De Novo Appearance and “Strain” Formation of Yeast Prion [PSI⁺]
Are Regulated By the Heat Shock Transcription Factor

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Running head:
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ABSTRACT

Yeast prions are non-Mendelian genetic elements that are conferred by altered and self-propagating protein conformations. Such a protein conformation-based transmission is similar to that of PrP Sc, the infectious protein responsible for prion diseases. Despite recent progress in understanding the molecular nature and epigenetic transmission of prions, the underlying mechanisms governing prion conformational switch and determining prion “strains” are not understood. We report here that the evolutionarily conserved heat shock transcription factor (HSF) strongly influences yeast prion formation and “strain” determination. An HSF mutant lacking the amino-terminal activation domain inhibits the yeast prion [PSI+] formation whereas a mutant lacking the carboxyl-terminal activation domain promotes [PSI+] formation. Moreover, specific [PSI+] “strains” are preferentially formed in these mutants, demonstrating the importance of genetic makeup in determining de novo appearance of prion “strains”. Although these hsfI mutants preferentially support the formation of certain [PSI+] “strains”, they are capable of receiving and faithfully propagating non-preferable “strains”, suggesting that prion initiation and propagation are distinct processes requiring different cellular components. Our findings establish the importance of HSF in prion initiation and “strain” determination and imply a similar regulatory role of mammalian HSFs in the complex etiology of prion disease.
INTRODUCTION

Prions or proteinaceous infectious particles are generally considered to be responsible for the class of fatal mammalian neurodegenerative diseases known as transmissible spongiform encephalopathies, including scrapie in sheep, bovine spongiform encephalopathy in cattle, and Creutzfeldt-Jakob disease in humans (PRUSINER 2004). In each case, it is thought that a normal host protein, PrPC, changes conformation to adopt a pathogenic or prion isoform, PrPSc. Remarkably, PrPSc can self-propagate by converting additional PrPC to its prion conformation, and is thus infectious (PRUSINER 1998). There are at least four epigenetic elements in yeast, [PSI+], [URE3], [RNQ+], and [NU+], which are transmitted from mother to daughter cell as particular self-propagating protein conformations. They are known as yeast prions since their transmission mechanism is similar to that of PrPSc (for reviews see (CHIEN et al. 2004; JONES and TUITE 2005; LIEBMAN and DERKATCH 1999; UPTAIN and LINDQUIST 2002; WICKNER et al. 2004)). Yeast prions share many features with their mammalian brethren, despite the absence of any structural or functional homologies among the implicated proteins. Similar to the mammalian prion protein, yeast prion proteins are capable of perpetuating particular conformational changes, forming amyloid (ordered protein aggregates) fibers under physiological conditions (GLOVER et al. 1997; KING et al. 1997), and existing as distinct prion “strains” (BRADLEY and LIEBMAN 2003; DERKATCH et al. 1996; SCHLUMPBERGER et al. 2001). In mammals, several distinct strains of prion disease have been described, which differ in symptoms, incubation times, and brain pathologies (PRUSINER 1998). Yeast prion “strains” are generally referred to as variants to distinguish them from the traditional classification of yeast strains (DERKATCH et al. 1996).
Yeast Sup35, the protein determinant of prion $[PSI^+]$, is a homologue of the highly conserved eukaryotic release factor 3 (eRF3). When Sup35 is in its native conformation, it binds to Sup45 to form a functional translational termination factor to direct ribosomes to stop faithfully at stop codons (STANSFIELD et al. 1995). When Sup35 enters an altered conformation, $[PSI^+]$, it is sequestered from the translation termination machinery to result in translational read-through. As a consequence, $[PSI^+]$ cells that contain ade1-14, a nonsense mutation in ADE1, are able to grow in medium lacking adenine because of sufficient read-through of ade1-14 (SERIO and LINDQUIST 1999). In contrast, isogenic $[psi^-]$ cells with the native Sup35 conformation cannot grow in adenine deficient media since translation faithfully terminates at the nonsense codon. Thus, isogenic $[PSI^+]$ and $[psi^-]$ cells carrying ade1-14 exhibit strikingly different appearances on YPD (rich media): $[psi^-]$ cells are red due to the accumulation of a pigment byproduct whereas $[PSI^+]$ cells are white due to the translational read-through. Such differences in growth and appearance provide a sensitive and convenient assay for $[PSI^+]$ (COX 1965).

Although there has been significant progress recently in understanding the “protein only” transmission of prions (CASTILLA et al. 2005; KING and DIAZ-AVALOS 2004; LEGNAME et al. 2004; LIEBMAN 2005; LIEBMAN and MASTRIANNI 2005; TANAKA et al. 2004), the molecular mechanisms underlying the prion conformational switch and “strain” determination remain a central problem of prion biology. In order to unveil the cellular machinery governing prion formation and transmission, we have investigated the role of yeast heat shock transcription factor (HSF) in prion formation and propagation. HSFs are
evolutionarily conserved transcription factors that play an essential role in protecting 
eukaryotic organisms against heat shock and other environmental stresses (MORANO and 
THIELE 1999). For example, upon heat shock, HSF is activated to increase the production 
of heat shock proteins (HSPs), most of which are molecular chaperones, a group of 
proteins that exercise protective functions in the cell by refolding or dis-aggregating 
denatured proteins produced during the stress (PIRKKALA et al. 2001). Several molecular 
chaperones, Hsp104, Ssa and Ssb, and Sis1, are implicated in playing important roles in 
prion propagation (ALLEN et al. 2005; CHERNOFF et al. 1995b; JUNG et al. 2000; JUNG and 
MASISON 2001; KUSHNIROV et al. 2000; SONDHEIMER et al. 2001). Hsp90 co-chaperones, 
Sti1 and Cpr7 were also shown to influence [\textit{PSI}^+] stability (JONES et al. 2004). Although 
studying the effect of individual factors and their simple combinations on yeast prion 
formation has proven to be fruitful, such an approach would likely fail to uncover factors 
with functionally redundant homologue(s) or protein networks that are required for prion 
formation. Since HSFs are the master regulators in controlling the expression of molecular 
chaperones, elucidating the link between prionogenesis and HSF would possibly allow us 
to identify such cellular components.

HSFs are structurally and functionally conserved from yeast to humans, containing 
a winged helix-turn-helix DNA binding domain, a hydrophobic stretch necessary for 
homo-trimerization, followed by a transcription activation domain that is also conserved 
(LITTLEFIELD and NELSON 2001; WU 1995). HSF binds to cis-acting DNA promoter 
elements known as heat shock elements (HSEs), which are highly conserved as well (AMIN 
et al. 1988). Unlike higher eukaryotes possessing multiple distinct HSF isomers, yeast has
a single essential gene, HSF1, containing both an amino-terminal activation domain (NTA, residue- 1-147) and a carboxyl-terminal activation domain (CTA, residue- 584-833), which are thought to differentially regulate the expression of HSF target genes (Chen and Parker 2002; Sorger 1990). Recently, a genome wide analysis using chromatin immunoprecipitation (CHIP) based DNA microarray technology identified approximately 165 direct HSF-target genes, which function in diverse processes including protein folding and degradation, transcription, energy generation, protein trafficking, and cell signaling (Hahn et al. 2004). In this study, we examined yeast prion formation and transmission in two HSF truncation mutants, \(\Delta\)NTA-HSF (147-833) and \(\Delta\)CTA-HSF (1-584) (Morano et al. 1999). We report here that both HSF truncation mutants have profound and distinct effects on protein aggregation, de novo formation as well as determination of the yeast prion \([PSI^+]\) variants. We have also shown that cellular factors essential for maintaining the yeast prion \([PSI^+]\) can be differentially expressed in strains containing different \(hsf1\) alleles. Our results demonstrate that elucidating the link between HSF and yeast prions will provide valuable information on the mechanisms of prion initiation and propagation.

