Published in final edited form as:

Mol Microbiol. 2014 September ; 93(6): 1156–1171. doi:10.1111/mmi.12725.

Structural variants of yeast prions show conformer-specific requirements for chaperone activity

Kevin C. Stein and Heather L. True*

Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, Missouri 63110

Summary

Molecular chaperones monitor protein homeostasis and defend against the misfolding and aggregation of proteins that is associated with protein conformational disorders. In these diseases, a variety of different aggregate structures can form. These are called prion strains, or variants, in prion diseases, and cause variation in disease pathogenesis. Here, we use variants of the yeast prions *[RNQ+]* and *[PSI+]* to explore the interactions of chaperones with distinct aggregate structures. We found that prion variants show striking variation in their relationship with Hsp40s. Specifically, the yeast Hsp40 Sis1, and its human ortholog Hdj1, had differential capacities to process prion variants, suggesting that Hsp40 selectivity has likely changed through evolution. We further show that such selectivity involves different domains of Sis1, with some prion conformers having a greater dependence on particular Hsp40 domains. Moreover, *[PSI+]* variants were more sensitive to certain alterations in Hsp70 activity as compared to *[RNQ+]* variants. Collectively, our data indicate that distinct chaperone machinery is required, or has differential capacity, to process different aggregate structures. Elucidating the intricacies of chaperone-client interactions, and how these are altered by particular client structures, will be crucial to understanding how this system can go awry in disease and contribute to pathological variation.

Keywords

Prions; Protein aggregation; Heat shock protein; Molecular chaperone; Yeast

Introduction

The aggregation of several different proteins is associated with the development of protein conformational disorders, including various neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and prion diseases (Chiti and Dobson, 2006). Interestingly, the proteins that aggregate in these disorders can misfold to form a wide range of aggregate structures that have distinct conformations (Toyama and Weissman, 2011). In prion diseases, these different self-perpetuating structures, called prion strains, cause changes in pathological phenotypes and dictate disease transmissibility. It is the responsibility of molecular chaperones, as the master regulators of protein quality control, to

*For correspondence, heather.true@wustl.edu; Tel.: (314) 362-3934; Fax: (314) 362-7463.

The authors declare no conflict of interest.

recognize and process these diverse misfolded substrates and mitigate the devastating consequences that these aggregates can have on cellular homeostasis (Hartl *et al.*, 2011). Yet, how chaperone machinery can handle a large array of both substrates and substrate conformers remains unclear.

Prion strains in tractable systems such as yeast (here called prion variants) have served as a valuable model for examining the interactions between chaperones and distinct amyloid structures (Liebman and Chernoff, 2012). The *[PSI+]* and *[RNQ+]* prions are two of the best-studied yeast prions. *[PSI+]* is the self-perpetuating prion form of the translation termination factor Sup35 (Wickner, 1994; Paushkin *et al.*, 1996; Patino *et al.*, 1996). Sequestration of Sup35 in prion aggregates reduces the efficiency of translation termination, causing readthrough of stop codons, called nonsense suppression (Serio and Lindquist, 1999). Different variants of the *[PSI+]* prion sequester Sup35 to different extents. Hence, *[PSI+]* variants are associated with different amounts of soluble Sup35, and consequently, distinct levels of nonsense suppression, which can be monitored phenotypically or biochemically (Derkatch *et al.*, 1996; Uptain *et al.*, 2001). Cells harboring stronger *[PSI+]* variants cause more nonsense suppression (less faithful translation termination) as compared to cells propagating weaker *[PSI+]* variants. The *[RNQ+]* prion, on the other hand, is formed from the Rnq1 protein that has no clear function in its non-prion form (Stein and True, 2011). However, *[RNQ+]* is responsible for inducing the *de novo* formation of the *[PSI+]* prion (Derkatch *et al.*, 2001; Oshervich and Weissman, 2001), and *[RNQ+]* variants are distinguished based on how readily *[PSI+]* forms in *[RNQ+]* cells (Bradley *et al.*, 2002; Kalastavadi and True, 2010).

Just as molecular chaperones play a pivotal role in processing substrates in humans, chaperones process the aggregates of the *[PSI+]* and *[RNQ+]* prions. Interestingly, chaperone-mediated processing is required for transmission of the prion state to daughter cells and the continued maintenance in a yeast population (Tuite and Cox, 2003). A combination of chaperones from the Hsp40 and Hsp70 families, along with the disaggregase Hsp104, is involved in the proper maintenance of yeast prions (Chernoff *et al.*, 1995; Newnam *et al.*, 1999; Sondheimer and Lindquist, 2000; Jung *et al.*, 2000; Allen *et al.*, 2005; Jones and Tuite, 2005; Aron *et al.*, 2007; Higurashi *et al.*, 2008; Tipton *et al.*, 2008). Hsp104 is a AAA+ ATPase that assembles into a ring-shaped hexamer having a central pore that threads a variety of substrates (Lum *et al.*, 2004; Tessarz *et al.*, 2008). Such substrate threading fragments large prion aggregates into smaller seeds that can be inherited by daughter cells (Paushkin *et al.*, 1996; Wegrzyn *et al.*, 2001; Kryndushkin *et al.*, 2003). Expression of Hsp104 is required for propagation of both *[PSI+]* and *[RNQ+]*, as fragmentation is a necessary feature for prion maintenance (Chernoff *et al.*, 1995; Sondheimer and Lindquist, 2000). In addition, proper processing of prion aggregates relies on Hsp70s, which act to transfer substrates to Hsp104 (Glover and Lindquist, 1998; Winkler *et al.*, 2012). Hsp70s like Ssa1, which plays a major role in prion propagation (Winkler *et al.*, 2012), are ATPases whose activity relies on a constant cycling between nucleotide binding states (Kampinga and Craig, 2010). Hsp70s will bind substrates in the ADP-bound state and release substrates in the ATP-bound state. Co-chaperones are required to mediate this ATPase cycle. Nucleotide exchange factors (NEFs) promote the ATP-bound state,

exchanging ADP for ATP and causing Hsp70 to have low affinity for non-native polypeptides. By contrast, Hsp40s are responsible for transferring substrates to Hsp70 and stimulating the Hsp70 ATPase activity (Kampinga and Craig, 2010). Thus, Hsp40s promote the ADP-bound state of Hsp70s, thereby causing Hsp70s to have higher affinity for non-native polypeptides and allowing further substrate processing. Interestingly, with a large number of diverse family members, Hsp40s are proposed to be the predominant regulator of Hsp70s by dictating both functionality and substrate selectivity (Kampinga and Craig, 2010).

Sis1 is the Hsp40 that is required for propagation of *[PSI+]* and *[RNQ+]*, and it is also the only Hsp40 essential for yeast viability (Zhong and Arndt, 1993; Aron *et al.*, 2007; Higurashi *et al.*, 2008; Tipton *et al.*, 2008). Sis1, like its human ortholog Hdj1, is a Type II Hsp40 that is divided into four major domains. The N-terminal J domain is required for Hsp70 interaction and stimulation, as well as for yeast viability (Cheetham and Caplan, 1998; Yan and Craig, 1999; Craig *et al.*, 2006). While the J domain is conserved in all Hsp40 proteins, there is tremendous sequence and structural divergence outside of this domain (Kampinga and Craig, 2010). In addition to the J domain, Sis1 has a C-terminal domain (CTD) responsible for binding substrates (Lu and Cyr, 1998), with the last 15 residues of the protein comprising a dimerization domain (DD) (Sha *et al.*, 2000). In between the J domain and CTD is a region rich in glycine and phenylalanine residues (G/F) followed by a region rich in glycine and methionine (G/M) residues. These regions are generally viewed as linker regions, but their function remains poorly understood. Interestingly, the G/F region is required for propagation of several different *[RNQ+]* variants (Stein *et al.*, 2014). Propagation of *[PSI+]* variants, on the other hand, differentially depends on this region (Stein *et al.*, 2014). *[PSI+]* variants also rely on Sis1 expression to different extents (Hines *et al.*, 2011). Yet, it remains unclear whether prion variants are differentially sensitive to changes in other Sis1 domains or vary in other chaperone requirements.

Here, we explored the relationship between molecular chaperones and their interactions with distinct aggregate structures. We found striking differences in the chaperone interactions required for propagation of the *[PSI+]* and *[RNQ+]* prions and their associated structural variants. Hdj1 was unable to propagate all of the prion variants of *[PSI+]* and *[RNQ+]* that Sis1 was capable of propagating. This is likely because propagation of different prion variants relies on the presence of different domains of Sis1. In general, the *[RNQ+]* variants were more sensitive to changes in Sis1 and how it interacts with Hsp70, while *[PSI+]* variants were more sensitive to particular changes in the activity of the Hsp70 Ssa1. These data indicate that distinct aggregate structures require different chaperone interactions or activities for proper processing. Hence, gaining insight into the complex nature of these interactions is necessary in order to fully understand how defects in the proteostasis network can lead to disease.

