Prion-like proteins sequester and suppress the toxicity of huntingtin exon 1

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<td>As Published</td>
<td><a href="http://dx.doi.org/10.1073/pnas.1412504111">http://dx.doi.org/10.1073/pnas.1412504111</a></td>
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<tr>
<td>Publisher</td>
<td>National Academy of Sciences (U.S.)</td>
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<tr>
<td>Version</td>
<td>Final published version</td>
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<tr>
<td>Accessed</td>
<td>Wed Apr 03 08:32:00 EDT 2019</td>
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Prion-like proteins sequester and suppress the toxicity of huntingtin exon 1

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Contributed by Susan Lindquist, July 3, 2014 (sent for review May 8, 2014)

Expansions of preexisting polyglutamine (polyQ) tracts in at least nine different proteins cause devastating neurodegenerative diseases. There are many unique features to these pathologies, but there must also be unifying mechanisms underlying polyQ toxicity. Using a polyQ-expanded fragment of huntingtin exon-1 (Htt103Q), the causal protein in Huntington disease, we and others have created tractable models for investigating polyQ toxicity in yeast cells. These models recapitulate key pathological features of human diseases and provide access to an unrivaled genetic toolbox. To identify toxicity modifiers, we performed an unbiased overexpression screen of virtually every protein encoded by the yeast genome. Surprisingly, there was no overlap between our modifiers and those from a conceptually identical screen recently reported, a discrepancy we attribute to an artifact of their overexpression plasmid. The suppressors of Htt103Q toxicity recovered in our screen were strongly enriched for glutamine- and asparagine-rich prion-like proteins. Separated from the rest of the protein, the prion-like sequences of these proteins were themselves potent suppressors of polyQ-expanded huntingtin exon-1 toxicity, in both yeast and human cells. Replacing the glutamates in these sequences with asparagines abolished suppression and converted them to enhancers of toxicity. Replacing asparagines with glutamines created stronger suppressors. The suppressors (but not the enhancers) coaggregated with Htt103Q, forming large foci at the insoluble protein deposit in which proteins were highly immobile. Cells possessing foci had fewer (if any) small diffusible oligomers of Htt103Q. Until such foci were lost, cells were protected from death. We discuss the therapeutic implications of these findings.

Significance

Expansion of polyglutamine tracts in at least nine proteins causes neurodegeneration. Although the pathology caused by each protein is different, there must be common features of the polyglutamine expansion that contribute to toxicity. We modeled polyglutamine toxicity in yeast by expressing a 103-glutamine expanded fragment of huntingtin (Htt103Q) and screened the yeast genome to identify proteins that alter this toxicity. Surprisingly, our suppressors were proteins containing glutamine- and asparagine-rich segments typical of prion proteins. We sequenced just these segments with Htt103Q, the two proteins formed large, coaggregated particles, and smaller, more toxic aggregated forms were absent. Proteins with such segments may interact with polyQ-expanded proteins and thereby modulate their toxicity. These interaction partners provide targets for therapeutic intervention.

protein misfolding | prions

Protein misfolding and aggregation underlie many neurodegenerative diseases. The causes of protein misfolding are numerous and include single amino acid changes and expansions of amino acid repeats. Polyglutamine (polyQ) expansions are an infamous example of the latter and are responsible for at least nine neurodegenerative conditions, including Huntington disease (HD). In most of these diseases the polyQ-expanded protein is expressed throughout the body, but tissue damage is restricted to the nervous system and, often, to a subset of cells depending on the causal protein. Thus, there are important cell-type-specific determinants of toxicity, and these are specific to particular proteins.

Despite these differences, disease severity is tightly linked to the number of glutamates. Progressively earlier onset occurs as the number of glutamates increases beyond a critical threshold of ∼35–45 residues (1). Moreover, in all cases, pathology is associated with misfolded forms of the polyQ-expanded protein. Thus, common features that arise from the expansions and result from shared aspects of polyQ-driven protein misfolding must contribute to pathology. Given the diverse nature of both the proteins bearing the polyQ tract and the pathologies they cause, understanding the nature of the interaction between misfolded polyQ-expanded proteins and the cellular environment is a complex but critical area of research.

