Modulation of Prion-dependent Polyglutamine Aggregation and Toxicity by Chaperone Proteins in the Yeast Model*

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In yeast, aggregation and toxicity of the expanded polyglutamine fragment of human huntingtin strictly depend on the presence of the endogenous self-perpetuating aggregated proteins (prions), which contain glutamine/asparagine-rich domains. Some chaperones of the Hsp100/70/40 complex, modulating propagation of yeast prions, were also reported to influence polyglutamine aggregation in yeast, but it was not clear whether they do it directly or via affecting prions. Our data show that although some chaperone alterations indeed act on polyglutamines via curing endogenous prions, other alterations decrease size and ameliorate toxicity of polyglutamine aggregates without affecting prion propagation. Therefore, the role of yeast chaperones in polyglutamine aggregation and toxicity is not restricted only to their effects on the endogenous prions. Moreover, chaperone interactions with prion and polyglutamine aggregates appear to be of a highly specific nature. One and the same chaperone alteration, substitution A503V in the middle region of the chaperone Hsp104, exhibited opposite effects on one of the endogenous prions (PSI*), the prion form of Sup35 and on polyglutamines, increasing aggregate size and toxicity in the former case and decreasing them in the latter case. On the other hand, different members of a single chaperone family exhibited opposite effects on one and the same type of aggregates: excess of the Hsp40 chaperone Ydj1 increased polyglutamine aggregate size and toxicity, whereas excess of the other Hsp40 chaperone, Sis1, decreased them. As many stress-defense proteins are conserved between yeast and mammals, these data shed light on possible mechanisms modulating polyglutamine aggregation and toxicity in mammalian cells.

Expansion of glutamine repeats (poly-Q)1 in certain proteins is responsible for neurodegenerative disorders. The hallmark of poly-Q diseases is the formation of insoluble cytosolic and nuclear inclusions (1). Huntington disease is one of the best known poly-Q disorders (2). It is caused by an expansion of the poly-Q stretch in the essential protein called huntingtin (Htt) to more than 37 amino acids. The length of the poly-Q stretch inversely correlates with the time of onset of the disease and the time of formation of Htt aggregates (3). The role of various types of aggregates in cell toxicity remains a matter of debate (4). Recent models propose that toxicity of poly-Q Htt arises from sequestration of certain essential proteins by Htt aggregates (5–7). The poly-Q stretch is located within the N-terminal (exon 1) region of Htt, which is involved in numerous protein-protein interactions (8). The N-terminal fragment of Htt with poly-Q extensions, when expressed in mice, aggregated and was sufficient to cause Huntington disease-like neurodegeneration (9, 10). This indicates that at least some parameters of poly-Q associated aggregation and toxicity could be reproduced in the experimental assays using only N-terminal poly-Q expanded fragments of Htt.

Several models for studying poly-Q aggregation and toxicity have been developed by using simple organisms such as fruit flies (11), nematode Caenorhabditis elegans (12), and yeast Saccharomyces cerevisiae. Yeast assays usually employ short constructs derived from the poly-Q expanded exon 1 of Htt. Although poly-Q aggregation has been readily observed in yeast (13–17), a yeast-based assay for poly-Q toxicity has not been available until recently. It turned out that efficient cytoplasmic aggregation and toxicity of the chimeric protein, containing the N-terminal region of Htt with expanded poly-Q stretch, fused to green fluorescent protein (GFP), could be detected only in the yeast strains bearing an endogenous yeast QN-rich protein, Rnq1, in its prion form, called [RNAQ+], or [PIN+](18). In the absence of a prion, poly-Q aggregates are rarely found, and toxicity is not seen.

Rnq1, a protein of unknown function, is one of several known yeast proteins containing QN-rich prion domains (19, 20). This group also includes Sup35, Ure2, and New1. Yeast prions are self-perpetuating amyloid-like protein aggregates, which are thought to propagate via a nucleated polymerization process, mediated by interactions between prion domains. By itself, the Rnq1 prion ([PIN+]) is not harmful to yeast cells, but it appears that QN-rich aggregates of Rnq1 “seed” aggregation of the heterologous poly-Q protein, leading to toxicity. The QN-rich domain of New1 in its prion form has also been shown to facilitate aggregation of a poly-Q construct originated from the mammalian mutant ataxin-3 involved in Machado-Joseph disease, although cell toxicity of that protein was not detected in raffinose, respectively, as a carbon source; FM4-64, lipophilic fluorescent dye; Sup35N, M and C, N-proximal, middle, and C-proximal regions of the Sup35 protein, respectively; CEN, centromeric; TRITC, tetramethylrhodamine isothiocyanate.
yeast (21). Further analysis has demonstrated that [PIN']-dependent toxicity of the Htt-derived poly-Q construct in yeast is associated with a defect of endocytosis, possibly via sequestration of some components of the vesicle-assembly machinery by poly-Q aggregates (22). As subcellular localization of Htt in mammalian cells has led to a suggestion that it may play a role in vesicle trafficking (23), it is possible that the yeast model reflects certain features of cell toxicity that are relevant to mammalian Huntington disease.

