

Commentary & View

Prion proteostasis

Hsp104 meets its supporting cast

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Infectious amyloid forms of the release factor, Sup35, comprise the yeast prion $[PSI^+]$. This protein-based unit of inheritance is an evolutionary capacitor able to release cryptic genetic variation during environmental stress and generate potentially beneficial phenotypes. Genetic data have uncovered a sophisticated proteostasis network that tightly regulates $[PSI^+]$ formation, propagation and elimination. Central to this network, is the AAA⁺ ATPase and protein disaggregase, Hsp104. Shifting the balance of the cytosolic Hsp70:Hsp40 chaperone machinery and associated nucleotide exchange factors also influences the $[PSI^+]$ prion cycle. Yet, a precise understanding of how these systems co-operate to directly modulate the protein folding events required for sustainable Sup35 prionogenesis has remained elusive. Here, we spotlight recent advances that begin to clarify this issue. We suggest that the Hsp70:Hsp40 chaperone machinery functions collectively as a rheostat that adjusts Hsp104's basic prion-remodeling activities.

The yeast prion $[PSI^+]$ arises when Sup35, a release factor, populates self-templating amyloid forms that transmit heritable increases in nonsense suppression.¹ This requires N-terminal regions of Sup35's prion domain to switch from an intrinsically disordered state to the cross- β form of amyloid fibers.¹ The modest reductions in translation termination fidelity conferred by $[PSI^+]$ alter mRNA stability, gene expression and protein function.¹⁻⁴ Consequently, complex multigenic traits develop that are often (but not always) advantageous.^{1,3,4} $[PSI^+]$ induction increases with increasing environmental stress,⁵ which has reinforced proposals that $[PSI^+]$ is a transient 'bet-hedging' adaptation that enhances survival in variable environments by promoting evolvability.^{1,3-6}

A sophisticated proteostasis⁷ network orchestrates $[PSI^+]$ formation, propagation and elimination.⁸ Central to this network is the hexameric AAA⁺ ATPase and protein-disaggregase, Hsp104.⁹ Either too much or too little Hsp104 can eliminate $[PSI^+]$.¹⁰ To understand this dosage relationship, pure components have been

used to define the direct effects of Hsp104 on Sup35 prionogenesis.^{11,12} The prion nature of various Hsp104-remodeled Sup35 conformers was stringently tested by infecting $[psi^-]$ cells.¹² At low concentrations, Hsp104 promotes Sup35 prion nucleation and severs Sup35 prions to expose new surfaces for prion growth¹¹⁻¹³ (Fig. 1A). At higher concentrations, Hsp104 converts Sup35 prions to SDS-soluble monomeric species and non-infectious amyloid-like material¹¹⁻¹⁵ (Fig. 1A). This 'amyloid-like' material is a non-fibrillar aggregated species, that retains SDS-resistance and the ability to bind Thioflavin-T, but has diminished capacity to template the prion conformation.^{11,12} Similar SDS-soluble and SDS-resistant Sup35 conformers appear to accumulate in $[PSI^+]$ cells expressing high levels of Hsp104.¹⁶ Moreover, SDS-resistant Sup35 polymers with reduced seeding activity can, in some circumstances, be isolated from $[psi^-]$ cells.¹⁷ The basic prion-remodeling activities of Hsp104 reconstituted in vitro would seem to explain how Hsp104 dictates $[PSI^+]$ inheritance patterns.^{11,12} Surprisingly, however, these activities did not require Hsp70 and Hsp40,^{11,12} which assist Hsp104 in renaturing chemically or thermally denatured aggregates.¹⁸ Furthermore, several aspects of the cellular milieu are not recreated in this minimal system and Hsp104 is unlikely to operate in isolation in vivo. Genetic data suggest that the Hsp70:Hsp40 chaperone machinery also affect $[PSI^+]$ induction, propagation and elimination.⁸ However, these data might reflect complex pleiotropic effects rather than direct effects on Sup35 folding. Thus, how Hsp104, Hsp70 and Hsp40 collaborate to directly affect the conformational states of Sup35 has remained unclear. Recent advances that combine pure protein biochemistry with prion infection studies have shed light on this issue.¹⁹

