Huntington's Disease

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Huntington's disease (HD) is the most common inherited neurodegenerative disease and is characterized by uncontrolled excessive motor movements and cognitive and emotional deficits. The mutation responsible for HD leads to an abnormally long polyglutamine (polyQ) expansion in the huntingtin (Htt) protein, which confers one or more toxic functions to mutant Htt leading to neurodegeneration. The polyQ expansion makes Htt prone to aggregate and accumulate, and manipulations that mitigate protein misfolding or facilitate the clearance of misfolded proteins tend to slow disease progression in HD models. This article will focus on HD and the evidence that it is a conformational disease.

HUNTINGTON'S DISEASE: A BRIEF HISTORY

In 1872 George Huntington, a young physician from Connecticut, published one of the first descriptions of the disorder that would come to bear his name (Huntington 1872). The publication, one of only two produced in his entire career, was based on families under the care of his father who was also a physician. The neurological disorder was dominantly inherited and characterized by excessive motor movements and neuropsychological deficits. Earlier descriptions of a neurological disorder that is almost certainly Huntington's disease (HD) can be found (Harper 2002; Lund 1860); however, George Huntington's is still considered by many to be the first fairly complete description of HD.

More than a century passed before the underlying genetic mutation in HD was identified (Bates 2005). A consortium of researchers engaged in one of the most ambitious gene hunting efforts of the time. They relied on DNA samples from families in the Lake Maracaibo region of Venezuela, an area with a high density of HD and significant consanguinity. In 1993, the consortium reported the successful discovery of an unstable triplet repeat expansion within *IT15* (interesting transcript #15) that cosegregated with coHD (Group 1993). *IT15*, later called the *HUNTINGTIN* (*HTT*) gene, had no significant homology to other genes in the genome and encoded an mRNA of approximately 10 kB and a protein of 350 kDa. A triplet nucleotide repeat, cytosine-adenonsine-guanine (CAG), is found near the 5′ end of the gene's coding region and is translated into a polyglutamine (polyQ) stretch.

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HUNTINGTON'S DISEASE: A CLINICAL OVERVIEW OF GENOTYPE–PHENOTYPE CORRELATIONS

Genetic Determinants of Symptom Onset and Disease Progression

With the discovery of *HTT*, the clinical features of HD could be better defined by investigating genotype-phenotype relationships with greater specificity. A major focus of these early studies was on understanding the relationship between the age when an individual first develops neurological symptoms and the length of the triplet repeat (Duyao et al. 1993; Rubinsztein et al. 1996; Snell et al. 1993) (Fig. 1A). Alleles of *HTT* with fewer than 35 CAGs were found to pose no risk for causing HD. Individuals harboring alleles with between 36–40 CAGs may or may not develop HD symptoms; those that do tend to become symptomatic late in life. However, those with alleles containing expansions greater than 40 CAGs repeats will eventually develop symptoms of HD if they live long enough. Curiously, individuals who harbor two HD-causing alleles (i.e., homozygous for mutant *HTT*) appear to develop symptoms about the same age as people with a single allele and the same CAG expansion. However, homozygosity for the CAG mutation has been reported to lead to a more severe clinical course (Squitieri et al. 2003). These observations should be considered cautiously because they are based on very small numbers of people: homozygosity is rare.

Interestingly, there is a striking and significant negative relationship between the length of the CAG expansion and the age of symptom onset; the longer the CAG stretch, the earlier symptoms typically appear (Fig. 1A). The most common HD alleles contain 40–50 polyQ. In that range, 50–70% of age of symptom onset appears to be explained by the length of the polyQ stretch, the remainder is determined by other modifying genes and environmental influences (Wexler et al. 2004). At longer polyQ stretches, an even greater proportion of age of symptom onset is explained by the length of the polyQ stretch. Length of the polyQ stretch also appears to influence the progression of

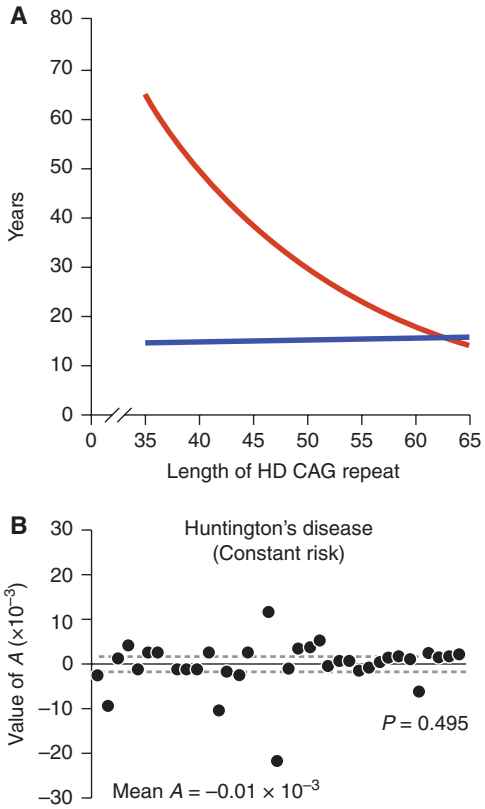

Figure 1. The role of the CAG expansion in *IT-15* on HD pathogenesis. (*A*) Correlation of HD CAG-repeat length with age at onset. Best-fit curves for age at neurological onset (red) and duration of disease from onset to death (blue), plotted against CAG-repeat length for the expanded mutant allele from Huntington disease (HD) patients. Age at onset is strongly correlated with the CAG-repeat length ($r^2 = 0.54$; $p < 0.001$), and duration of disease shows no correlation with the CAG-repeat length, suggesting that, after onset of HD, factors independent of the original trigger of pathogenesis determine the rate of progression. Adapted with permission from (Gusella and MacDonald 2006). (*B*) The kinetics of metabolic decline in Huntington's disease patients is best fit by a constant risk of cell loss. Values of A, the exponent of an equation relating longitudinal metabolic changes to time, do not differ significantly from zero (P = 0.495, Student's t-test). Each point represents the estimated A for an individual patient. Solid line, mean A (mean ± s.d.: $-0.01 \times 10^{-3} + 5.3 \times 10^{-3}$ mM/h^1, $n = 38$ patients) across patient population; dashed lines, 95% confidence interval for the mean value of A (-1.7×10^{-3}, 1.7×10^{-3}). Adapted with permission from (Clarke et al. 2000).

pathology, although the link between disease progression and the length of the polyQ stretch appears to be weaker than for age of symptom onset (Brandt et al. 1996; Penney et al. 1997) (Fig. 1A).

The ability to correlate the length of the CAG expansion to clinical phenotype uncovered the molecular basis for genetic anticipation (Telenius et al. 1993; Telenius et al. 1995). Anticipation in HD referred to the puzzling phenomenon whereby inheritance of the mutant *HTT* allele through the male germ line often led to a more severe clinical course than inheritance through the female germ line. On average, children experienced HD symptoms 8 years earlier than their fathers (Ranen et al. 1995). In those children, the CAG stretch had further expanded. The molecular mechanism responsible for the propensity of the CAG stretch to expand through the male germ line is debated (MacDonald et al. 1993; Telenius et al. 1994; Telenius et al. 1995).

Genetic Determinants of Symptom Profile and Neuropathology

The length of the CAG stretch also affects the types of symptoms. The most common HD alleles (40–50 CAGs) tend to produce classic HD symptoms that manifest during mid-life. The best-known symptoms are excessive uncontrolled jerky motor movements, called chorea, and gait disturbances. Some patients experience dystonia, an increase in muscle tone. Neuropsychiatric symptoms are common, especially depression and anxiety. Some patients report obsessive-compulsive symptoms and effects on cognition. Deficits in executive function appear early; a global dementia often ensues. Unusually long CAG stretches (>50) display symptoms so different that the syndrome is called juvenile HD (JHD), reflecting the earlier onset (Nance and Myers 2001). Unlike adult-onset HD, JHD is associated with a paucity of movements (bradykinesia) and an increased incidence of seizures.