Results

Sis1 and its human ortholog Hdj1 show distinct conformer specificity

Hsp40s have been suggested to dictate much of the substrate specificity of the chaperone network (Kampinga and Craig, 2010). As Sis1 is capable of propagating a large spectrum of

prion conformations (Higurashi *et al.*, 2008; Hines *et al.*, 2011; Stein *et al.*, 2014), we wondered whether its human ortholog, Hdj1, could functionally substitute for Sis1 in propagating different types of prion variants. To test this, we used four different prion conformers of the $[PSI^+]$ prion, representing two weaker $[PSI^+]$ variants (Sc37 and weak $[PSI^+]$) and two stronger $[PSI^+]$ variants (Sc4 and strong $[PSI^+]$) (Derkatch *et al.*, 1996; Tanaka *et al.*, 2004). Since $[PSI^+]$ modulates translation termination, $[PSI^+]$ variants are easily distinguished based on colony color using yeast strains containing the *ade1-14* allele, which has a premature stop codon in the *ADE1* gene (Serio and Lindquist, 1999). Cells propagating distinct $[PSI^+]$ variants have different amounts of soluble Sup35 (Uptain *et al.*, 2001), and thus differentially suppress the translation termination of *ade1-14* and cause different amounts of red pigment to accumulate. Stronger $[PSI^+]$ colonies, having less soluble Sup35 and more nonsense suppression, are whiter in color as compared to colonies propagating weaker $[PSI^+]$ variants that are darker pink. As Sup35 is soluble and functional in $[psi^-]$ cells, these colonies are red.

To determine if Hdj1 was capable of propagating these distinct $[PSI^+]$ variants, we used *sis1* cells that expressed *SIS1* from a *URA3*-marked plasmid, and used the plasmid shuffle technique to perform gene replacements and express Hdj1 or wild-type (WT) Sis1. Interestingly, the $[PSI^+]$ variants showed dramatic differences in their sensitivity to Hdj1 expression. While the weaker $[PSI^+]$ variants were darker pink when WT Sis1 was expressed, these cells were red and phenotypically $[psi^-]$ when Hdj1 was expressed (Fig. 1A). By contrast, expression of Hdj1 in stronger $[PSI^+]$ cells appeared to have no phenotypic consequences, as lighter pink colonies were seen when either Sis1 or Hdj1 was expressed. To confirm these results biochemically, we used semi-denaturing detergent gel electrophoresis (SDD-AGE), which not only resolves aggregated Sup35 from soluble Sup35, but also shows that $[PSI^+]$ variants can have different aggregate distributions: Sup35 aggregates in weaker $[PSI^+]$ cells typically have a larger average aggregate size as compared to stronger $[PSI^+]$ cells (Kryndushkin *et al.*, 2003). In agreement with our phenotypic results, both weak and Sc37 $[PSI^+]$ variants had no aggregates of Sup35 when Hdj1 was expressed (Fig. 1B). However, Hdj1 expression correlated with a minor shift in the Sup35 aggregate distribution in lysates from cells propagating the stronger $[PSI^+]$ variants, despite being phenotypically similar to cells expressing Sis1. Nevertheless, these results indicate that the $[PSI^+]$ variants show drastically different sensitivities to Hdj1 expression, suggesting that Hdj1 is unable to allow for propagation of the weaker $[PSI^+]$ variants.

Next, we asked if Hdj1 showed differential capabilities in handling $[RNQ^+]$ variants. Previously, it was shown that expression of Hdj1 was fully capable of propagating an uncharacterized prion variant of $[RNQ^+]$ (Lopez *et al.*, 2003). Here, we used five different $[RNQ^+]$ variants that have been shown to have drastic differences in their ability to induce the formation of the $[PSI^+]$ prion (Bradley *et al.*, 2002). Moreover, these $[RNQ^+]$ variants show differences in their *in vivo* distribution of aggregates using Rnq1-GFP, where single-dot (s.d.) $[RNQ^+]$ variants typically have one focus of fluorescence, while multiple-dot (m.d.) cells have multiple foci (Bradley and Liebman, 2003). Hence, cells propagating the s.d. low $[RNQ^+]$ variant show a single focus by Rnq1-GFP fluorescence and induce the formation of $[PSI^+]$ at low rates. As above, we used plasmid shuffle to express Hdj1 or Sis1

in cells propagating each of these [RNQ+] variants, followed by performing SDD-AGE to analyze prion propagation. As a complementary technique, because SDD-AGE does not reliably show Rnq1 monomer for unknown reasons, we used sedimentation assays to monitor the amount of soluble Rnq1 protein in these cells. With this assay, as almost all of the Rnq1 protein is sequestered in aggregates in WT [RNQ+] cells, Rnq1 is found in the insoluble fraction, whereas any impairment in prion propagation results in an increased soluble pool of Rnq1 (Sondheimer and Lindquist, 2000). Some prion variants, such as s.d. very high [RNQ+], have a slightly larger pool of soluble Rnq1 than others (Bradley *et al.*, 2002). Strikingly, both assays showed that Hdj1 had diverse capabilities in maintaining the [RNQ+] variants (Fig. 1C,D). Propagation of s.d. medium [RNQ+] was most severely affected by expression of Hdj1, as there were very few aggregates remaining, and a large pool of soluble protein was present. Moreover, propagation of the three other s.d. [RNQ+] variants was impaired to varying degrees, as indicated by the presence of fewer aggregates by SDD-AGE and a larger soluble pool of Rnq1 by the sedimentation assay. In stark contrast, m.d. high [RNQ+] appeared to be unaffected by Hdj1 expression. Collectively, these data suggest that Sis1 and Hdj1 have different specificity or functionality in processing distinct prion conformers.

Differential dependence on Hsp40 domains dictates conformer specificity

As Hdj1 and Sis1 showed differential ability to propagate particular prion variants, we hypothesized that there might be distinct differences in domain functionality of these Hsp40s. We next wanted to determine what functional domains might be responsible for these differences. To accomplish this, we created a suite of chimeras having various combinations of the four major domains present in Hdj1 and Sis1, and confirmed comparable expression as the WT proteins (Fig. S1). We refer to these chimeras by delineating the origins of each domain, with “S” for Sis1 and “H” for Hdj1 (Fig. 2A). As Hdj1 was unable to propagate certain prion variants, these chimeras allowed us to ask which Sis1 domains would rescue this defect.

To analyze the prion structures that were most severely altered when Hdj1 was expressed, we first replaced WT Sis1 with one of the chimeric proteins in cells propagating weak [PSI+] or Sc37. Interestingly, we found that the chimeras were differentially capable of propagating these structures, despite both being weaker [PSI+] variants (Fig. 2B,C). For instance, the chimera HSHH expressed the Sis1 G/F region in place of that of Hdj1. This chimera largely rescued propagation of Sc37, as indicated by the presence of less soluble Sup35 as compared to expression of Hdj1, based on phenotype (Fig. 2B) and SDD-AGE (Fig. 2C). However, HSHH provided very little rescue of propagation of weak [PSI+], as these cells were phenotypically similar to [psi-] cells and showed a greatly reduced population of aggregated species by SDD-AGE. Moreover, replacing additional Hdj1 domains with those of Sis1 (e.g. HSSH, HHSS, and SSSH) also partially rescued propagation of both prion variants. Yet, HHSS was more capable of propagating Sc37 than weak [PSI+], as there was a large soluble pool of Sup35 with weak [PSI+] cells expressing HHSS that was not seen with cells propagating Sc37. However, propagation of Sc37 was still impaired with each of the chimeras, as indicated by the darker pink to red colony color,

while SSSH appeared to fully promote propagation of weak [*PSI*⁺], as these cells phenocopied WT cells in both nonsense suppression and SDD-AGE assays (Fig. 2B,C).

