International Journal of Biomedical and Health Sciences
Vol. 8, No. 3, September 30, 2012
Printed in Nigeria

0794-4748/2012 $5.00 + 0.00

http://www.asopah.org

IJBHS 2012121/8303

Huntington's Disease Pathogenesis: Mechanisms and Pathways

Albert R. La Spada, Patrick Weydt, and Victor V. Pineda

Introduction

The discovery in 1993 of the gene responsible for Huntington's disease (HD) represented a crucial turning point in the HD research field. At the time of the discovery, no one could predict that HD would belong to a large class of inherited neurological diseases all caused by the same type of genetic mutation (i.e., polyglutamine [polyQ] expansion) or that the mechanistic basis of HD (i.e., protein misfolding) would emerge as a common theme linking together all the major neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), and the prion diseases. The study of how the mutant HD gene product, an unusually large 3,144 amino acid protein (huntingtin [htt]) with few recognizable motifs or obvious functional domains that results in the degeneration and death of neurons in the striatum and cortex, has been an enormous undertaking. Indeed, a PubMed search using the term "huntingtin" yields 1,124 hits at the time of writing this chapter. Suffice it to say that dozens of theories of pathogenesis have been proposed and studied. The goal of this chapter will be to present some of the most enduring lines of investigation, with an emphasis on the latest developments, and to highlight emerging notions likely to drive basic research on HD in the future.

HD displays the genetic feature of anticipation, defined as earlier disease onset and more rapid disease progression in successive generations of a pedigree segregating the disease gene. This feature was an important clue for discovery of the causal mutation, as a trinucleotide repeat expansion encoding an elongated glutamine tract in the htt protein was determined to be responsible for HD in 1993, and a relationship between the length of the expanded glutamine tract and the severity of the HD phenotype was uncovered at that time [1]. HD is one of nine inherited neurodegenerative disorders caused by CAG trinucleotide repeats that expand to produce disease by encoding elongated polyQ tracts in their respective protein products. Included in this CAG/polyQ repeat disease class are spinal and bulbar muscular atrophy (SBMA), dentatorubral-pallidoluysian atrophy (DRPLA), and six forms of spinocerebellar ataxia (SCA1, SCA2, SCA3, SCA6, SCA7, and SCA17) [2]. Based on work done on all these disorders, investigators have learned that once glutamine tracts exceed the mid-30s, the polyQ tract adopts a novel conformation that is pathogenic. An antipolyQ antibody (1C2) can specifically detect this structural transformation, as it will only bind to disease-length polyQ tracts from patients with different polyQ diseases [3]. The transition of polyQ-expanded proteins into this misfolded conformer is the crux of the molecular pathology in these disorders. Once in this conformation, however, it is unclear how polyQ tract expansions mediate the patterns of neuronal cell loss seen in each disease, as most of the polyQ disease gene products show overlapping patterns of expression within the central nervous system (CNS) but restricted pathology. In the case of HD, molecular explanations for disease pathogenesis must account for the selective vulnerability of the medium spiny neurons of the striatum and certain neuron subsets in the cortex.

Protein Aggregation and Degradation

A major turning point in the HD and polyQ disease field came in 1997 when independent groups detected visible proteinaceous aggregates (or inclusion bodies) in the nuclei of neurons from patients with SCA3 and from patients and mice with HD 4–6. These neuronal intranuclear inclusions (first abbreviated "NIIs" and then later

"NIs") appear before the onset of disease in mouse models of HD, suggesting a primary role in pathogenesis. Because the NIs displayed immunoreactivity to antibodies directed against the polyQ disease protein and the expanded polyQ tract epitope, the NIs were thought to be "aggregates" of the mutant disease protein. This led to the "aggregation theory" of polyQ disease pathogenesis that posited aggregation of expanded polyQ tracts as the crucial step in the cascade of events that leads to neurodegeneration in these diseases. As the kinetics of polyQ tract self-aggregation increases with the lengthening of the glutamine repeat [7], paralleling the genotype-phenotype relationship documented in HD, the aggregation theory accumulated a number of strong proponents. However, at the same time, other lines of investigation began to suggest otherwise. When the Orr and Zoghbi groups crossed their SCA1 transgenic mice with mice lacking E6-AP ubiquitin ligase function, they observed limited aggregate formation but with an earlier onset of SCA1 neurodegeneration [8]. HD yeast artificial chromosome (YAC) transgenic mice were then noted to develop a motoric phenotype and obvious neurodegeneration in the absence of protein aggregates [9]. Thus, a contentious debate ensued over the role of aggregate formation in polyQ disease pathogenesis—with some workers espousing the view that aggregates were responsible for disease pathology, others suggesting that the aggregation process was a protective coping mechanism of the cell and thereby beneficial, and still others insisting that aggregates were incidental and irrelevant. This debate was complicated by the fact that the absence of aggregates at the light microscope level could occur in the presence of so-called "microaggregates" at the electron microscope level [10].

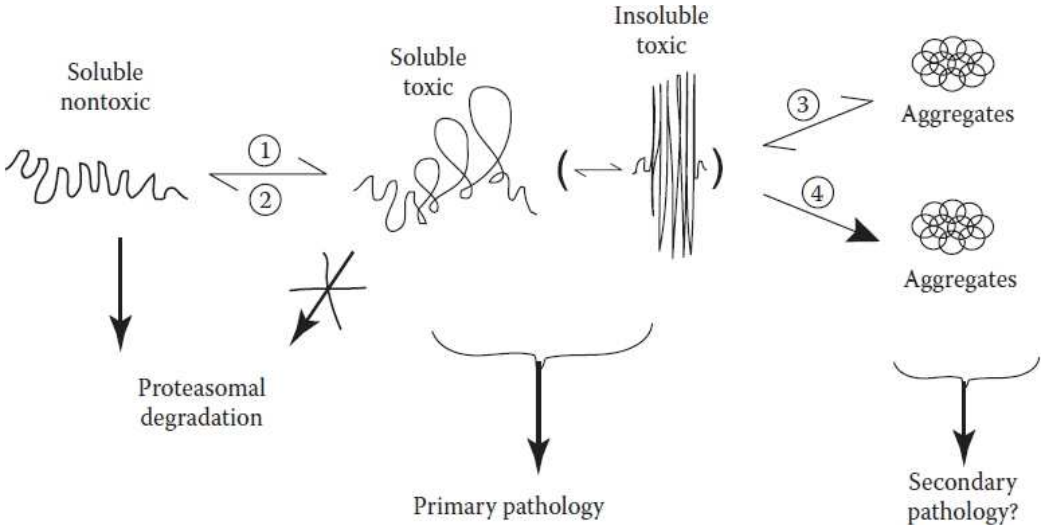

Figure 1: Model for polyglutamine neurotoxicity. Soluble (nontoxic) polyglutamine-expanded protein exists in equilibrium with a soluble toxic conformer/oligomer. Upon transition to the misfolded conformer, the polyglutamine-expanded protein can no longer be readily degraded. Note that certain processes, such as proteolytic cleavage (1), may favor transition to the misfolded conformer, whereas other processes, such as molecular chaperone interaction (2), likely favor refolding into a soluble, nontoxic, degradable conformation. Soluble toxic species become insoluble and ultimately coalesce into visible aggregates. Formation of visible aggregates may be protective (3), because they sequester soluble/insoluble toxic oligomers away; however, it is possible that excessive aggregate formation may cause secondary pathology. Current hypotheses of polyglutamine disease pathogenesis postulate that the microaggregates (i.e., the soluble and insoluble toxic species) are principally responsible for the neurotoxicity. (From Grote, S. K. and La Spada, A. R., *Cytogenet Genome Res* 100, 164, 2003. With permission.)

To deconstruct the nature of the microaggregates, investigators have used a variety of biophysical approaches—including transmission electron microscopy, Fourier transform infrared spectroscopy, and atomic force microscopy—to dissect the process of htt exon 1 peptide aggregation, and have found evidence for a number of sequential morphological and structural intermediates [11, 12]. Many have proposed that misfolding of expanded

polyglutamine tracts into insoluble aggregates involves transition through a number of steps, including the formation of oligomers, then assembly of oligomers into protofibrils, and followed by protofibril assembly into fibrils (Figure 2.1). The importance of such a model is that it accounts for how aggregation can at the same time be toxic and protective because views of aggregation had been dramatically oversimplified. To differentiate the toxicity of oligomeric precursor forms from the ultimate visible aggregates in an unbiased fashion, one group developed an automated microscope system for temporally tracking polyQ-htt-expressing cells over time and found that neuron cell death could not be attributed to visible inclusion formation [13]. Rather, levels of diffuse polyQ-htt expression were a significant negative predictor of neuron survival, and polyQ-htt neurons *lacking* visible inclusions had a higher cumulative risk of cell death, suggesting that visible aggregate formation can be protective. Hence work done on HD and other polyQ diseases suggests that aggregates may not be toxic *per se*, but rather may signify the presence of misfolded proteins whose toxic action is playing out in the soluble phase and/or at the level of oligomers or protofibril structures ("microaggregates").

Initial studies of protein aggregates in HD and the other polyQ diseases documented the presence of molecular chaperones and components of the ubiquitin-proteasome system (UPS) in polyQ inclusions by demonstrating intense immunoreactivity of the aggregates with antibodies directed against such factors [14]. As accurate folding of proteins is essential for the proper functioning of all cells, eukaryotic cells possess a highly efficient multistep protein quality control system that can eliminate misfolded proteins. Molecular chaperones are small scaffolding proteins that can facilitate proper folding of their client proteins or tag them for degradation [15]. Many molecular chaperones are heat shock proteins (Hsp), as their expression is induced by increased temperature [16], an environmental stress that elevates the likelihood of protein misfolding. The UPS is the main intracellular degradation pathway to remove short-lived proteins and to eliminate misfolded proteins [14]. The proteasome component of the UPS consists of a 19S entry ring where peptide unfolding occurs to permit delivery of the degradable substrate to a 20S barrel core with peptidase activity. A three-step conjugation system for ubiquitination of intended substrates is also required for the proper operation of this protein degradation pathway. Numerous studies have shown that inhibition of the UPS with pharmacological agents predisposes neuronal and non-neuronal cells to polyQ toxicity, whereas enhanced molecular chaperone activity (especially the Hsp40–Hsp70 combination) significantly ameliorates polyQ neurotoxicity [14]. However, although molecular chaperone and UPS function are important factors in countering misfolded polyQ protein toxicity, many aggregate-prone proteins, such as polyQ proteins, are inefficiently degraded by the proteasome 17–19. Failure of adequate degradation of aggregate-prone proteins activates alternative protein turnover pathways in the cell, particularly macroautophagy (typically referred to simply as "autophagy"). Autophagy is a degradative process that begins with engulfment of cytosolic materials and/or organelles and progresses through a series of steps involving production of a double membrane-bound structure, culminating in the delivery of the engulfed material to lysosomes [20]. In the CNS, basal levels of autophagy are required for the continued health and normal function of neurons, as conditional inactivation of the autophagy pathway in neural cells in mice yields neuronal dysfunction and neurodegeneration characterized by the accumulation of proteinaceous material 21, 22. A series of studies from the Rubinzstein laboratory has strongly implicated autophagy activation as an important compensatory pathway for countering htt toxicity in cell culture, *Drosophila*, and transgenic mouse models 23, 24. Whether pharmacological induction of autophagy can be achieved in the CNS as a therapeutic intervention for HD and related neurological proteinopathies remains to be determined.