Neuropathological changes are also related to CAG expansion length. The brain region believed to show the earliest changes is the striatum, and within the striatum, medium spiny neurons that contain the neuropeptide enkephalin and express the D2-subtype of dopamine receptor appear to be the most vulnerable (Augood et al. 1996; Ginovart et al. 1997; Le Moine et al. 1990; Marshall et al. 1983; Richfield et al. 1995; Sapp et al. 1995). Subregions of cortex are also significantly affected relatively early (Rosas et al. 2005; Sax et al. 1996). Other regions, such as the cerebellum, are relatively spared (Vonsattel et al. 1985). In JHD, the pathology is more widespread (Myers et al. 1988; Nance and Myers 2001); presumably, as the polyQ expansion increases in length, the mechanisms that restrict the specificity of neurodegeneration to a relatively small subpopulation of neurons in adult onset HD are progressively lost.

The Role of Aging in Huntington's Disease

That an individual harboring a genetic mutation could appear normal for decades before developing a mid-life neurodegenerative disease has puzzled investigators. Some interpret this to mean HD is caused by an underlying biochemical event—the nucleation of a misfolded form of polyQ-expanded HTT or the sufficient accumulation of protein deposits, which takes decades to occur (Chen et al. 2002b). However, it is probably incautious to assume a direct biochemical link between symptoms and biochemistry. In Parkinson's disease, for example, symptoms do not appear until most dopaminergic neurons in the substantia nigra are lost, suggesting that the nervous system has excess capacity before symptoms manifest (Bezard et al. 2003). The nervous system also has powerful cell-autonomous and cell-nonautonomous mechanisms to mitigate the effects of disease-associated mutations (Arrasate et al. 2004; Finkbeiner et al. 2006; Mitra et al. 2009; Palop et al. 2007). Indeed, some adaptive changes may contribute to symptoms under some circumstances (Graybiel 2004; Picconi et al. 2005). Finally, the use of symptoms to detect age of disease onset is somewhat arbitrary. Some clinical tests detect behavioral deficits in patients 15 years before the symptoms appear (Paulsen et al. 2008),

and a revision of what constitutes "early-stage" disease may be necessary. Presumably, more sensitive clinical tests could detect deficits earlier.

In 2000, a one-hit model of neurodegeneration was proposed. Clarke et al. carefully counted cells in HD brain sections collected at different disease stages. Surprisingly, they found cell numbers decline exponentially with time with genetic causes of neurodegeneration, such as HD (Clarke et al. 2000) (Fig. 1B). More recently, careful longitudinal live cell measurements in a primary neuron model of HD confirmed and extended the one-hit model by showing that the elevation in the risk of death was proportional to the length of the polyQ expansion beyond the threshold associated with HD (Arrasate et al. 2004; Miller et al. 2010b). The implication of these findings is that the risk that any particular cell would die is relatively constant and independent of the epoch of observation. In other words, the genetic mutation responsible for HD behaves as if it reduces the overall viability of cells by a certain amount, making them more susceptible to apparently random stresses. Although the risk of cell death is constant, cumulative cell loss over time eventually uses up the nervous system's excess capacity and exceeds its mechanisms to cope with cell loss. Network failure results and symptoms manifest (Ying 1996).

What then is the role of aging in HD? Aging is not an absolute requirement. The most common HD alleles (40–50 CAGs) produce symptoms in mid-life. However, longer CAG expansions produce earlier symptoms; the youngest person with HD developed symptoms at age 2. Indeed, electrophysiological abnormalities have been detected in cells derived from mouse models of HD at the embryonic stem cell stage. Aging has been associated with a decline in a variety of pathways relevant to neurodegenerative disease (Beal 1995; Cuervo et al. 2005; Gaczynska et al. 2001; Heydari et al. 1994; Keller et al. 2004; Klivenyi and Vecsei 1997; Massey et al. 2006), especially those that are critical for handling misfolded proteins (Zhou et al. 2003). Thus, aging may further reduce the overall ability of cells to withstand random stresses. The deficits in homeostatic mechanisms caused by aging could overlap or operate independently from those induced by disease-associated forms of HTT, but in either case, aged cells would be predicted to be more susceptible than younger ones. The reader is directed to an excellent article written by Taylor and Dillin (2011) on proteastasis and aging for a fuller account.

HUNTINGTON'S DISEASE AND PROTEOSTASIS

Huntington's Disease: Evidence of a Proteopathy

Having established the CAG expansion as the cause of HD, investigators turned toward understanding how it causes neurodegeneration. The first question was whether neurodegeneration caused by the CAG expansion was mediated at the level of the nucleic acids (DNA and mRNA) that contained them or through the polyQ expansion they encoded in the HTT protein.

CAG expansions might mediate neurodegeneration through abnormal polyQ expansions. Recently, an inducible transgenic mouse model of HD was created with the first exon of *HTT* (HTT^{ex1}), which contains the CAG expansion. In that model, behavioral and pathological deficits appeared when HTT^{ex1} was induced and could be reversed by removing the inducing agent and allowing HTT^{ex1} levels to decrease (Yamamoto et al. 2000). This result excludes a DNA-based mechanism for neurodegeneration. Perhaps the most definitive result was obtained serendipitously years earlier. Hayden and colleagues generated a transgenic mouse that constitutively expressed a human transcript encoding the human HD gene (Goldberg et al. 1996). However, an unplanned stop codon caused the *HTT* transcript to produce no protein. The animal showed no evidence of a disease-related phenotype, suggesting that a CAG expansion at the DNA or mRNA level was insufficient to produce neurodegeneration.

Others generated a transgenic mouse constitutively expressing the human HD gene but replaced the pure CAG expansion with a mixture of glutamine-encoding CAG and CAA

codons (Gray et al. 2008). The mixture did not affect the HTT protein, the polyQ stretch was identical to one encoded by a pure CAG stretch. However, the CAG expansion was less likely to change length during breeding. This mouse, which expressed polyQ-expanded HTT but lacked a pure CAG stretch, had behavioral and pathological features of neurodegeneration that resembled HD. The mix of CAA and CAG codons is significant for another reason. mRNAs containing pure triplet repeat expansions form double-stranded hairpins stabilized by intrastrand base-pairing. The use of alternating CAA and CAG codons disrupts hairpin formation. Because the formation of RNA hairpins has been proposed as a mechanism of toxicity mediated by mRNA-containing triple repeat expansions, the presence of a phenotype despite a manipulation designed to reduce mRNA hairpin formation is consistent with the conclusion that CAG-expansion-mediated neurodegeneration results primarily from the production of proteins that contain polyQ rather than from effects mediated by mRNA. These results strongly implicate proteotoxicity, but some mRNA-mediated toxicity cannot be excluded (Li et al. 2008).

Correlations to Protein Accumulation: HTT Aggregation and Inclusion Body Formation

The idea that HD might be a proteopathy arising from abnormal accumulation of mutant HTT got a considerable boost with the generation of the first mouse model of HD (Mangiarini et al. 1996). It expressed a transgene encoding HTT^{ex1} and contained a disease-associated CAG expansion. These mice showed behavioral phenotypes, reduced survival, and gene expression changes that parallel those seen in HD and other HD models based on the full-length HTT protein (Kuhn et al. 2007; Menalled et al. 2009). One neuropathological feature was nuclear accumulations of mutant HTT called intraneuronal nuclear inclusions (INNs) (Davies et al. 1997) (Fig. 2). The appearance of INNs correlated with the onset of behavioral deficits, and the overall burden of INNs correlated with the severity of symptoms. Thus, Davies et al. concluded that INN formation underlies HD. Soon after the observation of INNs were reported in HD models, they were described in HD brains (DiFiglia et al. 1997). Accumulations (or simply inclusion bodies [IBs]) of mutant HTT have also been reported in the cytoplasm, especially the perinuclear area and in dendrites (DiFiglia et al. 1997; Gutekunst et al. 1999). Indeed, dendritic IBs may be more common than those found elsewhere (Gutekunst et al. 1999).

