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Swa2, the yeast homolog of mammalian auxilin, is specifically required for the propagation of the prion variant [*URE3-1*]

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Summary

Yeast prions require a core set of chaperone proteins including Sis1, Hsp70 and Hsp104 to generate new amyloid templates for stable propagation, yet emerging studies indicate that propagation of some prions requires additional chaperone activities, demonstrating chaperone specificity beyond the common amyloid requirements. To comprehensively assess such prion-specific requirements for the propagation of the [*URE3*] prion variant [*URE3-1*], we screened 12 yeast cytosolic J-proteins, and here we report a novel role for the J-protein Swa2/Aux1. Swa2 is the sole yeast homolog of the mammalian protein auxilin, which, like Swa2, functions in vesicle-mediated endocytosis by disassembling the structural lattice formed by the protein clathrin. We found that, in addition to Sis1, [*URE3-1*] is specifically dependent upon Swa2, but not on any of the 11 other J-proteins. Further, we show that [*URE3-1*] propagation requires both a functional J-domain and the tetratricopeptide repeat (TPR) domain, but surprisingly does not require Swa2-clathrin binding. Because the J-domain of Swa2 can be replaced with the J-domains of other proteins, our data strongly suggest that prion-chaperone specificity arises from the Swa2 TPR domain and supports a model where Swa2 acts through Hsp70, most likely to provide additional access points for Hsp104 to promote prion template generation.

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Introduction

Transmissible spongiform encephalopathies including scrapie in sheep, Creutzfeldt–Jakob disease and mad cow disease, are now understood to be diseases transmitted by a misfolded and aggregated protein called PrP for prion protein (Prusiner, 2013). Because these aggregates are self-templating and infectious, they were first termed 'prions' denoting proteinaceous infectious particles (Prusiner, 1982). In *Saccharomyces cerevisiae*, prions confer heritable traits by acting as nonchromosomal genetic elements inherited through the cytoplasm (Liebman and Chernoff, 2012). Although numerous proteins are now thought to potentially form prions in yeast, the first two prions to be discovered were [*PSI*$^{+}$] and [*URE3*], which are the aggregated forms of the proteins Sup35 and Ure2 respectively (Wickner, 1994; Wickner *et al.*, 1995). Sup35 is a translation termination factor and Ure2 is involved in repression of nitrogen catabolism genes in poor nitrogen sources (Stansfield *et al.*, 1995; Cooper, 2002).

Yeast prions are self-propagating, such that soluble protein will misfold into the prion conformation in the presence of prion aggregates that serve as templates, joining the ends of the amyloid filaments by taking on the conformation dictated by the filament molecules (Serio *et al.*, 2000; Liebman and Chernoff, 2012). During cell division, these 'seeds,' now more commonly called 'propagons,' are passed from mother to daughter cell through the cytoplasm (Wickner, 1994; Cox *et al.*, 2003; Derdowski *et al.*, 2010). A set of molecular chaperone proteins that includes the disaggregase Hsp104, the Hsp70 Ssa, and the Hsp40/J-protein Sis1, is critical for the propagation of amyloid-based yeast prions (Chernoff *et al.*, 1995; Newnam *et al.*, 1999; Jung *et al.*, 2000; Sondheimer *et al.*, 2001; Higurashi *et al.*, 2008; Hines *et al.*, 2011b). Current models assert that Sis1 and Ssa bind directly to amyloids and unfold a portion of the amyloid-forming protein, which is then transferred to Hsp104 (Aron *et al.*, 2007; Satpute-Krishnan *et al.*, 2007; Tipton *et al.*, 2008; Haslberger *et al.*, 2010; Winkler *et al.*, 2012b). This polypeptide is then pulled through the central cavity of the Hsp104, at the expense of adenosine triphosphate (ATP), unfolding the monomeric

unit and resulting in the eventual fragmentation of amyloid aggregates (Tipton *et al.*, 2008; Winkler *et al.*, 2012a,b).

Hsp40s, hereafter called J-proteins, stimulate the ATPase activity of Hsp70, which stabilizes its interactions with client proteins (Cheetham and Caplan, 1998). The defining characteristic of all J-proteins is a small region of 60–80 residues known as a J-domain that is responsible for this interaction with Hsp70. J-domains contain a conserved histidine–proline–aspartic acid (HPD) motif that is responsible for stimulating Hsp70 ATPase activity (Kampinga and Craig, 2010). While a J-domain alone can enhance Hsp70 peptide binding, some J-proteins can also bind polypeptides directly and deliver them to Hsp70s. Thus, a single Hsp70 can participate in numerous distinct functions because J-proteins can act as specificity factors, directing and diversifying Hsp70 function (Kampinga and Craig, 2010). Sis1 is one of 13 cytosolic J-proteins found at least partially in the cytosol of *S. cerevisiae* and is specifically required for the continued propagation of the prions [*PSI*$^+$], [*RNQ*$^+$], [*SWI*$^+$], and [*URE3*] in cell populations (Sondheimer *et al.*, 2001; Sahi and Craig, 2007; Higurashi *et al.*, 2008; Hines *et al.*, 2011b). Despite its apparent universal involvement in the propagation of amyloid-based yeast prions, the role of Sis1 is biochemically complex, as distinct portions of the protein are required for the maintenance of different prions (Harris *et al.*, 2014; Reidy *et al.*, 2014; Stein and True, 2014; Sporn and Hines, 2015). Because J-proteins often act as targeting factors for Hsp70s, they may constitute the first response to the presence of amyloid *in vivo*, but a critical barrier to advancing the understanding of chaperone function in prion biology is that the fundamental chaperone requirements for most yeast prions remain unidentified, precluding a comprehensive understanding of how protein sequences give rise to amyloids with distinct patterns of chaperone interaction. The inadequacy of the current state of knowledge is underscored by our recent discovery of the first additional J-protein requirement for [*SWI*$^+$]. Specifically, in a previous investigation, we determined that [*SWI*$^+$] propagation requires not only Sis1 but also Ydj1, the yeast ortholog of bacterial DnaJ and the primary J-protein in the yeast cytosol (Caplan and Douglas, 1991; Ghaemmaghami *et al.*, 2003; Hines *et al.*, 2011b).

The discovery that [*SWI*$^+$] requires two J-proteins begs the question: *Do other yeast prions require multiple J-proteins for their propagation?* To directly address this issue, here we examine the potential for additional J-protein requirements by the prion [*URE3-1*], which is the strong [*URE3*] variant originally isolated by Aigle and Lacroute and the variant used in many previous investigations into [*URE3*]–chaperone interactions (Aigle and Lacroute, 1975; Fernandez-Bellot *et al.*, 2000; Higurashi *et al.*, 2008; Wickner *et al.*, 2014). While eliminating 11 cytosolic J-proteins, we discovered an essential role for the J-protein Swa2 and now report the first instance of the direct involvement of an auxilin homolog in prion biology. Swa2 (also called Aux1), is the fungal homolog of the mammalian protein auxilin, which is involved in protein trafficking, disassembling the lattice formed by the coat protein clathrin (Ungewickell *et al.*, 1995; Holstein *et al.*, 1996; Gall *et al.*, 2000; Pishvaee *et al.*, 2000). Auxilin recruits Hsp70 to clathrin cages and stimulates the ATPase activity of Hsp70 via its J-domain following vesicle formation at the trans-Golgi network, plasma membrane and endosomes (Holstein *et al.*, 1996; McMahon and Boucrot, 2011; Krantz *et al.*, 2013). Like auxilin, Swa2 contains N-terminal clathrin-binding domains, which bind clathrin molecules during the disassembly of clathrin lattices in endocytosis (Pishvaee *et al.*, 2000; Xiao *et al.*, 2006; Krantz *et al.*, 2013). Although Swa2 has three functional clathrin-binding domains, only one is required for clathrin binding *in vivo* (Xiao *et al.*, 2006). The N-terminus of the protein also contains a 41-residue long ubiquitin-associated (UBA) domain that contains two ubiquitin binding sites (Chim *et al.*, 2004; Matta-Camacho *et al.*, 2009). Although the precise function of this domain is unknown, it most likely interacts with ubiquitinated cargo proteins during endocytosis (Xiao *et al.*, 2006; Matta-Camacho *et al.*, 2009). Swa2 also has three tetratricopeptide repeats that form a TPR domain near its C-terminus; while TPR domains typically mediate protein–protein interactions, the relevant binding partner of the Swa2 TPR domain *in vivo* is unknown (Xiao *et al.*, 2006). Finally, like all J-proteins, Swa2 contains a J-domain, but its position at the extreme C-terminus is unusual as all other cytosolic J-proteins in *S. cerevisiae* have N-terminal J-domains (Gall *et al.*, 2000; Pishvaee *et al.*, 2000; Kampinga and Craig, 2010). The functional significance of the C-terminal location for the Swa2 J-domain remains unclear, but it may be important for proper geometric orientation of Swa2 on the clathrin lattice (Xing *et al.*, 2010).

Intriguingly, we found that the requirement for Swa2 by [*URE3-1*] does not involve the N-terminal domains necessary for clathrin-binding, but rather we localize the essential functions to the C-terminal J and TPR domains. We also show that the TPR domain is sufficient to dictate the specific requirement for Swa2 as the J-domain is replaceable with the J-domain from either the human ortholog or a yeast paralog. Finally, we clarify that the requirement for Swa2 is not shared by the prion [*PSI*$^+$] regardless of prion variant strength. The finding that Swa2 is required for [*URE3-1*] propagation in addition to Sis1, is now the second discovery of a 'secondary' J-protein requirement among yeast prions in just the past few years. The fact that these interactions remain to be discovered underscores the notion that we are far from fully understanding the breadth of prion–chaperone interactions responsible for amyloid propagation in yeast.

Results

11 J-proteins are dispensable for [URE3-1] propagation

In a previous investigation, we determined that only one of the 13 J-proteins present in the *S. cerevisiae* cytosol, Sis1, is required for the propagation of prions [*RNQ*$^{+}$] and [*PSI*$^{+}$] (Higurashi *et al*., 2008). The same study also demonstrated that Sis1 is also required for [*URE3-1*] propagation, but any potential requirements for the other 12 J-proteins in [*URE3-1*] maintenance remained undetermined. To examine whether [*URE3-1*] might also exhibit J-protein requirements in addition to the requirement for Sis1, a set of 12 [*RNQ*$^{+}$], [*psi*$^{-}$], [*ure-o*] strains, each carrying a deletion of one of the genes encoding the cytosolic J-proteins were crossed with a parental strain containing [*URE3-1*]. Following diploid sporulation and subsequent tetrad dissection, the presence or absence of [*URE3-1*] in the resulting haploids was determined using a red/white colony color assay that is based on the accumulation of a red-pigmented intermediate that is produced when there is a block in the adenine biosynthesis pathway; [*URE3*] colonies are white on rich medium while otherwise clonal [*ure-o*] (lacking [*URE3*]) colonies are red (Schlumpberger *et al*., 2001; Brachmann *et al*., 2005; Talarek *et al*., 2005; see *Experimental Procedures* for additional details). We were able to isolate [*URE3-1*]-maintaining haploids that stably propagated the prion in strains bearing individual deletions of 11 of the 12 J-proteins examined (Fig. 1A, first column). To confirm that the white color of these colonies is due to the presence of [*URE3-1*], rather than a spontaneous mutation that suppresses the accumulation of the red pigment, we assayed for the curability of the prion phenotype by growth on medium supplemented with GdnHCl. Each strain was grown for 48 h in media with 4 mM GdnHCl, which potently inhibits the action of Hsp104 and rapidly eliminates [*URE3*] (Wickner, 1994; Ferreira *et al*., 2001; Jung and Masison, 2001; Jung *et al*., 2002; Grimminger *et al*., 2004). A colony color change was observed in each case indicating the loss of the prion (Fig. 1A, second column). In contrast to the other 11 strains, when a *swa2-*Δ strain (*swa2::HIS3*) was crossed with the [*URE3-1*] parental strain, we were unable to isolate phenotypically [*URE3-1*] (white) cells in any haploids that inherited the *swa2::HIS3* allele. The colonies in Fig. 1B are representative of 96 haploid cells that were analyzed from 48 complete tetrads resulting from [*URE3-1*] diploids; in every case when the *swa2::HIS3* allele was present, the prion phenotype was absent. The [*URE3*] color phenotype is biochemically complex and alterations to the Ure2 binding partner Gln3 or to the relevant nuclear target of Gln3, the *DAL5* promoter (see *Experimental Procedures* for details), could alter or even block [*URE3*] detection by the colony color reporter assay