The most prevalent polyQ disease, HD, is caused by an expansion of the polyQ tract in the protein huntingtin (Htt). A fragment of polyQ-expanded Htt, roughly encompassing exon 1 and including the polyQ tract, characteristically aggregates and accumulates in the cortex and striatum, the regions most sensitive to degeneration in HD (1, 2). The expanded polyQ tract promotes aberrant splicing of HTT exon 1 and results in the production of an exon 1 fragment of Htt protein (3). Overexpression of an exon 1 fragment alone is sufficient to cause neurodegeneration in mice (4) and is the basis for polyQ toxicity models in many other organisms, including yeast.

Neuron-specific vulnerabilities cannot be addressed in yeast models. However, these simple cells possess many archetypal aspects of protein homeostasis and other fundamental cell biology that are central to eukaryotes and are known points of susceptibility in neurodegenerative diseases. These include the full suite of eukaryotic chaperones and protein remodeling factors, the ubiquitin/proteasome system, autophagy, vesicle trafficking and the secretory pathway, the nuclear envelope and chromatin structure, and mitochondrial biology. The relationship between the length and toxicity of the polyQ region observed in human diseases is recapitulated in yeast, providing a strong indication that some aspects of polyQ toxicity are conserved (5).
Generally, polyQ-expanded proteins have altered protein–protein interactions, which either buffer or propagate the deleterious conformational changes of the polyQ-containing protein (6–11). However, the characteristics that distinguish beneficial from toxic interactions at a molecular level remain enigmatic. Taking advantage of the genetic tools available in yeast, we performed a genome-wide overexpression screen for modifiers of polyQ toxicity. Of the 5,532 proteins we tested, 10 were highly reproducible suppressors. These proteins were disparate in their physiological functions, yet seven of them contained segments of primary sequence that were enriched for asparagine and glutamine residues (N and Q) and had prion-like character (12). Prion-like domains in several other proteins have recently been implicated in many neurodegenerative diseases, raising interest in these unusual polypeptides (13). We used a variety of cell-based assays to investigate how prion-like domains can suppress or enhance the toxicity of polyQ-expanded Htt exon-1.

**Results**

**Prion Domains Recovered from Genome-Wide Screen for Htt103Q Toxicity Modifiers.** To model polyQ-expanded proteotoxicity in yeast, we created a diploid strain carrying two chromosomally integrated polyQ-expanded fragment of huntingtin exon-1 (Htt103Q) genes (encoding a FLAG tag, the first 17 amino acids of Htt, 105 repeats of glutamine, and a fluorescent protein) under control of the galactose-inducible promoter. This resulted in an intermediate level of Htt103Q toxicity and enabled us to screen for both suppressors and enhancers (Fig. 1A). Our ORF library covered 95% of the proteins encoded by the yeast genome. Each ORF encoded the protein without either N- or C-terminal additions and was borne on a single-copy plasmid under the control of the same galactose-inducible promoter as Htt103Q (14). The growth of each strain was compared with an Htt103Q strain carrying the same plasmid lacking an insert. An ORF was deemed to be a suppressor or enhancer if it affected growth of the Htt103Q-expressing strain but not a control strain without Htt103Q.

We identified ten genes that suppressed Htt103Q toxicity (Fig. 1A). (All genes that enhanced Htt103Q toxicity were also toxic in the control strain lacking Htt103Q and were not further investigated.) No single cellular process logically connected all of the proteins. However, inspection of the amino acid sequences of the suppressors revealed in many a region strongly enriched for glutamine- and asparagine-rich prion-like sequences. This finding was surprising because other proteins with such domains are implicated in the pathology of various neurodegenerative diseases (13) and not in protection against proteotoxicity.

In yeast, an individual domain confers the prion characteristics of most prion proteins. These prion domains (PrDs), both on their own and in the context of the full-length proteins, are capable of self-perpetuating conformational changes and can exist in either soluble or aggregated states (15). To date, over 25 proteins that possess prion-like characteristics have been identified in yeast, and most of their PrDs are highly enriched for the amino acids glutamine (Q) and asparagine (N). Many of these were identified by computational algorithms for ranking the likelihood that a given protein might function as a prion (12). In this ranking, seven of our Htt103Q toxicity suppressors (all but Mbp1, Bre5, and Ykr075c) reside within the top 150 of all proteins in the yeast genome. The likelihood that this occurred by chance is less than 1 in $10^{10}$ (hypergeometric distribution).