We then tested whether the Sis1-Hdj1 chimeras would rescue propagation of the s.d. low, s.d. medium, and s.d. very high [*RNQ*⁺] variants that were most impaired by Hdj1 expression. Using SDD-AGE to monitor aggregate species after gene replacement, we found that the propagation of these variants showed dramatic differences in terms of which chimera was capable of mediating prion propagation (Fig. 2D). For s.d. medium [*RNQ*⁺], only the two chimeras that contained the Sis1 CTD (HHHS and HHSS) maintained propagation, as cells expressing these chimeras had a large aggregated species of Rnq1 as compared to Hdj1. Strikingly, HHHS did not rescue propagation of s.d. low and s.d. very high [*RNQ*⁺], but several other chimeras did (Fig. 2D). Yet, the chimeras that were capable of maintaining s.d. low and s.d. very high [*RNQ*⁺] were distinct. Collectively, these data indicate that propagation of both [*PSI*⁺] and [*RNQ*⁺] variants relies on distinct domains or functions of Hsp40.

Propagation of different prion variants relies on distinct regions of Sis1

As a complementary approach to asking whether particular prion variants differentially require the presence of certain Sis1 domains, we took advantage of a variety of Sis1 mutants that have previously been used to interrogate the importance of Sis1 regions on prion propagation (Sondheimer *et al.*, 2001; Lopez *et al.*, 2003; Kirkland *et al.*, 2011). This included deletion mutants of major Sis1 regions that supported viability in *sis1*⁻ cells (Sis1-G/F, Sis1-G/M, Sis1-CTD), the dimerization domain (Sis1-DD), smaller regions of the G/F region (Sis1-86–96 and Sis1-101–113), and a point mutation in the G/F region (Sis1-D110G). We transformed cells that propagated one of the [*PSI*⁺] or [*RNQ*⁺] variants with plasmids that expressed each of these mutants, performed plasmid shuffle to replace WT Sis1 in a *sis1*⁻ background, and confirmed expression by western blot (Fig. S2).

In testing the effect of these Sis1 mutants on prion propagation, for both weaker [*PSI*⁺] variants, we found that deletion of the G/F or G/M regions impaired prion propagation, as these cells were phenotypically darker pink as compared to cells expressing WT Sis1 (Fig. 3A), and there was an increase in soluble Sup35 as assessed by SDD-AGE (Fig. 3B). Additionally, deletion of the Sis1 CTD had a major impact on prion propagation, severely affecting the maintenance of both variants, while deletion of the DD or residues 86–96 had minimal effect. Paradoxically, however, deletion of residues 101–113 had a greater effect on the propagation of weak [*PSI*⁺] as compared to Sc37, with a complete loss of Sup35 aggregates in weak [*PSI*⁺] cells, but a point mutation within this stretch of Sis1, D110G, impaired propagation of Sc37 to a larger extent, as indicated by the darker pink color and a dramatic shift in the average Sup35 aggregate distribution (Fig. 3A,B). These data again suggest that the weaker [*PSI*⁺] variants are differentially sensitive to mutations in Sis1. By contrast, Sis1 mutants showed very minimal effect on the propagation of stronger [*PSI*⁺] variants, with the greatest impact being the toxicity of Sis1-CTD in the presence of stronger [*PSI*⁺] (Fig. S3), as previously shown (Kirkland *et al.*, 2011).

Next, we tested how the Sis1 mutants affected propagation of the [*RNQ*⁺] variants using the sedimentation assay that separates cell lysates into soluble and insoluble fractions. As we

found previously, deletion of the G/F region eliminated the propagation of all five [RNQ+] variants (Fig. 3C and (Stein *et al.*, 2014)). Interestingly, other than Sis1- G/F, both s.d. high [RNQ+] and m.d. high [RNQ+] were particularly resistant to changes in Sis1. There was some increased soluble Rnq1 when Sis1- 101–113 and Sis1- CTD were expressed in s.d. high [RNQ+] cells, but only deletion of the G/M region markedly impaired the propagation of these two [RNQ+] variants (Fig. 3C). Sis1- G/M also affected propagation of the three other [RNQ+] variants, thereby showing the general importance of this region in prion propagation. However, deletion of this region showed varying degrees of impairment, minimally increasing the soluble pool of Rnq1 in s.d. low [RNQ+] cells, while dramatically affecting the propagation of s.d. medium and s.d. very high [RNQ+] as most all of Rnq1 was soluble. Interestingly, s.d. medium and s.d. very high [RNQ+] were particularly sensitive to the deletion of the Sis1 CTD and the DD. Moreover, deletion of residues 86–96 resulted in an increase in soluble Rnq1 for only s.d. very high [RNQ+] cells, suggesting that this region is important for propagation of only the s.d. very high [RNQ+] prion variant. Collectively, these data suggest that propagation of a wide spectrum of aggregated structures requires distinct domains of Sis1 (Fig. 3D).

All [RNQ+] variants show greater dependence than [PSI+] on the bipartite interaction of Hsp40 with Hsp70

A complex of chaperones is often required to process misfolded substrates. It is thought that Hsp40s, in general, are responsible for recognizing substrates (Kampinga and Craig, 2010). In the case of yeast prion proteins, Sis1, in combination with an Hsp70, such as Ssa1, will transfer substrates to Hsp104 for fragmentation and continued prion propagation (Tipton *et al.*, 2008; Winkler *et al.*, 2012). While the Sis1 J domain is critical for the interaction of Sis1 with the nucleotide-binding domain of Hsp70s (Li *et al.*, 2009), part of the Sis1 CTD forms a bipartite interaction with the EEVD motif in the substrate-binding domain of Hsp70s (Freeman *et al.*, 1995; Qian *et al.*, 2002; Li *et al.*, 2006). Indeed, a mutation in the Sis1 CTD (L268P) was previously found to impair this secondary interaction of Sis1 and Ssa1 *in vitro*, and this mutant inhibited the propagation of an uncharacterized [RNQ+] variant *in vivo* (Aron *et al.*, 2005).

Using Sis1-L268P as a means of disrupting the Sis1-Hsp70 bipartite interaction, we asked whether prion variants similarly relied on the Sis1-Hsp70 bipartite interaction for proper maintenance by replacing WT Sis1 with Sis1-L268P in cells propagating each of the [RNQ+] or [PSI+] variants. Unexpectedly, despite the [RNQ+] variants showing dramatic differences in sensitivity to other changes in Sis1, the Sis1-L268P construct eliminated the propagation of all five [RNQ+] variants, as all of the Rnq1 protein accumulated in the soluble fraction after ultracentrifugation (Fig. 4A). By contrast, the propagation of the stronger [PSI+] variants was unchanged according to the nonsense suppression phenotype, while the propagation of the weaker [PSI+] variants was impaired, but not eliminated, as indicated by colonies that were redder in color, yet still distinct from [*psi*-] cells (Fig. 4B). This suggests that the propagation of all [RNQ+] variants depends on the bipartite interaction between Sis1 and Hsp70, but propagation of [PSI+] does not absolutely require this interaction, or at least does not require it to the same extent.

[RNQ+] variants do not show the same sensitivity as [PSI+] to certain modulations of the Hsp70 ATPase cycle

As Hsp70s clearly play an important role in substrate processing, we next wanted to determine whether altering Hsp70 function differentially affected propagation of prion variants. The Hsp70-dependent processing of substrates depends on an iterative nucleotide-binding cycle between ADP- and ATP-bound states that is facilitated by the activity of Hsp40 and NEFs (Kampinga and Craig, 2010). We asked whether modulation of Hsp70 activity might affect particular [RNQ+] variants to different extents, as has been observed with [PSI+] variants (Fan *et al.*, 2007).

To address this, we first utilized a mutant of Ssa1, called Ssa1–21, that is known to dominantly inhibit the propagation of the [PSI+] prion (Jung *et al.*, 2000). Ssa1–21 harbors the L483W mutation, which resides in the C-terminal substrate-binding domain of Ssa1 and causes elevated ATPase activity (Needham and Masison, 2008). In order to examine the effect of Ssa1–21, we created *ssa2* [RNQ+] strains as loss of Ssa2 enhances the effect of Ssa1–21 (Jung *et al.*, 2000; Sharma and Masison, 2008). We then transformed these cells with a plasmid expressing *SSA1–21* or *SSA1* from the endogenous *SSA1* promoter, or an empty vector control. To monitor the influence on prion propagation, we performed well-trap assays, which allows us to easily determine the amount of soluble Rnq1 in the cell, since aggregated Rnq1 is retained in the wells of an SDS-PAGE gel when cell lysate fractions are not boiled (Liebman *et al.*, 2006). Interestingly, there was no increased pool of soluble Rnq1 in cells propagating any of the [RNQ+] variants when Ssa1–21 was expressed (Fig. 5A). Importantly, as genetic background is a critical variable for revealing the effects of Ssa1–21 (Hines *et al.*, 2011) we found that an isogenic *ssa2* strain propagating strong [PSI+] showed that [PSI+] propagation was impaired with Ssa1–21 expression as compared to Ssa1-expressing cells and empty vector controls (Fig. 5B). However, [PSI+] was not cured as previous studies had shown (Jung *et al.*, 2000; Jones and Masison, 2003; Sharma and Masison, 2008).