Proteolytic Cleavage

Studies of HD suggest that proteolytic cleavage of the htt protein is a key step in the neurotoxicity pathway. The *htt* gene encodes a protein of 3,144 amino acids with the glutamine tract beginning at codon 18. An analysis of protein aggregates from *in vitro* models, *in vivo* models, and HD patients indicated that glutamineand amino-terminal epitopes are present in nuclear, cytosolic, and axonal aggregates [25]. In a landmark study, the Bates group used a 1.6-kb fragment of the *huntingtin* gene, containing only the first 2% of the huntingtin coding region, to derive lines of transgenic mice (R6/1 and R6/2) that showed a neurological phenotype that resembled HD [26]. This study demonstrated that a tiny amino-terminal portion of the htt protein (including the polyQ tract) was sufficient to produce an HD-like illness in mice. Other studies of the htt protein have shown that htt is a substrate for proteolytic cleavage by caspases and calpains 27–29. The elaboration of an amino-terminal truncation product in HD and in other polyQ diseases has led to the "toxic fragment hypothesis" [30]. According to this hypothesis, cleavage of the polyQ disease protein yields a polyQ-containing peptide that represents the principal toxic species at the molecular level. As the toxic fragment hypothesis is based on proteolytic cleavage of polyQ disease proteins by enzymes such as caspases and calpains, investigators have sought to define specific sites of proteolytic cleavage and the cleavage enzymes. Studies of HD mouse models indicate that not all aminoterminal proteolytic cleavage fragments are toxic,

as one HD YAC mouse model ("shortstop") expresses a polyQ-expanded amino-terminal truncation fragment that yields pronounced aggregates in the CNS but no neuronal toxicity [31]. In 2006, the Hayden group convincingly demonstrated that polyQ-expanded huntingtin protein with a cleavage site mutation (*putatively* for caspase-6) was incapable of causing neurotoxicity in HD YAC transgenic mice [32]. This work strongly supports a role for a specific proteolytic cleavage of htt as a required step in the HD pathogenic cascade. Identification of the enzyme mediating this cleavage will be an important goal toward a potential treatment for HD.

Transcriptional Dysregulation

The necessity of nuclear localization for HD disease pathogenesis highlighted nuclear pathology as a likely early step in the neurotoxicity cascade [33]. As glutamine tracts and glutamine-rich regions often occur in transcription factors and permit functional protein–protein interactions to produce transcription activation complexes in species as diverse as yeast, fruit flies, chicken, and humans, a hypothesis of "transcription interference" or "transcription dysregulation" was formulated. According to this hypothesis, polyQ-expanded disease proteins (or peptides), accumulating in the nucleus inappropriately, interact with transcription factors and regulators to disrupt normal transcriptional functions [34]. Studies of htt have implicated a number of important transcription factors and coactivators. One of the most studied and strongly implicated transcription factors is CREB-binding protein (CBP), a transcriptional coactivator involved in the regulation of multiple genes through its intrinsic histone acetyltransferase activity (that remodels chromatin to allow the transcription machinery to access target genes) 35, 36. A number of studies have shown htt interference of CBP-mediated transcription in a polyQ length-dependent fashion 37–39. Consequently, drugs that block histone deacetylation, and thereby favor the outcome of CBP action (i.e., histone acetylation), are therapeutically beneficial in *Drosophila* and mouse models of HD 40–42. Sp1, a ubiquitously expressed DNA binding factor that recruits the transcription factor IID (TFIID) complex, and TAFII130, a factor that mediates transcription activation complex assembly, have also been identified as targets of mutant htt protein [43]. Interaction of polyQ-expanded htt with CBP, Sp1, and TAFII130 has been shown to occur in the nucleus and to involve the amino-terminal region of htt. Roles for CREB-CBP and Sp1 gene targets in maintenance of normal neuronal function are suggested by other studies [44–47], supporting the conclusion that interference with CBP and Sp1 action could have deleterious effects on neuronal health and survival. Loss of brain-derived neurotrophic factor (BDNF) expression, via aberrant repressor protein localization, may also contribute to the transcription dysregulation in HD [48]. Finally, we and others have documented interference of htt with the peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) transcription factor [49, 50], a regulatory protein with a crucial role in modulating mitochondrial number and function [51, 52].

Mechanisms of Huntingtin-Mediated Transcriptional Interference

Remodeling of chromatin permits the RNA polymerase II complex to bind and initiate transcript synthesis at actively expressed gene loci. This occurs primarily through the covalent addition of acetyl moieties to lysine residues in the tails of core histone proteins—a process that is mediated by enzymes with histone acetyltransferase (HAT) activity [53]. CBP has intrinsic HAT activity, whereas other transcription factors recruit multiprotein coactivator complexes that contain one or more HAT components. PolyQ-expanded htt inhibits the HAT activities of CBP and the p300/CBP-associated factor (P/CAF). This effectively disrupts transcription of CBP targets and any other genes under the control of P/CAF- dependent transcription factors [54]. Transcriptional interference by mutant htt can also occur when key elements of the transcriptional machinery are not properly reconstituted at the site of transcription. Sp1 binds to specific *cis*-elements and directs core components of transcriptional complexes, such as TFIID, TATA box-binding protein (TBP), and other TBP-associated factors (TAFs), to initiate transcription at the Sp1 target gene [43]. Mutant htt disrupts the interaction between Sp1 and components of the TFIID and TFIIF transcriptional complex, thus causing transcriptional dysregulation of Sp1-dependent genes [55]. Finally, a different mechanism is observed when polyQ-expanded htt disrupts BDNF transcription. Normally, cytosolic sequestration of the RE1-silencing transcription factor/neuron-restrictive silencing factor (REST/NRSF) by full-length htt prevents REST/NRSF from binding to the neuron-restrictive silencer element (NRSE) found in the regulatory region of the *BDNF* gene [56]. Therefore, normal htt should sequester REST in the cytosol and allow transcription of *BDNF*; however, mutant htt, as it accumulates in the nucleus, may trap REST there, allowing it to repress NRSE-containing genes such as *BDNF* (Figure 2).

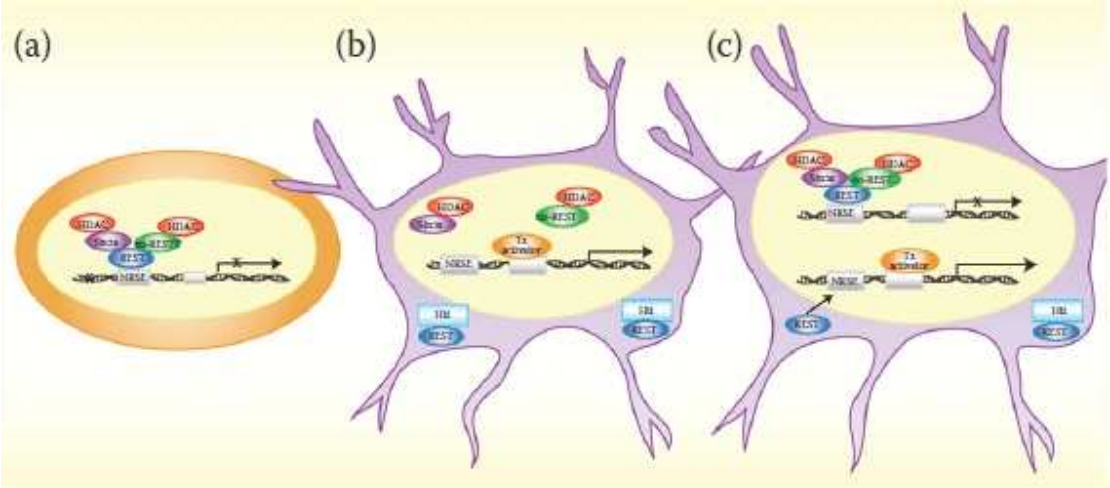

Figure 2: Model for polyglutamine-expanded huntingtin transcription interference of *BDNF* gene expression. Promoters containing a neuron-restrictive silencing element (a) are bound by transcription repressors that prevent expression of downstream genes, such as *BDNF*, in non-neural cell types. In normal neurons (b), normal huntingtin protein is localized to the cytosol and binds the transcription repressor REST there, allowing *BDNF* gene expression to occur. However, in HD neurons (c), polyglutamine-expanded huntingtin protein accumulates in the nucleus and does not sequester REST in the cytosol. REST thus inappropriately enters neuronal nuclei and represses neuronal expression of *BDNF* in HD. (From Thompson, L. M., *Nat Genet* 35, 13, 2003. With permission.)

Evidence for Mitochondrial Abnormalities and Defective Energy Metabolism in HD

Neurons in the brain have enormous demands for continued production of highenergy phosphate-bonded compounds such as ATP. In 1993, Beal et al. [57] reported that long-term administration of a mitochondrial toxin, 3-nitropropionic acid, resulted in a selective loss of medium spiny neurons in the striatum. This provocative finding suggested that mitochondrial dysfunction may underlie HD disease pathogenesis and perhaps account for the cell-type specificity in this neurodegenerative disorder. Follow-up studies performed on HD patient material have documented significant reductions in the enzymatic activities of complexes II, III, and IV of the mitochondrial oxidative phosphorylation pathway in caudate and putamen 58, 59 but have not detected such alterations in HD cerebella or fibroblasts [60]. Additional work has documented striatal-specific decreases in aconitase activity, a likely target of Ca^{++}-dependent, free radical-producing intramitochondrial enzymes [60]. Positron emission tomography (PET) scan analysis of HD patients also strongly supports the hypothesis of defective energy metabolism, as decreased rates of cerebral glucose metabolism are apparent in certain regions of the cortex and throughout the striatum [61]. Magnetic resonance spectroscopy corroborates such findings, revealing elevated lactate levels in striata of HD patients [62].

