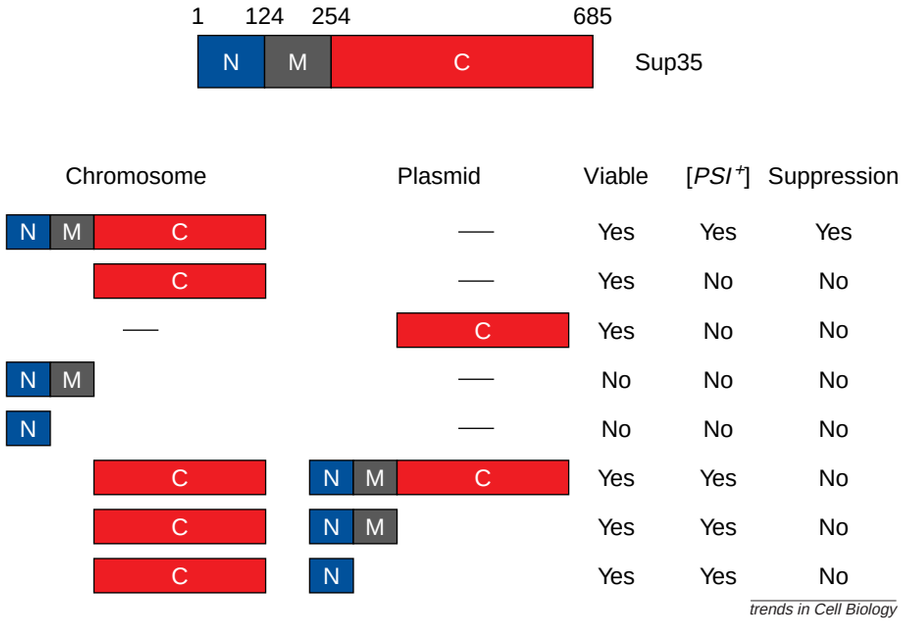

FIGURE 2

Regions of Sup35p required for viability and [*PSI*$^+$] propagation. Sup35p can be divided into three regions: N [amino acids (aa) 1–123)], M (aa 124–253) and C (aa 254–685). Full-length wild-type Sup35p supports both viability and [*PSI*$^+$] propagation in the absence of extrachromosomal sequences. C alone is sufficient to support viability when expressed from the chromosome or from a plasmid, but is unable to propagate [*PSI*$^+$] in either case. Neither NM nor N alone is sufficient to support viability, but, in the presence of a chromosome copy of C, any fragment of Sup35p containing N is sufficient to propagate [*PSI*$^+$]. However, in [*PSI*$^+$] strains expressing C, suppression is undetectable since the C region provides terminator function that cannot be modulated by [*PSI*$^+$].

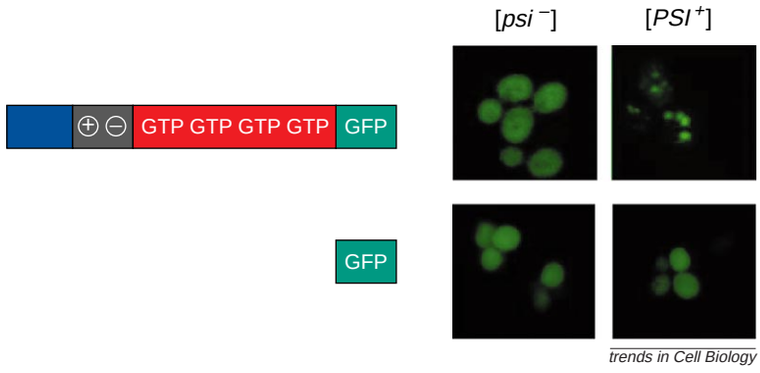

FIGURE 3

Self-propagation of the [*PSI*$^+$] and [*psi*$^-$] states. Green-fluorescent protein (GFP) tagging of Sup35p provides a method for visualizing the behaviour of Sup35p in [*PSI*$^+$] and [*psi*$^-$] cells. When the fusion protein is briefly expressed in [*psi*$^-$] strains, fluorescence is distributed diffusely throughout the cell (top, left panel). In [*PSI*$^+$] strains, it coalesces into discrete foci (top, right panel). Haemagluttinin tagging of pre-existing Sup35p demonstrates that newly synthesized protein is rapidly incorporated into pre-existing complexes in the [*PSI*$^+$] cytoplasm (J. Liu and S. Lindquist, unpublished). The fluorescence from GFP alone is unaffected by [*PSI*$^+$] status (bottom panels).

