Destabilizing Interactions Among [*PSI*$^+$] and [*PIN*$^+$] Yeast Prion Variants

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ABSTRACT

The yeast Sup35 and Rnq1 proteins can exist in either the noninfectious soluble forms, [*psi*$^-$] or [*pin*$^-$], respectively, or the multiple infectious amyloid-like forms called [*PSI*$^+$] or [*PIN*$^+$] prion variants (or prion strains). It was previously shown that [*PSI*$^+$] and [*PIN*$^+$] prions enhance one another's *de novo* appearance. Here we show that specific prion variants of [*PSI*$^+$] and [*PIN*$^+$] disrupt each other's stable inheritance. Acquiring [*PSI*$^+$] often impedes the inheritance of particular [*PIN*$^+$] variants. Conversely, the presence of some [*PIN*$^+$] variants impairs the inheritance of weak [*PSI*$^+$] but not strong [*PSI*$^+$] variants. These same [*PIN*$^+$] variants generate a single-dot fluorescence pattern when a fusion of Rnq1 and green fluorescent protein is expressed. Another [*PIN*$^+$] variant, which forms a distinctly different multiple-dot fluorescence pattern, does not impair [*PSI*$^+$] inheritance. Thus, destabilization of prions by heterologous prions depends upon the variants involved. These findings may have implications for understanding interactions among other amyloid-forming proteins, including those associated with certain human diseases.

THE notion of an infectious protein was first imagined more than 30 years ago to make sense of an odd infectious agent without nucleic acids that appeared to be causing sheep scrapie disease (Griffith 1967). Today infectious proteins are known as prions (Prusiner 1982) whether they cause infectious and fatal diseases in animals or heritable variations of traits in yeasts (Wickner 1994). Animal and fungal prions appear to be amyloid protein aggregates that propagate by capturing soluble proteins and converting them into infectious aggregated forms (Prusiner 2001; Uptain and Lindquist 2002). According to the "protein only" hypothesis (Prusiner 1982), the prion protein (PrP) is the sole agent responsible for causing numerous infectious diseases including scrapie, mad cow, kuru, and Creutzfeldt-Jakob, to name a few (Prusiner 1998; McKintosh *et al.* 2003). Many other amyloid-forming proteins are associated with various human neurodegenerative diseases and systemic amyloidoses and do not appear to be infectious.

Surprisingly, different strains of the PrP prion diseases infecting inbred animals have distinct and heritable characteristics such as incubation times and neural degeneration patterns (Bruce *et al.* 1989; Bessen and Marsh 1992). The notion of these disease strains has been difficult to reconcile with the protein only model, but is easily explained by a virino hypothesis proposing that the diseases and their strains are caused by a very small nucleic-acid-containing agent (Dickinson and Outram 1988). However, demonstrations of what appear to be differently aggregated PrP forms associated with distinct disease strains (Bessen and Marsh 1994; Telling *et al.* 1996; Safar *et al.* 1998) indicate that explaining the disease strains in terms of structural variations of PrP prion molecules may be possible.

The yeast *Saccharomyces cerevisiae* possesses several amyloid-forming proteins that are infectious, but have no sequence homology with PrP (Wickner *et al.* 2001). One of these prion proteins, Sup35, is a component of yeast's translation termination factor (Stansfield *et al.* 1995; Zhouravleva *et al.* 1995) and the determinant of the [*PSI*$^+$] prion (Ter-Avanesyan *et al.* 1994; Wickner 1994). Cox first described [*PSI*$^+$] as a non-Mendelian enhancer of stop codon readthrough (nonsense suppression) in yeast already containing a mutant tRNA suppressor (Cox 1965), and it was later shown that [*PSI*$^+$] could act as a nonsense suppressor on its own (Liebman and Sherman 1979). It is now known that [*PSI*$^+$] cells have prion aggregates of Sup35 that capture most of the soluble Sup35 molecules and reduce the yeast's translation termination efficiency (Patino *et al.* 1996; Paushkin *et al.* 1996). In [*PSI*$^+$] cells a fusion between Sup35 and green fluorescent protein (Sup35-GFP) forms a few dots visible by microscopy (Patino *et al.* 1996), but many more unseen aggregates are likely to be present in each cell (Bailleul-Winslett *et al.* 2000; Chernoff *et al.* 2002).

The normal propagation of the [*PSI*$^+$] prion is disrupted in a variety of ways. Mutations in the Sup35 N-terminal domain (Sup35N) interfere with [*PSI*$^+$] maintenance (Doel *et al.* 1994; Ter-Avanesyan *et al.* 1994). Deleting or overexpressing *HSP104* blocks [*PSI*$^+$] propagation (Chernoff *et al.* 1995) and various other

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chaperones are important for normal [*PSI*$^+$] propagation (Chernoff *et al.* 1999; Newnam *et al.* 1999; Jung *et al.* 2000; Kushnirov *et al.* 2000; Chacinska *et al.* 2001). Growth in the presence of guanidine hydrochloride (GuHCl) also cures cells of [*PSI*$^+$] (Tuite *et al.* 1981).

Multiple factors also influence the appearance of the [*PSI*$^+$] prion. Overproducing Sup35 (Chernoff *et al.* 1993; Derkatch *et al.* 1996) or just its N domain (Derkatch *et al.* 2000) induces the *de novo* appearance of [*PSI*$^+$]. After curing [*PSI*$^+$] by growth in GuHCl, some cells were inducible to the [*PSI*$^+$] state by overproducing Sup35, whereas other cells were completely refractory to [*PSI*$^+$] induction. Genetic analyses of these differences led to the hypothesis that another prion-like factor controls the appearance of [*PSI*$^+$] (Derkatch *et al.* 1997). That controlling factor, termed [*PIN*$^+$], exists independently of [*PSI*$^+$] (Derkatch *et al.* 2000) and has been identified (Derkatch *et al.* 2001) as the prion form of the Rnq1 protein (Sondheimer and Lindquist 2000). In this article, [*PIN*$^+$], also known as [*RNQ*$^+$], always refers to the prion form of Rnq1. Other prions or protein aggregates cause the Pin$^+$ phenotype because they facilitate the *de novo* appearance of [*PSI*$^+$] even in the absence of the [*PIN*$^+$] prion (Derkatch *et al.* 2001). It has been suggested that preexisting prions act as templates to occasionally facilitate the initial formation of heterologous prions (Derkatch *et al.* 2001; Osherovich and Weissman 2001), which would explain how the presence of one prion could enhance the formation of another. A negative interaction between [*PSI*$^+$] and another yeast prion, [*URE3*], has also been described (Schwimmer and Masison 2002).

In a phenomenon resembling PrP disease strains, excess Sup35 induces at least two distinct types of variants (or strains) of [*PSI*$^+$], weak and strong, in the same genetic background (Derkatch *et al.* 1996; King 2001; Kochneva-Pervukhova *et al.* 2001; Uptain *et al.* 2001). Weak [*PSI*$^+$] differs from strong [*PSI*$^+$] by causing less nonsense suppression and being less stably inherited (Derkatch *et al.* 1996). Furthermore, cells bearing weak [*PSI*$^+$] contain more nonprion soluble molecules of Sup35 compared to cells bearing strong [*PSI*$^+$] (Zhou *et al.* 1999; Uptain *et al.* 2001). In matings between weak and strong [*PSI*$^+$] haploids, strong [*PSI*$^+$] outcompetes weak [*PSI*$^+$] presumably because weak [*PSI*$^+$] aggregates propagate more slowly than strong [*PSI*$^+$] aggregates do. Furthermore, cells displaying weak [*PSI*$^+$] never reappear in the mitotic or meiotic progeny of these diploids (Bradley *et al.* 2002).

