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Review

Chaperone Effects on Prion and Nonprion Aggregates

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ABSTRACT

Exposure to high temperature or other stresses induces a synthesis of heat shock proteins. Many of these proteins are molecular chaperones and some of them help cells to cope with heat-induced denaturation and aggregation of other proteins. In the last decade, chaperones have received increased attention in connection with their role in maintenance and propagation of the *Saccharomyces cerevisiae* prions, infectious or heritable agents transmitted at the protein level. Recent data suggest that functioning of the chaperones in reactivation of heat-damaged proteins and in propagation of prions is based on the same molecular mechanisms but may lead to different consequences depending on the type of aggregate. In both cases the concerted and balanced action of "chaperones' team," including Hsp104, Hsp70, Hsp40 and possibly other proteins, determines whether a misfolded protein is to be incorporated into an aggregate, rescued to the native state or targeted for degradation.

CHAPERONES AND THERMOTOLERANCE

The role of Hsps in development of induced thermotolerance. A mild heat shock, which is nonlethal by itself, induces the synthesis of heat shock proteins (Hsps) and enhances the cell capacity to survive the subsequent severe heat shock exposure. This phenomenon is known as induced thermotolerance. It has been suggested that Hsp induction is essential for survival at elevated temperatures.[1] A correlation has been found between the dynamics of development of induced thermotolerance in *S. cerevisiae* and synthesis of the heat shock protein with the molecular mass of about 100 kDa, later designated as Hsp104.[2] Experiments with the deletion mutants clarified the role of Hsps in induced thermotolerance. Hsp104 is shown to play a crucial role in the induced thermotolerance in yeast,[1,3,4] while Hsps of the Hsp70,[4,5] Hsp40 and small Hsp[6,7] families perform auxiliary functions in this process. Our focus on the Hsp104, 70 and 40, which are shown to cooperate with each other in disaggregation and refolding of the stress-damaged proteins.

General characteristics of molecular chaperones: "holdases," "foldases" and "disagregases." Most Hsps are molecular chaperones facilitating protein folding, assembly and translocation across intracellular membranes. Roughly chaperones may be classified based on the mode of their interaction with substrate proteins. The ATP independent molecular chaperones, such as small heat shock proteins (sHsps) and proteins of Hsp40 family that can stabilize unfolded polypeptides but could not reactivate them are referred to as "holdases."[6,9] Some chaperones having ATP-binding domains that assist in folding of nonnative proteins via ATP-dependent binding and release are known as "foldases." Proteins of the Hsp70 family also assist in folding, so that Hsp70s also seem to possess "foldase" activity. However, there is an alternative view considering the Hsp70 chaperones as "unfoldases" that use free energy from ATP binding and/or hydrolysis to unfold or pull apart misfolded and aggregated proteins to yield productive folding intermediates.[8]

The Hsp100/ClpB proteins are proposed to use ATP hydrolysis to disentangle aggregated polypeptides and transfer partially folded species to the Hsp70-Hsp40 binary system for subsequent refolding.[4,10] In this context, Hsp100s act as "disaggregases."[11] However, *Escherichia coli* homologs of Hsp70 (DnaK) and Hsp40 (DnaJ) are thought to bind first to aggregated proteins, potentially helping Hsp100/ClpB to extract polypeptides from aggregates.[10] Such an interplay is also shown to occur between sHsps and Hsp100/ClpB in yeast and bacteria.[6,7,10]