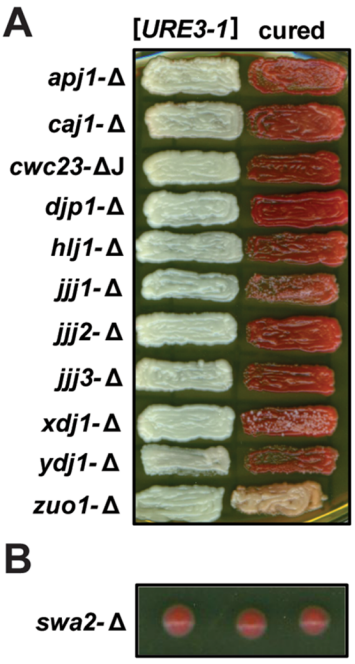

Fig. 1. 11 cytosolic J-proteins are dispensable for [*URE3-1*] propagation.
A. [*URE3-1*] is stably maintained in strains having any one of 11 J-protein deletions (first column, white color). Haploid W303 [RNQ^{+}], [*psi*$^{-}$] strains bearing J-protein gene deletions were crossed with white [*URE3-1*] (also [RNQ^{+}] and [*psi*$^{-}$]) strains. Following sporulation and tetrad dissection, J-protein-Δ haploids were assayed for the maintenance of [*URE3-1*] by colony color on rich glucose-based medium. Cells were cured by treatment with GdnHCl and plated onto rich medium to confirm that the observed color phenotype is prion-based (reddish color, second column). All strains have a complete gene deletion except *cwc23-ΔJ*, which has Cwc23 lacking its J-domain expressed from a plasmid in order to support cell viability (Higurashi *et al*., 2008; Sahi *et al*., 2010). Note: the cured *zuo1-Δ* strain is lighter red because of zuo1-dependent nonsense suppression (Higurashi *et al*., 2008).
B. Haploids isolated from crosses, which lack Swa2 (*swa2-Δ*) are invariably red indicating a possible inability to maintain the prion in these strains (three representative colonies shown). Colonies are representative of 96 haploid cells that were analyzed from 48 separate tetrads resultant of the cross between a *swa2-Δ* strain and a [*URE3-1*] strain.

without affecting the prion itself (Edskes *et al*., 2006; Puria *et al*., 2008). To rule out the possibility that the loss of Swa2 affects the ability of cells to manifest the [*URE3*] color phenotype, rather than the prion, we backcrossed these F1 haploids to cured versions of the parental [*URE3-1*] strains, now [*ure-o*] and phenotypically red. If [*URE3-1*] were hidden by the loss of the color phenotype in the *swa2-*Δ haploids, mating to these cured parental strains should result in a restored white phenotype in the resulting diploids. Consistent with the interpretation that [*URE3-1*] was actually lost, all resulting diploids, and *SWA2* haploids, isolated following sporulation and tetrad dissection of the diploids, formed red colonies (*data not*

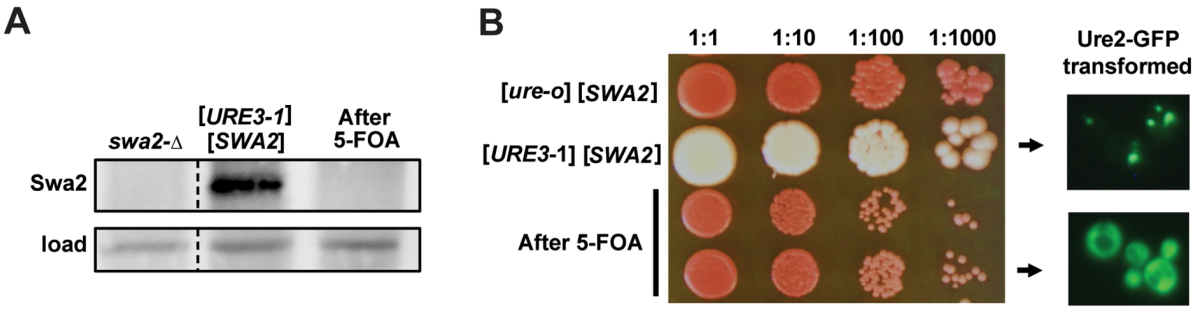

Fig. 2. Loss of Swa2 expression induces immediate loss of [*URE3-1*].
A. Lysates of a strain lacking Swa2 expression (*swa2-Δ*) and a [*URE3-1*] strain expressing Swa2 only from a *URA3*-marked plasmid before or after passage on medium containing 5-FOA were resolved by SDS-PAGE and subjected to immunoblot analysis using an antibody specific for Swa2. Dashed lines separate lanes taken from different parts of the same gel. A band-cross reacting with the Swa2 antibody is shown as a loading control.
B. Yeast cultures of equivalent density in log phase were serially diluted 10-fold and spotted in 5 μL drops on rich medium, and the image was collected after 72 h. of growth at 25°C to allow color development. [*ure-o*] and [*URE3-1*] strains expressing Swa2 only from a *URA3*-marked plasmid (rows 1 and 2 respectively) exhibit the slow-growth phenotype characteristic of *swa2-Δ* cells only following passage on 5-FOA medium (rows 3 and 4). Loss of [*URE3-1*] is complete and coincident with loss of Swa2 expression (compare rows 2 and 4). Results shown are for one representative of three identical replicates. The loss of the prion was also verified by characteristic fluorescence patterns. Strains in rows 2 and 4 were transformed by a vector expressing Ure2-GFP and visualized by fluorescence microscopy. Colonies from strains shown in rows 2 and 4 (before and after selection on 5-FOA medium) were transformed by a plasmid-bearing Ure2-GFP and the presence or absence of [*URE3-1*] was confirmed by examination of Ure2-GFP aggregation based on fluorescence (panel B, right side).

shown). These results strongly suggest that Swa2 is necessary for [*URE3-1*] propagation.

Swa2 is essential for [URE3-1] propagation in mitosis and prion loss is not likely due to an induced stress response

The observation that *swa2-Δ* strains isolated from crosses lack [*URE3-1*] indicates a potential role for Swa2 in [*URE3-1*] prion propagation. However, it is also possible that [*URE3-1*] simply becomes unstable during meiosis when these strains are crossed. To eliminate this possibly confounding factor, we next mated a [*URE3-1*] strain with a strain carrying both a genomic deletion of *SWA2* (*swa2::HIS3*) and a covering *URA3*-marked plasmid expressing Swa2 from its endogenous promoter ([*SWA2*-Swa2, *URA3*]). Following sporulation and tetrad dissection, we isolated a [*URE3-1*] haploid strain suitable for plasmid-shuffling experiments (see *Experimental Procedures*). Passage of this strain on medium containing 5-fluoroorotic acid (5-FOA) selects for cells in the population that have spontaneously lost the *URA3*-marked plasmid, and hence, have lost Swa2 protein expression. To confirm that Swa2 expression is indeed lost following passage on 5-FOA media, we subjected yeast cell lysates to SDS-PAGE followed by immunoblotting using a polyclonal antibody specific for Swa2. As expected, Swa2 expression is detectable in our plasmid-shuffling strain, but is undetectable following growth on 5-FOA medium (Fig. 2A), verifying that this treatment resulted in the complete loss of Swa2 protein expression.

Next, we examined [*URE3-1*] prion maintenance in this strain. Upon deletion of *SWA2*, cells exhibit a slow-growth phenotype, which is apparent when single colonies are grown on rich solid medium (Xiao *et al*., 2006). Passage onto medium containing 5-FOA resulted in the formation of red, slow-growing colonies, indicating immediate and complete prion loss coincident with loss of the Swa2-expressing plasmid (Fig. 2B, compare second and fourth rows). To confirm that 5-FOA treatment did not alter the strain color or growth phenotype independent of its effect on [*URE3-1*], we also included a genetically identical [*ure-o*] strain as a control (Fig. 2B, first and third rows). Indeed, following 5-FOA treatment no difference is evident between cured strains (Fig. 2B, compare third and fourth rows). To confirm that the color phenotypes are accurately reporting the presence or absence of the prion, strains were also transformed by a plasmid expressing a Ure2-green florescent protein (GFP) chimera. [*URE3-1*] cells exhibit a punctate pattern of florescence because the fluorophore is localized into prion aggregates, while [*ure-o*] cells have a diffuse florescence pattern indicative of soluble Ure2-GFP that is diffused throughout the cytoplasm (Edskes *et al*., 1999; Brachmann *et al*., 2006). As expected, cells expressing full-length Swa2 prior to 5-FOA treatment exhibited a punctate fluorescence pattern, while these same cells, following growth on 5-FOA, exhibit only diffuse fluorescence, confirming the total loss of [*URE3-1*] coincident with loss of the Swa2-expressing plasmid (Fig. 2B).

Like other yeast prions, [*URE3*] is sensitive to the ectopic expression of certain chaperones. Specifically,

overexpression of either Ydj1 or Sse1 cures [*URE3*] (Moriyama *et al.*, 2000, Kryndushkin & Wickner, 2007). Additionally, while overexpression of either Sis1 or Hsp104 destabilizes [*URE3*], the prion is rapidly cured by reductions in both Sis1 and Hsp104 activity (Wickner, 1994, Higurashi *et al.*, 2008, Reidy *et al.*, 2014, Moriyama *et al.*, 2000). To address whether the effects that we have observed thus far are due to a direct effect of the lack of Swa2 protein or to a secondary consequence such as a change in expression of one of these aforementioned chaperones because of an induced stress response, we next explored whether the levels of these other chaperones are altered in our strain lacking Swa2. Lysates of cells shown in Fig. 2A before and after 5-FOA treatment were resolved by SDS-PAGE followed by immunoblotting with antibodies for Ydj1, Sis1, Ssa1-4, Sse1 and Hsp104. Indeed no changes in expression levels of any of these proteins were apparent (Fig. S1), indicating that deletion of Swa2 is not likely resulting in a significant stress response. Taken together, these results strongly support the notion that Swa2 is essential for [*URE3-1*] propagation during normal mitotic cell division and that this genetic interaction is not likely due to alterations in other chaperone protein expression levels.

