

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

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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 *hsf1* 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 *hsf1* mutants preferentially support the formation of certain [*PSI*⁺] strains, they are capable of receiving and faithfully propagating nonpreferable 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.

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, PrP^C, changes conformation to adopt a pathogenic or prion isoform, PrP^{Sc}. Remarkably, PrP^{Sc} can self-propagate by converting additional PrP^C 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 PrP^{Sc} (for reviews see LIEBMAN and DERKATCH 1999; UPTAIN and LINDQUIST 2002; CHIEN *et al.* 2004; WICKNER *et al.* 2004; JONES and TUIITE 2005). 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” (DERKATCH *et al.* 1996; SCHLUMBERGER *et al.* 2001; BRADLEY and LIEBMAN 2003). 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 homolog 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* (COX 1965; FIROOZAN

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et al. 1991). 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 (KING and DIAZ-AVALOS 2004; LEGNAME *et al.* 2004; TANAKA *et al.* 2004; CASTILLA *et al.* 2005; LIEBMAN 2005; LIEBMAN and MASTRIANNI 2005), the molecular mechanisms underlying the prion conformational switch and strain determination remain unknown. 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 disaggregating 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 (CHERNOFF *et al.* 1995b; JUNG *et al.* 2000; KUSHNIROV *et al.* 2000; JUNG and MASISON 2001; SONDEHEIMER *et al.* 2001; ALLEN *et al.* 2005). Hsp90 cochaperones Sti1 and Cpr7 were also shown to influence [*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 a functionally redundant homolog(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 prion formation 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 homotrimerization, followed by a transcription activation domain that is also conserved (WU 1995; LITTLEFIELD and NELSON 2001). 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, residues 1–147) and a carboxyl-terminal activation domain (CTA, residues 584–833), which are thought to differentially regulate the expression of HSF target genes (SORGER 1990; CHEN and PARKER 2002). Recently, a genomewide analysis using chromatin immunoprecipitation (CHIP)-based DNA microarray technology identified ~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, Δ NTA-*HSF* (147–833) and Δ CTA-*HSF* (1–584). We report here that both HSF truncation mutants have profound and distinct effects on *de novo* formation as well as strain determination of the yeast prion [*PSI*⁺]. We have also shown that cellular factors essential for maintaining the yeast prion [*PSI*⁺] can be differentially expressed in different *hsf1* mutants. 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' TTGTTCCCGGGATGAATA ATGCTGCA 3' as the forward primer, and 5' TTGCCCTGA ATTCTATTTCTTAGC 3' as the reverse primer. After digestion with *Sma*I and *Eco*RI, the PCR fragment was ligated to pRS416GPD (*URA3*) that was predigested with *Sma*I/*Eco*RI. To create the *hsf1* disruption construct, we applied a PCR-based method using pFA6a-kanMX6 (*Kan*^R) as the template and two primers, *HSF1* F1 5' GAAACAAAAAGACAAAAAGA CAGCTGTATTGTTGGCGCCCGGATCCCCGGGTTAATTAA 3' and *HSF1* R1 5' AAATGATTATATACGCTATTTAATGAC CTTGCCCTGTGTAGAATTGCGAGCTCGTTTAAAC 3', to obtain a PCR product containing the *Kan*^R marker flanked with *HSF1* sequence. To create pRS305-HSP104 (*LEU2*), pYS104 (*URA3*) was digested with *Xho*I and the resulting *Xho*I-*Xho*I fragment containing the *HSP104* coding region as well as its flanking regions was ligated to pRS305 (*LEU2*) that was predigested with *Xho*I. To create pRS415-HSP104, pYS104 was digested with *Clal* and blunt ended by DNA polymerase I treatment followed by *Xho*I digestion. The resulting *Clal* (blunt-ended)-*Xho*I fragment containing HSP104 coding sequence and its flanking regions was ligated to p415GPD that was predigested with *Sac*I (blunt ended by DNA polymerase I treatment) and *Xho*I (the GPD promoter of p415GPD was thus removed). To create p425GPD-HSP104 (*LEU2*), p2HG-104 (*HIS3*) (LI and LINDQUIST 2000) was digested with *Bam*HI and the resulting *Bam*HI-*Bam*HI fragment containing the HSP104 coding region was ligated to p425GPD (*LEU2*) that was predigested with *Bam*HI.

Yeast strains: Yeast strains are listed in Table 2. To obtain the *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 the *Kan*^R marker flanked by *HSF1* sequence as described above through “one-step”

TABLE 1
Plasmids used in this study

Plasmid	Auxotrophic marker	Promoter	Copy number	Source
pRS416GPD-HSF1	URA3	<i>GPD</i>	<i>CEN</i> , low	This study
pRS314-HSF1	TRP1	<i>HSF1</i>	<i>CEN</i> , low	MORANO <i>et al.</i> (1999)
pRS314-HSF1 Δ NTA(148–833)	TRP1	<i>HSF1</i>	<i>CEN</i> , low	This study
pRS314-HSF1 Δ CTA(1–583)	TRP1	<i>HSF1</i>	<i>CEN</i> , low	MORANO <i>et al.</i> (1999)
pCUP1-GFP	URA3	<i>CUP1</i>	<i>CEN</i> , low	Lindquist lab
pCUP1-Sup35GFP	URA3	<i>CUP1</i>	<i>CEN</i> , low	Lindquist lab
pCUP1-NMGFP	URA3	<i>CUP1</i>	<i>CEN</i> , low	Lindquist lab
pCUP1-Rnq1GFP	URA3	<i>CUP1</i>	<i>CEN</i> , low	SONDHEIMER and LINDQUIST (2000)
pCUP1-Ure2(1–65)-GFP	URA3	<i>CUP1</i>	<i>CEN</i> , low	Lindquist lab
pRS313CUP1-NMGFP	HIS3	<i>CUP1</i>	<i>CEN</i> , low	DERKATCH <i>et al.</i> (2001)
pRS305-HSP104	LEU2	<i>HSP104</i>	Integrating, single	This study
pRS415-HSP104	LEU2	<i>HSP104</i>	<i>CEN</i> , low	This study
p425GPD-HSP104	LEU2	<i>GPD</i>	2 μ , high	This study
P426/PQ25	URA3	<i>GPD</i>	2 μ , high	KROBITSCH and LINDQUIST (2000)
P426/PQ47	URA3	<i>GPD</i>	2 μ , high	KROBITSCH and LINDQUIST (2000)
P426/PQ72	URA3	<i>GPD</i>	2 μ , high	KROBITSCH and LINDQUIST (2000)
P426/PQ103	URA3	<i>GPD</i>	2 μ , high	KROBITSCH and LINDQUIST (2000)

gene replacement (SHERMAN 1991). The resulting *hsf1* disruption strain was named 74D-*hsf1::Kan^R*.

