Annu. Rev. Cell Dev. Biol. 1999. 15:661–703

[*PSI+*]: An Epigenetic Modulator of Translation Termination Efficiency

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Key Words amyloid, prion, Sup35, nonsense suppression, epigenetic

■ **Abstract** The [*PSI+*] factor of the yeast *Saccharomyces cerevisiae* is an epigenetic regulator of translation termination. More than three decades ago, genetic analysis of the transmission of [*PSI+*] revealed a complex and often contradictory series of observations. However, many of these discrepancies may now be reconciled by a revolutionary hypothesis: protein conformation-based inheritance (the prion hypothesis). This model predicts that a single protein can stably exist in at least two distinct physical states, each associated with a different phenotype. Propagation of one of these traits is achieved by a self-perpetuating change in the protein from one form to the other. Mounting genetic and biochemical evidence suggests that the determinant of [*PSI+*] is the nuclear encoded Sup35p, a component of the translation termination complex. Here we review the series of experiments supporting the yeast prion hypothesis and provide another look at the 30 years of work preceding this theory in light of our current state of knowledge.

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INTRODUCTION

A new paradigm for the transmission of genetic information has recently been discovered in the yeast *Saccharomyces cerevisiae*. Phenotypic traits can be transmitted from generation to generation through self-perpetuating changes in the physical state of proteins with no underlying changes in nucleic acid. First described in *S. cerevisiae*, this new mode of inheritance is popularly known as the yeast prion hypothesis and is modeled after the protein-only prion mechanism first proposed to describe the infectious agent in a group of unusual neurodegenerative diseases in mammals (the transmissible spongiform encephalopathies or prion diseases) (Griffith 1967, Prusiner 1982). The nature of the change in protein state in *S. cerevisiae* appears related to those associated with several devastating human diseases (the amyloid diseases), and this phenomenon has sparked the interest of geneticists, cell biologists, protein chemists, and clinicians alike. Indeed, this mechanism of information transfer is likely to act in many different situations and in many other systems. Here we provide a detailed description of one proteinaceous genetic element, the [*PSI*+] factor.

[*PSI*+] is an epigenetic modulator of the fidelity of protein synthesis. The protein determinant of [*PSI*+], Sup35p, is required for the faithful termination of translation at stop (nonsense) codons in messenger RNAs (mRNAs). Sup35p can exist in at least two different stable physical states: the normal one is associated with accurate termination; the alternate state allows nonsense suppression. A self-perpetuating change in Sup35p from the normal (active) to the altered (inactive) state is proposed to be the mechanism by which [*PSI*+] is propagated.

We review the discovery of [*PSI*+], subsequent observations leading to the hypothesis that [*PSI*+] has a protein-only mode of transmission, and the strong

genetic, cell biological, and biochemical evidence that now supports this novel type of inheritance. We also provide a re-interpretation of the older [*PSI+*] literature in light of new findings. Unfortunately, the older literature is often difficult to read now because the same gene products and phenomena were described in different terms in different laboratories and in different strains, and it was, in many cases, not even clear that investigators were studying the same phenomenon. In the absence of a molecular framework in which to interpret this wealth of knowledge, a multiplicity of curiosities with possible insights remained uninvestigated. We hope that the interested reader will be encouraged to mine the invaluable resources this older literature provides.

THE PHENOMENON OF NONSENSE SUPPRESSION

Protein synthesis is regulated by a variety of factors to ensure that proteins contain the correct sequence of amino acids specified by their mRNAs. Included among these checks and balances are mechanisms for ensuring that ribosomes will terminate translation at stop codons. Changes in genetic or epigenetic regulators of translational termination can greatly decrease the fidelity of this process. In the laboratory, termination accuracy is monitored in cells carrying nonsense mutations in a gene encoding an auxotropic (nutrient-dependent) or color marker. Normally, protein will not be expressed from nonsense-containing messages. However, changes in the function or concentration of factors that govern translation termination can cause ribosomes to read through these stop codons frequently enough to synthesize sufficient protein to change the phenotype of the cell.

Several types of nonsense suppressors are known. For example, mutations in tRNA anticodon loops that allow recognition of ochre (UAA), amber (UAG), or opal (UGA) codons lead to enhanced nonsense suppression (stop codon read-through) (Sherman et al 1979). Similarly, mutations in some ribosomal proteins or in the translational termination factors *SUP35* and *SUP45* can also lead to nonsense suppression. In contrast with suppressor tRNAs, mutations in these general components of translation suppress all three nonsense codons (omnipotent suppression) (Hawthorne & Leupold 1974, Hinnebusch & Liebman 1991). The action of tRNA suppressors can be reversed by co-overexpression of wild-type Sup35p and Sup45p (Stansfield et al 1995). These observations support a competition hypothesis for translation termination: tRNA suppressors compete with termination factors for stop codons. Excess tRNAs or appropriate mutations in the anticodon loop shift the balance in favor of read-through. Conversely, read-through can be increased by loss of terminator function, which stalls the ribosome on the message and increases the likelihood of a tRNA inserting an amino acid at the stop codon.

The effects of tRNA and omnipotent suppressors can be modulated by both genetic and epigenetic factors. Genetic modulators act to either increase (allosuppressors) or decrease (antisuppressors) the efficiencies of nonsense suppression.

Epigenetic modulation by the [*PSI+*] factor was first described by Cox nearly 35 years ago (Cox 1965). This factor can enhance the efficiencies of both tRNA and omnipotent suppressors and can also cause suppression in the absence of known genetic suppressors. From the beginning, [*PSI+*] was found to be metastable, disappearing and reappearing in yeast strains with a baffling pattern that defied explanation.

DISCOVERY OF [*PSI+*]

[*PSI+*] was originally isolated in a genetic screen for nonsense suppressors in a strain containing a nonsense mutation in the *ADE2* gene (specifically, *ade2-1*). This allele prevents growth on medium without adenine and leads to the accumulation of a pigmented metabolic intermediate on rich medium (Cox 1965). (This is a particularly convenient marker for scoring nonsense suppression because it allows both a color screen and genetic selection by growth on medium lacking adenine. It has been used in many [*PSI+*] studies.) This red, adenine-requiring strain was irradiated with ultraviolet light (UV), and white, adenine-independent colonies that retained the *ade2-1* allele were isolated. The white derivatives contained a tRNA suppressor *SUQ5* (Olson et al 1981, Waldron et al 1981).

Cox noticed that the white *SUQ5* strain occasionally had red sectors (Cox 1965). Cells from red sectors or white sectors gave rise to stable red or white colonies, respectively, but white colonies could occasionally switch to red and vice versa. Surprisingly, *SUQ5* was retained in both red and white derivatives. This puzzling observation was explained when the white colonies proved to contain another factor that enhanced the efficiency of *SUQ5*-mediated suppression. In the absence of this factor, *SUQ5* suppression is too weak to be detected by this color assay. Cox's patience, curiosity, and resolve to characterize this metastable factor formed the foundation for the research by the many scientists described in this review.

A series of genetic crosses were undertaken to characterize the enhancer of *SUQ5* suppression (Cox 1965). If red *SUQ5* (172/9b) and white *SUQ5* strains are mated (Figure 1, cross 1), the resulting diploid is white and homozygous for *SUQ5*. That is, suppression is dominant over non-suppression. Surprisingly, upon sporulation of such diploids, the suppression phenotype segregates to all four meiotic progeny. Thus the factor that enhances *SUQ5* suppression segregates in a dominant non-Mendelian manner. Cox interpreted these results in the following logical way. Red sectors harbor *SUQ5* in an inactive (non-suppressing) state, and white sectors contain *SUQ5* in an active (suppressing) state. Mating red and white isolates reactivates *SUQ5* and, therefore, only active *SUQ5* is detected in haploid progeny. Failure of the non-suppressing *SUQ5* (red) to segregate to any of the progeny was taken as evidence that a "cytoplasmic mutation" rather than a nuclear lesion was responsible for inactivation.

Predicting the reactivation of *SUQ5* was not as straightforward as the original cross might have suggested. Surprisingly, crosses between certain non-suppressing

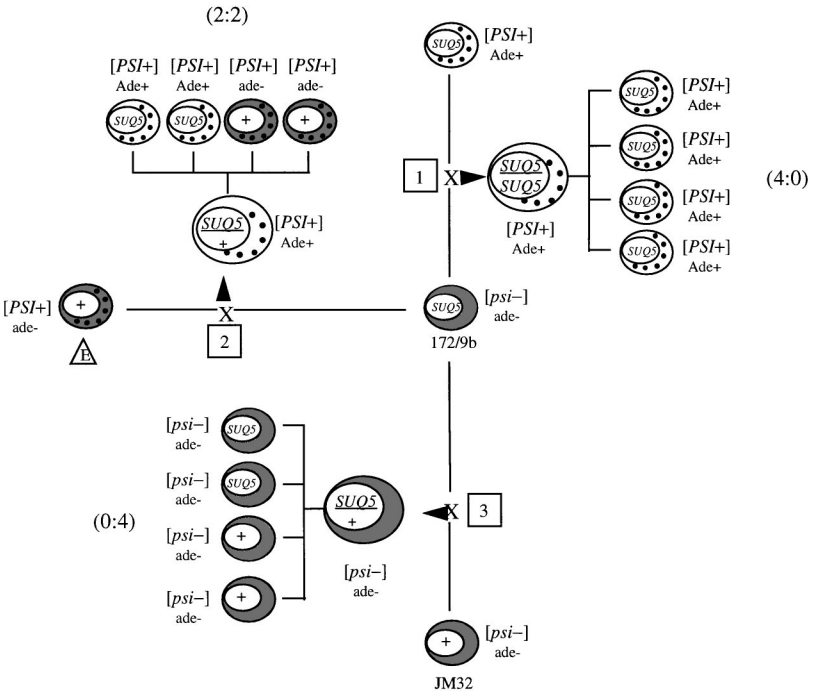

Figure 1 The genetics of [*PSI+*] inheritance. All strains carry the ochre allele *ade2-1*. Strains carrying the weak ochre tRNA suppressor *SUQ5* or the wild-type tRNA are distinguished by *SUQ5* or + in the nucleus. [*PSI+*] is denoted by black dots in the cytoplasm. Suppression of the *ade2-1* allele produces white colonies on rich medium (white cells). In the absence of suppression cells form red colonies on rich medium (shaded cells). X indicates matings (with cross numbers boxed for reference to the text and strain names of critical parental cells indicated). Arrowheads point toward the resulting diploids (larger cells) and their haploid meiotic progeny (brackets). Ratios of progeny with and without a suppression phenotype are indicated in the parentheses (figure adapted from Cox 1965).

red strains also led to *SUQ5* reactivation (Cox 1965). Mating the same red (non-suppressing) *SUQ5* strain (172/9b) to another red strain that lacked *SUQ5* (Figure 1, cross 2, strain E) produced a white, suppressing diploid, heterozygous for *SUQ5*. Upon sporulation, the *SUQ5* progeny were all white. Inexplicably, the mating of two inactive strains had somehow reactivated *SUQ5* in this cross.

Mating was not sufficient to activate *SUQ5*, however. Crosses between the red *SUQ5* strain (172/9b) and another red strain lacking *SUQ5* (Figure 1, cross 3, strain JM32), gave red diploids heterozygous for *SUQ5*. When this diploid was sporulated, all of the *SUQ5* progeny were red (non-suppressing) (Cox 1965). Reactivation of *SUQ5* and mating were apparently unlinked events.

Cox proposed a simple explanation for the complex genetics that he had observed: a cytoplasmic modifier of *SUQ5*. He referred to this modifier as [*PSI*] and defined active *SUQ5* strains as [*PSI+*] and inactive *SUQ5* strains as [*psi−*]. (The [ ] in this designation indicates that the determinant is extra-chromosomal, and the capital letters indicate that [*PSI+*] is dominant. The symbols $\psi+$ and $\psi-$ have also been used.) Revisiting the earlier crosses, it becomes apparent that 172/9b was [*psi−*]. The first strain crossed to 172/9b was [*PSI+*] (Figure 1, cross 2). The other non-suppressing strain (Figure 1, cross 3) was [*psi−*].