Swa2 is nonessential for [PSI$^+$] propagation regardless of prion variant strength

Structural variations in the amyloid-core structure and other adjacent regions can often result in distinct prion strains, more often termed 'variants' in yeast (Liebman and Chernoff, 2012). Prion variants are usually classified as 'strong' or 'weak' based on phenotypic strength and mitotic stability (Derkatch *et al.*, 1996). [*PSI*$^+$] variants are distinguished phenotypically by a colorimetric assay that depends upon the amount of soluble Sup35 within the cell (Zhou *et al.*, 1999). Prion variants can also have distinct chaperone requirements, as was recently demonstrated explicitly for both [*PSI*$^+$] and [*RNQ*$^+$] (Hines *et al.*, 2011a; Harris *et al.*, 2014; Stein and True, 2014; Sporn and Hines, 2015). A previous investigation demonstrated that Swa2 expression is dispensable for both [*PSI*$^+$] and [*RNQ*$^+$] propagation, and no phenotypic changes in colony color (for [*PSI*$^+$]) or aggregate size (for either prion) were observed upon *SWA2* deletion, indicating that the Swa2 is unlikely involved in the propagation of either prion (Higurashi *et al.*, 2008). However, this investigation utilized a single strong variant of [*PSI*$^+$], called [*PSI*$^+$]^{STR} (Higurashi *et al.*, 2008). Because different prion variants can exhibit distinct requirements for a J-protein, the question of whether weaker variants of either prion also require Swa2 activity for prion propagation remains unanswered. Despite only having a few well-studied yeast prions on which to draw comparisons, prion propagon number appears to be inversely correlated with co-chaperone requirements (Hines and Craig, 2011; Hines *et al.*, 2011b). Because [*URE3-1*] has a relatively low number of propagons per cell compared with other prions (Hines and Craig, 2011), we hypothesized that weak [*PSI*$^+$] variants, which also have low propagon numbers, may also share a requirement for Swa2. To test this hypothesis, we conducted another classical mating experiment. To create [*PSI*$^+$] cells with a genomic deletion of *SWA2*, cells bearing one of four different [*PSI*$^+$] variants (two strong variants called [*PSI*$^+$]^{Sc4} and [*PSI*$^+$]^{93S} and two weak variants called [*PSI*$^+$]^{Sc37} and [*PSI*$^+$]^{94W}) were mated with our strain lacking genomic Swa2 expression (*swa2::HIS3*), but expressing Swa2 from a *URA3*-marked plasmid ([*SWA2*-Swa2*, URA3*]) (Tanaka *et al.*, 2004; Harris *et al.*, 2014). Diploids now heterozygous for the *SWA2* allele (*SWA2*/*swa2*::*HIS3*) were isolated and then sporulated and subjected to tetrad dissection, resulting in the selection of haploid strains with the genotype: [*PSI*$^+$], *ade1-14*, *ADE2*, *swa2::HIS3*, [*SWA2*-Swa2*, URA3*]. Haploid genotypes were confirmed by prototrophic growth on selective media. The continued presence of the prion was confirmed by colony color assay and curability upon treatment with GdnHCl. In this case, [*psi*$^-$] haploids become red on rich medium because of the block in the adenine pathway that is due to a nonsense mutation of the *ADE1* gene (*ade1-14*). In [*PSI*$^+$] cells, the translation termination factor Sup35 is sequestered into [*PSI*$^+$] aggregates resulting in enhanced read-through of the nonsense codon producing colonies that are white or light pink (Liebman and Chernoff, 2012).

We next passaged these [*PSI*$^+$] strains on medium containing 5-FOA. A total of 12 isolates were examined for each [*PSI*$^+$] variant and representative strains are shown in Fig. 3. Despite the loss of the covering Swa2 expression plasmid, all strains remained [*PSI*$^+$] with no notable phenotypic changes, regardless of [*PSI*$^+$] variant identity or strength, as indicated by white/pink colony color as compared with the [*PSI*$^+$] parent-strain (pink) and cured [*psi*-] strain (red) used as a control for colony color (Fig. 3A). Once again to ensure that the color phenotype was correctly attributed to the presence of the prion, all cells were cured by growth on GdnHCl-containing medium (Fig. 3A, fourth column). Finally, to again rule out the possibility of unanticipated lingering Swa2 expression in these strains because of a rare homologous recombination event during the experimental manipulations, lysates of cells shown in Fig. 3A were subjected to immunoblotting. As expected, Swa2 expression was undetectable in all experimental strains as compared with a wild-type control strain (Fig. 3B), confirming that Swa2 is indeed dispensable for strong or weak [*PSI*$^+$] propagation.

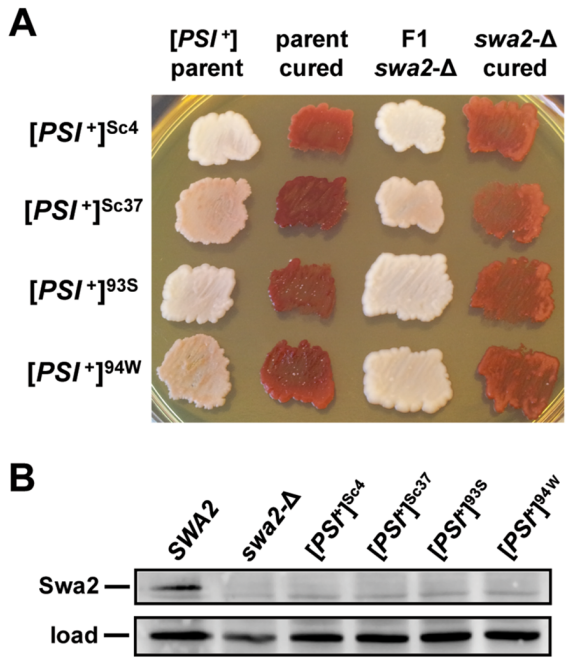

Fig. 3. Swa2 is not required for strong or weak [*PSI*$^+$] variant propagation.
A. Mating experiments to determine if Swa2 is essential for [*PSI*$^+$] variant propagation. Parental strong [*PSI*$^+$] strains ([*PSI*$^+$]^{Sc4} and [*PSI*$^+$]^{93S}) and weak [*PSI*$^+$] strains ([*PSI*$^+$]^{Sc37} and [*PSI*$^+$]^{94W}) (column 1, *[PSI*$^+$*] parent*) were mated with yeast only expressing Swa2 from a *URA3*-marked plasmid. Following sporulation and loss of the *URA3*-marked plasmid, F1 haploid isolates lacking Swa2 expression (column 3, *F1 swa2-Δ*) were patched onto rich medium to score for prion maintenance by colony color phenotype. Parent cells and F1 *swa2-Δ* progeny were then cultured in the presence of GdnHCl and plated (columns 2 and 4, *parent cured* and *swa2-Δ cured*) to ensure that colony color accurately reports the presence or absence of the prion.
B. Immunoblot showing Swa2 protein expression, or lack-thereof, in *swa2-Δ* haploid cells bearing [*PSI*$^+$] variants from panel A. Cell lysates were subjected to immunoblot analysis using antibody specific for Swa2. A band-cross reacting with the Swa2 antibody is shown as a loading control.

N-terminal Swa2 domains are dispensable for [URE3-1] propagation

Swa2 is a complex, multi-domain protein with regions that may interact *in vivo* with Hsp70, clathrin, ubiquitin and perhaps other proteins (Xiao *et al.*, 2006). To begin to determine the role of Swa2 in [*URE3-1*] prion propagation, we next investigated which domains of Swa2 might be essential to this process. Because Swa2 is the sole yeast homolog of the mammalian protein auxilin, we first hypothesized that Swa2 is required for [*URE3-1*] propagation through an uncharacterized clathrin-mediated process. To test this hypothesis, we transformed our plasmid-shuffling strain with various *LEU2*-marked plasmids containing well-characterized deletions of the Swa2 N-terminal domains (Fig. 4A) (Xiao *et al.*, 2006; Martineau *et al.*, 2010). Following passage on 5-FOA, cells were plated onto rich medium to assay for the continued presence of the prion by colony color. An empty vector and a vector expressing full-length Swa2 were included as negative and positive controls respectively. At least 10 individual transformants were scored for each plasmid examined and representative isolates are shown in Fig. 4A. As expected, [*URE3-1*] was lost (20 out of 20 shuffled transformants) or maintained (19 out of 20 shuffled transformants) when empty vector or vector expressing full-length Swa2 were used respectively (Fig. 4A). When a construct bearing a deletion of the UBA domain (Swa2ΔUBA) was used, [*URE3-1*] was maintained in 17 of 17 shuffled transformants. This result indicates that Swa2's role in [*URE3-1*] propagation is unlikely to require an interaction with ubiquitin. Similarly, cells shuffled with a construct bearing a deletion of the first two clathrin-binding domains, as well as the UBA domain (Swa2Δ2–302), maintained [*URE3-1*] in 17 of 17 shuffled transformants. Most importantly, [*URE3-1*] was maintained by 10 of 11 transformants even upon Swa2's replacement with a construct bearing a truncation of all four N-terminal domains, which includes all three clathrin-binding domains (Swa2Δ2–362). Because Swa2Δ2–362 was previously shown to be incapable of clathrin binding *in vitro* and *in vivo* (Xiao *et al.*, 2006), the ability of this construct in particular to maintain the prion strongly implies that clathrin binding is not necessary for Swa2's role in [*URE3-1*] propagation. Expression of each truncation construct was confirmed by SDS-PAGE followed by immunoblotting with a polyclonal Swa2 antibody (Fig. S2); the absence of a distinct band at the expected position of full-length Swa2 in each of these strains confirms that prion maintenance is accomplished by each truncated protein alone and not because of unexpected maintenance of the original plasmid, or a rare recombination event. Finally, to ensure that the white color of each of these Swa2 truncation strains is still due to the presence of [*URE3-1*], rather than a suppressing mutation, we again treated each strain with 4 mM GdnHCl. As expected, after just two days of growth in liquid culture, each strain lost the white phenotype and became phenotypically [*ure-o*] (Fig. S3). This experiment also demonstrates that the loss of the N-terminal domains of Swa2 does not affect the reliance of [*URE3-1*] on Hsp104 activity or the ability of GdnHCl to inhibit Hsp104 and prevent prion propagation. Together these results demonstrate that the Swa2 N-terminal domains are unnecessary for [*URE3-1*] propagation. Further, because all of the clathrin-binding domains are in the N-terminus, these results also surprisingly indicate that despite auxilin's well-established role in clathrin vesicle uncoating and Swa2 being the only known auxilin homolog in yeast, Swa2's role in [*URE3-1*] propagation is unlikely to involve clathrin.