MATERIALS AND METHODS

Plasmids: Plasmids described in this study are listed in table 1. To construct pRS416GPD-HSF (URA3), the \(HSF1\) ORF was obtained by a polymerase chain reaction (PCR) using pRS314 HSF (TRP1) as the template, 5’TTGTTCCCGGATGAATGCTGCA3’ as the forward primer, and 5’ TTGCCCTGAATTCTATTTCTAGC3’ as the reverse primer. After digestion with SmaI and EcoRI, the PCR fragment was ligated into pRS416GPD
(URA3) that was predigested with SmaI/EcoRI. To create the hsf1 disruption construct, we applied a PCR-based method using pFA6a-kanMX6 (KanR) as the template and two primers, HSF1 F1

5’GAAAACAAAAAAGACAAAAAGACAGCTGTATTGTTTGGCGGCCGCGATCCCCCGG
GTAAATTAAA’ and HSF1 R1

5’AAATGATTATATAAGCTATTTAATGACCTTGCCCTGTGTAGAATTTCGAGCTCG
TTAAAC3’, to obtain a PCR product containing the KanR marker flanked with HSF1 sequence. To create pRS305-HSP104 (LEU2), pYS104 (URA3) was digested with XhoI and the resulting XhoI-XhoI fragment containing HSP104 coding region as well as its flanking regions was ligated to pRS305 (LEU2) that was predigested with XhoI. To create pRS415-HSP104, pYS104 was digested with ClaI and blunt ended by DNA polymerase I treatment then followed by XhoI digestion. The resulting ClaI (blunt ended)-XhoI fragment containing HSP104 coding sequence and its flanking regions was ligated to p415GPD that was predigested with SacI (blunt ended by DNA polymerase I treatment) and XhoI (the GPD promoter of p415GPD was thus removed). To create p425GPD-HSP104 (LEU2), p2HG-104 (HIS3) (Li and Lindquist 2000) was digested with BamHI and the resulting BamHI-BamHI fragment containing the HSP104 coding region was ligated to p425GPD (LEU2) that was predigested with BamHI.

**Yeast strains:** Yeast strains are listed in Table 2. To obtain hsf1 disruption strain we transformed 74D-694 (MATa: ade1-14, trp1-289, his3Δ-200, ura3-52, leu2-3, 112, [psi], [RNQ⁺]) with pRS416GPD-HSF1 followed by chromosomal replacement of HSF1 with the PCR fragment containing KanR marker flanked by HSF1 sequence as described above
through “one step” gene replacement (SHERMAN 1991). The resulting \( hsf1 \) disruption strain was named 74D-\( hsf1::Kan^R \).

To create isogenic strains of \( wt-HSF \), \( \Delta NT A-HSF \), and \( \Delta C T A-HSF \), 74D-\( hsf1::Kan^R \) containing pRS416GPD-HSF1 was transformed with pRS413-\( wt-HSF \), pRS413-\( \Delta NT A-HSF \), or pRS413-\( \Delta C T A-HSF \). The resulting transformants were grown onto media containing 5-fluororotic acid to eliminate pRS416GPD-HSF1. The generated isogeneic strains, 74D-\( wt-HSF \), 74D-\( \Delta NT A-HSF \), and 74D-\( \Delta C T A-HSF \), express the full-length HSF, an NTA truncated HSF, and a CTA truncated HSF respectively under the control of the native \( HSF1 \) 5’ and 3’ flanking regions (Fig 1). The growth of yeast cultures and other yeast genetic manipulations were performed according to established protocols (SHERMAN 1991).

\([RNQ^+]\) sedimentation assay: Single colonies of each strain were inoculated in 3 mL liquid YPD and grown overnight at 30°C with shaking. Overnight yeast cultures were diluted into fresh media to a density of \( OD_{600} = 0.05 \) and grown to \( OD_{600} = 0.6 \) before harvesting. Spheroplasts were prepared, lysed, and centrifuged as described (SONDHEIMER and LINDQUIST 2000). The total lysate, soluble, and pellet fractions of each sample were analyzed by SDS-PAGE and immunoblot analysis using a polyclonal antibody, anti-Rnq1 (a kind gift from S. Lindquist’s lab, SONDHEIMER and LINDQUIST 2000).

\([PSI^+]\) induction and “strain” determination: Exponential-phase cells of \( wt-HSF \), \( \Delta NT A-HSF \), and \( \Delta C T A-HSF \) containing \( CEN/URA3 \) plasmids, pCUP1-GFP, pCUP1-
NMGFP, or pCUP1-Rnq1C-GFP were induced by CuSO₄ at a final concentration of 34µM. After 4 hr or 24 hr induction, cells were examined by fluorescence microscopy for aggregation and/or were spotted onto YPD and SC-ade to score for \([PSI]^+\) formation using a 5-fold serial dilution. Ade\(^+\) cells on SC-ade plates were randomly selected and streaked onto YPD to view their colors. Potential \([PSI]^+\)\(^S\) (white) and \([PSI]^+\)\(^W\) (pink) were streaked onto YPD and replica-plated onto YPD+5mM GdnHCl. Only ade\(^+\) colonies that were cured by GdnHCl were scored as \([PSI]^+\). Sometimes, sequential streaking was carried out in order to obtain stable \([PSI]^+\) variants as newly induced \([PSI]^+\) are often unstable (DERKATCH et al. 1996).

**Cytoduction:** L1976 (c10B-H49 MAT\(\alpha\), SUQ5, ade2-1, his3-11, lys1-1, leu1, kar1-1, cyhR, \([PIN]^+\)[\([PSI]^+\)\(^W\)]) and L1977 (c10B-H49 MAT\(\alpha\), SUQ5, ade2-1, his3-11, lys1-1, leu1, kar1-1, cyhR, \([PIN]^+\)[\([PSI]^+\)\(^S\)]) containing a CEN plasmid (HIS3) were used as donors to cytoduce 74D-\([psi^-]\) wt-HSF, \(\Delta\)NTA-HSF, and \(\Delta\)CTA-HSF cells (MAT\(\alpha\)), respectively. Individual pairs of donors and recipients were mixed in YPD for 5 hr at 30ºC and spread onto SC (-lys, -his) plates to select for diploids and cytoductants. Colonies grown on SC (-lys, -his) plates were transferred onto a 5-FOA plate, which select against the donor and diploid cells. Cytoductants were confirmed by a mating type test and the presence of the recipient’s auxotrophic nuclear markers, for example, the leu2 marker.

**RESULTS**
The HSF truncation mutants, ΔNTA-HSF and ΔCTA-HSF, do not affect the propagation of preexisting [RNQ⁺] prions. To examine the effect of HSF activity on yeast prions, we created a chromosomal hsf1 disruption mutant in yeast strain 74D-694 ([psi⁻][RNQ⁺]), which contains a premature stop codon in the ADE1 gene to allow sensitive and quantitative assays for [PSI⁺] formation (Cox 1965). In this particular strain, [PSI⁺] de novo appearance requires the presence of [RNQ⁺], which is also called [PIN⁺], [PSI⁺] inducibility (Derkatch et al. 2001). Since HSF is an essential gene, a CEN plasmid, pRS314-wt-HSF, pRS314-ΔNTA-HSF or pRS314-ΔCTA-HSF was maintained in the hsf1 disruption strain to give isogenic strains expressing wt-HSF, ΔNTA-HSF, or ΔCTA-HSF respectively (Fig 1).

We first investigated if [RNQ⁺] was faithfully maintained in the hsf1 truncation mutants. It has been shown that Rnq1, the protein determinant of [RNQ⁺], exists in an aggregated form in [RNQ⁺] cells but remains soluble in isogenic [mq⁻] cells (Sondheimer and Lindquist 2000). The difference in Rnq1 conformation in [RNQ⁺] and [mq⁻] cells can be detected by sedimentation assays (Sondheimer and Lindquist 2000). By doing so, we found that Rnq1 was in the pellet fraction in all ΔNTA-HSF, ΔCTA-HSF, and wt-HSF cells (Fig 2A), suggesting that deletion of the HSF NTA or CTA had no effect on preexisting [RNQ⁺]. Yeast prions such as [RNQ⁺] can be eliminated by treatment of cells with a low concentration of guanidine hydrochloride (GdnHCl) (Tuite et al. 1981). After GdnHCl treatment, Rnq1 was found in the soluble fractions (Fig 2A), indicating [RNQ⁺] was eliminated. Thus, the hsf1 alleles have no effect on the propagation of preexisting [RNQ⁺] or GdnHCl curing of [RNQ⁺].
Distinct HSF activation domains have profound effects on \([PSI^+]\) de novo formation.