Inclusion Bodies as a Mismatch of Protein Production and Clearance

Abnormal IB accumulations containing mutant HTT, particularly in brain regions affected in HD, provided prima facie evidence that HD is fundamentally associated with protein misfolding and inadequate protein clearance. IBs contain ubiquitin, molecular chaperones, and proteasome subunits, suggesting production of misfolded protein exceeds the ability of cells to clear them (Mitra and Finkbeiner 2008; Sieradzan et al. 1999; Stenoien et al. 1999; Waelter et al. 2001). Generation of an inducible HD mouse model allowed tests of the effects of terminating production of mutant HTT at an age that IBs had already formed and the animal had developed behavioral deficits (Yamamoto et al. 2000). The fact that IBs could be cleared and that behavioral deficits recovered were evidence that neurons must contain cellular mechanisms that metabolize IBs. More recently, experiments using siRNA or antisense oligonucleotides have targeted HTT in several HD models (Boudreau et al. 2009; DiFiglia et al. 2007; Harper et al. 2005). In every case, suppressing mutant HTT production was beneficial, leading to either slowed progression of pathology and behavioral deficits or partial reversal in some cases. Thus, IBs and behavioral deficits appear to result from an imbalance of production and clearance of mutant HTT (Waelter et al. 2001).

How polyQ expansion in HTT yields a mismatch of HTT production and clearance remains unresolved. Discovery of ubiquitin and proteasome subunits in IBs led to investigations of the effect of mutant HTT on

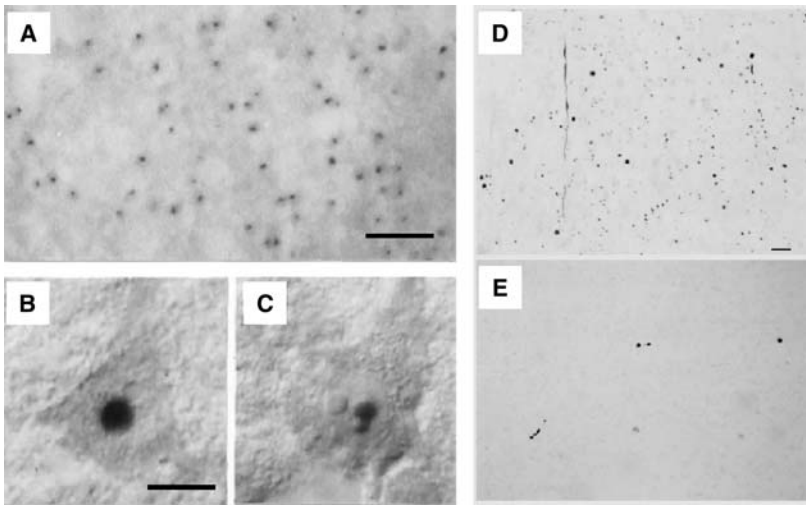

Figure 2. HD is characterized by abnormal protein deposits containing mutant huntingtin. (*A*) Huntingtin immunoreactivity in neuronal intranuclear inclusions (hNIIs) and dystrophic neurites in HD brain. Cortex of a juvenile patient shows numerous hNIIs prominently stained. (*B* and *C*) Cortical pyramidal neurons in a different juvenile patient shown with Nomarski optics contain one (*B*) and two (*C*) hNIIs. *A–C*, Adapted with permission from (DiFiglia et al. 1997). The nucleolus in each cell is unlabeled. (*D–E*) Huntingtin aggregates in human postmortem cerebral cortex and striatum from a presymptomatic case. Light micrographs are from the insular cortex (*D*) and dorsal striatum (*E*). Large numbers of EM48-immunoreactive aggregates of a wide variety of shapes and sizes are visible in cortex. All of these aggregates are in the neuropil. In contrast, striatal aggregates are exceedingly uncommon. Scale bar, 70 μm. *D–E*, Adapted with permission from (Gutekunst et al. 1999).

proteasome function (Chai et al. 1999). Pharmacological inhibition (Myung et al. 2001) of the proteasome increased IB formation (Wyttenbach et al. 2000), and protein aggregation inhibited proteasome function (Bence et al. 2001; Díaz-Hernández et al. 2006; Holmberg et al. 2004; Venkatraman et al. 2004). The implied feedback between accumulation of aggregation-prone proteins and proteasome inhibition suggested a mechanism (Ardley et al. 2005; Bennett et al. 2005; Ciechanover and Brundin 2003; Ding and Keller 2001; Ding et al. 2002; Gaczynska et al. 2001; Goellner and Rechsteiner 2003; Jana and Nukina 2003; Petrucelli and Dawson 2004): dysregulation of steady-state levels of vast numbers of cellular proteins whose degradation depended on the proteasome. However, other studies reported that the proteasome degrades polyQ-expanded proteins efficiently (Michalik and Van Broeckhoven 2004; Saric et al. 2004). Furthermore, several studies in mouse models of polyQ-induced neurodegeneration detected no proteasome inhibition (Bowman et al. 2005) or contribution of proteasome inhibition to disease progression (Bett et al. 2006).

The dynamics of proteasome function and IB formation have been measured longitudinally in single neurons expressing mutant HTT (Mitra et al. 2009). These studies revealed a transient increase in an artificial proteasome substrate, implying reduced flux through the proteasome, which preceded IB formation. Remarkably, substrate levels fell shortly after IBs formed, suggesting improved flux through the proteasome. Similar findings in vivo were reported recently (Ortega et al. 2010). A transient change in flux through the proteasome associated with polyQ-expanded proteins would likely be undetected using conventional measurements and might explain the conflicting data. However, these findings do not resolve

whether the change in flux arises from a direct inhibitory effect of polyQ expanded HTT on the proteasome. Alternatively, polyQ expanded HTT could lead to widespread stresses on cellular protein-fielding machinery and a pleiotropic increase in the load of misfolded proteins to the proteasome, which would compete with the artificial proteasome substrate, increasing its steady-state levels.

Directly measuring the effect of polyQ expansion on cellular clearance of proteins that contain them is difficult. For example, conventional approaches for measuring protein half-life are confounded by aggregation and toxicity from long polyQ expansions. Nevertheless, the available evidence suggests that HTT is normally a long-lived protein (Persichetti et al. 1996). In spinocerebellar ataxia 7, a disease-associated polyQ expansion was associated with overexpression of ataxin-7 (Yoo et al. 2003). The authors suggested that polyQ expansions might stabilize proteins, such as ataxin-7, that contain them, leading to their overproduction. However, steady state levels of HTT^{ex1} in neurons appear inversely correlated to the polyQ length (Arrasate et al. 2004; Miller et al. 2010a). Because different HTT^{ex1} versions were expressed from the identical heterologous promoter, polyQ-expanded HTT^{ex1} might be cleared faster than wild-type versions. Thus, neurons might selectively target proteins with disease-associated polyQ expansions for degradation.

Huntington's Disease, Protein Folding, and the Role of Molecular Chaperones

Scherzinger and colleagues reported polyQ expansions conferred to proteins the propensity to aggregate (Scherzinger et al. 1997) (Fig. 3A,B). Initially, aggregation was reported as an emergent property of polyQ expansions associated with disease. In fact, proteins with subthreshold polyQ stretches also aggregate (Hollenbach et al. 1999). However, the rate of aggregation dramatically increases as the polyQ expansion grows (Chen et al. 2002a; Georgalis et al. 1998). Moreover, aggregates of HTT with disease-associated polyQ expansions adopt relatively insoluble amyloid-like structures in vitro (Huang et al. 1998; Scherzinger et al. 1997; Scherzinger et al. 1999; Wanker et al. 1999). This relative insolubility helps to distinguish types of inclusions in situ (Arrasate et al. 2004; Kazantsev et al. 1999).