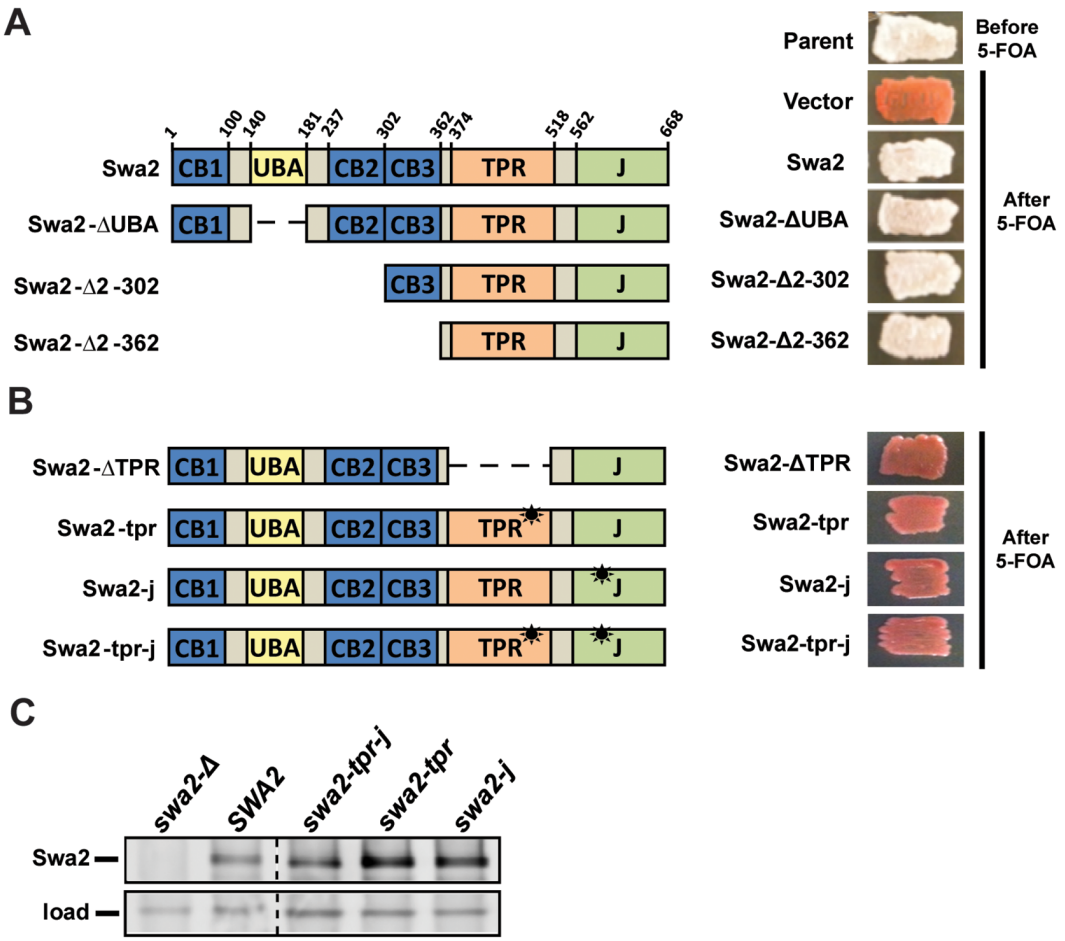

Fig. 4. Swa2 domain requirements for [*URE3-1*] propagation.
A. Swa2-plasmid-shuffling experiments to examine the potential effects of Swa2 N-terminal domain deletions on [*URE3-1*] propagation. (A and B) Primary sequence diagrams of Swa2 constructs used in the plasmid-shuffling experiments are illustrated on the left. Domains are denoted using the following notation: *CB1–3*, clathrin-binding domains 1–3; *UBA*, ubiquitin associated domain; *TPR*, tetratricopeptide repeat domain; *J*, J-domain (Xiao *et al*., 2006). Dashed lines indicate where a region had been deleted. Plasmid-shuffling strains were transformed by plasmids expressing Swa2 truncations, deletions, or point mutations and subjected to plasmid shuffling. Color phenotype assays are shown for representative transformants ($n \geq 10$ for each plasmid) following loss of the *URA3*-marked plasmid-bearing variants of *SWA2*. The parental [*URE3-1*] strain is included for a color control (parent). Cells expressing either empty *pRS315* (vector) or *pRS315-SWA2* (Swa2) were used as negative and positive controls, respectively, for the stability of the prion throughout the plasmid-shuffling assay.
B. Swa2-plasmid-shuffling experiments to examine the potential effects of Swa2 C-terminal domain deletions and mutations on [*URE3-1*] propagation.
C. Cell lysates from isolates shown in (B) as well as a wild-type strain (wt) and a strain lacking Swa2 expression (*swa2-Δ*) were resolved by SDS-PAGE and subjected to immunoblot analysis using polyclonal antibody raised against Swa2. Dashed lines separate lanes taken from different parts of the same gel. A band-cross reacting with the Swa2 antibody is shown as a loading control.

Swa2's C-terminal TPR and J-domains are both individually necessary and together sufficient for [URE3-1] propagation

Because the Swa2 N-terminal domains are dispensable for [*URE3-1*] propagation, we next turned our attention to the C-terminal domains of the protein. First we investigated constructs bearing mutations or a deletion of the TPR domain. When the entire TPR domain was deleted from Swa2, [*URE3-1*] was no longer maintained as 20 out of 20 shuffled transformants were red when plated onto rich medium (Fig. 4B). It is plausible that the inability of Swa2ΔTPR to support [*URE3-1*] may be due to either improper protein folding or low expression as the result of the removal of the entire domain, rather than because of the loss of some specific function of the TPR domain *per se*. Because our polyclonal Swa2 antibody does not recognize the Swa2ΔTPR construct, we were unable to rule out expression level as a causative factor with a simple Western blot. Rather, to address both of these issues simultaneously, we next considered whether a single-point mutation, which is expected to compromise TPR function,

would ablate [*URE3-1*] propagation. Based on structural comparisons with the highly conserved TPR domains of the human protein HOP (Hsp organizing protein), the Graham lab previously identified a conserved glycine-to-arginine mutation (Gly^{388}→Arg) that is predicted to disrupt a structurally conserved β-turn between two amphipathic helices within the TPR domain and thereby disrupt binding to client peptides (Gall *et al*., 2000; Xiao *et al*., 2006). This mutation (referred to here and also elsewhere as 'Swa2-tpr' or the *swa2-1* allele) is expressed at wild-type levels and both binds clathrin and stimulates Ssa1 ATPase activity equivalently to the wild-type protein, indicating that this point mutation affects only the function of the TPR domain and is unlikely to disrupt overall protein fold (Gall *et al*., 2000; Xiao *et al*., 2006). When used in our experimental system, 20 out of 20 shuffled transformants expressing this mutant protein lost [*URE3-1*] (Fig. 4B), indicating that the function of the Swa2-TPR domain, specifically, is important for [*URE3-1*] propagation.

Next, in order to determine if Swa2's J-domain needs to be functional for [*URE3-1*] propagation, we examined a variant of Swa2 lacking the canonical HPD motif that is responsible for stimulation of Hsp70 ATPase activity. This mutant, referred to here and elsewhere as 'Swa2-j', carries the triple alanine substitution HPD→AAA (Xiao *et al*., 2006). Like the Gly^{388}→Arg mutant Swa2-tpr, Swa2-j was previously found to be expressed at near wild-type levels (Xiao *et al*., 2006). Yet in our experiment, cells carrying this mutant as their sole version of Swa2 lost [*URE3-1*] in 20 of 20 shuffled transformants, indicating that Swa2's role in [*URE3-1*] propagation requires a functional J-domain and therefore, involves Hsp70 (Fig. 4B). Additionally, and as expected, when both the HPD→AAA and Gly^{388}→Arg mutations were combined (Swa2-tpr-j), the prion was also lost in all 20 shuffled transformants.

To confirm that the loss of the prion was the result of the particular alterations of the Swa2 protein and not due to poor protein expression in our strains, transformant lysates were again subjected to SDS-PAGE and immunoblotting using the antibody specific for Swa2. Bands corresponding to each Swa2 variant, with the exception of Swa2ΔTPR, were apparent and expressed at equal or higher levels than the wild-type protein (Fig. 4C). In summation, the results described thus far indicate that both Swa2 C-terminal domains are individually essential and together sufficient for [*URE3-1*] propagation and also strongly suggest that a functional interaction with Hsp70 is imperative.

The J-domain of either human auxilin or yeast Sis1 can replace that of Swa2 in [URE3-1] propagation

The requirement for Swa2 by [*URE3-1*] appears to be specific both to Swa2, as other J-proteins are not required, and to [*URE3-1*], as other prions tested thus far do not share this requirement for Swa2 activity. *What determines this prion-chaperone specificity?* Because Swa2's J-domain is essential for [*URE3-1*] propagation, we next wanted to determine if there is something specific and unusual about Swa2's own J-domain that is critical for [*URE3-1*] propagation and is responsible for the specific interaction with [*URE3-1*], or if the role of the Swa2 J-domain in [*URE3-1*] propagation is generic as has been shown to be the case for other yeast J-proteins (Lopez *et al*., 2003; Sahi and Craig, 2007; Higurashi *et al*., 2008). We speculated that if some necessary structural characteristic exists, it may be conserved in the human homolog. Indeed, Swa2 is the only J-protein of the yeast cytosol to have a C-terminal J-domain and auxilin shares this property (Kampinga and Craig, 2010). Likewise both auxilin and Swa2 share an extra N-terminal helix and long loop insertion in their J-domains, features that are not present in other yeast or human J-domains (Jiang *et al*., 2003). These distinctive features of the Swa2 J-domain, unique among all other cytosolic J-proteins in yeast, may be responsible for Swa2's specific role in [*URE3-1*] propagation. To test this, we first created a chimera replacing Swa2's own J-domain with that of human auxilin (Fig. 5A) and placed this chimera under the control of the *CYC1* promoter expressed from either single or multi-copy plasmids (*p415CYC1-Swa2-AuxJ and p425CYC1-Swa2-AuxJ*, Table 1). Following plasmid shuffling as described in previous sections above, these constructs produced near wild-type and above wild-type levels of protein expression relative to Swa2 respectively (Fig. 5B). *p315SWA2-SWA2* was used as a positive control for prion maintenance and color, resulting in [*URE3-1*] maintenance in 9 out of 10 transformants. Intriguingly, the Swa2-AuxJ chimera stably supported [*URE3-1*] propagation when expressed from either the single copy plasmid (20 out of 20 transformants) or the multi-copy plasmid (19 of 20 transformants) (Fig. 5C). These results indicate that the J-domain of Swa2 is not necessarily unique in that it can be replaced by the human homolog, but the possibility that there is an unusual characteristic conserved in the human homolog is still unresolved. Next, in order to determine if the Swa2 J-domain can be replaced by any J-domain, we constructed a similar chimera of Swa2 and Sis1. Sis1's J-domain was chosen because it has been found to be highly generic, being fully swappable with that of Ydj1 in numerous prion and non-prion assays (Yan and Craig, 1999; Gall *et al*., 2000; Johnson and Craig, 2001; Lopez *et al*., 2003; Sahi and Craig, 2007; Silva *et al*., 2011; Reidy *et al*., 2014). Because Sis1's J-domain is normally N-terminal, and because this J-domain has been previously shown to be functional when expressed alone with a nine-residue C-terminal human influenza hemagglutinin (HA) tag (Sahi and Craig, 2007; Hines *et al*., 2011b), we

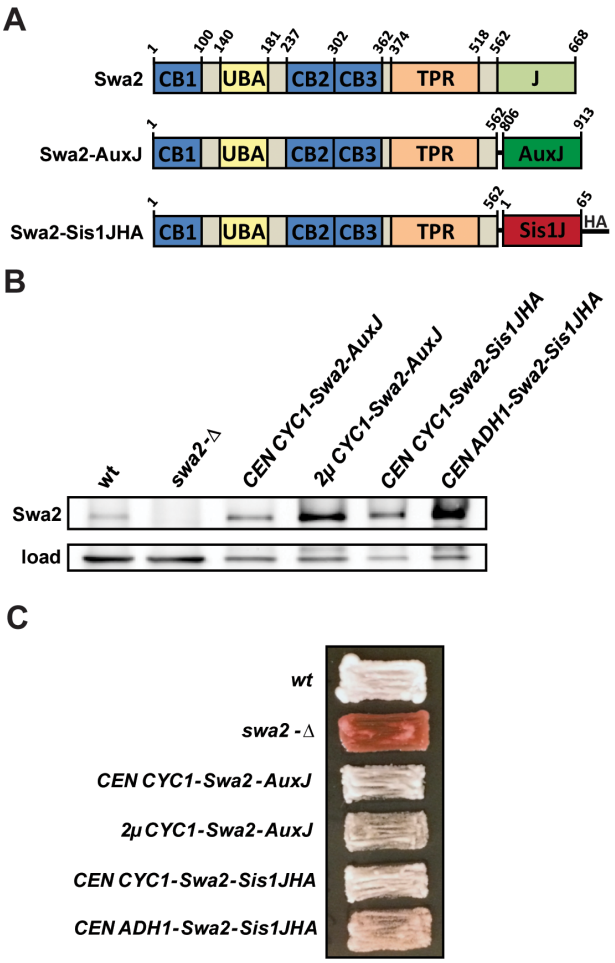

Fig. 5. The J-domain of Swa2 can be replaced by either that of human auxilin, or yeast Sis1, in [*URE3-1*] propagation.
A. Primary sequence diagrams of Swa2 and two chimeras: Swa2 with the J-domain of human auxilin (Swa2-AuxJ) and Swa2 with the J-domain of yeast Sis1 and a nine-residue C-terminal HA tag (Swa2-Sis1JHA); see Figure 4 legend for domain identities.
B and C. [*URE3-1*] plasmid-shuffling strains were transformed by plasmids expressing Swa2-AuxJ and Swa2-Sis1JHA chimeras. Cells were selected from 5-FOA containing medium and (B) lysates were resolved by SDS-PAGE followed by immunoblotting with polyclonal Swa2 antibody or (C) plated onto rich medium to assay for prion maintenance by colony color. Wild-type [*URE3-1*] cells (wt) and cells lacking Swa2 which are [*ure-o*] (*swa2*-Δ) are included as controls.

included a C-terminal HA tag (YPYDVPDYA) in the Swa2/Sis1 chimera anticipating that this would allow this domain to function at the Swa2 C-terminus (Fig. 5A). Swa2-Sis1JHA was expressed at near wild-type levels relative to Swa2 from the *CYC1* promoter or well above the wild-type levels from the *ADH1* promoter (*p415CYC1-Swa2-Sis1JHA* and *p415ADH1-Swa2-Sis1JHA*; Table 1). At both expression levels [*URE3-1*] was maintained in all (20 out of 20) shuffled transformants albeit with some colony sectoring apparent in the *p415ADH1-Swa2-Sis1JHA* samples (Fig. 5C). These results unambiguously demonstrate that [*URE3-1*] requires the TPR domain of Swa2 expressed in *cis* with a functional J-domain, but not necessarily the J-domain of Swa2. Most notably, these observations, combined with those from the domain analysis presented earlier, lead to the logical conclusion that the Swa2 TPR domain is capable of determining the specificity of the Swa2–[*URE3-1*] genetic interaction.