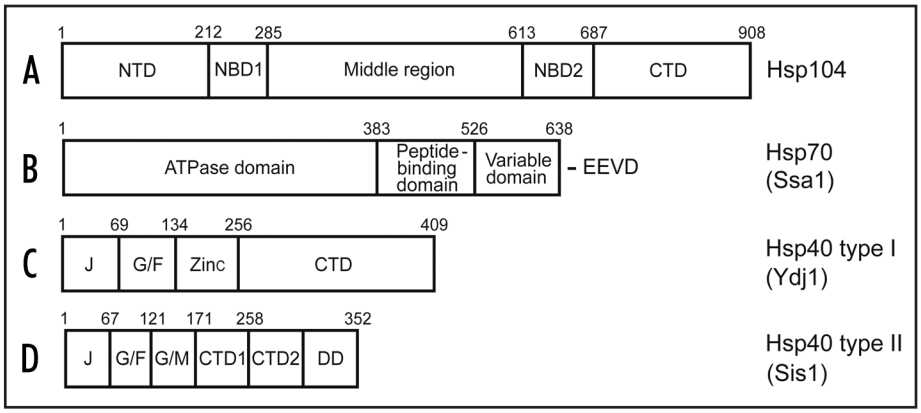

Figure 1. Structural organization of the yeast Hsp proteins involved in stress protection and prion propagation. (A) Hsp104; (B) Ssa1 as a representative of the Hsp70 family; (C) Hsp40 type I (Ydj1); (D) Hsp40 type II (Sis1). (The Hsp40 type III is not shown.) Designations: NBD, nucleotide-binding domain; NTD, N-terminal domain, middle region; CTD, C-terminal domain; J, J-domain; G/F, glycine and phenylalanine-rich region; Zinc, zinc-finger domain; G/M, glycine and methionine-rich region; DD, dimerization domain; E, glutamic acid; V, valine; D, aspartic acid. Numbers correspond to aa positions.

Yeast Hsp104. Hsp104 (Fig. 1A) is a yeast member of the Hsp100/ClpB family of AAA (ATPases associated with various cellular activities) superfamily of proteins, participating in a variety of cellular activities.[10,11] In the presence of ATP, ADP or ATPγS, Hsp104 monomers are assembled into the hexamer complexes with an axial channel.[12] Yeast Hsp104 has two nucleotide-binding domains, NBD1 and NBD2 with different catalytic properties.[12,13] Mutations in NBD1 have little effect on hexamerization, while mutations in NBD2 severely impair hexamerization.[14] There is an allosteric communication between NBD1 and NBD2[13] as well as communication between individual monomers in a hexamer, so that the ATP hydrolysis by Hsp104 is greatly influenced by hexamerization.[15]

Hsp104 is induced by a mild heat shock treatment, and is crucial for induced thermotolerance in *S. cerevisiae*.[1] It is also known to be induced in response to hydrogen peroxide,[16] ethanol and sodium arsenite,[1] and near-freezing cold shock.[17] The ability of the *hsp104*Δ cells pretreated at 37°C to survive a lethal heat shock at 50°C is severely impaired but not completely abolished, indicating that other heat induced proteins also play role in this process.[1] Specifically, Hsp70-Ssa and small heat shock protein Hsp26 become very important for thermotolerance in the *S. cerevisiae* cells lacking Hsp104.[5,7] Aggregation of cellular proteins is a major consequence of severe stress, and Hsp104 is thought to act directly on protein aggregates, leading to their resolubilization.[3] For efficient protein reactivation, Hsp104 requires the assistance of Hsp40 (Ydj1) and Hsp70 (Ssa).[4]

Abilities of Hsp104 to form a homohexamer and cooperatively bind and hydrolyze ATP are required for its functions in vivo.[12,13] When ATP binding to NBD1 is impaired by a mutation, Hsp104 is unable to interact with substrates both in vitro and in vivo.[18,19] Therefore, the ATP-bound state of NBD1 seems to be crucial for the chaperone-substrate interaction. Two models have been proposed for disaggregating action of Hsp104. In the first model, Hsp104 breaks up large aggregates into smaller ones in a crowbar-like activity. The second model suggests that a single polypeptide chain is extracted from an aggregate via translocation through the axial channel of Hsp104/ClpB hexamer, occurring by the unfolding/threading mechanism.[10,11] The structure of the pore entrance of the Hsp104 oligomeric complex was shown to be crucial for Hsp104 function,[20] supporting the latter mechanism.