As many studies suggest a critical relationship between poly-Q aggregation and cell toxicity, chaperone proteins countering aggregation are well positioned as likely antagonists of the poly-Q disorders. Indeed, some chaperones of the evolutionary conserved Hsp70 and Hsp40 families counteracted poly-Q aggregation in vitro (14) and antagonize poly-Q aggregation and, in some cases, toxicity in vivo, as seen in Drosophila and cultured mammalian cells (24–27). In yeast, aggregates of the heat-damaged proteins are solubilized and refolded by the chaperone complex, including Hsp104, Hsp70, and Hsp40 (28). Overproduction of some Hsp70 and Hsp40 proteins (14), simultaneous depletion of the Ssa1 and Ssa2 proteins of the Hsp70 family (18), or a point mutation in the Hsp40 protein Ydj1 (18) were shown to counteract poly-Q aggregation in yeast cells. Hsp104 was shown to be essential for poly-Q aggregation in yeast, whereas its overproduction eliminated or reduced poly-Q aggregation at least in some assays (13, 15, 16, 29).

However, one concern with most of these experiments is that they did not take into account the prion status of yeast cells. As subcellular localization of Htt in mammalian Huntington disease reflects certain features of cell toxicity that are relevant to poly-Q disorders. Indeed, some chaperones of the evolutionary conserved Hsp70 and Hsp40 families counteracted poly-Q aggregation simply by eliminating initial [PIN']-seeds (18).

Here, we have systematically examined the effects of various yeast chaperones of the Hsp104/Hsp70/Hsp40 complex on poly-Q aggregation and toxicity in the series of isogenic yeast strains that differ only by prion composition. Our data show that although some chaperone alterations certainly act on poly-Q aggregates indirectly by modulating prion propagation, other alterations appear to influence poly-Q without affecting endogenous yeast prions. Moreover, at least one Hsp104 alteration exhibited differential effects on toxicity of poly-Q aggregates and endogenous prion, and various members of the Hsp70 and Hsp40 families differed from each other in regard to their effects on poly-Q aggregation and toxicity.

EXPERIMENTAL PROCEDURES

Yeast Strains—The S. cerevisiae strains used in this study were derivatives of 74-D694, MATa ade1-14 his3 leu2 trpl ura3 (31). ‘Strong’ [PSI+] derivatives (see the description of the [PIN'] assay (35), and [psi'] derivative OT60 (34) also contain the [PIN'] prion. The [psi'] pin strain GT17 (34) contains no prions. The [PSI'] pin strain GT490 was OT56, cured of [PIN'] by expressing the dominant-negative Hsp104 derivative Hsp104-KT218,620 (35). For this purpose, OT56-derived colonies that retained [PSI'] after pulse induction of Hsp104-KT218,620 expression were then cured of [PSI'] by excess wild-type Hsp104 and checked for retention of [PSI+] (see the description of the [PIN'] assay (35), and [psi'] derivative wild-type Hsp104 usually does not cure [PIN'], isolates becoming [pin] after excess Hsp104 treatment were identified as originating from the [PSI'] [pin] derivatives. Lack of the aggregated (prion) form of Rnq1 in these derivatives was then confirmed by a fluorescent centrifugation assay (36).

The [psi'] pin strain GT234 (MATa ade1-14 his3 leu2 trpl ura3 lys2) (35) was used as a tester strain for the [PIN'] assay (see below). Yeast cultures were grown at 30 °C unless otherwise noted.
both plate assay and quantitative assay (for example, see Fig. 5) so that accurate comparisons could be made only between cultures growing in the same conditions. Assessments of yeast prion isoforms of the same proteins were designated as [psi] strains. The presence of the prion form of the Sup35 protein ([PSI] + ) was detected by its ability to cause readthrough of the ade1–14 (UGA) mutant allele (31). Sup35 is a translation termination factor, and its aggregation results in termination readthrough. The [psi] ade1–14 strains are unable to grow on adenine medium and exhibit a dark red color on organic complete (YPD) medium, whereas [PSI] ade1–14 strains are able to grow on adenine medium and exhibit a light pink color on YPD medium. The presence of [PIN] + in the [psi] background was tested on the basis of the ability of overproduced Sup35 to induce de novo formation of [PSI] + in the [PIN] + but not [pin] background (36). The [psi] strains, tested for [PIN] + , were mated to the [psi] pin strain GT234, bearing the multicopy plasmid pSTR7 with the SUP35 gene. The [PIN] + diploids, in contrast to the [pin] diploids, grew on adenine medium after 10–14 days of incubation due to induction of [PSI] + . In each experiment, 12 colonies originating from four independent cultures were tested for [PIN] + loss.