[*PIN*$^+$] also exists in distinct variants (or strains; Bradley *et al.* 2002). When a [*PIN*$^+$] derivative referred to as the "original" is cured to the [*pin*$^-$] state by growth on GuHCl (Derkatch *et al.* 1997), new [*PIN*$^+$] derivatives appear spontaneously following prolonged storage of the [*pin*$^-$] cells (Derkatch *et al.* 2000). The spontaneous appearance of [*PIN*$^+$] coincides with the appearance of aggregated Rnq1 (Derkatch *et al.* 2001), and a fusion of Rnq1 and green fluorescent protein (Rnq1-GFP) forms punctate dots in [*PIN*$^+$] cells but is distributed evenly throughout [*pin*$^-$] cells (Sondheimer and Lindquist 2000; Derkatch *et al.* 2001). It was recently shown that three independent spontaneous [*PIN*$^+$] together with the "original" [*PIN*$^+$] compose an array of distinct [*PIN*$^+$] variants in which low, medium, high, or very high levels of [*PSI*$^+$] are induced by overproducing Sup35, in which high [*PIN*$^+$] corresponds to the original [*PIN*$^+$] derivative (Bradley *et al.* 2002). In matings between different [*PIN*$^+$] haploids, the [*PIN*$^+$] variants maintaining less soluble Rnq1 outcompete those with more soluble Rnq1. "Very high" contains the most soluble Rnq1 and the remaining order is very high > low > medium > high (Bradley *et al.* 2002).

In this article we first describe how expressing Rnq1-GFP in different [*PIN*$^+$] derivatives produces two distinct fluorescence patterns. Cells harboring any of the spontaneously acquired [*PIN*$^+$] variants predominantly display a single-dot (s.d.) Rnq1-GFP pattern, while cells harboring the original [*PIN*$^+$] predominantly display a multiple-dot (m.d.) Rnq1-GFP pattern. These two patterns are properties of the [*PIN*$^+$] variants and are not due to Mendelian or non-Mendelian modifiers. We then show that the presence of s.d. [*PIN*$^+$], unlike m.d. [*PIN*$^+$], causes weak [*PSI*$^+$] to be very unstable. Likewise, the acquisition of [*PSI*$^+$] often eliminates specific s.d. [*PIN*$^+$] variants.

MATERIALS AND METHODS

Media and cultivation procedures: Standard yeast media and cultivation were employed (Sherman *et al.* 1986) and yeast were grown on yeast extract/peptone/dextrose media (YPD) at 30° unless indicated otherwise. To cure [*PSI*$^+$] and [*PIN*$^+$], yeast were grown on YPD containing 5 mm guanidine hydrochloride (Tuite *et al.* 1981; Derkatch *et al.* 1997). Transformants were grown on plasmid selective synthetic media with dextrose (SC), glycerol (SGly), or galactose (SGal). [*PSI*$^+$] variants were distinguished by white (strong [*PSI*$^+$]) or pink (weak [*PSI*$^+$]) color on YPD and by the amount of growth on SC medium lacking adenine (SC-Ade). [*RHO*$^+$] derivatives were made [*rho*$^-$] by growing on YPD plates containing 40 mg/liter of ethidium bromide (Goldring *et al.* 1970).

Cytoductions were performed between [*RHO*$^+$] donors and [*rho*$^-$] recipients and either the donor or the recipient contained a nonfunctional *KAR1* allele, which reduces the efficiency of nuclear fusion following mating (Conde and Fink 1976). The *KAR1* gene was disrupted to produce the *kar1-d15* allele as described (Vallen *et al.* 1992). Cytoductants were selected on SGly medium containing 3 mg/liter of cycloheximide (SGly+Cyh) if the recipient was cycloheximide resistant (cyh^R). Otherwise diploids and cytoductants were selected on SGly medium lacking a nutrient required by the donor, and cytoductants were isolated by a subsequent restreaking on YPD to identify colonies with the recipient's nuclear markers.

Scoring for [*pin*$^-$], s.d. [*PIN*$^+$], and m.d. [*PIN*$^+$]: Derivatives of 74-D694 (*MAT***a** *ade1-14 leu2-1 his3-Δ200 trp1-289 ura3-52*; Chernoff *et al.* 1993), BY4741 (*MAT***a** *his3*Δ *leu2*Δ *met15*Δ

*ura3*Δ; Research Genetics, Huntsville, AL), or c10B-H49 (*MAT*α *ade2-1 SUQ5 lys1-1 his3-11,15 leu1 kar1-1 cyh*^{R}; Kochneva-Pervukhova *et al.* 1998) were mated, respectively, to the [*psi*$^-$] [*pin*$^-$] tester strains SL1010-1A (*MAT*α *ade1-14 met8-1 leu2-1 his5-2 trp1-1 ura3-52*; Zhou *et al.* 1999) already transformed with a *URA3*-based centromeric vector containing a fusion of *RNQ1* and GFP (Rnq1-GFP) under the $CuSO_4$-inducible *CUP1* promoter (Sondheimer and Lindquist 2000) or A3099 (*MAT***a** *ade2-1 SUQ5 lys1-1 his3-11,15 leu1 kar1-1 ura3::KanMX4*; a kind gift of S. Lindquist, Whitehead Institute) already transformed with a *HIS3*-based centromeric vector containing Rnq1-GFP under the *CUP1* promoter (I. Derkatch and S. Liebman, unpublished results). Diploids containing the plasmid were selected on appropriate omission medium and observed on either glass slides using a Zeiss Axioskop 2 equipped with a ×40 Plan-Neofluar objective lens or poly-L-lysine-coated glass slides using a Zeiss Axioskop 200M deconvolution workstation equipped with a ×100 Plan-Apochromat objective lens. The differences among [*pin*$^-$] (all cells show diffuse fluorescence with no dots), s.d. [*PIN*$^+$] (~90% of cells display a single fluorescent dot), and m.d. [*PIN*$^+$] (~90% of cells have more than one fluorescent dot) are evident even when cells are grown on medium without supplemental $CuSO_4$ due to residual Cu in the synthetic medium.