this same region resulted in the irreversible loss of [*PSI*$^+$][17,24,27]. Thus, while required for viability, the C region of Sup35p alone was unable to propagate [*PSI*$^+$][27]. Notably, when the N-terminus of Sup35p is expressed from a plasmid in strains expressing the C domain from the chromosome, [*PSI*$^+$] can be propagated[27]. In contrast to wild-type strains, however, these strains do not exhibit a nonsense-suppressor phenotype[27].

Genetic and cell-biological support for [*PSI*$^+$] as a yeast prion

In 1994, Wickner proposed that [*PSI*$^+$] and [*URE3*], another cytoplasmically transmitted trait in *Saccharomyces cerevisiae* (see Ref. 28 for a detailed review of work on [*URE3*]), were propagated by alternative forms of Sup35p and Ure2p, respectively, rather than by changes in a nucleic acid determinant[29]. This suggestion, the yeast prion hypothesis, was based on the mammalian prion hypothesis originally proposed to explain transmission of the scrapie agent in sheep[30]. The hypothesis has been extended to all of the transmissible spongiform encephalopathies (TSEs), a group of devastating neurodegenerative diseases in mammals[31]. The prion or protein-only hypothesis suggests that a single protein can stably exist in two alternative physical states, each associated with a distinct phenotype. One of these states is rare, but, once formed, becomes predominant by directing newly synthesized protein to adopt the same state. This self-perpetuation of protein states 'replicates' the information contained in those states and is thereby analogous to replication for nucleic acid genetic determinants. This mechanism explains several otherwise mysterious attributes of [*PSI*$^+$], such as dominant non-mendelian inheritance unlinked to cytoplasmic nucleic acids, reversible curing with non-mutagenic agents and the ability to exist in a cryptic state.

A wealth of genetic, cell-biological and biochemical data substantiates [*PSI*$^+$] as a yeast prion. Ironically, the prion hypothesis is now far better established in yeast than it is in the organism for which it was first proposed. In addition to the power of yeast genetic analysis, it happens that the conformational transitions of Sup35p have proved more amenable to *in vitro* analysis than those of the mammalian prion protein (PrP). Furthermore, the function of Sup35p is known, and the conformational transitions that the protein undergoes fully account for the [*PSI*$^+$] phenotype. The function of mammalian PrP is, unfortunately, still unclear, as is the mechanism by which its misfolding might lead to disease.

The first physical analysis of Sup35p established that the protein is found mostly in large, sedimentable complexes in [*PSI*$^+$] strains, whereas, in [*psi*$^-$] strains, Sup35p remains mostly soluble[11,32]. Moreover, Sup35p isolated from [*PSI*$^+$] strains has increased resistance to proteolytic digestion[11,32]. Strikingly, these same two characteristics are used to distinguish between the prion and normal states of the mammalian PrP[33].

Self-propagation of the [*PSI*$^+$] and [*psi*$^-$] states was clearly demonstrated by Sup35p fusions to green fluorescent protein (GFP; Fig. 3)[11]. When Sup35p–GFP is briefly expressed in [*psi*$^-$] strains, its fluorescence is distributed diffusely throughout the cell. However, if Sup35p–GFP is expressed in [*PSI*$^+$] strains, the fluorescence coalesces into foci as soon as it can be visualized, suggesting that pre-existing complexes of Sup35p in [*PSI*$^+$] cells influence newly synthesized Sup35p to adopt the [*PSI*$^+$] state.