**GFP Detection by Fluorescence Microscopy**—GFP fluorescence images were taken using the Olympus BX41 microscope with a ×100 objective, with a narrow band GFP filter. Cultures were grown overnight in the synthetic Gal-Raf medium selective for the plasmid[s], as described above for quantitative assays. Only cells showing fluorescence were counted and grouped into different classes based on the patterns observed.

**Endocytosis Assay**—Mid-log phase cells grown in the Gal-Raf medium were concentrated 10 times, incubated for 12 min in the selective medium after 10–14 days of incubation due to induction of [PSI] + . The [pin] ade1–14 strains are unable to grow on adenine medium and exhibit a dark red color on organic complete (YPD) medium, whereas [PSI] ade1–14 strains are able to grow on adenine medium and exhibit a light pink color on YPD medium.

The presence of [PIN] + in the [psi] background was tested on the basis of the ability of overproduced Sup35 to induce de novo formation of [PSI] + in the [PIN] + but not [pin] background (36). The [psi] strains, tested for [PIN] + , were mated to the [psi] pin strain GT234, bearing the multicopy plasmid pSTR7 with the SUP35 gene. The [PIN] + diploids, in contrast to the [pin] diploids, grew on adenine medium after 10–14 days of incubation due to induction of [PSI] + . In each experiment, 12 colonies originating from four independent cultures were tested for [PIN] + loss.

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**Endocytosis Assay**—Mid-log phase cells grown in the Gal-Raf medium were concentrated 10 times, incubated for 12 min in the selective Gal-Raf medium with 8 μM FM4-64 (Molecular Probes), washed two times with the same medium without dye, and left at 30 °C with shaking for 10 min. FM4-64 staining was detected on the BX41 fluorescence microscope with TRITC filter.

**Protein Isolation and Differential Centrifugation**—Proteins were isolated from yeast cultures, grown overnight in plasmid-selective synthetic Gal-Raf medium, and analyzed according to the previously published protocol (22) as described below. At least two independent cultures (originated from independent transformants) were checked per each plasmid combination. Cells were collected by centrifugation and disrupted by 10-min vortexing with 600 μM acid-washed glass beads in the lysis buffer (40 mM HEPES (pH 7.5), 50 mM KCl, 1% Triton X-100, 1 mM Na3VO4, 50 mM β-glycerophosphate, 50 mM NaF, 5 mM EGTA, 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 mM benzamide, and 1/5 of one proteinase inhibitor tablet, Roche Diagnostics, predissolved in distilled water). Cell debris was removed by centrifugation at 200,000 ×g for 10 min. This step also precipitates some of the Q103 and Q25 protein; the fraction of protein precipitated in these conditions and not so when only [PIN] + is present (Fig. 1B) (30). The double Lys-to-Thr substitution at amino acid positions 218 and 620 of Hsp104 (Hsp104-KT; Fig. 2A) inactivates both nucleotide binding domains of Hsp104 (NBD1 and NBD2) and disrupts the function of wild-type Hsp104 in a dominant-negative fashion (31, 45). In contrast to overproduced wild-type Hsp104, mutant Hsp104-KT cures yeast cells not only of [PSI] + (31) but also of [PIN] + (35). As expected, expression of Hsp104-KT counteracts Q103 toxicity not only in the [PSI] + strains but also in the [psi] pin strains, apparently by curing cells of prions (data not shown). However, we have now demonstrated that [PSI] + , the prion form of Sup35, also promotes Q103 toxicity (Fig. 1A). Effects of [PIN] + and [PSI] + on Q103 toxicity appear to be additive (Fig. 1B).

Overproduction of the yeast chaperone Hsp104 is known to eliminate [PSI] + (31) but not [PIN] + (30). In agreement with these data, we have shown that overproduction of wild-type Hsp104 counteracts Q103 toxicity only in a [PSI] + background and not so when only [PIN] + is present (Fig. 1C).

**Mutant Alleles of Hsp104 Counteract Q103 Toxicity**—In yeast, toxicity of Q103 appears to be directly associated with its aggregation promoted by an endogenous yeast prion (18). Yeast chaperone protein Hsp104 is required for propagation of the yeast prions [PSI] + (31) and [PIN] + (30). The double Lys-to-Thr substitution at amino acid positions 218 and 620 of Hsp104 (Hsp104-KT; Fig. 2A) inactivates both nucleotide binding domains of Hsp104 (NBD1 and NBD2) and disrupts the function of wild-type Hsp104 in a dominant-negative fashion (31, 45). In contrast to overproduced wild-type Hsp104, mutant Hsp104-KT cures yeast cells not only of [PSI] + (31) but also of [PIN] + (35). As expected, expression of Hsp104-KT counteracts Q103 toxicity not only in the [PSI] + strains but also in the [psi] pin strains, apparently by curing cells of prions (data not shown), thus reproducing an effect of the Hsp104 deletion (18). Single dominant-negative NBD mutants KT218 and KT620 also exhibit antitoxicity (data not shown). Surprisingly, we have isolated a presumably wild-type HSP104 clone from a widely used cDNA yeast library, which counteracted Q103 toxicity in both [PSI] + (not shown) and [psi] pin (Fig. 2B) backgrounds. DNA sequencing revealed that this clone of HSP104 actually contained a G-to-C substitution at the nucle-
A509D (not shown), counteracted Q103 toxicity in the [psi-] strain (see in Fig. 2B). As expression of Hsp104-A503V increased the proportion of cells with dots and almost completely eliminated large clumps (Fig. 3B), confirming that in the absence of large aggregates, endocytosis is one of the likely causes for a defect of endocytosis, which is one of the likely causes for Q103 toxicity (22).