To create isogenic strains of *wt-HSF*, Δ NTA-*HSF*, and Δ CTA-*HSF*, 74D-*hsf1::Kan^R* containing pRS416GPD-HSF1 was transformed with pRS413-*wt-HSF*, pRS413- Δ NTA-*HSF*, or pRS413- Δ CTA-*HSF*. The resulting transformants were grown on media containing 5-fluoroorotic acid to eliminate pRS416GPD-HSF1. The generated isogenic strains, 74D-*wt-HSF*, 74D- Δ NTA-*HSF*, and 74D- Δ CTA-*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 (Figure 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° with shaking. Overnight yeast cultures were diluted into fresh media to a density of OD₆₀₀ = 0.05 and grown to OD₆₀₀ = 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*, Δ NTA-*HSF*, and Δ CTA-*HSF* containing *CEN/URA3* plasmids pCUP1-GFP, pCUP1-NMGFP, or pCUP1-Rnq1GFP were induced by CuSO₄ at a final concentration of 34 μ M. 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 fivefold serial dilution. Ade⁺ colonies 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 guanidine hydrochloride (GdnHCl). Only ade⁺ colonies that were cured by GdnHCl were scored as [PSI⁺]. Sometimes, sequential streaking was carried out to obtain stable [PSI⁺] variants as newly induced [PSI⁺] are often unstable (DERKATCH *et al.* 1996).

Cytoduction: L1976 (c10B-H49 *MAT α* , *SUQ5*, *ade2-1*, *his3-11*, *lys1-1*, *leu1*, *kar1-1*, *cyhR*, [PIN⁺][PSI⁺]^w) and L1977 (c10B-H49 *MAT α* , *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*, Δ NTA-*HSF*, and Δ CTA-*HSF* cells (*MAT α*), respectively. Individual pairs of donors and recipients were mixed in YPD for 5 hr at 30° and spread onto SC (-lys, -his) plates to select for diploids and cytoductants.

TABLE 2
***S. cerevisiae* strains used in this study**

Strain	Genotype description	Source
74D-694	<i>MATα</i> : <i>ade1-14</i> , <i>trp1-289</i> , <i>his3Δ-200</i> , <i>ura3-52</i> , <i>leu2-3</i> , <i>112</i> , [<i>psi</i> ⁻][PIN ⁺]	PATINO <i>et al.</i> (1996)
74D-694- <i>wt-HSF</i>	<i>MATα</i> : <i>ade1-14</i> , <i>trp1-289</i> , <i>his3Δ-200</i> , <i>ura3-52</i> , <i>leu2-3</i> , <i>112</i> , <i>hsf1::KAN^R</i> , [<i>psi</i> ⁻][PIN ⁺], containing pRS314-HSF	This study
74D-694- Δ NTA- <i>HSF</i>	<i>MATα</i> : <i>ade1-14</i> , <i>trp1-289</i> , <i>his3Δ-200</i> , <i>ura3-52</i> , <i>leu2-3</i> , <i>112</i> , <i>hsf1::KAN^R</i> , [<i>psi</i> ⁻][PIN ⁺], containing pRS314-HSF(148–833)	This study
74D-694- Δ CTA- <i>HSF</i>	<i>MATα</i> : <i>ade1-14</i> , <i>trp1-289</i> , <i>his3Δ-200</i> , <i>ura3-52</i> , <i>leu2-3</i> , <i>112</i> , <i>hsf1::KAN^R</i> , [<i>psi</i> ⁻][PIN ⁺], containing pRS314-HSF(1–583)	This study
L1976	<i>MATα</i> : <i>SUQ5</i> , <i>ade2-1</i> , <i>lys1-1</i> , <i>his3-11</i> , <i>15</i> , <i>leu1</i> , <i>kar1-1</i> , <i>cyh^R</i> , [PSI ⁺] ^w [PIN ⁺],	Liebman lab
L1977	<i>MATα</i> : <i>SUQ5</i> , <i>ade2-1</i> , <i>lys1-1</i> , <i>his3-11</i> , <i>15</i> , <i>leu1</i> , <i>kar1-1</i> , <i>cyh^R</i> , [PSI ⁺] ^s [PIN ⁺],	Liebman lab

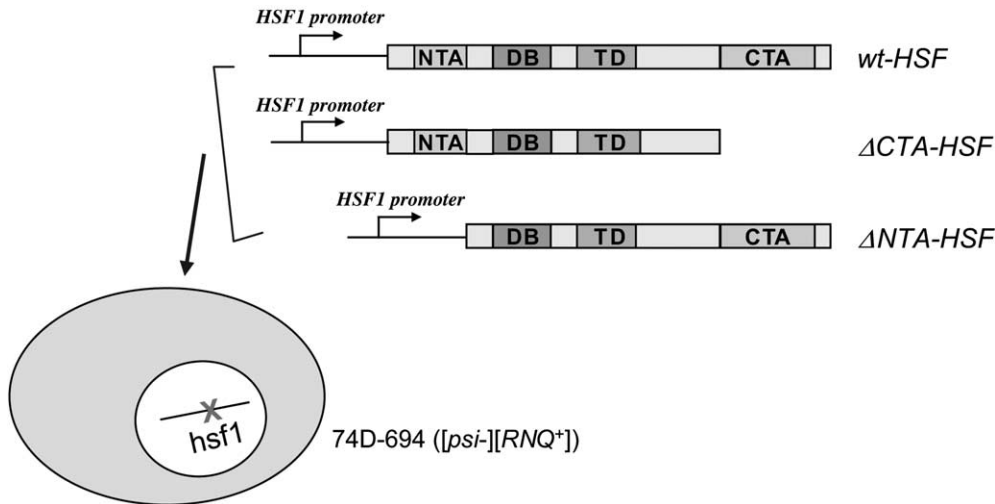


FIGURE 1.—Creating isogenic Δ NTA-HSF, Δ CTA-HSF, and *wt*-HSF cells in strain 74D-694. A *CEN* plasmid, p416GPD-HSF, expressing *wt* HSF1 with a *URA3* marker was transformed into a 74D-694 strain ($[psi^-][RNQ^+]$), whose chromosomal *HSF1* was subsequently disrupted with a *Kan^R* gene through one-step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

Colonies grown on SC (–lys, –his) plates were transferred onto a 5-FOA plate, which selects against the donor and diploid cells. Cytoductants were confirmed by a mating-type test and by the presence of the recipient's auxotrophic nuclear markers, for example, the *LEU2*.