**Reconciling Different Results from a Similar Screen.** Very recently, an apparently similar yeast overexpression screen for modifiers of Htt103Q toxicity was reported (16). Of the 317 hits reported, many were among our suppressors, nor was there a bias for proteins containing prion-like sequences. We investigated three properties of the Mason et al. (16) screen that differed from our own and might account for the disparate findings: (i) a haploid yeast strain from a different genetic background was used; (ii) each ORF carried three C-terminal tags, HA, ox His, and protein A; and (iii) both the Htt103Q expression construct and the ORF library were on high-copy 2μ plasmids. After reanalyzing their strongest suppressor, Gpx1, in a variety of strains (Fig. S1 and SI Results), we concluded that their suppressors were recovered due to an artifact of using two high-copy plasmids that both carried the same expression cassette-regulatory terminator (for the expression of both Htt103Q and the ORF library). Using a 2μ to express the ORF will create competition for limiting transcription factors and reduce the expression of Htt103Q. The variations in copy number that occur with 2μ libraries likely accounted for the apparent differences in suppression. We also note that while our manuscript was under review, another overexpression screen for modifiers of Htt103Q toxicity in yeast reported results highly consistent with our own (17).

**PrDs Themselves Can Suppress Htt103Q Toxicity.** The high percentage of proteins containing prion-like domains among our suppressors prompted us to examine whether the Q/N-rich PrDs might suppress toxicity on their own. First, we tested the PrD segments from four of the suppressors identified in our screen—Nup49, Pop2, Sup35, and Gts1 (see ref. 12 for the amino acid sequences of all PrDs), each expressed with a C-terminal EYFP fusion. Overexpression of just the PrDs of Nup49 and Pop2 did not suppress Htt103Q toxicity, indicating that it is not just Q-richness that determines suppression. It may be that the full-length protein or a longer fragment of the protein is required. [While our paper was under review, a slightly longer fragment of Pop2 was reported to suppress Htt103Q toxicity (17)]. More importantly, the PrDs of both Sup35 (known as NM) and Gts1 were effective suppressors, demonstrating that some PrDs can modify Htt103Q toxicity on their own.

Next, we tested 38 previously reported N- or Q-rich PrDs (Table S1) (12). Of 20 Q-rich PrDs tested, 12 suppressed Htt103Q toxicity, whereas only 2 of 22 N-rich PrDs were capable of suppression. Both N-rich PrD suppressors also possessed Q-rich segments.

To more rigorously test the relationship between the suppression of toxicity and the enrichment for Q/N residues, we used two sets of PrD variants (Table S2). In one set, all glutamine residues were changed to asparagines (Q→N). In the second set, all asparagine residues were changed to glutamines...
(N→Q). Each of the N→Q variants potently suppressed Htt103Q toxicity. In fact, the N→Q variants were much more potent than the wild-type PrDs and were stronger than any of the suppressors identified in the screen. The Q→N variants not only failed to suppress toxicity but in contrast often enhanced it (Fig. 1B and Fig. S2).

PrDs Can Also Reduce Htt Toxicity in Human Cells. The neurodegeneration caused by various polyQ expansion proteins is highly cell specific, despite the fact that these proteins are broadly expressed throughout the body. Thus, toxicity in any particular cell type must be influenced by the sequences flanking the polyQ segments and the unique proteomes of the susceptible cell types (5, 18, 19). We do not, therefore, suggest that our yeast suppressors can directly reveal a toxic signal relevant to human disease. Nor would we expect them to behave in any particular mammalian cell type exactly as they do in yeast or another mammalian cell type. Rather, we consider them “proteome-modifying agents” and wondered if some of them are capable of modifying the aggregation and/or toxicity of polyQ-expanded Htt exon-1 in any human cell type.