We next examined if the hsf1 alleles influence de novo formation of \([PSI^+]\). While \([PSI^+]\) and \([psi^-]\) cells interconvert at a low spontaneous rate of \(\sim 10^{-6}\) (TUITE et al. 1981; WICKNER 1994), overexpression of Sup35 (CHERNOFF et al. 1993) or its prion domain increases the rate of \([PSI^+]\) de novo formation up to 1,000 fold (DERKATCH et al. 1996). To determine if \(\Delta NTA\text{-}HSF\) and \(\Delta CTA\text{-}HSF\) affect the de novo formation of \([PSI^+]\), isogenic \(\Delta NTA\text{-}HSF\), \(\Delta CTA\text{-}HSF\), and \(wt\text{-}HSF\) cells were transformed with pCUP1-Sup35GFP or pCUP1-NMGFP, plasmids expressing GFP fusions of full-length \(SUP35\) or \(SUP35\text{NM}\) (the N-terminal and middle regions of Sup35) respectively. Freshly transformed cells were grown in SC-ura media and induced to form \([PSI^+]\) by CuSO4 addition. After 4-hrs, cells were spotted onto SC-ade plates to select for translation termination suppressors, indicative of potential \([PSI^+]\) cells. As shown in Fig 2B and Table 3, upon Sup35GFP overexpression, the number of ade\(^+\) colonies formed by \(\Delta CTA\text{-}HSF\) cells are approximately 7-fold more than that of isogenic \(wt\text{-}HSF\) cells, suggesting that the absence of the HSF-CTA promotes \([PSI^+]\) de novo formation. When fresh \(\Delta NTA\text{-}HSF\) transformants containing pCUP1-SUP35GFP were induced under identical conditions, \([PSI^+]\) formation was inhibited (Fig 2B and Table 3). As expected, control cells containing pCUP1-Rnq1C-GFP or pCUP1-GFP did not give rise to ade\(^+\) colonies (Fig 2B and Table 3). Upon NMGFP overexpression, \([PSI^+]\) induction was also inhibited in \(\Delta NTA\text{-}HSF\) cells but the \([PSI^+]\) promoting effect of \(\Delta CTA\text{-}HSF\) was lessened to \(\sim 2.5\) fold when compared to \([PSI^+]\) induction in \(wt\text{-}HSF\) (Fig 2B and Table 3). Since \(CUP1\) can be activated by HSF in response to a variety of growth and stress conditions, including heat shock, chemical stress and glucose starvation (HAHN et al. 2004; TAMAI et al. 1994), the observed differences in \([PSI^+]\) induction by hsf1 alleles could have
resulted from different expression levels of NMGFP or Sup35GFP (Hahn et al. 2004; Tamai et al. 1994). To test this possibility, we carried out immunoblot analysis using a polyclonal antibody specific to Sup35M to estimate the expression levels of NMGFP. As shown in Figure 2C, similar amounts of NMGFP was expressed in all cell types after a 4-hour induction. Comparable intensities of the endogenous Sup35 band (the slower migrating band) in each lane confirmed the equal loading (Fig 2C). The expression levels of Sup35GFP are also similar in these hsf1 alleles (data not shown). These results demonstrate that the observed differences in [PSI+] de novo formation in wt-HSF, ΔNTA-HSF, and ΔCTA-HSF cells are not due to differences in NMGFP or Sup35GFP expression levels.

Although [PSI+] is nearly undetectable after 5 days at 30°C for fresh ΔNTA-HSF transformants upon 4 hour Sup35-overexpression, [PSI+] formation can be detected upon a longer incubation period (data not shown). ΔNTA-HSF transformants stored for an extended period (>10 days) also give rise to [PSI+] with faster kinetics upon induction of Sup35 overexpression (Fig 2D). Presumably, the leaky CUP1 promoter, which is also induced in response to glucose deprivation (Hahn et al. 2004; Tamai et al. 1994), allowed weak but constitutive expression of Sup35GFP and NMGFP. These observations suggest that [PSI+] de novo formation in ΔNTA-HSF cells is severely impaired but not completely abolished. Under our experimental conditions, we have not observed any detectable differences in growth among the isogenic strains of ΔNTA-HSF, ΔCTA-HSF, and wt-HSF cells. As shown in Fig 2B, the number and size of colonies on YPD are similar in all cell types, demonstrating that they have a similar growth rate. Thus, the inhibitory effect of ΔNTA-HSF
or the stimulatory effect of ∆CTA-HSF on [PSI'] de novo formation was not caused by a difference in their growth rates.

**The effect of N-terminal or C-terminal domain deletion of HSF on prion protein and polyQ aggregation.** Consistent with our observation for [PSI'] induction, we observed that the deletion of HSF NTA or CTA has profound effects on Sup35 aggregation. We examined Sup35NMGFP fluorescent foci formation in isogenic *hsf1* strains that are [ψi−][RNQ+]. As shown in Fig 3A, fresh ∆NTA-HSF transformants containing pCUP1-NMGFP were unable to form detectable fluorescent foci after 4-hour CuSO4 addition. Approximately 7% of *wt-HSF* cells exhibited fluorescent foci after a 4-hour induction, similar to previously reported results (Patino et al. 1996). However, ∆CTA-HSF cells formed ~3 times more foci than that of *wt-HSF* cells (Fig 3A). For isogenic *hsf1* alleles that are [ψi−][rnq−] (after GdnHCl treatment), Sup35NMGFP exhibited minimal fluorescent foci in all cell types (Fig 3B), suggesting that deletion of HSF-NTA or -CTA does not evade the requirement of [RNQ+] for NMGFP aggregation. Indeed, [PSI'] induction in all *hsf1* alleles requires the presence of [RNQ+] (data not shown).

We next asked if the *hsf1* alleles influence Rnq1 aggregation. In agreement with the solubility assay, Rnq1C-GFP (a GFP fusion containing Rnq1 prion domain) formed fluorescent foci with a similar pattern of multiple dots (MD) (Bradley et al. 2002) in isogenic ∆NTA-HSF, ∆CTA-HSF, and wt-HSF cells that were derived from [ψi−][RNQ+], confirming their [RNQ+] status (Fig 3A). To examine de novo aggregation of Rnq1, we subjected cells ([ψi−][RNQ+]) containing pCUP1-Rnq1C-GFP as described in Fig 3A to
GdnHCl treatment to eliminate $[RNQ^+]$. After GdnHCl treatment, only a diffuse Rnq1C-GFP fluorescent pattern was seen for all $hsfl$ alleles upon 0.5-hour induction, confirming that $[RNQ^+]$ was cured (data not shown). After 4h induction, Rnq1C-GFP fluorescent patterns were strikingly different. $\Delta CT A$-HSF cells formed about 3 times more fluorescent foci (21% of 378 cells) than $wt$-HSF cells (7.5% of 349 cells). Rnq1C-GFP foci in $\Delta N T A$-HSF cells were almost undetectable (Fig 3B). Our results suggest that the $hsfl$ alleles have similar effects on the aggregation of both Sup35 and Rnq1.

To test if the $hsfl$ alleles specifically affect prion proteins, we examined the aggregation of Huntingtin protein. Huntingtin is a human protein containing a polyQ tract with variable lengths among individuals. PolyQ length is tightly associated with the etiology of Huntington disease: the longer the tract, the worse the symptoms and the earlier onset of the disease (Andrew et al. 1993; DuYao et al. 1993; Snell et al. 1993). There are at least nine polyQ-associated neurodegenerative diseases, including the Huntington’s disease (Walsh et al. 2005). Yeast has been a useful model for studying the aggregation and toxicity of polyQ proteins (Derkatch et al. 2004; Gokhale et al. 2005; Krobitsch and Lindquist 2000; Meriin et al. 2002; Osherovich and Weissman 2001). Huntingtin exon1, with a long polyQ tract, forms detectable aggregates in yeast cells and the aggregation strength and the associated cell toxicity are proportional to the length of the Q-tract (Krobitsch and Lindquist 2000; Meriin et al. 2002). We transformed the isogenic $\Delta N T A$-HSF, $\Delta C T A$-HSF, and $wt$-HSF cells with a set of high copy-GPD plasmids containing Huntingtin exon1-GFP with variable polyQ lengths: Q25, Q47, Q72, and Q103, respectively (Krobitsch and Lindquist 2000). As shown in Fig 3C, Q47-GFP formed no detectable
aggregates in \textit{wt-HSF}, in agreement with a previous report (KROBITSCH and LINDQUIST 2000). However, the formation of Q47-GFP aggregates was obvious in \textit{ΔCTA-HSF} cells (Fig 3C). For Huntingtin-Q72, ~ 30\% of \textit{ΔCTA-HSF} cells (92/296) formed fluorescent foci compared to 15\% of \textit{wt-HSF} cells (56/428) and none in \textit{ΔNTA-HSF} cells (0/392). For Q103, fluorescent foci were seen in 27\% of \textit{wt-HSF} cells (81/286), ~ 53\% of \textit{ΔCTA-HSF} cells (175/325) but only ~10\% of \textit{ΔNTA-HSF} cells (33/325) (Fig 3C). Thus, the effects of the distinct \textit{hsf1} alleles on protein aggregation are not specific to prion proteins.