Discussion

The C-terminal domains of Swa2 are required for [URE3-1] propagation and other uncharacterized cellular functions

In this study, we examined the potential for 12 J-proteins of the *S. cerevisiae* cytosol to perform critical functions in the propagation of the strong [*URE3*] variant [*URE3-1*]. Our cumulative results have simultaneously identified a new role for Swa2 in yeast prion propagation and confirmed that Swa2 and Sis1 are the only two cytosolic J-proteins that are essential for [*URE3-1*] propagation. Swa2 is also required for proper inheritance of the cortical endoplasmic reticulum and for mat formation on low-density agar plates, which is determined at least in part by the function of the Flo11 protein (Du *et al.*, 2001; Martineau *et al.*, 2010). Others have previously demonstrated that Swa2 must be at least bifunctional, for example while Swa2's function in clathrin recycling strictly requires both clathrin-binding and J-domain function, its role in the inheritance of the cortical ER can be fulfilled by a construct completely lacking the J-domain (Du *et al.*, 2001; Xiao *et al.*, 2006). Our investigation is consistent with this notion, and in fact, the domains that were here found to be required for prion propagation are distinct from those necessary for clathrin and Flo11 recycling or cortical ER inheritance, indicating the Swa2 may be a multifunctional protein with coordination of different sets of domains required for distinct cellular functions. For example, expression of a construct consisting of only the TPR and J-domains partially rescues the slow-growth phenotype of *swa2*-Δ cells despite this construct being entirely unable to bind clathrin, indicating that these domains also accomplish some still uncharacterized cellular function in yeast in addition to prion propagation (Xiao *et al.*, 2006).

Swa2 involvement in prion propagation is likely direct

Although it is challenging to definitively determine whether Swa2 is directly involved in prion propagation *in vivo*, our observations thus far support a direct role. Several factors are known to affect [*URE3*] prion propagation, most notably alterations in the expression levels of chaperone

Table 1. Plasmids used in this study.

Plasmid	Promoter	Marker	Copy number	Source
p416SWA2-SWA2	*SWA2*	*URA3*	CEN, low	Xiao *et al.*, 2006
p315SWA2-SWA2	*SWA2*	*LEU2*	CEN, low	Xiao *et al.*, 2006
p425SWA2-SWA2	*SWA2*	*LEU2*	2μ, high	Xiao *et al.*, 2006
p315SWA2-swa2-ΔUBA	*SWA2*	*LEU2*	CEN, low	Xiao *et al.*, 2006
p315SWA2-swa2-Δ2–302	*SWA2*	*LEU2*	CEN, low	Xiao *et al.*, 2006
p315SWA2-swa2-Δ2–362	*SWA2*	*LEU2*	CEN, low	Xiao *et al.*, 2006
p425SWA2-swa2-ΔTPR	*SWA2*	*LEU2*	2μ, high	This study
p425SWA2-swa2-TPR	*SWA2*	*LEU2*	2μ, high	Xiao *et al.*, 2006
p425SWA2-swa2-J	*SWA2*	*LEU2*	2μ, high	Xiao *et al.*, 2006
p425SWA2-SWA2-TPR-J	*SWA2*	*LEU2*	2μ, high	Xiao *et al.*, 2006
p415CYC1-Swa2-AuxJ	*CYC1*	*LEU2*	CEN, low	This study
p425CYC1-Swa2-AuxJ	*CYC1*	*LEU2*	2μ, high	This study
p415CYC1-Swa2-Sis1J-HA	*CYC1*	*LEU2*	CEN, low	This study
p415ADH1-Swa2-Sis1J-HA	*ADH1*	*LEU2*	CEN, low	This study
p415CYC1-Ure2-GFP	*CYC1*	*LEU2*	CEN, low	This study

proteins known to interact with [*URE3*] and the stress-induced sorting factors Btn2 and Cur1 (Kryndushkin *et al.*, 2008; Malinovska *et al.*, 2012; Wickner *et al.*, 2014). However, we found no detectable changes in chaperone expression levels upon deletion of *SWA2* indicating that even the complete deletion of Swa2 does not evoke a global stress response that could explain the total loss of [*URE3-1*] from the cell population. Further, the fact that [*URE3-1*] is maintained when all clathrin-binding domains are removed indicates that loss of the prion is not due to defects in clathrin dynamics or any of the consequences of such defects such as altered maturation of Flo11. Likewise because the domains required for prion maintenance do not correlate with those required for cortical ER inheritance, prion loss is also not a consequence of a loss of ER partitioning. Possibly the most compelling evidence for a direct role by Swa2 is the finding that single-point mutations in either the TPR domain or the J-domain, completely block [*URE3-1*] propagation. In further support of this idea, we have also begun to identify additional single side-chains within the Swa2 TPR domain that are important for [*URE3-1*] propagation; alanine substitutions of several different single residues within the predicted TPR binding cleft also destabilize the prion to differing degrees (e.g. E.M. Oliver and J.K. Hines, unpubl. obs.). Although we cannot at present rule out an indirect role, the notion that all of these alterations of Swa2 induce a common secondary effect that is responsible for curing [*URE3-1*] seems unlikely.

The involvement of Swa2 in [URE3-1] propagation may explain Hsp70 sensitivities

Our discovery that Swa2 is required for [*URE3-1*] propagation may tie together several different observations in the literature regarding the particular sensitivity of [*URE3*] propagation to Hsp70 activity in yeast. Multiple studies have shown that [*URE3*] propagation is preferentially dependent on Ssa2 rather than Ssa1 activity. For example, [*URE3*] is stably propagated in a strain expressing Ssa2 as the sole Ssa isoform, but unstable in a strain expressing only Ssa1 (Sharma and Masison, 2008). Furthermore overexpression of Ssa1, but not Ssa2, destabilizes [*URE3*], as do point mutations in Ssa2 (Schwimmer and Masison, 2002; Roberts *et al.*, 2004). Incredibly, these differences in Ssa1/2 have been localized to a single alanine/glycine polymorphism between the two isoforms, indicating that a single methyl-group dictates the functional specialization in prion propagation (Sharma and Masison, 2011). One intriguing hypothesis raised by the work presented here is that this difference in the ability to propagate [*URE3*] may be manifested through preferential cooperation with Swa2. Similarly in a recent investigation, Hsp70 from *Schizosaccharomyces pombe* (Sp-Hsp70) was found to support cell viability and [*PSI*$^+$] propagation in *S. cerevisiae* in place of the native isoforms, but was unable to support stable [*URE3*] propagation, leading those authors to conclude that [*URE3*] likely requires an uncharacterized and nonessential function of Hsp70 that Sp-Hsp70 was incapable of complimenting (Reidy *et al.*, 2013). In light of our investigation, it seems plausible to suggest that this nonessential function may have been cooperation with native Swa2 as an inability to perform this function would affect [*URE3*] only, and not [*PSI*$^+$], as those authors observed. Likewise the inability of the bacterial chaperones ClpB, DnaK and GrpE (BKE) from *Escherichia coli* to support [*URE3*] propagation in yeast may have been due to a similar incompatibility with native Swa2 (Reidy *et al.*, 2014). All of these observations would be explainable if Swa2 has a high degree of specificity for its Hsp70 partner, but whether Swa2 preferentially functions with Ssa1 or Ssa2 in this or other cellular

functions, or if it is incapable of partnering with the *S. pombe* or *E. coli* homologs of Ssa has yet to be explored.

Potential roles of the J and TPR domains in [URE3-1] propagation

The discovery that [*URE3-1*] requires Swa2 in addition to Sis1 is now the second example of a prion that requires at least two J-proteins, the first being [*SWI*$^{+}$], which requires both Sis1 and the most abundant J-protein of the yeast cytoplasm, Ydj1 (Ghaemmaghami *et al*., 2003; Hines *et al*., 2011b). Swa2's function in [*URE3-1*] prion propagation requires its TPR domain and a functional J-domain, yet despite having a distinctive extended J-domain, the J-domain of Swa2 is not specifically required as it can be replaced by J-domains from other orthologs or paralogs. This finding has led us to the logical conclusion that it is the pairing of a generic, but functional J-domain, with the TPR domain of Swa2 that leads to the functionality that allows stable [*URE3-1*] prion propagation. This finding explains the specific requirement for Swa2 among 11 other J-proteins as Swa2 is the only J-protein in *S. cerevisiae* with a TPR domain (Kampinga and Craig, 2010). *How might these two domains cooperate to promote prion propagation?* In the absence of additional empirical data, the most likely role for Swa2 is to supplement the action of Sis1 in [*URE3-1*] prion fragmentation, perhaps because [*URE3-1*] aggregates are less frangible, and therefore require greater intervention by molecular chaperones for prion fragmentation to keep pace with dilution by cell division. Because the J-domain is known to stimulate the ATPase activity of Hsp70, and because Hsp70 is known to be directly involved in the fragmentation process of at least four prions, including [*URE3*] (Kampinga and Craig, 2010; Liebman and Chernoff, 2012), we speculate that Swa2 interacts with Hsp70 during prion fragmentation with the TPR domain possibly serving to form a bipartite interaction with the C-terminus of Hsp70. Swa2's TPR region bears significant resemblance to the TPR1 domain from the human protein HOP and its yeast homolog Sti1. HOP mediates interactions between Hsp70 and Hsp90 by binding the two proteins at its TPR1 and TPR2A domains respectively (Scheufler *et al*., 2000). Others have suggested that because Swa2's TPR domain shares a higher degree of similarity to the TPR1 domain of HOP, which binds Hsp70, than to the TPR2A domain, which binds Hsp90, the likely interaction partner is Hsp70 (Xiao *et al*., 2006). Crystal structures showing the HOP TPR1 domain bound to the acidic C-terminal Glu-Glu-Val-Asp (EEVD) motif of Hsp70 reveal key basic residues necessary for this interaction (Scheufler *et al*., 2000). Also within the HOP TPR1 domain is a specific signature structure called the 'dicarboxylate clamp' that makes key interactions with the dicarboxylate of the aspartate at the extreme C-terminus of Hsp70 (Scheufler *et al*., 2000; Odunuga *et al*., 2003). Using the HOP TPR1 structure, bound to Hsp70, and pair-wise sequence alignments, we examined the conservation within the Swa2 sequence of critical residues that would be predicted to make up the binding surface of the Swa2 TPR domain by first cataloging the key interactions between the HOP TPR1 domain in its interaction with the C-terminal EEVD motif of Hsp70 (Table 2, Hsp70 EEVD residues are numbered, by convention, from the C-terminus). Based on this analysis, we confirmed that (i) all key residues necessary to bind an EEVD motif are conserved or conservatively mutated, indicating that the Swa2 TPR domain is competent to bind to an EEVD or EEVD-like acidic sequence and (ii) three of four residues necessary to form the dicarboxylate clamp structure are completely conserved and the fourth is conservatively mutated (Asn → Thr) (Table 2, clamp residues shown in bold text), indicating that the Swa2 binding partner is almost certainly a C-terminal EEVD or EEVD-like acidic sequence like that of Hsp70 or Hsp90. *How might a bipartite interaction with Hsp70 enhance fiber fragmentation?* One possibility is that it could allow the Swa2 J-domain to repeatedly stimulate the ATPase activity of Hsp70 through repeated binding and release while the two proteins remain associated via the interaction of the TPR domain and C-terminus of Hsp70. This would effectively increase the local concentration of J-domains at Hsp70 molecules bound to prion aggregates, which could potentially accelerate aggregate remodeling. Alternatively, the TPR domain may interact with other chaperone partners with C-terminal EEVD or EEVD-like motifs. Intriguingly, these candidates in yeast include not only Hsp90, which has no clearly defined role to date in yeast prion propagation, but also Hsp104, which has a C-terminal DDLD sequence that is known to bind to the TPR1 domain of Sti1 in *S. cerevisiae* (Abbas-Terki *et al*., 2001). In the case of Hsp104, a potential mechanism is obvious, as Swa2 could directly couple Hsp70 and