As another means of testing the sensitivity of the [RNQ+] variants to Hsp70 activity, we hypothesized that over-expression of Ssa1 might impair propagation of certain [RNQ+] variants. Additionally, as NEF activity is an important modulator of Hsp70 activity, we also wanted to determine whether the [RNQ+] variants would be differentially sensitive to the over-expression or deletion of the NEF *SSE1*, as is the case for other prions and prion variants (Fan *et al.*, 2007; Hines *et al.*, 2011). Using well-trap assays, we found that neither over-expression of Ssa1 nor Sse1 altered the amount of soluble Rnq1, as compared to empty vector controls, for any of the [RNQ+] variants (Fig. 5C). Furthermore, deletion of *SSE1* also had no effect on [RNQ+] propagation (Fig. 5D), despite the previous finding that [PSI+] variants are sensitive to deletion of *SSE1* to different extents (Fan *et al.*, 2007). These data suggest that [RNQ+] is less sensitive to at least a subset of alterations in Hsp70 activity as compared to [PSI+].

[RNQ+] variants similarly rely on Hsp104-ClpB chimeras

Along with Sis1, the other protein that is essential for the propagation of all known yeast prions is Hsp104 (Chernoff *et al.*, 1995; Sondheimer and Lindquist, 2000; Moriyama *et al.*,

2000; Du *et al.*, 2008). Upon transfer of substrates from Hsp40-Hsp70, Hsp104 fragments prion aggregates into smaller seeds that are transmissible to daughter cells and maintain the prion state (Derdowski *et al.*, 2010). Hsp104 has been divided into an N-terminal domain with no clear function, along with two ATPase domains that are separated by a coiled-coil middle (M) domain (Lee *et al.*, 2010). The M domain is responsible for mediating the physical interaction with the Hsp70 machinery (Sielaff and Tsai, 2010; Miot *et al.*, 2011; Seyffer *et al.*, 2012). Interestingly, the *E. coli* ortholog of Hsp104, ClpB, is unable to cooperate with the yeast co-chaperones to propagate *[PSI+]*, *[RNQ+]*, and *[URE3]*, unless the Hsp104 M domain is present (Tipton *et al.*, 2008; Reidy *et al.*, 2012). We have recently shown that modulating the activity of the Hsp104 M domain differentially affects *[RNQ+]* and *[PSI+]* variants (Dulle *et al.*, 2014), possibly through altering interactions with co-chaperones. Therefore, we wondered whether these differences between ClpB and Hsp104 would similarly affect all *[RNQ+]* variants. Hence, we took advantage of a set of chimeras that consisted of different combinations of domains from Hsp104 and ClpB (Tipton *et al.*, 2008) to ask if distinct prion structures showed differential dependence on other regions of Hsp104. As above, the origin of the domains is denoted with a “4” for Hsp104, or “B” for ClpB.

We used *[RNQ+]* *hsp104* yeast strains that expressed *HSP104* from a covering plasmid, and replaced WT Hsp104 by plasmid shuffle with one of the ClpB-Hsp104 chimeras, along with WT ClpB, WT Hsp104, and an empty vector as controls. We then monitored *[RNQ+]* propagation by the presence of aggregates using SDD-AGE. Unexpectedly, we found that only WT Hsp104 and the chimera 444B were able to maintain Rnq1 aggregates (Fig. 6). The chimera 444B was the only chimera that was previously shown to propagate a stronger *[PSI+]* variant (Tipton *et al.*, 2008). This indicates that the second ATPase domain of ClpB is able to functionally substitute for that of Hsp104. Moreover, these data suggest that the propagation of the *[RNQ+]* variants relies similarly on the different domains of Hsp104, or has similar requirements for Hsp104 activity.

Discussion

Our study highlights the extent to which molecular chaperones differentially interact with various conformations of protein aggregates. Previous work has focused on the fact that different prions, or prion variants, show distinct sensitivity to chaperone levels (Fan *et al.*, 2007; Hines and Craig, 2011; Hines *et al.*, 2011). We have recently shown that the propagation of several different *[RNQ+]* variants all require the presence of the Sis1 G/F region, while *[PSI+]* variants show differential dependence on this region (Stein *et al.*, 2014). Here, we show that other domains of Sis1 are also important for substrate recognition or processing in a prion variant-dependent manner. This suggests that substrate conformation, not simply the substrate protein, is a critical determinant of how the chaperone machinery interacts with and processes substrates. In fact, we recently found that the *[RNQ+]* variants likely have different regions of the Rnq1 protein that are available for chaperone binding (Stein and True, 2014).

Hsp40s are responsible for interacting with non-native or misfolded polypeptides and mediating the functional specificity of Hsp70s (Kampinga and Craig, 2010). We showed

that prion variants of [RNQ+] show differences in sensitivity to changes in the function of only Sis1, and not in Hsp70 or Hsp104. Moreover, we found that such specificity also extends to the direct orthologs of Sis1 and Hdj1 (Fig. 1). Hdj1 was unable to propagate either of the weaker [PSI+] variants, or the s.d. medium [RNQ+] variant, and impaired the propagation of the other s.d. [RNQ+] variants. Previous work with the bacterial DnaJ protein suggested that the G/F region may mediate substrate specificity (Perales-Calvo *et al.*, 2010). However, our work clearly shows that other domains of Sis1 also play an important role. In the case of the weaker [PSI+] variants, the Sis1 CTD is necessary for propagation (Fig. 3), which agrees with this being the substrate-binding domain (Sha *et al.*, 2000). However, the deletion of the Sis1 DD did not affect propagation of either of these variants, suggesting that dimerization is not essential for propagation. By contrast, we found that propagation of the [RNQ+] variants relied on the G/M region to different extents, which was particularly required for the maintenance of s.d. medium and s.d. very high [RNQ+]. Interestingly, these variants were the only structures eliminated by deletion of the CTD, and their propagation was even affected by the DD mutant (Fig. 3C). Moreover, only the Sis1-Hdj1 chimeras that maintained the Sis1 CTD allowed for propagation of s.d. medium [RNQ+] (Fig. 2D). Since the dimerization of Sis1 is proposed to increase the affinity of Sis1 for substrates or for transfer to Hsp70, we suggest that s.d. medium and s.d. very high [RNQ+] are particularly recalcitrant substrates, such that Sis1 must function as a dimer in order for these variants to be properly processed. Furthermore, as Sis1 can shuttle between the nucleus and cytosol (Douglas *et al.*, 2009; Park *et al.*, 2013), the possible disruption of Sis1 localization by certain Sis1 mutants or Sis1-Hdj1 chimeras might also contribute to altering prion propagation of certain variants.

Structurally, X-ray crystallography has shown that the structures of the J domain and CTD of Sis1 and Hdj1 are very similar (Li *et al.*, 2009). The CTD of these homo-dimeric proteins (Sha *et al.*, 2000), forms a U-shaped cleft that binds substrate proteins (Lu and Cyr, 1998) and part of the substrate-binding domain of Hsp70 (Li *et al.*, 2006). Interestingly, this cleft is smaller in Hdj1, in which the distance between the monomer subunits is shorter as compared to Sis1 (Hu *et al.*, 2008). This might indicate that the decreased ability of Hdj1 to process all the prion conformers that we tested is because its CTD has lower flexibility to process more recalcitrant substrates. Furthermore, the distinct set of clients for Hdj1 as compared to Sis1 might suggest that, through evolution from yeast to humans, which included a large increase in the number of Hsp40s (Kampinga and Craig, 2010), Hdj1 may have become more specialized.

A previous study had observed that expression of Hdj1 was synthetically lethal with the presence of strong [PSI+] (Kirkland *et al.*, 2011), an effect which we did not reproduce here. However, as shown in that same study (Kirkland *et al.*, 2011), we also found that expression of Sis1-CTD was toxic in strong [PSI+] cells (Fig. S3), as was expression of Sis1-L268P to some degree (Fig. 4B). We suggest that these different trends are likely due to variation in genetic background, which is an important consideration in analyzing chaperone-amyloid interactions (Hines *et al.*, 2011). Nevertheless, as aggregates can sequester various chaperone machinery (Tyedmers *et al.*, 2010; Yang *et al.*, 2013), it is feasible that Sup35 aggregates in strong [PSI+] cells sequester Sis1 more than other prion variants, such that the

already reduced capability (or expression) of Sis1- CTD (or Hdj1 in the previous work (Kirkland *et al.*, 2011)) cannot maintain the essential functions of Sis1 in yeast.