Whether HTT forms amyloid in vivo is more controversial. Fibrillar structures have been detected in situ ultrastructurally, and insoluble HTT-containing complexes can be biochemically extracted from brain (Scherzinger et al. 1997; Wanker et al. 1999). However, HD brains show little staining with Congo Red or Thioflavin T, which detect amyloid (DiFiglia et al. 1997). Also, ultrastructural analysis suggests IBs may be substantially amorphous granular material rather than mostly fibrils (DiFiglia et al. 1997; Miller et al. 2010a). Thus, the visible protein accumulations in situ may be mostly less-ordered nonamyloid protein aggregates.

The nomenclature used to describe protein aggregation in cells has sometimes led to confusion, especially in comparison to aggregation of purified proteins. Biochemical techniques can determine with some certainty whether assemblies of aggregated proteins are dimers, trimers, oligomers, or higher ordered species, the ability to make these determinations in cells is much more limited. With light microscopy, it is usually only possible to visualize large deposits, which we prefer to call IBs. Generally, it is not possible to distinguish monomers from dimers, trimers, or oligomers in most cases, so we prefer to avoid using the term "aggregates" altogether. For lack of a better word, we have chosen to describe intracellular forms of aggregation-prone proteins that are not bound up in an IB as "diffuse" to reflect the limitations of light microscopy. Similarly, we think it is prudent to avoid terms such as "soluble" to refer to diffuse pools of aggregation prone protein or "insoluble" to refer to visible protein deposits unless the necessary additional biochemical tests have been performed to make that determination. The mere collection of proteins into a visible protein deposit does not prove the proteins in the IBs are insoluble. Similarly, the "diffuse" pool of proteins might contain a substantial amount of SDS-resistant multimeric species.

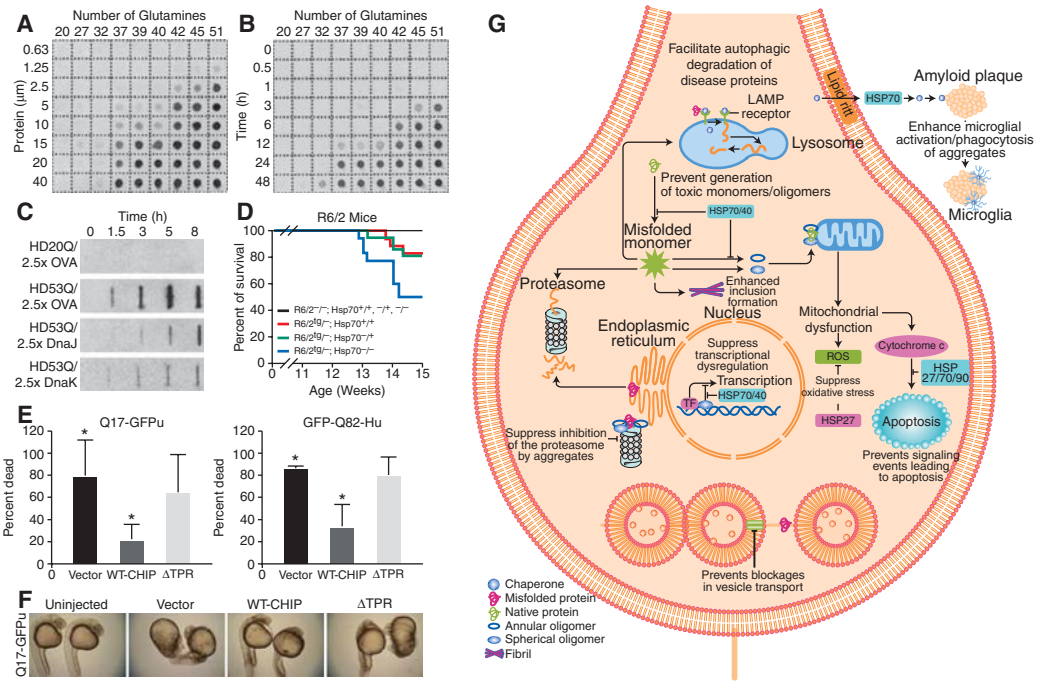

Figure 3. Aggregation of polyQ expansions is regulated by a network of chaperones and cochaperones. (*A*) Concentration dependence of polyQ-containing amino-terminal exon 1 peptide fragment of huntingtin (HDex1p) aggregation. Glutathione *S*-transferase (GST)-HDex1 proteins with polyQ tracts of different lengths were incubated at the indicated concentrations with trypsin for 24 h at 37°C. Aliquots (200 ng) of each protein were then diluted into 0.2 ml of 2% SDS/50 mM dithiothreitol, boiled for 3 min, and filtered through a cellulose acetate membrane. Captured aggregates were detected by incubation with antiAG51 serum (1:1000), followed by incubation with alkaline phosphatase-conjugated antirabbit secondary antibody and the fluorescent substrate Atto-Phos. (*B*) Time course of HDex1p aggregation. The various GST-HDex1 proteins were incubated at a concentration of 20 μM with trypsin. At the indicated times, aliquots (200 ng) of each protein were removed and analyzed by the filter retardation assay as in A. *A–B*, Adapted with permission from (Scherzinger et al. 1999). (*C*) Modulation of aggregation of amino-terminal exon 1 fragment of huntingtin (HDexon 1) by Hsp70 and Hsp40 in vitro. Time-dependent formation of SDS-insoluble aggregates of HD20Q and HD53Q (3 μM) in the presence and absence of ovalbumin (OVA) and DnaJ (Hsp40) or DnaK (Hsp70) of *Escherichia coli* (7.5 μM each) in the absence of ATP as detected in filter-trap assays. Chaperones were added when aggregation was initiated by proteolytic cleavage of GST-HD fusion proteins. OVA served as a nonchaperone control protein. Adapted with permission from Muchowski et al. (2000). (*D*) Deletion of *Hsp70.1* and *Hsp70.3* decreases survival in the R6/2 mouse model of HD. Kaplan–Meier survival curve for the indicated genotypes [$R6/2^{-/-}$; $Hsp70^{+/+}$ ($n = 21$), $R6/2^{tg/-}$; $Hsp70^{+/+}$ ($n = 18$), $R6/2^{-/-}$; $Hsp70^{-/+}$ ($n = 27$), $R6/2^{tg/-}$; $Hsp70^{-/+}$ ($n = 22$), $R6/2^{-/-}$; $Hsp70^{-/-}$ ($n = 18$), and $R6/2^{tg/-}$; $Hsp70^{-/-}$ ($n = 18$)] shows that the absence of *Hsp70.1/3* significantly decreased survival of R6/2 mice (log rank: $p = 0.033$). Nonontransgenic, Hsp70 heterozygous knock-out, or Hsp70 homozygous knock-out mice died during the 14 week time course. Adapted with permission from (Wacker et al. 2009). (*E*) Carboxy-terminal heat shock protein 70-interacting protein (CHIP) suppresses polyQ toxicity in developing zebrafish. Quantitation of embryo death 24 h after fertilization, after injection at the one-cell stage of Q71-GFPu (left) or GFP-Q82-Htt (right) together with the indicated CHIP plasmids. Death is decreased by coexpression of WT-CHIP but not of mutant CHIP-ΔTPR (deletion of tetratrico peptide repeat). The bars depict the mean and SD of four independent injections for Q71-GFPu (>120 total animals analyzed) and three independent injections for GFP-Q82-Htt (>80 total animals analyzed). $^{*}p<0.02$, significant difference between groups. (*F*) Representative bright-field images of 24-h-old survivors from (*E*) coinjected with Q71-GFPu and the indicated empty vector, WT-CHIP, or mutant CHIP. WT-CHIP partially restores normal embryo length, optical clarity, and development of head, tail, and somite structures. (*See facing page for legend.*)