In experiments monitoring Sup35p through sequential rounds of *de novo* [*PSI*$^+$] induction and curing, dynamic changes in physical state were linked to heritable changes in phenotype. In the first series of experiments, new [*PSI*$^+$] elements can be induced *de novo* in [*psi*$^-$] strains carrying episomal plasmids that express Sup35p from different regulatable promoters[11]. In these cases, [*PSI*$^+$] appears only in response to the appropriate induction stimulus, providing a genetic test of the prion hypothesis. In cells expressing Sup35p–GFP fusions, fluorescence begins to coalesce into discrete foci concomitantly with the *de novo* induction of [*PSI*$^+$].

In another group of experiments, the protein-remodelling factor Hsp104 was isolated as an extra-copy modifier of [*PSI*$^+$] in a genetic screen[10]. Either the deletion or the overexpression of Hsp104 heritably eliminates [*PSI*$^+$] from yeast strains, and this change in phenotype is accompanied by a change in the physical state of Sup35p[10,11]. In strains cured of [*PSI*$^+$], Sup35p was found in the soluble fraction of lysates[11]. Notably, [*PSI*$^+$] does not reappear in strains cured by elevated levels of Hsp104 once the overexpression plasmid is lost; that is, the determinant is eliminated rather than masked[10]. The only known function of Hsp104 is to alter the physical states of other proteins[34,35]. That a heritable change in phenotype in yeast can be induced by the transient overexpression of Hsp104 provides compelling support for [*PSI*$^+$] as a yeast prion.

Another important link between the level of soluble Sup35p and [*PSI*$^+$] was provided by a series of experiments analysing the effects of point mutations in Hsp104. Hsp104 contains two Walker-type nucleotide-binding sites that are crucial for its function in thermotolerance in yeast[36]. Mutation of both of these sites cures [*PSI*$^+$], and fluorescence from Sup35p–GFP fusion proteins is diffuse in these strains. When either site is mutated alone, [*PSI*$^+$] becomes cryptic; the nonsense-suppressor phenotype is lost, but reappears when the Hsp104 mutation is segregated away[10,11]. Strikingly, in strains containing single point mutations in Hsp104, the Sup35–GFP fluorescence pattern is intermediate between those of [*PSI*$^+$] and [*psi*$^-$] strains, with both foci and diffuse fluorescence being detected.

Biochemical support for [*PSI*$^+$] as a yeast prion

The ability of Sup35p to exist in distinct, heritable states *in vivo* has been modelled *in vitro*. Purified full-length Sup35p and fragments containing the prion-determining domain N form fibrous protein complexes that share structural characteristics with other amyloidogenic proteins that have been implicated in human disease[37,38]. Assembly of the NM fragment of Sup35p *in vitro* proceeds only after a lag phase. This time is reduced by the addition of preformed fibres or lysates from [*PSI*$^+$] strains but not from [*psi*$^-$] strains, modelling the ability of Sup35p complexes in [*PSI*$^+$] cells to continuously promote conversion of newly synthesized Sup35p to the [*PSI*$^+$] state[37,39].

Epigenetic modulation of translation termination by [*PSI*$^+$]

The link between inheritance of [*PSI*$^+$] and propagation of an alternative physical state of Sup35p provides the framework in which a molecular explanation of the [*PSI*$^+$] phenotype can be formulated (Fig. 1). [*PSI*$^+$] is a *cis*-acting epigenetic modulator of Sup35p translation termination activity. In [*psi*$^-$] strains, Sup35p exists as a soluble protein and provides an essential function in translation termination. In [*PSI*$^+$] strains, Sup35p exists in high-molecular-mass complexes and is precluded from performing its role as the yeast eRF3. Newly synthesized protein continues to join these complexes, which are passed through the cytoplasm from mother cells to their daughters where the self-propagation of protein states continues.

Two complex observations of [*PSI*$^+$] biology can also be explained by this model. In strains harbouring [*PSI*$^+$] in a cryptic state, the soluble Sup35p provides termination function and masks the [*PSI*$^+$] phenotype, but Sup35p complexes continue to self-propagate, ensuring [*PSI*$^+$] inheritance. Similarly, the [*PSI*$^+$] phenotype is reversed by overexpression of the C region, which provides eRF3 function that cannot be inactivated by incorporation into [*PSI*$^+$] complexes[27].