\textbf{The deletion of the N-terminal or C-terminal domain of HSF differentially affects [\textit{PSI}^+] variant formation.} The dramatic effect of \textit{hsf1} alleles on [\textit{PSI}^+] de novo formation encouraged us to examine whether they also influence [\textit{PSI}^+] variant establishment. Over-expression of the Sup35 prion domain in \textit{wt} cells typically gives rise to [\textit{PSI}^+] variants ranging from very weak to very strong (Derkatch, 1996 #10). This is also the case when we examined the induced [\textit{PSI}^+] in \textit{wt-HSF} cells upon NMGFP over-expression (Fig 4A, 4B). Although \textit{ΔCTA-HSF} significantly increased the de novo appearance of [\textit{PSI}^+], we were surprised to find that almost all ade^+ colonies of \textit{ΔCTA-HSF} cells appeared as very weak variants of [\textit{PSI}^+] upon streaking on YPD (Fig 4A, right panel). Detailed examination revealed unique mosaic colony morphology associated with [\textit{PSI}^+] induced in \textit{ΔCTA-HSF} cells (Fig 4B and 4C). Upon restreaking, these mosaic colonies gave rise to a low frequency of [\textit{psi}^-] cells (~15\%) and [\textit{PSI}^+]^S cells (~1\%) but [\textit{PSI}^+]^W was never seen under our experimental conditions. The majority remain their mosaic feature (Fig 4B and 4C). But once it becomes [\textit{psi}^-] or [\textit{PSI}^+]^S, it can be stably transmitted as [\textit{psi}^-] or [\textit{PSI}^+]^S upon restreaking. We named those mosaic [\textit{PSI}^+] induced in \textit{ΔCTA-HSF} cells as [\textit{PSI}^+]^V because
of their “unstable” and “undifferentiated” feature. In contrast to the preference of forming $[\text{PSI}^+]^\text{U}$ in $\Delta\text{CTA-HSF}$ cells, almost all $\text{ade}^+$ colonies formed in $\Delta\text{NTA-HSF}$ cells exhibited a uniform $[\text{PSI}^+]$ strength. They are apparently pinker than the control $[\text{PSI}^+]^\text{S}$ (Fig 4A middle panel & Fig 4B). Table 4 summarizes $[\text{PSI}^+]$ induction results from three consecutive experiments. Although $\text{wt-HSF}$ gave rise to mixed $[\text{PSI}^+]$ variants, 91% $[\text{PSI}^+]$ derived from $\Delta\text{NTA-HSF}$ cells were $[\text{PSI}^+]^\text{S}$ and 97% of $[\text{PSI}^+]$ induced from $\Delta\text{CTA-HSF}$ cells are $[\text{PSI}^+]^\text{U}$. Thus, specific $[\text{PSI}^+]$ variants are preferably formed in different $\text{hsf1}$ mutants.

The effect of N-terminal or C-terminal domain deletion of HSF on $[\text{PSI}^+]$

**propagation.** We next investigated if the $\text{hsf1}$ alleles are able to receive and propagate non-preferred $[\text{PSI}^+]$ variants. We carried out cytoduction experiments using $[\text{PSI}^+]^\text{S}$ and $[\text{PSI}^+]^\text{W}$ as donors and isogenic $\text{wt-HSF}$, $\Delta\text{NTA-HSF}$ and $\Delta\text{CTA-HSF} [\psi^-]$ cells as recipients. Cytoduction allows mating partners to share their cytoplasm but not their nuclear components (CONDÉ and FINK 1976). If a particular genetic trait is mediated by a prion, it should be transmissible to a mating partner without contributing its nuclear materials since it is “infectious.” This is indeed the case for all yeast prions identified to date (ÜPTAIN and LINDQUIST 2002). When $[\text{PSI}^+]^\text{S}$ was used as the donor and isogenic $\Delta\text{NTA-HSF}$, $\Delta\text{CTA-HSF}$, and $\text{wt-HSF}$ cells that are $[\psi^-]$ were used as recipients, cytoductants in all cell types were $[\text{PSI}^+]^\text{S}$ (Fig 5), indicating that deletion of the HSF NTA or CTA does not affect the ability of cells to faithfully receive and propagate $[\text{PSI}^+]^\text{S}$.

When $[\text{PSI}^+]^\text{W}$ was the donor, $\text{wt-HSF}$ and $\Delta\text{NTA-HSF}$ cells were able to receive and faithfully maintain $[\text{PSI}^+]^\text{W}$ whereas $\Delta\text{CTA-HSF}$ cytoductants of $[\text{PSI}^+]^\text{W}$ exhibited similar colony morphology to that of $[\text{PSI}^+]^\text{U}$ formed de novo in $\Delta\text{CTA-HSF}$ cells. They are mosaic
and sectored (compare the enlarged cell streak in Fig 4B and Fig 5). Their mosaic feature can be stably maintained upon multiple sequential streaking and even through plasmid transformation procedures. Table 5 summarizes results from three independent cytoduction experiments. All cytoductants of $[PSI^+]^S$ were $[PSI^+]^S$, confirming that all $hsl1$ alleles are capable of receiving and propagating $[PSI^+]^S$. Approximately 45% ΔNTA-HSF cytoductants of $[PSI^+]^W$ were $[psi^-]$, indicating a reduced ability of ΔNTA-HSF cells to receive and propagate $[PSI^+]^W$. The fact that ΔCTA-HSF cytoductants of $[PSI^+]^W$ displayed the same colony morphology as that of $[PSI^+]^U$ de novo formed in ΔCTA-HSF cells suggests that $[PSI^+]^U$ is the same as $[PSI^+]^W$ but with a unique phenotype in ΔCTA-HSF cells. To test this possibility, we crossed $[PSI^+]^U$ of ΔCTA-HSF with wt $[psi^-]$ cells of the opposite mating type. The resulting diploid cells were stable $[PSI^+]^W$, suggesting that the $[PSI^+]^U$ phenotype is not dominant (Fig 5B). Upon sporulation, a 2:2 ratio of stable $[PSI^+]^W$ (wt) to $[PSI^+]^U$ (ΔCTA-HSF) was obtained (Fig 5B). Upon streaking, the ΔCTA-HSF spores have the same colony morphology as that of $[PSI^+]^U$ (Fig 5B). Thus, our results demonstrate that the ΔCTA-HSF genetic background can not support a stable maintenance of $[PSI^+]^W$ and confirm that $[PSI^+]^U$ is essentially $[PSI^+]^W$ but with a different readout in ΔCTA-HSF cells.