Next, we checked whether Hsp104 mutations in regions other than the NBDs affect poly-Q toxicity. Indeed, two mutant alleles of HSP104 with substitutions in the middle region (Fig. 2A), previously isolated in a genetic screen at the S. Lindquist laboratory (37), HSP104-A503V (Fig. 2, C and D) and HSP104-A509D (not shown), counteracted Q103 toxicity in the [psi-] strain. Hsp104-A509D also counteracted Q103 toxicity in [PSI+] and cured yeast cells of prions (Table I), apparently due to inactivation of wild-type Hsp104.

Prion-dependent Q103 aggregation in yeast is associated with a defect of endocytosis, which is one of the likely causes for Q103 toxicity (22). We have monitored the effect of Q103 aggregation on endocytosis by using the lipophilic fluorescent dye FM4-64 (see “Experimental Procedures” for more details). This dye binds to the cell membrane and is internalized through the endocytic pathway. In cells with normal endocytosis, FM4-64 forms a distinct ring around the vacuole at 45 min after dye addition, reflecting the fusion of dye-stained endosomes with the vacuolar membrane. In contrast, cells with large Q103 clumps, and about 50% of cells with both small clumps and dots, contained no such fluorescent rings, indicating a defect in endocytosis. Cells with dots usually exhibited FM4-64 rings, confirming that in the absence of large aggregates, endocytosis was not affected (Fig. 3, C and D). As expression of Hsp104-A503V increased the proportion of cells with dots and almost eliminated large clumps (Fig. 3B), the endocytosis defect was essentially not seen in the presence of Hsp104-A503V (data not shown).

The differential centrifugation assay confirmed that co-expression of Hsp104-A503V with Q103 shifted a portion of the Q103 protein from the fraction pelletable at 10,000 g to the supernatant fraction (Fig. 3E). At a higher centrifugation speed (200,000 g), essentially all Q103 was precipitated independently of the presence or absence of Hsp104-A503V, whereas non-expanded Q25 control remained soluble (data not shown). These results are in agreement with the fluorescence microscopy data and show that expression of Hsp104-A503V does not solubilize Q103 aggregates but leads to a decrease in aggregate size so that a higher speed is needed for the efficient precipitation of aggregates. As the total amount of Q103 was not affected by Hsp104-A503V (data not shown), a decrease in aggregate size should be
accompanied by an increase in the number of aggregated units. Taken together, our data confirm that Hsp104-A503V ameliorates Q103 toxicity by counteracting formation of the large aggregates, which inhibit endocytosis.

Table I

| Overexpressor combinations | Colonies tested for | |
|----------------------------|---------------------|----------------|
|                            | [PSI−] | [psi−] | Total | [PIN−] | [pin−] | Total |
| Control                    | 48     | 0      | 48    | 48     | 0      | 48    |
| † WT Hsp104 + † Q103      | 0      | 48     | 48    | 48     | 0      | 48    |
| † Hsp104-K302N + † Q103   | 21     | 27     | 48    | 18     | 30     | 48    |
| † Hsp104-A509D + † Q103   | Not applicable*  | 48     | 0      | 48    |
| † Sis1 + † Q103           | Not tested      | 48     | 0      | 48    |

*Hsp104-A503V is toxic to [PSI−].

FIG. 3. Effects of the Hsp104-A503V mutant derivative on poly-Q aggregation and endocytosis in the [psi− PIN−] background. A, types of aggregates observed in Q103 overexpressing cells. Cultures were grown in the liquid Gal+Raf medium to induce the poly-Q construct. Three distinct classes of cells with aggregates were observed: black circle with curve, cells containing large clumps; black circle with two dots, cells containing dots; black circle with two dots and a curve, cells containing both clumps and dots. B, Expression of Hsp104-A503V decreases proportions of cells with clumps and increases the proportion of cells with dots. The average number from two repeats for each of two independent cultures is given in each case. The error bars indicate standard deviations. C and D, clumps but not dots block endocytosis. Ring-like staining of the vacuole with FM4-64 is a marker of normal endocytosis. The majority of cells with dots show rings, whereas the majority of cells with clumps and 50% of cells with both clumps and dots do not, indicating a defect in endocytosis. Note that residual fluorescence of the cells with large clumps in the red (FM4-64) filter does not represent endocytic vesicles but rather results from leakage of GFP fluorescence through the red filter. Data were homogenous for all plasmid combinations. Error bars indicate standard deviations. E, expression of Hsp104-A503V increases the proportion of soluble Q103 protein. Proteins were isolated after 24 h of growth in synthetic Gal+Raf medium selective for plasmids and fractionated as described under “Experimental Procedures.” No differences in the proportion of Q103 precipitated at 800 g were seen (not shown). Proportions of Q103-GFP protein in pellet (P) and supernatant (S) fractions obtained at 10,000 g were determined by densitometry. Standard deviations are shown.