Along with the mutations in Sis1, we had previously shown that mutations in the Hsp104 M domain affect propagation of the [RNQ+] variants differently (Dulle *et al.*, 2014). However, in this study, we found that the ClpB-Hsp104 chimeras did not show differential effects on the propagation of the [RNQ+] variants, with only the 444B chimera able to maintain Rnq1 aggregates. While a previous study had shown that multiple ClpB-Hsp104 chimeras were capable of propagating [RNQ+], this could be attributed to a difference in [RNQ+] variant, genetic background, or method of analysis (Reidy *et al.*, 2012). In light of the dramatic differences in sensitivity that the [RNQ+] variants have in response to alterations in Sis1, we now propose that the differential effects of mutations in the Hsp104 M domain are likely mediated through disrupting the interactions with co-chaperones, particularly Sis1. Indeed, the s.d. high and m.d. high [RNQ+] variants were the most resistant to changes in the Hsp104 M domain (Dulle *et al.*, 2014), just as they were the most resistant to mutations in Sis1 (Fig. 3). This would agree with the finding that co-chaperones bind to and mediate the activity of Hsp104 via the M domain (Seyffer *et al.*, 2012).

It has long been recognized that [PSI+] is the only prion that is cured by over-expression of Hsp104 (Hines and Craig, 2011), which has been attributed to Hsp104 outcompeting Ssa1 for binding to Sup35 aggregates and resulting in a non-productive interaction (Winkler *et al.*, 2012), although this remains controversial (Park *et al.*, 2014). Our data further indicate that [PSI+] is more sensitive to particular changes in Hsp70 activity as compared to [RNQ+] (Fig. 5). By contrast, while the weaker [PSI+] variants show some changes in their propagation due to altered functionality of Sis1, unlike the stronger [PSI+] variants, the [RNQ+] variants all show even more dependence on Sis1 as these structures were all eliminated by expression of Sis1- G/F. Furthermore, [PSI+] is less sensitive to Sis1-L268P as compared to [RNQ+], indicating that [RNQ+] propagation also depends on the Sis1-Ssa1 bipartite interaction to a greater extent than [PSI+]. Taken together, these data make a strong argument that Sis1 plays a more intimate role in propagation of [RNQ+] as compared to [PSI+], which depends more on Ssa1 and Hsp104.

Genetic and environmental changes can influence the formation and maintenance of distinct aggregate structures (Li and Kowal, 2012; Huang *et al.*, 2013; Stein and True, 2014; Westergard and True, 2014b; Stein *et al.*, 2014). Indeed, many of these changes are likely due to altering the ability of molecular chaperones to monitor protein quality control, which could help select for the different self-propagating structures that persist in natural environments (Westergard and True, 2014a). Our study indicates that substrate processing of not only different substrates, but also different substrate conformers, can depend on a distinct set of chaperone machinery. Therefore, with the vital role that molecular chaperones play in maintaining proteostasis, elucidating the complex nature of chaperone-aggregate interactions is crucial to understanding the appearance and progression of protein conformational disorders, as well as the basis for the pathological variation that is often observed.

Experimental procedures

Yeast strains and media

All yeast strains described in this study were derived from 74-D694 (*ade1-14 ura3-52 leu2-3,112 trp1-289 his3-200*) and are listed in Table S1. Standard culturing techniques were used throughout with ¼ YEPD (0.25% yeast extract, 2% peptone, 2% dextrose) or synthetic defined (SD) media (0.67% yeast nitrogen base without amino acids, 2% dextrose) lacking one or more nutrients to select for appropriate plasmids. Medium containing 1mg/mL 5-fluoroorotic acid was used to select against cells maintaining *URA3*-marked plasmids in order to replace wild-type proteins with mutant constructs using the plasmid shuffle technique (Guthrie and Fink, 2004).

WT yeast strains were kind gifts from S. Liebman (L1751, L1943, L1945, L1767 [*psi-*], L1953, L1749, S12600, S12606) (Derkatch *et al.*, 1996; Bradley *et al.*, 2002; Bagriantsev and Liebman, 2004) and J. Weissman (2397, 2398) (Tanaka *et al.*, 2004). Construction of the *sis1* strains containing pRS316-*SIS1* (see Table S2 for plasmids used in this study) and the *hsp104* strains containing pRS316-*HSP104p-HSP104* were described previously (Dulle *et al.*, 2014; Stein *et al.*, 2014). To create the *ssa2* strains, the *kanMX4* cassette having flanking homology to the *SSA2* promoter and terminator was amplified from pFA6a using oligonucleotides 0978 and 0979 (see Table S3 for oligonucleotides used in this study), followed by transforming yeast cells, and selecting for resistance to G418. Deletion of *SSA2* was confirmed by colony PCR. To create the *sse1* strains, the plasmid-based disruption cassette *sse1::LEU2* (kind gift from K. Morano (Shaner *et al.*, 2006)) was digested with *SacII*/*PstI*, then yeast cells were transformed with the digested plasmid, and Leu⁺ transformants were selected. Deletion of *SSE1* was confirmed by colony PCR and western blot using an α Sse1 antibody (gift from J. Brodsky).

Plasmid construction

The following plasmids were kind gifts (see Table S2): Hsp104-ClpB constructs from J. Weissman (Tipton *et al.*, 2008), Sis1 mutant constructs from E. Craig, S. Lindquist, and D. Masison (Sondheimer *et al.*, 2001; Lopez *et al.*, 2003; Aron *et al.*, 2005; Kirkland *et al.*, 2011), pRS424*GPD-HDJ1* from E. Craig (Lopez *et al.*, 2003), pRS315-*SSA1* and pRS315-*SSA1-21* from D. Masison (Jones and Masison, 2003), and pRS413*GPD-FLAG-SSE1* from K. Morano (Shaner *et al.*, 2006). Construction of pRS314-*SIS1* was described previously (Stein *et al.*, 2014). To create pRS415*GPD-SSA1*, *SSA1* was amplified using oligonucleotides 0219 and 0220, digested with *SpeI*/*HindIII*, ligated with pRS415*GPD* (kind gift of M. Funk (Mumberg *et al.*, 1995)) that was digested with the same enzymes, and confirmed by sequencing.

Construction of the Sis1-Hdj1 chimeras was performed using bridge PCR with the indicated oligonucleotides and pRS316-*SIS1* or pRS424*GPD-HDJ1* as templates, unless otherwise noted: *SHHH* (*S* with 0229 and 1581, *HHH* with 1580 and 1471), *HSHH* (*H* with 1466 and 1467, *S* with 1468 and 1469, *HH* with 1470 and 1471), *HSSH* (*HH* with 1466 and 1500, *S* with 1499 and 1566, *H* with 1565 and 1471), *HHHS* (*HHH* with 1466 and 1502, *S* with 1501 and 0230), *HSSH* (*HS* with 1466 and 1583 using pRS414*GPD-HSHH* as template, *SH* with

1582 and 1471 using pRS414*GPD-HHSH* as template), *HHSS* (*HH* with 1466 and 1500, *SS* with 1499 and 0230), *SSSH* (*SSS* with 0229 and 1566, *H* with 1565 and 1471). Sis1 domains were demarcated as follows: J domain (1–66), G/F (67–121), G/M (122–179), CTD (180–352). Hdj1 domains were demarcated as follows: J domain (1–66), G/F (67–108), G/M (109–161), CTD (162–340). All constructs were digested with SpeI/ClaI, ligated to pRS414*GPD* (kind gift of M. Funk (Mumberg *et al.*, 1995)) that was digested with the same enzymes, and confirmed by sequencing.

Phenotypic colorimetric analysis

Overnight yeast cultures were normalized to OD₆₀₀ 0.4, serially diluted five-fold, and spotted to the indicated media. Plates were grown at 30°C for 6 days for SD-ade-leu, and for 3 days for all other media, followed by overnight incubation at 4°C for additional color development. All results are representative of at least three independent experiments, each using at least five yeast transformants.