The effect of N-terminal or C-terminal domain deletion of HSF on the expression of Hsp104. The molecular chaperone Hsp104 plays an essential role not only in prion propagation but also in polyQ aggregation in S. cerevisiae (CHERNOFF et al. 1995b; KROBITSCH and LINDQUIST 2000). It has been shown that HSP104 expression is under the regulation of HSF (GRABLY et al. 2002; HALLADAY and CRAIG 1995). To examine if the
hsf1 alleles differentially regulate HSP104 expression, we conducted immunoblot analysis to assess the steady level of Hsp104 in isogenic wt-HSF, ∆NTA-HSF, and ∆CTA-HSF cells. As shown in Fig 6A, Hsp104 levels are similar in wt-HSF and ∆CTA-HSF cells. The level of Hsp104 in ∆NTA-HSF cells, however, is significantly elevated. To estimate the elevation level of Hsp104 in ∆NTA-HSF cells, we carried out a comparative immunoblot analysis to evaluate the Hsp104 levels of ∆NTA-HSF cells and wt cells containing various HSP104 expression plasmids. As shown in Fig 6B, the amount of Hsp104 in ∆NTA-HSF cells is slightly more than that of wt cells containing a single-copy integrated plasmid of HSP104 (lane 3) but less than that of wt cells containing a CEN or a multi-copy HSP104 expression plasmid (lane 4, 5). This is true in both cases of cells in early log phase and stationary phase (Fig 6B). We also examined the expression levels of Hsp70 and Hsp90, two additional HSF target genes (HAHN et al. 2004). The amount of Hsp70 is similar among all cell types (Fig 6A) as judged by immunoblot analysis using a monoclonal antibody against Drosophila Hsp70 (VELAZQUEZ and LINDQUIST 1984). The expression level of Hsp90 in ∆NTA-HSF cells is similar to that of wt cells. However, Hsp90 is significantly lower in ∆CTA-HSF cells, as reported previously (MORANO et al. 1999) (Fig 6A).

DISCUSSIONS

Our data demonstrates that the two truncation mutants of yeast HSF1, ∆NTA-HSF and ∆CTA-HSF, exhibit a striking difference in their ability to form [PSI+]: ∆CTA-HSF stimulates whereas ∆NTA-HSF inhibits the de novo formation of [PSI+]. This result implies
an important but complex role of HSF in prion formation. Early reports suggested that the NTA and CTA of HSF modulate distinct target genes upon different environmental stimuli (SORGER 1990). More recent studies suggest that the transcriptional activation domains of HSF are in a dynamic association with the DNA binding and oligomerization domains (BULMAN et al. 2001; CHEN and PARKER 2002). Maintaining such domain-domain interactions is important for preserving HSF in a repressive state under normal growth conditions (CHEN and PARKER 2002; HARDY et al. 2000). It is possible that the opposite effect of ∆NTA-HSF and ∆CTA-HSF on [PSI⁺] is due to their differences in perturbing such domain-domain interactions.

Our findings that ∆NTA-HSF and ∆CTA-HSF cells have a strikingly different ability with regard to [PSI⁺] de novo formation and variant determination suggest that important cellular factors required for prion formation are differently regulated in ∆NTA-HSF and ∆CTA-HSF cells. Indeed, we showed that HSP104 expression is significantly enhanced in ∆NTA-HSF, indicating that the N-terminal activation domain of HSF is inhibitory to HSP104 expression under non-stress conditions. The up-regulation of HSP104 in ∆NTA-HSF cells demonstrates that particular mutations in HSF1 are able to differentially regulate important cellular factors that are required for prion formation and propagation. It is possible that the elevated Hsp104 level is responsible for the observed inhibitory effect of ∆NTA-HSF on de novo [PSI⁺] formation since [PSI⁺] can be cured by overexpression of Hsp104 (CHERNOFF et al. 1995a). It has been shown and confirmed by us that Hsp90 level is significantly reduced in ∆CTA-HSF ((MORANO et al. 1999); Fig 6A). The expression levels of Sse1, a distant Hsp70 family member, and Sti1, an Hsp90 co-
chaperone, are also significantly lowered in ΔCTA-HSF cells (Morano, 1999). The involvement of Sti1 in [PSI+] propagation has been shown (Jones et al. 2004; Song and Masison 2005) but the role of Hsp90 and Sse1 in prion formation has not been reported. Comparative analysis of total gene expression profiles would allow identifying additional genes that are differentially expressed in these hsf1 mutants and are important for prion formation.

An unsolved mystery in prion biology is the “strain” phenomenon, a single protein molecule existing in multiple inheritable conformations that are “infectious” (Derkatch et al. 1996; Prusiner 1998). Although mutations within a specific prion protein have been linked to the formation of particular “strains” of the corresponding prion (Chien et al. 2003; King and Diaz-Avalos 2004; Vanik et al. 2004), cellular factors required for de novo appearance of a particular “strain” remain to be identified. Yeast cells derived from one single colony are able to form prions with a wide range of variants, from very strong to very weak upon Sup35 overexpression (Derkatch et al. 1996) (Fig 4B). These observations suggest that prion “strain” determinants are likely epigenetic modifiers. It has been shown that the cell cycle phase affects the number of [PSI+] seeding elements - “propagons” and their subsequent segregation (Cox et al. 2003). Environmental fluctuations, cell aging, and other unknown factors are also possible epigenetic modifiers influencing prion “strains” establishment. Our finding that the ΔNTA-HSF prefers [PSI+]S de novo formation whereas ΔCTA-HSF selectively gives rise to [PSI+]U variant demonstrates for the first time that specific prion variants can be preferentially formed in defined genetic backgrounds. Thus, there is also a genetic basis for prion variant
determination. Elucidating the relationship between HSF and \([PSI^+]\) variant formation will likely help us to reveal the identities of the variant determinants and solve the prion “strain” mystery.

We demonstrated that > 90% \([PSI^+]\) formed in \(\Delta \text{NTA-HSF}\) cells are \([PSI^+]^S\) whereas > 97% are \([PSI^+]^U\) in \(\Delta \text{CTA-HSF}\) cells (Fig 4 and Table 4). Both truncation mutants were, however, capable of receiving and faithfully propagating preformed \([PSI^+]^S\) through cytoduction (Fig 5 and table 5). Since \([PSI^+]^U\) is a special readout of \([PSI^+]^W\) in \(\Delta \text{CTA-HSF}\) cells, we consider that both \(\Delta \text{NTA-HSF}\) and \(\Delta \text{CTA-HSF}\) cells are also capable of receiving and propagating \([PSI^+]^W\) (Fig 5). These results strongly suggest that prion initiation and propagation are two separate processes that preferentially utilize distinct cellular machineries. The propagation process, including prion replication and subsequent segregation into daughter cells, has been extensively studied. For example, several cellular factors important for \([PSI^+]\) propagation, such as Hsp104, Ssa1 and Ssb1, Sis1, Sla1, Sti1, and Cpr7 have been identified (CHERNOFF et al. 1995b; JONES et al. 2004; KUSHNIROV et al. 2000; SONDHEIMER et al. 2001; SONG and MASISON 2005). In contrast, the initiation process is less understood. Our data suggest that the initiation process requires a more stringent cellular environment than that of propagation, since both \(\Delta \text{NTA-HSF}\) and \(\Delta \text{CTA-HSF}\) cells are able to receive and propagate preformed \([PSI^+]^S\) and \([PSI^+]^W\), but only a specific variant is preferably formed de novo in each mutant. Our finding demonstrates that a defined genetic background can give rise to a specific prion variant, offering a traceable system for identifying cellular factors that are important for prion initiation. Since both \(\Delta \text{NTA-HSF}\) and \(\Delta \text{CTA-HSF}\) cells we examined for \([PSI^+]\) de novo formation contained the
same [$RNQ^+$] variant, a multi dot (MD) form (Fig 2B) (BRADLEY and LIEBMAN 2003), the differences of $\Delta$NTA-HSF and $\Delta$CTA-HSF cells in [$PSI^+$] de novo formation are likely due to the influence of non-$[RNQ^+]$ factor(s) that remains to be identified.

Deposition of protein aggregates is a hallmark of several devastating protein-folding diseases, including Huntington’s disease, Alzheimer’s disease, Parkinson’s disease, and prion diseases (SOTO 2003). Although the underlying mechanisms that confer each disease are poorly understood, the involvement of molecular chaperones in their etiologies has been implicated, including the prion disease (MUCHOWSKI and WACKER 2005). Our results that HSF greatly affects the aggregation of polyQ strongly suggest that it is possible that mutations in mammalian HSF could lead to anti-aggregation or aggregation promoting phenotypes. Thus modulation of HSF activity can contribute to the etiology of protein misfolding diseases, e.g. prion disease, Huntington’s disease, and other amyloidogenic diseases. In this regard, the effect of HSF activity on the life span of C. elegans has been recently reported: overexpressing HSF prolonged the life span of C. elegans whereas loss-of-function mutations of hsf shortened it (HSU et al. 2003; MORLEY and MORIMOTO 2004).