RESULTS

The HSF truncation mutants, Δ NTA-HSF and Δ CTA-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 $[PSI^+]$ inducibility ($[PIN^+]$) (DERKATCH *et al.* 2001). Since *HSF* is an essential gene, a *CEN* plasmid, pRS314-*wt*-HSF, pRS314- Δ NTA-HSF, or pRS314- Δ CTA-HSF was maintained in the *hsf1* disruption strain to give isogenic strains expressing *wt*-HSF, Δ NTA-HSF, or Δ CTA-HSF, respectively (Figure 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 $[rnq^-]$ cells (SONDHEIMER and LINDQUIST 2000). The difference in Rnq1 conformation in $[RNQ^+]$ and $[rnq^-]$ 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 Δ NTA-HSF, Δ CTA-HSF, and *wt*-HSF cells (Figure 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 concentra-

tion of GdnHCl (TUIITE *et al.* 1981). After GdnHCl treatment, Rnq1 was found in the soluble fractions (Figure 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 $\sim 10^{-6}$ (TUIITE *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 1000-fold (TER-AVANESYAN *et al.* 1994). To determine if Δ NTA-HSF and Δ CTA-HSF affect the *de novo* formation of $[PSI^+]$, isogenic Δ NTA-HSF, Δ CTA-HSF, and *wt*-HSF cells were transformed with pCUP1-Sup35GFP or pCUP1-NMGFP, plasmids expressing GFP fusions of full-length *SUP35* or *SUP35NM* (the N-terminal and middle regions of Sup35), respectively. Freshly transformed cells were grown in SC-ura media and induced to form $[PSI^+]$ by $CuSO_4$ addition. After 4 hr, cells were spotted onto SC-ade plates to select for translation termination suppressors, indicative of potential $[PSI^+]$ cells. As shown in Figure 2B and Table 3, upon Sup35GFP overexpression, the number of *ade⁺* colonies formed by Δ CTA-HSF cells is approximately 7-fold more than that of isogenic *wt*-HSF cells, suggesting that the absence of the HSF-CTA promotes $[PSI^+]$ *de novo* formation. When fresh Δ NTA-HSF transformants containing pCUP1-SUP35GFP were induced under identical conditions, $[PSI^+]$ formation was inhibited (Figure 2B and Table 3). As expected, control cells containing pCUP1-Rnq1GFP or pCUP1-GFP did not give rise to *ade⁺* colonies (Figure 2B and Table 3). Upon NMGFP overexpression, $[PSI^+]$ induction was also inhibited in Δ NTA-HSF cells but the $[PSI^+]$ promoting effect of Δ CTA-HSF was lessened to

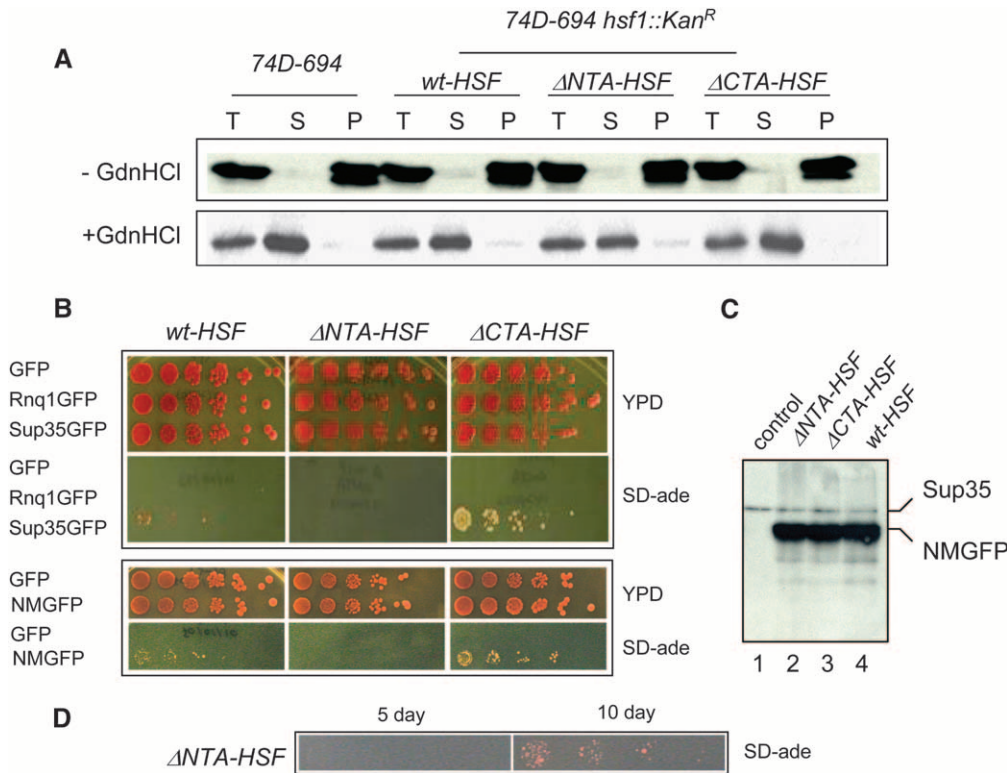


FIGURE 2.—The truncation mutants of *hsf1*, Δ NTA-*HSF* and Δ CTA-*HSF*, have no effect on preexisting $[RNQ^+]$ but dramatically influence the *de novo* formation of $[PSI^+]$. (A) $[RNQ^+]$ sedimentation assay of isogenic strains of Δ NTA-*HSF*, Δ CTA-*HSF*, and *wt-HSF*, which are derived from a 74D-694 ($[psi^-][RNQ^+]$) strain (see MATERIALS AND METHODS and Figure 1). 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 5 mM GdnHCl (+GdnHCl). (B) *De novo* formation of $[PSI^+]$ in isogenic Δ NTA-*HSF*, Δ 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-Rnq1GFP. Fresh transformants were grown in SC-ura media to early log phase before addition of $CuSO_4$ (final concentration, $34 \mu M$). After a 4-hr induction, cells were spotted onto either YPD or SC-ade in a fivefold dilution series. Pictures were taken after cells were grown for 3 days (YPD) or 5 days on SC-ade at 30° . (C) Immunoblot analysis of isogenic Δ NTA-*HSF*, Δ CTA-*HSF*, and *wt-HSF* cells after a 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. As shown: lanes 2, 3, and 4 are Δ NTA-*HSF*, Δ CTA-*HSF*, and *wt-HSF*, respectively. Lane 1 is a *wt* cell containing expression vector as a control. (D) Δ NTA-*HSF* cells were able to induce $[PSI^+]$ but with slower kinetics. The same Δ NTA-*HSF* transformant used for B was reassayed for its ability to induce $[PSI^+]$ after storage at 4° for 14 days. $[PSI^+]$ induction was carried out under identical conditions as described in B. The pictures were taken after 5 and 10 days of incubation at 30° on SC-ade.

