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Hsp104 and Prion Propagation

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Abstract

High-ordered aggregates (amyloids) may disrupt cell functions, cause toxicity at certain conditions and provide a basis for self-perpetuated, protein-based infectious heritable agents (prions). Heat shock proteins acting as molecular chaperones counteract protein aggregation and influence amyloid propagation. The yeast Hsp104/Hsp70/Hsp40 chaperone complex plays a crucial role in interactions with both ordered and unordered aggregates. The main focus of this review will be on the Hsp104 chaperone, a molecular “disaggregase”.

Keywords

Hsp104; yeast prion; *Saccharomyces cerevisiae*; chaperone; amyloid

1. INTRODUCTION

Transmissible spongiform encephalopathies (TSEs) include such neurodegenerative diseases as sheep scrapie, human Creutzfeldt-Jacob disease, bovine spongiform encephalopathy or “mad cow disease”, etc. Their common distinctive feature is that the infection is transmitted by protein particles, termed prions. The “protein only” model for TSE transmission, accepted by many scientists, states that an infectious particle is composed of a specific mammalian protein (named PrP) in an abnormal (“prion”) isoform, which promotes conversion of the normally folded protein of the same sequence into a prion isoform. Prion PrP molecules immobilize soluble PrP and form ordered aggregates [1]. These fibrous β -rich aggregates (called amyloids) were found in the brains of infected animals [2]. There is a strong similarity between amyloids formed by PrP and non-infectious amyloids as well as amyloid-like aggregates associated with such neurodegenerative diseases as Alzheimer disease (AD), Huntington disease (HD), Parkinson disease (PD), etc. (for review, see [3]). Most of these diseases remain incurable and fatal today, and some of them (including AD) are clearly age-dependent, that underlines the importance of studying these diseases.

Yeast and other fungi also contain self-perpetuating transmissible amyloids that possess prion-like properties (for review, see [4–6]). Yeast prions are heritable in cell generations and infectious via cytoplasmic exchange. Although recent data demonstrated that at least some of them are pathogenic to a certain extent (reviewed in [4,7]), they are not fatal for their carriers. It makes yeast, a classic object of genetic and molecular biology studies, also a convenient model organism for investigation of prion and amyloid diseases. In the yeast *Saccharomyces cerevisiae*, several proteins have been proven to generate self-perpetuating amyloid-based prions. These proteins include: (1) translational termination factor Sup35 (also called eRF3); (2) regulatory protein in the nitrogen metabolism pathway, Ure2; (3) protein of unknown

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function, Rnq1 (for review, see [4–7]), (4) chromatin-remodeling factor, Swi1 [8]. Prion isoforms of these proteins are named [*PSI*⁺], [*URE3*], [*PIN*⁺] (or [*RNQ*⁺]), and [*SWI*⁺], respectively.

Prion proteins contain prion domains (PrDs) that are required and sufficient for prion formation and propagation, and are usually dispensable for the normal cellular function of a prion protein (for review, see [5,6]). Although yeast prion proteins are homologous to neither mammalian PrP nor each other, some of them share common features of amino acid composition and organization. Yeast PrDs contain some sequence elements, resembling certain regions of mammalian amyloidogenic proteins: QN-rich stretches that are similar to the poly-Q stretch of mammalian huntingtin (a protein involved in Huntington disease), also some yeast PrDs have oligopeptide repeats (ORs) similar to those found in mammalian PrP (reviewed in [7]). Still it is worth mentioning that yeast can host not only Q/N rich prions, as prion domain protein HET-s from the fungus *Podospora anserina* fused to green fluorescent protein (GFP) could acquire and propagate the self-perpetuated prion state in *S. cerevisiae* [9]. This suggests a possibility for the existence of the endogenous non-Q/N-rich prions in yeast.

Even though amyloids are not always fatal for an organism, their toxic effects point to interference with certain cellular functions, therefore it is expected that cell defense systems could be activated in response to amyloid accumulation. One such system is the chaperone machinery that aids in proper folding of cellular proteins, and can be activated by stresses such as heat shock. Heat shock inducible chaperones belong to the so-called heat shock proteins (Hsps). In particular, the heat shock protein with the molecular mass of 104 kDa, later designated as Hsp104 [10], appeared to play a crucial role in the induced thermotolerance in yeast, that is, in the ability of yeast cells to survive severe heat shock exposure after pretreatment of mild heat shock [11–13]. Accumulation of protein aggregates at high temperature was detected in the mutant yeast cells lacking Hsp104. This suggested that the Hsp104 might counteract protein aggregation manifesting its role as molecular chaperone [11]. Further studies uncovered more facts about cellular activities of Hsp104, including its intimate involvement in propagation of prion aggregates. These data will be discussed in more detail below. Roles of the other components of the Hsp104/70/40 machinery are being addressed in other reviews (for example, see [14]), including this issue [15,16].