[*PSI+*] was proposed to be a non-Mendelian genetic element that allowed the *SUQ5* tRNA suppressor to function. In the absence of the *SUQ5* tRNA suppressor in this strain, [*PSI+*] had no phenotype (Figure 1, strain E). In crosses between [*PSI+*] and [*psi−*] strains (Figure 1, crosses 1 and 2), [*PSI+*] is always dominant. This initially suggested that [*psi−*] was a mutant loss-of-function form of [*PSI+*]. Most of these observations have held true, but subsequent work has shed new light on the molecular nature of [*PSI*], and, consequently, a new interpretation of these results has emerged. The protein determinant of [*PSI+*] is Sup35p, a cytoplasmic protein encoded by a nuclear gene (Crouzet & Tuite 1987, Kikuchi & Kikuchi 1988, Kushnirov et al 1988, Telkov & Surguchev 1986, Ter-Avanesyan 1981, Wilson & Culbertson 1988). Sup35p functions as a component of the translation termination complex (Stansfield et al 1995, Zhouravleva et al 1995). Following from this point, the [*psi−*] state is associated with normal Sup35p function: translational fidelity. The [*PSI+*] state is associated with an alteration in Sup35p activity, brought about by a change in the physical state of the protein. Once this state is achieved, it is self-perpetuating. [*PSI+*] is, therefore, dominant and segregates to all progeny in meiosis. The molecular mechanism proposed for this model is discussed further below.

[*PSI+*]-Directed Translational Ambiguity

[*PSI+*] was originally characterized by Cox as a translation infidelity factor required for ochre suppression by the weak tRNA suppressor *SUQ5* (Cox 1965). However, the mechanism of [*PSI+*] action was unclear. *SUQ5* could be inactive or, alternatively, too inefficient to be detected in [*psi−*] strains. Direct biochemical proof of the latter idea was provided by quantitation of nonsense read-through using two systems. In one, the levels of suppression of nonsense mutations in iso-1-cytochrome *c* was determined by a spectrophotometric assay for cytochrome *c* levels (Liebman et al 1975). In another, three fusions between phosphoglycerate kinase (*PGK1*) and *lacZ*, each with a different nonsense codon at the open reading frame (ORF) junction, allowed suppression to be quantified with an assay for β-galactosidase activity (Firoozan et al 1991). In both cases, *SUQ5* activity was measurable in [*psi−*] strains and was enhanced tenfold in the presence of [*PSI+*].

[*PSI+*] was later found to act in a remarkable breadth of circumstances. [*PSI+*] action is not specific to *SUQ5*, and the activities of several distinct ochre and opal tRNA suppressors are enhanced in the presence of [*PSI+*] (Table 1) (Firoozan et al

TABLE 1 Genetic interaction between dominant suppressors and [*PSI+*]

Dominant suppressor	[*PSI+*] Interaction	Reference
S_α S_β S_δ S_γ	Dominant lethal with [*PSI+*]	Cox 1971
SUP3	Recessive lethal with [*PSI+*]	Liebman & Sherman 1979
SUP11= S_d = S_{Q2}	Recessive lethal with [*PSI+*]	Cox 1971 Liebman & Sherman 1979
	Suppression enhanced by [*PSI+*]	Liebman & Sherman 1979
	Compatible with [*PSI+*]	Ono et al 1979
SUQ5 = *SUP16* = *SUP15*	Suppression enhanced by [*PSI+*]	Cox 1965 Liebman et al 1975 Liebman & Sherman 1979 Ono et al 1979
SUP17	Suppression enhanced by [*PSI+*]	Ono et al 1979
SUP19	Suppression enhanced by [*PSI+*]	Ono et al 1981
SUP22	Suppression enhanced by [*PSI+*]	Ono et al 1981
SUP7-**a**	Unaffected by [*PSI+*]	Liebman & Sherman 1979
S_{Q2}-**a**	Compatible with [*PSI+*]	Cox 1971
SUF1 *SUF4* *SUF6*	Frameshift suppression enhanced by [*PSI+*]	Culbertson et al 1977
SUP160	Lethal with [*PSI+*]	Ono et al 1988

1991, Liebman & Sherman 1979, Liebman et al 1975, Ono et al 1988). [*PSI+*] also enhances the nonsense suppression efficiency of mutated ribosomal proteins such as *SUP46* and a host of other protein translation factors that yet remain to be cloned, such as *SUP111, SUP112, SUP113, SUP114, SUP115, SUP138,* and *SUP139* (Table 2) (Ono et al 1991). However, [*PSI+*] action is not limited to nonsense suppressors. The activities of *SUF1, SUF4,* and *SUF6,* all glycl-tRNAs isolated as frame-shift suppressors, are enhanced in [*PSI+*] strains as well (Cummins et al 1980). This broad action spectrum can be explained in the framework of our current understanding of [*PSI*] biology. The [*PSI+*] phenotype results from an epigenetic loss of Sup35p terminator activity due to a self-perpetuating change in the physical state of this protein. Partial loss of terminator activity increases translational ambiguity, which can then be exploited by a variety of genetic factors.

[*PSI+*] can also suppress all three nonsense codons in the absence of known genetic suppressors, consistent with the breadth of its action on genetic modifiers of translational fidelity. Quantitation of [*PSI+*]-mediated read-through with the

TABLE 2 Genetic interaction between omnipotent suppressors and [*PSI+*]

Omnipotent suppressor	[*PSI+*] Interaction	Reference
sup111 *sup112* *sup113* *sup114* *sup115*	Isolated in [*PSI+*] strain Decreased or undetectable omnipotent suppression in [*psi−*] strains	Ono et al 1982 Ono et al 1986 Ono et al 1991
sal2 *sal6*	Unaffected by [*PSI+*]	Song & Liebman 1987
SUP43 *SUP44*	Isolated in [*PSI+*] background [*psi−*] phenotype unknown	Wakem & Sherman 1990
SUP46 *SUP138* *SUP139*	Decreased suppression in [*psi−*] strains	Ono et al 1991
SUP35	Overexpression growth defect	Dagkesamanskaya & Ter-Avanesyan 1991 Chernoff et al 1992 Derkatch et al 1996
sal3.5	Recessive lethal with [*PSI+*]	Cox 1977
sup35-2	Recessive lethal with [*PSI+*]	Liebman & All-Robyn 1984
SUP45	Heterozygous disruption in [*PSI+*] diploid has growth and sporulation defect	Dagkesamanskaya & Ter-Avanesyan 1991 Ter-Avanesyan et al 1989
	Overexpression allo-suppressor phenotype is enhanced in [*PSI+*]	Chernoff et al 1992 Derkatch et al 1998
sal4.1 *sal4.2*	Recessive lethal with [*PSI+*]	Cox 1977

PGK1-lacZ system demonstrated that ochre (UAA) suppression was the weakest, followed by amber (UAG) and then opal (UGA) (Firoozan et al 1991). Independently, [*PSI+*] was shown to suppress different nonsense codons at various loci including *trp5-48* (UAA) (Liebman & Sherman 1979, Liebman et al 1975, Singh 1979), *met8-1* (UAG) (Chernoff et al 1995, Ishiguro et al 1981, Ono et al 1986), *lys2-101* (UGA) (Ishiguro et al 1981, Ono et al 1986), *ade1-14* (UGA) (Chernoff et al 1995), and *lys2-187* (UGA) (Chernoff et al 1995).

In light of our current understanding of [*PSI+*] as an epigenetic modulator of terminator activity, autonomous suppression by [*PSI+*] in the absence of known genetic suppressors is somewhat perplexing. Even in the background of diminished terminator activity, an amino acid must still be inserted into the nascent polypeptide. Naturally occurring wild-type tRNAs with low intrinsic suppressor activity may contribute to autonomous [*PSI+*] suppression (Edelman & Culbertson 1991,

Lin et al 1986, Pure et al 1985, Tuite et al 1981a, Tuite & McLaughlin 1982, Weiss & Friedberg 1986). Alternately, weak uncharacterized suppressors may be present in the strains used. Indeed, low suppressor background strains have been isolated by virtue of their cold resistance, and [*PSI*+] is unable to suppress nonsense mutations in these backgrounds (Cox et al 1988), although it does so efficiently in others (Liebman & Sherman 1979, Liebman et al 1975, Singh 1979).

In Vitro [*psi*−] Is Dominant Over [*PSI*+]

Using a cell-free translation system from *S. cerevisiae*, several aspects of [*PSI*+] suppression could be reproduced in vitro (Tuite et al 1981a). For example, read-through of all three nonsense codons by suppressor tRNAs occurs only when the translation lysates are prepared from [*PSI*+] but not [*psi*−] strains (Tuite et al 1981a). The relative suppression efficiencies for the three nonsense codons in vitro corresponds to the in vivo results (Firoozan et al 1991), with opal read-through being the most efficient, followed by amber and then ochre (Tuite et al 1981a).

Although the suppressor phenotype of [*PSI*+] strains is reproduced in vitro, the genetic interaction between [*PSI*+] and [*psi*−] strains is not. Recall that [*PSI*+] is dominant in all genetic crosses; only [*PSI*+] diploids are obtained when [*PSI*+] and [*psi*−] haploids are mated (Cox 1965). However, mixing as little as 20% of a [*psi*−] lysate with a [*PSI*+] lysate abolishes nonsense suppression in vitro. This (Tuite et al 1983) and other work (Colby & Sherman 1981) suggested the presence of a protein inhibitor of [*PSI*+] in the [*psi*−] cytosol (Tuite et al 1981a).

SEARCH FOR THE [*PSI*+] DETERMINANT

[*PSI*+] Is Cytoplasmically Transmitted

One possible explanation for the non-Mendelian inheritance of [*PSI*+] is a cytoplasmic mode of transmission. The isolation of karogamy mutations, which allow cytoplasmic mixing in the absence of nuclear fusion (cytoduction), provided a rigorous test of this possibility (Conde & Fink 1976). In such experiments, the transmission of mitochondrial function is a positive control for efficient cytoplasmic mixing, and the failure to transfer nuclear markers provides a negative control for diploidization. Strains that exhibit the transfer of cytoplasmic but not nuclear markers also show transfer of [*PSI*+] (Conde & Fink 1976, Cox et al 1980). Extrachromosomal nuclear plasmids can also be transferred at low frequency in such experiments (Sigurdson et al 1981). However, coincidence of transfer for [*PSI*+] and mitochondrial function is nearly 100%, strongly suggesting a cytoplasmic localization for [*PSI*+]. The efficiency of this transfer suggests that the [*PSI*+] factor is present in high copy in the yeast cytosol.

Once cytoplasmic inheritance was established, attempts to characterize the [*PSI*+] determinant centered on known cytoplasmic nucleic acids, most obviously the mitochondrial genome. However, [*PSI*+] and mitochondrial DNA have

different sensitivities to ethidium bromide and to UV irradiation in repair-deficient strains (Tuite & Cox 1980, Tuite et al 1981b). [*PSI+*] inheritance is also unlinked to the mitochondrial marker for erythromycin resistance (Young & Cox 1972), and [*PSI+*] can be maintained in strains lacking mitochondrial DNA (Tuite et al 1982). Finally, certain nuclear mutations eliminate [*PSI+*] (Young & Cox 1971) or the mitochondrial genome independently of each other (Leibowitz & Wickner 1978).

In similar experiments [*PSI+*] was shown to be distinct from the other known cytoplasmic nucleic acids: The L and M dsRNAs associated with the killer phenotype of yeast and the sporulation-induced 20S RNA. [*PSI+*] strains lacking dsRNA were isolated (Leibowitz & Wickner 1978, Tuite et al 1982), and spontaneously arising [*psi−*] strains were found to retain the killer phenotype (Wickner 1976). These genetic elements are further distinguished by differing sensitivities to heat shock (Cox et al 1988, Lindquist et al 1995, Singh 1979, Tuite et al 1981b, Wickner 1976) and cycloheximide (Wickner 1976). Finally, both [*PSI+*] and [*psi−*] strains produce 20S RNA (Garvik & Haber 1978).