Unstable [*PSI*$^+$] cytoductions and matings: The L1767 unstable [*PSI*$^+$] derivative of 74-D694 was cytoduced into derivatives of L2595, a *kar1-d15* cyh^R derivative of L1845 (*MAT*α *ade1-14 leu2-1 his3-*Δ*200 trp1-289 ura3-52*; Bradley *et al.* 2002). Individual cytoductants were then used as donors in a second round of cytoduction. Each was transformed with pRS415, a *LEU2*-based centromeric vector (Sikorski and Hieter 1989), and mated with various [*psi*$^-$] [*PIN*$^+$] derivatives of 74-D694 already transformed with pFL44, a *URA3*-based 2μ vector (Bonneaud *et al.* 1991) or a *URA3*-based centromeric vector expressing Rnq1-GFP. The recipients were [*RHO*$^+$] in this set of cytoduction experiments only. Cytoductants were selected on SC-Ura-Leu medium and distinguished by scoring for mating type. Transfer of the pRS415 plasmid, known as plasmiduction (Natsoulis *et al.* 1994), and [*PSI*$^+$] from the donor distinguished the cytoductant from the original recipient. Cytoductants were then scored for [*PSI*$^+$] stability by spreading on YPD. The presence of red and sectoring colonies and the absence of pink or white colonies indicated that [*PSI*$^+$] was unstable.

[*PSI*$^+$] induction: To induce the *de novo* appearance of [*PSI*$^+$], the four derivatives of 74-D694 harboring different [*PIN*$^+$] variants (low, medium, high, and very high) were transformed with the pGAL::SUP35 plasmid (Derkatch *et al.* 1996). Three transformants of each of the four derivatives were grown in a patch on plasmid-selective SGal-Leu medium for approximately seven generations and then diluted in water and plated on YPD medium at ~200 colonies per plate. After 7 days, the numbers of red and non-red (white, pink, or sectored) colonies were counted to give the percentage of [*PSI*$^+$] induction. Approximately 70 non-red colonies from the three transformants of each of the four derivatives were spread on YPD plates. To score these colonies as [*psi*$^-$], weak [*PSI*$^+$], or strong [*PSI*$^+$], at least three colonies from each plate were patched on YPD master plates, which were incubated for 3 days and then replica plated on YPD, YPD+GuHCl, SC-Leu, and SC-Ade medium. Patches that became red on YPD+GuHCl were scored as [*PSI*$^+$], and all newly induced [*PSI*$^+$] failed to grow on SC-Leu because they had lost the inducing plasmid. In addition to the color on YPD, the amount of growth on SC-Ade was used to score weak *vs.* strong [*PSI*$^+$]. Red on YPD and no growth on SC-Ade indicated [*psi*$^-$]. Pink on YPD and poor growth on SC-Ade indicated weak [*PSI*$^+$]. White on YPD and good growth on SC-Ade indicated strong [*PSI*$^+$]. To determine if the [*PSI*$^+$] were unstable, additional colony spreads on YPD medium were performed so that no more than ~100 colonies were grown on each plate. This was done to ensure that the final colonies were large enough to observe the red sectoring that occurs only in the unstable [*PSI*$^+$] condition.

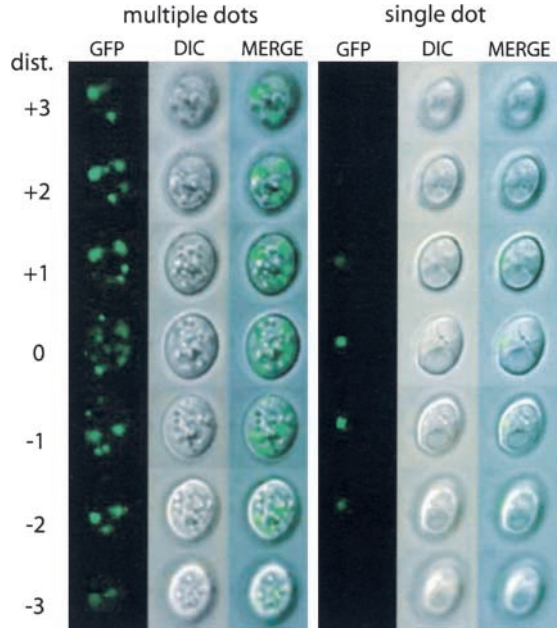

Figure 1.—Phenotypes of m.d. [*PIN*$^+$] and s.d. [*PIN*$^+$] Rnq1-GFP patterns. Derivatives of 74-D694 harboring m.d. [*PIN*$^+$] (high) or s.d. [*PIN*$^+$] (the low variant is shown here) were mated with a [*psi*$^-$] [*pin*$^-$] derivative of SL1010-1A bearing a plasmid with the Rnq1-GFP fusion under a $CuSO_4$-inducible promoter. The diploids were grown in medium containing 50 μM $CuSO_4$ prior to imaging. One typical cell is shown filtered for GFP-emitted light (GFP), in differential interference contrast mode (DIC), and as an overlay of the GFP and DIC images (MERGE). Each cell is shown at seven different focal points separated by 1-μm distances in the *z*-axis (dist.). GFP images were deconvolved with the Zeiss nearest-neighbor algorithm from an original Z-stack of 75 images collected every 200 nm. Note that ~10% of cells containing s.d. [*PIN*$^+$] displayed two or more dots, while cells containing m.d. [*PIN*$^+$] displayed a uniform range of dots from 1 up to ~10.

RESULTS

Two distinct Rnq1-GFP patterns are not caused by Mendelian modifiers: When Rnq1-GFP was expressed in derivatives bearing the original high [*PIN*$^+$], multiple fluorescent dots (m.d.'s) formed in ~90% of the cells (Figure 1). In contrast, Rnq1-GFP formed a single fluorescent dot (s.d.) in ~90% of the cells bearing any one of the eight spontaneously acquired [*PIN*$^+$], including low, medium, and very high [*PIN*$^+$]. Therefore, we use s.d. [*PIN*$^+$] to collectively refer to these eight spontaneously acquired [*PIN*$^+$] variants.

To determine whether the distinction between the m.d. and s.d. Rnq1-GFP patterns was controlled by nuclear or cytoplasmic factors, we utilized cytoduction,