As mitochondrial energy production and metabolic pathways supply energy for ion exchange pumps, whose function is to maintain an electrochemical gradient across the mitochondrial membrane, defective energy metabolism could translate into an enhanced susceptibility of HD mitochondria to undergo depolarization. A number of studies have evaluated this and have indeed found that mitochondria from HD patients are exquisitely sensitive to depolarizing stresses. In one study, treatment of HD lymphoblasts with complex IV inhibitors resulted in mitochondrial depolarization and apoptotic cell death involving caspase activation [63]. In an independent study, electrical measurements of HD lymphoblast mitochondria yielded lower than normal membrane potentials and depolarization in response to modest Ca^{++} loads [64]. As mitochondrial membrane depolarization results in caspase activation and cleavage of htt protein appears to be mediated in part by caspases (and Ca^{2+}-activated calpains), mitochondrial dysfunction may represent an early step in the HD neurotoxicity cascade.

Linking Transcription Interference with Mitochondrial Bioenergetic Abnormalities

Although more than a decade of study of HD has consistently implicated mitochondrial dysfunction as a central feature of disease pathogenesis, the molecular basis of the mitochondrial abnormality has remained elusive. At the same time, evidence continues to accumulate that nuclear pathology is likely preeminent in the polyQ diseases and that polyQ diseases displaying nuclear accumulation of mutant peptides are in reality "transcriptionopathies" [34]. Recent work on PGC-1α has suggested a connection between htt-mediated transcription dysregulation and mitochondrial abnormalities. PGC-1α is a transcription coactivator that lacks any apparent chromatin-remodeling enzymatic activity such as HAT activity [52]. It was cloned from a brown fat library and subsequently found to be a master regulator of complex transcriptional programs involved in the response to cold temperatures and to high caloric intake through its coactivation of peroxisome proliferator-activated receptor γ (PPARγ)-mediated gene expression. PGC-1α stimulates mitochondrial oxidative phosphorylation respiration and mitochondrial uncoupled respiration in brown fat and skeletal muscle cells [65]. Interestingly, PGC-1α also coordinates mitochondrial biogenesis by up-regulating the expression of the nuclear respiratory factors (NRF)-1 and NRF-2 [66]. After boosting the expression level of NRF-1, PGC-1α directly interacts with NRF-1 to coactivate expression of mitochondrial transcription factor A (mtTFA), whose function is to transcribe and replicate the mitochondrial genome, permitting the production of increased numbers of mitochondria [51]. PPARγ also participates in mitochondrial biogenesis with PGC-1α by driving the expression of mitochondrial fatty acid oxidation enzymes [67]. All these findings indicate that PGC-1α is the key regulatory node in a complex network of transcription programs that culminate in adaptive thermogenesis or mitochondrial biogenesis. PGC-1α is very highly expressed in brain, where its role in mitochondrial biogenesis and uncoupling protein expression may be critical to neuron health and survival.

To determine the role of PGC-1α in metabolism and thermoregulation, the Spiegelman laboratory generated PGC-1α knockout mice [68]. Although these workers anticipated that PGC-1$\alpha^{-/-}$ mice would display a predisposition to obesity, they instead noted that the mice were lean. The explanation for their enigmatic leanness turned out to be their phenotype of pronounced hyperactivity. Further analysis of the PGC-1$\alpha^{-/-}$ mice revealed neurological abnormalities, including myoclonus, dystonia, exaggerated startle responses, and clasping (which is a stereotypical finding in all polyQ and HD mouse models). Neuropathology examination of the PGC-1$\alpha^{-/-}$ mice yielded evidence of degeneration in cortex, thalamus, basal ganglia, and hippocampus, with the most pronounced degeneration in the striatum. The striatal degeneration was spongiform in nature and resulted from a significant drop-out of neurons. Interestingly, real-time reverse transcriptionpolymerase chain reaction (RT-PCR) analysis of hyperactive PGC-1$\alpha^{-/-}$ mice documented significant reductions in the expression of mitochondrial genes. In addition to their phenotype of hyperactivity and striatal neurodegeneration, the PGC-1$\alpha^{-/-}$ mice displayed reduced thermogenic capacity as a result of a failure of induction of uncoupling protein 1 (UCP1) gene expression. An independently generated PGC-1α knockout mouse model also developed a neurological phenotype with degeneration of the striatum, although this PGC-1α knockout model could properly regulate its body temperature when subjected to cold challenge for most of its lifespan [69]. In 2006, we reported that HD N171-82Q transgenic mice display profound thermoregulatory and metabolic defects [50]. Our discovery of deranged thermoregulation in HD mice led us to evaluate the PGC-1α pathway in the brain and the periphery of these HD mice and to survey PGC- 1α-regulated target genes in the striatum of HD patients. We documented altered mitochondrial function in brown adipose tissue from HD N171-82Q mice and noted that the expression levels of PGC-1α target genes, whose protein products mediate oxidative metabolism in the mitochondria, were significantly reduced [50]. When we analyzed the expression levels of PGC-1α target genes in the striatum of HD patients by gene set enrichment analysis (GSEA) of microarray data, we observed significant reductions in 24 of these 26 PGC-1α target genes and confirmed these findings by RT-PCR (Figure 3). Thus, reduced expression and function of PGC-1α and its targets may be central to HD striatal degeneration, and PGC-1α transcription interference may provide a crucial link between two established aspects of HD molecular pathology: transcription dysregulation and mitochondrial dysfunction.

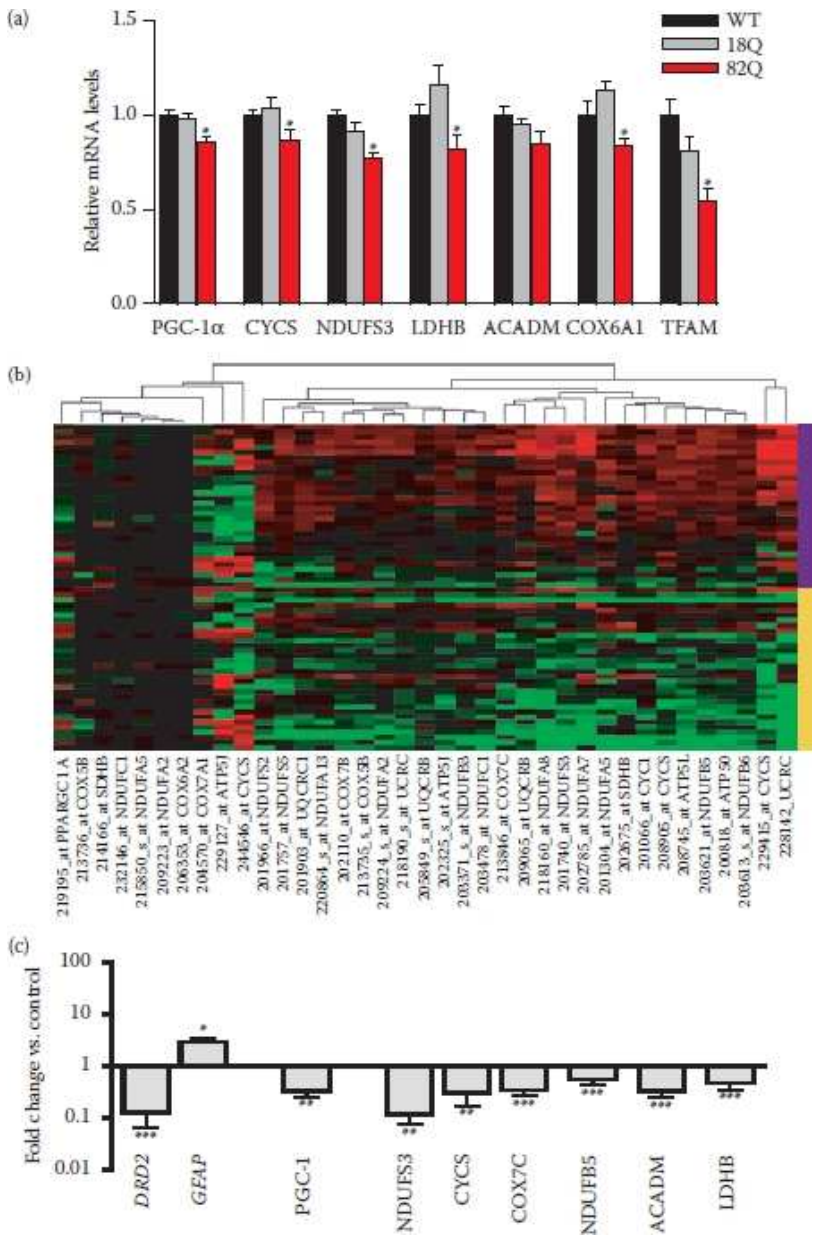

Figure 3: PGC-1α transcription interference in HD mice and HD patients. (a) Real-time RT-PCR analysis of striatal RNAs from HD 82Q mice (red), 18Q mice (gray), and wild-type mice (black) reveals decreased mitochondrial gene expression in the HD mouse model. (b) Microarray expression analysis of PGC-1α- regulated genes in human caudate. Here we see a heat map comparing the caudate nucleus expression of 26 PGC-1α target genes for 32 Grade 0–2 HD patients (adjacent to gold bar) and 32 matched controls (adjacent to blue bar). Most PGC-1α target genes are down-regulated. (c) Confirmation of expression reduction of PGC-1α-regulated genes in human caudate. We measured RNA expression levels for six PGC-1α targets (NDUFS3, CYCS, COX7C, NDUFB5, ACADM, and LDHB), PGC-1α, and two control genes (*GFAP* and *DRD2*). In this way, we confirmed significant expression reductions in PGC-1α targets and detected reduced PGC-1α in human HD striatum from early-grade patients. Statistical comparisons were performed with the *t*-test (*, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.0005$). (From Weydt, P., *Cell Metab* 4, 349, 2006. With permission.)