Several laboratories also analyzed the relationship between [*PSI+*] and small extra-chromosomal nucleic acids of the nucleus, such as the 2μ (McCready & McLaughlin 1977, Tuite et al 1982) and 3μ DNAs (Dai et al 1986, McCready & McLaughlin 1977). [*PSI+*] is maintained in strains cured of the 2μ plasmid, eliminating it as a possible [*PSI+*] determinant (Tuite et al 1982); however, a potential link between [*PSI+*] and 3μ circles was observed (Dai et al 1986). The 3μ-enriched fractions from [*PSI+*] lysates converted [*psi−*] strains to [*PSI+*] at a frequency that was slightly greater than the spontaneous rate of change from [*psi−*] to [*PSI+*] (Dai et al 1986). These results were met with skepticism, however, since 3μm circles had been previously detected in [*psi−*] lysates (McCready & McLaughlin 1977). 3μ circles are extra-chromosomal genes for ribosomal RNA (rRNA) (Clark-Walker & Azad 1980, Larionov et al 1980), and they were eliminated later as the [*PSI+*] determinant because the [*PSI+*] phenotype and its inheritance, unlike rRNA expression, are not dependent upon RNA polymerase I (Nierras & Cox 1994).

Effects of Nucleic Acid Mutagens on [*PSI+*]

Mutagenic studies were undertaken to probe the nature of the [*PSI+*] determinant. Certain nucleic acid mutagens, including ethylmethane sulfonate (EMS), *N*-methyl-*N*′-nitrosoguanidine (NTG), and UV irradiation, can restore translational fidelity in [*PSI+*] strains (Lund & Cox 1981, Tuite & Cox 1980, Tuite et al 1981b). Further characterization of these strains revealed that the most frequent mutations were nuclear lesions that masked the [*PSI+*] suppressor phenotype without disrupting [*PSI+*] propagation (Tuite & Cox 1980). That is, although [*PSI+*] is, in effect, hidden from view, its phenotype reappears when genetic crosses segregate out the mutation. Mutations specifically curing [*PSI+*] are observed at a tenfold lower frequency, and induction of these lesions by UV irradiation follows linear kinetics. This suggests that the mutation of a single locus is sufficient to eliminate [*PSI+*] (Tuite & Cox 1980). Surprisingly, both the excision and error

prone repair pathways, which are active in the nucleus, protect this locus from mutation (Tuite & Cox 1980), although [*PSI*+] itself clearly resides in the cytoplasm (Conde & Fink 1976, Cox 1965, Cox et al 1980). These observations led to considerable confusion and the mistaken belief that [*PSI*+] was conferred by a nucleic acid determinant.

In light of current work indicating that [*PSI*+] is an alternate physical state of Sup35p, the UV mutagenesis results can be re-interpreted in different ways. First, Sup35p is encoded by a nuclear gene, and thus mutations at this locus can disrupt [*PSI*+] inheritance (DePace et al 1998, Doel et al 1994). Second, mutations in extra-genic modifiers of [*PSI*+] such as *HSP104* (see below) can eliminate [*PSI*+] (Chernoff et al 1995). Moreover, Hsp104 levels are changed in response to several mutagens that are effective in eliminating [*PSI*+] (Lindquist et al 1995).

Other Agents Effective in Eliminating [*PSI*+]

In contrast to these treatments, another group of agents not active on nucleic acids had a surprisingly strong influence on [*PSI*+] to [*psi*−] conversion rates. This important work began serendipitously. In the course of plating a [*PSI*+] strain, a drop of methanol from Mundy's spreader inadvertently fell onto the plate. After incubation, the area "treated" with methanol surprisingly contained [*psi*−] colonies (MF Tuite, personal communication).

A systematic study of the effects of organics, salts, sugars, and other osmolytes on [*PSI*+] → [*psi*−] conversion rates then ensued (Table 3). Because these agents were included in the growth media, care was taken to differentiate treatments that would induce conversion from those that would preferentially select for growth of sponanteously arising [*psi*−] isolates (Tuite et al 1981b). Guanidium hydrochloride (1–5 mM), methanol (10% v/v), and ethylene glycol (1.8 M) were shown to be particularly efficient in inducing [*PSI*+] to [*psi*−] conversions (Table 3) (Cox et al 1988, Lund & Cox 1981, Singh 1979, Tuite et al 1981b).

Guanidium hydrochloride (GdnHCl) has been the most extensively studied curing agent by virtue of its unique ability to induce nearly 100% conversion at very low concentrations (Cox et al 1988, Tuite et al 1988). GdnHCl is effective only after several generations of exponential growth (Tuite et al 1981b). Once [*psi*−] cells begin to appear, their numbers gradually increase, while the number of [*PSI*+] cells decreases over the course of exposure to GdnHCl. Consequently, if GdnHCl is removed from a culture before it is completely cured, a mixed population of [*PSI*+] and [*psi*−] cells will be present (Cox 1993; S Eaglestone & MF Tuite, personal communication). These observations suggest that GdnHCl inhibits [*PSI*+] propagation: In the presence of GdnHCl, new [*PSI*+] elements are not produced, but existing [*PSI*+] elements are not affected (Cox 1993; S Eaglestone & MF Tuite, personal communication). Cell division eventually dilutes existing [*PSI*+] elements, leading to curing of the culture, but if GdnHCl is removed before this process is complete, the existing [*PSI*+] elements can resume propagation. These

TABLE 3 Efficiencies of [*PSI+*] to [*psi−*] conversions by various treatments[a]

Treatment	Dose	Efficiency (%)
Guanidine HCl	1–5 mM	100
Ethylene glycol	1.8 M	100
Methanol	10% v/v	90
Sodium glutamate	1 M	50
Magnesium chloride	1 M	45
Potassium chloride	$\leq$2 M	30
Dimethylsulfoxide	5–10% v/v	25
Glycerol	1.8 M	20
Mannose	2 M	NR
Sucrose	1.8 M	3
Fructose	1.8 M	1
Ethanol	10% v/v	NR
Sodium succinate	1 M	NR
Sodium pyruvate	1 M	NR
Erythromycin	250 μg/ml	NR
Heat shock	50°C up to 45 min 42°C, 15 min	0.5 2
Ethidium bromide	10 μg/ml	NE
Acriflavine	10 μg/ml	NE
5FU	50 μg/ml	NE
Manganese chloride	8.8 mM	NE
Thymidine starvation		
Elevated temperature	37°C 39°C, 1–2 days	NE
Glucose	2 M	NE
Galactose	2 M	NE

[a]Adapted from Cox 1988; Data taken from Singh 1979, Tuite et al 1981b, Lund & Cox 1981. NR = not reported; NE = not effective.

observations are consistent with a plasmid determinant for [*PSI+*] and, therefore, the UV mutagenesis results. However, this result was later re-interpreted in light of the hypothesis that the [*PSI+*] determinant was an alternate physical state of Sup35p. In this case, Sup35p in the [*PSI+*] form is eliminated from yeast cells through cell division and is replaced by newly synthesized Sup35p which adopts the [*psi−*] state (S Eaglestone & MF Tuite, personal communication).

Is [*PSI+*] Transmitted as a Gene Product?

At the time, the observations described above were baffling (Cox et al 1988). Segregation of [*PSI+*] to all meiotic progeny and efficient cytoduction suggested the determinant was present in high copy and resided in the cytoplasm (Conde & Fink 1976, Cox 1965, Cox et al 1980). However, this was at odds with the UV mutagenesis studies suggesting that the [*PSI+*] determinant was maintained as a single copy (Tuite & Cox 1980). As a possible resolution to these inconsistencies, a unique role for the gene product of the [*PSI+*] determinant was proposed (Tuite et al 1982):

> . . .the possibility still exists that the [*PSI*] phenomenon may in fact represent a self-sustaining regulatory system governing the activity of a nuclear gene (the "[*PSI+*] determinant") coding for a component of the yeast translation system.

This prescient statement was the first suggestion that the product of the "[*PSI+*] gene" influenced the inheritance of the suppressor trait. This model was later elaborated on by Strathern (Cox et al 1988):

> "the 'determinant' for the purposes of maintaining the phenotype is not the gene but the gene product."

These suggestions were embedded within complex models designed to explain the switch between the [*PSI+*] and [*psi−*] states, most elements of which have been discarded. Nevertheless, the simple core idea of a gene product directing inheritance would gain great support in the next decade.

RELATIONSHIP BETWEEN [*PSI+*] AND SUP35p

Over-Expression of Sup35p Increases the Frequency of [*PSI+*] Appearance

A group of scientists, including Cox, Chernoff, Ter-Avanesyan, and Inge-Vechtomov, provided the first link between [*PSI+*] and its true determinant *SUP35* (Chernoff et al 1988, 1993; Doel et al 1994). *SUP35* had been previously implicated in translational accuracy[1] (Hawthorne & Leupold 1974, Inge-Vechtomov & Andrianova 1975). A study examining the effect of changes in *SUP35* copy number on the fidelity of protein synthesis led directly to the seminal findings implicating a protein-only (prion) model of inheritance for [*PSI+*].

[1]It should be noted that earlier work from several laboratories suggested a role for *SUP35* in translational fidelity. The gene is variously known as *SUP40* (Hawthorne & Leupold 1974), *SUPP* (Gerlach 1975), *SUP36* (Ono et al 1984), *SUP2(S$_2$)* (Inge-Vechtomov 1964, 1965; Inge-Vechtomov & Andrianova 1970), *SAL3* (Cox 1977), *SUF12* (Culbertson et al 1982), and *GST1* (Kikuchi et al 1988).

In these studies, it was determined that an extra copy of *SUP35* (carried on a plasmid) causes nonsense suppression in [*psi*−] strains. This nonsense suppression continues even after the plasmid is lost (Chernoff et al 1988, 1993) but can be reversed by GdnHCl treatment. This nonesense suppression phenotype cannot be induced when the *SUP35* coding sequence carries a nonsense mutation, strongly suggesting that Sup35 protein (Sup35p) rather than the mere presence of the plasmid or transcript, governs these phenomena (Derkatch et al 1996). Taken together, these data indicate that transient over-expression of Sup35p can convert cells from [*psi*−] to [*PSI*+]. [Rigorous proof that the new, curable suppressor elements are bona fide [*PSI*+] elements was later provided by (Derkatch et al 1996, Ter-Avanesyan et al 1994)]. Another curiosity noted in these experiments was that "the effect of plasmid-mediated amplification of *SUP35* was similar to that of mutations in this gene" (Chernoff et al 1993). This statement linked the [*PSI*+] phenotype to loss of *SUP35* function for the first time. Further work by these investigators, both collaboratively and independently, characterized the regions of *SUP35* capable of inducing [*PSI*+] de novo.

The *SUP35* sequence predicts a protein of 685 residues. Three regions are immediately apparent from their distinct amino-acid compositions and homologies to other proteins (Kikuchi & Kikuchi 1988; Kikuchi et al 1988; Kushnirov et al 1987, 1988; Telkov & Surguchev 1986; Wilson & Culbertson 1988). The amino-terminal region (N), amino acids 1–123, and the middle region (M), amino acids 124–253, have no homologies to proteins of known function. The carboxy-terminal region (C), amino acids 254–685, is homologous to translation elongation factor EF-1α, and contains four putative GTP binding sites. A series of experiments with various Sup35p fragments on plasmids and in the genome, in the presence and in the absence of wild-type Sup35p, defined the role of these three regions in cell viability and [*PSI*+] metabolism (Table 4).

The region encompassing N and M is dispensable for viability (Ter-Avanesyan et al 1993). However, plasmids containing this region induce nonsense suppression in [*psi*−] cells that carry a wild-type *SUP35* gene in the genome (Table 4) (Ter-Avanesyan et al 1993, Derkatch et al 1996). By several criteria it is now clear that the suppression phenotype induced by these fragments is associated with de novo [*PSI*+] induction (Derkatch et al 1996, Kochneva-Pervakhova 1998b, Ter-Avanesyan et al 1994) and that a subfragment of N, encoding just the first 114 amino acids is sufficient for this effect (Ter-Avanesyan et al 1993). Nearly all fragments capable of inducing [*PSI*+] in [*psi*−] strains cause a growth defect when expressed in cells that are already [*PSI*+] (Table 4) (Chernoff et al 1988, 1993; Dagkesamanskaya & Ter-Avanesyan 1991; Derkatch et al 1996; Ter-Avanesyan et al 1994, 1993b). The precise molecular reason for this toxicity is still unclear, but it is consistent with earlier observations indicating that excessive nonsense suppression is incompatible with growth (Gilmore & Mortimer 1966, Gilmore 1967, Gilmore et al 1971, Kakar 1963, Liebman & Sherman 1979, Liebman et al 1975, Mortimer & Gilmore 1968, Ono et al 1988).