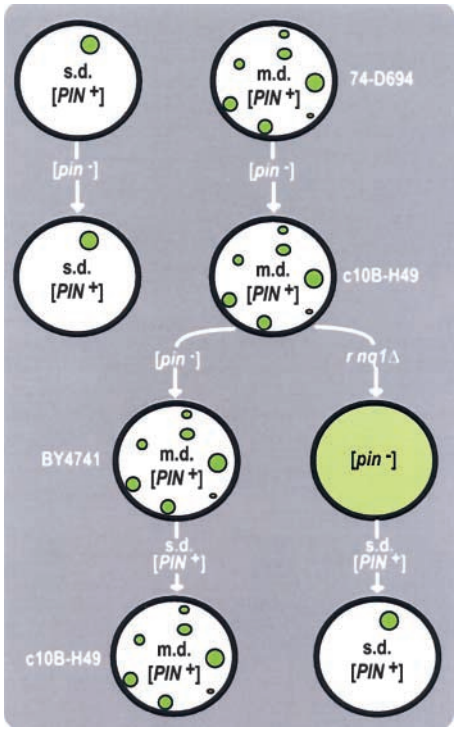

FIGURE 2.—Genetic analyses of m.d. [*PIN*$^+$] and s.d. [*PIN*$^+$] Rnq1-GFP patterns. At each step cells were scored as m.d. [*PIN*$^+$] or s.d. [*PIN*$^+$] by mating with a [*pin*$^-$] strain expressing the Rnq1-GFP fusion (see MATERIALS AND METHODS). Derivatives of 74-D694 including the "original" high m.d. [*PIN*$^+$] (L1749 in the laboratory collection) and eight spontaneous s.d. [*PIN*$^+$] including low, medium, and very high (L1941, L1943, L1945, L1947, L1949, L1951, L1953, and L1955), were cytoduced into a [*psi*$^-$] [*pin*$^-$] derivative of c10B-H49. Next (left), m.d. [*PIN*$^+$] derivatives of c10B-H49 were cytoduced into a [*psi*$^-$] [*pin*$^-$] derivative of BY4741. The resulting m.d. [*PIN*$^+$] derivatives of BY4741 were grown for >20 generations and then cytoduced into the eight s.d. [*PIN*$^+$] derivatives of c10B-H49, including low, medium, and very high (L2337, L2338, L2339, L2340, L2341, L2343, L2347, and L2348). Also (right) the m.d. [*PIN*$^+$] derivatives of c10B-H49 were cytoduced into a [*psi*$^-$] *rnq1*Δ derivative of BY4741. The resulting [*pin*$^-$] (*rnq1*Δ) derivatives of BY4741 were grown for >20 generations and then cytoduced into the eight s.d. [*PIN*$^+$] derivatives of c10B-H49.

an incomplete form of yeast mating that results in the transfer of cytoplasm, but not of the nucleus, from a donor cell into a recipient cell (CONDE and FINK 1976). Because the m.d. and s.d. patterns were faithfully transferred by cytoduction into [*pin*$^-$] recipients, the difference between them is not controlled by a nuclear gene: from the eight spontaneous s.d. [*PIN*$^+$] donors, 24 of 24 cytoductants were s.d. [*PIN*$^+$], and from the original high m.d. [*PIN*$^+$] donor 3 of 3 cytoductants were m.d. [*PIN*$^+$] (Figure 2). The distinct Rnq1-GFP patterns could reflect differences among the m.d. and s.d. [*PIN*$^+$] variants themselves (*i.e.*, different prion forms of Rnq1). Alternatively, the m.d. [*PIN*$^+$] pattern might result from the presence of a non-Mendelian modifier, *e.g.*, "prion X," which was cured on the GuHCl medium along with the original high [*PIN*$^+$] prior to the appearance of s.d. [*PIN*$^+$].

The two distinct Rnq1-GFP patterns are not caused by other non-Mendelian modifiers: When s.d. [*PIN*$^+$] cells received cytoplasm from m.d. [*PIN*$^+$] donors, 54 of 54 cytoductants from 22 independent trials were scored as m.d. [*PIN*$^+$] (Figure 2). Also, in a "reverse" experiment (not shown) in which an m.d. [*PIN*$^+$] [*rho*$^-$] derivative of 74-D694 (L2345) received cytoplasm from five different s.d. [*PIN*$^+$] donors (L2397, L2398, L2399, L2401, and L2402), 15 of 15 cytoductants remained m.d. [*PIN*$^+$]. To control for the positive transfer of s.d. [*PIN*$^+$] in the reverse experiment, a [*pin*$^-$] [*rho*$^-$] derivative of 74-D694 (L2346) received cytoplasm from five different s.d. [*PIN*$^+$] donors and 14 of 15 cytoductants became s.d. [*PIN*$^+$]. Therefore, m.d. [*PIN*$^+$] always outcompeted s.d. [*PIN*$^+$] in cytoplasmic mixing experiments.

This result is consistent with either the prion X hypothesis or the [*PIN*$^+$] variants hypothesis. On the one hand, the hypothetical prion X in the m.d. [*PIN*$^+$] cells would be expected to be dominant over the absence of prion X in s.d. [*PIN*$^+$] cells. But on the other hand, the m.d. [*PIN*$^+$] Rnq1-GFP pattern would be expected to overshadow the s.d. [*PIN*$^+$] pattern if the two [*PIN*$^+$] variants coexisted in the same cell. The m.d. [*PIN*$^+$] variant probably even propagates faster and thus outcompetes s.d. [*PIN*$^+$] variants since the high *de novo* [*PSI*$^+$] induction phenotype associated with the m.d. [*PIN*$^+$] variant outcompeted the low, medium, or very high phenotypes associated with three different s.d. [*PIN*$^+$] variants (BRADLEY *et al.* 2002).

To distinguish between these possibilities, m.d. [*PIN*$^+$] cytoplasm was donated to an *rnq1*Δ derivative that is capable of maintaining the putative prion X but not [*PIN*$^+$]. We then tested whether these *rnq1*Δ cytoductants indeed contained a prion X that could convert s.d. [*PIN*$^+$] into m.d. [*PIN*$^+$] (Figure 2). However, when the *rnq1*Δ cytoductants were used as donors to cytoduce s.d. [*PIN*$^+$] recipients, 99 of 99 cytoductants from 33 independent trials remained s.d. [*PIN*$^+$]. Therefore, because m.d. [*PIN*$^+$] always dominates over s.d. [*PIN*$^+$] in cytoplasmic mixing experiments and requires continuous expression of *RNQ1* to do so, the m.d. pattern is a property of the "original" high [*PIN*$^+$] variant and is not due to a non-Mendelian modifier of s.d. [*PIN*$^+$].

Certain [*PSI*$^+$] isolates that carry s.d. [*PIN*$^+$] are very unstable: We used the premature stop codon allele *ade1-14* (CHERNOFF *et al.* 1995) to monitor nonsense suppression caused by [*PSI*$^+$]. In [*psi*$^-$] *ade1-14* cells, translation is efficiently terminated at the premature stop codon and therefore adenine is not produced. Consequently, [*psi*$^-$] cells do not grow on medium lacking adenine, and they accumulate a red-colored by-product of adenine biosynthesis on rich medium (FISHER 1969). [*PSI*$^+$] cells occasionally read through (suppress) the premature stop codon in *ade1-14*, and thus they are able

TABLE 1

Frequency of [*PSI*$^+$] loss in the unstable [*PSI*$^+$] condition

	Colonies				
Strain	[*psi*$^-$]	Unstable	Stable	Total	% [*psi*$^-$] ± SD
L1767	2199	2221	0	4420	49.7 ± 19.3
L1768	947	1360	0	2307	41.1 ± 8.7
L1769	1214	740	0	1955	62.1 ± 6.7

The percentage of [*PSI*$^+$] loss and the standard deviation (±SD) were calculated for three 74-D694 unstable [*PSI*$^+$] by spreading at least three independent colonies from each to YPD plates and scoring the resulting colonies as [*psi*$^-$], unstable [*PSI*$^+$] (sectored), or stable [*PSI*$^+$].

to grow on medium lacking adenine and become white or pink on YPD, depending on the amount of nonsense suppression.