2. GENERAL CHARACTERIZATION OF Hsp104

Hsp104 is a yeast member of the Clp/Hsp100 family of AAA (ATPases associated with various cellular activities) protein superfamily [17,18]. Its production is in response to heat shock and other stressors including hydrogen peroxide [19], ethanol and sodium arsenite [11], and near-freezing cold shock [18,20]. Although induced thermotolerance of the yeast strain lacking Hsp104 is severely impaired, it is not completely abolished, suggesting that other heat shock stimulated factors may also contribute to this phenomenon [11]. While Hsp104 is conserved in prokaryotes (where it is called ClpB), fungi and plants, as well as in animal mitochondria, its cytoplasmic orthologs were not found in multicellular animals thus far (for review, see [21]). Nevertheless, the phenomenon of induced thermotolerance is detected in animal cells, indicating that other animal proteins may serve as functional analogs of Hsp104.

Molecular basis of the induced thermotolerance phenomenon is apparently related to the fact that one of the detrimental consequences of severe heat shock, and other similar stresses, is aggregation of stress-damaged cellular proteins. Hsp104 is thought to act directly on protein aggregates, leading to their resolubilization [12]. The presence of ATP, ADP or ATP γ S (slowly hydrolyzable analog of ATP) is required for assembly of Hsp104 monomers into the hexamer complexes (see Fig. (1)) [24]. Hsp104 has two nucleotide-binding domains, NBD1 and NBD2, which have an allosteric communication between each other [25], also there is communication

between individual monomers in the hexamer, so that the ATP hydrolysis by Hsp104 is greatly influenced by hexamerization [26]. It was shown that cooperative binding and hydrolysis of ATP are required for Hsp104 function in thermotolerance [24,25]. *In vitro* and *in vivo* experiments demonstrated that impairing of ATP binding to NBD1 by a mutation in this domain prevents Hsp104 from interaction with substrates [27,28]. Therefore, the ATP-bound state of NBD1 seems to be crucial for the chaperone-substrate interaction. Mutation analysis of Hsp104 has shown that the structure of the pore entrance of the Hsp104 homohexamer is crucial for Hsp104 function [29].

Two models have been proposed to explain the disaggregating role of Hsp104. In the first model, Hsp104 breaks large aggregates into smaller ones in a crowbar-like fashion [13]. The second model suggests unfolding/threading mechanism, implying that disaggregation occurs due to extracting a single polypeptide chain from an aggregate through the central pore of the Hsp104 hexamer [17,18].

While Hsp104 is playing a cytoprotective role in regard to aggregated heat-damaged proteins, independent studies of other aggregation-related phenomenon, that is, yeast prions, uncovered the crucial importance of this molecular disaggregase for amyloid propagation.

3. ROLE OF Hsp104 IN PRION PROPAGATION

In yeast, prions are apparently propagated from generation to generation by transmitting aggregated “seeds” from mother to daughter cells via cytoplasm. This initiates new rounds of aggregation, as seeds are capable of immobilizing a newly synthesized protein of the same amino acid sequence and converting it into a prion state. A prion isoform of the yeast protein Sup35, $[PSI^+]$, was shown to be maintained only at certain levels of Hsp104 [30]. Inactivation or overproduction of Hsp104 resulted in $[PSI^+]$ “curing”, meaning that the initially prion-containing culture produced a culture containing primarily (in case of overproduction) or exclusively (in case of inactivation) so-called $[psi^-]$ cells with a non-prion isoform of Sup35. Thus, the yeast model provided the first evidence connecting the chaperone system to a prion. Remarkably, intact Hsp104 was later confirmed to be required for the maintenance of all other known yeast prions, even though prions other than $[PSI^+]$ are not “cured” by Hsp104 overproduction (for review, see [14,31]).