Table 2. Conservation of key EEVD binding residues between human HOP and yeast Swa2.

Hsp70	HOP TPR1	Interaction	SWA2 TPR
D^{0}	**K^{8}**	salt bridge	**K^{378}**
D^{0}	**N^{43}**	ion-dipole	**N^{417}**
D^{0}	**N^{12}**	ion-dipole	**$\rightarrow T^{382}$**
D^{0}	**K^{73}**	salt bridge	**K^{468}**
V^{-1}	L^{15}	hydrophobic	$\rightarrow F^{385}$
V^{-1}	A^{46}	hydrophobic	A^{420}
E^{-2}	K^{73}	ion-dipole	K^{468}
E^{-2}	R^{77}	ion-dipole	R^{472}
E^{-3}	R^{77}	ion-dipole	R^{472}

Hsp104 interaction via docking the two proteins at the J-domain and TPR domains, respectively, much in the same manner as HOP docks Hsp70 and Hsp90. In this manner, Swa2 could potentially directly recruit additional Hsp104 hexamers to [*URE3*] aggregates to promote prion fragmentation, again by virtue of binding to already prion-associated Hsp70 molecules via the J-domain. Finally, given the recent discovery that another TPR-containing protein Sgt2 mediates direct interactions with yeast prions and amyloids, it is also possible that Swa2 interacts directly with [*URE3*] aggregates, perhaps in some way mediated or stabilized by interactions in the TPR domain as is the case for Sgt2 (Kiktev *et al*., 2012). Efforts to explore these and other hypotheses regarding the function of the Swa2 TPR domain are underway with the hope that understanding the role of Swa2 in prion biology will enlighten both our understanding of both amyloid biology and molecular chaperone function.

Implications for human protein misfolding disorders

As noted previously, a serious problem with advancing the understanding of chaperone function in yeast prion biology is that the fundamental chaperone requirements for most yeast prions remain unidentified. A molecular understanding of prion–chaperone interactions has the potential to inform the development of interventions for protein misfolding disorders as manipulations of chaperone biology are already being explored as treatments of amyloid and prion diseases (Howarth *et al*., 2007; Vashist *et al*., 2010; Hosokawa *et al*., 2012; Smith and Gestwicki, 2012). Investigations in yeast also have the potential to reveal previously unappreciated connections in other systems (Tribouillard-Tanvier *et al*., 2008; Stein *et al*., 2014). With respect to the work presented here, because auxilin does not possess a TPR domain homologous to that of Swa2, whether auxilin might function similarly in mammalian amyloid biology is unclear. On the other hand, two other human J-proteins, the ER-associated protein DNAJC3 (also called P58^{IDK}) and the cytosolic protein DNAJC7 (also called CCRP or TPR2) have exactly the gross structural characteristics identified here as critical for Swa2 function in prion propagation: both proteins have extensive TPR domains with signature residues for binding EEVD motifs followed by functional C-terminal J-domains (Lee *et al*., 1994; Murthy *et al*., 1996; Kampinga and Craig, 2010). Both proteins are indeed involved in protein homeostasis and intriguingly, alterations in the expression of either protein impacts models of neurodegenerative disease, for example loss of DNAJC3 expression ameliorated neurodegeneration in a mouse model of Marinesco–Sjögren syndrome while overexpression of DNAJC7 suppressed polyglutamine toxicity in a *Drosophila* model of Huntington's disease (Kazemi-Esfarjani and Benzer, 2000; Zhao *et al*., 2010). Our observations reported here raise the interesting possibility that either of these proteins may also be functionally orthologous to the minimal construct of Swa2 found here to participate in amyloid biology. While J-proteins are increasingly implicated in protein misfolding disorders (Gibbs and Braun, 2008; Maiti *et al*., 2014), the discovery of two new J-protein requirements for yeast prions in the last few years makes plain that we have only begun to unravel the complexity of prion–chaperone interactions. Our discovery of a role for Swa2 in prion biology suggests that other previously unassessed J-proteins may impact amyloid dynamics in other systems in ways that have yet to be explored.

Experimental procedures

Yeast strains and plasmids

Haploid *S. cerevisiae* W303-derived strains were used throughout this investigation. To assay for [*URE3-1*] maintenance in strains with individual deletions of 12 cytosolic J-proteins, strains bearing each gene deletion and derived from PJ513a ([*RNQ*$^{+}$], [*psi*-], *trp1-1*, *ura3-1*, *leu2-3,112*, *his3-11,15*, *ade2-1*, *can1-100*, *GAL2*, *met2-1*, *lys2-2*,) were crossed with the [*URE3-1*] strain Y2086 (*trp1-1, leu2-3,112*, *his3-11,15*, *ade2-1*, *URA3*, *ura2::kanMX*, *dal5::PDAL5-ADE2*, [*URE3-1*], [*RNQ*$^{+}$], [*psi*-], [*nu*-]) (Sahi and Craig, 2007; Higurashi *et al*., 2008). To create a [*URE3-1*] strain competent for Swa2-plasmid shuffling, a haploid *swa2-Δ* strain, Y1723 (*trp1-1, ura3-1, leu2-3,112 his3-11,15, ade2-1*, *can1-100*, *GAL+*, *met2-Δ1, lys2-Δ2, swa2*::*HIS3*) was transformed with a *URA3*-marked Swa2 plasmid (*p416SWA2-SWA2*) and then crossed with Y2086. The resulting diploids were sporulated on potassium acetate minimal medium, and subjected to tetrad dissection. The desired haploid strain (*trp1-1, ura3-1, leu2-3,112, ade2-1*, [*URE3-1*], *swa2::HIS3, his3-11,15, dal5::PDAL5-ADE2*) was selected for by prototrophic growth on selective media. To create [*PSI*$^{+}$] strains with genomic *SWA2* deletions, strains Y2459, Y2462, Y2469, and Y2471 ([*PSI*$^{+}$] [*rnq*$^{-}$] *ade1-14 ADE2*$^{+}$ *trp1-1 ura3-1 leu2-3,112 his3-11,15 can1-100 GAL2*$^{+}$) bearing distinct [*PSI*$^{+}$] variants: [*PSI*$^{+}$]^{Sc4}, [*PSI*$^{+}$]^{Sc37}, [*PSI*$^{+}$]^{93S}, [*PSI*$^{+}$]^{94W} respectively (Hines *et al*., 2011a; Harris *et al*., 2014), were crossed with Y1723 bearing [*Swa2-SWA2, URA3*]. Resulting diploids were selected by prototrophic growth on selective medium lacking adenine, sporulated on potassium acetate minimal medium, and subjected to tetrad dissection. Haploids were genotyped by replica plating onto selective media.

Plasmids used in this study are based on the pRS series (Mumberg *et al*., 1995). *p425SWA2-SWA2* was used as a template to create *p425SWA2-swa2-ΔTPR* by site-directed mutagenesis (Quikchange) polymerase chain reaction (PCR) using the primers *delTPR-for* and *delTPR-rev* (Table S1). Template plasmid was selectively digested with *DpnI* (New England Biolabs, Ipswich, MA) and the nicked-circular PCR product used to transform chemically competent DH5α *E. coli*. All plasmids were harvested from isolated *E. coli* colonies using QIAprep Spin Miniprep Kit (Qiagen, Valencia, CA)

and sequenced by Sanger dideoxyterminator sequencing (Genewiz, South Plainfield, NJ).

In order to create Swa2/auxilin and Swa2/Sis1 chimeras, gene sequences for two Swa2 chimeras (*Swa2-Sis1J-HA* and *Swa2-AuxJ*) were codon optimized for expression in *S. cerevisiae* and synthesized *de novo* from overlapping oligos (GenScript, Piscataway Township, NJ). The open-reading frames of each chimeric gene were amplified by PCR introducing a 5′ *BamHI* site and a 3′ *SalI* site. Amplified inserts were then digested with *BamHI* and *SalI* and ligated into double-cut *p415CYC1*, *p425CYC1*, and/or *p415ADH*.

Yeast growth assays

Cells used for yeast growth assays were grown in liquid culture in log phase for at least five generations and collected at equal density in log phase (~0.3 OD) prior to spotting. Collected cells were serially diluted in 10-fold increments in liquid-rich medium (YPD) and spotted in 5 μL drops on solid medium. Cells were allowed to form colonies and develop color at 25°C for 3–4 days prior to image acquisition.

Plasmid shuffling and assays for prion loss

Plasmids bearing Swa2 constructs (Table 1) were used to transform plasmid-shuffling strains and transformants selected by growth on selective medium. Typically at least 20 colonies were isolated in each experiment and plated onto medium containing 5-FOA, counter-selecting against the *URA3*-marked *SWA2* plasmid. *URA3* encodes orotidine 5-monophosphate decarboxylase, which converts harmless 5-FOA into cytotoxic 5-fluorouracil, a potent inhibitor of thymidylate synthase; only cells that have lost the *URA3*-marked *SWA2* plasmid survive to form colonies. Loss of the *URA3*-marked plasmid was further confirmed in all cases by the failure to grow on synthetic medium lacking uracil. Shuffled cells were plated onto rich medium to detect for [*URE3*] maintenance by colony color assay. The [*URE3*] strains used in our experiments have a genomic mutation (*ade2-1*) at the *ADE2* gene locus and have an insertion of *ADE2* at the *DAL5* gene locus resulting in Ade2 protein expression under the control of the *DAL5* promoter (*pDAL5-ADE2*). *DAL5* encodes an important component of the allantoate transport system (Rai *et al.*, 1987). Ure2 binds the transcription factor Gln3, preventing its translocation into the nucleus, which is required to activate *pDAL5* (Kulkarni *et al.*, 2001). As such, in our strains when Ure2 is soluble, as in the [*ure-o*] colonies, *pDAL5-ADE2* is inactive, resulting in a block in adenine biosynthesis because of the lack of functional Ade2 enzyme and a buildup of the red-pigmented intermediate in those cells. When Ure2 is aggregated into [*URE3*], *pDAL5-ADE2* is active and colonies are white due to normal adenine biosynthesis. Prion maintenance was routinely confirmed by the curability of the color phenotype following brief growth in liquid medium containing 4 mM GdnHCl, and/or by subsequent transformation of cells with a plasmid expressing a Ure2-GFP chimera (*p415CYC1-Ure2-GFP*, Table 1) to visualize [*URE3-1*] aggregates. [*ure-o*] and [*URE3-1*] cells consistently exhibited distinct diffuse or punctate florescence patterns, respectively, following transformation by *p415CYC1-Ure2-GFP*.