Neurotrophic Factors

Neurotrophic factors are signaling molecules that mediate important physiological processes in the CNS and peripheral nervous system (PNS). The paracrine or autocrine effects are transduced through membrane receptor tyrosine kinases that mediate a number of phosphorylation events, culminating in *de novo* transcription in the nucleus. These neurotrophic factor-induced changes in gene expression regulate calcium homeostasis, modulate synaptic efficiency, and promote neuron survival. BDNF is a member of the neurotrophic factor family that has been strongly implicated in the pathogenesis of HD. In postmortem HD brains, levels of this neurotrophic factor are decreased in the striatum but not in all the cortical samples analyzed 70, 71. A recent report also noted that serum levels of BDNF are lower in HD patients [72]. Loss of BDNF has been noted in a number of mouse and cellular models for HD [73]. In a study where the level of BDNF was genetically modulated in mice, strong evidence for a role of BDNF in HD pathogenesis emerged, as earlier disease onset was observed in HD mice that were heterozygous-null for BDNF gene dosage [74]. Indeed, when BDNF expression was ablated in cortical pyramidal cells, age-associated dendrite degeneration, followed by loss of medium-sized spiny neurons, occurred [75]—a pattern of results that closely mimics HD striatal degeneration. Microarray analysis of HD transgenic mice yields a pattern of gene expression alterations that closely parallels the gene expression alterations observed in BDNF knockout mice [76], lending further support to the importance of BDNF loss of function in HD pathogenesis.

Although BDNF immunoreactivity is rather intense in the striatum, the BDNF transcript is barely detectable there, especially compared with other regions of the CNS (such as the hippocampus and neocortex), where both BDNF mRNA and protein levels are very high. Striatal BDNF originates in corticostriatal projection neurons that deliver the signaling molecule to medium-sized spiny neurons via anterograde transport (Figure 2.4). Experimental evidence points to two possible molecular mechanisms that impair BDNF production in the corticostriatal pathway: (1) dysregulation of BDNF transcription may result from mutant htt-mediated neural sequestration of REST/NRSF, a repressor that resides in the cytosol with normal htt [75]; and (2) a decrease in striatal BDNF could reflect the disruption of anterograde axonal transport by polyQ-expanded htt. Normal htt has been shown to bind HAP1 and $p150^{Glued}$ to mediate axonal transport of BDNF, but this process may be disrupted when mutant htt is present [71]. Whatever the mechanism, there can be little doubt that impaired cortical BDNF release would deleteriously impact striatal neuron survival. However, other pathological factors may compromise the prosurvival function of this neurotrophic factor. For example, reduced corticostriatal expression of TrkB, the BDNF receptor tyrosine kinase, has been observed in the brains of HD patients and was also found in two different HD mouse models [77]. Loss of this signal transduction receptor would negatively impact the target cell and the presynaptic neuron because BDNF has both autocrine and paracrine effects (Figure 4).

Cytoskeletal Defects and Axonal Transport

Neurons have a unique problem because of their unusual cellular geometry and specialized cellular morphology. The cell body, where gene transcription and most protein translation occur, is usually a considerable distance from the synaptic terminals, as a single axon can be up to a meter long in humans and extend much further in larger mammals. Axons not only propagate electrical signals throughout the cell, they also serve as the main transport corridor for proteins and other metabolic components needed to maintain proper synaptic function. Hence a two-way transport system exists whose purpose is to shuttle ion channel components, membrane receptors, synaptic vesicle precursors, mitochondria, and signaling molecules, including neurotrophic factors and peptide neurotransmitters. The process is microtubule dependent and is powered by kinesin and dynein family members. Cargo is conveyed toward the synaptic terminal in an anterograde direction by kinesins, whereas transport toward the soma by dyneins occurs in a retrograde orientation 78, 79. Disruption of axonal transport leads to aggregation of accumulated cargo, resulting in neuronal dysfunction and degeneration [reviewed in 78–80. Charcot-Marie-Tooth type 2A1 (CMT2A1) and hereditary spastic paraplegia type 10 (SPG10) result from mutations in kinesin subunits, whereas familial and sporadic forms of amyotrophic lateral sclerosis (ALS) and lower motor neuron disease have been linked to mutations in the dynactin subunit $p150^{Glued}$ 81, 82. Defects in axonal transport have also been implicated in AD and in familial ALS type 1 as a result of Cu/Zn superoxide dismutase 1 (SOD1) mutations. The pathogenic protein associated with these neurodegenerative disorders is posited to interact with the transport machinery and perturb normal axonal transport in the disease state.

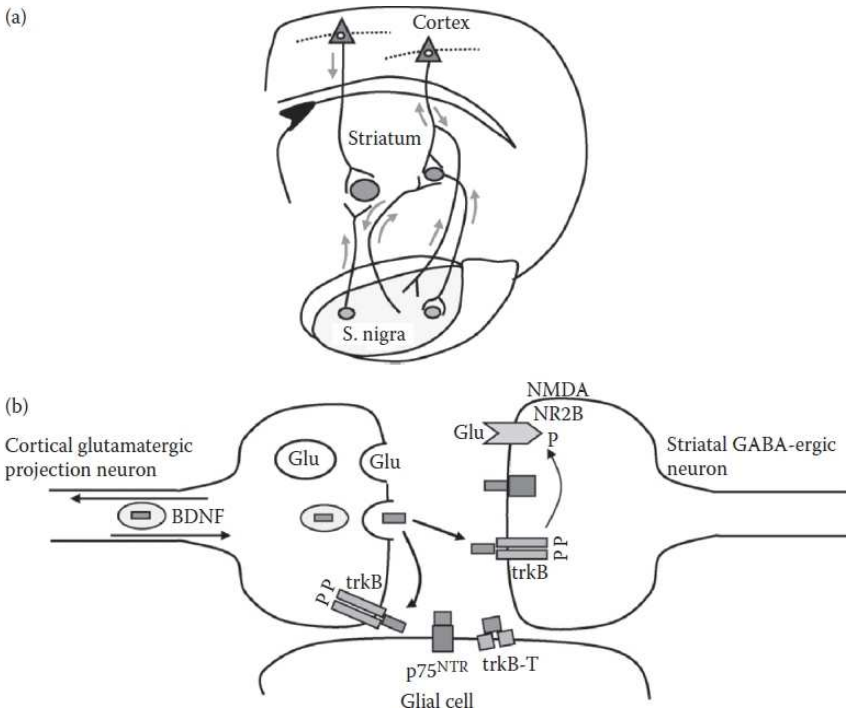

Figure 4: Cortico-striatal delivery and function of BDNF. (a) The cortico-striatal-nigral pathway. Anterograde and retrograde delivery of BDNF is indicated by the orange arrows. (b) Action of BDNF at the cortico-striatal synapse. BDNF vesicles fuse with the presynaptic membrane of the cortical projection neuron, causing BDNF to activate TrkB receptors on the postsynaptic membrane of striatal neurons, TrkB receptors on the presynaptic membrane, and TrkB-T or p75 receptors on glial cells. (From Zuccato, C. and Cattaneo, E., *Prog Neurobiol* 81, 294, 2007. With permission.)

In HD, two mechanisms for the axonal trafficking dysfunction have been proposed [83]. Based on studies in invertebrate models, normal htt was shown to play a role in fast axonal transport. Corollary studies of axonal transport in the face of polyQ-expanded htt or on reduced expression of an htt orthologue revealed marked reductions in the fast axonal transport pathway. The role of htt in axonal transport may depend on a presumed direct physical interaction with the axonal transport machinery. HAP1, a huntingtin interacting protein that associates with BDNF- containing vesicles in the cytosol, facilitates the interaction of htt with $p150^{Glued}$ [71]. The resulting htt/$p150^{Glued}$/HAP1 protein complex interacts with microtubules to facilitate transport of the tethered vesicle along the axon (Figure 2.5). RNA interference-mediated knockdown of htt *or* polyQ expansion of full-length htt can disrupt binding of the HAP1/$p150^{Glued}$ complex to microtubules and motor complexes and thereby can depress BDNF axonal transport and release [71]. The second proposed mechanism of polyQ-expanded htt axonal transport dysfunction posits titration of axonal transport components and/or physical disruption of microtubule-dependent movement of cargo secondary to cytosolic aggregation of mutant htt 84, 85. This steric blockage of axonal transport function in HD could involve the accumulation of autophagosomes whose maturation is blocked during dysfunctional autophagy.

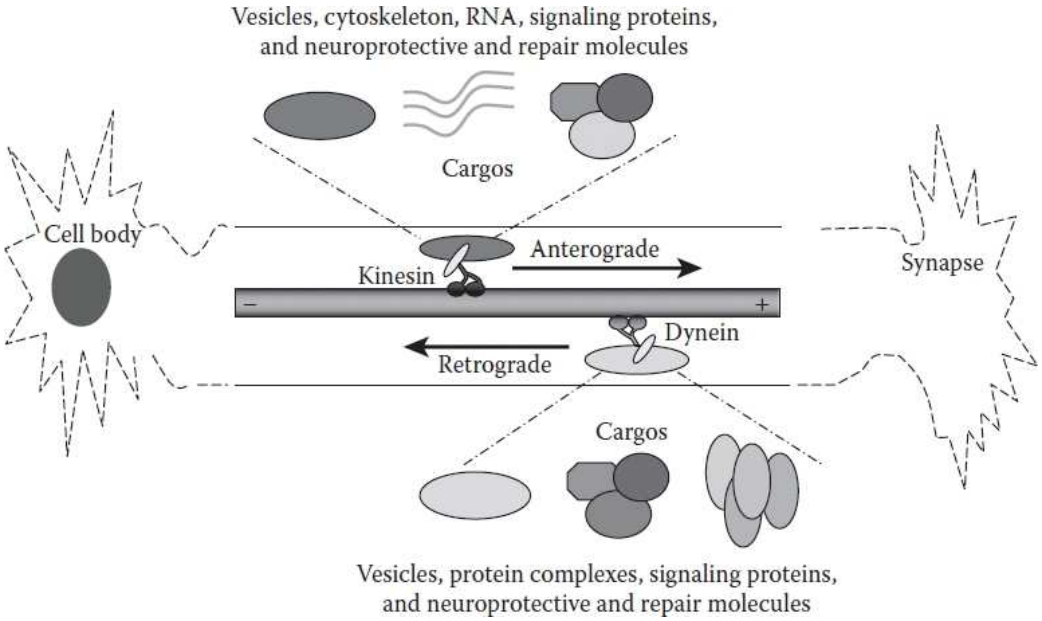

Figure 5: Microtubule-based axonal transport pathways. The plus-end motor protein kinesin transports Golgi-derived vesicles, cytosolic proteins, RNAs, and other molecules anterogradely, whereas the minus-end motor protein dynein is principally responsible for retrograde transport. The huntingtin protein may directly interact with the dynein motor protein complex, suggesting that polyglutamine-expanded huntingtin protein could interfere with the normal functioning of this axonal transport pathway. Alternatively, misfolded huntingtin protein may form aggregates and create blockages in the pathway. (From Gunawardena S. and Goldstein, L. S., *Arch Neurol* 62, 46, 2005. With permission.)