[*ETA*$^+$] and other [*PSI*$^+$] strains

The initial molecular characterization of [*PSI*$^+$] discussed above suggested that Sup35p can exist in two states (Sup35p$^{[PSI^+]}$ or Sup35p$^{[psi^-]}$) associated with two phenotypes (suppression or termination). However, [*PSI*$^+$] variants exist. In his initial study, Cox noted that cells with heritably different levels of nonsense suppression could arise from a single [*PSI*$^+$] colony[1]. More recently, such heritable differences in nonsense suppression were demonstrated to be epigenetic in nature[9]. Strong [*PSI*$^+$] strains have robust nonsense suppression and transmit [*PSI*$^+$] to ~100% of their daughters; weak [*PSI*$^+$] strains have lower levels of nonsense suppression and lose [*PSI*$^+$] at a higher rate upon cellular division. [*ETA*$^+$] is an extremely weak variant of [*PSI*$^+$] in which suppression is sometimes undetectable, and [*ETA*$^+$] is only transmitted to 70% of meiotic progeny[40,41]. These [*PSI*$^+$] variants collectively are called [*PSI*$^+$] strains; however, they are distinct from genetic strains in that [*PSI*$^+$] strains can be interconverted by sequential rounds of curing and *de novo* induction without changes in nucleic acid[9]. Similarly, prion strains with distinct aetiologies have been described for mammalian PrP (the TSE determinant), and, as is the case with [*PSI*$^+$] strains (see below), the mammalian prion strains are associated with differences in the physical state of PrP[42].

In the framework of the molecular model for [*PSI*$^+$] described above, Sup35p should exist in a distinct state in weaker [*PSI*$^+$] variants such as [*ETA*$^+$] strains. Indeed, the intermediate nonsense-suppressor phenotypes of weak [*PSI*$^+$] and [*ETA*$^+$] are accompanied by an intermediate level Sup35p solubility[41]. Since a large portion of Sup35p is not incorporated into the Sup35p complexes in these strains, the weak [*PSI*$^+$] or [*ETA*$^+$] states must be transferred less efficiently to newly synthesized Sup35p. In support of this notion, self-perpetuating morphological differences in Sup35p fibres *in vitro* suggest that Sup35p does have the capacity to pack into more than one distinct structure[37].

Intragenic modifiers of [*PSI*$^+$]

Characterization of a number of either spontaneously arising or engineered mutations in *SUP35* has provided some insight into the dynamics of [*PSI*$^+$] replication (e.g. self-perpetuation of the Sup35p$^{[PSI^+]}$ state). These mutations have been divided into two groups: [*PSI*$^+$]-no-more mutations that are dominant (*PNM*) or recessive (*pnm*).

The first *PNM* mutation described at the molecular level (*PNM2*)[26,43] is a glycine-to-glutamic acid substitution at amino acid position 58 in the second nonapeptide repeat in the N region[26]. In [*PSI*$^+$] strains heterozygous for *PNM2*, accurate translation termination is restored; thus, *PNM2* causes [*PSI*$^+$] to be lost progressively from these strains after several generations of growth under normal conditions. Since *PNM2* exerts its curing effect even in the presence of a wild-type copy of *SUP35* in some strains[43,44], it is considered a dominant [*PSI*$^+$]-no-more mutation. Additional *PNM* mutations have been derived from a random mutagenic screen of the N region[45]. Isolated mutations were limited to residues 8–24 within the first nonapeptide repeat, and most involved a change from glutamine or asparagine to a charged residue. Notably, when residues 8–24 were replaced by polyglutamine, the altered proteins entered complexes in [*PSI*$^+$] cells and remained soluble in [*psi*$^-$] cells, as determined by fusion to GFP. Together, these results suggest that polar residues in the N region, rather than a specific sequence motif, are crucial for the self-perpetuation of the Sup35p$^{[PSI^+]}$ state and inheritance of the [*PSI*$^+$] phenotype.