SDS-PAGE and immunoblot analysis

Total protein extracts for SDS-PAGE were prepared by harvesting ~1.0 OD units of yeast cells followed by vortexing in 0.2 M NaOH at 25°C. Cells were spun at 14,500 r.p.m. on a table-top centrifuge at 25°C and the supernatant was removed. Pellets were resuspended in a sample buffer containing SDS and boiled for 5 min before resolving in a 10% or 11% polyacrylamide gel. The protein was transferred to a nitrocellulose membrane at 1 A for 1 h at 25°C in tris-glycine/methanol buffer and probed with polyclonal antibodies against: Swa2 (Graham Lab, Vanderbilt University, Nashville, TN), Hsp104 (Cayman Chemicals, Ann Arbor, MI), Sse1/2 (Brodsky Lab, University of Pittsburgh, Pittsburg, PA), Sis1 and Ydj1 (Craig lab, University of Wisconsin-Madison, Madison, WI). Western ladder from New England Biolabs was used as a marker to detect relative protein sizes.

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References

Abbas-Terki, T., Donze, O., Briand, P.A., and Picard, D. (2001) Hsp104 interacts with Hsp90 cochaperones in respiring yeast. *Mol Cell Biol* **21:** 7569–7575.

Aigle, M., and Lacroute, F. (1975) Genetical aspects of [URE3], a non-mitochondrial, cytoplasmically inherited mutation in yeast. *Mol Gen Genet* **136:** 327–335.

Aron, R., Higurashi, T., Sahi, C., and Craig, E.A. (2007) J-protein co-chaperone Sis1 required for generation of [RNQ+] seeds necessary for prion propagation. *EMBO J* **26:** 3794–3803.

Brachmann, A., Baxa, U., and Wickner, R.B. (2005) Prion generation *in vitro*: amyloid of Ure2p is infectious. *EMBO J* **24:** 3082–3092.

Brachmann, A., Toombs, J.A., and Ross, E.D. (2006) Reporter assay systems for [URE3] detection and analysis. *Methods* **39:** 35–42.

Caplan, A.J., and Douglas, M.G. (1991) Characterization of YDJ1: a yeast homologue of the bacterial dnaJ protein. *J Cell Biol* **114:** 609–621.

Cheetham, M.E., and Caplan, A.J. (1998) Structure, function and evolution of dnaJ: conservation and adaptation of

chaperone function. *Cell Stress Chaperones* **3:** 28–36.
Chernoff, Y.O., Lindquist, S.L., Ono, B., Inge-Vechtomov, S.G., and Liebman, S.W. (1995) Role of the chaperone protein Hsp104 in propagation of the yeast prion-like factor [psi+]. *Science* **268:** 880–884.
Chim, N., Gall, W.E., Xiao, J., Harris, M.P., Graham, T.R., and Krezel, A.M. (2004) Solution structure of the ubiquitin-binding domain in Swa2p from *Saccharomyces cerevisiae*. *Proteins* **54:** 784–793.
Cooper, T.G. (2002) Transmitting the signal of excess nitrogen in *Saccharomyces cerevisiae* from the Tor proteins to the GATA factors: connecting the dots. *FEMS Microbiol Rev* **26:** 223–238.
Cox, B., Ness, F., and Tuite, M. (2003) Analysis of the generation and segregation of propagons: entities that propagate the [PSI+] prion in yeast. *Genetics* **165:** 23–33.
Derdowski, A., Sindi, S.S., Klaips, C.L., DiSalvo, S., and Serio, T.R. (2010) A size threshold limits prion transmission and establishes phenotypic diversity. *Science* **330:** 680–683.
Derkatch, I.L., Chernoff, Y.O., Kushnirov, V.V., Inge-Vechtomov, S.G., and Liebman, S.W. (1996) Genesis and variability of [PSI] prion factors in *Saccharomyces cerevisiae*. *Genetics* **144:** 1375–1386.
Du, Y., Pypaert, M., Novick, P., and Ferro-Novick, S. (2001) Aux1p/Swa2p is required for cortical endoplasmic reticulum inheritance in *Saccharomyces cerevisiae*. *Mol Biol Cell* **12:** 2614–2628.
Edskes, H.K., Gray, V.T., and Wickner, R.B. (1999) The [URE3] prion is an aggregated form of Ure2p that can be cured by overexpression of Ure2p fragments. *Proc Natl Acad Sci USA* **96:** 1498–1503.
Edskes, H.K., Naglieri, B.M., and Wickner, R.B. (2006) Nitrogen source and the retrograde signalling pathway affect detection, not generation, of the [URE3] prion. *Yeast* **23:** 833–840.
Fernandez-Bellot, E., Guillemet, E., and Cullin, C. (2000) The yeast prion [URE3] can be greatly induced by a functional mutated URE2 allele. *EMBO J* **19:** 3215–3222.
Ferreira, P.C., Ness, F., Edwards, S.R., Cox, B.S., and Tuite, M.F. (2001) The elimination of the yeast [PSI+] prion by guanidine hydrochloride is the result of Hsp104 inactivation. *Mol Microbiol* **40:** 1357–1369.
Gall, W.E., Higginbotham, M.A., Chen, C., Ingram, M.F., Cyr, D.M., and Graham, T.R. (2000) The auxilin-like phosphoprotein Swa2p is required for clathrin function in yeast. *Curr Biol* **10:** 1349–1358.
Ghaemmaghami, S., Huh, W.K., Bower, K., Howson, R.W., Belle, A., Dephoure, N., *et al*. (2003) Global analysis of protein expression in yeast. *Nature* **425:** 737–741.
Gibbs, S.J., and Braun, J.E. (2008) Emerging roles of J proteins in neurodegenerative disorders. *Neurobiol Dis* **32:** 196–199.
Grimminger, V., Richter, K., Imhof, A., Buchner, J., and Walter, S. (2004) The prion curing agent guanidinium chloride specifically inhibits ATP hydrolysis by Hsp104. *J Biol Chem* **279:** 7378–7383.
Harris, J.M., Nguyen, P.P., Patel, M.J., Sporn, Z.A., and Hines, J.K. (2014) Functional diversification of hsp40: distinct j-protein functional requirements for two prions allow for chaperone-dependent prion selection. *PLoS Genet* **10:** e1004510.
Haslberger, T., Bukau, B., and Mogk, A. (2010) Towards a unifying mechanism for ClpB/Hsp104-mediated protein disaggregation and prion propagation. *Biochem Cell Biol* **88:** 63–75.
Higurashi, T., Hines, J.K., Sahi, C., Aron, R., and Craig, E.A. (2008) Specificity of the J-protein Sis1 in the propagation of 3 yeast prions. *Proc Natl Acad Sci USA* **105:** 16596–16601.
Hines, J.K., and Craig, E.A. (2011) The sensitive [SWI (+)] prion: new perspectives on yeast prion diversity. *Prion* **5:** 164–168.
Hines, J.K., Higurashi, T., Srinivasan, M., and Craig, E.A. (2011a) Influence of prion variant and yeast strain variation on prion-molecular chaperone requirements. *Prion* **5:** 238–244.
Hines, J.K., Li, X., Du, Z., Higurashi, T., Li, L., and Craig, E.A. (2011b) SWI], the prion formed by the chromatin remodeling factor Swi1, is highly sensitive to alterations in Hsp70 chaperone system activity. *PLoS Genet* **7:** e1001309.
Holstein, S.E., Ungewickell, H., and Ungewickell, E. (1996) Mechanism of clathrin basket dissociation: separate functions of protein domains of the dnaJ homologue auxilin. *J Cell Biol* **135:** 925–937.
Hosokawa, M., Arai, T., Masuda-Suzukake, M., Nonaka, T., Yamashita, M., Akiyama, H., and Hasegawa, M. (2012) Methylene blue reduced abnormal tau accumulation in P301L tau transgenic mice. *PLoS ONE* **7:** e52389.
Howarth, J.L., Kelly, S., Keasey, M.P., Glover, C.P., Lee, Y.B., Mitrophanous, K., *et al*. (2007) Hsp40 molecules that target to the ubiquitin-proteasome system decrease inclusion formation in models of polyglutamine disease. *Mol Ther* **15:** 1100–1105.
Jiang, J., Taylor, A.B., Prasad, K., Ishikawa-Brush, Y., Hart, P.J., Lafer, E.M., and Sousa, R. (2003) Structure-function analysis of the auxilin J-domain reveals an extended Hsc70 interaction interface. *Biochemistry* **42:** 5748–5753.
Johnson, J.L., and Craig, E.A. (2001) An essential role for the substrate-binding region of Hsp40s in *Saccharomyces cerevisiae*. *J Cell Biol* **152:** 851–856.
Jung, G., and Masison, D.C. (2001) Guanidine hydrochloride inhibits Hsp104 activity *in vivo*: a possible explanation for its effect in curing yeast prions. *Curr Microbiol* **43:** 7–10.
Jung, G., Jones, G., Wegrzyn, R.D., and Masison, D.C. (2000) A role for cytosolic hsp70 in yeast [PSI(+)] prion propagation and [PSI(+)] as a cellular stress. *Genetics* **156:** 559–570.
Jung, G., Jones, G., and Masison, D.C. (2002) Amino acid residue 184 of yeast Hsp104 chaperone is critical for prion-curing by guanidine, prion propagation, and thermotolerance. *Proc Natl Acad Sci USA* **99:** 9936–9941.
Kampinga, H.H., and Craig, E.A. (2010) The HSP70 chaperone machinery: J proteins as drivers of functional specificity. *Nat Rev Mol Cell Biol* **11:** 579–592.
Kazemi-Esfarjani, P., and Benzer, S. (2000) Genetic suppression of polyglutamine toxicity in *Drosophila*. *Science* **287:** 1837–1840.
Kiktev, D.A., Patterson, J.C., Muller, S., Bariar, B., Pan, T., and Chernoff, Y.O. (2012) Regulation of chaperone effects