First, we used a neuroblastoma-derived cell line (SH-EP) where the expression of polyQ expansion proteins is not toxic and examined changes in the aggregation state of Htt (20). We chose to limit this analysis to the prion domains in which we had exchanged all of the Qs or all of the Ns because these produced the strongest phenotypes. Cell cultures were cotransfected with Htt79Q (tagged with YFP) and our PrD modifiers (tagged with mCherry). The aggregation of Htt79Q in cells that expressed both proteins was scored 48 h posttransfection, using high-content fluorescence microscopy and an automated imaging system. This instrument assesses the mean intensity, area, and number of aggregates per cell, normalized to a neutral control (beta-galactosidase from Escherichia coli, uidA). Two human proteins lacking PrDs—the G1T1 protein, which increases polyQ-expanded Htt exon-1 aggregation, and the Pfn2 protein, which decreases it (21)—were used as additional controls. Because these were used as additional controls and were expressed throughout the body, we chose to limit this analysis to the prion domains in which we had exchanged all of the Qs or all of the Ns because these produced the strongest phenotypes. NM had no visible effect, NM→Q (N→Q→Q), and NM→N (N→N→N) variants not only possessed both colocalized and independent Htt103Q aggregates in almost every cell, but also the colocalized aggregates were often maintained, whereas those that lost it (29% of deaths) constituted 69% of deaths. The neurodegeneration caused by various polyQ expansion proteins is highly cell specific, despite the fact that these proteins are broadly expressed throughout the body. Thus, toxicity in any particular cell type must be influenced by the sequences flanking the polyQ segments and the unique proteomes of the susceptible cell types (5, 18, 19). We do not, therefore, suggest that our yeast suppressors can directly reveal a toxic signal relevant to human disease. Nor would we expect them to behave in any particular mammalian cell type exactly as they do in yeast or another mammalian cell type. Rather, we consider them “proteome-modifying agents” and wondered if some of them are capable of modifying the aggregation and/or toxicity of polyQ-expanded Htt exon-1 in any human cell type.

To examine cells with a different tissue of origin and hence a different proteome, we used HEK293 cells. These cells are commonly used to study both the toxicity and aggregation of various disease-related proteins (23). We cotransfected cells with Htt72Q (tagged with GFP) and several of the yeast PrD modifiers (tagged with mCherry). After 48 h, we fixed the cells and scored the aggregation of Htt72Q and cell death (Fig. 1D and Fig. S4). Only four of the PrDs were expressed at sufficient levels to include in the analysis (NM, NM→N, Ure2N→Q, and Gal11). The effects of these proteins on Htt72Q aggregation varied. NM had no visible effect, NM→Q significantly decreased aggregation, and Gal11 and Ure2N→Q increased aggregation. Nevertheless, all four of these Q-rich PrDs suppressed Htt72Q toxicity. NM and Ure2N→Q reduced cell death by over 40%, whereas NM→N→Q and Gal11 attenuated toxicity by ~20%. Although we neither expected nor found identical outcomes in the two different human cell types and in yeast, clearly, the PrD modifiers can affect both the aggregation and the toxicity of polyQ-expanded Htt exon-1 in the context of human proteomes.

Paradoxically, PrD Suppressors Increase Htt103Q Protein Levels. To investigate further, we returned to the more tractable yeast cells. These cells provide genetically identical, genomically stable populations that are subject to only minor epigenetic modifications of gene activity in culture. Further, each and every cell carries an identical complement of inducible genes, either integrated in the genome or on a plasmid. This ensures more homogeneous and controlled gene expression across the entire population.

We first asked if coexpression of PrD modifiers altered the amount of Htt103Q protein in the yeast cells. Western blotting with antibodies against GFP simultaneously revealed the Htt103Q protein and the PrD modifiers because both were modified with a GFP variant (Fig. 2A). Enhancers did not increase the level of Htt103Q protein (if anything, they decreased it). Even more surprisingly, suppressors of Htt103Q toxicity actually increased Htt103Q protein levels (Fig. 2B). Experiments in which cycloheximide was used to abruptly terminate all protein synthesis demonstrated that the half-life of the Htt103Q protein was dramatically increased by the presence of a PrD suppressor (Fig. 2B). Because coexpression of PrD suppressors does not decrease the amount of Htt103Q, they must (directly or indirectly) reduce its toxicity.

PrD Suppressors Form Large Coaggregates with Htt103Q. We turned to fluorescence microscopy to examine the relative localization of Htt103Q and the PrD modifiers in the yeast cells. In the absence of a modifier, the toxic Htt103Q protein formed irregular agglomerations of various sizes and shapes distributed throughout the cytoplasm (Fig. 3A). As expected, a coexpressed EYFP control (EYFP protein alone) was diffusely distributed in these cells. When cells coexpressed EYFP-tagged enhancers, the aggregation pattern of Htt103Q did not change in any obvious way, and the enhancer protein either remained diffuse or formed independent aggregates that excluded Htt103Q. In the presence of a suppressor, however, Htt103Q collected in only one or two large foci in the cytoplasm of each cell, where it colocalized with the suppressor protein (Fig. 3A and Fig. S5).