Identification of *HTT* facilitated the creation of cell and animal disease models. HD models in yeast (Krobitsch and Lindquist, 2000; Muchowski et al. 2000; Willingham et al. 2003), *Caenorhabditis elegans* (Faber et al. 1999; Kim et al. 2002; Satyal et al. 2000), and *Drosophila* (Jackson et al. 1998) are amenable to unbiased screens for genes that modify HTT toxicity. Because the polyQ expansion is believed to confer a toxic function to HTT and the toxic function might be unrelated to normal function, unbiased screens might reveal critical biological networks that have eluded conventional hypothesis-driven research. In the first screen for genetic enhancers of polyQ toxicity in *Drosophila*, two molecular chaperones, dHDJ1, a homolog of heat shock protein 40 (HSP40), and dTPR2, a homolog of tetratricopeptide repeat protein 2, were potent suppressors of HTT toxicity (Kazemi-Esfarjani and Benzer 2000). In the first screen for genetic enhancers of the toxicity of an amino-terminal fragment of mutant HTT in yeast, host proteins in the protein folding, stress response, and ubiquitin-dependent protein catabolism pathways were significantly overrepresented among the list of modifiers of mutant HTT toxicity (Willingham et al. 2003). For example, loss of cytoplasmic or ER versions of the molecular chaperone HSP40 significantly enhanced HTT toxicity. Similarly, a screen was performed for genes whose suppression led to the premature appearance of inclusion bodies by a polyQ containing polypetide in *Caenorhabditis elegans* (Nollen et al. 2004). Five classes of genes were discovered, including those involved in RNA metabolism, protein synthesis, protein folding, protein degradation and protein trafficking.

Before molecular chaperones were implicated in HTT toxicity, they already had been suspected in pathogenesis. Several studies reported the effects of manipulating chaperone function on polyQ aggregation, inclusion formation, and toxicity in vitro and in vivo. Hsp40 and/or Hsp70 suppress polyQ-dependent aggregation and neurodegeneration in a variety of systems (Carmichael et al. 2000; Jana et al. 2000; Kim et al. 2003; Kobayashi et al. 2000; Krobitsch and Lindquist 2000; Muchowski et al. 2000; Wacker et al. 2004; Warrick et al. 1999) (Fig. 3C). A yeast chaperone, Hsp104, without a known mammalian ortholog, disaggregates polyQ and suppresses cell death (Glover and Lindquist 1998; Krobitsch and Lindquist 2000; Parsell et al. 1994; Satyal et al. 2000; Wyttenbach et al. 2000). Other chaperones also disrupt polyQ aggregation and toxicity (Kitamura et al. 2006; Tam et al. 2006). Overexpressing or reducing Hsp70 in mice, for instance, has been associated with suppressing or enhancing polyQ toxicity, respectively (Cummings et al. 2001; Wacker et al. 2009) (Fig. 3D). Additionally, overexpressing Hsp104 in a mouse HD model was associated with reduced protein deposition and better survival (Vacher et al. 2005). The effects of chaperones on neurodegeneration are partly mediated by direct interactions that govern protein misfolding, but probably also involve additional more complex and indirect effects (Muchowski and Wacker 2005) (Fig. 3E).

The Significance of Inclusion Bodies to Neurodegeneration

The correlation of onset and severity of behavioral deficits and IB burden in mouse disease models implicated IBs themselves as the pathogenic species for neurodegeneration (Davies et al. 1997) (Fig. 4). IBs might sequester

Figure 3. (*Continued*) (*E–F*) Adapted with permission from (Miller et al. 2005). (*G*) Direct and indirect effects of molecular chaperones on disease protein toxicity. Molecular chaperones might prevent toxicity by blocking inappropriate protein interactions, by facilitating disease protein degradation or sequestration, and by blocking downstream signaling events that lead to neuronal dysfunction and apoptosis. ER, endoplasmic reticulum; ERAD, endoplasmic reticulum-associated degradation; HSP, heat shock protein; LAMP, lysosomal-associated membrane protein; ROS, reactive oxygen species; TF, transcription factor. Adapted with permission from (Muchowski and Wacker, 2005).

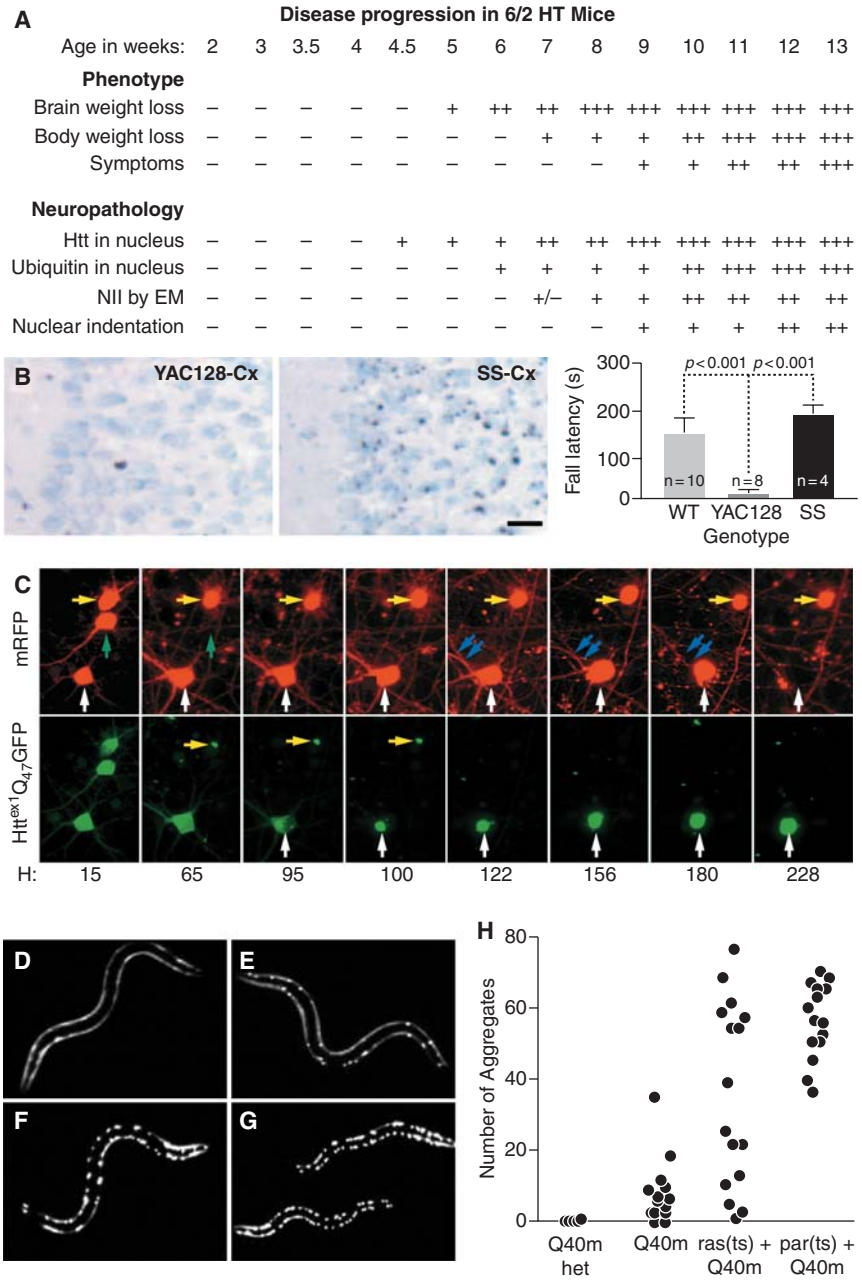

Figure 4. HD pathogenesis unfolds as a complex temporal dynamic, instigated by polyQ-expanded huntingtin but shaped by a network of cellular adaptive responses. (*A*) In the R6/2 mouse model of HD, IB formation precedes and correlates to behavioral deficits. Adapted with permission from (Davies et al. 1997). However, the Shortstop mouse model of HD (*B*) has more IBs (left, micrographs) but fewer behavioral deficits (right, graph) than the YAC128 mouse model of HD. Adapted with permission from (Slow et al. 2005). (*C*) The paradox could be explained if IB formation behaves as a coping response to more diffuse forms of mutant huntingtin. Here striatal neurons were transfected with mutant huntingtin tagged with GFP and followed longitudinally. Levels of diffuse mutant huntingtin decrease to nearly baseline levels after IB formation and, at the same, begin to show a lower risk of death (i.e., better survival). (*See facing page for legend.*)