Protein analysis

Semi-denaturing detergent agarose gel electrophoresis (SDD-AGE) for both Sup35 and Rnq1 proteins was performed as described previously with generated polyclonal αSup35 (kind gift of S. Lindquist) and αRnq1 antibodies, respectively (Huang *et al.*, 2013; Dulle *et al.*, 2014). The solubility assay of Rnq1 was performed as described (Bardill and True, 2009). Briefly, cell lysates in detergent-containing lysis buffer were subjected to ultracentrifugation at 80,000 rpm for 30 min at 4°C to separate into soluble and insoluble fractions that were analyzed by SDS-PAGE and western blot with a polyclonal αRnq1 antibody. Well-trap assays were performed as described (Stein and True, 2014). Additional antibodies used to analyze protein expression included: two different αSis1 antibodies (here referred to as αSis1.1 (COP-COP-080051, Cosmo Bio Co.) and αSis1.2 (a kind gift from E. Craig)), αHdj1 (ADI-SPA-400, Enzo Life Sciences), and αPgk1 (A6457, Molecular Probes). All results are representative of at least three independent experiments, each using at least five yeast transformants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful to the following for reagents: J. Brodsky, E. Craig, M. Funk, S. Liebman, S. Lindquist, D. Masison, K. Morano, and J. Weissman. We thank the members of the True lab for helpful discussions and comments. We thank the National Institutes of Health for funding: F31AG040899 and T32GM007067 to KCS and GM072778 to HLT.

References

- Allen KD, Wegrzyn RD, Chernova TA, Müller S, Newnam GP, Winslett PA, et al. Hsp70 chaperones as modulators of prion life cycle: novel effects of Ssa and Ssb on the *Saccharomyces cerevisiae* prion [PSI⁺]. *Genetics*. 2005; 169:1227–1242. [PubMed: 15545639]
- Aron R, Higurashi T, Sahi C, Craig EA. J-protein co-chaperone Sis1 required for generation of [RNQ⁺] seeds necessary for prion propagation. *EMBO J*. 2007; 26:3794–3803. [PubMed: 17673909]

- Aron R, Lopez N, Walter W, Craig EA, Johnson J. In vivo bipartite interaction between the Hsp40 Sis1 and Hsp70 in *Saccharomyces cerevisiae*. *Genetics*. 2005; 169:1873–1882. [PubMed: 15687271]
- Bagriantsev S, Liebman SW. Specificity of prion assembly in vivo. [PSI+] and [PIN+] form separate structures in yeast. *J Biol Chem*. 2004; 279:51042–51048. [PubMed: 15465809]
- Bardill JP, True HL. Heterologous prion interactions are altered by mutations in the prion protein Rnq1p. *J Mol Biol*. 2009; 388:583–596. [PubMed: 19324054]
- Bradley ME, Liebman SW. Destabilizing interactions among [PSI(+)] and [PIN(+)] yeast prion variants. *Genetics*. 2003; 165:1675–1685. [PubMed: 14704158]
- Bradley ME, Edskes HK, Hong JY, Wickner RB, Liebman SW. Interactions among prions and prion "strains" in yeast. *Proc Natl Acad Sci USA*. 2002; 99(Suppl 4):16392–16399. [PubMed: 12149514]
- Cheetham ME, Caplan AJ. Structure, function and evolution of DnaJ: conservation and adaptation of chaperone function. *Cell Stress Chaperones*. 1998; 3:28–36. [PubMed: 9585179]
- Chernoff YO, Lindquist SL, Ono B, Inge-Vechtomov SG, Liebman SW. Role of the chaperone protein Hsp104 in propagation of the yeast prion-like factor [psi+]. *Science*. 1995; 268:880–884. [PubMed: 7754373]
- Chiti F, Dobson CM. Protein misfolding, functional amyloid, and human disease. *Annu Rev Biochem*. 2006; 75:333–366. [PubMed: 16756495]
- Craig EA, Huang P, Aron R, Andrew A. The diverse roles of J-proteins, the obligate Hsp70 co-chaperone. *Rev Physiol Biochem Pharmacol*. 2006; 156:1–21. [PubMed: 16634144]
- Derdowski A, Sindi SS, Klaips CL, DiSalvo S, Serio TR. A size threshold limits prion transmission and establishes phenotypic diversity. *Science*. 2010; 330:680–683. [PubMed: 21030659]
- Derkatch IL, Bradley ME, Hong JY, Liebman SW. Prions affect the appearance of other prions: the story of [PIN(+)]. *Cell*. 2001; 106:171–182. [PubMed: 11511345]
- Derkatch IL, Chernoff YO, Kushnirov VV, Inge-Vechtomov SG, Liebman SW. Genesis and variability of [PSI] prion factors in *Saccharomyces cerevisiae*. *Genetics*. 1996; 144:1375–1386. [PubMed: 8978027]
- Douglas PM, Summers DW, Ren H-Y, Cyr DM. Reciprocal efficiency of RNQ1 and polyglutamine detoxification in the cytosol and nucleus. *Mol Biol Cell*. 2009; 20:4162–4173. [PubMed: 19656852]
- Du Z, Park K-W, Yu H, Fan Q, Li L. Newly identified prion linked to the chromatin-remodeling factor Swi1 in *Saccharomyces cerevisiae*. *Nat Genet*. 2008; 40:460–465. [PubMed: 18362884]
- Dulle JE, Stein KC, True HL. Regulation of the Hsp104 middle domain activity is critical for yeast prion propagation. *PLoS ONE*. 2014; 9:e87521. [PubMed: 24466354]
- Fan Q, Park K-W, Du Z, Morano KA, Li L. The role of Sse1 in the de novo formation and variant determination of the [PSI+] prion. *Genetics*. 2007; 177:1583–1593. [PubMed: 18039878]
- Freeman BC, Myers MP, Schumacher R, Morimoto RI. Identification of a regulatory motif in Hsp70 that affects ATPase activity, substrate binding and interaction with HDJ-1. *EMBO J*. 1995; 14:2281–2292. [PubMed: 7774586]
- Glover JR, Lindquist S. Hsp104, Hsp70, and Hsp40: a novel chaperone system that rescues previously aggregated proteins. *Cell*. 1998; 94:73–82. [PubMed: 9674429]
- Guthrie, C.; Fink, GR. *Guide to Yeast Genetics and Molecular and Cell Biology*. Gulf Professional Publishing; 2004.
- Hartl FU, Bracher A, Hayer-Hartl M. Molecular chaperones in protein folding and proteostasis. *Nature*. 2011; 475:324–332. [PubMed: 21776078]
- Higurashi T, Hines JK, Sahi C, Aron R, Craig EA. Specificity of the J-protein Sis1 in the propagation of 3 yeast prions. *Proc Natl Acad Sci USA*. 2008; 105:16596–16601. [PubMed: 18955697]
- Hines JK, Craig EA. The sensitive [SWI (+)] prion: New perspectives on yeast prion diversity. *Prion*. 2011; 5:164–168. [PubMed: 21811098]
- Hines JK, Higurashi T, Srinivasan M, Craig EA. Influence of prion variant and yeast strain variation on prion-molecular chaperone requirements. *Prion*. 2011; 5:238–244. [PubMed: 22156732]