Overproducing Sup35 induces [*PSI*$^+$] (Chernoff *et al.* 1993). If newly induced [*PSI*$^+$] colonies are observed immediately after induction, many show red sectoring indicating some loss of [*PSI*$^+$]. Upon restreaking, however, these colonies achieve full stability (I. Derkatch and S. Liebman, unpublished data). Indeed, while experimenting on the *de novo* appearance of [*PSI*$^+$] in m.d. [*PIN*$^+$] and [*pin*$^-$] derivatives of the yeast strain 74-D694 (Derkatch *et al.* 1997, 1998, 1999, 2000, 2001), we induced only three [*PSI*$^+$] derivatives that never achieved full stability among thousands of stable [*PSI*$^+$] isolates. We refer to these three [*PSI*$^+$] derivatives as unstable [*PSI*$^+$] since approximately half of their progeny failed to inherit [*PSI*$^+$] (Table 1), an ~100-fold increase in [*PSI*$^+$] loss compared to the 0.5% loss observed for normal weak [*PSI*$^+$] (Derkatch *et al.* 2000). These unstable [*PSI*$^+$] derivatives never achieved the stable [*PSI*$^+$] state (Table 1). This was true even after streaking one of the derivatives 10 consecutive times (Figure 3A).

Among the three unstable [*PSI*$^+$] isolates, two appeared upon overproducing Sup35 in the m.d. [*PIN*$^+$] derivative and the other one appeared after overproducing Sup35 following prolonged incubation of a [*pin*$^-$] derivative. Surprisingly though, each of the three unstable [*PSI*$^+$] derivatives had acquired an s.d. [*PIN*$^+$] factor. This is the first of many correlations that we report between the unstable [*PSI*$^+$] condition and the presence of an s.d. [*PIN*$^+$]. Although [*PSI*$^+$] was unstable in these derivatives, the s.d. [*PIN*$^+$] was stably maintained. The s.d. [*PIN*$^+$] was found in all mitotic progeny examined (30 unstable [*PSI*$^+$] and 30 [*psi*$^-$] colonies) after 10 consecutive streakings of an unstable [*PSI*$^+$] derivative.

Weak [*PSI*$^+$] prions are destabilized by s.d. [*PIN*$^+$] variants: The unstable [*PSI*$^+$] condition was maintained upon cytoduction of the three unstable [*PSI*$^+$] derivatives into [*psi*$^-$] [*pin*$^-$] recipients (Figure 3B). This eliminates the possibility that the unstable [*PSI*$^+$] condition

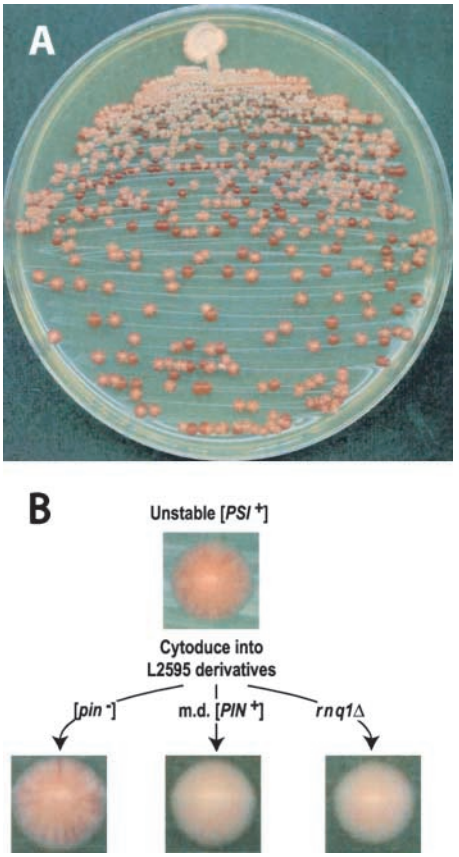

Figure 3.—Unstable [*PSI*$^+$]. (A) The final plate in a series of 10 consecutive streakings of unstable [*PSI*$^+$] L1767. Each colony is either pink-red sectored or wholly red. (B) Outline of the cytoductions demonstrating that unstable [*PSI*$^+$] is caused by s.d. [*PIN*$^+$]. The L1767 unstable [*PSI*$^+$] was cytoduced into [*pin*$^-$], m.d. [*PIN*$^+$], or *rnq1*Δ derivatives of L2595. The result of each en masse cytoduction into the L2595 derivatives was analyzed by streaking cells from SGly + Cyh to YPD to check the stability of many [*PSI*$^+$] cytoductants; one representative colony is shown. Cytoduction into a [*pin*$^-$] derivative of L2595 (L2598) always resulted in retention of the unstable [*PSI*$^+$] condition because s.d. [*PIN*$^+$] was transmitted and maintained in the cytoductants. Cytoduction of unstable [*PSI*$^+$] into either m.d. [*PIN*$^+$] (L2595) or its *rnq1*Δ derivative (L2667) always resulted in conversion to a stable [*PSI*$^+$] state because s.d. [*PIN*$^+$] is outcompeted by m.d. [*PIN*$^+$] and s.d. [*PIN*$^+$] cannot be maintained in *rnq1*Δ derivatives. Cytoducing the L1768 and L1769 unstable [*PSI*$^+$] and four more unstable [*PSI*$^+$] induced in the presence of low [*PIN*$^+$] (see below) into the three L2595 derivatives shown here gave identical results.

results from a Mendelian mutation. Upon cytoduction of the unstable [*PSI*$^+$] derivatives into a [*psi*$^-$] m.d. [*PIN*$^+$] recipient, a stable weak [*PSI*$^+$] state emerged (Figure 3B). Since the recipient's m.d. [*PIN*$^+$] outcompetes the donor's s.d. [*PIN*$^+$], one way to explain these results is that the unstable [*PSI*$^+$] condition results from a destabilizing force associated with s.d. [*PIN*$^+$]. Alternatively, m.d. [*PIN*$^+$] might actively stabilize the [*PSI*$^+$]. To distinguish between these possibilities, we cytoduced the unstable [*PSI*$^+$] derivatives into a [*psi*$^-$] *rnq1*Δ recipi-

TABLE 2

The s.d. [*PIN*$^+$] variants destabilize weak [*PSI*$^+$] introduced by cytoduction

	L2668 donor				L2692 donor			
	Weak [*PSI*$^+$]		Unstable [*PSI*$^+$]		Weak [*PSI*$^+$]		Unstable [*PSI*$^+$]	
Recipient	[*PIN*$^+$]	[*pin*$^-$]	[*PIN*$^+$]	[*pin*$^-$]	[*PIN*$^+$]	[*pin*$^-$]	[*PIN*$^+$]	[*pin*$^-$]
s.d.	0	0	8	0	0	0	5	0
Low s.d.	0	0	3	0	0	0	4	0
Medium s.d.	0	5[a]	5 + 5[a]	0	0	4	0	0
Very high s.d.	0	8[a]	8[a]	0	0	4[a]	4[a]	0
High m.d.	4	0	0	0	5	0	0	0
[*pin*$^-$]	0	7	0	0	0	4	0	0

Numbers represent independent cytoductant colonies obtained from each donor and recipient pair. Recipients were 74-D694 derivatives with different [*PIN*$^+$] variants. Donors contained stable weak [*PSI*$^+$]. L2668 is a derivative of L1845 *rnq1*Δ that was cytoduced with the L1767 original unstable [*PSI*$^+$]. L2692 is a L1845 *rnq1*Δ derivative that was cytoduced with the N21 stable weak [*PSI*$^+$] (Derkatch *et al.* 1996). Each cytoductant was scored for [*PSI*$^+$] stability (weak *vs.* unstable) and for the ability to form Rnq1-GFP dots ([*PIN*$^+$] or [*pin*$^-$]). The unnamed s.d. [*PIN*$^+$] recipient in the first row is from the L1767 unstable [*PSI*$^+$] derivative.