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Effects of Hsp on yeast prions in the presence of Q103

Cultures were grown in the conditions inducing both constructs. Aliquots were plated onto non-inducing medium. Resulting colonies were scored for prions as described under “Experimental Procedures.”
grown in the liquid prion (Fig. 4, A). As expected, Sup35C counteracted toxicity of Hsp104-A503V in the [PSI+] background (Fig. 4 C). Differential Effects of the Hsp40 and Hsp70 Chaperones on Q103 Toxicity—

However, in the presence of Hsp104-A503V, the same P\text{SUP35}-SUP35NM-GFP construct produced large detectable clumps or dots (Fig. 4 D). Thus, expression of Hsp104-A503V increased the size of Sup35 aggregates. Possibly, increased Sup35 aggregation contributes to cell toxicity. Such an effect of Hsp104-A503V would be similar to consequences of Sup35 (or Sup35/N/NN) overproduction in the [PSI+] background, which results in both the appearance of large detectable Sup35 clumps (47) and cell toxicity (43, 46, 48). Therefore, it appears that Hsp104-A503V exhibits opposite effects on the Q103 and Sup35 aggregates, decreasing aggregate size and toxicity in the former case and increasing them in the latter case.

The yeast Hsp70 proteins tested (Ssa1, -2, -3, and -4, and Ssb1), Hsps on the prion-dependent Q103 toxicity in yeast. Among all yeast Hsp70 proteins of the Ssa subfamily aid in propagation of the yeast prion [PSI+] (33, 40, 50) and exhibit differential effects on the other yeast prion, [URE3] (41, 51). Proteins of the other cytosolic Hsp70 subfamily, Ssb, consistently antagonize [PSI+] in various assays (38, 40, 52). In contrast, Hsp26 and Hsp90 chaperones did not show any detectable effects on the other yeast prion, [PSI+] (33).

We have checked the effects of individually overproduced Hsps on the prion-dependent Q103 toxicity in yeast. Among all yeast Hsp70 proteins tested (Ssa1, -2, -3, and -4, and Ssh1), only overproduction of Ssa4 counteracted Q103 toxicity in the

OT60, confirming that it is specific to [PSI+] rather than to any prion (Fig. 4, A and B). Moreover, the severity of the toxic effect depended on the variant of [PSI+]. The [PSI+] strain OT55, which is isogenic to OT56 but bears a weak variant of [PSI+], characterized by the larger proportion of the Sup35 protein remaining in the soluble fraction (40, 46), was not inhibited by expression of Hsp104-A503V to such an extent as was OT56 (data not shown). One possibility is that toxicity of Hsp104-A503V is due to increased sequestration of the soluble Sup35 protein by prion aggregates. As Sup35 is an essential protein working in termination of translation, such sequestration may inhibit growth of the strong [PSI+] variants in which the amount of the remaining soluble Sup35 is already low. To test this hypothesis, a high copy plasmid expressing only the Sup35M (prion) and Sup35N (mid-
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FIG. 5. Effects of Hsp40 and Hsp70 chaperones on Q103 aggregation and toxicity. A–C, plate assays. Plates were photographed after 7 days of incubation (second passage) on the UrA-Leu/Gal medium (Saa4), 7 days of incubation (second passage) on the Ura-His/Gal medium (Ydj1), or 3 days of incubation (second passage) on the Ura-Trp/Gal medium (Sis1). These media select for the plasmids and induce the Q103 (and in panel A, also Saa4) constructs; Ydj1 and Sis1 constructs are constitutively expressed. No differences in growth were detected on Glu media (not shown). No effects of Ydj1 or Sis1 on toxicity were detected in the presence of [PSI+] (not shown). Saa1, -2, and -3 overexpressors did not exhibit any detectable effect on toxicity in the same conditions, and none of the Ssa constructs influenced toxicity in the isogenic [psi PIN+] and [PSI pin] strains at detectable levels (data not shown). D and E, quantitative assay. Cultures were grown in the liquid Ura-His/Gal+Raf (Ydj1) or Ura-Trp/Gal+Raf (Sis1) media, which are selective for the plasmids and induce the poly-Q constructs. Differences between Q103 cultures and cultures co-expressing Ydj1 or Sis1 with Q103 were statistically significant after 3 and 6 days of incubation, respectively. The numbers show the concentration of viable cells at these time points. F and G, excess Ydj1 increases the proportion of cells with Q103 clumps, whereas excess Sis1 decreases it. Cultures were grown in the liquid Ura-His/Gal+Raf (Ydj1) or Ura-Trp/Gal+Raf (Sis1) medium overnight. Designations are the same as in Fig. 3. Error bars indicate standard deviations. H and I, excess Ydj1 decreases the proportion of soluble Q103 (H), whereas excess Sis1 decreases it (I). Proteins were isolated from the [psi PIN+] strain. Procedure was same as described in the legend for Fig. 3 (for panel E). Differences between the proportion of soluble protein in Q103 controls in H and I are apparently due to different media used.