The functions and effects of the C region contrast sharply with those of NM. First, the C region is essential for viability (Kushnirov et al 1990a, Ter-Avanesyan

TABLE 4 Properties of Sup35p fragments

Fragment (amino acids)	[*PSI*+] induction/ propagation	Growth suppression in [*PSI*+]	[*PSI*+] antisuppression
1–685	+[a,c,d]	+[a,e]	−[b]
125–685 (Δ2ATG)	−[d]	−[d]	+[b]
147–685	−[a]	−[a]	NA
254–685 (Δ3ATG)	−[d]	−[d]	−[b]
1–35	−[a]	−[a]	NA
1–114 (Δ*Eco*)	+[c]	NA	−[b]
1–154 (Δ*Bal2*)	+[d]	+[d]	−[b]
1–239 (Δ*Bcl*)	+[c,d]	+[c,d]	−[b]
1–482 (Δ*Sal*)	+[c,d]	+[c,d]	−[b]
1–21, 70-685 (Δ*BstE*II)	−[c,d]	+[d] −[b]	+[b]

[a]Chernoff et al 1988, 1993.

[b]Ter-Avanesyan et al 1993b.

[c]Ter-Avanesyan et al 1994.

[d]Derkatch et al 1996.

[e]Dagkesamanskaya et al 1991.

et al 1993b) and is highly conserved (Figure 2*A*) (Hoshino et al 1989; Ito et al 1998; Kushnirov et al 1990b, c; Samsonova et al 1991). Mutations in this region confer a recessive nonsense suppression phenotype (Doel et al 1994, Tarunina et al 1994; MF Tuite, personal communication). Unlike fragments containing N, overexpression of C does not induce nonsense suppression in [*psi*−] cells (Kushnirov et al 1990a, Ter-Avanesyan et al 1993b) and is not toxic to [*PSI*+] cells, but it eliminates the suppressor phenotype in these strains (Derkatch et al 1996; Ter-Avanesyan et al 1993b, 1994). Suppression is again observed, however, when the plasmid expressing the C region is lost. That is, excess C masks the [*PSI*+] phenotype without curing the [*PSI*+] determinant (Ter-Avanesyan et al 1993b).

Regions of Sup35p Required for Propagation of [*PSI*+]

The over-expression experiments described above delineated a clear role for the N terminus of *SUP35* in the de novo induction of [*PSI*+]. To determine the role of this region in [*PSI*+] propagation, the endogenous *SUP35* gene was replaced with a series of N-terminal deletion mutants (Ter-Avanesyan et al 1994). These replacements cause haploid [*PSI*+] cells to revert to [*psi*−]. Thus the N-terminal region is required for the propagation of [*PSI*+]. In [*PSI*+] diploids carrying one wild-type copy of *SUP35* and one N-terminal deletion copy, the nonsense suppression phenotype is lost. That is, the N-terminal deletion acts dominantly to mask the [*PSI*+] suppression phenotype. Upon sporulation, [*PSI*+] segregates to haploid progeny carrying the wild-type *SUP35* gene. Therefore, both integrated

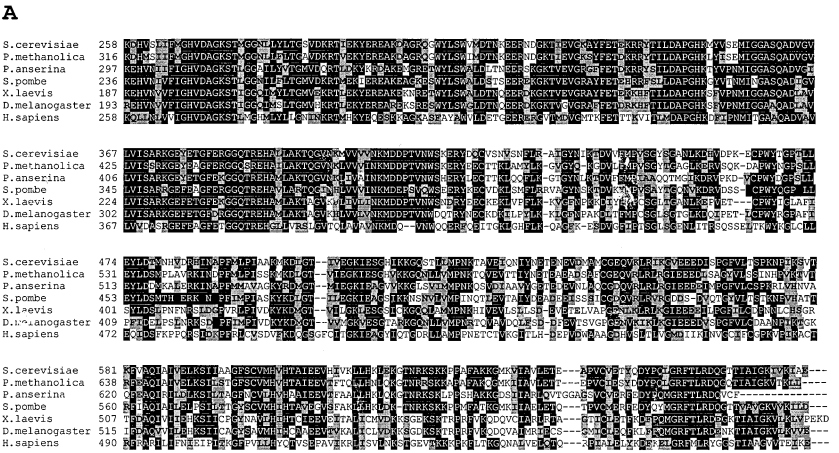

Figure 2 (*A*) Alignment of the C-terminal regions of *SUP35* from various organisms. Alignment was created with Clustal W using the entire *SUP35* sequence. GenBank accession numbers are as follows: Z46727 *(S. cerevisiae)*, X56910 *(P. methanolica)*, AF045014 *(P. anserina)*, D79214 *(S. pombe)*, L37045 (*X. laevis*), U88868 *(D. melanogaster)*, U87791 *(H. sapiens)*. Identical sequences are boxed in black; homologous sequences are shown in gray. (*B*) The unusual distribution of amino acids in the N-terminal (N and M) regions of Sup35 proteins. Glutamine (Q), asparagine (N), glycine (G), and tyrosine (Y) residues are boxed in black; lysines (K), aspartate (D) and glutamate (E) residues are highlighted in gray. The four fungal proteins from *S. cerevisiae (S. cer)*, *P. methanolica (P. meth)*, *P. anserina (P. ans)*, and *S. pombe* share an unusual distribution of amino acids, with Q, N, Y, and G residues concentrated toward the N termini and K, D, and E residues concentrated between this region and the highly conserved C terminus shown in (*A*). Alignment was created with Clustal W using the N-terminal regions of the Sup35ps. Note, however, that the divergence of the primary sequences makes the precise alignment questionable. For comparison, the *X. laevis* (*X. lav*) sequence is shown. GenBank accession numbers are as in (*A*). Sequence numbering for both A and B corresponds to the predicted ORFs.

and extra-chromosomal N-terminal truncations conceal the [*PSI+*] phenotype but they do not dominantly interfere with [*PSI+*] propagation or transmission. Consequently, deletions in N are considered to be recessive [*PSI+*]-no-more (*pnm*) mutations.

The role of the C region in [*PSI+*] propagation was particularly difficult to determine since it is required for viability, and N-terminal truncations mask the [*PSI+*] phenotype. These difficulties were circumvented in an elegant series of experiments (Ter-Avanesyan et al 1994) employing cytoduction (abortive matings that mix cytoplasmic factors without nuclear fusion and produce progeny with haploid parental genotypes). [*PSI+*] was transferred by cytoduction to strains in which wild-type Sup35p has been replaced by the Sup35p C region and different

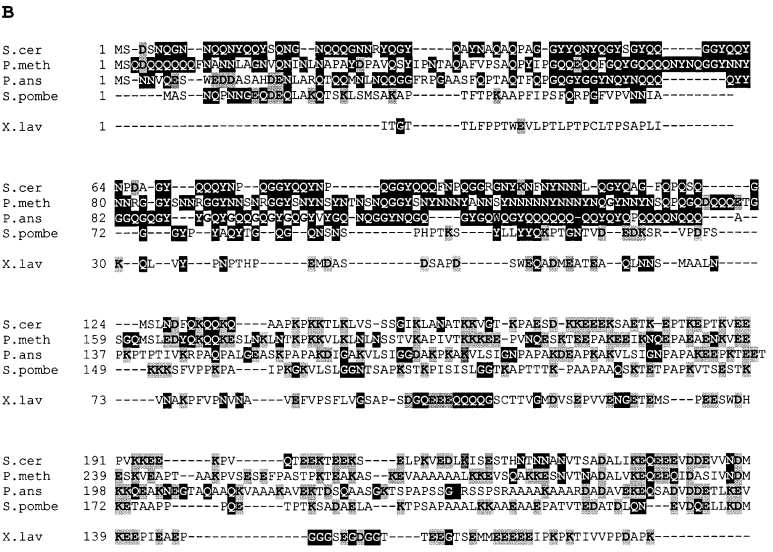

Figure 2 (*Continued*)

fragments of Sup35p were expressed from a plasmid. Because the C region of Sup35p is incapable of propagating [*PSI+*] (Ter-Avanesyan et al 1994), retention of [*PSI+*] depends upon fragments expressed from the plasmid. [*PSI+*] propagation was then scored in a second round of cytoduction to another [*psi−*] strain expressing wild-type Sup35p. Using this system, all fragments capable of inducing [*PSI+*] de novo when over-expressed are competent to propagate [*PSI+*] in the absence of full-length Sup35p (Table 4). Taken together, the over-expression and replacement experiments confirmed that the N region of Sup35p is both necessary and sufficient for [*PSI+*] propagation while the C region is dispensable.

[*PSI+*] IS A PRION-LIKE FORM OF SUP35p

In 1994 Wickner provided a conceptual breakthrough that tied the pieces of this complex puzzle together. Wickner had been studying another cytoplasmically transmitted phenotype in yeast called [*URE3*], which shares many of the baffling genetic features of [*PSI+*] (discussed below). Based on genetic arguments, Wickner suggested that [*URE3*] and [*PSI+*] were examples of yeast prions (Wickner 1994).

The prion model was initially developed to explain the peculiar nature of the infectious agent responsible for a group of unusual neurodegenerative diseases including kuru, Creutzfeldt-Jakob disease (CJD), fatal familial insomnia (FFI), and Gerstmann-Sträussler-Scheinker syndrome (GSS) of humans, bovine spongiform encephalopathy (BSE) of cows, and scrapie of sheep (Griffith 1967, Prusiner

1982). The term prion derives from proteinacious infectious particle (Prusiner 1982), and the model holds that the protein is itself the infectious agent. The essential features of the hypothesis are as follows. The prion protein, PrP, exists in at least two functionally distinct but stable conformations, the normal cellular form PrP^{C}, and the pathogenic form PrP^{Sc}. PrP^{Sc} appears rarely, but once present, this conformer becomes predominant by influencing other PrP protein to adopt the PrP^{Sc} state. This both generates new infectious material and causes pathogenic changes in neurons. Propagation of the disease is dependent upon the continued synthesis of the protein and, therefore, indirectly upon the gene encoding it (Prusiner 1998).

As Wickner pointed out, diverse aspects of the [*PSI+*] phenomenon can be explained by this hypothesis (Wickner et al 1996a, Wickner 1994). [*PSI+*] is suggested to arise from a rare change in the physical state of Sup35p ($Sup35p^{[PSI+]}$). $Sup35p^{[PSI+]}$ becomes predominant by influencing other Sup35p to adopt the same state. This both generates new [*PSI+*] elements to be passed by mothers to their daughters and changes the phenotype of the cell. The N region of Sup35p is essential to this change in state, and therefore the propagation of [*PSI+*] is dependent on N. Over-expression of Sup35p or the N region alone increases the likelihood that $Sup35p^{[PSI+]}$ will appear, and therefore induce new [*PSI+*] elements in [*psi−*] cells. Once this state has appeared, it is self-perpetuating, and over-expression is not required to sustain it. Thus transient over-expression is sufficient to induce a heritable change in phenotype. Furthermore, this model explains the cytoplasmic inheritance of [*PSI+*]: Sup35p resides in the cytoplasm where it can be transferred by cytoduction and to all four progeny in meiosis. This revolutionary hypothesis has now been supported by a wealth of additional genetic, cell biological, and biochemical data.

GENETIC INTERACTIONS BETWEEN [*PSI+*] AND MOLECULAR CHAPERONES

The Molecular Chaperone Hsp104

As the prion hypothesis was being formulated, strong support came from an unexpected source (Chernoff et al 1995). Heat-shock protein Hsp104, a molecular chaperone (Parsell et al 1991, Sanchez & Lindquist 1990, Sanchez et al 1992), was isolated in a screen for extra-copy plasmids that could inhibit the suppression phenotype of [*PSI+*].[2] *HSP104* is a highly conserved member of the ClpB/Hsp100 family of molecular chaperones (Parsell et al 1991). The function of this class of Hsps in stress tolerance is distinct. Most HSP chaperones bind other proteins when they are unfolded and prevent them from aggregating. In contrast, Hsp104,

[2][*PSI+*] can be cured by *HSP104* over-expression or deletion in multiple, unrelated strains. (Chernoff et al 1995, Newman et al 1999).

disaggregates proteins that have already begun to aggregate (Glover & Lindquist 1998, Parsell et al 1994). This biochemical function correlates with Hsp104's distinct biological role in stress tolerance. Unlike other HSPs, Hsp104 is not required for growth, even at high temperatures. It is, however, required for cells to survive sudden extreme stresses (Sanchez & Lindquist 1990, Sanchez et al 1992), when the rate of protein unfolding might be expected to outpace the capacity of other chaperones to prevent aggregation.