At the molecular level, decreased production of Hsp104 or its inactivation by introduction of the dominant-negative mutant Hsp104 derivative in the prion-containing cells increased size and decreased number of the cytologically detectable Sup35 aggregates [32]. Treatment of yeast cells with guanidine hydrochloride (GuHCl), an agent known to cure yeast prions [8, 33–35] presumably via Hsp104 inhibition [36], or down regulation of Hsp104 production, also increased the average size of oligomeric units, into which aggregates are disassembled in the semi-denaturing conditions and which hypothetically correspond to prion seeds [37]. These effects can be explained by the assumption that Hsp104 breaks prion polymers into smaller oligomers. Therefore, deletion of the *HSP104* gene or inhibition of the Hsp104 activity will result in the generation of a small number of large polymers [38] (see Fig. (2)). A decreased abundance of polymers per cell would decrease overall efficiency of prion conversion, as active conversion sites are associated with the ends of the fibers. Besides, transmission of the huge aggregates to the daughter cells could be impaired, leading to the eventual loss of $[PSI^+]$ in cell divisions [39].

In some $[PSI^+]$ derivatives with abnormally large Sup35 aggregates [40,41], the prion state can be maintained only at high levels of Hsp104. At the normal Hsp104 level, these prion derivatives behave like “normal” Sup35 prions upon GuHCl inactivation of Hsp104, accumulating large non-transmissible aggregated structures. In addition to confirming the “shearing” model of the Hsp104 effect on $[PSI^+]$, these data also point to the possible existence

of metastable (“quasi-prion”) prion states that can be maintained only in specific physiological conditions characterized by altered Hsp levels. It has been hypothesized that such “quasi-prions” may potentially play a biologically positive role by controlling feedback regulation (see [7]) or protecting proteins assembled into reversibly aggregated structures from the damaging effects of certain stresses [42].

Same disaggregation-based model could potentially explain curing of $[PSI^+]$ by Hsp104 overproduction. It is possible that excess Hsp104 eliminates $[PSI^+]$ by dissolving Sup35 prion polymers to monomers. However, direct evidence for such a mechanism is lacking thus far. Fluorescence microscopy indicates that excess Hsp104 leads to diffusion of large Sup35-GFP aggregated structures visible in the $[PSI^+]$ cells [32]. But it is not clear whether diffused fluorescence pattern reflects monomerization of Sup35-GFP or simply a decrease in the aggregate size. Biochemical assays confirm that $[PSI^+]$ cells overexpressing Hsp104 accumulate a larger proportion of soluble Sup35 [43], that behaves as a monomeric protein in the semi-denaturing conditions [37]. However, it is not clear whether this monomeric Sup35 is generated *via* disaggregation of prion complexes or is synthesized *de novo* when prion propagation is inhibited and/or the number of remaining active seeds is decreased. Even if excess Hsp104 produces monomeric Sup35, it is likely to be misfolded. It is therefore possible that Sup35 produced by Hsp104 disaggregase is eliminated by proteolytic systems rather than (or in addition to) being refolded into a native form. It is worth mentioning that in case if only a fraction of prion protein actively participates in prion proliferation, degradation of this fraction would not necessarily cause a detectable change in the steady state levels of prion protein.

Interestingly, the remaining Sup35 polymers that can be detected by the semi-denaturing gel electrophoresis in the presence of excess Hsp104 are generally larger in size, compared to those found in the extracts of the same strain expressing Hsp104 at normal levels [37]. Based on these data, an alternative model has been proposed suggesting that excess Hsp104 “cures” $[PSI^+]$ by converting prion aggregates into larger non-transmissible structures (see in [44]). Overproduced GFP-tagged Sup35 indeed formed large filaments in $[PSI^+]$ cells when Hsp104 was overexpressed, as detected by fluorescent microscopy [45]. Such filamentous structures have not been detected in the $[PSI^+]$ cells expressing Hsp104 at normal levels; however, they have been seen in the initially $[psi^-]$ cultures overproducing GFP-tagged Sup35 constructs [45]. It was shown that these filaments are associated with some cytoskeletal components of the endocytic/vacuolar network and are interpreted as the intermediates in the prion *de novo* formation pathway [46]. It is possible that similar structures may serve as prion curing intermediates as well. For instance, excess Hsp104 might initiate conversion of amyloids into amorphous agglomerates associated with cytoskeletal structures that can be then degraded *via* autophagy and vacuolar proteolysis (for review, see [14]). However, it is not known if such agglomerates can be formed when prion protein is not overproduced.