[a] These cytoductants gave rise to unstable [*PSI*$^+$] colonies containing [*PIN*$^+$], as well as weak [*PSI*$^+$] colonies lacking [*PIN*$^+$]. To reflect this, these cytoductants have been tabulated twice, once in the weak [*PSI*$^+$]/[*pin*$^-$] column and once in the unstable [*PSI*$^+$]/[*PIN*$^+$] column. Note that the only derivatives (medium and very high) to lose [*PIN*$^+$] in this manner were also the only derivatives that sometimes lost [*PIN*$^+$] during [*PSI*$^+$] induction (see Table 3).

ent, where neither m.d. nor s.d. [*PIN*$^+$] can be maintained. Since the unstable [*PSI*$^+$] condition was converted to the stable weak [*PSI*$^+$] state in the *rnq1*Δ recipients (Figure 3B), m.d. [*PIN*$^+$] is not required to stabilize [*PSI*$^+$]. Rather, s.d. [*PIN*$^+$] appears to be the destabilizing force.

Furthermore, one such *rnq1*Δ-passaged stable weak [*PSI*$^+$] remained stable upon cytoduction into [*psi*$^-$] recipients that were either [*pin*$^-$] or m.d. [*PIN*$^+$], but emerged as unstable [*PSI*$^+$] with varying degrees of instability upon cytoduction into [*psi*$^-$] recipients carrying s.d. [*PIN*$^+$] variants (Table 2). Similar results were obtained using an *rnq1*Δ-passaged weak [*PSI*$^+$] that had never before been unstable. Interestingly, introducing either of these weak [*PSI*$^+$] often caused the loss of medium and very high s.d. [*PIN*$^+$] (Table 2).

An unstable [*PSI*$^+$] derivative harboring an s.d. [*PIN*$^+$] was also mated with [*psi*$^-$] derivatives that were [*pin*$^-$], s.d. [*PIN*$^+$], or m.d. [*PIN*$^+$] (data not shown). Mating with the m.d. [*PIN*$^+$] resulted in conversion to a stable weak [*PSI*$^+$] state as expected since the m.d. [*PIN*$^+$] outcompetes the s.d. [*PIN*$^+$] from the unstable [*PSI*$^+$] parent. Mating with the [*pin*$^-$] or with any of the s.d. [*PIN*$^+$] derivatives resulted in retention of s.d. [*PIN*$^+$] and the unstable [*PSI*$^+$] condition. Thus, these results together with the cytoduction experiments shown in Figure 3B and Table 2 indicate that weak [*PSI*$^+$] prions are destabilized by the presence of an s.d. [*PIN*$^+$].

Unstable [*PSI*$^+$] can be induced in the presence of s.d. [*PIN*$^+$]: We hypothesized that since the unstable [*PSI*$^+$] condition results from a destabilizing force associated with s.d. [*PIN*$^+$], unstable [*PSI*$^+$] should arise in derivatives containing s.d. [*PIN*$^+$], but not in derivatives containing m.d. [*PIN*$^+$]. To test this, [*PSI*$^+$] was induced by overproducing Sup35 in four [*psi*$^-$] derivatives that each contained a different [*PIN*$^+$] variant. As expected, the frequency of induced [*PSI*$^+$] correlated with the previously determined strengths of the different [*PIN*$^+$] variants (Bradley *et al.* 2002). In addition, only stable weak or strong [*PSI*$^+$] states appeared in the presence of m.d. [*PIN*$^+$], while unstable [*PSI*$^+$] and stable strong [*PSI*$^+$] appeared in the presence of low and medium s.d. [*PIN*$^+$] (Table 3). Levels of instability varied among unstable [*PSI*$^+$] induced in the presence of low and medium s.d. [*PIN*$^+$] (Figure 4). Strong [*PSI*$^+$] isolates that were induced and propagated in the presence of s.d. [*PIN*$^+$] were no more unstable than those of m.d. [*PIN*$^+$] origin (data not shown). Thus only weak [*PSI*$^+$] are subject to destabilization by s.d. [*PIN*$^+$]. We refer to cells that are weak [*PSI*$^+$] and unstable simply as unstable [*PSI*$^+$] and we refer to cells that are weak [*PSI*$^+$] and stable simply as weak [*PSI*$^+$].

Paradoxically, weak [*PSI*$^+$] were frequently induced in cells carrying medium s.d. [*PIN*$^+$]. We found, however, that these weak [*PSI*$^+$] had always become [*pin*$^-$], while all newly induced unstable [*PSI*$^+$] retained the s.d. [*PIN*$^+$] (Table 3). The appearance of [*PSI*$^+$] also frequently caused the loss of very high [*PIN*$^+$]. The loss of low or high [*PIN*$^+$] never occurred. In addition, loss of [*PIN*$^+$] was strictly associated with the appearance of [*PSI*$^+$] since [*psi*$^-$] colonies from the same experiment, which had been treated with excess Sup35 but did not become [*PSI*$^+$], never lost [*PIN*$^+$] (36 of 36 from each of the four [*PIN*$^+$] derivatives). Upon cytoduction into

TABLE 3

[*PSI*$^+$] variants induced in the presence of different [*PIN*$^+$] variants

		No. of [*PSI*$^+$] colonies							
		Strong		Weak		Unstable		Mixed	
[*PIN*$^+$]	% [*PSI*$^+$]	[*PIN*$^+$]	[*pin*$^-$]	[*PIN*$^+$]	[*pin*$^-$]	[*PIN*$^+$]	[*pin*$^-$]	[*PIN*$^+$]	[*pin*$^-$]
Low s.d.	2.6	35	0	0	0	27[a]	0	3	0
Medium s.d.	6.6	10	23	0	15	18[a]	0	2	1
High m.d.	12.4	28	0	25	0	0	0	9	0
Very high s.d.	35.0	29	17	24[b]	0	0	0	0	0

Sup35 was overproduced in four different 74-D694 [*PIN*$^+$] derivatives (see MATERIALS AND METHODS). The percentage of [*PSI*$^+$] is equal to the non-red colonies divided by ~7000 (low), 2400 (medium), 2400 (high), and 1200 (very high) total colonies. Approximately 70 colonies from each derivative were scored as strong, weak, or unstable [*PSI*$^+$]. Some colonies were scored as mixed because their three to six descendent colonies displayed a nonhomogenous combination of the [*PSI*$^+$] states normally induced in that derivative. One explanation for the mixed events is that clumps of cells (more than two) were independently induced to different [*PSI*$^+$] states and formed a colony. Colonies were also scored for [*PIN*$^+$].

[a] Of 27 unstable [*PSI*$^+$] induced in cells containing low s.d. [*PIN*$^+$], 17 were extremely unstable and 10 were slightly unstable. In medium s.d. [*PIN*$^+$], 17 of 18 induced were extremely unstable and one was slightly unstable.

[b] Of 24 weak [*PSI*$^+$] induced in the presence of very high [*PIN*$^+$], 23 were unusual in that they frequently converted to strong [*PSI*$^+$] (see text).