Yeast Hsp70. All Hsp70 family proteins have three functionally separated domains: N-terminal 45 kDa ATP-binding domain, 15 kDa peptide-binding domain and C-terminal variable domain (Fig. 1B). Hsp70 transiently holds unfolded substrates in an intermediate state, preventing irreversible aggregation and catalyzing folding in the ATP dependent manner.[21] However, the precise mechanism by which Hsp70 promotes folding is unclear thus far. There are at least two possible models of Hsp70's action, which are not mutually exclusive. Model 1 ('kinetic partitioning' model) suggests that Hsp70 plays a rather passive role. Via repetitive substrate binding and release cycles, it decreases the concentration of a free substrate. This prevents aggregation and allows more time for the substrate to fold into the native state. Model 2 ('local unfolding' model) proposes that Hsp70 induces local unfolding in the substrate, e.g., the untangling of a misfolded β-sheet, which helps to overcome kinetic barriers for folding to the native state. ATP energy may be needed either to induce such conformational changes or alternatively, to drive the ATPase cycle in the right direction.[21] *S. cerevisiae* genome contains at least fourteen genes coding for the Hsp70 proteins. These proteins are localized in a variety of cellular compartments including the cytosol (subfamilies Ssa and Ssb), mitochondria (Ssc1 and Ssq1), endoplasmic reticulum (Kar2 and Lhs1), etc.[22]

Ssa subfamily is encoded by four genes: *SSA1*, *2*, *3* and *4* (reviewed in ref. 22). *SSA3* and *SSA4* genes are expressed only at very low level in the exponentially growing cells, but are drastically induced after the temperature upshift, as well as by the stationary phase and other stresses. *SSA2* is constitutively expressed, while *SSA1* is normally expressed at moderate levels and induced by stresses. Deletion of any individual *SSA* gene does not affect induced thermotolerance. Double *ssa1*Δ *ssa2*Δ mutants grew slower than the parent at all temperatures and were unable to form colonies at 37°C, but their ability to induce tolerance to heat shock at 37°C was not changed. Moreover, double mutant displayed a higher level basal thermotolerance that is apparently due to upregulation of other Hsps.[22,23] At least one of the Ssa proteins must be present to preserve the cell viability.[22] Ssa proteins are implicated in protein translocation across intracellular membrane, prevention of aggregation of denatured proteins[24] and cotranslational folding.[25]

Another yeast cytosolic Hsp70 family, Ssb, is not stress-inducible, and is encoded by two almost identical genes, *SSB1* and *SSB2*. Strains with a single *SSB* gene disrupted exhibit no phenotypic change. However, a mutant with both genes disrupted grows slowly at all temperatures, and is cold sensitive.[22] The major fraction of Ssb proteins has been found in association with the translating ribosomes, although some Ssb is distributed freely in the yeast cytosol. It is postulated that Ssb aids in folding of the emerging newly synthesized proteins.[25] It is also possible that this protein is involved

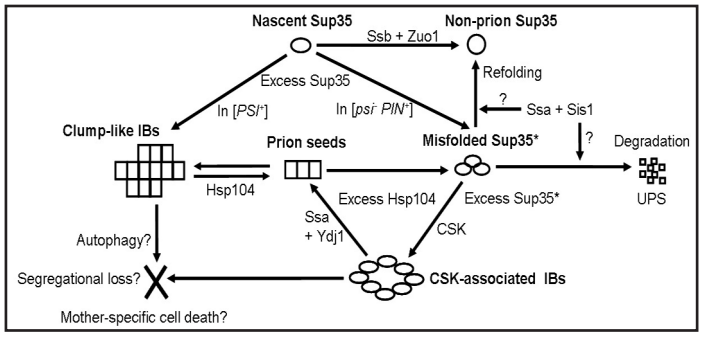

Figure 2. Model for the role of molecular chaperones in formation and propagation of the [*PSI*+] prion. CSK, cytoskeleton; UPS, ubiquitin-proteasome system; IBs, inclusion bodies. See comments in text.

in protein turnover in the yeast by targeting misfolded proteins for degradation, as overexpression of Ssb1 suppressed the growth defects caused by some proteasome mutations.[26]

Yeast Hsp40 or J-Proteins. The Hsp40 family, subdivided into three subtypes, includes the structurally and functionally diverse proteins with one common feature, N-terminal J-domain (Fig. 1C and D).[9] According to the current model,[9,21] J-proteins first bind unfolded protein substrates in order to prevent their aggregation, and then transfer them to Hsp70, simultaneously stimulating the Hsp70 ATPase activity and thus stabilizing the Hsp70 interaction with the unfolded protein.