It is also possible that larger aggregates detected in the presence of excess Hsp104 are simply selected because they are less sensitive to the disaggregating action of this chaperone. This agrees with the observation that prion derivatives, characterized by an abnormally large aggregate size, can be maintained only at high Hsp104 levels (see above and [40,41]). On the other hand, some weak $[PSI^+]$ variants are hypersensitive to excess Hsp104 [47] despite the fact that they accumulate large aggregates. This suggests that either sensitivity to Hsp104 may also depend on parameters other than aggregate size, or the majority of large aggregates do not participate in prion proliferation in such variants.

These and other controversies indicate that despite remarkable progress, molecular basis of the Hsp104 role in prion propagation is not yet completely understood. Indeed, it is not clear why

prions other than [*PSI*⁺] (i.e. [*URE3*] or [*PIN*⁺]) require normal level of Hsp104 for propagation but are not eliminated by excess Hsp104 [35,48–50].

One possibility could be that such prion aggregates are not disaggregated as efficiently as Sup35 aggregates, either due to their larger size (compared to Sup35 aggregates), or a larger proportion of small polymers involved in active prion proliferation. However, direct evidence for this is lacking thus far.

Some data contradicting the proposed “fragmentation” model are coming from *in vitro* experiments. *In vitro* studies of the bacterial homolog of Hsp104, ClpB, do not support its ability to act as a crowbar producing smaller oligomers from larger aggregates. Rather, these data indicate that ClpB can extract monomers from aggregated structures one by one [51]. Although evidence for the ability of yeast Hsp104 to fragment prion protein polymers into oligomers *in vitro* has been produced [52], Hsp104 has also been reported to facilitate *de novo* amyloid assembly by PrD-containing fragments of Sup35 in at least some *in vitro* assays [52,53]. It remains to be seen whether this effect is relevant to the processes occurring *in vivo*, as evidence for the Hsp104 role in *de novo* prion formation in the yeast cell is lacking.

Notably, all existing models of the Hsp104 effects propose direct physical interactions between Hsp104 and amyloid fibers. While such interactions were detected by some *in vitro* assays [52,54,55], no evidence of *in vivo* interactions between Hsp104 and yeast prion proteins has been produced until recently (for example, see [56]). This could be explained by a high specificity of Hsp104 to the amyloid isoform, and the transient nature of interaction leading to a rapid release of Hsp104 from the complex. Recent paper by Bagriantsev *et al.* [57] describes co-localization of Hsp104 (as well as some other chaperones) with the cytologically detectable aggregates of yeast prion proteins that points to the direct *in vivo* interaction.

It is also unclear which specific regions of prion proteins are recognized by Hsp104. Some data implicate the Sup35 PrD region specifically located between positions 40 and 97 and including 5.5 imperfect copies of the oligopeptide repeat (OR) as a “propagation element” responsible for [*PSI*⁺] transmission in cell divisions [58,59]. As prion propagation is achieved *via* interactions with Hsp104, one could expect that these ORs represent an interacting region. However, Sup35 derivatives with a “shuffled” PrD, which maintain the same amino acid composition but lack the repetitive organization of PrD, retain the capability of generating and propagating the prion state, which remains Hsp104-dependent [60]. Also, not all yeast prions contain OR-like regions; for example, Ure2 lacks it. Moreover, some data indicate that the “middle” region of Sup35 located outside of the PrD may influence effects of Hsp104 on the prion [61]. It is therefore likely that either Hsp104 may recognize a variety of sequences in an amyloid conformation, or the role of ORs (or other regions with similar function) is rather to make the amyloid structure accessible for Hsp104 rather than interact with a chaperone directly.

Some discrepancies between the effects observed in case of direct inactivation of Hsp104 by genetic manipulations and its inactivation by GuHCl also need to be explained. While role of Hsp104 inactivation in prion curing by GuHCl is not in doubt [62] it is worth mentioning that kinetic parameters of [*PSI*⁺] loss in the presence of GuHCl are different from those observed after direct inactivation of Hsp104 [32]. Attempts to explain these differences by variations in experimental conditions (see [63]) fail (for review, see [64]). Probably, either inactivation of Hsp104 by GuHCl is incomplete, that is in agreement with some other results obtained *in vitro* [65], or GuHCl is also affecting some other proteins participating in prion curing. Nevertheless, one should note that the most recent data [66] confirm the previous observation [36] that GuHCl-induced loss of the [*PSI*⁺] prion occurs only in the growing yeast cultures and can be explained by a progressive decrease in the seed concentration, eventually resulting in

a failure to segregate to the daughter cells. These data support the “fragmentation” model of the Hsp104 effect.