When Hsp104 is over-expressed at a modest level in [*PSI*+] cells, it reduces their capacity for nonsense suppression but does not cure them of [*PSI*+]. However, [*PSI*+] is efficiently cured by higher levels of Hsp104 (Chernoff et al 1995, Lindquist et al 1995, Newman et al 1999, Patino et al 1996, Zhou et al 1999). Indeed, the strength of [*PSI*+] suppression and the level of *HSP104* expression required to cure it appear to be directly correlated (Newman et al 1999). These effects of Hsp104 are not due to an effect on translation per se, but are specific to the maintenance of [*PSI*+] (Chernoff et al 1995). Most remarkably, transient over-expression of Hsp104 is sufficient to cure cells of [*PSI*+]. Because the only known function of Hsp104 is to change the conformational state of other proteins, this strongly argues that the inheritance of [*PSI*+] is due to the transmission of a protein with an altered physical state. Once the alternate state is eliminated, [*PSI*+] is lost. (That over-expression of Hsp104 leads to a heritable change in the physical state of Sup35p was later established in cell biological studies; see below).

Surprisingly, *HSP104* deletions also cure cells of [*PSI*+] (Chernoff et al 1995). In heterozygous diploids containing a wild-type *HSP104* gene and an *hsp104* deletion, [*PSI*+] is stable. However, when the diploid is sporulated, the two spores that receive the *hsp104* deletion chromosome are [*psi*−]. Thus Hsp104 deletions define a second class of recessive [*PSI*+]-no-more (*pnm*) mutations. [The first class consists of deletions and mutations in the N region of *SUP35* (Doel et al 1994, Ter-Avanesyan et al 1994)].

Taken together, these observations demonstrate that the inheritance of [*PSI*+] depends on the expression of intermediate levels of Hsp104. Either too much or too little Hsp104 can cure cells of [*PSI*+]. It is not common for over-expression and loss-of-function mutations to have the same phenotype, but when they do, this situation usually betokens a requirement for the protein to interact with another protein in a dosage-dependent way. It is therefore particularly interesting that Sup35p behaves in a similar manner. Over-expression of Sup35 has the same phenotype as partial loss-of-function mutations: nonsense suppression.

Two Walker-type ATP binding sites are required for *HSP104* function in stress tolerance (Parsell et al 1991), and mutations in these sites act dominantly to interfere with [*PSI*+] (Chernoff et al 1995, Patino et al 1996), suggesting a common mechanism of action. In vitro, Hsp104p has been reported to alter the circular dichroism spectrum of Sup35p, but the relationship between this observation and [*PSI*+] propagation is unclear (Schirmer & Lindquist 1997). Possible models for Hsp104's effects on [*PSI*+] will be considered after we discuss the physical changes in Sup35p that are associated with [*PSI*+].

Other Chaperones

Another chaperone protein that interacts genetically with [*PSI+*] is Ssa1, a member of the Hsp70 family. Extra-copy plasmids carrying the *SSA1* gene inhibit suppression in some [*PSI+*] strains (Chernoff et al 1995) and enhance it in others (Newman et al 1999). Extra-copy *SSA1* plasmids also interfere with the curing of [*PSI+*] by Hsp104 over-expression (Newman et al 1999). These effects of Hsp70 on [*PSI+*] are intriguing since *HSP104* and *SSA1* cooperate in the rescue of heat-damaged aggregated proteins (Glover & Lindquist 1998, Sanchez et al 1993). With respect to [*PSI+*] propagation, however, their relationship appears to be antagonistic. Given that our understanding of Hsp104's function in [*PSI+*] propagation is rudimentary, it is difficult to model the role of Hsp70. In addition, Hsp70 is problematic to manipulate experimentally in vivo and its effects on cell physiology are complex. Deletion or over-expression of Hsp104 has no general effects on growth and no detectable effects on the synthesis or accumulation of other proteins in the cell (Sanchez & Lindquist 1990). In contrast, Hsp70 levels can be altered by only a few-fold without toxicity, and even these alterations dramatically change the expression of many proteins in the cell (Werner-Washburne et al 1987).

A precise understanding of Hsp70's effects on Sup35p and [*PSI+*] must await the development of a system that reconstructs their essential features in vitro, just as reactivation of denatured protein substrates in vitro (Glover & Lindquist 1998) has begun to elucidate the roles of Hsp104 and Hsp70 in stress tolerance in vivo (Sanchez et al 1993). Nevertheless, the simple fact that the net effect of Hsp70 over-expression in vivo is to counteract the action of high levels of Hsp104 in curing cells of [*PSI+*] (Newman et al 1999) may explain one puzzling aspect of [*PSI+*] biology. Hsp104p levels are naturally elevated following heat shock, during the transition to stationary phase growth and during sporulation, yet these conditions do not cure [*PSI+*] (Cox 1965, Singh 1979, Tuite et al 1981b). The cellular physiology is vastly different during these stresses, and Hsp104p interaction with other factors such as Hsp70, which is also induced under these conditions, may attenuate its ability to cure [*PSI+*]. Alternately, these conditions are also accompanied by a cessation of cellular division, a crucial requirement for [*PSI+*] curing by at least two different methods (Cox 1993, McCready et al 1977, Young & Cox 1971; S Eaglestone & MF Tuite, personal communication).

BIOCHEMICAL SUPPORT FOR [*PSI+*] AS A YEAST PRION

The initial suggestion that [*PSI+*] is inherited through a protein-only mechanism was based solely on genetic considerations. General acceptance of the hypothesis would require biochemical evidence for a physical difference between $Sup35p^{[PSI+]}$ and $Sup35p^{[psi-]}$ and an explanation for how that difference could account for nonsense suppression. Two key elements were quick to appear.

First, a striking difference in the physical state of Sup35p was discovered in [*PSI*+] and [*psi*−] cells. There are no apparent differences in Sup35p$^{[PSI+]}$ and Sup35p$^{[psi-]}$ migration on two-dimensional gels from isogenic [*PSI*+]/[*psi*−] strain pairs (Patino et al 1996) nor are there consistent differences in Sup35p levels in isogenic [*PSI*+] /[*psi*−] strain pairs (Patino et al 1996, Paushkin et al 1996). However, when cell lysates are subjected to centrifugation or gel filtration, most Sup35p$^{[PSI+]}$ is found in large, insoluble complexes while most Sup35p$^{[psi-]}$ remains in small soluble complexes (Patino et al 1996, Paushkin et al 1996). This difference in Sup35p solubility is accompanied by a difference in sensitivity to digestion with proteinase K: Sup35p$^{[PSI+]}$ is more resistant than Sup35p$^{[psi-]}$. Insolubility and protease resistance are two features that distinguish the mammalian PrP protein in its prion state from its normal cellular state (Prusiner 1998).

Rounds of [*PSI*+] curing and re-introduction demonstrated the relevance of these differences to [*PSI*+] biology. In cells switched from [*PSI*+] to [*psi*−] by Hsp104 manipulations, Sup35p is soluble.[3] Similarly, in cells switched from [*psi*−] to [*PSI*+] by over-expression of NM, Sup35p is found in the insoluble fraction (Patino et al 1996). To monitor protein dynamics in living cells, the NM region of Sup35p was fused to the green fluorescent protein (GFP) (Patino et al 1996). After brief induction, the fusion protein exhibits diffuse fluorescence throughout the cytoplasm of [*psi*−] cells but coalesces into discrete foci in [*PSI*+] cells. (Fusions between GFP and full-length Sup35p behave similarly; JJ Liu & S Lindquist, unpublished data). The differences in fluorescence pattern between [*PSI*+] and [*psi*−] cells expressing GFP fusion proteins for the same length of time suggest that newly synthesized Sup35p is rapidly assembled into the pre-existing higher order complexes of Sup35p in [*PSI*+] cells (Patino et al 1996). Changes in the fluorescence patterns of GFP fusions are an accurate indicator of the [*PSI*] status of strains. In cells switched from [*PSI*+] to [*psi*−], GFP remains diffuse; in cells switched from [*psi*−] to [*PSI*+], GFP coalesces into intense foci (Patino et al 1996).

These experiments also provided our first glimpse of [*PSI*+] induction in real time (Patino et al 1996). When the NM-GFP fusion protein is induced in [*psi*−] cultures for longer periods, coalescing fluorescent foci begin to appear in some of the cells. This is accompanied by the appearance of [*PSI*+] cells, as determined by platings to selective media. Finally, the GFP experiments provide a simple explanation for the curious ability of exogenous factors to suppress the [*PSI*+] phenotype, while maintaining the [*PSI*+] element. In cells expressing an Hsp104 point mutant that has such an effect (Chernoff et al 1995), the NM-GFP fluorescence pattern is intermediate. A portion of the protein coalesces while a substantial fraction remains diffuse; this correlates with the intermediate distribution of Sup35p between pellet and supernatant fractions of lysates isolated from these cells (Patino et al 1996). Thus some of the protein is in the active state, allowing efficient

[3]The *HSP104* dependence of Sup35p self-assembly in vivo has been observed in multiple unrelated strains (Patino et al 1996, Paushkin et al 1996).

termination of nonsense codons, whereas some remains in the prion state, allowing [*PSI+*] to reappear when the Hsp104 point mutant is no longer present.

Both the sedimentation and GFP experiments directly link the physical state of Sup35p in [*PSI+*] cells to the region required for [*PSI+*] propagation. Fragments of Sup35p lacking all or part of N are not assembled into large complexes when expressed in [*PSI+*] strains (Paushkin et al 1996; 1997a, b). Similarly, the coalesced pattern of fluorescence in [*PSI+*] cells from GFP is dependent on its fusion to this region (Patino et al 1996).

At about the same time that the prion hypothesis was proposed to explain [*PSI+*] inheritance, the function of Sup35p was described (Tuite & Stansfield 1994). As noted above, the amino-acid sequence of Sup35p has homology to elongation factor EF-1α (Kikuchi et al 1988; Kushnirov et al 1987, 1988; Samsonova et al 1991; Wilson & Culbertson 1988), but early experiments were inconsistent with a role for Sup35p in elongation (Surguchov et al 1980; Smirnov et al 1973, 1974). A direct link between *SUP35* and termination emerged when a homologue of *SUP45* was purified from rabbit reticulocyte lysates as the polypeptide chain release factor (Frolova et al 1994). Perplexingly, *SUP45* had no homology to GTP-binding proteins, but the process of translation termination was known to be GTP dependent (Beaudet & Caskey 1971, Frolova et al 1994, Konecki et al 1977). A genetic interaction between *SUP35* and *SUP45* was well-established (Cox 1977; Inge-Vechtomov & Andrianova 1975; Inge-Vechtomov et al 1994, 1988; Tarunina et al 1994; Ter-Avanesyan et al 1993a), and Sup35p was predicted to bind to GTP (Kushnirov et al 1987, Wilson & Culbertson 1988). Consequently, it was proposed (Tuite & Stansfield 1994) and later shown in a *Xenopus laevis* in vitro termination system that Sup35p stimulated Sup45p-directed polypeptide release in a GTP-dependent manner (Zhouravleva et al 1995). Because *X. laevis SUP35* can complement mutations in *S. cerevisiae SUP35*, it is very likely that the proteins function similarly (Zhouravleva et al 1995). Indeed, co-over-expression of wild-type *SUP35* and *SUP45* reverses the nonsense suppression phenotype of a mutant tRNA in yeast, as expected for the termination complex (Stansfield et al 1995).