Reconstructing these events using pure components¹⁹ poses a challenge due to the complexity of the cytosolic Hsp70:Hsp40 chaperone network. Two cytosolic Hsp70 subfamilies affect $[PSI^+]$: Ssa and Ssb. Ssa can promote²⁰⁻²⁴ or antagonize^{21,25,26} $[PSI^+]$, whereas Ssb is a consistent $[PSI^+]$ antagonist.^{22,25,27} These functional differences are conferred by the distinct substrate binding domains of Ssa and Ssb.²² The four members of the Ssa subfamily are found throughout the cytoplasm.²⁸ By contrast, the two Ssb proteins are mostly associated with the ribosome, although a considerable portion can be found throughout the cytoplasm.²⁹ Hsp70s couple their ATPase cycle to rounds of substrate binding and release.³⁰ Substrates are typically bound via extended polypeptide segments that become exposed in non-native forms.³⁰ In the ATP-bound state, substrate binding is dynamic, while in the ADP state substrate is stably bound.³⁰

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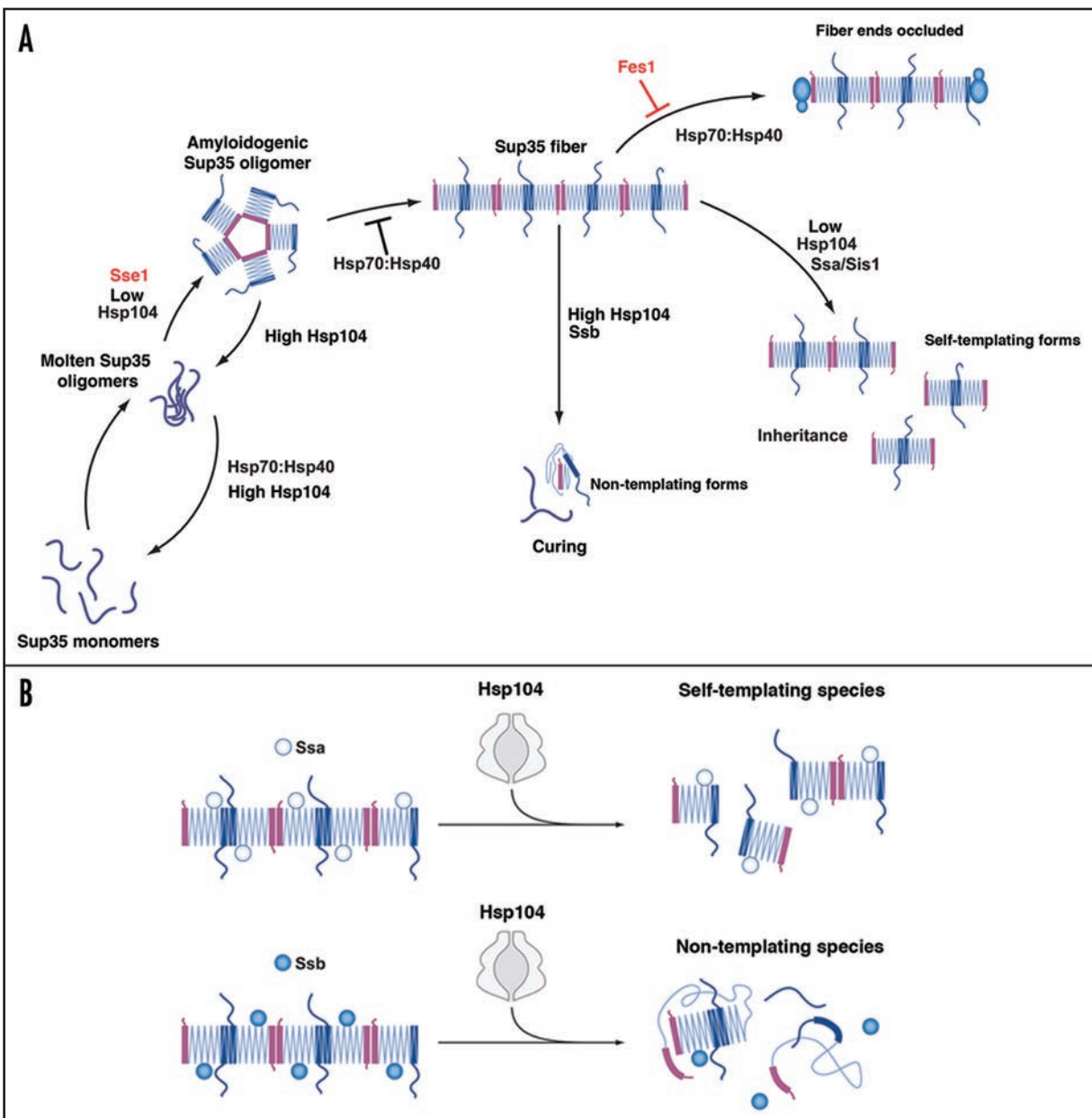