In spite of the fact that the fragmentation model is accepted by many scientists, there is an important issue which usually escapes attention. According to the fragmentation model, Hsp104 is most important for prion propagation in cell divisions. However, prions are not lost in the exponentially dividing yeast cells, although cellular levels of Hsp104 are almost negligible in these conditions. Estimates of the number of prion seeds per cell vary between several dozens and several hundred [44]. As average numbers remain constant between cell divisions, there should be at least as many fragmentation events per division. It needs to be determined whether number of Hsp104 molecules, remaining in exponential cells, is sufficient to produce that many fragmentation events.

4. MUTATIONAL ANALYSIS OF HSP104 EFFECTS ON PRIONS AND OTHER AMYLOID AGGREGATES

A number of mutations in Hsp104 were identified that affect both thermotolerance and prion propagation, i.e. 33F (a frameshift mutation caused by a 2-bp deletion at nucleotide positions 98 and 100), INV_{110–121} (an internal inversion mutation caused by inversion from nucleotide positions 330 to 363), G212D, P389L and G661D mutants, exhibited no thermotolerance, similar to that of the *hsp104Δ* strain [22]. Also some mutations in Hsp104 impairing prion propagation but not thermotolerance were investigated. These included L462R [22], located in the middle region, and P557L [62], D704N [22], and T317A [22,25], located near the middle region in D1 small region, NBD2, and NBD1, respectively (see also Fig. (1) and Table 1). All amino acids altered by these mutations situated on the lateral channel of the Hsp104 hexamer according to alignment with the *Thermus thermophilus* ClpB sequence [22,23]. This channel is ~10-to-30 Å wide, thus it was proposed that such a channel could be sufficient to thread amyloid fibers, pointing to the possible ability of Hsp104 to use the lateral channels for disaggregation of prion polymers, while the central pore is used for disaggregating heat-damaged proteins [22,23,67]. This might in principle explain the above-mentioned discrepancy between *in vitro* results indicating that ClpB extracts monomers from the aggregates of heat damaged proteins [51], while Hsp104 fragments amyloids into oligomers [37,52].

Attempts were also made to compare effects of Hsp104 on yeast prions to its effects on other amyloid-forming proteins, for example on polyglutamines expressed in yeast. Amyloid like aggregation of proteins with expanded polyQ sequence has been reproduced in yeast cells by using constructs bearing the expanded polyQ stretch from human huntingtin fused to GFP. It appeared that polyQ aggregates can not propagate in yeast cells on their own, however their aggregation is being promoted (and possibly nucleated) by pre-existing endogenous prions, such as [*PIN*⁺] (Rnq1) and/or [*PSI*⁺] (Sup35), resulting in cytotoxicity [68,69]. Although Hsp104 was shown to be essential for polyQ aggregation in yeast, and its overproduction eliminated or reduced polyQ aggregation [70–73], these effects of Hsp104 are most likely mediated by its influence on prion “seeds” [68]. For example, the dominant negative mutations Hsp104-KT218,620 and Hsp104-K302N, located within the NBD domains, and Hsp104-A509D, located in the middle domain, inhibited both polyQ toxicity and prion maintenance [30,69,74]. However, one of the middle domain mutations, Hsp104-A503V, which impaired neither thermotolerance [75] nor prion maintenance in the presence of wild type Hsp104, significantly decreased polyQ toxicity in these conditions [69]. This indicates that Hsp104 may exhibit effects on polyQ aggregates that can not be solely explained by its interaction with endogenous prions.

5. HSP104 AND OTHER CELLULAR COMPONENTS

It is also shown that other cellular components may influence effects of Hsp104 on prion aggregates. For example, the $[PSI^+]$ prion is not lost in the stationary phase or during growth at high temperature despite the fact that Hsp104 levels are greatly elevated in these conditions. This is probably due to the fact that other stress-induced proteins “protect” the prion from excess Hsp104. Indeed, it is known that Hsp104 closely interacts with members of Hsp70 and Hsp40 families (in yeast, with Ssa and Ydj1 proteins, respectively) in the process of disaggregation and recovery of heat-damaged proteins. While Hsp104 acts as a disaggregase, Hsp70 and Hsp40 probably participate in refolding of the disaggregated proteins into the native conformation, also it was shown that if Ssa1 (Hsp70) and Ydj1 (Hsp40) were present, non-native luciferase was stabilized in a state from which it could be refolded by subsequent addition of Hsp104 [13]. Hsp70-Ssa is also shown to modulate Hsp104 effects on $[PSI^+]$. Interestingly, in this system excess Ssa prevents elimination of the Sup35 aggregates in the presence of excess Hsp104, therefore favoring the amyloid state [56,76]. Possibly, Ssa recognizes amyloids as stable highly ordered structures that need to be protected from destruction. Indeed, it is known that Hsp70 proteins are involved in protection of some intracellular structures during stresses in higher eukaryotes [77].