A Model to Explain the [*PSI+*] Phenotype

The discovery of [*PSI+*] complexes containing Sup35p provided the foundation for a model to explain the [*PSI+*] phenotype in molecular terms (Patino et al 1996, Paushkin et al 1996). Sup35p is present in a soluble state in the [*psi−*] cytosol, available to fulfill its function as a component of the yeast termination complex. Incorporation of Sup35p into large complexes in [*PSI+*] strains sequesters it, effectively depleting the concentration of functional Sup35p. Consistent with this hypothesis, mutations that decrease the levels or activity of Sup35p cause a nonsense suppressor phenotype (Cox 1977; Gerlach 1975; Hawthorne & Leupold 1974; Inge-Vechtomov 1964, 1965; Inge-Vechtomov & Andriavnova 1970; Ono et al 1984; Zhou et al 1999)

Inactivation of Sup35p by a change in physical state is also consistent with the ability of the C region to reduce nonsense suppression in [*PSI+*] strains. Because the N domain is required for [*PSI+*] propagation (Ter-Avanesyan et al 1994) and Sup35p incorporation (Patino et al 1996, Paushkin et al 1996), the C domain should be resistant to inactivation and remain available for translation termination. This model also explains the disparate phenotypes resulting from expression of the N and C domains in *cis* or in *trans*. Over-expression of full-length Sup35p induces [*PSI+*] and its associated suppressor phenotype. If the N and C regions are expressed as two separate proteins in the same cell, the [*PSI+*] phenotype is masked by the terminator activity of the C region (Ter-Avanesyan et al 1994). Conversion of the N domain into the prion state, therefore, acts as an epigenetic modulator of the C domain's translation-termination activity.

At the core of this model is the theory that [*psi−*] is the normal state, whereas [*PSI+*] is a loss-of-function state. While many details of the model remain to be tested, this aspect is solidly supported by the available data. Strikingly, for nearly 30 years, the opposite scenario was predicted. Genetically, dominance is most commonly associated with a gain-of-function. From in vivo experiments it had been predicted that [*PSI+*] cells produce an activator of nonsense suppressors (Cox 1965), and in vitro mixing experiments with cell free translation lysates predicted that [*psi−*] cells contain an inhibitor of [*PSI+*] activity. It now seems likely that it is the reduction in termination factor activity that leads to increased nonsense suppression by mutant tRNAs and that [*psi−*] lysates restore translational fidelity in vitro by providing functional Sup35p. Simply stated, [*PSI+*] is a dominant, epigenetic loss-of-function in Sup35p.

LINK BETWEEN [*PSI+*] STATE IN VIVO AND SUP35p SELF-ASSEMBLY IN VITRO

To determine if the various regions of Sup35p might have biochemical properties that could explain their in vivo activities, different fragments of the protein were purified from *Escherichia coli* (Glover et al 1997, King et al 1997). Fragments containing the N region alone (amino acids 1–114 or 1–124) are insoluble in physiological buffers but rapidly assemble into fibers rich in β-sheet structure in 40% acetonitrile or 2 M urea (Glover et al 1997, King et al 1997). A fragment encompassing both the N and the highly charged M domain (NM), in contrast, is soluble in non-denaturing buffers and also assembles into fibers, but only after a considerable lag (Glover et al 1997). These fibers share characteristics with protein amyloids implicated in several human diseases (Serpell et al 1997). For example, Sup35p fibers are protease resistant and bind to the dye Congo red, producing the characteristic spectral shift and apple-green birefringence under polarized light (King et al 1997; AG Cashikar, G Westermark & S Lindquist, unpublished observation). Most convincingly, by X-ray diffraction, NM fibers

exhibit the dominant reflections characteristic of the cross-pleated β-sheets of other amyloid proteins (L Serpell, M Sunde, T Serio & S Lindquist, unpublished observations).

The M region and the C region of Sup35p alone do not form fibers in vitro, but the whole protein does. (Whole Sup35 does not form fibers as reproducibly as NM, perhaps because the large C region is not in its native state after purification and is prone to other types of aggregation.) In high-salt buffers, fibers formed from the whole protein exhibit an interesting structural relationship to those formed by N and NM alone. By electron microscopy, N and NM fibers are smooth, with diameters of ~7 and ~10 nm, respectively. Sup35p fibers exhibit shaggy protuberances from a central fiber core of ~10 nm (Glover et al 1997). Thus just as the N region is necessary and sufficient for the induction of [*PSI*+] in vivo, it seems to be the primary component directing fiber formation in vitro, with the M and C domains entering the fiber only because they are attached to it.

In Vitro Seeding

The delay in NM assembly into amyloid fibers in vitro provides a window in which the effects of known modifiers of [*PSI*+] propagation can be assessed. The prion hypothesis and the behavior of NM-GFP fusions in vivo predict that conversion to the [*PSI*+] state will be accelerated by protein already in that state. Indeed, in physiological buffers, small quantities of pre-formed fibers greatly accelerate the rate at which NM forms fibers (Glover et al 1997). Preformed fibers also accelerate the rate at which N (amino acids 1–114) forms fibers in 40% acetonitrile (King et al 1997). Second, when NM fragments contain a deletion in the N region of Sup35p, which inhibits [*PSI*+] formation in vivo (Δ*BstE*II), fibers form only after a very prolonged lag phase, even with the addition of preformed fibers (Glover et al 1997). Third, point mutations in the N region, which interfere with [*PSI*+] propagation in vivo (DePace et al 1998, Kochneva-Pervakhova 1998a), reduce the rate of Sup35p self-assembly in vitro. Finally, a mutant that enhances the rate at which [*PSI*+] elements form in vivo, enhances the rate at which NM forms fibers in vitro (Liu & Lindquist 1999).

Experiments with yeast cell lysates established a direct link between the self-assembly properties of Sup35p and its derivatives in vitro and the propagation of [*PSI*+] in vivo. Cell lysates from three unrelated [*PSI*+] strains (YC13-6C, 74-D694, and D1142-1A) can substitute for preformed fibers in accelerating the assembly of purified, soluble NM. Lysates from their isogenic [*psi*−] counterparts cannot (Glover et al 1997). Related experiments examined the behavior of Sup35p present in whole cell lysates. When full-length Sup35p and a C-terminal truncation mutant are expressed together in [*psi*−] cells, both species can be converted to aggregated, proteinase K-resistant forms, when mixed with lysates of [*PSI*+] cells (Paushkin et al 1997a).

Remaining Mechanistic Questions

Taken together, these genetic, cell biological, and biochemical experiments provide very strong support for the protein-only or prion model of [*PSI*+] inheritance and considerable insight into the biochemical nature of the process. In fact, additional supporting experiments are continuing to be reported. For example, Sup35p expressed from promoters regulated by different stimuli, such as galactose, glucocorticoid, or copper, induce the de novo appearance of [*PSI*+] only in response to the appropriate signal. These observations strongly support a protein-only mode of inheritance for [*PSI*+] (Patino et al 1996).

However, many issues remain to be resolved. For example, although there are multiple correlations between [*PSI*+] in vivo and amlyoid formation in vitro, it is by no means clear that the Sup35 protein in [*PSI*+] cells is in the amyloid state. The critical change in conformation in vivo that is determinative for [*PSI*+] might involve a distinct folding intermediate that is unstable in the purified system in vitro and simply progresses on to the amyloid state under these conditions.

Another unanswered question is which form of Sup35p converts to the [*PSI*+] state in vivo. Can fully mature Sup35p complexed with its partner Sup45p be converted? Or is only immature or nascent protein susceptible? In vitro conversion reactions do not answer these questions. In fact, the nature of the physical change associated with the [*psi*−] to [*PSI*+] conversion is itself unclear. The structure of Sup35p in its native [*psi*−] state has yet to be defined. Indeed it is not even clear that the N and M regions have a unique native state; computer programs for predicting structure indicate a low propensity to form either α-helix or β-sheet, and when purified denatured NM is diluted into buffers, it retains a random coil configuration for extended periods (Glover et al 1997).

The molecular mechanisms by which Hsp104 and GdnHCl cure cells of [*PSI*+] are another focus of speculation. Many models can be proposed to explain the interaction between *HSP104* and [*PSI*+]. We consider two that have been discussed in the literature (Figure 3) (Patino et al 1996, Paushkin et al 1996).

The first suggests that Hsp104p deletions cure cells of [*PSI*+] because the chaperone is required to help Sup35p reach a susceptible folding intermediate (Patino et al 1996). From this state, Sup35p can either fold to its native form, (active in translation termination), or be captured by pre-existing Sup35p in the [*PSI*+] state. Excess Hsp104p might cure cells of [*PSI*+] either by disaggregating [*PSI*+] assemblages, rebinding folding intermediates and precluding their assembly, or by reducing the number of Sup35p folding intermediates interacting with a single Hsp104p molecule, thereby decreasing the efficiency of self-assembly. The second model suggests that *HSP104* is required for the propagation of [*PSI*+] because its dissaggregation activity maintains the Sup35p assemblages of these cells within a specific size range, optimizing seeding and partitioning to progeny upon cell division (Kushnirov 1998, Paushkin et al 1996). In this model, excess Hsp104p

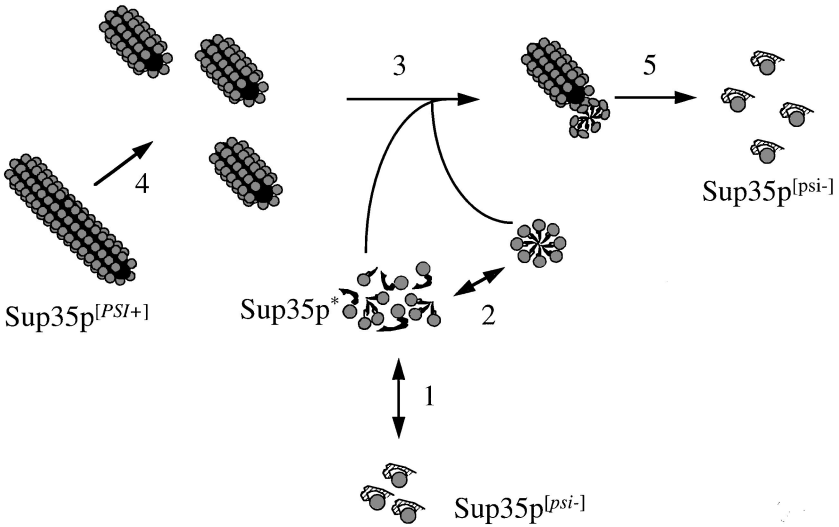

Figure 3 Models for Hsp104 regulation of [*PSI+*] metabolism. As predicted from model I (see text), Hsp104 is required for Sup35p$^{[psi-]}$ to reach a transition state (1) Sup35p* that is susceptible to self-assembly (2) but can also return to the native state Sup35p$^{[psi-]}$. The ratio of Hsp104 to Sup35p may determine whether small complexes (2), which act as intermediates in the assembly process, will form. Hsp 104 may facilitate the addition of transition state Sup35p* or intermediate complexes to pre-existing complexes (Sup35p$^{[PSI+]}$) (3). When over-expressed, Hsp104 can block any of these steps by interacting with transition states, intermediate complexes, or disaggregating pre-existing Sup35p$^{[PSI+]}$ complexes (5). As predicted from model 2 (see text), Hsp104 acts only to disaggregate Sup35p$^{[PSI+]}$ complexes. A low level of Hsp104 keeps the complexes in a size range (4) that maximizes seeding efficiency and partitioning to daughter cells during cell division. A high level of Hsp104 cures by dissociating the complexes (5).

would cure cells of [*PSI+*] by dissociating protein from the complexes more rapidly than it is added to them.

The first model predicts that Hsp104p can act to promote a [*PSI+*] conformation de novo as well as to eliminate it. The second predicts a single mode of action. Unfortunately, no real-time information is available regarding *HSP104* curing, either by over-expression or deletion. GFP fluorescence assays while correlative cannot be considered definitive for monitoring the [*PSI+*] status of the cell. The current acceptable method for determining the [*PSI+*] state requires detection of a heritable nonsense suppression phenotype. Unfortunately, the read-out for this assay requires many generations of growth and is far removed from the curing stimulus. Furthermore, a full reconstruction in vitro is likely to require Sup35, Sup45, Hsp104, Hsp70, and perhaps other factors. While there is genetic

(Chernoff et al 1995, Patino et al 1996, Paushkin et al 1996) and some biochemical evidence (Schirmer & Lindquist 1997) that Hsp104 directly alters the physical state of Sup35p, the possibility remains that *HSP104* modulates [*PSI+*] indirectly. Yet another complication is the common difficulty of isolating stable complexes between any proteins in the ClpB/Hsp100 family and their substrates (Schirmer et al 1996). New advances are clearly required to accurately address the role of *HSP104* in [*PSI+*] metabolism.