Figure 1. The role of Hsp104, Hsp70 and Hsp40 in Sup35 prionogenesis. (A) Sup35 prions assemble after a lag phase during which a dynamic ensemble of monomeric and molten oligomeric species form. The intermolecular contacts that nucleate prion assembly are likely established within amyloidogenic oligomers. Sup35 fibers are held together by an alternating sequence of Head-to-Head (pink) or Tail-to-Tail (dark blue) intermolecular contacts that are separated by a central core region (light blue) comprised of intramolecular contacts. Once formed, fibers stimulate their own assembly by recruiting and converting monomers at their ends. Various steps are promoted or antagonized by Hsp104, Hsp70, Hsp40 and the NEFs Fes1 and Sse1 as indicated. At high concentrations, Hsp70 and Hsp40 can bind Sup35 prions and occlude prion recognition elements. The C-terminal domain of Sup35 is not depicted for clarity. (B) Incorporation of Ssa1 and Ssb1 into Sup35 prions has distinct effects on subsequent Hsp104 remodeling. Ssa1 incorporation protects fibers against remodeling by Hsp104 to non-infectious conformations, perhaps by maintaining active fiber ends. Ssb1 incorporation promotes the remodeling of fibers to non-infectious conformations, perhaps by promoting inactivation of fiber ends. The majority of Ssb1 is associated with the ribosome.²⁹ However, a considerable fraction is found throughout the cytoplasm,²⁹ which is likely to be able to interact with Sup35 prions.

Yet, basal Hsp70 ATPase activity is too slow to drive chaperone activity.³⁰ Therefore, Hsp70s function with their obligate Hsp40 cochaperones, which via their J-domain accelerate Hsp70 ATPase activity and stabilize substrate binding.³⁰ For Ssa1 two key Hsp40s are Ydj1 and Sis1.³¹ By contrast, Ssb1 requires a heterodimer of Zuo1, an Hsp40 and Ssz1, an atypical Hsp70.³² Nucleotide exchange factors (NEFs) facilitate exchange of ADP for ATP and

promote substrate release.³⁰ Fes1 and Sse1 serve as NEFs for both Ssa1 and Ssb1.³³⁻³⁶ Recently, it became clear that Ssa1/2 are major components of ex vivo Sup35 prion polymers, with ~one Ssa molecule per two Sup35 molecules. Ssb1, Ydj1 and Sis1 are also found to be physically associated, although in smaller amounts.³⁷

First, the direct effects of the Hsp70:Hsp40 chaperone system on Sup35 prionogenesis were determined.¹⁹ Sup35 prions form after