[*psi*$^-$] *rnq1*Δ recipients, each of four tested unstable [*PSI*$^+$] isolates induced in the presence of an s.d. [*PIN*$^+$] emerged as stable weak [*PSI*$^+$], as did the three original unstable [*PSI*$^+$] (see Figure 3 legend).

An unusual type of weak [*PSI*$^+$] was frequently induced in the very high [*PIN*$^+$] derivative. These extraordinary weak [*PSI*$^+$] frequently converted to strong [*PSI*$^+$]. A similar phenomenon in which weak [*PSI*$^+$] apparently give rise to strong [*PSI*$^+$] has been observed previously (KOCHNEVA-PERVUKHOVA *et al.* 2001). Streaking colonies containing the unusual weak [*PSI*$^+$] gave two types of colonies: white strong [*PSI*$^+$] colonies, which, upon restreaking, gave rise only to the same white colonies; and pink-white sectored colonies, which, upon restreaking, gave rise to nearly equal amounts of both pink-white sectored colonies and white colonies. The unusual pink-white sectoring [*PSI*$^+$] was maintained upon cytoduction into [*psi*$^-$] recipients that were [*pin*$^-$], m.d. [*PIN*$^+$], or *rnq1*Δ. Thus, the very high [*PIN*$^+$] was not required to maintain this [*PSI*$^+$] state.

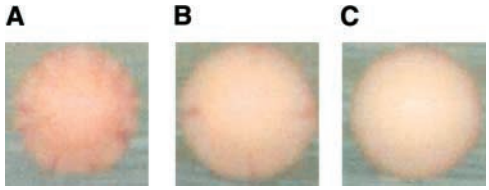

FIGURE 4.—Colonies harboring newly induced [*PSI*$^+$]. (A and B) Unstable [*PSI*$^+$] induced in the presence of low s.d. [*PIN*$^+$] display various degrees of instability as indicated by the amount of red sectors in each colony. (C) Weak [*PSI*$^+$] induced in the presence of the m.d. [*PIN*$^+$] variant are always stable and never display red sectors.

Possibly, this unusual [*PSI*$^+$] is a [*PSI*$^+$] variant that requires the specific seeding of very high [*PIN*$^+$] to appear, but that once established can be propagated independently of very high [*PIN*$^+$].

DISCUSSION

Previous work has shown that the presence of one prion, such as [*PIN*$^+$], can positively affect the appearance of other prions like [*PSI*$^+$] (DERKATCH *et al.* 2001; BRADLEY *et al.* 2002). Four different [*PIN*$^+$] variants that each allow for different amounts of [*PSI*$^+$] induction upon Sup35 overproduction maintain distinct ratios of soluble to aggregated Rnq1 protein (BRADLEY *et al.* 2002). We have now shown that these [*PIN*$^+$] direct the formation of either s.d. or m.d. Rnq1-GFP patterns. The possibility that these differences are not due to inherent differences between the prion variants, but rather reflect the influence of other heterologous prions, has been eliminated, addressing for the first time the possibility that multiple heterologous prions cause the apparent difference between prion strains. The presence of s.d. [*PIN*$^+$] drastically destabilizes weak [*PSI*$^+$], and likewise the acquisition of [*PSI*$^+$] often eliminates s.d. [*PIN*$^+$] variants. These findings indicate that the [*PSI*$^+$] and [*PIN*$^+$] prions interact more extensively than previously thought. Curiously, these interactions occur only for specific combinations of prion variants (Table 4). Previous descriptions of antagonistic interactions affecting the phenotypes of two prions, [*URE3*] and [*PSI*$^+$], including an increase in the frequency of [*URE3*] loss from undetectable loss in the absence of [*PSI*$^+$] to an ~1% loss in the presence of [*PSI*$^+$]

TABLE 4

Summary of interactions among [*PIN*$^+$] and [*PSI*$^+$] variants

	[*PSI*$^+$]		
[*PIN*$^+$]	Induced[a]	Destabilized	Eliminated [*PIN*$^+$][b]
Low s.d.	Strong, unstable	Weak	No
Medium s.d.	Strong, unstable	Weak	Yes
Very high s.d.	Strong, unusual	Weak	Yes
High m.d.	Strong, weak	None	No
[*pin*$^-$]	None	None	Not applicable

[a] When [*PIN*$^+$] is not lost.
[b] When [*PSI*$^+$] is induced by overproducing Sup35 or acquired through cytoduction.

(Schwimmer and Masison 2002), may represent a phenomenon similar to that reported here.

The numbers of Sup35-GFP or Rnq1-GFP dots detected upon overproduction of the fusions do not appear to correlate with the actual numbers of heritable prion seeds. For example, while only one or two Sup35-GFP dots are often visible in [*PSI*$^+$] cells (Bailleul-Winslett *et al.* 2000; Chernoff *et al.* 2002), ~60 heritable seeds have been predicted by genetic tests (Eaglestone *et al.* 2000). Possibly, the heritable seeds of [*PSI*$^+$] and [*PIN*$^+$] are numerous smaller aggregates that either coalesce into one or two large dots in the presence of overproduced GFP fusion proteins (Chernoff *et al.* 2002) or are mostly not associated with the GFP fusions since the fusion proteins might concentrate around only a few seeds in each cell. Nevertheless, the Rnq1-GFP patterns do suggest that more heritable seeds are associated with m.d. [*PIN*$^+$] compared to s.d. [*PIN*$^+$]. The members of the s.d. [*PIN*$^+$] collection were each outcompeted by m.d. [*PIN*$^+$] and were indistinguishable by the Rnq1-GFP test. Although each s.d. [*PIN*$^+$] tested did destabilize weak [*PSI*$^+$] in cytoplasmic mixing experiments, some differences in their interactions with [*PSI*$^+$] were observed (Table 4). The induction of [*PSI*$^+$] led to the appearance of unstable [*PSI*$^+$] in low and medium s.d. [*PIN*$^+$], but induction in very high s.d. [*PIN*$^+$] did not. Furthermore, the *de novo* induction of [*PSI*$^+$] and the introduction of [*PSI*$^+$] through cytoduction frequently caused the elimination of medium and very high s.d. [*PIN*$^+$]. In contrast, low s.d. [*PIN*$^+$], like high m.d. [*PIN*$^+$], remained stable under these conditions.

That [*PIN*$^+$] aggregates allow for the *de novo* induction of [*PSI*$^+$] (Derkatch *et al.* 2001; Bradley *et al.* 2002) and yet cause the elimination of weak [*PSI*$^+$] suggests that the interactions between Sup35 and Rnq1 may persist over a longer period than previously thought (Derkatch *et al.* 2000). Two models have been proposed to explain how [*PIN*$^+$] aggregates could allow for the *de novo* appearance of [*PSI*$^+$] (Derkatch *et al.* 2001; Osherovich and Weissman 2001). The seeding model invokes direct interactions between different prion proteins by proposing that preexisting prions template the conversion of a heterologous protein into its prion form. In the titration model, instead of invoking direct interactions among different prion aggregates, preexisting prions are portrayed as sequestering a protein that inhibits the conversion of the other protein into its prion form. Current evidence favors the seeding model. Even a large excess of Sup35 is not sufficient to induce the appearance of [*PSI*$^+$] in the absence of other prions (Derkatch *et al.* 1997). Also, genome-wide mutagenesis failed to inactivate the hypothesized inhibitor protein (Derkatch *et al.* 2001). Moreover, there is no general correlation between how frequently different [*PIN*$^+$] variants enhance [*PSI*$^+$] and [*URE3*] induction, a correlation that would be expected if each [*PIN*$^+$] variant simply sequestered an inhibitor protein (Bradley *et al.* 2002). In addition, colocalization of [*PIN*$^+$] prion aggregates and newly induced [*PSI*$^+$] prion aggregates has been detected using cyan fluorescent protein and yellow fluorescent protein tagged alleles of Rnq1 and Sup35 (I. Derkatch and S. Liebman, unpublished data).