A role for the loss of Huntingtin normal function in HD?

Although polyQ expansion mutations produce a dominant gain-of-function toxicity, gain-of-function and loss-of-function mechanisms are not mutually exclusive in these diseases. There is considerable evidence for a pathogenic role of decreased normal function of disease proteins containing polyQ tract expansions [71, 86–90], including especially the htt protein. In a seminal study in 2000, postnatal elimination of htt protein expression yielded striatal degeneration in conditional knockout mice [91]. In the HD YAC128 mouse, the absence of endogenous huntingtin expression was achieved by crossing the HD YAC128 transgene onto an htt-null background, and this was shown to accelerate HD neuropathology [92, 93]. Similar studies of SBMA, a polyQ disorder with obvious disease protein-dependent non-neural loss-of-function phenotypes, have demonstrated that androgen receptor (AR) YAC100 mice display a more severe neuromuscular disease phenotype when placed on an AR-null background [94].

Given the evidence for a likely contribution of decreased htt normal function to HD pathogenesis, a crucial question is what is the normal function of htt, whose partial loss factors into the corticostriatal degeneration in HD? Different investigators have reported different potential normal htt functions [reviewed in 95]. Various *in vitro* studies have found that cells with depressed levels of htt expression are more susceptible to polyQ toxicity and have argued that htt is an important antiapoptotic factor 96, 97. Dissection of htt's potential antiapoptotic actions has suggested that htt may inhibit procaspase-9 processing, perhaps by preventing the interaction of a proapoptotic initiator with the htt interacting protein-1 [98]. As noted previously, htt may be involved in the transcription regulation of BDNF production [95], and as htt possesses a functional nuclear export signal and has been shown to shuttle into and out of the nucleus [99, 100], htt could be regulating a process/factor that occurs in or originates in the nucleus. That htt is involved in the TFIID and TFIIF transcription factor complex through its interaction with TAFII130 indicates that one of its functions is to be a transcription cofactor [55]. Yet another function of htt is to mediate vesicular transport of BDNF [71], as discussed in the axonal transport section of this chapter. Htt has also

been implicated in synaptic function, as it is highly enriched in synaptic terminals and may be involved in synaptic neurotransmission through its interaction with postsynaptic density protein-95 [101]. Although such divergent roles for htt may seem implausible, one must remember that the htt protein is very large (3,144 amino acids) and is subject to proteolytic cleavage; hence, different protein isoforms of htt may exist in neurons and carry out distinct functions in diverse subcellular compartments. The presence of three clusters of HEAT repeats indicates that one very likely normal function of htt is to serve as a scaffold protein, on which other sets of protein–protein interactions take place. Thus, it is reasonable to envision that htt (and peptide fragments thereof) are performing diverse tasks in neurons and other cell types; however, determining which function(s) are biologically relevant to HD pathogenesis remains a daunting challenge.

Non-Cell Autonomous Degeneration in HD

The major neurodegenerative diseases, including HD, are defined by the neuronal population that is preferentially vulnerable: motor neurons in ALS; cortical neurons in AD; neurons of the substantia nigra in PD; and medium spiny neurons of the striatum (as well as cortical neurons) in HD [102]. In HD and in other neurodegenerative diseases, such as familial ALS, this pronounced selective vulnerability of neurons is an important conundrum, as expression of the offending protein occurs not only in neurons throughout the CNS but also in non-neural cells in the CNS and often throughout the rest of the body [103–105]. A fundamental insight that has emerged from the study of transgenic mouse models of neurodegenerative diseases in general and HD in particular is that the degeneration of neurons can be *non-cell autonomous*—meaning that cell types other than the dying neurons themselves will be critically involved in the degenerative process [106].

In HD, there is compelling evidence that both projecting neurons and surrounding glial cells are essential mediators of mutant htt toxicity. Studies of conditional transgene expression, using the Cre-lox system, have revealed that widespread expression of mutant htt in the brain, extending well beyond the primarily affected corticostriatal neurons, is necessary to produce motor dysfunction and brain pathology in HD mice [106]. Because mutant htt expression results in reduced BDNF gene transcription [48], one specific non-cell autonomous effect of the HD mutation is to deprive medium spiny neurons of their trophic input from cortical neurons. The relative importance of this scenario is underscored by a recent study from Strand et al. [76], in which targeted knockdown of BDNF expression in the forebrain yielded gene expression changes in the striatum that resemble the gene expression changes in human HD. However, non-neural cells are also impacted by the expression of mutant htt. The best evidence for this thesis comes from studies of astrocytes and microglia. (The role of microglia in HD is discussed under "Neuroinflammation in HD: How Important Are Microglia?"). In HD, a role for glutamate excitotoxicity has long been postulated and is supported by extensive literature [50–55]. Most recently, astrocytes derived from R6/2 HD transgenic mice or astrocytes infected with viral vectors encoding mutant huntingtin protein displayed a notable reduction in the glutamate transporter GLT-1/Slc1a2, as well as a limited ability to protect cocultured neurons from glutamatergic insult [56]. In *Drosophila*, the presence of polyQ-expanded peptides prevented the reactive up-regulation of glial glutamate transporters [107]. That polyQ expansions can impair the glutamate transport capacity of astrocytes *in vivo* has also been shown for SCA7, another polyQ repeat disease [108]. A likely contribution of impaired glial glutamate uptake to polyglutamine neurodegeneration implies that modulators of glutamate transporter expression could be vetted as potential therapeutic agents in these diseases. Although impaired glutamate uptake may primarily result from glial dysfunction in HD, it is also worth noting that cell autonomous factors may determine which types of neurons in the striatum are susceptible to the excitotoxic stress, as medium spiny striatal neurons in HD YAC transgenic mice display greater sensitivity to NMDA receptor activation, apparently because of preferential expression of the NR2B subunit in this neuronal population [109]. In the striatum of patients with early HD, evidence also exists for deficient *in vivo* glycolysis, a predominant function of astrocytes, suggesting yet another pathway by which glial dysfunction may contribute to the metabolic abnormalities occurring in HD [110].

For oligodendrocytes, the situation is less clear. White matter changes are a wellrecognized feature of HD pathology. In postmortem HD brains, oligodendrocyte densities are increased independent of the presence of manifest astrocytosis [111, 112]. Also, white matter changes are found in imaging studies of presymptomatic HD patients [113]. Finally, PGC-1$\alpha^{-/-}$ mice, which recapitulate many aspects of the mouse HD phenotype, display significant oligodendrocyte abnormalities [68, 114]. Studies of the effect of mutant htt expression on oligodendrocyte function and their interactions with neurons are lacking; therefore, the role of oligodendrocyte dysfunction in HD pathogenesis is yet to be addressed.

Neuroinflammation in HD: How Important Are Microglia?

Microglia, resembling peripheral tissue macrophages, are the resident immune cells of the CNS and are the primary mediators of neuroinflammation. The past two decades have brought compelling evidence that microglia are important determinants of the microenvironment of the brain and are involved in many acute and chronic neurological diseases, including neurodegeneration [115]. In the unperturbed adult brain, microglia exist as so-called "resting" or "quiescent" microglia [116]. In this state, they have a small cell body with fine, ramified processes and minimal expression of surface antigens. Far from what the terminology of "quiescent" and "resting" would suggest, however, microglia in the healthy CNS are in fact busy "patrolling" the brain for lesions and intruders [117]. In the event of CNS injury, these cells are swiftly activated and therefore heavily involved in the pathology of almost all neurological disorders. The net result of neuroinflammation reflects the outcome of a delicate balance between the neurotoxic and neuroprotective factors that microglia release into their immediate environment [115]. Microglial effects on neurons and glia (astrocytes and oligodendrocytes) are mediated by the release of toxic substances such as nitric oxide, oxygen radicals, glutamate, proteases, and neurotoxic cytokines, as well as protective agents such as growth factors and neuroprotective cytokines [118]. These effects are modulated by cytokines and neurotransmitters released from astrocytes and neurons, giving rise to complex interactions between microglia, neurons, and astrocytes.

A large and growing body of evidence implicates microglia in the pathogenesis of the major sporadic neurodegenerative diseases: AD, PD, and ALS [115, 119]. For example, in AD, activated microglia are found near amyloid plaques and neurofibrillary tangles—abnormalities that are central to the pathogenesis of the disease [115]. In ALS mouse models, microglia are an important determinant of disease progression [120]. Recent evidence also suggests that microglial activation is detrimental for the generation of endogenous stem cells in the brain [121]. Microglial activation involves the up-regulation of the peripheral benzodiazepine binding site on the outer mitochondrial membrane. Through recent advances in brain imaging, it is possible to readily detect and visualize this process *in vivo* in experimental animals and in humans using the ^{11}C-labeled benzodiazepine receptor ligand PK-11195 [122]. Pathological imaging studies in HD show that microglial activation is an integral and remarkably early event in the disease process [123,124]. Postmortem studies have revealed activated microglia mainly in the striatum and cortex of HD brains. The level of activation is a function of the degree of neuronal pathology [123]. As the distribution of the activated microglia extends well into the white matter, axonal pathology rather than neuronal loss may trigger and sustain neuroinflammation in HD. Indeed, PK-11195 imaging studies indicate that microglial activation is present not only in symptomatic HD patients but also in presymptomatic gene carriers [124, 125]. In presymptomatic gene carriers, microglial activation was closely associated anatomically with subclinical striatal dysfunction as measured by raclopide-PET. Striatal PK-11195 binding was also significantly correlated with a shorter "predicted time to symptomatic onset" of HD. In a gene array study of HD brain, mRNA expression revealed generalized activation of inflammatory pathways [126]. Using proteomics approaches, a systemic inflammatory response is also detectable in plasma and CSF of HD patients [127], whereas the transcriptome of peripheral blood did not show consistent inflammation [128]. Although microglial activation is also a feature of transgenic mouse models of HD at the histological level 129, 130], experimental treatments with inhibitors of microglial activation (namely, minocycline) have yielded conflicting results [131–134].