[PSI+ PIN+] strain (Fig. 5A), although it exhibited no detectable antitoxicity in the [psi PIN+] or [PSI+ pin] backgrounds and did not cure [PSI+] at a significant level (Ref. 40 and data not shown). Overproduction of Hsp26 or Hsp90 did not exhibit any antitoxicity effect on Q103, independently of which prions were present (data not shown). The Hsp40 chaperones Ydj1 and Sis1 did not affect Q103 toxicity at any detectable level in the [PSI+] strains but exhibited opposite effects on Q103 toxicity when overproduced in the [psi PIN+] strain. An extra copy of YDJ1, expressed from either the strong constitutive P_{cyc} promoter (Fig. 5, B and D) or the endogenous P_{YDJ1} promoter (not shown), increased Q103 toxicity. In contrast, an extra copy of SIS1, expressed from either P_{cyc} (Fig. 5, C and E) or endogenous P_{SIS1} promoter (not shown), counteracted Q103 toxicity.

Fluorescence microscopy analysis confirmed that co-expression of Ydj1 with Q103 in the [psi PIN+] background increased the proportion of the endocytosis-defective cells with large Q103 clumps (Fig. 5F). Likewise, the proportion of Q103 protein that is insoluble and pelletable at 10,000 × g was increased in the presence of excess Ydj1 (Fig. 5H). In contrast, the [psi PIN+] cultures co-expressing Sis1 with Q103 exhibited a decrease in the proportion of the endocytosis-defective cells with large clumps, in comparison with those expressing Q103 alone. This was accompanied by an increase in the proportion of the cells with dots or with both dots and small clumps (Fig. 5G) and by a decrease in the proportion of the Q103 protein pelletable at 10,000 × g (Fig. 5I).

Neither excess Ydj1 nor excess Sis1 affected total levels of Q103 (data not shown). Excess Sis1 did not lead to any detectable loss of [PIN+] (Table I), confirming that the effect of Sis1 is not due to loss of a prion. Taken together, these data suggest that excess Ydj1 exacerbates the toxic effect of Q103 by increasing the size of Q103 aggregates, whereas excess Sis1 counteracts toxicity by decreasing aggregate size.
Role of Endogenous Prions in Poly-Q Aggregation and Toxicity—Our data confirm previous observations showing that the presence of endogenous yeast QN-rich proteins in a prion form promotes poly-Q aggregation (18, 21) and toxicity (18) and demonstrate that [PSI⁺], a prion form of Sup35, is promoting toxicity of the Htt-derived poly-Q construct in the same way as does [PIN⁺], a prion form of Rnq1 (Fig. 1A). These results, implicating endogenous QN-rich prions as susceptibility factors in the poly-Q disorders, could be relevant to mammalian cells, containing a lot of proteins with potentially prionogenic QN-rich domains (see Ref. 53).

Prion domains of Sup35, and possibly of Rnq1, are composed of “aggregation modules,” or QN-rich stretches, and “propagation modules,” in the case of Sup35, oligopeptide repeats (20, 54). QN-rich stretches form stable intermolecular β-sheets, leading to the generation of aggregates with fibrillar morphology (1), characteristic for both prions and poly-Q proteins such as Htt. It is possible that QN-rich prion aggregates serve as propagation seeds for the poly-Q derivatives of Htt (18). Propagation modules are required for propagation of aggregates in the cell divisions via repetitive cycles of the chaperone-mediated aggregate “shearing,” generating new seeds (20, 54). Poly-Q fragments of Htt apparently lack propagation capabilities of their own (54) so that in the absence of endogenous prions, poly-Q aggregates are not only rarely formed but also incapable of persisting in yeast cells. Possibly, association with prions confers a self-perpetuating capability to the whole prion/poly-Q complex. Direct biochemical assays confirm that aggregated Q103 is associated with Rnq1 in the yeast cells (22).

Role of the Chaperone Hsp104 in Poly-Q Aggregation and Toxicity—Numerous previous studies have shown that the presence of Hsp104 is required for poly-Q aggregation in yeast (13, 15, 16, 18). Hsp104 overproduction reduced poly-Q aggregation at least in some yeast strains (13) and in the heterologous model of C. elegans (29). However, Hsp104 is required for propagation of all known endogenous yeast prions, including [PSI⁺] (31) and [PIN⁺] (30). Apparently, it serves as a major factor promoting aggregate shearing (20). Therefore, previously reported effects of Hsp104 on poly-Q aggregates could be mediated by its effects on the endogenous prions.