Type I Hsp40s, for instance yeast Ydj1, also contain the glycine and phenylalanine-rich (G/F) region, zinc finger-like domain, and conserved C-terminal domain (CTD). Type II Hsp40s, for example yeast Sis1, lack the zinc finger-like domain but contain extended glycine-rich region. The first 55 amino acids (aa) of this region are also rich in phenylalanines (G/F); the last 49 aa are rich in methionine residues (G/M). The C-terminal 181 aa of Sis1 contain the proposed polypeptide binding site (CTD1), a domain of unknown function (CTD2), and a dimerization domain.[9,27]

Both Ydj1 (type I) and Sis1 (type II) interact with Ssa, but not with Ssb. Ssb has its own Hsp40 cochaperone, zuotin or Zuo1.[9] Ydj1 is not essential, but *ydj1*Δ deletion causes severe growth defects. In cooperation with Ssa, Ydj1 promotes the protein translocation across the intracellular membranes, and participates in refolding of the heat-damaged proteins. C-terminal domain of Ydj1 has been implicated in binding unfolded polypeptides.[23] Apparently, zinc finger-like domain is necessary for transferring the nonnative polypeptides from Ydj1 to Hsp70.[28] Ydj1 is also required for ubiquitin-dependent degradation of certain abnormal proteins.[23]

The essential protein Sis1 is shown to be less effective than Ydj1 in helping Ssa to suppress aggregation of stress damaged proteins but is linked to other processes, for example initiation of protein synthesis.[25] Functions of Ydj1 and Sis1 are overlapping but not identical. Although excess Sis1 complements the slow growth phenotype of *ydj1*Δ, Ydj1 cannot complement the lethal phenotype of *sis1*Δ.[24]

CHAPERONE EFFECTS ON PRION PROPAGATION

The role of Hsp104 in propagation of the yeast prions. Yeast prions [*PSI*+], [*PIN*+] and [*URE3*] are self-perpetuating amyloid-like polymers of the proteins Sup35, Rnq1 and Ure2, respectively reviewed in refs. 29–31. Role of chaperones in prion propagation was first demonstrated for [*PSI*+].[32] Search for genes that antagonize [*PSI*+] when present in the increased number of copies produced *HSP104*.[33] Further investigation revealed that both overproduction and inactivation of Hsp104 cause loss of [*PSI*+].[32] Surprisingly, overproduction of Hsp104 does not prevent de novo [*PSI*+] appearance.[34,35] Hsp104 is also required for propagation of [*PIN*+][36] and [*URE3*],[37] although high levels of Hsp104 do not antagonize these prions.

Recent experimental evidence[38-41] (reviewed in ref. 29) supports a model[35] postulating that the major role of Hsp104 in prion propagation in vivo is to break prion amyloids into smaller "seeds," initiating new rounds of prion production (Fig. 2). This mechanism essentially means that disaggregating activity of Hsp104 converts amyloids into self-perpetuating prions.[30,31] Variants of [*PSI*+] producing large aggregates that are relatively insensitive to Hsp104 require excess of this protein for efficient propagation.[35,39]

Dominant negative point mutations in either NBD (Fig. 1) disturb the Hsp104 ability to perform its role in both induced thermotolerance[3,4,12,13] and prion propagation,[13,32,38,42] indicating that both functions are associated with hexamerization and ATP hydrolysis. However, some mutant derivatives of Hsp104 that function efficiently in prion propagation can not protect yeast from extreme thermal stress.[19] On the other hand, the dominant mutant derivative of Hsp104, bearing the A503V substitution in the middle region, increases size of the Sup35 aggregates in [*PSI*+] cells, leading to accumulation of cytologically detectable clumps and cytotoxicity, but decreases aggregate size and cytotoxicity of the polyglutamine fragment of human huntingtin expressed in yeast, and does not affect thermotolerance.[43] A503V substitution impairs coordinated regulation of the NBD action without completely eliminating ATPase activity,[44] that possibly leads to different consequences depending on with which type of aggregate that Hsp104 is interacting.