Mammals have multiple members of HSFs, each of which differentially responds to distinct environmental stimuli (MORIMOTO 1998). Although the role of each member in protein aggregation is unclear, our finding that yeast hsf1 mutations profoundly affect the aggregation of both prion protein and polyQ suggest that deciphering the regulatory mechanism of HSF on protein aggregation might provide valuable information to facilitate the development of novel therapeutic drugs for protein misfolding diseases.
ACKNOWLEDGEMENTS

We thank Professor Susan Liebman for kindly providing the 74D-MATα strains of \([PSI^+]^S\) and \([PSI^+]^W\) used as cytoduction donors in our study. We are grateful to Professor Susan Lindquist for the gifts of Sup35, Rnq1, Hsp70, Hsp90, and Hsp104 antibodies. We also thank Ms Mohanity for editing the manuscript. This work was partially supported by grants from US ARMY (0650-370-R744) and Ellison Medical Foundation for L.L. and National Institutes of Health (GM59911) to D.T.

FIGURE LEGENDS

Figure 1. Creating isogenic \(\Delta NTA\text{-}HSF\), \(\Delta CTA\text{-}HSF\), and \(wt\text{-}HSF\) cells in strain 74D-694. A \(CEN\) plasmid, p416GPD-HSF, expressing \(wt\) \(HSF1\) with a \(URA3\) marker was transformed into a 74D-694 strain \([psi][RNQ^+])\), whose chromosomal \(HSF1\) was subsequently disrupted with a Kan\(^R\) gene through one step gene replacement (see Materials and Methods). After transformation with a \(CEN/HIS3\) plasmid, pRS314\(\Delta NTA\text{-}HSF(148\text{-}833)\), pRS314\(\Delta CTA\text{-}HSF(1\text{-}583)\), or pRS314\(wt\text{-}HSF\), p416GPD-HSF was eliminated by growing the transformants in the presence of 5-FOA to obtain isogenic strains with \(\Delta NTA\text{-}HSF\), \(\Delta CTA\text{-}HSF\), or \(wt\text{-}HSF\) as the sole copy of HSF.

Figure 2. The truncation mutants of \(hsg1\), \(\Delta NTA\text{-}HSF\) and \(\Delta CTA\text{-}HSF\), have no effect on preexisting \([RNQ^+]\) but dramatically influence the de novo formation of \([PSI^+]\).
A). [$RNQ^+$] sedimentation assay of isogenic strains of $\Delta NTA-HSF$, $\Delta CTA-HSF$, and $wt-HSF$, which are derived from a 74D-694 ($[psi^+][RNQ^+]$) strain (see Materials and Methods and Fig 1). Total protein extract was prepared and centrifuged as described previously (SONDHEIMER and LINDQUIST 2000). T: total protein extract; S: soluble fraction; P: pellet fraction. Cells were treated either without (-GdnHCl) or with 5mM GdnHCl (+GdnHCl).

B). De novo formation of $[PSI^+]$ in isogenic $\Delta NTA-HSF$, $\Delta CTA-HSF$, and $wt-HSF$ cells. Yeast cells with specific genetic background indicated were transformed with pCUP1-GFP (vector control), pCUP1-Sup35GFP, pCUP1-NMGFP, or pCUP1-Rnq1Q-GFP. Fresh transformants were grown in SC-ura media to early log phase before addition of CuSO$_4$ (final concentration, 34µM). After 4-hour induction, cells were spotted onto either YPD or SC-ade in a 5-fold dilution series. Pictures were taken after cells were grown for 3 days (YPD) or 5 days on SC-ade at 30°C. C). Immunoblot analysis of isogenic $\Delta NTA-HSF$, $\Delta CTA-HSF$, and $wt-HSF$ cells after 4-hr induction of Sup35NMGFP. Crude extracts prepared using the ethanol lysis method were analyzed by SDS-PAGE and immunoblot analysis using Sup35-1B, a polyclonal antibody against the Sup35M region (Li and LINDQUIST 2000). As shown: lanes 2, 3, 4 are $\Delta NTA-HSF$, $\Delta CTA-HSF$, and $wt-HSF$ respectively. Lane 1 is wt cell containing expression vector as control. D). $\Delta NTA-HSF$ cells were able to induce $[PSI^+]$ but with slower kinetics. The same $\Delta NTA-HSF$ transformant used for B was reassayed for its ability to induce $[PSI^+]$ after storage at 4°C for 14 days. $[PSI^+]$ induction was carried out under identical conditions as described in B). The pictures were taken after 5 and 10 days incubation at 30°C on SC-ade.
Figure 3: Fluorescent microscopic assay of NMGFP, Rnq1C-GFP, and polyQ-GFP aggregation. Isogenic ΔNTA-HSF, ΔCTA-HSF, and wt-HSF cells were transformed respectively with pCUP1-GFP (vector control), pCUP1-NMGFP, pCUP1-Rnq1C-GFP, or p426-GPD based polyQ-GFP expression plasmids varying in Q-lengths (KROBITSCH and LINDQUIST 2000). Expression of GFP-fusion proteins was induced by addition of CuSO4 (final concentration, 34μM). Data shown was acquired after 4h induction. A).

Visualization of NMGFP and Rnq1C-GFP aggregations in yeast cells ([psi][RNQ⁺]) with specific hsf1 alleles indicated (left) and a quantitative summary of three independent experiments of NMGFP and Rnq1C-GFP aggregation (right). B). Fluorescent microscopic assay of prion protein aggregation in [psi][rnq⁻] cells with different hsf1 alleles. Cells from A) were treated with 5mM GdnHCl to eliminate [RNQ⁺]. The resulting [psi][rnq⁻] cells containing the respective plasmids were grown in SC-Ura to early log phase and induced to express GFP-fusions by addition of CuSO4 (final concentration, 34μM) as described in A). Data shown was acquired after 4h induction. C). Visualization of polyQ-GFP aggregation in isogenic yeast cells of wt-HSF, ΔNTA-HSF, and ΔCTA-HSF (upper) and a quantitative summary of three independent experiments of polyQ-GFP aggregation (lower).

Figure 4: [PSI⁺] induced in isogenic ΔNTA-HSF, ΔCTA-HSF, and wt-HSF cells exhibits distinct phenotypes. A). Individual ade⁺ colonies of ΔNTA-HSF, ΔCTA-HSF, and wt-HSF formed by overexpression of Sup35NMGFP (see Figure 2) were randomly picked and streaked onto YPD plates to show their color. Also shown are [PSI⁺] (+) and [psi⁻] (-) control variants derived from wt 74D-694 cells. B). Representative single colonies from A)
were enlarged to show their colony morphology. C). A representative streak of \([PSI^+]^U\) induced in \(\Delta{CTA-HSF}\) background.

**Figure 5. The transmissibility of preexisting \([PSI^+]\) to \(hsf1\) mutants.** A). Cytoduction: \(\alpha\) cells (c10B-H49) of \([PSI^+]^S\) and \([PSI^+]^W\) containing a \(kar-1\) mutation (gifts of Professor Susan Liebman) were used as donors to cytodeuce isogenic \(\alpha\) cells of \(\Delta{NTA-HSF}\), \(\Delta{CTA-HSF}\), and \(wt-HSF\) (74D-694) that are \([psi^-]\). Cytoduction was carried out as described in Materials and Methods. Cytoductants were verified by the presence of the recipient’s nuclear marker (leu-2) and mating type, \(\alpha\). The \([PSI^+]\) status of each cytoductant was confirmed by its curability by GdnHCl. Also shown are patches of individual cytoductants on YPD for color visualization. Note: \(\Delta{CTA-HSF}\) cytoductants of \([PSI^+]^W\) exhibit \([PSI^+]^U\) phenotype (see the enlarged cell streak), identical to the de novo formed \([PSI^+]\) in \(\Delta{CTA-HSF}\) cells upon overexpression of Sup35NMGFP. B). Mating and sporulation: Wild type \(\alpha\) cells (74D-694 \([psi^-][rnq^-]\)) were crossed with \(\Delta{CTA-HSF}\) \(\alpha\) cells that are \([PSI^+]^U\). The diploids were picked and incubated at 30°C for 4 days on YPD plates before subjected to sporulation (30°C for 7 days on sporulation plate). The tetrads were dissected and incubated at 30°C for 4 days before streaking. The genome typing of spores was done by testing their ability to grow in 37°C since \(wt-HSF\) cells are viable whereas \(\Delta{CTA-HSF}\) cells are not at 37°C (MORANO et al. 1999).