on a yeast prion by cochaperone Sgt2. *Mol Cell Biol* **32:** 4960–4970.
Krantz, K.C., Puchalla, J., Thapa, R., Kobayashi, C., Bisher, M., Viehweg, J., *et al.* (2013) Clathrin coat disassembly by the yeast Hsc70/Ssa1p and auxilin/Swa2p proteins observed by single-particle burst analysis spectroscopy. *J Biol Chem* **288:** 26721–26730.
Kryndushkin, D., and Wickner, R.B. (2007) Nucleotide exchange factors for Hsp70s are required for [URE3] prion propagation in *Saccharomyces cerevisiae*. *Mol Biol Cell* **18:** 2149–2154.
Kryndushkin, D.S., Shewmaker, F., and Wickner, R.B. (2008) Curing of the [URE3] prion by Btn2p, a Batten disease-related protein. *EMBO J* **27:** 2725–2735.
Kulkarni, A.A., Abul-Hamd, A.T., Rai, R., El Berry, H., and Cooper, T.G. (2001) Gln3p nuclear localization and interaction with Ure2p in *Saccharomyces cerevisiae*. *J Biol Chem* **276:** 32136–32144.
Lee, T.G., Tang, N., Thompson, S., Miller, J., and Katze, M.G. (1994) The 58,000-dalton cellular inhibitor of the interferon-induced double-stranded RNA-activated protein kinase (PKR) is a member of the tetratricopeptide repeat family of proteins. *Mol Cell Biol* **14:** 2331–2342.
Liebman, S.W., and Chernoff, Y.O. (2012) Prions in yeast. *Genetics* **191:** 1041–1072.
Lopez, N., Aron, R., and Craig, E.A. (2003) Specificity of class II Hsp40 Sis1 in maintenance of yeast prion [RNQ+]. *Mol Biol Cell* **14:** 1172–1181.
McMahon, H.T., and Boucrot, E. (2011) Molecular mechanism and physiological functions of clathrin-mediated endocytosis. *Nat Rev Mol Cell Biol* **12:** 517–533.
Maiti, P., Manna, J., Veleri, S., and Frautschy, S. (2014) Molecular chaperone dysfunction in neurodegenerative diseases and effects of curcumin. *Biomed Res Int* **2014:** 495091.
Malinovska, L., Kroschwald, S., Munder, M.C., Richter, D., and Alberti, S. (2012) Molecular chaperones and stress-inducible protein-sorting factors coordinate the spatiotemporal distribution of protein aggregates. *Mol Biol Cell* **23:** 3041–3056.
Martineau, C.N., Melki, R., and Kabani, M. (2010) Swa2p-dependent clathrin dynamics is critical for Flo11p processing and 'Mat' formation in the yeast *Saccharomyces cerevisiae*. *FEBS Lett* **584:** 1149–1155.
Matta-Camacho, E., Kozlov, G., Trempe, J.F., and Gehring, K. (2009) Atypical binding of the Swa2p UBA domain to ubiquitin. *J Mol Biol* **386:** 569–577.
Moriyama, H., Edskes, H.K., and Wickner, R.B. (2000) [URE3] prion propagation in *Saccharomyces cerevisiae*: requirement for chaperone Hsp104 and curing by overexpressed chaperone Ydj1p. *Mol Cell Biol* **20:** 8916–8922.
Mumberg, D., Muller, R., and Funk, M. (1995) Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds. *Gene* **156:** 119–122.
Murthy, A.E., Bernards, A., Church, D., Wasmuth, J., and Gusella, J.F. (1996) Identification and characterization of two novel tetratricopeptide repeat-containing genes. *DNA Cell Biol* **15:** 727–735.
Newnam, G.P., Wegrzyn, R.D., Lindquist, S.L., and Chernoff, Y.O. (1999) Antagonistic interactions between yeast chaperones Hsp104 and Hsp70 in prion curing. *Mol Cell Biol* **19:** 1325–1333.
Odunuga, O.O., Hornby, J.A., Bies, C., Zimmermann, R., Pugh, D.J., and Blatch, G.L. (2003) Tetratricopeptide repeat motif-mediated Hsc70-mSTI1 interaction. Molecular characterization of the critical contacts for successful binding and specificity. *J Biol Chem* **278:** 6896–6904.
Pishvaee, B., Costaguta, G., Yeung, B.G., Ryazantsev, S., Greener, T., Greene, L.E., *et al.* (2000) A yeast DNA J protein required for uncoating of clathrin-coated vesicles *in vivo*. *Nat Cell Biol* **2:** 958–963.
Prusiner, S.B. (1982) Novel proteinaceous infectious particles cause scrapie. *Science* **216:** 136–144.
Prusiner, S.B. (2013) Biology and genetics of prions causing neurodegeneration. *Annu Rev Genet* **47:** 601–623.
Puria, R., Zurita-Martinez, S.A., and Cardenas, M.E. (2008) Nuclear translocation of Gln3 in response to nutrient signals requires Golgi-to-endosome trafficking in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* **105:** 7194–7199.
Rai, R., Genbauffe, F., Lea, H.Z., and Cooper, T.G. (1987) Transcriptional regulation of the DAL5 gene in *Saccharomyces cerevisiae*. *J Bacteriol* **169:** 3521–3524.
Reidy, M., Sharma, R., and Masison, D.C. (2013) *Schizosaccharomyces pombe* disaggregation machinery chaperones support *Saccharomyces cerevisiae* growth and prion propagation. *Eukaryot Cell* **12:** 739–745.
Reidy, M., Sharma, R., Shastry, S., Roberts, B.L., Albino-Flores, I., Wickner, S., and Masison, D.C. (2014) Hsp40s specify functions of Hsp104 and Hsp90 protein chaperone machines. *PLoS Genet* **10:** e1004720.
Roberts, B.T., Moriyama, H., and Wickner, R.B. (2004) [URE3] prion propagation is abolished by a mutation of the primary cytosolic Hsp70 of budding yeast. *Yeast* **21:** 107–117.
Sahi, C., and Craig, E.A. (2007) Network of general and specialty J protein chaperones of the yeast cytosol. *Proc Natl Acad Sci USA* **104:** 7163–7168.
Sahi, C., Lee, T., Inada, M., Pleiss, J.A., and Craig, E.A. (2010) Cwc23, an essential J protein critical for pre-mRNA splicing with a dispensable J domain. *Mol Cell Biol* **30:** 33–42.
Satpute-Krishnan, P., Langseth, S.X., and Serio, T.R. (2007) Hsp104-dependent remodeling of prion complexes mediates protein-only inheritance. *PLoS Biol* **5:** e24.
Scheufler, C., Brinker, A., Bourenkov, G., Pegoraro, S., Moroder, L., Bartunik, H., *et al.* (2000) Structure of TPR domain-peptide complexes: critical elements in the assembly of the Hsp70–Hsp90 multichaperone machine. *Cell* **101:** 199–210.
Schlumpberger, M., Prusiner, S.B., and Herskowitz, I. (2001) Induction of distinct [URE3] yeast prion strains. *Mol Cell Biol* **21:** 7035–7046.
Schwimmer, C., and Masison, D.C. (2002) Antagonistic interactions between yeast [PSI(+)] and [URE3] prions and curing of [URE3] by Hsp70 protein chaperone Ssa1p but not by Ssa2p. *Mol Cell Biol* **22:** 3590–3598.
Serio, T.R., Cashikar, A.G., Kowal, A.S., Sawicki, G.J., Moslehi, J.J., Serpell, L., *et al.* (2000) Nucleated conformational conversion and the replication of conformational

information by a prion determinant. *Science* **289:** 1317–1321.
Sharma, D., and Masison, D.C. (2008) Functionally redundant isoforms of a yeast Hsp70 chaperone subfamily have different antiprion effects. *Genetics* **179:** 1301–1311.
Sharma, D., and Masison, D.C. (2011) Single methyl group determines prion propagation and protein degradation activities of yeast heat shock protein (Hsp)-70 chaperones Ssa1p and Ssa2p. *Proc Natl Acad Sci USA* **108:** 13665–13670.
Silva, J.C., Borges, J.C., Cyr, D.M., Ramos, C.H., and Torriani, I.L. (2011) Central domain deletions affect the SAXS solution structure and function of yeast Hsp40 proteins Sis1 and Ydj1. *BMC Struct Biol* **11:** 40.
Smith, M.C., and Gestwicki, J.E. (2012) Features of protein–protein interactions that translate into potent inhibitors: topology, surface area and affinity. *Expert Rev Mol Med* **14:** e16.
Sondheimer, N., Lopez, N., Craig, E.A., and Lindquist, S. (2001) The role of Sis1 in the maintenance of the [RNQ+] prion. *EMBO J* **20:** 2435–2442.
Sporn, Z.A., and Hines, J.K. (2015) Hsp40 function in yeast prion propagation: amyloid diversity necessitates chaperone functional complexity. *Prion* **9:** 80–89.
Stansfield, I., Jones, K.M., Kushnirov, V.V., Dagkesamanskaya, A.R., Poznyakovski, A.I., Paushkin, S.V., *et al*. (1995) The products of the SUP45 (eRF1) and SUP35 genes interact to mediate translation termination in *Saccharomyces cerevisiae*. *EMBO J* **14:** 4365–4373.
Stein, K.C., and True, H.L. (2014) Structural variants of yeast prions show conformer-specific requirements for chaperone activity. *Mol Microbiol* **93:** 1156–1171.
Stein, K.C., Bengoechea, R., Harms, M.B., Weihl, C.C., and True, H.L. (2014) Myopathy-causing mutations in an HSP40 chaperone disrupt processing of specific client conformers. *J Biol Chem* **289:** 21120–21130.
Talarek, N., Maillet, L., Cullin, C., and Aigle, M. (2005) The [URE3] prion is not conserved among *Saccharomyces* species. *Genetics* **171:** 23–34.
Tanaka, M., Chien, P., Naber, N., Cooke, R., and Weissman, J.S. (2004) Conformational variations in an infectious protein determine prion strain differences. *Nature* **428:** 323–328.
Tipton, K.A., Verges, K.J., and Weissman, J.S. (2008) *In vivo* monitoring of the prion replication cycle reveals a critical role for Sis1 in delivering substrates to Hsp104. *Mol Cell* **32:** 584–591.
Tribouillard-Tanvier, D., Beringue, V., Desban, N., Gug, F., Bach, S., Voisset, C., *et al*. (2008) Antihypertensive drug guanabenz is active *in vivo* against both yeast and mammalian prions. *PLoS ONE* **3:** e1981.
Ungewickell, E., Ungewickell, H., Holstein, S.E., Lindner, R., Prasad, K., Barouch, W., *et al*. (1995) Role of auxilin in uncoating clathrin-coated vesicles. *Nature* **378:** 632–635.
Vashist, S., Cushman, M., and Shorter, J. (2010) Applying Hsp104 to protein-misfolding disorders. *Biochem Cell Biol* **88:** 1–13.
Wickner, R.B. (1994) [URE3] as an altered URE2 protein: evidence for a prion analog in *Saccharomyces cerevisiae*. *Science* **264:** 566–569.
Wickner, R.B., Masison, D.C., and Edskes, H.K. (1995) [PSI] and [URE3] as yeast prions. *Yeast* **11:** 1671–1685.
Wickner, R.B., Bezsonov, E., and Bateman, D.A. (2014) Normal levels of the antiprion proteins Btn2 and Cur1 cure most newly formed [URE3] prion variants. *Proc Natl Acad Sci USA* **111:** E2711–E2720.
Winkler, J., Tyedmers, J., Bukau, B., and Mogk, A. (2012a) Chaperone networks in protein disaggregation and prion propagation. *J Struct Biol* **179:** 152–160.
Winkler, J., Tyedmers, J., Bukau, B., and Mogk, A. (2012b) Hsp70 targets Hsp100 chaperones to substrates for protein disaggregation and prion fragmentation. *J Cell Biol* **198:** 387–404.
Xiao, J., Kim, L.S., and Graham, T.R. (2006) Dissection of Swa2p/auxilin domain requirements for cochaperoning Hsp70 clathrin-uncoating activity *in vivo*. *Mol Biol Cell* **17:** 3281–3290.
Xing, Y., Bocking, T., Wolf, M., Grigorieff, N., Kirchhausen, T., and Harrison, S.C. (2010) Structure of clathrin coat with bound Hsc70 and auxilin: mechanism of Hsc70-facilitated disassembly. *EMBO J* **29:** 655–665.
Yan, W., and Craig, E.A. (1999) The glycine–phenylalanine-rich region determines the specificity of the yeast Hsp40 Sis1. *Mol Cell Biol* **19:** 7751–7758.
Zhao, L., Rosales, C., Seburn, K., Ron, D., and Ackerman, S.L. (2010) Alteration of the unfolded protein response modifies neurodegeneration in a mouse model of Marinesco–Sjogren syndrome. *Hum Mol Genet* **19:** 25–35.
Zhou, P., I., Derkatch, L., Uptain, S.M., Patino, M.M., Lindquist, S., and Liebman, S.W. (1999) The yeast non-Mendelian factor [ETA+] is a variant of [PSI+], a prion-like form of release factor eRF3. *EMBO J* **18:** 1182–1191.

Supporting information

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