~ 2.5 -fold when compared to $[PSI^+]$ induction in *wt-HSF* (Figure 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 (TAMAI *et al.* 1994; HAHN *et al.* 2004), the observed differences in $[PSI^+]$ induction by *hsf1* alleles could have resulted from different expression levels of NMGFP or Sup35GFP (TAMAI *et al.* 1994; HAHN *et al.* 2004). To test this possibility, we carried out immu-

noblot 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-hr induction. Comparable intensities of the endogenous Sup35 band (the slower migrating band) in each lane confirmed the equal loading (Figure 2C). The expression levels of Sup35GFP are also similar in these *hsf1* alleles (data not shown). These results demonstrate that the observed differences

TABLE 3

The effects of *hsf1* alleles on $[PSI^+]$ *de novo* formation

		$[PSI^+]$ appearance %			
		GFP	Sup35GFP	NMGFP	Rnq1GFP
74D-694	<i>wt-HSF</i>	<0.1	2.1 ± 0.9	2.5 ± 0.6	<0.1
		(0/2242)	(48/2353)	(50/2020)	(0/2569)
	Δ NTA- <i>HSF</i>	<0.1	<0.1	<0.1	<0.1
		(0/2387)	(0/2278)	(0/1905)	(0/2302)
	Δ CTA- <i>HSF</i>	<0.1	15.4 ± 3.1	6.1 ± 2.0	<0.1
		(0/2206)	(330/2217)	(124/2155)	(0/2153)

in $[PSI^+]$ *de novo* formation in *wt-HSF*, $\Delta NTA-HSF$, and $\Delta CTA-HSF$ cells are not due to differences in NMGFP or Sup35GFP expression levels.

Although $[PSI^+]$ is nearly undetectable after 5 days at 30° for fresh $\Delta NTA-HSF$ transformants upon 4 hr Sup35 overexpression, $[PSI^+]$ formation can be detected upon a longer incubation period (data not shown). $\Delta NTA-HSF$ transformants stored for an extended period (>10 days) also give rise to $[PSI^+]$ with faster kinetics upon induction of Sup35 overexpression (Figure 2D). Presumably, the leaky *CUP1* promoter, which is also induced in response to glucose deprivation (TAMAI *et al.* 1994; HAHN *et al.* 2004), allowed weak but constitutive expression of Sup35GFP and NMGFP. These observations suggest that $[PSI^+]$ *de novo* formation in $\Delta NTA-HSF$ 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 $\Delta NTA-HSF$, $\Delta CTA-HSF$, and *wt-HSF* cells. As shown in Figure 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 $\Delta NTA-HSF$ or the stimulatory effect of $\Delta CTA-HSF$ 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 poly(Q) aggregation: Consistent with our observation for $[PSI^+]$ induction, we observed that deletion of HSF NTA or CTA has a profound effect on Sup35 aggregation. We examined Sup35NMGFP fluorescent foci formation in isogenic *hsf1* strains that are $[psi^-][RNQ^+]$. As shown in Figure 3A, fresh $\Delta NTA-HSF$ transformants containing pCUP1-NMGFP were unable to form detectable fluorescent foci after a 4-hr $CuSO_4$ addition. Approximately 7% of *wt-HSF* cells exhibited fluorescent foci after a 4-hr induction, similar to previously reported results (PATINO *et al.* 1996). However, $\Delta CTA-HSF$ cells formed approximately three times more foci than did *wt-HSF* cells (Figure 3A). For isogenic *hsf1* alleles in $[psi^-][rnq^-]$ strains (after GdnHCl treatment), Sup35NMGFP exhibited minimal fluorescent foci in all cell types (Figure 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, Rnq1GFP (a GFP fusion containing the Rnq1 prion domain) formed fluorescent foci with a similar pattern of multiple dots (m.d.) (BRADLEY *et al.* 2002) in isogenic $\Delta NTA-HSF$, $\Delta CTA-HSF$, and *wt-HSF* cells that were derived from $[psi^-][RNQ^+]$, confirming their $[RNQ^+]$ status (Figure 3A). To examine *de novo* aggregation of Rnq1, we subjected cells ($[psi^-][RNQ^+]$) containing pCUP1-Rnq1GFP as described in Figure 3A to GdnHCl treatment to eliminate $[RNQ^+]$. After GdnHCl treat-

ment, only a diffuse Rnq1GFP fluorescent pattern was seen for all *hsf1* alleles upon a 0.5-hr induction, confirming that $[RNQ^+]$ was cured (data not shown). After a 4-hr induction, Rnq1GFP fluorescent patterns were strikingly different. $\Delta CTA-HSF$ cells formed about three times more fluorescent foci (21% of 378 cells) than did *wt-HSF* cells (7.5% of 349 cells). Rnq1GFP foci in $\Delta NTA-HSF$ cells were almost undetectable (Figure 3B). Our results demonstrate that the *hsf1* alleles have similar effects on the aggregation of both Sup35 and Rnq1.

To test if the *hsf1* alleles specifically affect prion proteins, we examined the aggregation of Huntingtin protein, which contains a poly(Q) tract with variable lengths among different individuals. Poly(Q) length is tightly associated with the etiology of Huntington's disease: the longer the tract is, the worse the symptoms are, and the earlier the onset of the disease is (ANDREW *et al.* 1993; DUYAO *et al.* 1993; SNELL *et al.* 1993). There are at least nine poly(Q)-associated neurodegenerative diseases, including Huntington's disease (WALSH *et al.* 2005). Yeast has been a useful model for studying the aggregation and toxicity of poly(Q) proteins (KROBITSCH and LINDQUIST 2000; OSHEROVICH and WEISSMAN 2001; MERIIN *et al.* 2002; DERKATCH *et al.* 2004; GOKHALE *et al.* 2005). Huntingtin exon-1, with a long poly(Q) 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 $\Delta NTA-HSF$, $\Delta CTA-HSF$, and *wt-HSF* cells with a set of high-copy GPD plasmids containing Huntingtin exon-1-GFP with variable poly(Q) lengths: Q25, Q47, Q72, and Q103, respectively (KROBITSCH and LINDQUIST 2000). As shown in Figure 3C, Q47-GFP formed no detectable aggregates in *wt-HSF* cells, in agreement with a previous report (KROBITSCH and LINDQUIST 2000). However, the formation of Q47-GFP aggregates was obvious in $\Delta CTA-HSF$ cells (Figure 3C). For Huntingtin-Q72, ~30% of $\Delta CTA-HSF$ cells (92/296) formed fluorescent foci compared to 15% of *wt-HSF* cells (56/428) and none in $\Delta NTA-HSF$ cells (0/392). For Q103, fluorescent foci were seen in 27% of *wt-HSF* cells (81/286), in ~53% of $\Delta CTA-HSF$ cells (175/325), but only in ~10% of $\Delta NTA-HSF$ cells (33/325) (Figure 3C). Thus, the effects of the distinct *hsf1* alleles on protein aggregation are not specific to prion proteins.