The complex phenotypes of *PNM* mutations have also been dissected at the molecular level[44–46]. The ability of *PNM2* to cure [*PSI*$^+$] only after several generations of growth was initially interpreted as a cessation in replication of the [*PSI*$^+$] element and a gradual dilution of [*PSI*$^+$] particles from cells upon division[47]. Experimental support for these principles exists within the framework of our current understanding of [*PSI*$^+$] propagation. *PNM2* joins pre-existing Sup35p [*PSI*$^+$] complexes more slowly than does wild-type Sup35p[46], and thus a larger pool of Sup35p remains soluble in the cell to function in translation termination. Moreover, the ability of *PNM2* to join Sup35p aggregates, albeit at a reduced rate, might explain its dominant [*PSI*$^+$] curing phenotype: [*PSI*$^+$] aggregates containing *PNM2* might have a reduced capacity to impart the [*PSI*$^+$] state onto newly synthesized Sup35p. The other *PNM* mutations derived by random mutagenesis behave similarly[45].

The most extensively characterized intragenic *pnm* mutations are deletions of all or part of the N region of *SUP35*[17,27]. Unlike *PNM2*, the *pnm* mutations do not dominantly cure [*PSI*$^+$] in the heterozygous state, but they have a dominant antisuppressor phenotype (*ASU*). Framed within our current understanding of [*PSI*$^+$] propagation, these observations are consistent with the failure of N deletion mutants to enter [*PSI*$^+$] complexes, allowing a soluble pool of functional Sup35p to accumulate in the cytoplasm. Indeed, this is the case for at least two mutants: *ΔBstEII*, which removes residues 22–69 (nonapeptide repeats 1 and 2), and *RΔ2–5*, which removes residues 57–93 (nonapeptide repeats 2–5)[32,48]. Moreover, a series of single amino acid substitutions in this region (aa 8–24) have an antisuppressor phenotype in [*PSI*$^+$] cells expressing wild-type Sup35p[45]. Although these mutants have not been used to replace wild-type *SUP35* in the genome, they are predicted to be *pnm* as well by virtue of their increased solubility in [*PSI*$^+$] strains.

Notably, a Sup35p mutation has been described that increases the rate of spontaneous [*PSI*$^+$] appearance by four orders of magnitude[48]. This mutant (*R2E2*) has two extra copies of the second nonapeptide repeat (aa 57–65), and the increased rate of [*PSI*$^+$] induction *de novo* for this mutant is accompanied by the appearance of Sup35p complexes. The increased propensity of *R2E2* for self-assembly has also been established *in vitro*; purified NM protein containing the same nonapeptide expansion forms fibres at an increased rate, again linking Sup35p assembly to [*PSI*$^+$] inheritance.

Hsp104 and Hsp70

To date, there is one known extragenic *pnm* locus: *HSP104*. As discussed above, intermediate levels of Hsp104 are required for the continued propagation of [*PSI*$^+$]. Unlike *pnm* mutations mapping to the *SUP35* locus, deletion of *HSP104* does not have an antisuppressor phenotype in heterozygotes[11]. However, when homozygous, this lesion leads to [*PSI*$^+$] curing by increasing the pool of soluble Sup35p in cells[11,32,49].

Two different modes of action have been proposed for the role of Hsp104 in [*PSI*$^+$] metabolism (Fig. 4)[11,32]. The first model proposes that Hsp104 is required for Sup35p to reach a transition state efficiently, from which it can fold into the [*psi*$^-$] state and function in translation termination or be captured by pre-existing complexes in [*PSI*$^+$] strains (Fig. 4, arrows 1–3)[11]. The second model posits that Hsp104 is not required for Sup35p to reach the [*PSI*$^+$] state, but instead partially disaggregates Sup35p$^{[PSI^+]}$ complexes to maximize partitioning to daughter cells upon division (Fig. 4, arrow 4)[32]. The core distinction between these models is whether or not Sup35p continues to join [*PSI*$^+$] complexes when Hsp104 function is lost. Unfortunately, the available information regarding Hsp104 curing is at the level of colony formation, where the [*PSI*$^+$] status can be assessed. Because colony phenotype is

detected several generations after the curing event, neither of the two models can be eliminated by current data. However, Hsp104 is required for the *de novo* formation of complexes in [*psi*⁻] cells, providing support for the first model[11].