Another pathway central to neurodegeneration is oxidative stress and the concomitant production of reactive oxygen species (ROS). Glial cells likely play a role in propping up the antioxidant defenses of adjacent neurons. In PD, the drug rasagiline was identified based on its ability to block monoamine oxidase B metabolism of dopamine neurotransmitter, but it actually prevents the accumulation of iron in glial cells [135], suggesting that it reduces ROS production. Although glial cells are capable of relieving ROS stress, they could also conceivably be the source of it. In HD, kynurenine 3-monooxygenase was identified as a potent suppressor of huntingtin toxicity in yeast [136]. As the kynurenine pathway drives production of metabolites (3-hydroxy-kynurenine and quinolinic acid) known to increase ROS, and the kynurenine pathway operates in microglia, a model for microglia-induced non-cell autonomous degeneration of HD has been proposed. The availability of small molecules to inhibit the kynurenine 3-monooxygenase enzyme will permit investigators to evaluate efficacy in preclinical trials in HD mice.

In summary, there is compelling evidence from histopathological and imaging studies in human patients and transgenic mouse models that microglial activation is part of HD. In theory, this represents an alluring therapeutic target; however, further research is needed to determine how the complex neuroinflammatory response can be best tweaked to improve the clinical course of HD. Independently of the therapeutic implications, neuroinflammation and microglial activation may provide an opportunity to track disease progression with imaging and other biomarker techniques.

Conclusion

There can be little doubt that we have come a long way since interesting transcript 15 was elevated to the status of the HD gene in 1993. Through considerable funding support from patient groups and many governments, we have attacked the molecular basis of HD pathogenesis with an onslaught of basic research approaches that cut across an amazingly diverse range of disciplines. The net result of this truly global research enterprise has been to provide important insights into how polyQ expansions cause pathology. An important step has been the realization that HD presents a fascinating paradox: the protein misfolding process that is the crux of the pathology in HD is a fundamental feature of practically all neurodegenerative diseases, whereas the explanation for the selective loss of corticostriatal neurons in HD almost assuredly reflects some HD-specific process, most likely stemming from the normal biology of the huntingtin protein. The challenge now is to delineate which cellular pathologies and molecular abnormalities are central to HD onset *or* progression so as to better guide therapeutic efforts. Nuclear transcription interference remains a strong candidate for a principal role in HD pathogenesis, as does proteolytic cleavage. The linkage of transcription interference with mitochondrial dysfunction may prove fundamental. However, axonal transport and neurotrophic factor abnormalities also seem to be strong candidates for contributing to the ultimate demise of the affected neurons in HD and thus could make very valuable therapeutic targets. The relatively recent ascendancy of a role for astrocytes and microglia in HD has become widely accepted in the HD field and more broadly in the entire field of neurodegeneration. This paradigm shift has deepened our understanding of HD pathogenesis and has important implications for devising therapeutic strategies, especially stem cell–based approaches. The progress of the past 15 years has been significant enough that we now stand poised to apply translational tools to identified target pathways and players, in the hope that some of these endeavors will yield meaningful new treatments for this devastating disorder.

ACKNOWLEDGMENTS: We apologize to the many investigators whose work could not be discussed in this paper because of space limitations. We also thank the Hereditary Disease Foundation and CHDI Foundation, Inc. for their support of our HD research program, as well as for their encouragement to all of us in the HD and polyglutamine disease field.

References

1. Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell. 1993;72:971–83.
2. Zoghbi HY, Orr HT. Glutamine repeats and neurodegeneration. Annu Rev Neurosci. 2000;23:217–47.
3. Trottier Y, Lutz Y, Stevanin G, Imbert G, Devys D, Cancel G, et al. Polyglutamine expansion as a pathological epitope in Huntington's disease and four dominant cerebellar ataxias. Nature. 1995;378:403–06.
4. Paulson HL, Perez MK, Trottier Y, Trojanowski JQ, Subramony SH, Das SS, et al. Intranuclear inclusions of expanded polyglutamine protein in spinocerebellar ataxia type 3. Neuron. 1997;19:333–44.
5. Davies SW, Turmaine M, Cozens BA, DiFiglia M, Sharp AH, Ross CA, et al. Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. Cell. 1997;90:537–48.
6. DiFiglia M, Sapp E, Chase KO, Davies SW, Bates GP, Vonsattel JP, et al. Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science. 1997;277:1990–93.
7. Scherzinger E, Lurz R, Turmaine M, Mangiarini L, Hollenbach B, Hasenbank R, et al. Huntingtin-encoded polyglutamine expansions form amyloid-like protein aggregates in vitro and in vivo. Cell. 1997;90:549–58.
8. Cummings CJ, Reinstein E, Sun Y, Antalffy B, Jiang Y, Ciechanover A, et al. Mutation of the E6-AP ubiquitin ligase reduces nuclear inclusion frequency while accelerating polyglutamine-induced pathology in SCA1 mice. Neuron. 1999;24:879–92.
9. Hodgson JG, Agopyan N, Gutekunst CA, Leavitt BR, LePiane F, Singaraja R, et al. A YAC mouse model for Huntington's disease with full-length mutant huntingtin, cytoplasmic toxicity, and selective striatal neurodegeneration. Neuron. 1999;23:181–92.
10. Ross CA, Poirier MA. Protein aggregation and neurodegenerative disease. Nat Med. 2004 10 Suppl:S10–17.
11. Poirier MA, Li H, Macosko J, Cai S, Amzel M, Ross CA. Huntingtin spheroids and protofibrils as precursors in polyglutamine fibrilization. J Biol Chem. 2002;277:41032–37.
12. Wacker JL, Zareie MH, Fong H, Sarikaya M, Muchowski PJ. Hsp70 and Hsp40 attenuate formation of spherical and annular polyglutamine oligomers by partitioning monomer. Nat Struct Mol Biol. 2004;11:1215–22.
13. Arrasate M, Mitra S, Schweitzer ES, Segal MR, Finkbeiner S. Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. Nature. 2004;431:805–10.

14. Sherman MY, Goldberg AL. Cellular defenses against unfolded proteins: a cell biologist thinks about neurodegenerative diseases. Neuron. 2001;29:15–32.
15. Bukau B, Weissman J, Horwich A. Molecular chaperones and protein quality control. Cell. 2006;125:443–51.
16. Sherman MY, Goldberg AL. Cellular defenses against unfolded proteins: a cell biologist thinks about neurodegenerative diseases. Neuron. 2001;29:15–32.
17. Bence NF, Sampat RM, Kopito RR. Impairment of the ubiquitin-proteasome system by protein aggregation. Science. 2001;292:1552–55.
18. Holmberg CI, Staniszewski KE, Mensah KN, Matouschek A, Morimoto RI. Inefficient degradation of truncated polyglutamine proteins by the proteasome. Embo J. 2004;23:4307–18.
19. Venkatraman P, Wetzel R, Tanaka M, Nukina N, Goldberg AL. Eukaryotic proteasomes cannot digest polyglutamine sequences and release them during degradation of polyglutamine-containing proteins. Mol Cell. 2004;14:95–104.
20. Shintani T, Klionsky DJ. Autophagy in health and disease: a double-edged sword. Science. 2004;306:990–95.
21. Hara T, Nakamura K, Matsui M, Yamamoto A, Nakahara Y, Suzuki-Migishima R, et al. Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. Nature. 2006;441:885–89.
22. Komatsu M, Waguri S, Chiba T, Murata S, Iwata J, Tanida I, et al. Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature. 2006;441:880–84.
23. Ravikumar B, Acevedo-Arozena A, Imarisio S, Berger Z, Vacher C, O'Kane CJ, et al. Dynein mutations impair autophagic clearance of aggregate-prone proteins. Nat Genet. 2005;37:771–76.
24. Ravikumar B, Vacher C, Berger Z, Davies JE, Luo S, Oroz LG, et al. Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. Nat Genet. 2004;36:585–95.
25. DiFiglia M. Huntingtin fragments that aggregate go their separate ways. Mol Cell. 2002;10:224–25.
26. Mangiarini L, Sathasivam K, Seller M, Cozens B, Harper A, Hetherington C, et al. Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. Cell. 1996;87:493–506.
27. Gafni J, Ellerby LM. Calpain activation in Huntington's disease. J Neurosci. 2002;22:4842–49.
28. Goldberg YP, Nicholson DW, Rasper DM, Kalchman MA, Koide HB, Graham RK, et al. Cleavage of huntingtin by apopain, a proapoptotic cysteine protease, is modulated by the polyglutamine tract. Nat Genet. 1996;13:442–49.
29. Wellington CL, Ellerby LM, Gutekunst CA, Rogers D, Warby S, Graham RK, et al. Caspase cleavage of mutant huntingtin precedes neurodegeneration in Huntington's disease. J Neurosci. 2002;22:7862–72.
30. Ross CA. Polyglutamine pathogenesis: emergence of unifying mechanisms for Huntington's disease and related disorders. Neuron. 2002;35:819–22.
31. Slow EJ, Graham RK, Osmand AP, Devon RS, Lu G, Deng Y, et al. Absence of behavioral abnormalities and neurodegeneration in vivo despite widespread neuronal huntingtin inclusions. Proc Natl Acad Sci U S A. 2005;102:11402–07.
32. Graham RK, Deng Y, Slow EJ, Haigh B, Bissada N, Lu G, et al. Cleavage at the caspase-6 site is required for neuronal dysfunction and degeneration due to mutant huntingtin. Cell. 2006;125:1179–91.
33. Sisodia SS. Nuclear inclusions in glutamine repeat disorders: are they pernicious, coincidental, or beneficial? Cell. 1998;95:1–4.
34. La Spada AR, Taylor JP. Polyglutamines placed into context. Neuron. 2003;38:681–84.
35. Bannister AJ, Kouzarides T. The CBP co-activator is a histone acetyltransferase. Nature. 1996;384:641–43.
36. Ogryzko VV, Schiltz RL, Russanova V, Howard BH, Nakatani Y. The transcriptional coactivators p300 and CBP are histone acetyltransferases. Cell. 1996;87:953–59.
37. Cong SY, Pepers BA, Evert BO, Rubinsztein DC, Roos RA, van Ommen GJ, et al. Mutant huntingtin represses CBP, but not p300, by binding and protein degradation. Mol Cell Neurosci. 2005;30:560–71.
38. Nucifora FC Jr, Sasaki M, Peters MF, Huang H, Cooper JK, Yamada M, et al. Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity. Science. 2001;291:2423–28.
39. Steffan JS, Kazantsev A, Spasic-Boskovic O, Greenwald M, Zhu YZ, Gohler H, et al. The Huntington's disease protein interacts with p53 and CREB-binding protein and represses transcription. Proc Natl Acad Sci U S A. 2000;97:6763–68.
40. Ferrante RJ, Kubilus JK, Lee J, Ryu H, Beesen A, Zucker B, et al. Histone deacetylase inhibition by sodium butyrate chemotherapy ameliorates the neurodegenerative phenotype in Huntington's disease mice. J Neurosci. 2003;23:9418–27.
41. Hockly E, Richon VM, Woodman B, Smith DL, Zhou X, Rosa E, et al. Suberoylanilide hydroxamic acid, a histone deacetylase inhibitor, ameliorates motor deficits in a mouse model of Huntington's disease. Proc Natl Acad Sci U S A. 2003;100:2041–46.
42. Steffan JS, Bodai L, Pallos J, Poelman M, McCampbell A, Apostol BL, et al. Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in Drosophila. Nature. 2001;413:739–43.
43. Dunah AW, Jeong H, Griffin A, Kim YM, Standaert DG, Hersch SM, et al. Sp1 and TAFII130 transcriptional activity disrupted in early Huntington's disease. Science. 2002;296:2238–43.
44. Li SH, Cheng AL, Zhou H, Lam S, Rao M, Li H, et al. Interaction of Huntington disease protein with transcriptional activator Sp1. Mol Cell Biol. 2002;22:1277–87.
45. Mantamadiotis T, Lemberger T, Bleckmann SC, Kern H, Kretz O, Martin Villalba A, et al. Disruption of CREB function in brain leads to neurodegeneration. Nat Genet. 2002;31:47–54.