Indeed, NBD mutations (both the previously described Hsp104-K218L620 and the newly found Hsp104-K302N) and at least one mutation in the middle region of Hsp104 (Hsp104-A509D) inhibited both poly-Q toxicity and Hsp104 function in prion maintenance (Fig. 2B and Table I). Loss of poly-Q toxicity was accompanied by a loss of the endogenous prion(s), suggesting prion elimination as a major cause of the toxicity relief. In contrast, overproduction of wild-type Hsp104, which is known (30, 31) and confirmed (Table I) to solubilize and eliminate [PSI⁺] but not [PIN⁺], reduced poly-Q toxicity only in the [PSI⁺] strains but not in the [pin⁺] strains (Fig. 1C), suggesting that excess Hsp104 influenced poly-Q via prion elimination.

However, in the case of Hsp104-A503V mutant, toxicity relief (Fig. 2, C and D) was not accompanied by elimination of a prion (Table I) or loss of the thermotolerance function of Hsp104 (37). The antitoxicity effect of Hsp104-A503V was apparently due to a decreased size of poly-Q aggregates (Fig. 3, B, C, and E); expression of Hsp104-A503V reduced the proportion of the cells with large poly-Q clumps (Fig. 3B), which exhibit a defect in endocytosis (Fig. 3, C and D), a landmark of prion-dependent poly-Q toxicity in yeast (22). Thus, our data provide the first evidence that at least some mutant derivatives of Hsp104 are capable of modulating poly-Q aggregation without eliminating yeast prions.

Differential Effects of Hsp104-A503V on Poly-Q and [PSI⁺] Aggregates—In contrast to the observations Schirmer et al. (37) made in a different genotypic background, expression of the Hsp104-A503V protein in our strains did not lead to temperature sensitivity (data not shown). However, Hsp104-A503V was incompatible with the presence of the strong variant of [PSI⁺] prion (Fig. 4, A and B). This effect was counteracted by simultaneous expression of the Sup35C region, incapable of aggregation due to the lack of prion domain (Fig. 4C). Thus, incompatibility between Hsp104-A503V and [PSI⁺] was most likely due to increased sequestration of functional Sup35 by prion aggregates. Indeed, the size of the Sup35 aggregates is increased in the [PSI⁺] strain expressing Hsp104-A503V (Fig. 4D). It is known that decreased Hsp104 function increases the size of the Sup35 prion clumps without detectable inhibition of growth (35). Probably, this is due to the fact that shearing-defective Sup35 aggregates are quickly lost from the population. As such loss apparently does not occur in the case of Hsp104-A503V, it seems likely that this mutant protein derivative promotes aggregate growth by facilitating prion conversion rather than by inhibiting aggregate shearing. The gain-of-function effect of Hsp104-A503V also agrees with the observations that it remains functional in thermotolerance (37) and does not impair [PIN⁺] maintenance (Table I). Recent data suggest that in addition to aggregate shearing, Hsp104 promotes Sup35 aggregation in certain in vitro assays (55).

Possible Molecular Mechanisms of the Hsp104-A503V Effects—According to previous data from the S. Lindquist laboratory (56), the A503V mutant exhibits increased background ATPase activity but cannot further increase ATP hydrolysis at NBD1 in response to substrate binding at the C terminus. We propose that these alterations of the Hsp104 function have different consequences, depending on the type of aggregates Hsp104 encounters. In the case of non-perpetuating poly-Q aggregates, increased background ATPase activity may lead to increased aggregate shearing, and therefore, to decreased aggregate size. Hsp104 interaction with the self-perpetuating (prion) aggregates of Sup35 is more complex. One possibility is that the inability of mutant Hsp104 to undergo fine tuning of the NBD action results in shifting the balance between its shearing and “conversion-promoting” activities, which facilitates aggregate growth and sequestration of the newly synthesized Sup35 protein by aggregates.

Differential Effects of Hsp40 Chaperones on Poly-Q Aggregation and Toxicity—Yeast Sis1 and Ydj1, functionally distinct heat shock proteins of the Hsp40 family, are homologs of the human Hsp40 proteins Hdj1 and Hdj2, respectively. In the Drosophila model of a poly-Q disease, both proteins suppressed neurodegeneration, but Hdj1 had a stronger effect than Hdj2 (25). Interestingly, overexpression of Hdj2 increased inclusion formation by Htt in the mammalian COS7 cells (57). However, yeast Ydj1, a homolog of Hdj2, has previously been shown to suppress rather than increase formation of insoluble aggregates of poly-Q expanded exon 1 of Htt in yeast (14). The effect of Sis1 on poly-Q aggregation in yeast has not been studied previously.