The deletion of the N-terminal or C-terminal domain of HSF differentially affects $[PSI^+]$ variant formation: The dramatic effect of *hsf1* alleles on $[PSI^+]$ *de novo* formation encouraged us to examine whether they also influence $[PSI^+]$ variant establishment. Overexpression of the Sup35 prion domain in wild-type (wt) cells typically gives rise to $[PSI^+]$ variants ranging from very weak to very strong (DERKATCH *et al.* 1996). This is also the case when we examined the induced $[PSI^+]$ in *wt-HSF* cells upon NMGFP overexpression (Figure 4, A and B). Although $\Delta CTA-HSF$ significantly increased the *de novo*

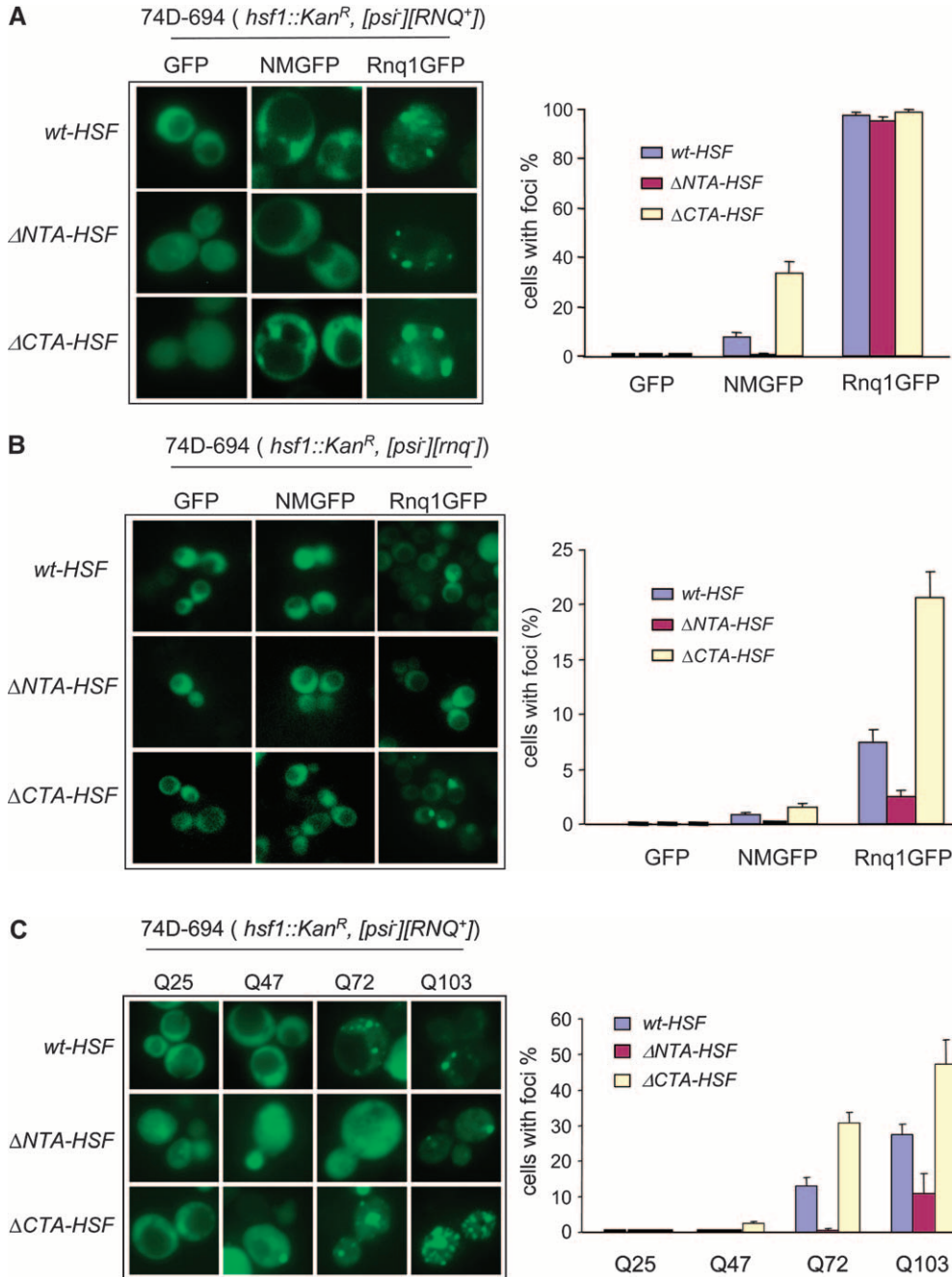


FIGURE 3.—Fluorescent microscopic assay of NMGFP, Rnq1-GFP, and poly(Q)-GFP aggregation. Isogenic Δ NTA-*HSF*, Δ CTA-*HSF*, and *wt-HSF* cells were transformed, respectively, with pCUP1-GFP (vector control), pCUP1-NMGFP, pCUP1-Rnq1GFP, or p426-GPD-based poly(Q)-GFP expression plasmids varying in Q-lengths (KROBITSCH and LINDQUIST 2000). (A) Visualization of NMGFP and Rnq1GFP aggregations in yeast cells (*[psi⁻][RNQ⁺]*) with specific *hsf1* alleles indicated (left) and a quantitative summary of three independent experiments of NMGFP and Rnq1GFP aggregation (right). (B) Fluorescent microscopic assay of prion protein aggregation in *[psi⁻][rnq⁻]* cells with different *hsf1* alleles. Cells from A were treated with 5 mM GdnHCl to eliminate *[RNQ⁺]*. Cells containing the respective plasmids were grown in SC-Ura to early log phase and induced to express GFP fusions by addition of CuSO_4 (final concentration, 34 μM). Data shown were acquired after a 4-hr induction. (C) Visualization of poly(Q)-GFP aggregation in isogenic yeast cells of *wt-HSF*, Δ NTA-*HSF*, and Δ CTA-*HSF* (top) and a quantitative summary of three independent experiments of poly(Q)-GFP aggregation (bottom).

appearance of $[\text{PSI}^+]$, we were surprised to find that almost all *ade⁺* colonies of Δ CTA-*HSF* cells appeared as very weak $[\text{PSI}^+]$ upon streaking on YPD (Figure 4A, right). Detailed examination revealed unique mosaic colony morphology associated with $[\text{PSI}^+]$ induced in Δ CTA-*HSF* cells (Figure 4, B and C). Upon restreaking, these mosaic colonies gave rise to a low frequency of *[psi⁻]* cells ($\sim 15\%$) and $[\text{PSI}^+]^s$ cells ($\sim 1\%$). The majority of them retain their mosaic feature (Figure 4, B and C). Intriguingly, $[\text{PSI}^+]^w$ was not observed. Once they become *[psi⁻]* or $[\text{PSI}^+]^s$, they can be stably transmitted as *[psi⁻]* or $[\text{PSI}^+]^s$. We named those mosaic $[\text{PSI}^+]$ induced in Δ CTA-*HSF* cells $[\text{PSI}^+]^u$ because of their “unstable” and “undifferentiated” features. In contrast to the preference

of forming $[\text{PSI}^+]^u$ in Δ CTA-*HSF* cells, almost all *ade⁺* colonies formed in Δ NTA-*HSF* cells exhibited a uniform $[\text{PSI}^+]$ strength. They are apparently pinker than the control $[\text{PSI}^+]^s$ [Figure 4, A (middle) and B]. Table 4 summarizes $[\text{PSI}^+]$ induction results from three consecutive experiments. Although *wt-HSF* gave rise to mixed $[\text{PSI}^+]$ variants, 91% of $[\text{PSI}^+]$ derived from Δ NTA-*HSF* cells were $[\text{PSI}^+]^s$ and 97% of $[\text{PSI}^+]$ induced from Δ CTA-*HSF* cells are $[\text{PSI}^+]^u$. Thus, specific $[\text{PSI}^+]$ variants are preferably formed in different *hsf1* mutant strains.