Cells with Suppressor/PrD Coaggregates Grow More Rapidly. To determine whether the formation of large Htt103Q aggregates was directly related to the suppression of toxicity, we used a PrD with only moderate suppressor activity (NM) and which colocalized with Htt103Q in many but not all cells. Time-lapse video microscopy of yeast in microfluidic chambers allowed us to both monitor protein aggregation within each cell and unambiguously track the lineages of the cells as they divided over the course of the experiment. Thus, any difference in the growth rates of cells with and without aggregates would be apparent.

As expected, some founder cells possessed colocalized foci of NM and Htt103Q. In others, the foci contained only Htt103Q but not the suppressor. As the colonies grew, cells descended from founders with colocalized foci often maintained that distribution and repeatedly divided, eventually taking over the entire field of view. Cells in which NM was not colocalized with Htt103Q grew poorly and soon died (Fig. 3B). Cells that initially possessed both colocalized and independent Htt103Q aggregates were particularly informative. In these, the focus in which NM and Htt103Q colocalized was occasionally lost. When this happened, it was quickly followed by cell death (Fig. 3C). In total, cells that did not inherit a colocalized focus (40% of deaths) and those that lost it (29% of deaths) constituted 69% of deaths.

Suppressor PrD/Htt103Q Coaggregates Accumulate at the IPOD. In eukaryotes, there are two cytoplasmic sites for the deposition of misfolded proteins: the insoluble protein deposit (IPOD) and the juxtanuclear quality control compartment (JUNQ) (24). The
IPOD is located near the vacuole and serves as a site for the sequestration of aggregated proteins. The JUNQ is located adjacent to the nucleus and contains misfolded proteins that are destined for proteasomal degradation. To test whether Htt103Q localizes to either of these structures, we coexpressed Htt103Q and some of our PrD modifiers in three separate yeast strains carrying markers for different subcellular compartments. These labeled either the IPOD with Rnq1, the JUNQ with von Hippel-Lindau tumor suppressor (VHL), or the nucleus with NHP6a. Htt103Q formed many small puncta when expressed on its own or together with an enhancer. These aggregates were broadly distributed and often around, but not within, the nucleus. In the majority of cells (82 ± 9%), a small portion of Htt103Q was colocalized with VHL at the JUNQ (Fig. S6). However, much of the aggregated Htt103Q was outside the JUNQ and never localized to the IPOD (Fig. 4). In contrast, when Htt103Q was coexpressed with a suppressor PrD, both proteins consistently colocalized at the IPOD (Fig. 4).

Proteins localized to the IPOD characteristically have very low mobility (24). To determine if Htt103Q and suppressor proteins in the IPOD had these dynamics, we used fluorescence recovery after photobleaching (FRAP) to quantify the mobility of Htt103Q molecules inside and outside the foci. We photobleached a small region of the cell with a brief, intense laser pulse and then determined how quickly fluorescence was restored by the migration of unaffected proteins into this spot. In cells coexpressing Htt103Q and NM, photobleached regions outside of foci regained roughly 60% of their signal within a minute and eventually recovered more than 80% of their original fluorescence, indicating that the Htt103Q molecules outside of foci were highly mobile. In contrast, when we photobleached Htt103Q protein inside foci, recovery was negligible for at least 5 min (Fig. 5A). Thus, Htt protein localized to the IPOD is highly immobile.

**PrD Suppressors Eliminate Diffusible Oligomers of Htt103Q.** Small oligomers have been implicated as the causal agents in many proteinopathies. To monitor changes in the population of Htt103Q oligomers caused by our PrD enhancers and suppressors in yeast, we used fluorescence correlation spectroscopy (FCS) (25). To concentrate our analysis on the Htt103Q protein that was not localized to large aggregates, cell lysates were subjected to centrifugation to remove them. The supernatant was recovered and two-photon excitation was exploited to generate a very small excitation volume. We measured the fluctuations of fluorescence caused by the diffusion of single particles into, and out of, this volume. The brightness of these events (i.e., the number of fluorophores per diffusing particle) provides a measure of the oligomerization state of the Htt103Q. To limit artifacts from intermolecular quenching, which would result in lower fluorescence, we used mCherry tags on the PrD modifiers. We also measured the fluorescence intensity of the lysates to ensure that the number of fluorophores in the excitation volume was within acceptable limits. Because some cultures expressing moderate suppressors contained a mixed population of cells experiencing varying degrees of toxicity (see above for the example of NM), they were excluded from the analysis.