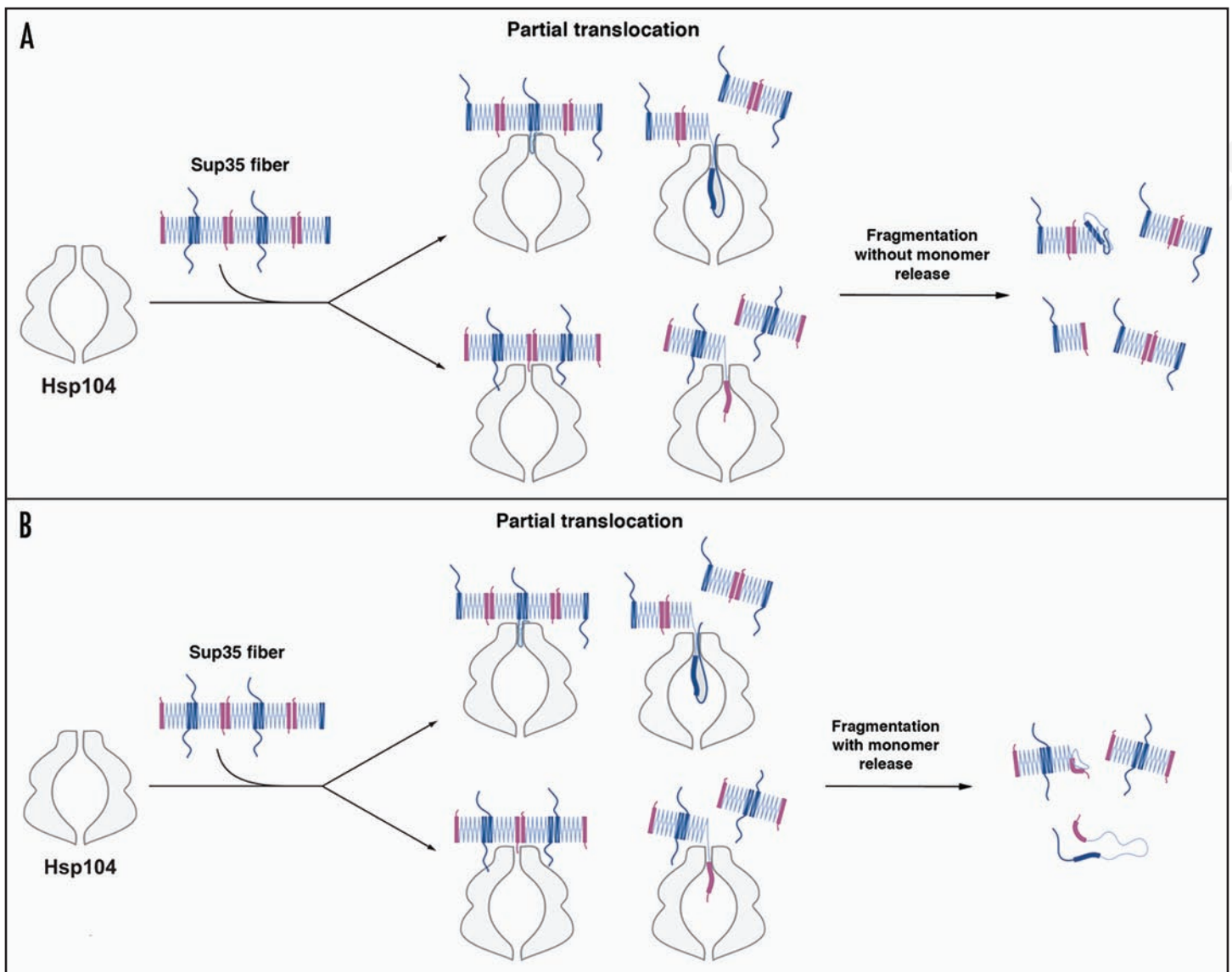


Figure 2. Possible modes of Sup35 prion fragmentation by Hsp104. (A, B) Partial translocation might disrupt Head-to-Head (pink) or Tail-to-Tail (blue) contacts without (A) or with (B) monomer release. Partial translocation might occasionally leave some fiber ends inactivated. The C-terminal domain of Sup35 is not depicted for clarity.

a lag phase during which a dynamic ensemble of monomers and molten oligomers form³⁸ (Fig. 1A). Molten oligomers mature into amyloidogenic oligomers that nucleate prion assembly^{11,12,38} (Fig. 1A). During this process, prion recognition elements within the N-terminal prion domain termed the ‘Head’ and ‘Tail’ are proposed to make homotypic intermolecular contacts. Thus, fibers are held together by an alternating sequence of ‘Head-to-Head’ and ‘Tail-to-Tail’ interactions, which are separated by a central core comprised of intramolecular contacts³⁹⁻⁴¹ (Fig. 1A). Once formed, fibers seed their own assembly by recruiting and converting monomers at their growing ends.³⁸ Hsp70:Hsp40 pairs were potent antagonists of de novo Sup35 prion assembly, particularly the ribosome-associated Ssb1:Zuo1:Ssz1 complex.¹⁹ Ydj1 and Sis1 selectively bound Sup35 oligomers and fibers, but not monomers, and facilitated Ssa1 and Ssb1 binding.¹⁹ Hsp70:Hsp40 pairs blocked prion nucleation by disassembling molten oligomers and by binding mature oligomers¹⁹