Similarly, elevated Hsp104 levels might lead to [*PSI*⁺] loss, either by blocking the incorporation of newly synthesized Sup35p into [*PSI*⁺] complexes or by directly disaggregating these complexes[11,32]. For example, when Hsp104/Sup35p levels become unbalanced, the number of Sup35p molecules in the vicinity of a single Hsp104 hexamer[50] might decrease, reducing the rate at which self-assembly occurs (Fig. 4, arrow 5)[11]. Alternatively, excess Hsp104 might rebind folding intermediates and preclude their assembly (Fig. 4, arrow 6)[11]. Finally, if the rate of disaggregation of [*PSI*⁺] complexes exceeds the rate of assembly in the presence of elevated levels of Hsp104, the net effect would be particle disassembly, and [*PSI*⁺] would be lost from growing cultures over time (Fig. 4, arrow 7). Additional experiments are required to distinguish between the models presented for [*PSI*⁺] curing by excess Hsp104.

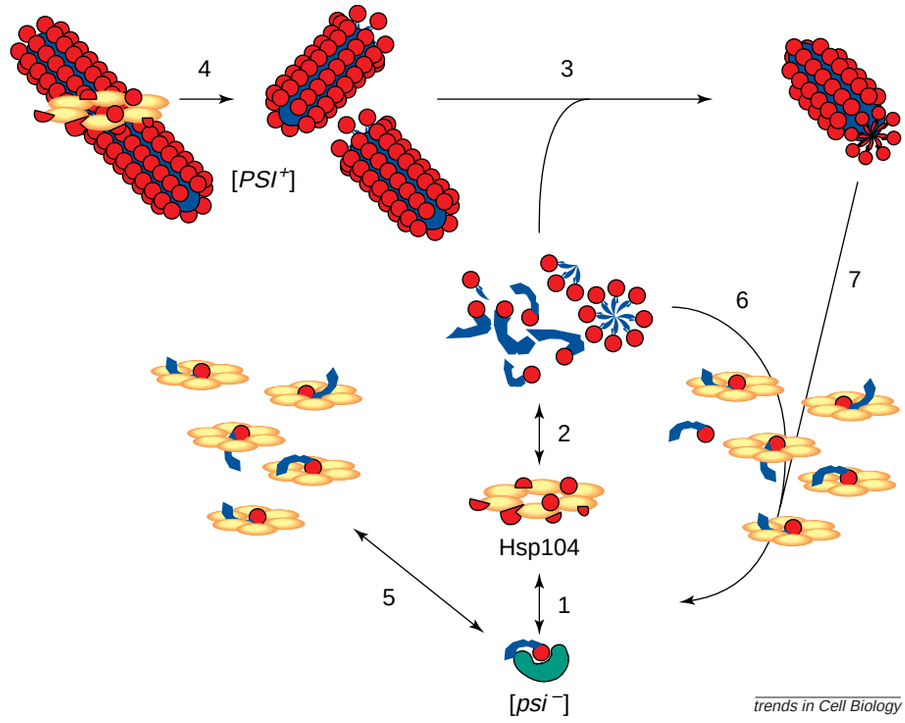

FIGURE 4

Models of Hsp104 action in [*PSI*⁺]. Model 1. Hsp104 (yellow hexamer) interacts with Sup35p (red and blue particle) in the [*psi*⁻] state (1, forward) allowing the protein to reach a conformational 'transition state' (2, forward). This state is unstable and might convert to the [*PSI*⁺] state by interacting with pre-existing [*PSI*⁺] particles (large red and blue complexes) as either monomers or oligomeric intermediates (3), or simply revert to the [*psi*⁻] form (2 and 1, reverse) in which it complexes with Sup45 (green crescent). Elevated Hsp104 levels might cure [*PSI*⁺] by changing the stoichiometry required for efficient self-assembly (5), rebinding transition-state Sup35 and facilitating reversion to the [*psi*⁻] state (6), or directly disaggregating pre-existing Sup35p [*PSI*⁺] complexes (7). Model 2. The only function of Hsp104 is to disaggregate Sup35p [*PSI*⁺] particles. At low levels of Hsp104, this creates small particles that efficiently partition to daughter cells (4). At high levels of Hsp104, the rate of disaggregation exceeds the rate of self-assembly, leading to the eventual loss of [*PSI*⁺] (7).