It remains unknown why does excess Hsp104 cause loss of [*PSI*+] but not of the other yeast prions. [*PSI*+] loss in the presence of excess Hsp104 probably requires other Hsp104 activities in addition to (or instead of) those involved in prion propagation, as N-terminal region of Hsp104 is required for [*PSI*+] curing by excess Hsp104 but not for [*PSI*+] maintenance.[45] It is possible that excess Hsp104 solubilizes Sup35 prion polymers into monomers, that may require a mode of action distinct from one involved in oligomeric "seed" production. Alternatively, it was proposed that excess Hsp104 may impair prion segregation in cell divisions.[46] Indeed, average size of remaining Sup35 polymers is increased in the presence of excess Hsp104,[41] although this could be due to the fact that larger polymers are less sensitive to the Hsp104 disaggregating effect. The [*PSI*+] cells overexpressing Hsp104 accumulate cytologically detectable ring-like Sup35 structures that are not usually found in the [*PSI*+] cells with normal levels of Hsp104.[34] Rings are also observed in the cells undergoing de novo [*PSI*+] induction in the presence of excess Sup35,[34] and are shown to be associated with some components of the cortical actin cytoskeleton (CSK) involved in endocytosis, and/or with vacuolar membrane.[47] It is possible that rings represent intermediates that arise in the process of either de novo prion formation or prion elimination, and are attempted by the cell to be targeted for elimination via autophagy and vacuolar proteolysis.

EFFECTS OF OTHER CHAPERONES ON PRIONS AND POLYGLUTAMINE AGGREGATES IN YEAST

Ssa effects. Increased levels of Ssa1[48] or any other member of Ssa subfamily[49] enhanced phenotypic manifestation of [*PSI*$^+$] and antagonized [*PSI*$^+$] curing by excess Hsp104. Moreover, excess Ssa facilitated de novo [*PSI*$^+$] induction in [*psi*$^-$] cells by overproduced Sup35.[49] Several semi-dominant mutations in the *SSA1* gene have been obtained that decrease the mitotic stability of [*PSI*$^+$].[51,52] Strains with the mutant alleles of *SSA1* were unable to propagate [*PSI*$^+$] in the absence of the wild-type alleles of both *SSA* genes normally expressed in the absence of stress, *SSA1* and *SSA2*.[50] The second-site mutations in *SSA1* restored normal prion propagation.[51] Some (but not all) of the distantly related Hsp70 homologs from other organisms (plants and mammals) partly compensated the defect in [*PSI*$^+$] maintenance observed in the presence of mutant Ssa.[45] This shows that Ssa functions involved in [*PSI*$^+$] maintenance are at least to a certain extent conserved in evolution.

Both calculations based on kinetics of [*PSI*$^+$] curing,[50] and biochemical assays such as size fractionation by chromatography and efficiency of fluorescence recovery after photobleaching indicated that size of the Sup35 aggregated structures is increased in the [*PSI*$^+$] strains with mutant Ssa.[53] It has been interpreted as an evidence of that Ssa helps to disassemble large aggregated structure into smaller polymers that become a target for the "shearing" action of Hsp104. On the other hand, Ssa overproduction slightly but reproducibly increased average size of prion polymers produced from large structures in the semi-denaturing conditions and visualized by semi-denaturing gel electrophoresis, that was also accompanied by an increase in the proportion of monomeric Sup35.[49] As phenotypic manifestation of [*PSI*$^+$] was enhanced rather than antagonized by excess Sup35, one could suggest that a significant fraction of the monomeric Sup35 generated in these cultures remained in the nonfunctional (possibly misfolded) state. Physical association between Ssa and Sup35 has been confirmed both in vivo and in vitro, suggesting that effects of Ssa on [*PSI*$^+$] are due to its direct interaction with the prion-forming protein.[49]