(Fig. 1A). Although able to disassemble molten oligomers, Hsp70:Hsp40 could not disassemble preformed Sup35 prions.¹⁹ Rather, recruitment of Ssa1 or Ssb1 to Sup35 fibers occluded prion recognition elements and prevented seeded assembly¹⁹ (Fig. 1A). These biochemical data help explain several genetic correlates, including why overexpression of Hsp70:Hsp40 can cure certain [PSI⁺] variants,^{25,26} and why deletion of Ssb, the most potent antagonist (in combination with Zuo1 and Ssz1) of synthetic Sup35 prionogenesis, increases [PSI⁺] induction ~10-fold.²⁷

How do NEFs affect these Hsp70 activities? Curiously, Sse1 directly stimulated nucleation of the prion domain of Sup35²⁴ (Fig. 1A). Indeed, Sse1 facilitates [PSI⁺] induction even in the absence of [PIN⁺].²⁴ By contrast, Fes1 exerted no direct effects on Sup35 prionogenesis,¹⁹ allowing direct assessment of how nucleotide exchange activity affected Hsp70 activities. Fes1 partially antagonized the inhibitory activities of Hsp70:Hsp40 (Fig. 1A), which

provides a direct explanation for why Fes1 deletion destabilizes [*PSI*⁺] and Fes1 overexpression promotes [*PSI*⁺].²¹ Fes1 NEF activity promotes substrate release by Ssa1 and Ssb1, which may remove Hsp70 from Sup35 prions and allow conformational replication to proceed. This is consistent with the transient and iterative interactions between Hsp70 and polyglutamine aggregates observed *in vivo*, where Hsp70 rapidly cycles on and off the aggregate surface.⁴²

In general, the Hsp70:Hsp40 chaperone machinery directly inhibits *de novo* and templated Sup35 prion assembly. Yet, how does this machinery affect prion remodeling by Hsp104? At low concentrations, Hsp104 accelerates Sup35 prionogenesis.¹² This activity was able to override the inhibitory activities of Ssa1:Sis1 or Ssb1:Sis1, but not Ssa1:Ydj1 and Ssb1:Ydj1.¹⁹ Thus, opposing Hsp40-dependent chaperone machineries balance the promotion (Sis1) or inhibition (Ydj1) of Sup35 prionogenesis.

Incorporation of Hsp70 and Hsp40 into nascent Sup35 prions made them better substrates for remodeling by high concentrations of Hsp104.¹⁹ This supports genetic data that Hsp70 assists prion fragmentation by Hsp104.^{20,21} Importantly, the consequences of Hsp104-remodeling depended upon the incorporated Hsp70. Ssb1 promoted the elimination of Sup35 prions, whereas Ssa1 inhibited it (Fig. 1A and B). Thus, Ssb1 likely increases the Hsp104 activity that converts Sup35 prions to non-replicating amyloid-like species, perhaps by ensuring inactivation of fiber ends (Fig. 1B). This might involve destabilization of fiber end structure or by sequestration of fiber ends to make them inaccessible (Fig. 1B). By contrast, Ssa1 likely maintains active fiber ends despite remodeling by Hsp104 (Fig. 1B). These differences in Ssa1 and Ssb1 activity likely reflect differences in their interactions with Sup35 prions conferred by their distinct substrate binding domains.²² Furthermore, these biochemical observations help explain why high levels of Ssa1 inhibit curing by overexpression of Hsp104, whereas high levels of Ssb1 promote it.^{23,27} The protection of self-replicating activity by Ssa1 despite remodeling by Hsp104 helps explain several genetic correlates that Ssa1 can promote [*PSI*⁺].^{23,24,27} The presence of Ssa1/2 as a major component of Sup35 prions *in vivo*³⁷ might help ensure long-term prion stability. Taken together, these observations suggest that the Hsp70:Hsp40 chaperone machinery functions collectively as a rheostat that finely tunes Hsp104's basic prion-remodeling activities.