46. Qiu Z, Norflus F, Singh B, Swindell MK, Buzescu R, Bejarano M, et al. Sp1 is up-regulated in cellular and transgenic models of Huntington disease, and its reduction is neuroprotective. J Biol Chem. 2006;281:16672–80.
47. Ryu H, Lee J, Zaman K, Kubilis J, Ferrante RJ, Ross BD, et al. Sp1 and Sp3 are oxidative stress-inducible, antideath transcription factors in cortical neurons. J Neurosci. 2003;23:3597–606.
48. Zuccato C, Ciammola A, Rigamonti D, Leavitt BR, Goffredo D, Conti L, et al. Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. Science. 2001;293:493–98.
49. Cui L, Jeong H, Borovecki F, Parkhurst CN, Tanese N, Krainc D. Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration. Cell. 2006;127:59–69.
50. Weydt P, Pineda VV, Torrence AE, Libby RT, Satterfield TF, Lazarowski ER, et al. Thermoregulatory and metabolic defects in Huntington's disease transgenic mice implicate PGC-1alpha in Huntington's disease neurodegeneration. Cell Metab. 2006;4:349–62.
51. Kelly DP, Scarpulla RC. Transcriptional regulatory circuits controlling mitochondrial biogenesis and function. Genes Dev. 2004;18:357–68.
52. Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM. A coldinducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell. 1998;92:829–39.
53. Fry CJ, Peterson CL. Transcription. Unlocking the gates to gene expression. Science. 2002;295:1847–48.
54. Landles C, Bates GP. Huntingtin and the molecular pathogenesis of Huntington's disease. Fourth in molecular medicine review series. EMBO Rep. 2004;5:958–63.
55. Zhai W, Jeong H, Cui L, Krainc D, Tjian R. In vitro analysis of huntingtin-mediated transcriptional repression reveals multiple transcription factor targets. Cell. 2005;123:1241–53.
56. Thompson LM. An expanded role for wild-type huntingtin in neuronal transcription. Nat Genet. 2003;35:13–14.
57. Beal MF, Brouillet E, Jenkins BG, Ferrante RJ, Kowall NW, Miller JM, et al. Neurochemical and histologic characterization of striatal excitotoxic lesions produced by the mitochondrial toxin 3-nitropropionic acid. J Neurosci. 1993;13:4181–92.
58. Browne SE, Bowling AC, MacGarvey U, Baik MJ, Berger SC, Muqit MM, et al. Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia. Ann Neurol. 1997;41:646–53.
59. Gu M, Gash MT, Mann VM, Javoy-Agid F, Cooper JM, Schapira AH. Mitochondrial defect in Huntington's disease caudate nucleus. Ann Neurol. 1996;39:385–89.
60. Tabrizi SJ, Cleeter MW, Xuereb J, Taanman JW, Cooper JM, Schapira AH. Biochemical abnormalities and excitotoxicity in Huntington's disease brain. Ann Neurol. 1999;45:25–32.
61. Stoessl AJ, Martin WR, Clark C, Adam MJ, Ammann W, Beckman JH, et al. PET studies of cerebral glucose metabolism in idiopathic torticollis. Neurology. 1986;36:653–57.
62. Harms L, Meierkord H, Timm G, Pfeiffer L, Ludolph AC. Decreased N-acetylaspartate/choline ratio and increased lactate in the frontal lobe of patients with Huntington's disease: a proton magnetic resonance spectroscopy study. J Neurol Neurosurg Psychiatry. 1997;62:27–30.
63. Sawa A, Wiegand GW, Cooper J, Margolis RL, Sharp AH, Lawler JF Jr, et al. Increased apoptosis of Huntington disease lymphoblasts associated with repeat lengthdependent mitochondrial depolarization. Nat Med. 1999;5:1194–98.
64. Panov AV, Gutekunst CA, Leavitt BR, Hayden MR, Burke JR, Strittmatter WJ, et al. Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines. Nat Neurosci. 2002;5:731–36.
65. Puigserver P, Spiegelman BM. Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator. Endocr Rev. 2003;24:78–90.
66. Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, et al. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell. 1999;98:115–24.
67. Vega RB, Huss JM, Kelly DP. The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor alpha in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. Mol Cell Biol. 2000;20:1868–76.
68. Lin J, Wu PH, Tarr PT, Lindenberg KS, St-Pierre J, Zhang CY, et al. Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1alpha null mice. Cell. 2004;119:121–35.
69. Leone TC, Lehman JJ, Finck BN, Schaeffer PJ, Wende AR, Boudina S, et al. PGC-1alpha deficiency causes multi-system energy metabolic derangements: muscle dysfunction, abnormal weight control and hepatic steatosis. PLoS Biol. 2005;3
70. Ferrer I, Goutan E, Marin C, Rey MJ, Ribalta T. Brain-derived neurotrophic factor in Huntington disease. Brain Res. 2000;866:257–61.
71. Gauthier LR, Charrin BC, Borrell-Pages M, Dompierre JP, Rangone H, Cordelieres FP, et al. Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. Cell. 2004;118:127–38.
72. Ciammola A, Sassone J, Cannella M, Calza S, Poletti B, Frati L, et al. Low brain-derived neurotrophic factor (BDNF) levels in serum of Huntington's disease patients. Am J Med Genet B Neuropsychiatr Genet. 2007;144:574–77.
73. Zuccato C, Cattaneo E. Role of brain-derived neurotrophic factor in Huntington's disease. Prog Neurobiol. 2007;81:294–330.
74. Canals JM, Pineda JR, Torres-Peraza JF, Bosch M, Martin-Ibanez R, Munoz MT, et al. Brain-derived neurotrophic factor regulates the onset and severity of motor dysfunction associated with enkephalinergic neuronal degeneration in Huntington's disease. J Neurosci. 2004;24:7727–39.