While excess Ssa normally aids in propagation of the "conventional" variants of [*PSI*$^+$], this effect could be reversed depending on the features of prion isolate and/or conditions affecting aggregate size and seed number. When Sup35 is overproduced in the [*PSI*$^+$] strains, this leads to [*PSI*$^+$] loss at low but detectable frequency, probably in result of accumulation of the large nontransmissible aggregates due to impairment of the balance between Sup35 and Hsp104.[49] Notably, this effect is exacerbated in the presence of excess Ssa. Likewise, excess Ssa antagonizes propagation of the [*PSI*$^+$] derivatives that are characterized by the abnormally large aggregate size and require increased levels of Hsp104 for their propagation.[35,39]

In contrast to its effect on conventional [*PSI*$^+$], overproduction of Hsp70-Ssa1 impaired propagation of the yeast prion [*URE3*].[54] Strangely enough, overproduction of the highly homologous Ssa2 protein did not show the same effect, and moreover, deletion of *SSA2* impaired propagation of [*URE3*].[55] Overproduction of some members of the Ssa subfamily counteracted poly-Q aggregation and/or toxicity in some yeast-based assays.[43,56] However, at least in one genotypic background poly-Q aggregation was also decreased by double *ssa1*Δ *ssa2*Δ deletion.[57]

Ssb effects. In a strong contrast to Ssa, Ssb proteins consistently act as [*PSI*$^+$] antagonists. Excess Ssb increases [*PSI*$^+$] curing by Hsp104 overproduction,[58] inhibits [*PSI*$^+$]-mediated suppression in certain [*PSI*$^+$] isolates,[58] and causes loss of [*PSI*$^+$] upon prolonged incubation in certain genotypic backgrounds.[59,60] Simultaneous deletion of both *SSB1* and *SSB2* genes decreases efficiency of [*PSI*$^+$] curing by excess Hsp104 and increases the frequency of the spontaneous [*PSI*$^+$] formation in [*psi*$^-$] cells even in the absence of Sup35 overproduction.[58] Ssb, like Ssa, can directly interact with Sup35.[49] No effect of overproduced Ssb on poly-Q toxicity was detected.[43,56]

Difference between the Ssb and Ssa proteins in respect to [*PSI*$^+$] curing is in significant part determined by their peptide-binding domains. The presence of Ssb peptide-binding domain is sufficient for an antiprion effect even when it is combined with the ATPase and variable domains of Ssa origin.[49]

Hsp40 effects. Little is known about the effects of Hsp40 chaperones on yeast prions. Excess Ydj1 promoted loss of [*URE3*][37] and some variants of [*PIN*$^+$],[61] and somewhat antagonized the chimeric prion [*PSI*$^+$]^{PS} generated by the Sup35 protein with a prion domain from the distantly related yeast *Pichia methanolica*.[62,63] The simultaneous overproduction of Ydj1 and Ssa1 cured some weak [*PSI*$^+$] variants, but propagation of the strong variants remained unaffected.[62] On the other hand, Ydj1 deficiency did not affect maintenance of [*PIN*$^+$][64] or [*PSI*$^+$][51] in the absence of other chaperone mutations. In the cells carrying the semi-dominant mutation *SSA1-21*, lack of Ydj1 further impaired mitotic stability of [*PSI*$^+$].[51]

Another yeast cochaperone of the Hsp40 family, Sis1, appears to be required for propagation of the [*PIN*$^+$] prion.[64] Although Sis1 is an essential protein and therefore viable yeast cells lacking Sis1 cannot be constructed, in frame deletion within the *SIS1* gene eliminated [*PIN*$^+$], although it did not affect [*PSI*$^+$]. In contrast, overproduction of Sis1 had no detectable effect on [*PIN*$^+$] propagation.[65] Sis1 is coprecipitated with the aggregated but not with the soluble form of Rnq1.[64] An extended glycine-rich region of Sis1, including a region rich in phenylalanine residues (G/F) is critical for prion maintenance.[64-66]