Hsp70 and Hsp40 likely help, but are not absolutely required, to present specific polypeptide loops of Sup35 fibers to Hsp104 to promote prion remodeling. Such a role is consistent with a function for Hsp70 and Hsp40 early in protein disaggregation.⁴³ Accumulating evidence suggests that Hsp104 drives protein disaggregation and prion propagation by translocating substrates across its large central cavity after they are fed into its N-terminal entrance.^{15,43-49} Recent genetic data reinforce the facilitatory role of the Hsp70:Hsp40 machinery in Sup35 prion severing by Hsp104. Depletion of Sis1 leads to gradual increases in polymer size and loss of [*PSI*⁺] over ~60 generations,^{46,50} suggesting that severing of Sup35 prions by Hsp104 is partially impaired by the absence of Sis1. Similarly, incorporation of Sis1 into Sup35 prions increases their susceptibility to Hsp104-driven remodeling *in vitro*.¹⁹ Intriguingly, depletion of Sis1 causes a rapid loss of [*RNQ*⁺] and [*URE3*] indicating a greater importance of the Hsp70:Hsp40 chaperone machinery for the propagation of these prions.⁵⁰ Little is known about the direct effects of Hsp104, Hsp70 or Hsp40 on synthetic Rnq1 prionogenesis. However, Hsp104 is able

to effectively fragment Ure2 prions *in vitro*, but is not able to eliminate their self-templating activity.¹² Therefore, *in vivo*, additional factors associated with Ure2 prions may make the Hsp70:Hsp40 system more essential for Hsp104 activity. Unfortunately, in contrast to Sup35 and Rnq1,^{37,51} little is known about the factors that associate with Ure2 prions *in vivo*.

Within the framework of the 'Head-to-Head' and 'Tail-to-Tail' model of Sup35 prion structure³⁹⁻⁴¹ (Fig. 2A), Hsp104 need only break the 'Head-to-Head' or 'Tail-to-Tail' intermolecular contact to fragment prions. This might only require partial translocation across the central channel of Hsp104 hexamers. For example, Hsp104 might pull on the extreme N-terminal region of Sup35, which is partially accessible in Sup35 prions,^{39,52} to melt only the Head-to-Head contact and then release (Fig. 2A). Alternatively, Hsp104 might translocate polypeptide loops just C-terminal to the Tail to forcibly separate only the Tail-to-Tail contact and then release (Fig. 2A). In this way, fragmentation of Sup35 prions at low Hsp104 concentrations may not involve the liberation of Sup35 monomers (Fig. 2A). In either case, the released portion of Sup35 may occasionally fail to fold back into the appropriate β -sheet conformation leading to inactivation of the fiber end (Fig. 2A). This event may be promoted or inhibited by Hsp70 (Fig. 1B). At higher Hsp104 concentrations, Hsp104 hexamers likely co-operate to drive Sup35 fiber disassembly,^{11,12} which facilitates the release of Sup35 monomers (Fig. 2B) and perhaps more frequent inactivation of fiber ends. Even with Sup35 monomer release, translocation might be partial, such that the Head-to-Head and Tail-to-Tail contacts of individual Sup35 molecules are broken, but the large C-terminal GTPase domain of Sup35 is not translocated across the channel (Fig. 2B). The large central cavity of Hsp104 might facilitate such an activity.¹⁵ Indeed, this mode of partial threading is sufficient for Hsp104 to promote the disaggregation of denatured aggregates of model substrates.⁵³ In this manner, Hsp104 might release Sup35 with a functional C-terminal GTPase domain and rapidly reverse the [*PSI*⁺] phenotype.

Our model outlined above emphasizes the importance of the large central cavity of Hsp104, which has been revealed by cryo-EM reconstructions.¹⁵ Several interesting Hsp104 mutants (L462R, P557L and D704N) have been described that confer defects in [*PSI*⁺] propagation, but not thermotolerance.⁵⁴ These mutations were all suggested to be close to the 'lateral channels' of Hsp104.⁵⁴ However, these assignments were based on cryo-EM reconstructions and domain fitting of tClpB hexamers,⁵⁵ which appear unlikely to accurately reflect Hsp104 hexamer structure.¹⁵ Indeed, in the cryo-EM reconstruction and domain fitting of Hsp104, L462 and D704 do not appear to be so close to the lateral channels.¹⁵ In this model, L462 resides on helix L2 of Hsp104's coiled-coil domain in proximity to nucleotide in nucleotide binding domain (NBD) 1 and D704 is located on NBD2 at the contact interface with the coiled-coil of the adjacent protomer.¹⁵ P557 is quite close to the lateral channels but faces the central cavity on a short linker between the NBDs.¹⁵ Hence, the importance of the lateral channels and how mutations at these positions selectively affect Hsp104 prion-remodeling activity remains unclear.