The mechanism of curing by GdnHCl also remains a mystery. GdnHCl induces a moderate increase in Hsp104p levels in vivo (Patino et al 1996), and Hsp104p function in vitro is extremely sensitive to GdnHCl (Glover & Lindquist 1998). Thus it is possible that GdnHCl cures by increasing or decreasing Hsp104 function in the cell. If so, however, it is difficult to understand why [*URE3*], the other well-characterized yeast prion (Wickner 1994), is readily cured by GdnHCl but not by over-expression or deletion of Hsp104 (YO Chernoff, personal communication).

EFFECTS OF *SUP35* MUTATIONS ON [*PSI+*] PROPAGATION

[*PSI+*]-No-More Mutations in the N Region of Sup35p

In an effort to identify nuclear factors important for [*PSI+*] propagation, Young & Cox isolated strains unable to propagate [*PSI+*] following UV irradiation (Young & Cox 1971). The only characterized isolate, U_{16}, is a dominant, [*PSI+*]-no-more mutation, *PNM2*[4] (Doel et al 1994). This mutation reduces [*PSI+*]-mediated nonsense suppression and cures cells of [*PSI+*], but only after several generations of growth. *PNM2* maps to the *SUP35* locus, and is a glycine-to-glutamic acid substitution at amino acid position 58 (Cox 1977, Doel et al 1994). The inability of *PNM2* strains to propagate [*PSI+*] provides independent proof of the importance of N in this process.[5]

[4]In the literature, it is also referred to as *R* (Young & Cox 1971) and *PNM* (McCready et al 1977).

[5]It is common in [*PSI+*] phenomenology for strain differences to affect the outcome of an experiment. In the case of *PNM2*, these are particularly strong. Yeast strain differences appear to contribute to the [*PSI+*] eliminating activity of *PNM2* (Derkatch et al 1999, Kochneva-Pervakhova 1998, McCready et al 1977, Young & Cox 1971). Although *PNM2* can dominantly eliminate [*PSI+*] through mating (McCready et al 1977) or when expressed from a plasmid (Doel et al 1994) in BSC483/1a, BSC412/3c, or BSC170/2c strains, the over-expression of *PNM2* in 74-D694 or SL1010-1A has no effect on [*PSI+*] propagation (Derkatch et al 1999). In fact, over-expression of *PNM2* can even induce [*PSI+*] de novo in some wild-type strains (Derkatch et al 1999) and can also support [*PSI+*] propagation on its own in other strains lacking NM (Kochneva-Pervakhova 1998a). Table 5 lists the genotypes of commonly used [*PSI*] strains.

TABLE 5 Common [*PSI*] strains

Strain name[a]	Genotype[b]	Reference
33G-D373	*pheA10 his7-1 (UAA) lys9-A21 (UAA) trpl-289 (UAG) ade2-144, 717 ura3-52 leu2-3,112*	Chernoff et al 1992
74-D694	*ade1-14 (UGA)trp1-289 his3-Δ200 ura3-52 leu2-3,112*	Chernoff et al 1995
SL1010-1A	*ade1-14 (UGA) met8-1 (UAG)*[c] *leu2-1 his5-2 trp1-1 ura3-52*	Zhou et al 1999
SL1010-6B	*ade1-14 (UGA) met8-1 (UAG)*[c] *leu2-1 his5-2 trp1-1 ura3-52, lys2-1*	Zhou et al 1999
L28-2V-P3982	*ade1-14 (UGA)*[d] *his7-1 lys2-87 thr4-B15*	Inge-Vechtomov & Adrianova 1970
64-D697	*ade1-14 (UGA)trp1-289 lys9-A21(UAA) ura3-52 leu2-3,112*	Derkatch et al 1997
5V-H19	*ade2-1 (UAA) can1-100 leu2-3,112 ura3-52 SUP16*	Ter-Avanesyan et al 1993b
1-5V-H19	5V-H19 *SUP35ΔNM*	Ter-Avanesyan et al 1994
2-5V-H19	5V-H19 *SUP35ΔN*	Ter-Avanesyan et al 1994
3-5V-H19	5V-H19 *SUP35ΔBstEII*	Ter-Avanesyan et al 1994
1A-H19	*ade2-1(UAA) lys1-1 his3-11, 15 leu2-3, 112 SUQ5*	Ter-Avanesyan et al 1994
BSC783/4a	*ade2-1(UAA)SUQ5 leu2-3,112 ura3-1his3-11 his3-15*	Eaglestone et al 1999
BSC468/1a	*ade2-1(UAA) SUQ5 lys1-1 can1-100 ura3-1*	Tuite & Cox 1980

BSC483/2d	*ade2-1(UAA) SUQ5 lys1-1 can1-100*	Dagkesamanskaya & Ter-Avanesyan 1991
MT182/8d	*ade2-1(UAA) SUQ5 his5-2 can1-100 ura3-1*	Tuite et al 1981b
170/2c	*ade2-1(UAA) SUQ5*	Cox 1965
10B-H49	*ade2-1(UAA) lys1-1 his3-11,15 leu2-3,122 kar1-1*	Ter-Avanesyan et al 1994
7G-H66	*ade2-1(UAA) SUQ5 trp1-289 leu2-3,112 ura3-52*	Czaplinski et al 1998
2G-DM8	*his7-1(UAA) lys2-187 (UAA) ura3-52 trp1-1 pheA10 ade2-144,717*	Ter-Avanesyan et al 1993b
YC13-6C	*ade2 aro1 ilv1-2 (UAA)*[e] *leu2-1 (UAA) met8-1(UAG) trp1 ura3 can1 sup111*	Ono et al 1991
D1142-1A	*aro7 cyc1 his4 leu2 lys2-187(UGA) met8-1(UAG) trp5-48(UAA) ura3*	Wakem & Sherman 1990 Eustice et al 1986
SL611-17A	*met8-1 leu2-1 hsi5-2 trp1-1 ura3-52 lys2-1*[*ETA+*]	Zhou et al 1999

[a]Related strains are grouped.

[b]The stop codon is indicated for [*PSI+*] suppressible markers.

[c]met8-1 is suppressed only in strong [*PSI+*] variants in these strains.

[d]*ade1-14* is suppressible at 20 °C but not at 30 °C in this strain.

[e]In this strain, suppression of the markers is dependent upon *sup111*.

The observation that several generations of growth are required to cure *PNM2* cells of [*PSI+*] led to the prescient suggestion that the elements are removed by dilution in these cells (Cox 1993, McCready et al 1997; S Eaglestone & MF Tuite, personal communication). Recent molecular analysis suggests this occurs because Sup35p^{PNM2} has a reduced capacity to join pre-existing Sup35p$^{[PSI+]}$ complexes. Sup35p^{PNM2} converts to a sedimentable form more slowly than wild-type protein in vitro (Kochneva-Pervakhova 1998a). Moreover, fusions between GFP and NM^{PNM2} exhibit fluorescent foci in [*PSI+*] strains, but a substantial amount of diffuse non-incorporated fluorescence can also be detected (Derkatch et al 1999). The ability of this mutant to dominantly cure [*PSI+*] also suggests that the Sup35^{PNM2} protein interferes with pre-existing Sup35p complexes and reduces their ability to incorporate newly synthesized Sup35p.

In an effort to expand our knowledge of specific sequences in N required for [*PSI+*] inheritance, two groups mutagenized this region and analyzed isolates for in vivo suppression and in vitro amyloid formation. Before detailing the results of these studies, we briefly review the structural features of N, and the lesser-studied M region.

The N region has a highly unusual amino acid composition, 28% glutamine, 16% asparagine, 17% glycine, and 16% tyrosine. The M region has a very high concentration of charged amino acids and their representation is strongly biased: 24 lysines, 0 arginines, 23 glutamates, and 7 aspartates. Sup35 proteins from other fungi contain N-terminal regions of variable lengths. In contrast to the highly conserved C region, the primary sequences of N and M are poorly conserved. The distribution of residues, however, strikingly parallels that of *S. cerevisiae* Sup35p (Figure 2*B*). Glutamine, asparagine, glycine, and tyrosine cluster toward the N terminus, and charged amino acids fall in the region immediately preceeding the highly conserved C terminus. Metazoan Sup35ps contain N-terminal extensions of varying lengths, but the fungal pattern of amino acid distributions is not conserved.

Random PCR mutagenesis of the N region of *SUP35* (amino acids 1–125) isolated substitutions that confer two different phenotypes. One class of mutants has an antisuppressor phenotype (*ASU*), inhibiting [*PSI+*]-mediated nonsense suppression. The second class confers a dominant [*PSI+*]-no-more (*PNM*) phenotype, causing cells to lose the [*PSI+*] element (DePace et al 1998). Remarkably, the vast majority of the 28 *ASU* and 13 *PNM* mutants sequenced, contain single amino acid substitutions in glutamine or asparagine residues located between position 8 and 24. Furthermore, a GFP-NM fusion protein with amino acids 8–24 replaced by polyglutamine retains the ability to induce [*PSI+*]. These observations suggest that the crucial attribute of this region is its high concentration of polar amino acids (Q and N) rather than its specific sequence. The purified NM regions of representative mutants exhibit a reduced capacity to form amyloid in vitro, providing another link between the self-assembly properties of Sup35p and the propagation of [*PSI+*].

The second study employed site-directed mutagenesis to investigate the role of the oligopeptide repeats in N (Liu & Lindquist 1999). The motivation for this

study was that the only obvious relationship between the primary amino acid sequences of Sup35p and the mammalian prion protein PrP, is the presense of five oligopeptide repeats in each protein, PQGGYQQYN in Sup35p (Kikuchi et al 1998, Kushnirov et al 1998, Wilson & Culbertston 1998) and PHGGGWGQ in PrP (Prusiner 1998). Expansion of these repeats in PrP produces heritable forms of the spongiform encephalopathies. When the wild-type *SUP35* gene is replaced with a repeat expansion variant (containing two extra copies), [*PSI+*] elements were spontaneously produced in the strain at 5,000 times the rate of wild-type cells (Liu & Lindquist 1999). A deletion of the oligopeptide repeats blocked the de novo formation of [*PSI+*] even when the protein was over expressed. In vitro, fully denatured repeat-expansion peptide formed β-sheet-rich fibers much more rapidly than wild-type peptides. These data provide an extaordinary link between the protein-based mechanism of [*PSI+*] inheritance in yeast and protein-based mechanism of the neurodegenerative prion diseases in mammals. They also provide the first case in which a mutation in an amyloidogenic protein has been shown to increase the rate at which amyloid forms from fully unfolded protein in vitro. Work on mammalian amyloid-disease proteins has established that some mutations promote amyloid formation by destabilizing the native state (Colon et al 1996, Booth et al 1997). Clearly mutations might contribute to this process by a broader array of mechanisms than is currently envisioned.

EFFECTS OF SUP45p AND OTHER SUP35p INTERACTING FACTORS ON [*PSI+*]

Because the propagation of [*PSI+*] depends on the continued self-assembly of Sup35p, other proteins that interact with Sup35 will undoubtedly influence this process. The characterization of these factors is just beginning, but their importance is clear. *SUP35* and *SUP45* interact genetically (Cox 1977; Inge-Vechtomov & Andrianova 1975; Inge-Vechtomov et al 1994, 1988; Tarunina et al 1994; Ter-Avanesyan et al 1984, 1993a) and biochemically (Paushkin et al 1997b, Stansfield et al 1995, Ter-Avanesyan et al 1995). Two separate binding sites on Sup35p for Sup45p are found in vitro; the first located between amino acids 1–253, the second between amino acids 254–483 (Paushkin et al 1997b). Interestingly, a fragment of Sup35p containing amino acids 1–254 interacts with Sup45p if expressed in yeast but not in *E. coli*. Addition of yeast lysate to purified protein does not restore binding, suggesting the need for a yeast-specific post-translational modification, structural alteration, or perhaps the presence of an inhibitory bacterial modification (Paushkin et al 1997b).

Over-expression of Sup45p decreases the efficiency of de novo [*PSI+*] induction by extra-copy *SUP35*. Likewise, extra-copy *SUP45* ameliorates the toxicity of plasmids encoding *SUP35* in [*PSI+*] strains (Derkatch et al 1998). These results suggest a competition between Sup45p and Sup35p$^{[PSI+]}$ for newly synthesized Sup35p. However, over-expression of Sup45p does not alter the [*PSI+*] phenotype

or its propagation. The inability of Sup45p to cure [*PSI+*] suggests that once Sup35p is in a [*PSI+*] complex, it does not interact with Sup45p and/or does not dissociate and rebind to the complexes present in [*PSI+*] strains.