- Hines JK, Li X, Du Z, Higurashi T, Li L, Craig EA. [SWI], the prion formed by the chromatin remodeling factor Swi1, is highly sensitive to alterations in Hsp70 chaperone system activity. *PLoS Genet.* 2011; 7:e1001309. [PubMed: 21379326]
- Hu J, Wu Y, Li J, Qian X, Fu Z, Sha B. The crystal structure of the putative peptide-binding fragment from the human Hsp40 protein Hdj1. *BMC Struct Biol.* 2008; 8:3. [PubMed: 18211704]
- Huang VJ, Stein KC, True HL. Spontaneous Variants of the [RNQ+] Prion in Yeast Demonstrate the Extensive Conformational Diversity Possible with Prion Proteins. *PLoS ONE.* 2013; 8:e79582. [PubMed: 24205387]
- Jones GW, Masison DC. *Saccharomyces cerevisiae* Hsp70 mutations affect [PSI+] prion propagation and cell growth differently and implicate Hsp40 and tetratricopeptide repeat cochaperones in impairment of [PSI+]. *Genetics.* 2003; 163:495–506. [PubMed: 12618389]
- Jones GW, Tuite MF. Chaperoning prions: the cellular machinery for propagating an infectious protein? *BioEssays.* 2005; 27:823–832. [PubMed: 16015602]
- Jung G, Jones G, Wegrzyn RD, Masison DC. A role for cytosolic hsp70 in yeast [PSI(+)] prion propagation and [PSI(+)] as a cellular stress. *Genetics.* 2000; 156:559–570. [PubMed: 11014806]
- Kalastavadi T, True HL. Analysis of the [RNQ+] prion reveals stability of amyloid fibers as the key determinant of yeast prion variant propagation. *J Biol Chem.* 2010; 285:20748–20755. [PubMed: 20442412]
- Kampinga HH, Craig EA. The HSP70 chaperone machinery: J proteins as drivers of functional specificity. *Nat Rev Mol Cell Biol.* 2010; 11:579–592. [PubMed: 20651708]
- Kirkland PA, Reidy M, Masison DC. Functions of yeast Hsp40 chaperone Sis1p dispensable for prion propagation but important for prion curing and protection from prion toxicity. *Genetics.* 2011; 188:565–577. [PubMed: 21555396]
- Kryndushkin DS, Alexandrov IM, Ter-Avanesyan MD, Kushnirov VV. Yeast [PSI+] prion aggregates are formed by small Sup35 polymers fragmented by Hsp104. *J Biol Chem.* 2003; 278:49636–49643. [PubMed: 14507919]
- Lee S, Sielaff B, Lee J, Tsai FTF. CryoEM structure of Hsp104 and its mechanistic implication for protein disaggregation. *Proc Natl Acad Sci USA.* 2010; 107:8135–8140. [PubMed: 20404203]
- Li J, Qian X, Sha B. Heat shock protein 40: structural studies and their functional implications. *Protein Pept Lett.* 2009; 16:606–612. [PubMed: 19519518]
- Li J, Wu Y, Qian X, Sha B. Crystal structure of yeast Sis1 peptide-binding fragment and Hsp70 Ssa1 C-terminal complex. *Biochem J.* 2006; 398:353–360. [PubMed: 16737444]
- Li L, Kowal AS. Environmental regulation of prions in yeast. *PLoS Pathog.* 2012; 8:e1002973. [PubMed: 23166488]
- Liebman SW, Chernoff YO. Prions in yeast. *Genetics.* 2012; 191:1041–1072. [PubMed: 22879407]
- Liebman SW, Bagriantsev SN, Derkach IL. Biochemical and genetic methods for characterization of [PIN+] prions in yeast. *METHODS.* 2006; 39:23–34. [PubMed: 16793281]
- Lopez N, Aron R, Craig EA. Specificity of class II Hsp40 Sis1 in maintenance of yeast prion [RNQ+]. *Mol Biol Cell.* 2003; 14:1172–1181. [PubMed: 12631732]
- Lu Z, Cyr DM. Protein folding activity of Hsp70 is modified differentially by the hsp40 co-chaperones Sis1 and Ydj1. *J Biol Chem.* 1998; 273:27824–27830. [PubMed: 9774392]
- Lum R, Tkach JM, Vierling E, Glover JR. Evidence for an unfolding/threading mechanism for protein disaggregation by *Saccharomyces cerevisiae* Hsp104. *J Biol Chem.* 2004; 279:29139–29146. [PubMed: 15128736]
- Miot M, Reidy M, Doyle SM, Hoskins JR, Johnston DM, Genest O, et al. Species-specific collaboration of heat shock proteins (Hsp) 70 and 100 in thermotolerance and protein disaggregation. *Proc Natl Acad Sci USA.* 2011; 108:6915–6920. [PubMed: 21474779]
- Moriyama H, Edskes HK, Wickner RB. [URE3] prion propagation in *Saccharomyces cerevisiae*: requirement for chaperone Hsp104 and curing by overexpressed chaperone Ydj1p. *Mol Cell Biol.* 2000; 20:8916–8922. [PubMed: 11073991]
- Mumberg D, Müller R, Funk M. Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds. *Gene.* 1995; 156:119–122. [PubMed: 7737504]

- Needham PG, Masison DC. Prion-impairing mutations in Hsp70 chaperone Ssa1: effects on ATPase and chaperone activities. *Arch Biochem Biophys*. 2008; 478:167–174. [PubMed: 18706386]
- Newnam GP, Wegrzyn RD, Lindquist SL, Chernoff YO. Antagonistic interactions between yeast chaperones Hsp104 and Hsp70 in prion curing. *Mol Cell Biol*. 1999; 19:1325–1333. [PubMed: 9891066]
- Osherovich LZ, Weissman JS. Multiple Gln/Asn-rich prion domains confer susceptibility to induction of the yeast [PSI(+)] prion. *Cell*. 2001; 106:183–194. [PubMed: 11511346]
- Park S-H, Kukushkin Y, Gupta R, Chen T, Konagai A, Hipp MS, et al. PolyQ Proteins Interfere with Nuclear Degradation of Cytosolic Proteins by Sequestering the Sis1p Chaperone. *Cell*. 2013; 154:134–145. [PubMed: 23791384]
- Park Y-N, Zhao X, Yim Y-I, Todor H, Ellerbrock R, Reidy M, et al. Hsp104 overexpression cures *Saccharomyces cerevisiae* [PSI+] by causing dissolution of the prion seeds. *Eukaryotic Cell*. 2014; 13:635–647. [PubMed: 24632242]
- Patino MM, Liu JJ, Glover JR, Lindquist S. Support for the prion hypothesis for inheritance of a phenotypic trait in yeast. *Science*. 1996; 273:622–626. [PubMed: 8662547]
- Paushkin SV, Kushnirov VV, Smirnov VN, Ter-Avanesyan MD. Propagation of the yeast prion-like [psi+] determinant is mediated by oligomerization of the SUP35-encoded polypeptide chain release factor. *EMBO J*. 1996; 15:3127–3134. [PubMed: 8670813]
- Perales-Calvo J, Muga A, Moro F. Role of DnaJ G/F-rich domain in conformational recognition and binding of protein substrates. *J Biol Chem*. 2010; 285:34231–34239. [PubMed: 20729526]
- Qian X, Hou W, Zhengang L, Sha B. Direct interactions between molecular chaperones heat-shock protein (Hsp) 70 and Hsp40: yeast Hsp70 Ssa1 binds the extreme C-terminal region of yeast Hsp40 Sis1. *Biochem J*. 2002; 361:27–34. [PubMed: 11743879]
- Reidy M, Miot M, Masison DC. Prokaryotic Chaperones Support Yeast Prions and Thermotolerance and Define Disaggregation Machinery Interactions. *Genetics*. 2012; 192:185–193. [PubMed: 22732191]
- Serio TR, Lindquist SL. [PSI+]: an epigenetic modulator of translation termination efficiency. *Annu Rev Cell Dev Biol*. 1999; 15:661–703. [PubMed: 10611975]
- Seyffer F, Kummer E, Oguchi Y, Winkler J, Kumar M, Zahn R, et al. Hsp70 proteins bind Hsp100 regulatory M domains to activate AAA+ disaggregase at aggregate surfaces. *Nat Struct Mol Biol*. 2012; 19:1347–1355. [PubMed: 23160352]
- Sha B, Lee S, Cyr DM. The crystal structure of the peptide-binding fragment from the yeast Hsp40 protein Sis1. *Structure*. 2000; 8:799–807. [PubMed: 10997899]
- Shaner L, Sousa R, Morano KA. Characterization of Hsp70 binding and nucleotide exchange by the yeast Hsp110 chaperone Sse1. *Biochemistry*. 2006; 45:15075–15084. [PubMed: 17154545]
- Sharma D, Masison DC. Functionally redundant isoforms of a yeast Hsp70 chaperone subfamily have different antiprion effects. *Genetics*. 2008; 179:1301–1311. [PubMed: 18562668]
- Sielaff B, Tsai FTF. The M-Domain Controls Hsp104 Protein Remodeling Activity in an Hsp70/Hsp40-Dependent Manner. *J Mol Biol*. 2010; 402:30–37. [PubMed: 20654624]
- Sondheimer N, Lindquist S. Rnq1: an epigenetic modifier of protein function in yeast. *Mol Cell*. 2000; 5:163–172. [PubMed: 10678178]
- Sondheimer N, Lopez N, Craig EA, Lindquist S. The role of Sis1 in the maintenance of the [RNQ+] prion. *EMBO J*. 2001; 20:2435–2442. [PubMed: 11350932]
- Stein KC, True HL. The [RNQ+] prion: a model of both functional and pathological amyloid. *Prion*. 2011; 5:291–298. [PubMed: 22052347]
- Stein KC, True HL. Extensive diversity of prion strains is defined by differential chaperone interactions and distinct amyloidogenic regions. *PLoS Genet*. 2014; 10:e1004337. [PubMed: 24811344]
- Stein KC, Bengoechea R, Harms MB, Weihl CC, True HL. Myopathy-Causing Mutations in an Hsp40 Chaperone Disrupt Processing of Specific Client Conformers. *J Biol Chem*. 2014
- Tanaka M, Chien P, Naber N, Cooke R, Weissman JS. Conformational variations in an infectious protein determine prion strain differences. *Nature*. 2004; 428:323–328. [PubMed: 15029196]