We must also note that the precise atomic structure of Sup35 prions remains unknown and several distinct models of Sup35 prion structure have been proposed.^{39,40,52,56-58} Regardless of the exact prion structure, fragmentation by Hsp104 requires separation

of intermolecular prion contacts. However, the very nature of these intermolecular contacts can also vary as Sup35 can assemble into multiple structurally distinct amyloid forms or 'strains', which encode distinct $[PSI^+]$ variants.^{1,39,59-61} $[PSI^+]$ variants range from 'weak' to 'strong' as the extent of Sup35 aggregation and inactivation increases.^{1,59} In vitro, Sup35's prion domain forms fibers at 4°C that encode strong $[PSI^+]$ variants, whereas fibers formed at 25°C encode weak $[PSI^+]$ variants.^{39,60,61} These distinct prion conformations are distinguished by the amount of primary sequence that is sequestered in cross- β structure.^{39,52} More specifically, the central core is longer,³⁹ the position of the Tail-to-Tail contact is more C-terminal³⁹ and residues N-terminal to the Head are more structured in 25°C fibers than in 4°C fibers.^{39,52} This renders 4°C fibers more fragile and sensitive to detergents.^{60,61} Fragmentation of these distinct prion conformations likely makes distinct demands on Hsp104. For example, in 4°C fibers, the shorter stretch of cross- β structure is likely to increase the accessibility of intermolecular contacts and require less ATP hydrolysis by Hsp104 to unfold. By contrast, the intermolecular contacts of 25°C fibers are likely to be less accessible and the longer stretch of cross- β structure likely requires more ATP hydrolysis by Hsp104 to unfold. Thus, prion fragmentation by Hsp104 with or without monomer release (Fig. 2A and B) is likely to be more difficult for Sup35 prion conformations that encode weak $[PSI^+]$ and perhaps leads to more frequent fiber end inactivation. This is in keeping with weak $[PSI^+]$ variants possessing fewer, longer Sup35 polymers per cell.^{16,61} The sensitivity of $[PSI^+]$ variants to curing by excess Hsp104 is inversely proportional to their strength.⁶² That is, weak $[PSI^+]$ variants are more readily cured by Hsp104 overexpression than strong $[PSI^+]$ variants.⁶² We suspect that excess Hsp104 remodels prion conformations that encode weak $[PSI^+]$ in a manner that more rapidly generates various non-templating species (Fig. 1A and B). Future biochemical studies will address the foregoing possibilities, as well as how the Hsp70:Hsp40 machinery affects different Sup35 prion strains.

It is also important to consider that the protein-remodeling activities of Hsp104 might have downstream consequences for prions in vivo that are not reconstituted in vitro. Several recent studies emphasize the importance of spatial quality control of protein aggregates in yeast.⁶³⁻⁶⁶ For example, Hsp104 appears to be intimately involved in the retention of carbonylated, aggregated proteins in the mother cell, which are associated with aging.⁶³ Moreover, yeast lacking Hsp104 have reduced longevity and fail to retain carbonylated proteins in the mother.⁶³ Overexpressed Sup35, Ure2 and Rnq1 form aggregates that are found throughout the cytoplasm, but a subset are partitioned to a discrete perivacuolar compartment.^{64,65} This partitioning of prion aggregates may play an important and currently unappreciated role in yeast prion protein regulation.⁶⁷ For example, the cell may be unable to recover Sup35 from non-replicating amyloid-like aggregates generated by excessive remodeling by Hsp104. These terminally aggregated conformers might then accumulate at a perivacuolar site⁶⁵ and be targeted for retention and possibly autophagic degradation.

Finally, Hsp104 rapidly remodels amyloid and preamyloid oligomers.^{11,12} Curiously, there is no known orthologue or analogue of Hsp104 in metazoa.⁹ Therefore, reintroduction of Hsp104 might have therapeutic utility for several protein-misfolding disorders.⁹ Indeed, expression of Hsp104 has ameliorative effects in rodent models of Huntington^{68,69} and Parkinson disease.⁷⁰ Moreover, Hsp104 directly disassembles α -synuclein fibers and oligomers

connected with Parkinson disease.^{70,71} Hsp104 also directly antagonizes A β 42 amyloidogenesis, which is connected with Alzheimer's disease.⁷² An accurate understanding of Hsp104 structure and function might enable enhancements that potentiate its activity against the specific protein-misfolding events that distinguish several fatal neurodegenerative disorders.⁹

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