Data on the effects of Hsp40 proteins on poly-Q aggregation in yeast are somewhat contradictory. In some assays, overproduction of Ydj1 counteracted aggregation of some poly-Q constructs in yeast.[56] In the assay using prion-dependent poly-Q aggregation,[57] Ydj1 and Sis1 exhibited opposite effects: excess Ydj1 increased the size and toxicity of poly-Q aggregates generated in the [*PIN*$^+$] strain, while excess Sis1 decreased them.[42] Likewise, mutation in the *YDJ1* gene decreased poly-Q aggregation.[57] Interestingly, these effects of Ydj1 and Sis1 somewhat parallel at least some observations made with their human homologs (Hdj2 and Hdj1, respectively) in the mammalian cells.[67] Our preliminary data also indicate that overproduced Ydj1 and Sis1 proteins influence [*PSI*$^+$] curing by excess Hsp104 in the opposite ways (S. Müller, J. Patterson and Y. Chernoff, unpublished).

Model for the chaperone effects. Data reviewed above show that one and the same group of yeast chaperones is involved in protection against misfolded proteins and in prion propagation. We propose that effects of these chaperones on prion and nonprion aggregates are determined by one and the same molecular mechanism, while differences in effects are due to different parameters of aggregates. The model summarizing our current view is shown on Figure 2.

We propose that at normal levels, Hsp104 is responsible for propagation of the prion polymers via the subsequent cycles of breakage and growth. When the Hsp104/Sup35 ratio is shifted towards Sup35 in the [*PSI*$^+$]-containing cells, polymer size is increased leading to the accumulation of large clump-shaped inclusion bodies (IBs) that are eliminated from the population either due to a segregation defect, followed by the death of IB-accumulating cells, or via autophagy. Abundance of such IBs is increased in the [*PSI*$^+$] variants with the decreased sensitivity to Hsp104 (such as [*PSI*$^+$] ≠ 104d)[35] resulting in frequent loss of these variants in the normal conditions and their rescue at high levels of Hsp104.

Increase in the Hsp104 levels results in disruption of the ordered structure of prion aggregates. However, if overexpression of Hsp104 is not accompanied by the overexpression of the Hsp70 and Hsp40 chaperones, Hsp104 is not capable of solubilizing aggregates into the properly refolded monomers on its own. Therefore, disruption of the aggregate structure and increased hydrophobic exposure, induced by Hsp104, are followed by amorphous agglomeration of the misfolded prion protein molecules, rather than by solubilization. We propose that these misfolded agglomerated proteins can be recognized by Sis1, which in combination with Ssa, targets them for either refolding or degradation via the proteasomal pathway.

Alternatively, agglomerates are recognized by the CSK networks involved in endocytosis, including such proteins as Sla1, Sla2 and End3.[47] This results in formation of the different kind of IBs, frequently having the ring-shaped morphology and associated with CSK. These IBs are intended to be targeted for degradation via autophagy and vacuolar pathway, but they can also be recognized by Ydj1, which in combination with Ssa stabilizes proteins in the misfolded state and promotes disassembly of IBs to oligomers, capable of reentering the prion propagation cycle. Therefore, Ssa effects depend on the participating cochaperones.

The Ssb protein is acting on the nascent Sup35 polypeptide, and its major function is to promote folding of the newly synthesized Sup35 into a nonprion form. In this way, Ssb is antagonizing prion formation and propagation, and its effects are additive to all other anti-prion factors as it is working in a separate pathway.

The same model also explains chaperone effects on de novo [*PSI*$^+$] formation, as shown on Figure 2. Some parts of this model should certainly apply to the other prions as well, although other steps could turn out to be protein specific. E.g., effects of specific Hsps may vary depending on the prion, for example due to different affinities of the Hsp40s to different prion proteins. In case of polyglutamine aggregates lacking the prion propagating activity on their own, prevention of the aggregate degradation in the presence of excess Ydj1 leads to an increase in their size and toxicity.

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