**Figure 6. The effect of \(hsf1\) alleles on the expression of molecular chaperones.** A). Isogenic \(\Delta{NTA-HSF}\), \(\Delta{CTA-HSF}\), and \(wt-HSF\) cells were grown in YPD to OD\(_{600}\) = 0.5. Whole cell lysates were prepared and analyzed by SDS/PAGE followed by immunoblot
using a polyclonal antibody of yeast Hsp104. The same membrane was probed with a monoclonal antibody of Drosophila Hsp70, a polyclonal antibody of yeast Hsp90, or a polyclonal antibody of Rnq1 (as loading control) following sequential stripping. B) ΔNTA-HSF cells (lane 1) and isogenic wt 74D-694 cells containing p415GPD (lane 2), integrated single copy HSP104 (pRS305-HSP104) with HSP104 promoter and terminator (lane 3), p415-HSP104 with HSP104 promoter and terminator (lane 4), and p425GPD-HSP104 with GPD promoter and CYC1 terminator were grown under identical conditions and harvested at either early log phase or stationary phase (overnight). Whole cell lysates were prepared and analyzed by SDS/PAGE followed by immunoblot using a polyclonal antibody of yeast Hsp104. (All antibodies used in this figure were gifts from Lindquist’s lab).

Table 3. [PSI⁺] de novo formation upon Sup35NMGFP overexpression. Isogenic 74D-694 ΔNTA-HSF, ΔCTA-HSF, and wt-HSF cells containing pCUP1-GFP (vector control), pCUP1-Sup35GFP, pCUP1-NMGFP, or pCUP1-Rnq1C-GFP were grown and induced to form [PSI⁺] as described in Materials and Methods. The rate of [PSI⁺] formation was calculated by dividing number of [PSI⁺] colonies with the total number of cells spotted on. Note, only ade⁺ colonies cured by 5mM GdnHCl were counted as [PSI⁺]. Results from five independent experiments are shown.

Table 4. Distinctive [PSI⁺] variants were induced from isogenic ΔNTA-HSF, ΔCTA-HSF, and wt-HSF cells. Results from three independent induction experiments are shown. Only ade⁺ colonies that could be cured by GdnHCl were scored as [PSI⁺], ([PSI⁺]S: white; [PSI⁺]W: pink; [PSI⁺]U: mosaic and/or sectored; [psi-]: red).
Table 5. [\textit{PSI}^+] variants of cytoductants in isogenic \(\Delta NT A-HSF\), \(\Delta C T A-HSF\), and \textit{wt-HSF} cells. Cytoduction experiments were carried out as described in Figure 5. Results of three independent cytoduction experiments are summarized. [\textit{PSI}^+] variants of cytoductants were scored by their color in YPD, [\textit{PSI}^+]\textsuperscript{S}: white; [\textit{PSI}^+]\textsuperscript{W}: pink; [\textit{PSI}^+]\textsuperscript{U}: mosaic and/or sectored; [\textit{psi}^{-}]: red.

\textbf{Allen, K. D., R. D. Wegorzyn, T. A. Chernova, S. Muller, G. P. Newnam et al., 2005} Hsp70 chaperones as modulators of prion life cycle: novel effects of Ssa and Ssb on the Saccharomyces cerevisiae prion [PSI\textsuperscript{+}]. Genetics 169: 1227-1242.


STANSFIELD, I., K. M. JONES, V. V. KUSHNIROV, A. R. DAKESAMANSKAYA, A. I. POZNYAKOVSKI et al., 1995 The products of the SUP45 (eRF1) and SUP35 genes interact to mediate translation termination in Saccharomyces cerevisiae. EMBO J 14: 4365-4373.

TANAKA, M., P. CHIEN, N. NABER, R. COOKE and J. S. WEISSMAN, 2004
Conformational variations in an infectious protein determine prion strain

TER-AVANESYAN, M. D., A. R. DAGKESAMANSKAYA, V. V. KUSHNIROV and V. N.
SMIRNOV, 1994 The SUP35 omnipotent suppressor gene is involved in the
maintenance of the non-Mendelian determinant [PSI+] in the yeast

TUITE, M. F., C. R. MUNDY and B. S. COX, 1981 Agents that cause a high frequency of
 genetic change from [PSI+] to [psi-] in Saccharomyces cerevisiae. Genetics 98:
691-711.


for interspecies transmissibility of mammalian prions. Mol Cell 14: 139-145.

VELAZQUEZ, J. M., and S. LINDQUIST, 1984 hsp70: nuclear concentration during
environmental stress and cytoplasmic storage during recovery. Cell 36: 655-
662.

WALSH, R., E. STOREY, D. STEFANI, L. KELLY and V. TURNBULL, 2005 The roles of
proteolysis and nuclear localisation in the toxicity of the polyglutamine

WICKNER, R. B., 1994 [URE3] as an altered Ure2 protein: evidence for a prion analog

WICKNER, R. B., H. K. EDSKES, E. D. ROSS, M. M. PIERCE, U. BAXA et al., 2004 Prion

## Table 1. Plasmids used in this study

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## Table 2. S. cerevisiae strains used in this study

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<td>MATα: ade1-14, trp1-289, his3Δ-200, ura3-52, leu2-3, 112, hsf1::KANΔ, [psi][PIN+]</td>
<td>This study</td>
</tr>
<tr>
<td>74D-694-ΔNTA-HSF</td>
<td>MATα: ade1-14, trp1-289, his3Δ-200, ura3-52, leu2-3, 112, hsf1::KANΔ, [psi][PIN+]</td>
<td>This study</td>
</tr>
<tr>
<td>74D-694-ΔCTA-HSF</td>
<td>MATα: ade1-14, trp1-289, his3Δ-200, ura3-52, leu2-3, 112, hsf1::KANΔ, [psi][PIN+]</td>
<td>This study</td>
</tr>
<tr>
<td>L1976</td>
<td>MATα: SUQ5, ade2-1, lys1-1, his3-11, 15, leu1, kar1-1, cyhR, [PSI]m [PIN+]</td>
<td>Liebman lab</td>
</tr>
<tr>
<td>L1977</td>
<td>MATα: SUQ5, ade2-1, lys1-1, his3-11, 15, leu1, kar1-1, cyhR, [PSI]s [PIN+]</td>
<td>Liebman lab</td>
</tr>
</tbody>
</table>
Table 3. The effects of *hsf1* alleles on [*PSI*⁺] de novo formation

<table>
<thead>
<tr>
<th></th>
<th>GFP</th>
<th>Sup35GFP</th>
<th>NMGFP</th>
<th>Rnq1GFP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>74D-694</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>wt-HSF</em></td>
<td>&lt; 0.1</td>
<td>2.1 ± 0.9</td>
<td>2.5 ± 0.6</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td></td>
<td>(0/2242)</td>
<td>(48/2353)</td>
<td>(50/2020)</td>
<td>(0/2569)</td>
</tr>
<tr>
<td><strong>ΔNTA-HSF</strong></td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td></td>
<td>(0/2387)</td>
<td>(0/2278)</td>
<td>(0/1905)</td>
<td>(0/2302)</td>
</tr>
<tr>
<td><strong>ΔCTA-HSF</strong></td>
<td>&lt; 0.1</td>
<td>15.4 ± 3.1</td>
<td>6.1 ± 2.0</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td></td>
<td>(0/2206)</td>
<td>(330/2217)</td>
<td>(124/2155)</td>
<td>(0/2153)</td>
</tr>
</tbody>
</table>

Table 4. The effects of *hsf1* alleles on [*PSI*⁺] variant formation

<table>
<thead>
<tr>
<th></th>
<th><em>wt-HSF</em></th>
<th>ΔNTA-HSF</th>
<th>ΔCTA-HSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>[<em>PSI</em>⁺]⁰</td>
<td>34 (55±10%)</td>
<td>29 (91±8%)</td>
<td>2 (3±3%)</td>
</tr>
<tr>
<td>[<em>PSI</em>⁺]⁰</td>
<td>28 (45±10%)</td>
<td>3 (9±8%)</td>
<td>0</td>
</tr>
<tr>
<td>[<em>PSI</em>⁺]⁰</td>
<td>0</td>
<td>0</td>
<td>64 (97±3%)</td>
</tr>
<tr>
<td><strong>Total # examined</strong></td>
<td>62</td>
<td>32</td>
<td>66</td>
</tr>
</tbody>
</table>
Table 5. The effects of *hsf1* alleles on [*PSI*'] variant propagation.