The effect of N-terminal or C-terminal domain deletion of HSF on $[\text{PSI}^+]$ propagation: We next investigated if the *hsf1* alleles are able to receive and propagate nonpreferred $[\text{PSI}^+]$ variants. We carried out

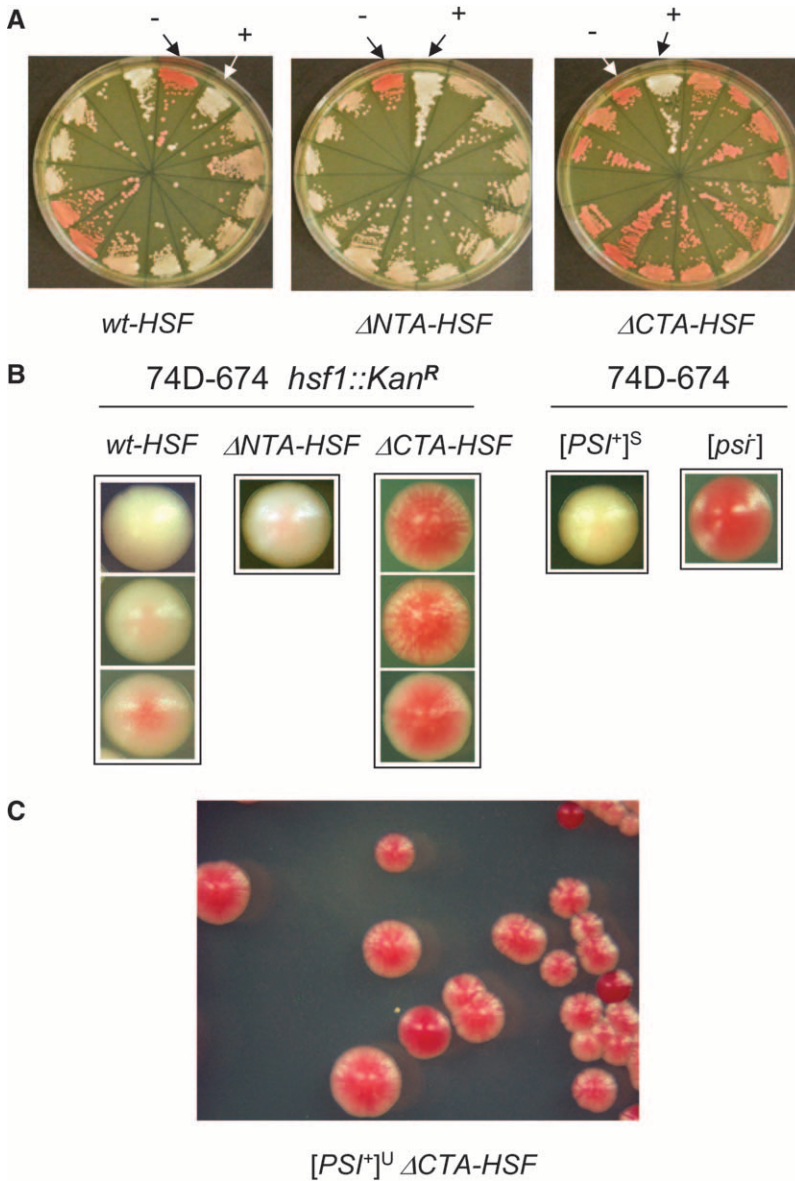


FIGURE 4.— $[PSI^+]$ induced in isogenic Δ *NTA-HSF*, Δ *CTA-HSF*, and *wt-HSF* cells exhibits distinct phenotypes. (A) Individual *ade*⁺ colonies of Δ *NTA-HSF*, Δ *CTA-HSF*, and *wt-HSF* formed by overexpression of Sup35NMGFP (see Figure 2) were randomly picked and streaked onto YPD plates to show 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 a Δ *CTA-HSF* background.

cytoduction experiments using $[PSI^+]^S$ and $[PSI^+]^W$ as donors and isogenic *wt-HSF*, Δ *NTA-HSF*, and Δ *CTA-HSF* $[psi^-]$ cells as recipients. Cytoduction allows mating partners to share their cytoplasm but not their nuclear components (CONDE 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 (UPTAIN and LINDQUIST 2002). When $[PSI^+]^S$ was used as the donor and isogenic $[psi^-]$ cells of Δ *NTA-HSF*, Δ *CTA-HSF*, and *wt-HSF* were used as recipients, cytoductants in all cell types were $[PSI^+]^S$ (Figure 5), indicating that deletion of the HSF NTA or CTA does not affect the ability of cells to faithfully receive and propagate $[PSI^+]^S$. When $[PSI^+]^W$ was the donor, *wt-HSF* and Δ *NTA-HSF* cells were able to receive and faithfully maintain $[PSI^+]^W$ whereas Δ *CTA-HSF* cytoductants of

$[PSI^+]^W$ exhibited similar colony morphology to that of $[PSI^+]^U$ formed *de novo* in Δ *CTA-HSF* cells. They are mosaic and sectored (compare the enlarged cell streak in Figures 4B and 5). Their mosaic feature can be stably maintained upon multiple sequential streaking and even through plasmid transformation procedures.

TABLE 4

The effects of *hsf1* alleles on $[PSI^+]$ variant formation

	<i>wt-HSF</i> (%)	Δ <i>NTA-HSF</i> (%)	Δ <i>CTA-HSF</i> (%)
$[PSI^+]^S$	34 (55 ± 10)	29 (91 ± 8)	2 (3 ± 3)
$[PSI^+]^W$	28 (45 ± 10)	3 (9 ± 8)	0
$[PSI^+]^U$	0	0	64 (97 ± 3)
Total no. examined	62	32	66