Hsp104 and Ssa1p (Hsp70) act in concert to rescue aggregated proteins following thermal stress[35,51]. Intriguingly, *SSA1* genetically interacts with [*PSI*⁺]; however, the effects are complex. Extra-copy *SSA1* acts as an antisuppressor in some [*PSI*⁺] strains[10] and as an allosuppressor in others[49]. These disparate phenotypes could be explained by an antagonistic relationship between Hsp104 and Ssa1 with regard to [*PSI*⁺]; elevated levels of Ssa1 block the ability of extra-copy *HSP104* to cure [*PSI*⁺][49]. Although this interaction between Hsp104 and Ssa1 seems mechanistically complex, it could provide some insights into the persistence of [*PSI*⁺] during times of stress. Neither heat shock nor sporulation alters the inheritance of [*PSI*⁺], although Hsp104 levels are elevated under both conditions[1,8]. Ssa1p might serve fortuitously to protect [*PSI*⁺] in such situations by cooperating with Hsp104 in the rescue of aggregated proteins. In addition, cellular division does not proceed under these stresses, and pre-existing [*PSI*⁺] complexes might persist unaltered until conditions return to normal.

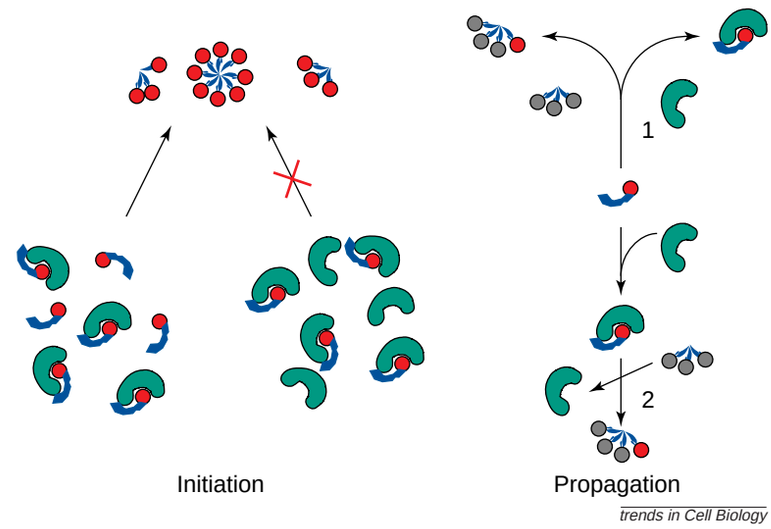

FIGURE 5

Potential roles for Sup45p (green crescent) in [*PSI*⁺] initiation and propagation. Excess Sup45p might block the initiation of [*PSI*⁺] conversion by binding to free Sup35p (blue and red particle) and decreasing the rate of conformational conversion and/or self-assembly (X). Once established, [*PSI*⁺] propagation is unaffected by excess Sup45p (see text). This observation suggests that [*PSI*⁺] particles (blue and red complexes) can effectively compete with Sup45p for binding to nascent Sup35p (1) or, alternatively, Sup35p existing as a complex with Sup45p can still undergo self-assembly in the presence of a pre-existing [*PSI*⁺] particle (2).

Sup35p-interacting proteins

A genetic interaction between *SUP35* and *SUP45* was characterized as early as 1975[2], and recent experiments indicate that Sup35p and Sup45p interact physically as well[52–54]. The significance of this interaction with regard to ribosome targeting, translation termination and viability is unclear[53]; however, the Sup35p–Sup45p interaction might have important