- Tessarz P, Mogk A, Bukau B. Substrate threading through the central pore of the Hsp104 chaperone as a common mechanism for protein disaggregation and prion propagation. *Mol Microbiol.* 2008; 68:87–97. [PubMed: 18312264]
- Tipton KA, Verges KJ, Weissman JS. In vivo monitoring of the prion replication cycle reveals a critical role for Sis1 in delivering substrates to Hsp104. *Mol Cell.* 2008; 32:584–591. [PubMed: 19026788]
- Toyama BH, Weissman JS. Amyloid Structure: Conformational Diversity and Consequences. *Annu Rev Biochem.* 2011; 80:557–585. [PubMed: 21456964]
- Tuite MF, Cox BS. Propagation of yeast prions. *Nat Rev Mol Cell Biol.* 2003; 4:878–890. [PubMed: 14625537]
- Tyedmers J, Mogk A, Bukau B. Cellular strategies for controlling protein aggregation. *Nat Rev Mol Cell Biol.* 2010; 11:777–788. [PubMed: 20944667]
- Uptain SM, Sawicki GJ, Caughey B, Lindquist S. Strains of [PSI(+)] are distinguished by their efficiencies of prion-mediated conformational conversion. *EMBO J.* 2001; 20:6236–6245. [PubMed: 11707395]
- Wegrzyn RD, Bapat K, Newnam GP, Zink AD, Chernoff YO. Mechanism of prion loss after Hsp104 inactivation in yeast. *Mol Cell Biol.* 2001; 21:4656–4669. [PubMed: 11416143]
- Westergard L, True HL. Wild yeast harbour a variety of distinct amyloid structures with strong prion-inducing capabilities. *Mol Microbiol.* 2014a; 92:183–193. [PubMed: 24673812]
- Westergard L, True HL. Extracellular environment modulates the formation and propagation of particular amyloid structures. *Mol Microbiol.* 2014b; 92:698–715. [PubMed: 24628771]
- Wickner RB. [URE3] as an altered URE2 protein: evidence for a prion analog in *Saccharomyces cerevisiae*. *Science.* 1994; 264:566–569. [PubMed: 7909170]
- Winkler J, Tyedmers J, Bukau B, Mogk A. Hsp70 targets Hsp100 chaperones to substrates for protein disaggregation and prion fragmentation. *J Cell Biol.* 2012; 198:387–404. [PubMed: 22869599]
- Yan W, Craig EA. The glycine-phenylalanine-rich region determines the specificity of the yeast Hsp40 Sis1. *Mol Cell Biol.* 1999; 19:7751–7758. [PubMed: 10523664]
- Yang Z, Hong JY, Derkatch IL, Liebman SW. Heterologous Gln/Asn-Rich Proteins Impede the Propagation of Yeast Prions by Altering Chaperone Availability. *PLoS Genet.* 2013; 9:e1003236. [PubMed: 23358669]
- Zhong T, Arndt KT. The yeast SIS1 protein, a DnaJ homolog, is required for the initiation of translation. *Cell.* 1993; 73:1175–1186. [PubMed: 8513501]

Fig. 1. Hdj1 can maintain propagation of some, but not all prion variants of [*PSI*+] and [*RNQ*+] (A) Normalized numbers of yeast *sis1*⁻ cells expressing Sis1 or Hdj1 and propagating the indicated [*PSI*+] variant, as compared to [*psi*-] cells, were serially diluted five-fold and spotted on ¼ YEPD to monitor prion-dependent phenotypes and propagation by colony color. (B) [*PSI*+] and [*psi*-] cell lysates expressing Sis1 or Hdj1 were subjected to SDD-AGE and western blot using an α Sup35 antibody to assess average aggregate size. (C and D) Cells propagating the indicated [*RNQ*+] variant and expressing Sis1 or Hdj1 were subjected to (C) SDD-AGE or (D) separation by high-speed ultracentrifugation into total

(T), soluble (S), and insoluble (I) fractions and SDS-PAGE, followed by western blot using an α Rnq1 antibody.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 2. Prion propagation shows variant-specific dependence on Sis1-Hdj1 chimeras
 (A) Schematic diagram of domain structure of Sis1, Hdj1, and one of the Sis1-Hdj1 chimeras, HSSH. (B) Normalized numbers of yeast *sis1*⁻ cells expressing Sis1, Hdj1, or the indicated Sis1-Hdj1 chimera and propagating weak [*PSI*+]⁻ or Sc37, with [*psi*-] *SIS1* cells as a control, were serially diluted five-fold and spotted onto ¼ YEPD solid medium to monitor prion propagation by colony color. (C) Lysates of cells propagating weak [*PSI*+]⁻ or Sc37 and expressing the indicated constructs were subjected to SDD-AGE and western blot using

an α Sup35 antibody. (D) As in (C) with cells propagating the s.d. low, s.d. medium, and s.d. very high [*RNQ+*] variants and using an α Rnq1 antibody.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 3. Distinct *Sis1* domains are involved in the propagation of specific prion conformers
 (A) Normalized numbers of yeast *sis1*⁻ cells expressing WT *Sis1* or the indicated *Sis1* mutant, and propagating weak [*PSI*⁺] or Sc37, with [*psi*⁻] *SIS1* cells as a control, were serially diluted five-fold and spotted onto 1/4 YEPD solid medium to monitor prion propagation by colony color. (B) Lysates from cells propagating weak [*PSI*⁺] or Sc37 and expressing the indicated constructs, along with [*psi*⁻] cells expressing WT *Sis1*, were subjected to SDD-AGE and western blot using an α Sup35 antibody. (C) Lysates from cells propagating the indicated [*RNQ*⁺] variant and expressing WT *Sis1* or the indicated *Sis1*

mutant, were separated by high-speed ultracentrifugation into total (T), soluble (S), and insoluble (I) fractions, followed by SDS-PAGE and western blot using an α Rnq1 antibody. (D) Summary of effects of Sis1 mutants on propagation of prion variants. Mutations are characterized as having no effect on propagation (white), mild effect (yellow), moderate effect (orange), or severe effect (red).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 4. [RNQ+] variants have a greater dependence on the Sis1-Hsp70 bipartite interaction as compared to [PSI+] variants

(A) Lysates from cells propagating the indicated [RNQ+] variant, and expressing the Sis1-L268P mutant in place of WT Sis1, were separated by high-speed ultracentrifugation into total (T), soluble (S), and insoluble (I) fractions, followed by SDS-PAGE and western blot using an α Rnq1 antibody. (B) Normalized numbers of yeast *sis1* cells expressing WT Sis1 or Sis1-L268P, and propagating the indicated [PSI+] variant, with [psi-] *SIS1* cells as a control, were serially diluted five-fold and spotted onto 1/4 YEPD plates to monitor prion propagation by colony color.

Fig. 5. [PSI⁺] variants, but not [RNQ⁺] variants, are sensitive to certain alterations in the Hsp70 ATPase cycle

(A) The amount of soluble Rnq1 protein in lysates of *ssa2* cells propagating the indicated [RNQ⁺] variant and expressing an extra copy of Ssa1 or Ssa1-21, or an empty vector (EV) control, was analyzed by well-trap assay. Cell lysates were incubated at 100°C (+) or room temperature (-) and subjected to SDS-PAGE and western blot using an αRnq1 antibody. (B) Normalized numbers of *ssa2* cells propagating strong [PSI⁺] and expressing an extra copy of Ssa1 or Ssa1-21, or an EV control, were serially diluted five-fold and spotted onto the

indicated media to monitor prion propagation by colony color (SD-leu) or cell growth (SD-leu-ade). (C) WT cell lysates propagating the indicated [*RNQ+*] variant and over-expressing Ssa1 or Sse1, as compared to an EV control, were analyzed by well-trap assay as in (A). (D) *SSE1* and *sse1* cell lysates propagating the indicated [*RNQ+*] variant, as compared to [*rnq* −] *sse1* cells, were analyzed by well-trap assay as described in (A).

Fig. 6. [RNQ+] variants show similar reliance on Hsp104 function, as assessed by Hsp104-ClpB chimeras

Yeast *hsp104* cells harboring an *HSP104* covering plasmid and propagating the indicated [RNQ+] variant, had WT Hsp104 replaced by either ClpB or the indicated Hsp104-ClpB chimeras, with WT Hsp104 and an empty vector (EV) as controls. Cell lysates were subjected to SDD-AGE and western blot using an α Rnq1 antibody.