The m.d. [*PIN*$^+$] was previously reported to not affect any phenotypes of weak or strong [*PSI*$^+$], including stability of inheritance (Derkatch *et al.* 2000). The unstable [*PSI*$^+$] condition described here demonstrates that s.d. [*PIN*$^+$], in contrast to m.d. [*PIN*$^+$], does impair weak [*PSI*$^+$] inheritance. What might be the difference among [*PIN*$^+$] variants that determines whether or not they cause weak [*PSI*$^+$] to become unstable? Using the framework of the seeding model, the unstable [*PSI*$^+$] condition could be explained by a prolonged direct interaction between the prion proteins in addition to the initial seeding event. Possibly, s.d. [*PIN*$^+$] aggregates sequester [*PSI*$^+$] seeds into larger co-aggregates, thus inhibiting their segregation into daughter cells and resulting in instability. Indeed, an allele of Sup35 lacking residues 22–69 was recently shown to lose [*PSI*$^+$] in the absence of selection because it formed much larger aggregates than normal [*PSI*$^+$] (Borchsenius *et al.* 2001). Since aggregation-prone proteins sharing common features such as polyglutamine stretches have been

shown to co-aggregate (Kazantsev *et al.* 1999), the modest similarity between the Sup35 and Rnq1 prion proteins (Sondheimer and Lindquist 2000) may enable them to directly interact. However, s.d. [*PIN*$^+$] variants are stably inherited when propagated in the presence of [*PSI*$^+$] even though they cause the frequent loss of [*PSI*$^+$]. This could be explained within the direct interaction model if the [*PSI*$^+$] aggregates concentrate around only a few of the [*PIN*$^+$] aggregates, thus leaving the rest of the [*PIN*$^+$] seeds to continue propagating. Furthermore, if [*PSI*$^+$] aggregates also maintain long-term interactions with m.d. [*PIN*$^+$], the [*PSI*$^+$] instability may not be observed simply because m.d. [*PIN*$^+$] seeds are much more widely distributed throughout the cytoplasm compared to s.d. [*PIN*$^+$] seeds.

Even though there is good evidence that prions directly interact to facilitate each other's appearance, the same phenomenon does not necessarily explain the unstable [*PSI*$^+$] condition. An equally valid model depicts s.d. [*PIN*$^+$] sequestering a factor, "protein Y," which is necessary for the stable propagation of weak [*PSI*$^+$]. Possibly, protein Y is a chaperone protein that both weak [*PSI*$^+$] and s.d. [*PIN*$^+$] compete for, and must interact with, to propagate normally. For example, protein Y might be required for partitioning large prion aggregates into heritable seeds, a function proposed to explain why Hsp104 (Chernoff *et al.* 1995) is essential for [*PSI*$^+$] maintenance (Paushkin *et al.* 1996; Wegrzyn *et al.* 2001). Alternatively, protein Y could aid the conversion from the [*psi*$^-$] to [*PSI*$^+$] form in the presence of [*PSI*$^+$] seeds or protect [*PSI*$^+$] from being completely disaggregated by Hsp104. Both have been proposed to explain why the Ssa proteins are required for proper [*PSI*$^+$] maintenance and why they protect [*PSI*$^+$] from elimination by Hsp104 (Newnam *et al.* 1999; Jung *et al.* 2000). In fact, three chaperone proteins, Ssa1, Sis1, and Ydj1, are known to co-immunoprecipitate with the prion form of Rnq1, and Sis1 mutations render cells incapable of maintaining [*PIN*$^+$] or cause a switch from an s.d.-like pattern to an extreme form of an m.d.-like pattern with numerous fast-moving dots (Sondheimer *et al.* 2001; Lopez *et al.* 2003).

The strong [*PSI*$^+$] form of Sup35, which is inherently more stable than weak [*PSI*$^+$] (Derkatch *et al.* 1996, 2000), is not destabilized by s.d. [*PIN*$^+$]. Possibly, strong [*PSI*$^+$] still interacts with s.d. [*PIN*$^+$] but not in a complete enough manner to lead to its destabilization, or strong [*PSI*$^+$] is not prone to destabilization caused by reduced protein Y activity. Both of these possibilities are consistent with the idea that strong [*PSI*$^+$] variants, because they are more stable than weak [*PSI*$^+$] variants (Derkatch *et al.* 1996, 2000), are likely to have more heritable seeds than weak [*PSI*$^+$] (Eaglestone *et al.* 2000). Weak [*PSI*$^+$] was recovered from unstable [*PSI*$^+$] whenever s.d. [*PIN*$^+$] was eliminated. In addition, the unstable [*PSI*$^+$] condition was generated whenever s.d. [*PIN*$^+$] was combined with weak [*PSI*$^+$] even if the weak [*PSI*$^+$] had never before been unstable. Therefore, cells in the unstable [*PSI*$^+$] condition contain the weak [*PSI*$^+$] variant, not a new unstable [*PSI*$^+$] variant. The s.d. [*PIN*$^+$] variants caused weak [*PSI*$^+$] to become unstable whether [*PSI*$^+$] was induced *de novo* in s.d. [*PIN*$^+$] derivatives or introduced by cytoduction into s.d. [*PIN*$^+$] derivatives. Interestingly, although stable weak [*PSI*$^+$] isolates were frequently obtained among cells originally bearing either medium or very high s.d. [*PIN*$^+$], in each such isolate the initial medium or very high s.d. [*PIN*$^+$] had been lost.

In parallel to the above results, *de novo* induction of strong [*PSI*$^+$] or introduction of weak [*PSI*$^+$] through cytoduction was often associated with the loss of medium and very high s.d. [*PIN*$^+$]. Low s.d. [*PIN*$^+$], like high m.d. [*PIN*$^+$], was not eliminated upon acquiring [*PSI*$^+$]. Since all [*PIN*$^+$] variants stably propagate in [*psi*$^-$], stable, or unstable [*PSI*$^+$] backgrounds, it appears that the loss of these specific medium and very high s.d. [*PIN*$^+$] variants is promoted during the early stages of [*PSI*$^+$] appearance.

As noted elsewhere (Bradley *et al.* 2002), methods to eliminate prions may be evolutionary adaptations that allow organisms to avoid acquiring too many prions. The fact that the interactions described here are highly specific for different prion variants suggests that prion variants provide additional levels to the control of prion proliferation. These results suggest that complex interactions may exist among various other amyloid-forming proteins such as those associated with Alzheimer's, Huntington's, and Parkinson's diseases.

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