implications for [*PSI*$^+$]. Overexpression of Sup45p inhibits the *de novo* induction of [*PSI*$^+$] by elevated levels of Sup35p[55]. These observations suggest that, when Sup35p is complexed with Sup45p, it is less susceptible to conversion to the [*PSI*$^+$] conformation (Fig. 5, initiation). Notably, excess Sup45p does not reverse the nonsense-suppression phenotype of [*PSI*$^+$] strains nor does it dominantly cure [*PSI*$^+$][55]. This observation suggests that, when Sup35p is already present in the [*PSI*$^+$] state, it can compete effectively with Sup45p for binding to newly synthesized Sup35p (Fig. 5, propagation, arrow 1). Alternatively, Sup45p might be able to inhibit the initiation of Sup35p self-assembly but not its propagation (Fig. 5, propagation, arrow 2). In support of this hypothesis, Sup45p is not found in [*PSI*$^+$] complexes in at least three unrelated strains[11,56]. However, Sup45p is found associated with [*PSI*$^+$] complexes in two other strains that are related to each other[52,57]. Whether these differences are a consequence of genetic distinctions between the strains, or of assay or growth conditions, has yet to be resolved.

Other proteins have recently been identified as Sup35p partners. These include a series of proteins that interact with the N-terminal 113 residues of Sup35p by two-hybrid analysis: Reg1p and Eno2p (two proteins involved in glucose metabolism), the translation elongation factor EF-2 and the cytoskeletal assembly protein Sla1p[58]. The Sup35p–Sla1 interaction is the most extensively characterized. The Sup35p–Sla1p interaction is eliminated by both the *PNM2* and *ΔBstEII* mutations, as well as by disruption of *HSP104*. Disruption of *SLA1* does not cure [*PSI*$^+$]; rather, it decreases the efficiency of [*PSI*$^+$] curing by elevated levels of Hsp104 or treatment with dimethylsulphoxide (DMSO). Perhaps Sla1p can compete weakly with [*PSI*$^+$] complexes for binding to newly synthesized Sup35p. In any case, a direct link between Sup35p and the cytoskeleton will surely provide new avenues to explore the role of [*PSI*$^+$] in yeast cell biology as well as the complex and still enigmatic relationship between the cytoskeleton and translational regulation.

In yeast lysates, Sup35p interacts with Upf1p, a component of the nonsense-mediated mRNA decay pathway in yeast[57]. As is the case for *SLA1*, disruption of *UPF1* does not cure [*PSI*$^+$] strains, but the effects of extra-copy *UPF1* on *de novo* [*PSI*$^+$] induction or [*PSI*$^+$] curing have not been assessed. In addition, the human homologue of Sup35p has recently been shown to interact with poly-A binding protein[59], suggesting another possible modulator of [*PSI*$^+$] metabolism in yeast.

Future directions

The molecular-genetic experiments described above have begun to elucidate the mechanism by which the Sup35p protein can exist stably in alternative physical states and act as an element of inheritance in yeast. The yeast prion proteins share many characteristics with the mammalian PrP, and the lessons learned through the study of [*PSI*$^+$] might be applicable to the transmission of disease in higher eukaryotes, and vice versa. For example, a nonapeptide repeat expansion mutation that increases the spontaneous rate of [*PSI*$^+$] formation was designed to mimic repeat expansions in the mammalian PrP protein that increased the spontaneous appearance of spongiform encephalopathy[48]. However, repeat expansion mutations in the mammalian protein were thought to act by destabilizing the native fold. *In vitro* characterization of the analogous change in Sup35p revealed that this mutation acts by increasing the rate of self-assembly and suggests an alternative interpretation for the mammalian observations. Future work might reveal similarly acting mutations in the mammalian protein. In addition, characterization of the mammalian prion strains provided a molecular explanation for an unusual and previously inexplicable variation in [*PSI*$^+$] phenotype[17].

Although the work detailed above collectively provides a convincing argument for protein-only inheritance, the molecular mechanics of this process remain a mystery waiting to be solved. Undoubtedly, new insights will be provided with an increased understanding of how [*PSI*$^+$] metabolism is modulated by factors that interact with *SUP35*, both genetically and biochemically.

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Acknowledgements

We thank the many researchers whose work has collectively revealed the richness of [*PSI*$^+$] biology. We apologize for the limited citations allowed owing to space constraints. We also thank Julie Feder for invaluable help with the figures. This work was supported by the Cancer Research Fund of the Damon Runyon–Walter Winchell Foundation Fellowship DRG-1436 to T.R.S. and NIH GM025874 to S.L.L. and the Howard Hughes Medical Institute.