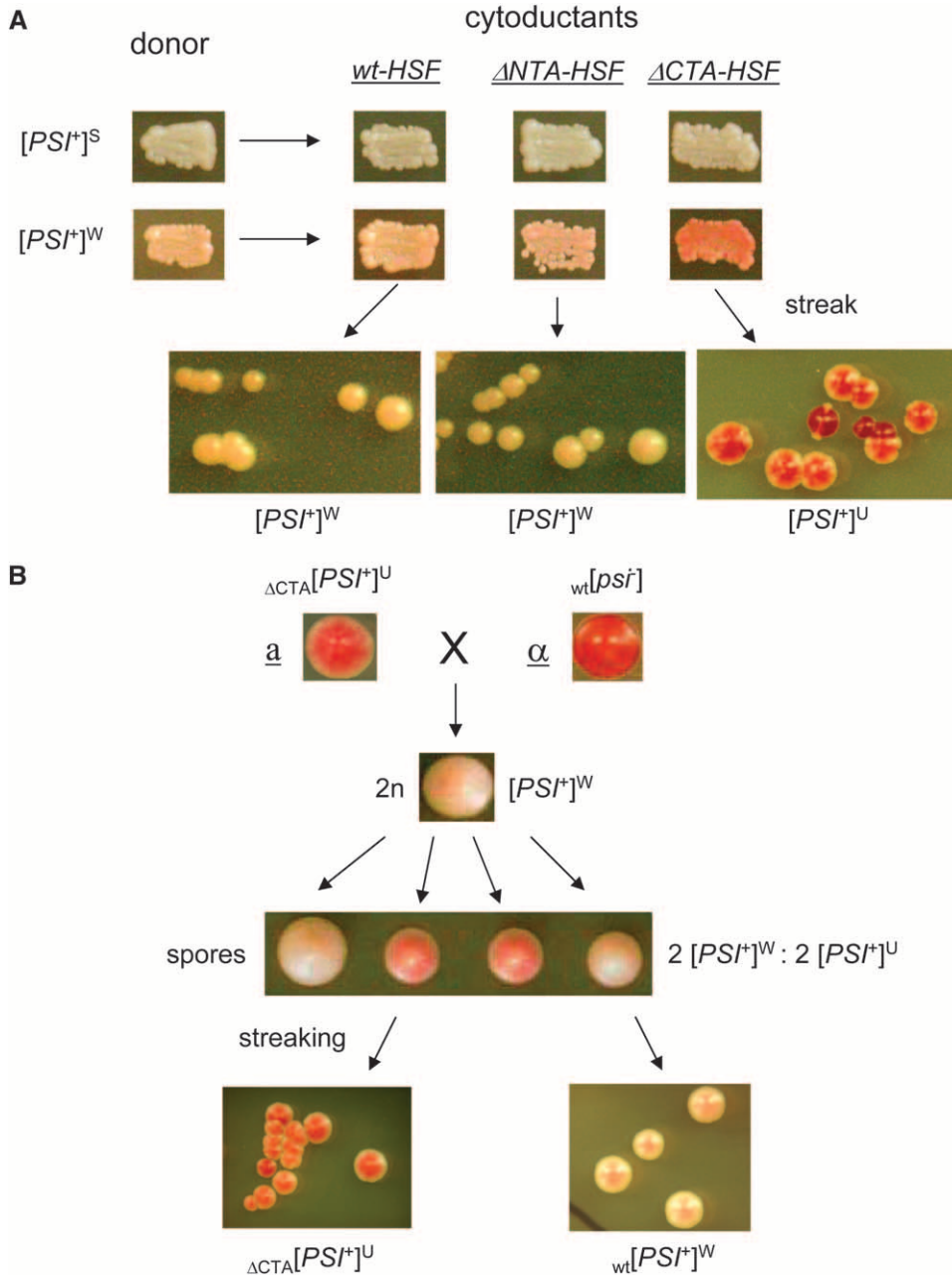


FIGURE 5.—The transmissibility of preexisting $[PSI^+]$ to *hsf1* mutants. (A) Cytoduction: α -cells (c10B-H49) of $[PSI^+]^S$ and $[PSI^+]^W$ containing a *kar-1* mutation (gifts of Susan Liebman) were used as donors to cytoduce isogenic α -cells of Δ *NTA-HSF*, Δ *CTA-HSF*, and *wt-HSF* (74D-694) that are $[psi^-]$. Cytoduction was carried out as described in MATERIALS AND METHODS. Cytoductants were verified by the presence of the recipient's nuclear marker (LEU2) and mating type, **a**. 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 that Δ *CTA-HSF* cytoductants of $[PSI^+]^W$ exhibit $[PSI^+]^U$ phenotype (see the enlarged cell streak), identical to the *de novo* formed $[PSI^+]$ in Δ *CTA-HSF* cells upon overexpression of Sup35NMGFP. (B) Mating and sporulation: wild-type α -cells (74D-694 [*psi*⁻][*rnq*⁻]) were crossed with Δ *CTA-HSF* α -cells that are $[PSI^+]^U$ [pRS305-HSF(1–583) was integrated at *leu2* locus]. The diploids were picked and incubated at 30° for 4 days on YPD plates before being subjected to sporulation (30° for 7 days on a sporulation plate). The tetrads were dissected and incubated at 30° for 4 days before streaking. The genome typing of spores was done by testing their ability to grow at 37° since *wt-HSF* cells are viable whereas Δ *CTA-HSF* cells are not at 37° (MORANO *et al.* 1999).

Table 5 summarizes results from three independent cytoduction experiments. All cytoductants of $[PSI^+]^S$ were $[PSI^+]^S$, confirming that all *hsf1* alleles are capable of receiving and propagating $[PSI^+]^S$. Approximately 45% Δ *NTA-HSF* cytoductants of $[PSI^+]^W$ were $[psi^-]$, indicating a reduced ability of Δ *NTA-HSF* cells to receive and propagate $[PSI^+]^W$. The fact that Δ *CTA-HSF* cytoductants of $[PSI^+]^W$ displayed the same colony morphology as that of $[PSI^+]^U$ *de novo* formed in Δ *CTA-HSF* cells suggests that $[PSI^+]^U$ is the same as $[PSI^+]^W$ but with a unique phenotype in Δ *CTA-HSF* cells. To test this possibility, we crossed $[PSI^+]^U$ of Δ *CTA-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 (Figure 5B). Upon sporulation, a 2:2 ratio of stable $[PSI^+]^W$ (*wt*) to $[PSI^+]^U$ (Δ *CTA-HSF*) was obtained (Figure 5B). Upon streaking, the Δ *CTA-HSF* spores have the same colony morphology as that of $[PSI^+]^U$ (Figure 5B). Thus, our results demonstrate that the Δ *CTA-HSF* genetic background cannot support a stable maintenance of $[PSI^+]^W$ and confirm that $[PSI^+]^U$ is essentially $[PSI^+]^W$ but with a different readout in Δ *CTA-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 poly(Q) aggregation in *Saccharomyces cerevisiae* (CHERNOFF *et al.* 1995b;

TABLE 5
The effects of *hsf1* alleles on $[PSI^+]$ variant propagation

Cytoductant phenotype	Donor: Recipient:	$[PSI^+]^W$			$[PSI^+]^S$		
		<i>wt-HSF</i>	$\Delta NTA-HSF$	$\Delta CTA-HSF$	<i>wt-HSF</i>	$\Delta NTA-HSF$	$\Delta CTA-HSF$
$[PSI^+]^S$		0	0	0	18	14	13
$[PSI^+]^W$		15	21	0	0	0	0
$[PSI^+]^U$		0	0	23	0	0	0
$[psi^-]$		0	17	0	0	0	0
No. of cytoductants examined		15	38	23	18	14	13