<table>
<thead>
<tr>
<th>cytoductant phenotype</th>
<th>[<em>PSI</em>']&lt;sup&gt;S&lt;/sup&gt;</th>
<th>[<em>PSI</em>']&lt;sup&gt;W&lt;/sup&gt;</th>
<th>[<em>PSI</em>']&lt;sup&gt;U&lt;/sup&gt;</th>
<th>[<em>psi</em>]</th>
<th>(donor)</th>
<th>(recipient)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[wt-&lt;br&gt;HSF]</td>
<td>[ΔNTA-&lt;br&gt;HSF]</td>
<td>[ΔCTA-&lt;br&gt;HSF]</td>
<td>[wt-&lt;br&gt;HSF]</td>
<td>[ΔNTA-&lt;br&gt;HSF]</td>
<td>[ΔCTA-&lt;br&gt;HSF]</td>
</tr>
<tr>
<td>[<em>PSI</em>']&lt;sup&gt;S&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>18</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>[<em>PSI</em>']&lt;sup&gt;W&lt;/sup&gt;</td>
<td>15</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>[<em>PSI</em>']&lt;sup&gt;U&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>23</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>[<em>psi</em>]</td>
<td>0</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td># of cytoductant examined</td>
<td>15</td>
<td>38</td>
<td>23</td>
<td>18</td>
<td>14</td>
<td>13</td>
</tr>
</tbody>
</table>
**Figure 1.**

Diagram showing the HSF1 promoter with three regions: NTA, DB, TD, and CTA. The regions are labeled as follows:
- **wt-HSF**: Full promoter with all regions.
- **ΔCTA-HSF**: Promoter without the CTA region.
- **ΔNTA-HSF**: Promoter without the NTA region.

In the center of the diagram, there is a circle labeled hsf1, indicating the location of the hsf1 gene.

The diagram also shows the 74D-694 ([psi-][RNQ']) strain.

**Figure 2**

**A**

<table>
<thead>
<tr>
<th></th>
<th>74D-694</th>
<th>wt-HSF</th>
<th>ΔNTA-HSF</th>
<th>ΔCTA-HSF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T</strong></td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td><strong>S</strong></td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
</tbody>
</table>

A gel image showing the effect of GdnHCl (-GdnHCl, +GdnHCl).

**B**

Images of colonies of strains with different promoters and GFP expression:
- **wt-HSF**, **ΔNTA-HSF**, **ΔCTA-HSF**
- Strains include: GFP, Rnq1QGFP, Sup35GFP, GFP, Sup35GFP, NMGFP, GFP, NMGFP, GFP, NMGFP

**C**

A gel image showing the protein expression of Sup35 and NMGFP.

**D**

Images showing the growth of ΔNTA-HSF strains after 5 and 10 days on SD-ade medium.
Figure 3

A 74D-694 (hsf1::Kan^R, [psi][RNQ^])

<table>
<thead>
<tr>
<th></th>
<th>GFP</th>
<th>NMGFP</th>
<th>Rnq1GFP</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt-HSF</td>
<td><img src="wt-HSF" alt="Image" /></td>
<td><img src="wt-HSF" alt="Image" /></td>
<td><img src="wt-HSF" alt="Image" /></td>
</tr>
<tr>
<td>ΔNTA-HSF</td>
<td><img src="%CE%94NTA-HSF" alt="Image" /></td>
<td><img src="%CE%94NTA-HSF" alt="Image" /></td>
<td><img src="%CE%94NTA-HSF" alt="Image" /></td>
</tr>
<tr>
<td>ΔCTA-HSF</td>
<td><img src="%CE%94CTA-HSF" alt="Image" /></td>
<td><img src="%CE%94CTA-HSF" alt="Image" /></td>
<td><img src="%CE%94CTA-HSF" alt="Image" /></td>
</tr>
</tbody>
</table>

B 74D-694 (hsf1::Kan^R, [psi][rnq])

<table>
<thead>
<tr>
<th></th>
<th>GFP</th>
<th>NMGFP</th>
<th>Rnq1GFP</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt-HSF</td>
<td><img src="wt-HSF" alt="Image" /></td>
<td><img src="wt-HSF" alt="Image" /></td>
<td><img src="wt-HSF" alt="Image" /></td>
</tr>
<tr>
<td>ΔNTA-HSF</td>
<td><img src="%CE%94NTA-HSF" alt="Image" /></td>
<td><img src="%CE%94NTA-HSF" alt="Image" /></td>
<td><img src="%CE%94NTA-HSF" alt="Image" /></td>
</tr>
<tr>
<td>ΔCTA-HSF</td>
<td><img src="%CE%94CTA-HSF" alt="Image" /></td>
<td><img src="%CE%94CTA-HSF" alt="Image" /></td>
<td><img src="%CE%94CTA-HSF" alt="Image" /></td>
</tr>
</tbody>
</table>
C

74D-694 (hsf1::KanR, [psi][RNQ^+])

<table>
<thead>
<tr>
<th></th>
<th>Q25</th>
<th>Q47</th>
<th>Q72</th>
<th>Q103</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt-HSF</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>ΔNTA-HSF</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
<tr>
<td>ΔCTA-HSF</td>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Cell with foci %

- wt-HSF
- ΔNTA-HSF
- ΔCTA-HSF

bars: Q25, Q47, Q72, Q103
Figure 4

A

wt-HSF  ΔNTA-HSF  ΔCTA-HSF

B

74D-674  hsf1::Kan^R

<table>
<thead>
<tr>
<th></th>
<th>wt-HSF</th>
<th>ΔNTA-HSF</th>
<th>ΔCTA-HSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>74D-674</td>
<td><img src="wild-type.png" alt="Image of wild-type HSF" /></td>
<td><img src="DeltaNTA.png" alt="Image of ΔNTA-HSF" /></td>
<td><img src="DeltaCTA.png" alt="Image of ΔCTA-HSF" /></td>
</tr>
</tbody>
</table>

C

[PSI]^U  ΔCTA-HSF
Figure 5

A

<table>
<thead>
<tr>
<th>Donor</th>
<th>Cytoductants</th>
</tr>
</thead>
<tbody>
<tr>
<td>[PSI⁺]S</td>
<td>wt-HSF</td>
</tr>
<tr>
<td>[PSI⁺]W</td>
<td>wt-HSF</td>
</tr>
</tbody>
</table>

B

\[ \Delta_{CTA}[PSI⁺]^U \times wt[psi] \]

[2n] [PSI⁺]W

Spores


Streaking

\[ \Delta_{CTA}[PSI⁺]^U \]

\[ wt[PSI⁺]^W \]
Figure 6

A

<table>
<thead>
<tr>
<th></th>
<th>wt-HSF</th>
<th>ΔNTA-HSF</th>
<th>ΔCTA-HSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hsp104</td>
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<tr>
<td>Hsp70</td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
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<tr>
<td>Hsp90</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
</tr>
<tr>
<td>Rnq1</td>
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<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
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</tbody>
</table>

B

<table>
<thead>
<tr>
<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
</tr>
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<tbody>
<tr>
<td><img src="image13.png" alt="Image" /></td>
<td><img src="image14.png" alt="Image" /></td>
<td><img src="image15.png" alt="Image" /></td>
<td><img src="image16.png" alt="Image" /></td>
<td><img src="image17.png" alt="Image" /></td>
</tr>
</tbody>
</table>

early log phase
stationary phase