The effect of [*PSI+*] on the association of Sup35p and Sup45p is a point of controversy. In some [*PSI+*] yeast strains (74-D694, D1142-1A, and BSC783/4c), Sup45p is not present in Sup35p$^{[PSI+]}$ complexes (Eaglestone et al 1999, Patino et al 1996), while in other backgrounds (5V-H19 and 7G-H66), Sup45p is found in the insoluble fraction of [*PSI+*] lysates (Czaplinski et al 1998, Paushkin et al 1997b). This disparity may result from genetic differences in the strains or perhaps differences in growth or assay conditions. Unfortunately, the inability of extra-copy Sup35p to reverse the [*PSI+*] phenotype or cure [*PSI+*] was analyzed in strains that do not incorporate Sup45p into Sup35p$^{[PSI+]}$ complexes (Derkatch et al 1998), and, consequently, the functional significance of this association is ambiguous.

Sup35p forms complexes with Upf1, a component of the nonsense-mediated mRNA decay pathway (Czaplinski et al 1998), and two-hybrid analysis suggests an interaction with Sla1p, a cytoskeletal organizing protein (Bailleul et al 1999). The association between Sup35p and Sla1p is dependent upon *HSP104*, and Sup35p-containing point mutations in N that block the propagation of [*PSI+*] do not interact with Sla1p. Disruption of *SLA1* has no effect on the propagation of [*PSI+*], however. [*PSI+*] propagation is also unaltered in strains disrupted for *UPF1*, although Upf1p is found in [*PSI+*] complexes in a strain that also incorporates Sup45p into such complexes. Whereas de novo induction of [*PSI+*] by extra-copy *SUP35* is reduced in Δ*SLA1* strains, the effects of *UPF1* on [*PSI+*] metabolism have not been evaluated. However, no [*psi+*]-dependent differences in mRNA turnover rates have been observed in at least one strain (Lieberman & Derkatch 1999).

OTHER EPIGENETIC EFFECTS ON [*PSI+*]

Non-Mendelian Regulation of [*PSI+*] Induction

A fascinating, but still baffling non-Mendelian regulator of [*PSI+*], known as PIN, has recently been described. GdnHCl curing of [*PSI+*] strains produces two types of [*psi−*] variants: PIN+ and pin−. PIN+ [*psi−*] isolates can revert to [*PSI+*] either spontaneously or following over-expression of either full-length Sup35p or the NM fragment. In contrast, pin− [*psi−*] strains do not revert to [*PSI+*] under the same conditions (Derkatch et al 1997, Lund & Cox 1981). Disruption of *HSP104* produces only pin− [*psi−*] strains (Chernoff et al 1995, Derkatch et al 1997), but re-introduction of *HSP104* on a plasmid does not convert these strains to PIN+ (Derkatch et al 1997). This observation suggests that PIN+ is not conferred by *HSP104*.

The pin− state is not absolute, however. A fragment at the N terminus of Sup35p (amino acids 1–154), but not a larger fragment (amino acids 1–238), can induce [*PSI+*] even in pin− strains (Derkatch et al 1997). This observation may reflect the

enhanced activity of C-terminal truncated fragments of Sup35p to induce [*PSI*+] (Kochneva-Pervakhova 1998b). Despite the ability of shorter Sup35p fragments to induce [*PSI*+] in pin− strains, PIN+ is linked neither to the N nor M regions of Sup35p (Derkatch et al 1996).

Mating studies provided additional insights into the nature of PIN+. For example, PIN+ is dominant over pin− in diploids and segregates as a non-Mendelian trait to all haploid progeny upon sporulation (Derkatch et al 1997, Lund & Cox 1981). However, PIN+ cannot be transferred to pin− strains by cytoduction (Cox et al 1988). While the induction of [*PSI*+] de novo is inhibited in pin− strains, pin− has no effect on existing [*PSI*+] elements (Derkatch et al 1997). PIN+, therefore, provides the first distinction between de novo formation and propagation of [*PSI*+]. Just as the baffling character of [*PSI*+] led to remarkable new understanding, it seems likely that the as yet inexplicable nature of [PIN] will provide crucial insights into the nature of protein conformation–based inheritance once its molecular mechanism has been unraveled.

[*PSI*+] Variants

In his original paper, Cox described three different colony colors derived from [*PSI*] strains—white, pink, and red—corresponding to strong, weak, or no suppression, respectively (Cox 1965). By monitoring colony sectoring, Cox noted that these states could interconvert at a low frequency. However, colonies derived from particular sectors stably retained their color characteristics. Recent work from the Liebman laboratory has provided strong evidence that this phenomenon is due to the existance of distinct epigenetic variants of [*PSI*+].

When overexpression of Sup35p is employed to induce new [*PSI*+] elements in a [*psi*−] strain containing a suppressible adenine mutation, white, pink, and light pink colonies are obtained. The suppression strength of these variants for other suppressible markers, for example *lys2-87*, correlates with the strength of *ade1-14* suppression and with their mitotic stability (Derkatch et al 1996). Most remarkably, when strains with different suppression efficiencies were cured of the [*PSI*+] element, each [*psi*−] derivative could give rise to the same spectrum of new [*PSI*+] elements. That is, the difference in suppression strength of the original isolates was not from genetic polymorphism, but from a stochastic difference in the epigenetic state induced by Sup35p over-expression. [*PSI*+] variants isolated from a single genetic background are referred to as "strains" of [*PSI*+]. Note however, that these strains are genetically identical and should not be confused with the typical nomenclature for yeast strains, which indicates distinctions in the genetic background.

Another variant of [*PSI*+] known as [*ETA*+] or [*EPSILON*+] has also been reported (All-Robyn & Liebman 1983, Liebman & All-Robyn 1984). [*ETA*+] strains were originally isolated by their recessive lethality with certain alleles of *SUP35* and *SUP45*, at least one of which is recessively lethal with [*PSI*+] (All-Robyn & Liebman 1983, Liebman & All-Robyn 1984, Zhou et al 1999). [*ETA*+]

is now considered to be a weak variant of [*PSI+*], having a very low but detectable ability to suppress nonsense mutations (Liebman & All-Robyn 1984, Zhou et al 1999). [*ETA+*] is less meiotically stable than other [*PSI+*] isolates, passing to only 70% of haploid progeny (All-Robyn & Liebman 1983, Liebman & All-Robyn 1984). Like [*PSI+*], however, the maintenance of [*ETA+*] depends upon the N-terminal region of Sup35p. [*ETA+*] can be induced de novo by over-expression of Sup35p, and aggregates of Sup35p are found in [*ETA+*] strains by both GFP fluorescence and differential centrifugation of cell lysates. Notably [*ETA+*] cells have more soluble Sup35 protein than most [*PSI+*] strains, accounting for their weaker levels of nonsense suppression (Zhou et al 1999).

This work provides another correlate between the yeast and mammalian prions. One of the most baffling features of the prion diseases is the existence of strains, with distinct rates and patterns of disease progression, and distinct pathological lesions in the brain. The existence of these strains has been a major source of contention in the mammalian prion field. Many have argued that it indicates that a nucleic acid must be a component of the infectious agent. However, there is a growing acceptance of the notion that mammalian prion strains derive from the capacity of PrP to exist in several distinct, self-perpetuating conformational states. That a similar phenomenon exists in yeast may provide an opportunity to gain a more rapid understanding of mammalian prion strains. More importantly, it reveals a new richness and complexity in this novel form of genetic inheritance in yeast.

BIOLOGICAL ROLE FOR [*PSI+*]

The biological significance of the epigenetic regulation of translational termination has only begun to be addressed. To date for the strains tested, [*PSI+*] has no marked influence on exponential growth rates but, for some, it decreases viability in deep stationary phase in expired medium (Chernoff et al 1998, Eaglestone et al 1999). Other experiments have revealed that some [*PSI+*] strains have a mild increase in basal thermo- and chemo-tolerance levels, whereas other strains show no such phenotype (Eaglestone et al 1999).

One might predict that [*PSI+*] would cause occasional read-through of natural stop codons to produce C-terminal extensions from a few residues to a novel domain, depending on the locus (Lindquist 1997). Both could have a broad impact on physiology. For example, even short C-terminal extensions could alter protein turnover rates, whereas additional domains could impart a new functionality. Interestingly, computer analysis has revealed several continuous open reading frames (ORFs) in yeast that are interrupted by a single nonsense codon (Lindquist 1997). These loci might represent multi-domain proteins whose synthesis is altered in a [*PSI+*]- dependent maner.

Although [*PSI+*] is not generally required under laboratory growth conditions, one obligate [*PSI+*] strain is known (Lindquist et al 1995). This strain contains a nonsense mutation in the heat shock transcription factor (HSF), an essential gene

(Schneiter et al 1995). [*PSI*+] allows synthesis of functional HSF and, therefore, maintains viability. Naturally occurring nonsense mutations have been described in loci other than the auxotrophic markers used to monitor nonsense suppression as well. The *FLO8* locus, required for filamentous growth, is a well-characterized example in the strain S288C, although the effect of [*PSI*+] has not been evaluated (Liu et al 1996). These examples raise the interesting possibility that [*PSI*+] might act as a buffer for nonsense mutations, thereby increasing the genomic flexibility of the organism and influencing the rate at which it evolves.

OTHER FUNGAL PRIONS

Four other prion-like traits with diverse phenotypes have been described in different fungi. Three are not well characterized (Coustou et al 1997, Kunz & Ball 1977, Silar et al 1999, Talloczy et al 1998). The fourth [URE3], has been studied for nearly as many years as [*PSI*+] (Lacroute 1971). It shares many of the unusual genetic features of [*PSI*+] and mystified yeast geneticists to a similar degree, until Wickner proposed in 1994 that it replicates through a protein-only, prion mechanism (Wickner 1994). Biochemical support for this hypothesis is also very strong. Although the phenotype conferred by [URE3], a defect in nitrogen-mediated repression of catabolic enzymes (Lacroute 1971), is very different from that conferred by [*PSI*+] and the underlying protein determinant, Ure2p (Aigle & Lacroute 1975, Wickner 1994), is unrelated to Sup35p, there are many striking similarities. For example, Ure2p has an N-terminal domain with an unusual amino acid composition (in this case particularly rich in asparagines) that is dispensable for the normal function of Ure2p (Masison & Wickner 1995). Overexpression of this domain can induce a dominant cytoplasmically inherited phenotype that mimics loss-of-function mutations in Ure2p (Edskes et al 1999, Masison & Wickner 1995, Masison et al 1997, Wickner 1994). Furthermore, Ure2p and the N-terminal domain self-assemble in vivo and form amyloid fibers in vitro that bind to Congo red and exhibit green birefringence under polarized light (Taylor et al 1999). [URE3] has been extensively reviewed elsewhere, and the interested reader is urged to consult these sources (Wickner 1996, Wickner et al 1996).

CONCLUDING REMARKS

In the last few years we have achieved a remarkable new understanding of mysterious genetic elements that had long puzzled geneticists for the preceding 30 years. A new paradigm for the inheritance of information has been unveiled in yeast and is shown to have extraordinary parallels to an unusual mechanism of pathogenesis described for the mammalian prion diseases: both operate through self-perpetuating changes in protein conformation. However, many challenges still remain. We have no real understanding of the biophysical or biological forces that

govern the inheritance of [*PSI+*] or [URE3]. We have hints that this mechanism of heredity may be widespread but we do not know in how many organisms it might operate, how many types of biological processes might be affected by it, nor whether it provides a selective advantage or is simply an accident of nature. These questions will keep us busy for years to come.

ACKNOWLEDGMENTS

We are indebted to the many scientists who have contributed to the study of [*PSI+*] over the past three decades, and whose work is described herein. We also thank Julie Feder, Tony Kowal, and Kathryn Osen for their invaluable help in the preparation of this manuscript, and Susan Uptain and Heather True for critical comments. This work was supported by the Cancer Research Fund of the Damon Runyon-Walter Winchell Foundation Fellowship, DRG-1436 to TRS, the National Institutes of Health GM025874 to SLL, and the Howard Hughes Medical Institute.

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Annual Review of Cell and Developmental Biology
Volume 15, 1999

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