KROBITSCH and LINDQUIST 2000). It has been shown that *HSP104* expression is under the regulation of HSF (HALLADAY and CRAIG 1995; GRABLY *et al.* 2002). 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*, $\Delta NTA-HSF$, and $\Delta CTA-HSF$ cells. As shown in Figure 6A, Hsp104 levels are similar in *wt-HSF* and $\Delta CTA-HSF$ cells. The level of Hsp104 in $\Delta NTA-HSF$ cells, however, is significantly elevated. To estimate the Hsp104 level in $\Delta NTA-HSF$ cells, we carried out a comparative immunoblot analysis using $\Delta NTA-HSF$ cells and isogenic wt cells containing various *HSP104* expression plasmids. As shown in Figure 6B, the amount of Hsp104 in $\Delta NTA-HSF$ cells is slightly more than that in wt cells containing a single-copy integrated plasmid of *HSP104* (lane 3) but less than that in wt cells containing a *CEN* or a multicopy *HSP104* expression plasmid (lanes 4 and 5). This is true for cells harvested at both the early log phase and stationary phase (Figure 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 (Figure 6A) as judged by immunoblot analysis using a monoclonal antibody against *Drosophila* Hsp70 (VELAZQUEZ and LINDQUIST 1984). The expression level of Hsp90 in $\Delta NTA-HSF$ cells is similar to that in *wt-HSF* cells. However, Hsp90 expression is significantly lower in $\Delta CTA-HSF$ cells, as reported previously (MORANO *et al.* 1999) (Figure 6A).

DISCUSSION

We have observed a striking difference in $[PSI^+]$ formation between two truncation mutants of yeast *HSF1*, $\Delta NTA-HSF$ and $\Delta CTA-HSF$: $\Delta CTA-HSF$ stimulates whereas $\Delta NTA-HSF$ inhibits the *de novo* appearance 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 (HARDY *et al.* 2000; CHEN and PARKER 2002). It is possible that the opposite effects of $\Delta NTA-HSF$ and $\Delta CTA-HSF$ on $[PSI^+]$ are due to their differences in perturbing such domain–domain interactions.

Our findings that $\Delta NTA-HSF$ and $\Delta CTA-HSF$ cells have a strikingly different ability with regard to $[PSI^+]$ *de novo*

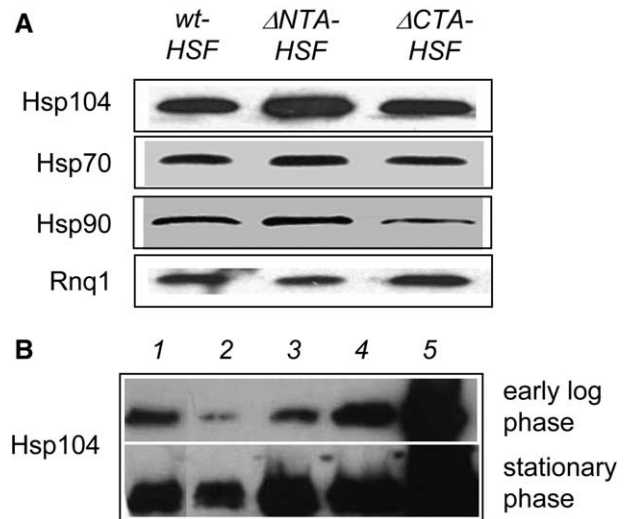


FIGURE 6.—The effect of *hsf1* alleles on the expression of molecular chaperones. (A) Isogenic $\Delta NTA-HSF$, $\Delta CTA-HSF$, and *wt-HSF* cells were grown in YPD to $OD_{600} = 0.5$. Whole cell lysates were prepared and analyzed by SDS–PAGE followed by immunoblot using a polyclonal antibody of 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) $\Delta NTA-HSF$ 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 were gifts from Susan Lindquist's lab).

formation and variant determination suggest that important cellular factors required for prion formation are differently regulated in $\Delta NTA-HSF$ and $\Delta CTA-HSF$ cells. Indeed, we showed that *HSP104* expression is significantly enhanced in $\Delta NTA-HSF$, indicating that the N-terminal activation domain of HSF is inhibitory to *HSP104* expression under nonstress conditions. The upregulation of *HSP104* in $\Delta NTA-HSF$ cells demonstrates that particular mutations in *HSF1* are able to differentially regulate 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 $\Delta NTA-HSF$ 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 the Hsp90 level is significantly reduced in $\Delta CTA-HSF$ (MORANO *et al.* 1999) (Figure 6A). The expression levels of Sse1, a distant Hsp70 family member, and Sti1, an Hsp90 cochaperone, are also significantly lowered in $\Delta CTA-HSF$ cells (MORANO *et al.* 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 us to identify 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) (Figure 4B). These observations suggest that prion strain determinants can be 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 $\Delta NTA-HSF$ prefers $[PSI^+]^S$ *de novo* formation whereas $\Delta CTA-HSF$ selectively gives rise to the $[PSI^+]^U$ 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 might help us to reveal the identities of the variant determinants.

We demonstrated that >90% of $[PSI^+]$ formed in $\Delta NTA-HSF$ cells are $[PSI^+]^S$ whereas >97% are $[PSI^+]^U$ in $\Delta CTA-HSF$ cells (Figure 4 and Table 4). Both truncation mutants were, however, capable of receiving and faithfully propagating preformed $[PSI^+]^S$ through cytoduction (Figure 5 and Table 5). Since $[PSI^+]^U$ is a special readout of $[PSI^+]^W$ in $\Delta CTA-HSF$ cells, we consider that both $\Delta NTA-HSF$ and $\Delta CTA-HSF$ cells are also capable of receiving and propagating $[PSI^+]^W$ (Figure 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; KUSHNIROV *et al.* 2000; SONDEHEIMER *et al.* 2001; JONES *et al.* 2004; SONG and MASISON 2005). In contrast, the initiation process is less understood. Our data suggest that the initiation process requires more stringent cellular environmental conditions than that of propagation, since both $\Delta NTA-HSF$ and $\Delta CTA-HSF$ 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 $\Delta NTA-HSF$ and $\Delta CTA-HSF$ cells we examined for $[PSI^+]$ *de novo* formation contained the same $[RNQ^+]$ variant, an m.d. form (Figure 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 a 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 poly(Q) strongly suggest that it is possible that mutations in mammalian HSF can lead to anti-aggregation or aggregation-promoting phenotypes. Thus modulation of HSF activity can contribute to the etiology of protein-misfolding diseases. In this regard, the effect of HSF activity on the life span of *Caenorhabditis 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 responds differentially

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 poly(Q) suggests 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.

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