75. Baquet ZC, Gorski JA, Jones KR. Early striatal dendrite deficits followed by neuron loss with advanced age in the absence of anterograde cortical brain-derived neurotrophic factor. J Neurosci. 2004;24:4250–58.
76. Strand AD, Baquet ZC, Aragaki AK, Holmans P, Yang L, Cleren C, et al. Expression profiling of Huntington's disease models suggests that brain-derived neurotrophic factor depletion plays a major role in striatal degeneration. J Neurosci. 2007;27:11758–68.
77. Gines S, Bosch M, Marco S, Gavalda N, Diaz-Hernandez M, Lucas JJ, et al. Reduced expression of the TrkB receptor in Huntington's disease mouse models and in human brain. Eur J Neurosci. 2006;23:649–58.
78. Duncan JE, Goldstein LS. The genetics of axonal transport and axonal transport disorders. PLoS Genet. 2006;2
79. Roy S, Zhang B, Lee VM, Trojanowski JQ. Axonal transport defects: a common theme in neurodegenerative diseases. Acta Neuropathol. 2005;109:5–13.
80. Gerdes JM, Katsanis N. Microtubule transport defects in neurological and ciliary disease. Cell Mol Life Sci. 2005;62:1556–70.
81. Munch C, Sedlmeier R, Meyer T, Homberg V, Sperfeld AD, Kurt A, et al. Point mutations of the p150 subunit of dynactin (DCTN1) gene in ALS. Neurology. 2004;63:724–26.
82. Schymick JC, Talbot K, Traynor BJ. Genetics of sporadic amyotrophic lateral sclerosis. Hum Mol Genet. 2007:R233–42. 16 Spec No. 2.
83. Gunawardena S, Goldstein LS. Polyglutamine diseases and transport problems: deadly traffic jams on neuronal highways. Arch Neurol. 2005;62:46–51.
84. Lee WC, Yoshihara M, Littleton JT. Cytoplasmic aggregates trap polyglutamine-containing proteins and block axonal transport in a Drosophila model of Huntington's disease. Proc Natl Acad Sci U S A. 2004;101:3224–29.
85. Trushina E, Dyer RB, Badger JD 2nd, Ure D, Eide L, Tran DD, et al. Mutant huntingtin impairs axonal trafficking in mammalian neurons in vivo and in vitro. Mol Cell Biol. 2004;24:8195–209.
86. Li F, Macfarlan T, Pittman RN, Chakravarti D. Ataxin-3 is a histone-binding protein with two independent transcriptional corepressor activities. J Biol Chem. 2002;277:45004–12.
87. Palhan VB, Chen S, Peng GH, Tjernberg A, Gamper AM, Fan Y, et al. Polyglutamine-expanded ataxin-7 inhibits STAGA histone acetyltransferase activity to produce retinal degeneration. Proc Natl Acad Sci U S A. 2005;102:8472–77.
88. Chai Y, Berke SS, Cohen RE, Paulson HL. Poly-ubiquitin binding by the polyglutamine disease protein ataxin-3 links its normal function to protein surveillance pathways. J Biol Chem. 2004;279:3605–11.
89. Donaldson KM, Li W, Ching KA, Batalov S, Tsai CC, Joazeiro CA. Ubiquitin-mediated sequestration of normal cellular proteins into polyglutamine aggregates. Proc Natl Acad Sci U S A. 2003;100:8892–97.
90. Satterfield TF, Jackson SM, Pallanck LJ. A Drosophila homolog of the polyglutamine disease gene SCA2 is a dosage-sensitive regulator of actin filament formation. Genetics. 2002;162:1687–702.
91. Dragatsis I, Levine MS, Zeitlin S. Inactivation of Hdh in the brain and testis results in progressive neurodegeneration and sterility in mice. Nat Genet. 2000;26:300–06.
92. Leavitt BR, Guttman JA, Hodgson JG, Kimel GH, Singaraja R, Vogl AW, et al. Wild-type huntingtin reduces the cellular toxicity of mutant huntingtin in vivo. Am J Hum Genet. 2001;68:313–24.
93. Van Raamsdonk JM, Pearson J, Rogers DA, Bissada N, Vogl AW, Hayden MR, et al. Loss of wild-type huntingtin influences motor dysfunction and survival in the YAC128 mouse model of Huntington disease. Hum Mol Genet. 2005;14:1379–92.
94. Thomas PS Jr, Fraley GS, Damien V, Woodke LB, Zapata F, Sopher BL, et al. Loss of endogenous androgen receptor protein accelerates motor neuron degeneration and accentuates androgen insensitivity in a mouse model of X-linked spinal and bulbar muscular atrophy. Hum Mol Genet. 2006;15:2225–38.
95. Cattaneo E, Zuccato C, Tartari M. Normal huntingtin function: an alternative approach to Huntington's disease. Nat Rev Neurosci. 2005;6:919–30.
96. Leavitt BR, Raamsdonk JM, Shehadeh J, Fernandes H, Murphy Z, Graham RK, et al. Wild-type huntingtin protects neurons from excitotoxicity. J Neurochem. 2006;96:1121–29.
97. Rigamonti D, Bauer JH, De-Fraja C, Conti L, Sipione S, Sciorati C, et al. Wild-type huntingtin protects from apoptosis upstream of caspase-3. J Neurosci. 2000;20:3705–13.
98. Gervais FG, Singaraja R, Xanthoudakis S, Gutekunst CA, Leavitt BR, Metzler M, et al. Recruitment and activation of caspase-8 by the Huntingtin-interacting protein Hip-1 and a novel partner Hippi. Nat Cell Biol. 2002;4:95–105.
99. Truant R, Atwal RS, Burtnik A. Nucleocytoplasmic trafficking and transcription effects of huntingtin in Huntington's disease. Prog Neurobiol. 2007;83:211–27.
100. Xia J, Lee DH, Taylor J, Vandelft M, Truant R. Huntingtin contains a highly conserved nuclear export signal. Hum Mol Genet. 2003;12:1393–403.
101. Sun Y, Savanenin A, Reddy PH, Liu YF. Polyglutamine-expanded huntingtin promotes sensitization of N-methyl-D-aspartate receptors via post-synaptic density 95. J Biol Chem. 2001;276:24713–18.
102. Martin JB. Molecular basis of the neurodegenerative disorders. N Engl J Med. 1999;340:1970–80.
103. Li SH, Schilling G, Young WS 3rd, Li XJ, Margolis RL, Stine OC, et al. Huntington's disease gene (IT15) is widely expressed in human and rat tissues. Neuron. 1993;11:985–93.
104. Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A, et al. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature. 1993;362:59–62.

105. Strong TV, Tagle DA, Valdes JM, Elmer LW, Boehm K, Swaroop M, et al. Widespread expression of the human and rat Huntington's disease gene in brain and nonneural tissues. Nat Genet. 1993;5:259–65.
106. Lobsiger CS, Cleveland DW. Glial cells as intrinsic components of non-cell-autonomous neurodegenerative disease. Nat Neurosci. 2007;10:1355–60.
107. Lievens JC, Rival T, Iche M, Chneiweiss H, Birman S. Expanded polyglutamine peptides disrupt EGF receptor signaling and glutamate transporter expression in Drosophila. Hum Mol Genet. 2005;14:713–24.
108. Custer SK, Garden GA, Gill N, Rueb U, Libby RT, Schultz C, et al. Bergmann glia expression of polyglutamine-expanded ataxin-7 produces neurodegeneration by impairing glutamate transport. Nat Neurosci. 2006;9:1302–11.
109. Zeron MM, Hansson O, Chen N, Wellington CL, Leavitt BR, Brundin P, et al. Increased sensitivity to N-methyl-D-aspartate receptor-mediated excitotoxicity in a mouse model of Huntington's disease. Neuron. 2002;33:849–60.
110. Powers WJ, Videen TO, Markham J, McGee-Minnich L, Antenor-Dorsey JV, Hershey T, et al. Selective defect of in vivo glycolysis in early Huntington's disease striatum. Proc Natl Acad Sci U S A. 2007;104:2945–49.
111. Gomez-Tortosa E, MacDonald ME, Friend JC, Taylor SA, Weiler LJ, Cupples LA, et al. Quantitative neuropathological changes in presymptomatic Huntington's disease. Ann Neurol. 2001;49:29–34.
112. Myers RH, Vonsattel JP, Paskevich PA, Kiely DK, Stevens TJ, Cupples LA, et al. Decreased neuronal and increased oligodendroglial densities in Huntington's disease caudate nucleus. J Neuropathol Exp Neurol. 1991;50:729–42.
113. Thieben MJ, Duggins AJ, Good CD, Gomes L, Mahant N, Richards F, et al. The distribution of structural neuropathology in pre-clinical Huntington's disease. Brain. 2002;125:1815–28.
114. Leone TC, Lehman JJ, Finck BN, Schaeffer PJ, Wende AR, Boudina S, et al. PGC-1alpha deficiency causes multi-system energy metabolic derangements: muscle dysfunction, abnormal weight control and hepatic steatosis. PLoS Biol. 2005;3
115. Wyss-Coray T, Mucke L. Inflammation in neurodegenerative disease—a doubleedged sword. Neuron. 2002;35:419–32.
116. Kreutzberg GW. Microglia: a sensor for pathological events in the CNS. Trends Neurosci. 1996;19:312–18.
117. Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science. 2005;308:1314–18.
118. Hanisch UK. Microglia as a source and target of cytokines. Glia. 2002;40:140–55.
119. Weydt P, Moller T. Neuroinflammation in the pathogenesis of amyotrophic lateral sclerosis. Neuroreport. 2005;16:527–31.
120. Boillee S, Yamanaka K, Lobsiger CS, Copeland NG, Jenkins NA, Kassiotis G, et al. Onset and progression in inherited ALS determined by motor neurons and microglia. Science. 2006;312:1389–92.
121. Monje ML, Toda H, Palmer TD. Inflammatory blockade restores adult hippocampal neurogenesis. Science. 2003;302:1760–65.
122. Banati RB. Visualising microglial activation in vivo. Glia. 2002;40:206–17.
123. Sapp E, Kegel KB, Aronin N, Hashikawa T, Uchiyama Y, Tohyama K, et al. Early and progressive accumulation of reactive microglia in the Huntington disease brain. J Neuropathol Exp Neurol. 2001;60:161–72.
124. Tai YF, Pavese N, Gerhard A, Tabrizi SJ, Barker RA, Brooks DJ, et al. Microglial activation in presymptomatic Huntington's disease gene carriers. Brain. 2007;130:1759–66.
125. Pavese N, Gerhard A, Tai YF, Ho AK, Turkheimer F, Barker RA, et al. Microglial activation correlates with severity in Huntington disease: a clinical and PET study. Neurology. 2006;66:1638–43.
126. Hodges A, Strand AD, Aragaki AK, Kuhn A, Sengstag T, Hughes G, et al. Regional and cellular gene expression changes in human Huntington's disease brain. Hum Mol Genet. 2006;15:965–77.
127. Dalrymple A, Wild EJ, Joubert R, Sathasivam K, Bjorkqvist M, Petersen A, et al. Proteomic profiling of plasma in Huntington's disease reveals neuroinflammatory activation and biomarker candidates. J Proteome Res. 2007;6:2833–40.
128. Runne H, Kuhn A, Wild EJ, Pratyaksha W, Kristiansen M, Isaacs JD, et al. Analysis of potential transcriptomic biomarkers for Huntington's disease in peripheral blood. Proc Natl Acad Sci U S A. 2007;104:14424–29.
129. Ma L, Morton AJ, Nicholson LF. Microglia density decreases with age in a mouse model of Huntington's disease. Glia. 2003;43:274–80.
130. Simmons DA, Casale M, Alcon B, Pham N, Narayan N, Lynch G. Ferritin accumulation in dystrophic microglia is an early event in the development of Huntington's disease. Glia. 2007;55:1074–84.
131. Chen M, Ona VO, Li M, Ferrante RJ, Fink KB, Zhu S, et al. Minocycline inhibits caspase-1 and caspase-3 expression and delays mortality in a transgenic mouse model of Huntington disease. Nat Med. 2000;6:797–801.
132. Hersch S, Fink K, Vonsattel JP, Friedlander RM. Minocycline is protective in a mouse model of Huntington's disease. Ann Neurol. 2003;54:842–43. author reply.
133. Smith DL, Woodman B, Mahal A, Sathasivam K, Ghazi-Noori S, Lowden PA, et al. Minocycline and doxycycline are not beneficial in a model of Huntington's disease. Ann Neurol. 2003;54:186–96.
134. Stack EC, Smith KM, Ryu H, Cormier K, Chen M, Hagerty SW, et al. Combination therapy using minocycline and coenzyme Q10 in R6/2 transgenic Huntington's disease mice. Biochim Biophys Acta. 2006;1762:373–80.
135. Youdim MB, Fridkin M, Zheng H. Bifunctional drug derivatives of MAO-B inhibitor rasagiline and iron chelator VK-28 as a more effective approach to treatment of brain ageing and ageing neurodegenerative diseases. Mech Ageing Dev. 2005;126:317–26.
136. Giorgini F, Guidetti P, Nguyen Q, Bennett SC, Muchowski PJ. A genomic screen in yeast implicates kynurenine 3-monooxygenase as a therapeutic target for Huntington disease. Nat Genet. 2005;37:526–31.