In our system, excess Sis1 decreased Q103 aggregate size (Fig. 5, G and J) and counteracted Q103 toxicity (Fig. 5, C and E), similar to the effect of its mammalian homolog Hdj1 in other models. Intact Sis1 is required for the maintenance of the [PIN⁺] prion (49); however, excess Sis1 did not cure cells of [PIN⁺] (Table I), ruling out a possibility that antitoxicity was due to loss of a prion. In contrast to Sis1, excess Ydj1 increased Q103 aggregate size (Fig. 5, F and H), similar to the effect of its mammalian homolog Hdj2 at least in some assays (57), and increased Q103 toxicity (Fig. 5, B and D). This agrees with the previous observation that Q103 aggregation is decreased in the
strain carrying the ydj1Δ-159 mutation (18). Interestingly, our preliminary data indicate that Sis1 and Ydj1 also differ from each other in regard to their effects on the yeast prion [PSI+]. Differences between our results and previous reports on the effects of yeast Ydj1 on poly-Q (14) are probably due to differences in the poly-Q constructs employed, yeast strains, and/or prion compositions. For instance, our plate assay has not detected any effects of Hsp40s on poly-Q toxicity in the presence of both [PSI+] and [PIN+] prions simultaneously, possibly because toxicity was too strong to see an effect clearly (data not shown). As differential effects of yeast Hsp40s on poly-Q aggregation and toxicity, observed in our [psi+ PIN+] strain, parallel differences between their human homologs detected in at least some higher eukaryotic models, one could argue that the yeast assay used in our work better reproduces patterns of interactions between poly-Q aggregates and chaperones in the homologous mammalian systems, in comparison with the yeast assays used by other groups. Also, our data suggest that at least some differences between members of the Hsp40 family in patterns of their interactions with poly-Q aggregates could be conserved throughout evolution from yeast to humans.

Do Hsp70 Chaperones Influence Poly-Q Toxicity?—Both yeast Hsp40 proteins studied in this report, Sis1 and Ydj1, are thought to be co-factors of the Hsp70 chaperones of the Ssa subfamily (58). Yeast Ssa1 was shown to suppress the formation of insoluble poly-Q aggregates in yeast cells (14). However, simultaneous deletion of two members of the Ssa subfamily, Ssa1 and Ssa2, decreased prion-dependent Q103 aggregation in our system (18), and excess of any Ssa protein promoted rather than decreased aggregation of the yeast prion protein Sup35 in our hands (33, 40).

One could suggest that Hsp40 chaperones act on poly-Q aggregates via modulating activity of the Hsp70 proteins, which are known to interact with Hsp40s. However, our data show that only one of the four members of the S. cerevisiae Hsp70-Ssa subfamily, Ssa4, counteracts Q103 toxicity when overproduced. Moreover, the antitoxic effect of Ssa4 could be seen clearly only in the yeast strains containing both prions, [PIN+] and [PSI+] simultaneously (Fig. 5A). Differential effects of various members of the Hsp40 and Hsp70 families on Q103 confirm that chaperone interactions with poly-Q aggregates are of a highly specific nature and suggest caution in interpreting the previous data in which only some members of the family were studied. For example, the effect of the ssa1Δ/2 deletion (18) could be not due to elimination of Ssa1 and Ssa2 per se but due to the increased fraction of Ssa4 in the total Ssa pool. Interestingly, although all Ssa proteins act in the same direction on [PIN+] (40), Ssa1 and Ssa2 differ from each other in their effects on the other yeast prion, [URE3] (41). Further investigations are needed to establish the molecular basis of the differential effects of Ssa chaperones on protein aggregates.

Conclusions—Not only [PIN+], a prion form of Rnq1 protein, but also [PSI+], a prion form of Sup35 protein, promote toxicity of poly-Q protein in the yeast model. This suggests that pre-existing endogenous QN-rich aggregates manifest themselves as susceptibility factors in poly-Q diseases, probably by providing nuclei for poly-Q aggregation. Effects of the yeast chaperone Hsp104 on poly-Q aggregation and toxicity are not restricted to its role in propagation of endogenous yeast prions. Expression of the Hsp104-A503V mutant decreases the size of poly-Q aggregates and ameliorates poly-Q toxicity without eliminating an endogenous yeast prion. In contrast, Hsp104-A503V increases the size of the Sup35 prion aggregates, leading to synthetic lethality between the A503V mutation and [PSI+], a prion form of Sup35. Our data provide the first evidence for the synthetic lethality between the chaperone mutation and a prion.

Chaperone interactions with poly-Q aggregates are of a highly specific nature as different members of one and the same chaperone family may exhibit different effects. Ydj1, a chaperone of the yeast Hsp40 family, increased prion-dependent poly-Q aggregation and toxicity, whereas Sis1, another yeast Hsp40 protein, decreased the size of poly-Q aggregates and antagonized poly-Q toxicity. Among yeast Hsp70 proteins, only Ssa4 was capable of counteracting poly-Q toxicity.

Taken together, our data establish yeast prion-based experimental model as a cheap and effective experimental assay for studying the chaperone modulation of poly-Q aggregation and toxicity. As many stress-defense proteins are conserved between yeast and mammals, our data shed light on possible mechanisms modulating poly-Q aggregation and toxicity in mammalian cells.

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