In lysates of cells expressing Htt103Q and a neutral protein (EYFP), we detected slowly diffusing particles of ECFP-tagged Htt103Q. The molecular brightness of these particles was much greater than monomeric fluorophores (i.e., that of ECFP without Htt103Q), consistent with an oligomeric species (Fig. 5B). In lysates of cells coexpressing Htt103Q and PrD enhancers, Htt particles had diffusion coefficients similar to those of cells expressing Htt103Q and the neutral control (Fig. S7). As determined by their molecular brightness, these particles were smaller on average but more broadly distributed in sizes. In lysates of cells coexpressing strong suppressors, Htt103Q particles had the fastest diffusion coefficients and molecular brightness comparable to that of monomeric fluorophores, indicating that Htt103Q was monomeric.

**Discussion**

Starting with an unbiased genome-wide screen, we discovered that glutamine (Q)-rich protein domains can act as potent suppressors of Htt103Q toxicity. These suppressors coaggregated with Htt103Q. The resulting Htt103Q aggregates were much less toxic, and small oligomers of Htt103Q were undetectable. Thus, interactions between polyQ-expanded disease proteins and other Q-rich proteins do not always contribute to pathology. Some of these interactions may instead have the beneficial effect of capturing the misfolded polyQ-expanded protein and preventing it from inactivating more essential proteins. The complexities such interactions are formidable, but understanding them may offer new therapeutic strategies.

What features of Q-rich domains might determine their capacity to suppress Htt103Q toxicity? Strikingly, this ability is inversely related to their capacity to form prions. Prion proteins have the unusual ability to convert into self-perpetuating amyloids, which can act as protein-based elements of inheritance (15). The majority of known prion proteins have a distinct prion-determining region (PrD) that is rich in N and Q residues. In

![Fig. 3. PrD suppressors alter the intracellular localization of Htt103Q. (A) Strains expressing Htt103Q (green) along with a neutral control (EYFP) or an EYFP-tagged PrD enhancer of toxicity (NM N=N, red) or an EYFP-tagged suppressor of toxicity (NM N=NM, red). Far right graph shows quantitation of the percentage of cells with Htt103Q aggregated in one or two foci. (B) Time course of a single microcolony coexpressing Htt103Q and NM. (C) The fate of the cell indicated by the black arrow in B is shown in greater time resolution. The white arrow indicates the location of a focus with colocalized Htt103Q and suppressor PrD. This focus was spontaneously lost between 12.5 h and 14.5 h, after which the cell died. (All scale bars: 5 μm.)](image)

![Fig. 4. Localization of Htt103Q in the presence of PrD modifiers in yeast. (A) The relative localization of Htt103Q (green) with the nucleus (magenta) or the insoluble protein deposit marker Rnq1 (red) upon coexpression with a PrD modifier. (B) Quantitation of the colocalization of Htt103Q and Rnq1 in the IPOD. (All scale bars: 5 μm.)](image)
As noted above, interaction between Htt logically fits another report, the sequestration of Htt103Q into Sti1 foci, a juxta-oligomeric structure. In another report, the sequestration of Htt103Q aggregates consisted of large foci at the IPOD. In our yeast model, cells experiencing the greatest toxicity harbored many small oligomers. In cells experiencing the lowest toxicity, similar oligomers were undetectable. Because the suppressors greatly increased the total accumulation of Htt103Q, our PrD suppressors must prevent the formation of oligomers and/or promote their sequestration. PolyQ-expanded Htt exon-1, in vitro, forms an α-helical oligomer early in the aggregation process (40). It is possible that the suppressor PrDs intercalate into such α-helix-rich oligomers and promote their assembly into the large coaggregated foci.