Remarkably, another member of the yeast Hsp70 family, Ssb (which is efficiently expressed at normal conditions and is not heat-shock inducible), facilitates $[PSI^+]$ curing by excess Hsp104 [78]. Differences in the effects of Ssa and Ssb proteins on prions are primarily determined by peptide-binding domains of these proteins, responsible for substrate recognition [56]. Roles of Hsp70 proteins in prion propagation are reviewed in more detail by Guinan and Jones, and Sharma and Masison in this issue [15,16]. Less is known about effects of the Hsp40 proteins, however, it is shown that different members of this family may exhibit differential effects on yeast prions [48,79] and on prion-dependent polyQ toxicity [68,69,80]. Specifically, Sis1 protein of the Hsp40 family has been suggested to play a similar role for $[PIN^+]$ as Hsp104 plays for $[PSI^+]$ [81]. Other cellular proteins modulating effects of Hsp104 on yeast prions include some components of the ubiquitin/proteasome proteolytic pathway, e. g. ubiquitin conjugating enzyme Ubc4 [82]. Precise mechanisms of interactions between Hsp104 and the ubiquitin/proteasome system remains to be uncovered. To date, most of our understanding of the chaperone role in prion propagation is coming from the experiments aimed at the analysis of individual components of the chaperone machinery, e. g. Hsp104 itself. While importance of this “reductionist” approach should not be underestimated, the emerging picture of complexity makes calls for the systematic *in vivo* analysis of the amyloid propagating machinery in the yeast cell that is yet to be developed.

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Figure 1. Domain structure of yeast Hsp104

A. NTD – N-terminal domain; NBD1 and NBD2 – the nucleotide-binding domains; D1 - small domain; MR – middle region; CTD – C-terminal peptide-binding domain. Numbers indicate amino acid positions of domain boundaries in yeast Hsp104, determined from alignment to the *Thermus thermophilus* ClpB sequence [22,23]. Most mutations impairing Hsp104 activities are located within NBD and MR domains. Effects of mutations are summarized in Table 1 (for details, see Section 4). **B.** Location of some mutations that impaired prion propagation, but retained thermotolerance on the Hsp104 homoheamer (based on [22,23]). Central channel, and lateral channels are shown.

Figure 2. Role of Hsp104 in Sup35 prion propagation

The model presented postulates that at moderate levels of Hsp104 the initial polymer is being broken into oligomeric “seeds”, that initiate new rounds of prion propagation. At low levels (or activity) of Hsp104, or in case of the amyloid aggregate which is insensitive to Hsp104, a polymer is growing in an uncontrollable fashion, possibly resulting in the eventual death of the polymer-containing cell. At high levels of Hsp104, amyloid polymers are hypothesized to be solubilized into presumably monomeric misfolded intermediates, whose fate remains unknown; they could be refolded into a non-prion conformation, probably with help of other chaperones, or degraded. Large aggregates (amorphous agglomerates?) also appear. See the text, Section 3.

Table 1

Effects of Hsp104 Mutations on Prion Propagation ([PSI⁺]), polyQ Toxicity, and Thermotolerance (Based on [22,25,32,69,75])

HSP104 Allele	Mutation Location (Hsp104 Domain)	[PSI ⁺] Propagation	Thermotolerance	PolyQ Toxicity
WT control	-	+	+++	+
33F ^a	NTD	-	-	n/t
INV ₁₁₀₋₁₂₁	NTD	-	-	n/t
G212D	NBD1	-	-	n/t
KT218,620	NBD1 NBD2	-*	-*	-*
G259D	NBD1	-	+	n/t
K302N	NBD1	-*	-*	-*
T317A	NBD1	-	+++	n/t
P389L	D1	-	-	n/t
L462R	MR	-	+++	n/t
A503V	MR	+	+++	-*
A509D	MR	-	-	-*
P557L	NBD2	-	+++	n/t
G661D	NBD2	-	-	n/t
D704N	NBD2	-	+++	n/t

Symbols:

^a33F- a frameshift mutation caused by a 2-bp deletion at nucleotide positions 98 and 100;

n/t not tested;

* only dominant effect tested;

otherwise - both dominant and recessive effects tested.