critical proteins to cause neurodegeneration (Chai et al. 2002; Donaldson et al. 2003; McCampbell et al. 2000; Preisinger et al. 1999). However, several findings did not fit a simple causal connection. In a primary neuron model, IB formation could be reduced by expressing a ubiquitin ligase, but cell death was exacerbated (Saudou et al. 1998). Also, several trophic factors suppressed neurodegeneration induced by mutant HTT while simultaneously increasing IB formation. Orr, Zoghbi and colleagues reported analogous findings from a mouse model of a related polyQ disorder, spinocerebellar ataxia 1 (SCA1) (Klement et al. 1998). They showed that deletion of a self-association domain in SCA1, which reduced IB formation, worsened pathology, and behavioral deficits. Subsequent neuropathology studies implied that IB formation could be dissociated from neurodegeneration (Gutekunst et al. 1999; Kuemmerle et al. 1999; Slow et al. 2005).

These reports (Saudou et al. 1998)' (Klement et al. 1998) spurred a controversy about IB formation in neurodegeneration (Davies et al. 1998; Floyd and Hamilton 1999; Ross 1997; Rubinsztein et al. 1999; Sisodia 1998). Defining the role of IBs in HD exemplifies a general problem in understanding the significance of changes to neurodegenerative disease (Finkbeiner et al. 2006). How can a disease-relevant phenotype, such as death or dysfunction, be related to an earlier change, such as the formation of an IB? The conventional approach of collecting snapshots of disease progression and making gross correlations is fundamentally flawed. It can mislead investigators to interpret their data exactly opposite to the underlying pathobiology. No wonder the study of neurodegenerative disease is fraught with controversies; the potential for misinterpretation is great.

Inclusion Body Formation as a Regulated Mechanism to Cope with Misfolded Proteins

An automated microscope system provides an innovative solution to this dilemma. It follows individual neurons as they express mutant HTT and show neurodegeneration and analyzes images in an automated user-independent fashion (Arrasate and Finkbeiner 2005; Arrasate et al. 2004). The images record individual neurons until their fate is known, and powerful statistical tools used for clinical studies can be applied to these data to identify factors that predict a particular fate. Importantly, the tool addresses thorny questions in neurodegenerative disease (Orr 2004). Do particular changes, such as IB formation, have prognostic value? Which are the most important? Do they predict a better outcome or a worse one? Which changes are causal and which are effects? What "system" of factors determines neurodegeneration?

This new system allowed us to revisit the role of IBs in HD. Surprisingly, striatal neurons that formed IBs had lower risks of death; they survived better than neurons without IBs (Arrasate et al. 2004). This finding has been replicated by others (Miller et al. 2010a; Mitra et al. 2009; Takahashi et al. 2008; Taylor et al. 2003). The factor that predicted neuronal death was the level of "diffuse" mutant HTT, all forms of mutant HTT other than those bound in an IB. By 24–48 h after IB formation, levels of diffuse mutant HTT fell to nearly baseline levels along with the risk of death (Arrasate et al. 2004; Miller et al. 2010a). A simple model integrated IB formation as a coping response to more toxic

Figure 4. (*Continued*) Adapted with permission from Arrasate et al. (2004). (*D–H*) Progressive disruption of cellular folding capacity by misfolded proteins. Fluorescent images of representative L2 larvae at the permissive temperature of heterozygous (*D*) or homozygous (*E*) Q40 m, ras (ts)+Q40 m (*F*), and paramyosin (ts)+Q40 m (*G*) strains. (*H*) Number of visible aggregates in L2 larvae expressing indicated proteins. ras (ts)+Q40 m in (*F*) and (*G*) denotes the fluorescent progeny of an F2 ras (ts) animal expressing Q40 m; these progeny could be either homozygous or heterozygous for Q40 m. Adapted with permission from Gidalevitz et al. (2006).

forms of diffuse mutant HTT (Fig. 5). IBs were proposed to mitigate the effects of diffuse mutant HTT by sequestering it, thereby reducing its chemical activity and/or possibly refolding it into a more inert form (Arrasate et al. 2004; Miller et al. 2010a).

Why then does IB formation grossly correlate positively with toxicity in some cases and negatively in others? The lesson from single-cell longitudinal work is that inferences made about the significance of pathological changes from gross correlations are unreliable and can be misleading (Table 1). A manipulation that increases IB formation because it increases the load of misfolded protein is going to be associated with greater toxicity because, like any homeostatic response, it typically will be only partially effective at mitigating the perturbation to the equilibrium. In other words, a homeostatic response makes things less bad than they would

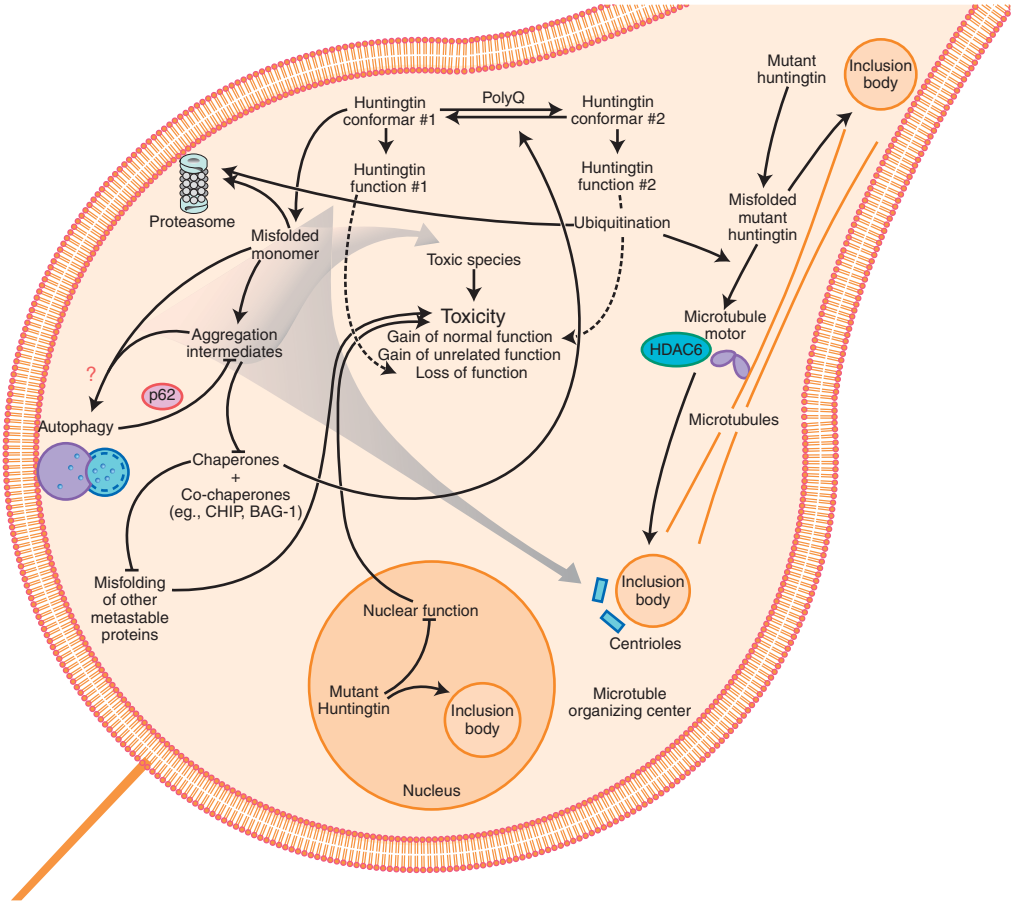

Figure 5. Mechanisms of neurodegeneration induced by protein malfolding in HD. A diagram depicting some of the pathways by which misfolded mutant huntingtin is collected into IBs or cleared. Toxicity could result from direct aberrant interactions between mutant huntingtin and myriad specific effector proteins. Mutant huntingtin could also cause neurodegeneration indirectly by stressing the protein homeostasis system sufficiently that other metastable proteins fail to fold properly and widespread dysfunction of the proteome ensues. Mutant huntingtin induces neurodegeneration predominantly through gain-of-function (GOF) mechanisms, although huntingtin loss-of-function (LOF) may have a role. GOF mechanisms may involve functions unrelated to huntingtin's normal function, but simultaneous GOF and LOF might also result if polyQ expansion increased a normal function of huntingtin to supraphysiological levels at the expense of another normal function of huntingtin.