When overexpressed separately from the rest of the protein, the PrDs of some of the suppressors recovered from our screen could not suppress Htt103Q toxicity. In some cases, the PrD and at least part of the rest of the protein must work together to relieve Htt103Q toxicity. It is notable that four of these particular suppressors—Sis1, Nup49, Bre5, and Upb3—fit logically into a nuclear trafficking and degradation pathway recently identified in yeast and implicated in Htt103Q toxicity (41, 42). In previous literature, most Q-rich proteins that modify Htt toxicity have been shown to be enhancers (7, 8, 10). Indeed, many of the PrDs we tested that had very strong suppressor activity on their own were not recovered as suppressors in our screen of the full-length proteins. The Q-rich segments, as well as the rest of the protein to which they are attached, can each influence Htt toxicity and can do so in different ways. Thus, the physical interaction of Q-rich PrDs with Htt103Q, although highly significant, is not the sole determinant of toxicity suppression or enhancement.

The interaction between Q-rich PrDs and the polyQ-expanded disease protein is not necessarily specific to a given cell type or even specific to Htt. The Sup35 PrD, NM, not only suppresses polyQ-expanded Htt exon-1 toxicity in yeast and human cells as shown in this report but also suppresses polyQ-expanded SCA3 toxicity in Drosophila (43). As noted above, interaction between Q-rich proteins and polyQ-expanded disease proteins frequently leads to enhanced toxicity, but clearly, there are examples of beneficial interactions like those reported here in yeast and previously in C. elegans (44). No doubt the phenotypic outcome will depend on the expression patterns of the Q-rich proteins, as well as their posttranslational modifications, in the context of a particular proteome. Variability in this interaction network must be a key contributor to the vulnerability of different cell types to different polyQ expansion proteins. The complexity of these effects is daunting. Nevertheless, learning to control these interactions might prove a valuable strategy in the treatment of polyQ expansion diseases.

**Materials and Methods**

**Overexpression Screen and PrD Modifiers.** The overexpression library consisted of 5,332 full-length, untagged, sequence-verified, galactose-inducible yeast ORFs in the plasmid pBY001 in a BY4741 yeast strain (14). PrD modifiers were cloned into Gateway destination vectors with C-terminal fluorescent fusion proteins and transformed into the BY4741 strain. These strains were individually mated with a [RNQ+] w303 strain containing Htt103Q integrations of pRS303GAL-FLAG-Htt103Q-ECFP and pRS305GAL-FLAG-Htt103Q-DsRed (see SI Materials and Methods for more details).

**HEK-293 Assays.** Approximately 200 ECFP-Htt72Q and PrD-mCherry expressing cells were counted per modifier and assessed for cell death and aggregation. Nuclei were stained with 4,6-diamidino-2-phenylindole, and those that showed morphology indicative of necrosis or apoptosis (for example, fragmentation or pyknosis) were considered dead. Each experiment was carried out in triplicate and performed at least twice. Thus, cell death and the formation of large Htt103Q foci seems to be protective against toxicity.

As evidence for the protective nature of IBs has mounted, a consensus has emerged implicating small oligomeric particles as the toxic species. For polyQ-containing proteins, oligomers form before IBs, their formation is polyQ length-dependent, and their presence is well correlated with cell death (25, 37). Both chaperones that remodel oligomers and peptides that inhibit oligomer formation suppress toxicity (38, 39). In our yeast model, cells experiencing the greatest toxicity harbored many small oligomers. In cells experiencing the lowest toxicity, similar oligomers were undetectable. Because the suppressors greatly increased the total accumulation of Htt103Q, our PrD suppressors must prevent the formation of oligomers and/or promote their sequestration. PolyQ-expanded Htt exon-1, in vitro, forms an α-helical oligomer early in the aggregation process (40). It is possible that the suppressor PrDs intercalate into such α-helix-rich oligomers and promote their assembly into the large coaggregated foci.