Table 1. As a coping response, inclusion body formation should correlate better with levels of misfolded protein than neurodegeneration.

Manipulation	Possible examples	Levels of diffuse misfolded protein	Inclusion body formation	Neurodegeneration
↑ Load of misfolded protein	1. Chaperone deficiency leading to increased production (Wacker et al. 2009) 2. Inhibition of proteasome (Chai et al. 1999; Mitra et al. 2009; Wyttenbach et al. 2000) or autophagic clearance (Pandey et al. 2007) 3. Oxidative stress (Charvin et al. 2005)	↑	↑/-	↑
↓ Load of misfolded protein	1. Suppression of mutant Htt expression (Boudreau et al. 2009; DiFiglia et al. 2007; Harper et al. 2005; Yamamoto et al. 2000) 2. Induction of autophagic clearance (Rideout et al. 2004; Sarkar et al. 2007a; Sarkar et al. 2007b; Yamamoto et al. 2006)	↓	↓	↓
↑ Extent of inclusion body formation	1. Small-molecule inducers (Bodner et al. 2006) 2. Htt mutations (Slow et al. 2005) 3. NMDAR-dependent synaptic activity (Okamoto et al. 2009)	↓	↑	↓
↓ Extent of inclusion body formation	1. Intereference with Ubiquitin ligase (Saudou et al. 1998) 2. Mutation of dimerization domain (Klement et al. 1998) 3. Rhes (Subramaniam et al. 2009)	↑	↓	↑
↑ Ability to withstand toxic misfolded protein/↑ cellular survival and longevity	1. Growth factors (Saudou et al. 1998; Yamamoto et al. 2006) 2. Activation of prosurvival pathways (Humbert et al. 2002)	↑/-	↑/-	↓

If a manipulation reduces the susceptibility of a cell to the toxicity of misfolded protein, the cell may live longer and may withstand accumulation of higher levels of toxic protein leading to more IBs formation. In such cases, gross neurodegeneration may be reduced although levels of diffuse mutant protein are higher.

be without the response but never as good as they would be if the instigating problem were eliminated altogether. On the other hand, a manipulation might somehow accelerate IB formation without increasing the load of misfolded protein. It might act on the process of IB formation itself and downstream of the initial misfolding, leading to even more reduced levels of diffuse misfolded protein than in the absence of the manipulation and, therefore, a reduction in toxicity. In the first case, toxicity and IB formation go in the same direction, and in the second, they go in the opposite direction. Yet, both would be consistent with the simple model that incorporating misfolded proteins into IBs reduces their toxicity (Table 1). The roles of IB formation in pathogenesis may someday be shown to be more complex, but no published findings indicate that it has to be.

Several groups showed IB formation is regulated, at least for IBs in the perinuclear area. In nonneuronal cells, these inclusions or aggresomes are associated with the microtubule-organizing center (MTOC) (Johnston et al. 1998; Lelouard et al. 2004; Shimohata et al. 2002). Indeed, IB formation itself appears to be microtubule dependent, suggesting misfolded proteins are gathered to the MTOC by microtubule-based transport (Muchowski et al. 2002). HDAC6 might function in this process, possibly in some adapter capacity (Kawaguchi et al. 2003) or as part of a complex process involving p62 and ubiquitin proteasome and autophagic mechanisms of protein clearance (Babu et al. 2005; Iwata et al. 2005; Nagaoka et al. 2004; Pandey et al. 2007; Webb et al. 2004; Zatloukal et al. 2002). Activating Ca^{2+} influx through the NMDA receptor promotes IB formation, suggesting that the process in neurons is regulated by synaptic activity (Okamoto et al. 2009). Intriguingly, pharmacological manipulations of the NMDA receptor that promoted IB formation reduced disease phenotypes. Conversely, Rhes, a protein implicated in HTT sumoylation, mediates mutant HTT-induced cytotoxicity, apparently by interfering with IB formation (Subramaniam et al. 2009). The complex process and regulation of IB formation may also explain why many proteins aggregate readily in a test tube, but form IBs in cells with significantly different propensities.

Neurons also clear IBs in vitro. In a few neurons, IBs cleared spontaneously despite the continued production of mutant HTT (Arrasate et al. 2004). Because proteins must be unfolded to be degraded in proteasomes, other protein clearance pathways might clear IBs. Macroautophagy can, in principle, clear aggregation-prone proteins (Ravikumar and Rubinsztein 2004; Shacka et al. 2008). In this process, double-membrane organelles called autophagosomes engulf cellular cargo and fuse with lysosomes to form autophagolysosomes, which then degrade the organelle's contents (Kim and Klionsky 2000; Meijer and Codogno 2004). HTT expression is associated with up-regulated autophagy (Kegel et al. 2000; Petersén et al. 2001; Rogers and Leavitt 2004), and autophagy degrades polyQ-expanded proteins (Qin et al. 2003; Ravikumar et al. 2002; Yamamoto et al. 2006). Induction of autophagy is associated with reductions in IBs, but it is controversial whether IBs are engulfed in toto and targeted to autophagosomes. Autophagy might reduce IBs by clearing IBs precursors and eventually depleting them of mutant HTT (Fortun et al. 2003; Rideout et al. 2004; Sarkar et al. 2007b; Wong et al. 2008a).

AN INTEGRATED VIEW OF PROTEIN MISFOLDING AND NEURODEGENERATION IN HUNTINGTON'S DISEASE

A Network for Protein Homeostasis

Reductionist approaches to mechanisms of neurodegeneration in HD have been helpful for uncovering important disease-related molecular and cellular changes. However, the initial insights have often been revised after additional factors were analyzed. Including temporal dynamics and simultaneous measurements of HTT levels, IB formation, and fate, for example, revealed that proteasome inhibition occurred transiently and was time-linked to IB formation, and that IB formation led to reduced levels of diffuse forms of mutant HTT and improved survival (Arrasate et al. 2004; Mitra et al.

2009). Now excellent evidence links the molecular chaperone system, ubiquitin proteasome pathway, and autophagic protein clearance pathway. These are mediated by specific molecules (e.g., HDAC6, BAG1, and CHIP) in some cases, and the proteasome and autophagic pathways communicate (Al-Ramahi et al. 2006; Jana and Nukina 2003; Lüders et al. 2000; Massey et al. 2006; Miller et al. 2005; Pandey et al. 2007). The new view is that cells maintain healthy proteins through an adaptable network of interconnected pathways that sense misfolded proteins and either refold them or target them for clearance.

This idea was shown convincingly in *C. elegans.* Morimoto and colleagues (Gidalevitz et al. 2006) examined effects of expression of a polyQ expansion fused to a fluorescent protein on the folding of other unrelated proteins that harbored temperature-sensitive (ts) mutations. At low temperatures, these proteins remain folded and functioned normally. At slightly elevated temperatures, they misfolded and aggregated. Remarkably, polyQ expansions with lengths close to or slightly above the threshold for human disease led to misfolding of the ts mutant proteins at normal temperatures, even though the two proteins did not interact directly. The likeliest explanation is that the polyQ proteins competed with the ts mutant proteins for access to the chaperone system. The capacity of the chaperone system, which was sufficient to prevent aggregation of either misfolded protein alone, was exceeded with both. If these findings apply to humans, the implications are profound. The human chaperone system may have evolved to match the average load of metastable proteins in humans. If so, any polymorphisms affecting protein stability could be genetic modifiers of protein misfolding diseases, whether or not those polymorphisms affect function. Moreover, small sequence differences in proteins among species, which are often ignored in disease models, might be important if they affect protein stability.