When overexpressed separately from the rest of the protein, the PrDs of some of the suppressors recovered from our screen could not suppress Htt103Q toxicity. In some cases, the PrD and at least part of the rest of the protein must work together to relieve Htt103Q toxicity. It is notable that four of these particular suppressors—Sis1, Nup49, Bre5, and Upb3—fit logically into a nuclear trafficking and degradation pathway recently identified in yeast and implicated in Htt103Q toxicity (41, 42). In previous literature, most Q-rich proteins that modify Htt toxicity have been shown to be enhancers (7, 8, 10). Indeed, many of the PrDs we tested that had very strong suppressor activity on their own were not recovered as suppressors in our screen of the full-length proteins. The Q-rich segments, as well as the rest of the protein to which they are attached, can each influence Htt toxicity and can do so in different ways. Thus, the physical interaction of Q-rich PrDs with Htt103Q, although highly significant, is not the sole determinant of toxicity suppression or enhancement. The interaction between Q-rich PrDs and the polyQ-expanded disease protein is not necessarily specific to a given cell type or even specific to Htt. The Sup35 PrD, NM, not only suppresses polyQ-expanded Htt exon-1 toxicity in yeast and human cells as shown in this report but also suppresses polyQ-expanded SCA3 toxicity in Drosophila (43). As noted above, interaction between Q-rich proteins and polyQ-expanded disease proteins frequently leads to enhanced toxicity, but clearly, there are examples of beneficial interactions like those reported here in yeast and previously in C. elegans (44). No doubt the phenotypic outcome will depend on the expression patterns of the Q-rich proteins, as well as their posttranslational modifications, in the context of a particular proteome. Variability in this interaction network must be a key contributor to the vulnerability of different cell types to different polyQ expansion proteins. The complexity of these effects is daunting. Nevertheless, learning to control these interactions might prove a valuable strategy in the treatment of polyQ expansion diseases.
aggregation are expressed in terms of odds ratio (see SI Materials and Methods for more details).

Epifluorescence, Time Lapse, and Confocal Imaging. For epifluorescence imaging, we used the original screening strain described above with EYFP-tagged PrD modifiers. The ECFP-tagged Htt103Q strain proved superior for fluorescence microscopy and was used as a general indicator of the fate of Htt103Q in our yeast cells. Cells were grown overnight in liquid culture, and images were acquired with a Nikon Plan Apo 100x: oil objective (NA 1.4). Data from two biological replicates and three for suppressors were quantitated.

The strains used for time-lapse and confocal imaging experiments were identical except that all modifiers were integrated into the genome at the URA locus. VHL was tagged with EYFP and expressed from a galactose-inducible vector integrated in the URA locus. Rnp1 and NHP6a were both endogenously tagged with IRFP. For time-lapse imaging, mKate2 was integrated into the HO locus to serve as a cytoskeletal marker. Time-lapse bright field and RFP images were captured every 15 min, and CFP and YFP channels were acquired every 30 min (see SI Materials and Methods for more details).

High-Content Microscopy. High-content microscopy of SH-EP cells coexpressing EYFP-tagged PrD modifiers growing in log phase was subjected to FRAP on an Andor Revolution spinning disc confocal microscope using a 100× 1.49 NA Nikon Plan Apo objective. ECFP–mCherry-tagged PrD modifiers. Yeasts were spheroplasted and gently lysed with 2% Igepal at 4 °C and centrifuged for 30 min at 14,000 × g to remove large aggregates (see SI Materials and Methods for details).

ACKNOWLEDGMENTS. We thank M. Duennwald for extensive assistance during the initial phases of this project; W. Salmon and the Whitehead Keck Microscopy Facility for assistance with microscopy; A. S. Khali for his assistance with the microfluidic chambers; D. Landgraf for assistance with the time-lapse microscopy and R. Kamm, M. Lang, and members of the S.L. laboratory for their insights. This work was supported by funding from the Howard Hughes Medical Institute (S.L.), National Institutes of Health Grant GM52874 (to S.L.), National Science Foundation Fellowship 1122374 (to W.R.H.), the Harold and Leila Y. Mathers Foundation (S.L.), the Beckman Laser Institute Foundation (J.V.S.), the Eleanor Schwartz Charitable Foundation (S.L.), and the Whitehead Institute for Biomedical Research. E.E.W. was supported by grants from the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich [SFB] 740, 740/11 and SFB 618: 618/6-1), Bundesministerium für Bildung und Forschung (Nationalen Genomforschungsnetz-Plus, NeuroNet: 01GS08169-73), EU (EuroSpin: Health-F2-F2009-241498 and SynSys: HEALTH-F2-F2009-242167), and the Helmholtz Association (The Max Delbrück Centre for Molecular Medicine Systems Biology Network and The Helmholtz Alliance for Mental Health in an Ageing Society: HA-215).

FRAP. The Htt103Q strain coexpressing EYFP-tagged PrD modifiers growing in log phase was subjected to FRAP on an Andor Revolution spinning disc confocal microscope using a 100× 1.49 NA Nikon Plan Apo objective. ECFP–mCherry-tagged PrD modifiers were performed as previously described (20).