From a network perspective, a cogent model might combine the dynamics of IB formation, proteasome inhibition, and IB clearance. A protein prone to misfold, such as mutant HTT, presumably would be sensed initially by the chaperone system. If the load exceeds the capacity of the cell's chaperone system, it may deprive other endogenous metastable proteins of the chaperones they need for proper folding and lead to a substantial increase in the amount of catastrophically misfolded proteins requiring clearance. The increased load would lead to a "traffic jam" of proteins requiring proteasome-dependent degradation and might be detected experimentally as an accumulation of an artificial proteasome substrate. The stress on the chaperone system and protein clearance machinery could signal the cell that emergency measures are needed to avoid apoptosis, including the induction of IB formation and the longer-term up-regulation of protein clearance pathways, including the ubiquitin proteasome pathway and the autophagic clearance pathway. To the extent that IB formation results in homotypic aggregation of a protein and quantitative reduction in its levels through storage in inert structures, it would reduce the nonnative surfaces requiring chaperone protection. The resulting stress relief on the chaperone system would reduce the load of misfolded proteins requiring proteasome degradation. Experimentally, this might look like improved flux of an artificial proteasome substrate. If IB formation was part of a broader cellular coping response, which involved an up-regulation of autophagy and proteasome function, levels of mutant HTT might remain low after IB formation and some IBs might spontaneously be cleared.

How Does Protein Misfolding Lead to Neurodegeneration?

Several lines of evidence indicate that HD is fundamentally a proteopathy and that measures to mitigate protein misfolding or to promote the clearance of misfolded proteins reduce behavioral deficits and neuropathological abnormalities. Still, the fundamental question remains. How do misfolded proteins lead to neurodegeneration? The fact that the polyQ expansion confers to HTT (1) the propensity to aggregate and form IBs and (2) one or more toxic functions led early on to the idea that those two

properties were directly linked: aggregation somehow mediated toxicity in HD (Thakur et al. 2004).

The early focus on the role of IB formation and amyloid led to the concept of a toxic protein species, a particular form of aggregated HTT that was envisioned to be responsible for a disproportionate amount of neuronal toxicity. Initially, that species was proposed to be IBs (Becher et al. 1998; Davies et al. 1997; DiFiglia et al. 1997; Yang et al. 2002). Toxicity was proposed to arise secondarily from sequestration of other critical proteins (Donaldson et al. 2003; McCampbell et al. 2000; Preisinger et al. 1999). The amyloid hypothesis of neurodegeneration has occupied a special place in the field for decades (Tanzi and Bertram, 2005; Wirths et al. 2004), although it has decreased on hard times now. Sequestration of key proteins by IBs is insufficient to explain the observed dysfunction (Bennett et al. 2005; Yu et al. 2002). Moreover, functional amyloids exist in mammals and appear to serve useful functions, indicating that amyloid formation per se is not toxic (Fowler et al. 2006).

More recently, attention shifted to submicroscopic molecular assemblies called oligomers (Behrends et al. 2006; Glabe and Kayed, 2005; Kayed et al. 2003; Nagai et al. 2003; Nandi, 1996; Onodera et al. 1997; Sánchez et al. 2003; Sathasivam et al. 2010; Takahashi et al. 2008; Wacker et al. 2004; Wong et al. 2008b) or malfolded monomers (Nagai et al. 2007; Wang et al. 2006; C Peters-Libeu, E Rutenber, J Miller, et al. unpubl.). How oligomers or malfolded monomers are toxic and trigger neurodegeneration remains a matter of speculation. Tools to resolve these questions are limited. No tools detect specific protein species in situ and link functions to particular proteins, especially in the context of and as distinct from other submicroscopic species. HTT appears to adopt many conformations, even as a monomeric protein, and an equilibrium likely exists among the conformers and assemblies. Proteins cannot be produced with a particular homogeneous conformation and remain in that pure conformation after they are delivered to cells. Despite these limitations, oligomers might cause toxicity by creating pores in membranes (Kayed et al. 2004) or by binding to specific cellular proteins, such as receptors, and altering their function (Trommer et al. 2005; Walsh et al. 2002). Monomers may also bind to transcription factors or other targets and degrade their function (Schaffer et al. 2004).

Because the effects of protein misfolding might be mediated through a proteostasis network, the concept of a toxic species may require revision. Chaperones recognize nonnative folds in proteins through unfolded-polypeptide-binding domains, which prefer to bind short sequences (~5–7 amino acids) enriched in hydrophobic residues (Gething 1996). From the perspective of the cell's molecular chaperone system, it may be more productive to think of "toxic folds" (or "unfolds") rather than "toxic species" (Ignatova and Gierasch 2006; Poirier et al. 2005). By the same logic, the most stressful forms of aggregation-prone proteins would be predicted to be those that deliver the largest dose of binding sites for chaperones per protein and are the most difficult for the cell to clear. The key unanswered question, therefore, is whether certain monomeric, oligomeric, or aggregated forms of HTT are particularly effective at binding chaperones and stressing the proteostasis system. Because protein aggregation often buries hydrophobic surfaces of polypeptides so they are unavailable to chaperones, it is possible, in principle, to understand how malfolded monomers might confer the greatest toxicity per protein and how aggregation might mitigate toxicity under some circumstances. Stress on the proteostasis system from mutant HTT could reduce cellular folding capacity sufficiently that myriad other metastable proteins misfold, producing a complex loss-of-function phenotype and eventual neurodegeneration.

Finally, enhancing a normal function might explain how polyQ expansions confer toxicity (Duvick et al. 2010; Kratter and Finkbeiner 2010; Nedelsky et al. 2010). Proteins containing polyQ expansions might adopt two or more conformations, and each might be linked to a specific function. Different conformers might exist in equilibrium, but disease-associated polyQ expansions shift this equilibrium so

that one conformation and function (e.g., gene transcription, protein trafficking, etc.) is effectively overexpressed. In this model, neurodegeneration effectively is the result of "too much of a good thing." It has been challenging to test this hypothesis for HD—HTT functions are poorly defined. Nevertheless, structural studies suggest that the polyQ expansion is flexible (Klein et al. 2007; Masino et al. 2002) and that it can adopt multiple conformations (Kim et al. 2009). For SCA1, SBMA, and SCA7, a disease-associated polyQ expansion might affect normal functions that contribute to disease (Bowman et al. 2007; Duvick et al. 2010; Kratter and Finkbeiner 2010; Lam et al. 2006; Lim et al. 2008; Nedelsky et al. 2010; Yoo et al. 2003).

CONCLUDING REMARKS

After 140 years, many questions remain about HD. The genetic mutation that causes all HD leads to an abnormal expansion of a polyQ tract in the HTT protein. In turn, the polyQ expansion confers to HTT one or more toxic functions and the propensity to aggregate and accumulate intracellularly. Cells contain complex interconnected molecular networks to prevent proteins from misfolding and to correct or degrade those that become misfolded. Those networks might sense mutant HTT as a threat and initiate beneficial coping responses, but ultimately these responses only delay HD. How a polyQ expansion in mutant HTT induces neurodegeneration is a mystery, but monomeric or aggregated protein species might form structures that confer particular toxic functions, and sufficient nonnative folds intracellularly might overwhelm the molecular chaperone system and cause a collapse of the cellular proteostasis system or promote too much of one of the normal functions of HTT. Regardless, efforts to pharmacologically reduce HTT misfolding or mutant HTT levels by inducing proteasome function or autophagy could have therapeutic potential.

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